Oxygen supply to cells by the cardiovascular system involves multiple physical and chemical processes that aim to satisfy fluctuating metabolic demand. Regulation mechanisms range from increased heart rate to minute adaptations in the microvasculature. The challenges and limitations of experimental studies in vivo make computational models an invaluable complement. In this thesis, oxygen transport from capillaries to tissue is investigated using a new numerical model that is tailored for validation with experimental data. On this basis, theoretical analyses of tissue oxygenation and intravascular oxygen distribution are conducted with applications to blood flow regulation mechanisms and heterogeneity in microvascular networks.

The computational model developed here employs moving red blood cells (RBCs) in the frame of reference of the tissue. This key feature enables direct result comparison to oxygen measurements in capillaries and tissue. Thus, the first model validation with micrometric-resolution measurements of oxygen partial pressure (PO₂) in vivo could be performed. This novel technique is used to describe the complex relation between hemoglobin saturation and plasma PO₂ which manifests itself by the presence of erythrocyte-associated transients.

Dynamic regulation mechanisms of microvascular blood flow in the brain were modeled. The effect of individual pericyte-induced capillary dilations was investigated in a simple capillary network. Although a slight increase in tissue PO₂ was observed, multiple dilations need to simultaneously occur to produce a response comparable to the effect of arteriolar dilations. The dependence of tissue oxygenation on hematocrit and blood velocity was also described. Based on analytical tools supported by the computational model, the relative influence of hematocrit was demonstrated to be stronger.

The complex topology of microvascular networks entails a certain degree of heterogeneity in oxygen supply. Excessive variations in RBC transit times through the microvasculature have been associated to conditions such as diabetes, Alzheimer's disease and hypertension. Their relation with hemoglobin saturation heterogeneity was modeled based on the computational and analytical tools developed here. Diffusive interaction within and between capillaries was shown to lead to a considerable reduction of hemoglobin saturation heterogeneity at the scale of neighboring capillaries. These findings may substantially affect the interpretation of RBC transit time measurements in health and disease.
Cover: simulation of oxygen transport from a microvascular network reconstructed from the mouse somatosensory cortex. See also Fig. 4.3 on p. 92.

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Computational Modeling of Oxygen Transport in the Microcirculation
—
From an Experiment-Based Model to Theoretical Analyses

A thesis submitted to attain the degree of
DOCTOR OF SCIENCES of ETH ZURICH
(Dr. sc. ETH Zurich)

presented by
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2017
Abstract

Oxygen supply to cells by the cardiovascular system involves multiple physical and chemical processes that aim to satisfy fluctuating metabolic demand. Regulation mechanisms range from increased heart rate to minute adaptations in the microvasculature. The challenges and limitations of experimental studies in vivo make computational models an invaluable complement. In this thesis, oxygen transport from capillaries to tissue is investigated using a new numerical model that is tailored for validation with experimental data. On this basis, theoretical analyses of tissue oxygenation and intravascular oxygen distribution are conducted with applications to blood flow regulation mechanisms and heterogeneity in microvascular networks.

The computational model developed here employs moving red blood cells (RBCs) in the frame of reference of the tissue. This key feature enables direct result comparison to oxygen measurements in capillaries and tissue. Thus, the first model validation with micrometric-resolution measurements of oxygen partial pressure (PO$_2$) in vivo could be performed. This novel technique is used to describe the complex relation between hemoglobin saturation and plasma PO$_2$ which manifests itself by the presence of erythrocyte-associated transients.

Dynamic regulation mechanisms of microvascular blood flow in the brain were modeled. The effect of individual pericyte-induced capillary dilations was investigated in a simple capillary network. Although a slight increase in tissue PO$_2$ was observed, multiple dilations need to simultaneously occur to produce a response comparable to the effect of arteriolar dilations. The dependence of tissue oxygenation on hematocrit and blood velocity was also described. Based on analytical tools supported by the computational model, the relative influence of hematocrit was demonstrated to be stronger.

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hemoglobin saturation heterogeneity was modeled based on the computational and analytical tools developed here. Diffusive interaction within and between capillaries was shown to lead to a considerable reduction of hemoglobin saturation heterogeneity at the scale of neighboring capillaries. These findings may substantially affect the interpretation of RBC transit time measurements in health and disease.

The state-of-the-art technique for high-resolution oxygen measurements in vivo is currently two-photon phosphorescence lifetime microscopy (2PLM). To address uncertainties related to photochemical consumption of oxygen, a numerical model for 2PLM that includes oxygen diffusion and removal by organic molecules was devised. The ensuing potential underestimation of $P_O_2$ can be quantified, thereby allowing to choose experimental parameters to avoid systematic errors and photodamage of the region of interest. This model, along with the rest of this thesis, considerably improves the synergy between theoretical modeling and experimental studies in the context of microvascular oxygen transport.
Résumé


Le modèle mathématique développé ici emploie des globules rouges mobiles dans le référentiel du tissu. Cette propriété essentielle permet de directement comparer les résultats à des mesures d’oxygène dans les capillaires et le tissu. Ainsi, la première validation d’un modèle à l’aide de mesures de la pression partielle d’oxygène ($P_{O_2}$) avec une résolution micrométrique a pu être effectuée. Cette technique innovante est employée pour décrire la relation complexe entre la saturation de l’hémoglobine et la $P_{O_2}$ dans le plasma qui se manifeste par la présence de fluctuations associées aux érythrocytes.

Des mécanismes dynamiques de régulation du débit sanguin à travers les microvaisseaux du cerveau ont été modélisés. Les effets de la dilatation de capillaires causée par des pérycytes ont été étudiés dans un réseau simple de capillaires. Bien qu’une légère augmentation de la $P_{O_2}$ dans le tissu ait été observée, de multiples dilatations doivent survenir simultanément afin de produire une réponse comparable à l’effet de la dilatation d’une artériole. La dépendance de l’oxygénation vis-à-vis de l’hématocrite et de la vitesse du sang est également décrite. Une analyse mathématique, elle-même renforcée par le modèle numérique, démontre que l’influence relative de l’hématocrite
La topologie complexe des réseaux microvasculaires provoque un certain niveau d’hétérogénéité dans le transport d’oxygène. Les fluctuations excessives du temps de transit des globules rouges à travers le système microvasculaire ont été précédemment reliées à des affections telles que le diabète, la maladie d’Alzheimer et l’hypertension. Leur relation avec la saturation de l’hémoglobine a été modélisée sur la base des outils numériques et analytiques développés dans cette thèse. L’interaction diffusive entre capillaires provoque une importante réduction de l’hétérogénéité de la saturation d’hémoglobine à l’échelle de capillaires voisins. Ces conclusions pourraient considérablement affecter l’interprétation des temps de transit de globules rouges mesurés expérimentalement dans des sujets sains et malades.

La technique de pointe pour mesurer la PO$_2$ in vivo avec haute résolution est actuellement la microscopie biphotonique à durée de vie de phosphorescence (2PLM). Dans le but d’investiguer les incertitudes liées à la consommation photochimique d’oxygène, un modèle numérique de la 2PLM qui inclut la diffusion d’oxygène et sa consommation par des molécules organiques a été développé. La sous-estimation de la PO$_2$ qui s’ensuit peut être quantifiée, ce qui permet de choisir les paramètres expérimentaux afin d’éviter des erreurs systématiques et des lésions photoniques de la région étudiée. Ce modèle, à l’instar du reste de cette thèse, améliore considérablement la synergie entre modèles théoriques et études expérimentales dans le contexte du transport d’oxygène dans les microvaisseaux.
In the course of this thesis, I have received support from many people, the most important of whom I wish to mention here.

I would like to sincerely thank **Prof. Patrick Jenny** for supervising my PhD thesis and providing continuous support and guidance. I could benefit from his trust, allowing me to work independently, and his availability whenever I needed advice. Needless to say, many ideas developed in this work stem from him. The great working atmosphere at Institute of Fluid Dynamics and the related good memories that I now have owe much to him. I also wish to thank **Prof. Bruno Weber** who co-supervised this thesis. His guidance and expertise were vital to orient myself in the world of biomedical research. This work would not have been possible without him.

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# Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contents</td>
<td>I</td>
</tr>
<tr>
<td>List of Figures</td>
<td>IV</td>
</tr>
<tr>
<td>List of Tables</td>
<td>VI</td>
</tr>
<tr>
<td>Nomenclature</td>
<td>VII</td>
</tr>
<tr>
<td>1 Introduction</td>
<td>1</td>
</tr>
<tr>
<td>1.1 Basic terminology</td>
<td>1</td>
</tr>
<tr>
<td>1.2 Energy metabolism and oxygen supply</td>
<td>2</td>
</tr>
<tr>
<td>1.3 History of oxygen transport modeling</td>
<td>6</td>
</tr>
<tr>
<td>1.4 Experimental methods</td>
<td>8</td>
</tr>
<tr>
<td>1.5 Open questions</td>
<td>10</td>
</tr>
<tr>
<td>1.6 Outline</td>
<td>11</td>
</tr>
<tr>
<td>2 Numerical modeling of oxygen transport from capillaries</td>
<td>13</td>
</tr>
<tr>
<td>2.1 Introduction</td>
<td>14</td>
</tr>
<tr>
<td>2.2 Finite-volume model with moving red blood cells</td>
<td>16</td>
</tr>
<tr>
<td>2.2.1 Governing equations</td>
<td>16</td>
</tr>
<tr>
<td>2.2.2 Overlapping mesh method</td>
<td>19</td>
</tr>
<tr>
<td>2.2.3 Time integration</td>
<td>22</td>
</tr>
<tr>
<td>2.2.4 Stable advection scheme</td>
<td>25</td>
</tr>
<tr>
<td>2.2.5 Reconstructed capillary networks</td>
<td>28</td>
</tr>
<tr>
<td>2.2.6 Model parameters</td>
<td>31</td>
</tr>
<tr>
<td>2.3 Results</td>
<td>33</td>
</tr>
<tr>
<td>2.3.1 Erythrocyte-associated transients</td>
<td>33</td>
</tr>
<tr>
<td>2.3.2 Capillary dilations</td>
<td>38</td>
</tr>
<tr>
<td>2.4 Discussion</td>
<td>41</td>
</tr>
<tr>
<td>2.5 Conclusion</td>
<td>47</td>
</tr>
</tbody>
</table>
### 3 The relative influence of hematocrit and RBC velocity on tissue oxygenation

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>Introduction</td>
<td>50</td>
</tr>
<tr>
<td>3.2</td>
<td>Methods</td>
<td>51</td>
</tr>
<tr>
<td>3.2.1</td>
<td>Ordinary differential equation model</td>
<td>51</td>
</tr>
<tr>
<td>3.2.2</td>
<td>Model parameters</td>
<td>56</td>
</tr>
<tr>
<td>3.3</td>
<td>Results</td>
<td>57</td>
</tr>
<tr>
<td>3.3.1</td>
<td>Tissue oxygenation</td>
<td>57</td>
</tr>
<tr>
<td>3.3.2</td>
<td>Intravascular drop in oxygen partial pressure</td>
<td>62</td>
</tr>
<tr>
<td>3.4</td>
<td>Discussion</td>
<td>69</td>
</tr>
<tr>
<td>3.5</td>
<td>Conclusion</td>
<td>76</td>
</tr>
</tbody>
</table>

### 4 The heterogeneity of hemoglobin saturation in capillary networks

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1</td>
<td>Introduction</td>
<td>77</td>
</tr>
<tr>
<td>4.2</td>
<td>Methods</td>
<td>80</td>
</tr>
<tr>
<td>4.2.1</td>
<td>Diffusive interaction models</td>
<td>80</td>
</tr>
<tr>
<td>4.2.1.1</td>
<td>Diffusive interaction between RBCs in a single capillary</td>
<td>81</td>
</tr>
<tr>
<td>4.2.1.2</td>
<td>Diffusive interaction between parallel capillaries</td>
<td>84</td>
</tr>
<tr>
<td>4.2.2</td>
<td>Flow reconstruction algorithm based on sparse measurements</td>
<td>87</td>
</tr>
<tr>
<td>4.2.3</td>
<td>Model parameters</td>
<td>90</td>
</tr>
<tr>
<td>4.2.4</td>
<td>Experimental procedures</td>
<td>92</td>
</tr>
<tr>
<td>4.3</td>
<td>Results</td>
<td>93</td>
</tr>
<tr>
<td>4.3.1</td>
<td>Diffusive interaction between RBCs</td>
<td>93</td>
</tr>
<tr>
<td>4.3.2</td>
<td>Diffusive interaction between parallel capillaries</td>
<td>97</td>
</tr>
<tr>
<td>4.3.3</td>
<td>Reconstructed microvascular networks</td>
<td>104</td>
</tr>
<tr>
<td>4.4</td>
<td>Discussion</td>
<td>108</td>
</tr>
<tr>
<td>4.5</td>
<td>Conclusion</td>
<td>114</td>
</tr>
</tbody>
</table>

### 5 Modeling oxygen measurements with two-photon phosphorescence lifetime microscopy

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.1</td>
<td>Introduction</td>
<td>115</td>
</tr>
<tr>
<td>5.2</td>
<td>Methods</td>
<td>119</td>
</tr>
<tr>
<td>5.2.1</td>
<td>Mathematical model</td>
<td>119</td>
</tr>
<tr>
<td>5.2.2</td>
<td>Numerical method</td>
<td>122</td>
</tr>
<tr>
<td>5.2.3</td>
<td>Model parameters</td>
<td>124</td>
</tr>
<tr>
<td>5.3</td>
<td>Results</td>
<td>126</td>
</tr>
<tr>
<td>5.4</td>
<td>Discussion</td>
<td>133</td>
</tr>
</tbody>
</table>
## Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.5 Conclusion</td>
<td>137</td>
</tr>
<tr>
<td>6 Conclusion and outlook</td>
<td>139</td>
</tr>
<tr>
<td>6.1 Conclusion</td>
<td>139</td>
</tr>
<tr>
<td>6.2 Outlook</td>
<td>140</td>
</tr>
<tr>
<td>Bibliography</td>
<td>143</td>
</tr>
</tbody>
</table>
List of Figures

1.1 Schematic of aerobic cellular respiration .......................... 3
1.2 Heart, lungs and alveoli ........................................... 5
1.3 Brain arteries and microvasculature .............................. 6
1.4 Schematic representation of erythrocyte-associated transients .. 7

2.1 Overlapping meshes for an axisymmetric geometry ............ 20
2.2 Schematic for the improved scheme for the advection term .... 27
2.3 Meshes used in the model extension to capillary networks ...... 29
2.4 Axisymmetric computational domain with moving RBCs ....... 32
2.5 Instantaneous PO$_2$ longitudinal profiles ......................... 34
2.6 Time-averaged PO$_2$ levels on the vessel centerline .......... 35
2.7 Dependence of RBC PO$_2$ and inter-RBC PO$_2$ on linear density 37
2.8 Tissue PO$_2$ for different standard deviations of linear density 37
2.9 Computational domain for the capillary dilation study ........ 40
2.10 Temporal profiles of PO$_2$ with capillary dilation and CBF increase 42
2.11 Oxygen profiles with a dilated capillary and CBF increase .... 42

3.1 Sketches of the computational domains ......................... 57
3.2 Tissue PO$_2$ as a function of LD and RBC velocity .......... 59
3.3 Radial PO$_2$ profiles from the analytical and numerical models 60
3.4 Longitudinal tissue PO$_2$ profiles with and without axial diffusion 61
3.5 Values of the function $Z$ defined in Eq. (3.22) ............ 63
3.6 Nusselt number as a function of LD and RBC velocity ...... 66
3.7 MTC as a function of LD and RBC velocity .................... 67
3.8 Sketch of the plasma region between two cylindrical RBCs . 68
3.9 IVR coefficient for different RBC and capillary radii ........ 69
3.10 Difference between RBC and plasma PO$_2$ .................... 70

4.1 Schematics for RBC diffusive interaction ...................... 82
4.2 Schematics for capillary diffusive interaction ............... 84
4.3 Reconstructed MVNs from the mouse cerebral cortex ....... 92
<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.4</td>
<td>Hemoglobin saturation profiles with alternating inlet values</td>
<td>94</td>
</tr>
<tr>
<td>4.5</td>
<td>Hemoglobin saturation profiles with a uniform distribution of $S_a$</td>
<td>95</td>
</tr>
<tr>
<td>4.6</td>
<td>Relative errors from the RBC diffusive interaction models</td>
<td>96</td>
</tr>
<tr>
<td>4.7</td>
<td>Decay time scale $\tau_{RI}$ for RBC diffusive interaction</td>
<td>97</td>
</tr>
<tr>
<td>4.8</td>
<td>Model coefficient $K_{OS}$ for RBC diffusive interaction</td>
<td>98</td>
</tr>
<tr>
<td>4.9</td>
<td>Hemoglobin saturation profiles in parallel capillaries</td>
<td>99</td>
</tr>
<tr>
<td>4.10</td>
<td>Relative errors from the capillary diffusive interaction models</td>
<td>100</td>
</tr>
<tr>
<td>4.11</td>
<td>Decay time scale $\tau_{CI}$ for capillary diffusive interaction</td>
<td>101</td>
</tr>
<tr>
<td>4.12</td>
<td>Diffusive interaction with concurrent and countercurrent flow</td>
<td>102</td>
</tr>
<tr>
<td>4.13</td>
<td>Diffusive interaction with different RBC velocities</td>
<td>103</td>
</tr>
<tr>
<td>4.14</td>
<td>Diffusive interaction with different linear densities</td>
<td>103</td>
</tr>
<tr>
<td>4.15</td>
<td>Vessel diameters and RBC velocities in both reconstructed MVNs</td>
<td>105</td>
</tr>
<tr>
<td>4.16</td>
<td>Hemoglobin saturation downstream of a converging bifurcation</td>
<td>106</td>
</tr>
<tr>
<td>4.17</td>
<td>Profiles of hemoglobin saturation in three selected inlet vessels</td>
<td>107</td>
</tr>
<tr>
<td>4.18</td>
<td>Evolution of the standard deviation of hemoglobin saturation</td>
<td>108</td>
</tr>
<tr>
<td>5.1</td>
<td>Optical path of the scan optics and detection path</td>
<td>118</td>
</tr>
<tr>
<td>5.2</td>
<td>Chemical reaction model for 2PLM</td>
<td>119</td>
</tr>
<tr>
<td>5.3</td>
<td>Notation for the grid cell coordinates and spacings</td>
<td>123</td>
</tr>
<tr>
<td>5.4</td>
<td>Probe concentration and $PO_2$ in the computational domain</td>
<td>128</td>
</tr>
<tr>
<td>5.5</td>
<td>Simulated oxygen calibration curves and Stern-Vollmer plots</td>
<td>130</td>
</tr>
<tr>
<td>5.6</td>
<td>Influence of the probe concentration and laser power</td>
<td>131</td>
</tr>
<tr>
<td>5.7</td>
<td>Influence of the acquisition time and cycle length</td>
<td>132</td>
</tr>
<tr>
<td>5.8</td>
<td>Influence of the rate of oxygen consumption by organic molecules</td>
<td>132</td>
</tr>
<tr>
<td>5.9</td>
<td>Influence of the oxygen diffusion coefficient</td>
<td>133</td>
</tr>
<tr>
<td>5.10</td>
<td>Influence of the domain size and the boundary condition</td>
<td>134</td>
</tr>
</tbody>
</table>
# List of Tables

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Algorithm accuracy with coarse time steps and grid cells</td>
<td>25</td>
</tr>
<tr>
<td>2.2</td>
<td>Parameter values</td>
<td>32</td>
</tr>
<tr>
<td>2.3</td>
<td>Specific parameters used in Subsection 2.3.1</td>
<td>33</td>
</tr>
<tr>
<td>2.4</td>
<td>Longitudinal variation of the time-averaged PO$_2$ over 50µm</td>
<td>36</td>
</tr>
<tr>
<td>3.1</td>
<td>Resistance coefficients for different RBC and capillary radii</td>
<td>70</td>
</tr>
<tr>
<td>4.1</td>
<td>Topology and flow characteristics of the two reconstructed MVNs</td>
<td>91</td>
</tr>
<tr>
<td>5.1</td>
<td>Physical properties of the medium and laser parameters</td>
<td>126</td>
</tr>
<tr>
<td>5.2</td>
<td>Phosphorescent nanoprobe characteristics</td>
<td>127</td>
</tr>
</tbody>
</table>
The following nomenclature applies to all chapters except Chapter 5 where symbols are defined separately.

### Abbreviations

- **COSH**: capillary outflow saturation heterogeneity
- **CMRO$_{2}$**: cerebral metabolic rate of oxygen consumption
- **CTH**: capillary transit time heterogeneity
- **EAT**: erythrocyte-associated transient
- **IVR**: intravascular resistance
- **LD**: red blood cell linear density
- **MVN**: microvascular network
- **MTC**: mass transfer coefficient
- **PO$_{2}$**: oxygen partial pressure
- **SD**: standard deviation
- **RBC**: red blood cell
- **2PLM**: two-photon phosphorescence lifetime microscopy

### Roman

- **$C$**: oxygen concentration
- **$C_0$**: volume ratio of oxygen bound to hemoglobin ($= N_{Hb} V_{mol, O_2}$)
- **$D$**: diffusion coefficient
- **$d$**: diameter
- **$H_D$**: discharge hematocrit
- **$H_T$**: tube hematocrit
- **$j$**: oxygen flux
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>$J$</td>
<td>cost function</td>
</tr>
<tr>
<td>$k$</td>
<td>mass transfer coefficient (Eq. (3.31))</td>
</tr>
<tr>
<td>$k_{\text{m}}$</td>
<td>reaction rate between hemoglobin and oxygen</td>
</tr>
<tr>
<td>$K$</td>
<td>resistance coefficient</td>
</tr>
<tr>
<td>$L$</td>
<td>length</td>
</tr>
<tr>
<td>$M_0$</td>
<td>maximal metabolic oxygen consumption rate</td>
</tr>
<tr>
<td>$n$</td>
<td>Hill exponent</td>
</tr>
<tr>
<td>$N_{\text{Hb}}$</td>
<td>heme group concentration</td>
</tr>
<tr>
<td>$\text{Nu}$</td>
<td>Nusselt number (Eq. (3.32))</td>
</tr>
<tr>
<td>$p$</td>
<td>hydraulic pressure</td>
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<tr>
<td>$P$</td>
<td>oxygen partial pressure</td>
</tr>
<tr>
<td>$P_{50}$</td>
<td>oxygen partial pressure at half hemoglobin saturation</td>
</tr>
<tr>
<td>$q$</td>
<td>flow</td>
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<tr>
<td>$Q_{O_2}$</td>
<td>convective oxygen capacity (Eq. (3.9))</td>
</tr>
<tr>
<td>$P_{\text{crit}}$</td>
<td>oxygen partial pressure at half consumption</td>
</tr>
<tr>
<td>$r$</td>
<td>radius, radial coordinate</td>
</tr>
<tr>
<td>$R$</td>
<td>hydraulic resistance</td>
</tr>
<tr>
<td>$S$</td>
<td>hemoglobin saturation</td>
</tr>
<tr>
<td>$V_{\text{mol, }O_2}$</td>
<td>molar volume of oxygen</td>
</tr>
<tr>
<td>$v_{\text{rbc}}$</td>
<td>red blood cell velocity</td>
</tr>
<tr>
<td>$V_{\text{rbc}}$</td>
<td>red blood cell volume</td>
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<tr>
<td>$w$</td>
<td>interpolation weight</td>
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<tr>
<td>$x$</td>
<td>axial coordinate</td>
</tr>
<tr>
<td>$Z$</td>
<td>nondimensional function (Eq. (3.22))</td>
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</table>

**Greek**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha$</td>
<td>solubility coefficient</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>volume fraction</td>
</tr>
<tr>
<td>$\epsilon$</td>
<td>relative error</td>
</tr>
<tr>
<td>$\mu$</td>
<td>mean quantity, viscosity</td>
</tr>
<tr>
<td>$\sigma$</td>
<td>standard deviation</td>
</tr>
<tr>
<td>$\tau$</td>
<td>transit time</td>
</tr>
<tr>
<td>$\omega$</td>
<td>weighting factor</td>
</tr>
<tr>
<td>$\Omega$</td>
<td>computational domain</td>
</tr>
</tbody>
</table>
Indices

\( (\cdot)_a \) arterial
\( (\cdot)_c \) red blood cell
\( (\cdot)_{CI} \) capillary interaction
\( (\cdot)_{eq} \) in chemical equilibrium
\( (\cdot)_{EV} \) extravascular
\( (\cdot)_{\text{Euler}} \) Eulerian frame of reference
\( (\cdot)_i, (\cdot)_j \) vertex indices
\( (\cdot)_I, (\cdot)_J \) grid cell indices
\( (\cdot)_{OS} \) oscillation spreading
\( (\cdot)_p \) plasma
\( (\cdot)_{\phi}, (\cdot)_{\psi} \) capillary indices
\( (\cdot)_{RI} \) red blood cell interaction
\( (\cdot)_t \) tissue
\( (\cdot)_v \) venous
\( (\cdot)_w \) capillary endothelium
Chapter 1

Introduction

The vertebrate cardiovascular system is responsible for supplying cells with all molecules required for energy metabolism. This comprises oxygen and nutrients such as glucose, fatty acids, amino acids, electrolytes and vitamins. Among these, oxygen transport is the most sensitive to disturbed supply due to a comparatively high demand and low storage capacity in bodily tissues. Thus, a local interruption of blood flow can decrease oxygen concentration to a critical level within seconds. This is crucial in the brain where metabolism is almost entirely dependent on the presence of oxygen. For instance, after a cerebral stroke, cell death can follow within minutes. Impaired oxygen transport occurs in a wide range of diseases and conditions such as hypertension, diabetes, heart failure, respiratory failure and traumatic injury. The study of these phenomena is largely dependent on the ability to measure oxygen concentration in vivo. Although considerable progress in experimental methods has been made in the last decades, their temporal and spatial resolution is still limited. The complexity of certain techniques also entails substantial uncertainty and high costs. Therefore, computational modeling is a valuable complement to experiments as it provides full control on physiological variables. In this introduction, the fundamentals of oxygen transport to tissue are presented, as well as the contributions of modeling that are sought after in this thesis. Special attention is devoted to the brain microvasculature.

1.1 Basic terminology

Terms that will be frequently used are now introduced.

Blood is a suspension composed of a liquid part called plasma and a solid part that consists of three cell types. Red blood cells (RBCs), also called erythrocytes, are the most abundant cells in the blood. RBCs contain hemoglobin, a protein with four iron cores that chemically bind in a reversible way with oxygen. The two other cell types are white blood cells,
which are cells of the immune system, and platelets, which are involved in coagulation. The volume fraction of RBCs in the blood is called hematocrit and its systemic value generally lies between 40 and 45 percent.

Blood vessels fall into three categories. Arteries carry blood away from the heart; capillaries are the smallest blood vessels and have a diameter between 4 and 9 µm in humans [48]; veins bring blood back to the heart. The microcirculation is composed of those vessels with diameter less than 0.1–0.2 mm. The arteries and veins in the microcirculation are called arterioles and venules, respectively.

The presence of oxygen can be quantified either by its concentration or its partial pressure. In this context, the oxygen partial pressure is often called oxygen tension and abbreviated by PO2. The standard unit for gas pressure is the pascal. In the medical community, it is very often quantified with the unit millimeter of mercury (mmHg) which is equal to 133.3 pascals. The standard atmospheric pressure is 760 mmHg and, under standard conditions, oxygen constitutes 21% of the air volume, thus its partial pressure is 160 mmHg. The gas concentration is the product of its partial pressure and a proportionality factor called solubility coefficient.

In the brain, most neuron somata are located in the cerebral cortex which is the superficial layer of gray matter on the brain hemispheres. For brevity, we will employ the word “cortex” to denote the cerebral cortex.

1.2 Energy metabolism and oxygen supply

The supply and demand systems for oxygen are briefly summarized in this section. The description of the cardiovascular system is focused on mammals and humans. For a comprehensive introduction to cell energy metabolism and to the human cardiovascular and respiratory systems, the reader is referred to [7] and [48], respectively.

Cell metabolism requires a continuous supply of free energy to synthesize biomolecules, to actively transport ions and molecules, and to perform muscular contraction. The achievement of these tasks relies on a single common currency of energy in the form of adenosine triphosphate (ATP). In animals, the energy required for its synthesis results from breaking down carbofuels such as carbohydrates and fats. It is then used to produce ATP from adenosine diphosphophate (ADP) and phosphate. Glucose is one of the most important energy sources for the body. During cellular respiration, glucose is broken down during glycolysis into two pyruvate molecules with a net production of two ATPs. In aerobic respiration, oxygen is involved as a final electron acceptor in the breakdown of pyruvate. The theoretical
maximum amount of energy production is 38 ATP molecules per glucose. The steps involved in aerobic cellular respiration are shown in Fig. [1.1]. By contrast, in anaerobic respiration, no oxygen is used and pyruvate is degraded into lactic acid by fermentation, so each glucose molecule yields only two ATPs.

![Figure 1.1: Schematic of aerobic cellular respiration. Oxygen is used in complex IV at the end of the electron transport chain. File downloaded from https://commons.wikimedia.org/wiki/File%3ACellRespiration.svg on January 9, 2016. Image under Creative Commons license CC BY-SA 3.0.](https://commons.wikimedia.org/wiki/File%3ACellRespiration.svg)

In the presence of oxygen, the largest fraction of ATP is created by oxidative phosphorylation. This results from a biochemical process where pyruvate enters a mitochondrion and is entirely broken down into carbon dioxide and water. Oxygen is needed during the last step of the electron transport chain, after pyruvate oxidation and the citric acid cycle. This chain occurs at the inner mitochondrial membrane where high energy electrons gradually release their energy in a series of reactions. During these
reactions, protons are pumped from the mitochondrion matrix to the intermembrane space, which creates an electrochemical potential that is used by the enzyme ATP synthase to produce ATP from ADP and phosphate. In the final step, the enzyme cytochrome c oxidase, also called complex IV, transfers electrons from the protein cytochrome c to oxygen molecules, thereby producing water and pumping additional protons across the inner membrane. Although oxygen is only involved in the last of many reactions, the whole chain is dependent on its function of final electron acceptor.

The oxygen required by energy metabolism needs to be transported from the environment of the organism to the individual cells. In mammals, it is extracted from the ambient air by the lungs. The path from the lungs to the mitochondrial matrix involves diffusion, chemical binding and transport through the cardiovascular system. We now give a brief summary of these steps. During breathing, air flows through the respiratory tree which is composed of the trachea, bronchi, bronchioles, and alveolar ducts, up to the alveoli where gas exchange occurs. While oxygen is transported by convection in bronchi and bronchioles, diffusive transport takes over in the alveolar sacs due to the small airway size and air velocity \[108\]. As a result of the oxygen gradient required by diffusion, alveolar \(P_{O_2}\) is approximately 100 mmHg in humans \[27\], well below the value of 160 mmHg in ambient air. A capillary network with inflowing oxygen-poor and carbon dioxide-rich blood surrounds the alveoli (Fig. 1.2). Oxygen diffuses across the alveolar-capillary membrane to the blood where its largest fraction chemically binds to hemoglobin. Its presence increases the oxygen transport capacity of blood by a factor of approximately 60, compared to pure plasma which has a low oxygen solubility. After leaving the lungs, oxygen-rich blood flows through the pulmonary veins into the left atrium of the heart and is then ejected from the left ventricle into the aorta. A network of arteries with decreasing diameter transports blood to all parts of the body. The typical value of oxygen partial pressure in arterial blood is 95 mmHg \[48\]. The comparatively low surface area of arteries implies that no significant fraction of oxygen leaves these vessels.

Due to the sheer number of microvessels, their total surface area completely outweighs that of arteries and veins in the macrocirculation. Therefore, most gas exchange between blood and tissue occurs in the microcirculation which forms dense microvascular networks that perfuse almost all tissues. Oxygen is transported from microvessels to cell mitochondria by diffusion. Metabolic oxygen consumption drives a gradient in oxygen partial pressure that creates a diffusive flux from the microvessels to the cells in the perfused tissue. When oxygen is extracted from blood vessels, chem-
1.2. Energy metabolism and oxygen supply

Figure 1.2: Left: Front view of heart and lungs. Right: Schematic longitudinal section of a primary lobule of the lung; r. b., respiratory bronchiole; al. d., alveolar duct; at., a.; atria; a. s., alveolar sac; a, alveolus or air cell; p. a., pulmonary artery; p. v., pulmonary vein; l., lymphatic; l. n. lymph node. Images and captions adapted from Gray [42].

Bically bound oxygen molecules dissociate from hemoglobin and dissolved oxygen diffuses through the RBC membrane, blood plasma, the capillary wall, and interstitial fluid until it reaches the cells where it is consumed. The carbon dioxide produced by cellular respiration diffuses from the tissue to the vasculature and is transported by the venous vasculature to the right heart and then to the lungs where it is removed from the blood. Due to the comparatively high solubility of carbon dioxide, its transport away from cells does not impose as tight constraints on the vasculature as oxygen transport.

The human brain consumes approximately 20% of the total oxygen supply, although it is only 2% of the body’s weight [100], and its metabolism depends almost exclusively on oxidative phosphorylation. Cerebral blood flow is tightly linked to neural activity by a set of mechanisms called neurovascular coupling. The complexity of these processes as well as the brain’s vulnerability make the study of oxygen transport in this organ particularly relevant. A brief overview of the cerebral vasculature is now given. The brain is supplied by a pair of arteries on either side, the internal carotid artery and the vertebral artery. From a ring-like structure known as the circle of Willis, the anterior, middle and posterior cerebral arteries branch off (Fig. 1.3). Further branchings give rise to a redundant network of pial arteries. These vessels lie on the pia mater which is the innermost layer of the meninges (Fig. 1.3). Penetrating arterioles plunge through the pia
mater into the cortical gray matter and divide into a tree-like structure that leads to the capillary bed which has a mesh-like topology. Ascending venules collect blood from the capillary bed and are drained into pial veins. Oxygen-poor blood finally flows back to the heart through the venous circulation.

Figure 1.3: Brain arteries and microvasculature. Left: arteries of the base of the brain. Image source: Gray [42]. Right: electron micrograph of a vascular corrosion cast from the monkey visual cortex. The arrow heads indicate penetrating arterioles (A) and ascending venules (V). The larger vessels on the cortex surface are pial vessels. The dotted line shows the boundary between gray and white matter. Image source: Weber et al. [139].

1.3 History of oxygen transport modeling

The developments of oxygen transport modeling from its beginnings are shortly summarized. For a more complete account, the reader is referred to the reviews by Popel [94] and Goldman [35]. Specific aspects pertaining to individual chapters of this thesis will be reviewed separately. The first model for oxygen transport dates back to the work by August Krogh in 1919 [63]. As a basis of his study, he considered capillaries in muscles that are parallel to the muscle fibers and used an idealized cylindrical tissue domain around these capillaries, now known as Krogh cylinder. Using a series of assumptions, he could derive with the help of the mathematician K. Erlang a formula for the drop in partial pressure from the capillary wall to the
1.3. History of oxygen transport modeling

outer border of the tissue cylinder. The Krogh-Erlang equation indicates the minimal oxygen partial pressure at the capillary wall required to sustain oxygen consumption in the tissue cylinder. In spite of the numerous underlying assumptions, this equation is still used nowadays to model the oxygen tension drop in tissues.

For almost half a century after August Krogh’s work, most authors have neglected the oxygen distribution in capillaries and tacitly assumed that the variation in intravascular oxygen tension was dominated by the \( P_{O_2} \) drop in the tissue. In other words, it was assumed that the resistance of capillaries to oxygen transport was negligible compared to that of the tissue. In 1977, Hellums [51] was the first to recognize the importance of the particulate nature of blood, in other words, of the presence of individual erythrocytes. He showed that the capillary resistance to oxygen transport was 50% of the total resistance when RBCs were modeled as discrete cells and 20% when blood was treated as a continuum with the same hemoglobin content. He also coined the term “erythrocyte-associated transients” for the predicted fluctuations in oxygen tension due to individual RBCs (Fig. 1.4).

![Figure 1.4: Schematic representation of erythrocyte-associated transients. The terminology from Parpaleix et al. [86] is used. \( P_c \): average oxygen partial pressure in the RBC.](image)

The remaining hypotheses of the Krogh model have been examined since then and led to various model extensions that were still based on the Krogh cylinder geometry. The considered phenomena include convective transport of oxygen in the capillary, axial diffusion, facilitated diffusion by hemoglobin and myoglobin, time-dependent transport, \( P_{O_2} \)-dependent oxygen
consumption, oxygen-hemoglobin kinetics and the influence of carbon dioxide [35].

The development of oxygen transport models in more realistic geometries represented a major advance. Starting in the late 1970s with the work by Popel [93], several authors investigated parallel capillary arrays with significant differences from the Krogh cylinder. The heterogeneity of oxygen supply was found to decrease the average oxygen tension in the tissue [95] and irregular capillary spacings were observed to substantially increase the heterogeneity in tissue oxygenation [54]. In the late 1980s, the first oxygen transport model for microvascular networks without assumptions on the tissue regions supplied by individual vessels was proposed by Secomb and Hsu [113]. Their method based on Green's functions reduces the three-dimensional oxygen diffusion problem in the tissue to a one-dimensional problem for the source strengths in the capillaries. A decade later, Goldman and Popel [34] introduced a model for oxygen transport from complex capillary networks based on the finite difference method. These models shed further light on the consequences of vascular network heterogeneity on oxygen transport. Additionally, multiple models for oxygen transport with arterioles and venules were developed, as reviewed in Goldman [35].

The role of individual erythrocytes in intravascular oxygen transport was further investigated after the work by Hellums [51] and reviewed in Hellums et al. [50]. Early studies restricted their attention to capillaries with individual RBCs and considered various boundary conditions at the capillary wall. Eggleton et al. [23] simulated the coupled equations for intravascular and extravascular oxygen transport in the frame of reference of the RBCs. Their results provided hematocrit-dependent mass transfer coefficients (which are inversely proportional to the intravascular resistance) that were subsequently used in the microvascular network model by Goldman et al. [36]. Thus, progress in the modeling of intravascular and extravascular oxygen distributions could be combined.

In spite of these numerous advances, there are still considerable challenges. As pointed out by Goldman [35], most models have not been validated by experimental data. It is therefore essential for modeling approaches to be adapted to experimental techniques for oxygen measurements.

### 1.4 Experimental methods

Computational models require experimental data acquired in vivo as input parameters and as a reference for validation. To allow meaningful compar-
isons of intravascular oxygen gradients, the employed technique should have a spatial resolution on the order of the vessel diameters, or even lower. The first in vivo measurements of oxygen partial pressure at this scale were performed in the 1960s using recessed-tip microelectrodes, also called Whalen electrodes [143]. The diameter of their tip is 1–2 μm and they provide excellent spatial and temporal resolution. However, this technique is invasive as the electrodes can damage the tissue or the wall of blood vessels and simultaneous measurements at different locations are challenging. Micro-spectrophotometry for hemoglobin saturation in the microvasculature was introduced in 1975 by Pittman and Duling [91, 92]. The distinct light absorption of oxygenated and deoxygenated hemoglobin at different wavelengths is used to determine the saturation of RBCs in microvessels. This technique requires the transillumination of a thin tissue sample on the microscope stage. While certain muscles can be adequately prepared, the vasculature of organs such as the brain cannot be observed with this method.

Noninvasive measurements of oxygen partial pressure in vivo were made possible by phosphorescence lifetime microscopy which was introduced in 1988 by Wilson and coworkers [103]. This technique relies on a phosphorescent dye that produces a decaying light signal after a short illumination. Certain phosphorescent molecules are “quenched” by the presence of oxygen, which shortens the decay time of the light signal. Values of oxygen partial pressure are obtained using a calibration curve that relates $P_{O_2}$ to the measured decay time. Among numerous applications, a transient drop in tissue oxygenation that follows sensory stimulation and precedes the increase in blood flow could be observed [137], as well as the erythrocyte-associated transients predicted by Hellums [51]. However, this method causes photochemical oxygen consumption in potentially large volumes and generates highly reactive oxygen species that can damage organic molecules in the phosphorescent dye’s environment.

The introduction of two-photon oxygen sensors by Vinogradov and coworkers in 2008 [28] solved these issues to a large extent. While the principle of phosphorescence quenching is unchanged, these sensors are excited by the simultaneous absorption of two photons. This property enables the achievement of micrometer resolution, which drastically reduces the produced amount of reactive oxygen species and the background signal. Additionally, two-photon phosphorescent sensors are excited with infrared light, which increases the achievable penetration depth. Values of oxygen tension can be acquired in the microvasculature or in the tissue. As a further advantage, this method can be combined with quantitative measurements of RBC flow acquired with two-photon fluorescence. Simultaneous blood flow and
oxygen measurements have already substantially improved our knowledge of oxygen transport and opened up new opportunities for model validation \[65, 86, 104\].

1.5 Open questions

This section gives a non-exhaustive overview of open questions related to oxygen transport in the microcirculation with possible contributions from computational modeling.

Microvascular networks need to provide tissue with a sufficient amount of oxygen for energy metabolism, but the precise objectives of the vasculature development are unclear. Are they optimized to homogenize tissue oxygenation or merely to avoid oxygen concentrations below a critical level? What role does fault tolerance play, for example with respect to vessel occlusion?

Oxygen transport is regulated to satisfy the varying energy requirements of cells. The increased heart rate during a physical effort is one example that we are all familiar with. However, these regulation mechanisms act on a wide scale range. In the microcirculation, arterioles and possibly capillaries have the ability to actively alter their diameter \[129, 47\]. How do the resulting changes in blood flow affect tissue oxygenation? Does the response of tissue $P_{O_2}$ in a specific region depend on its location with respect to the microvasculature?

In the brain, neurovascular coupling involves a sequence of changes in microvessel diameters \[53\]. In the typical activation scenario, an increase in metabolism is followed by a higher increase in cerebral blood flow, which causes an overshoot in hemoglobin saturation and oxygen partial pressure. The interpretation of functional magnetic resonance imaging data critically depends on this phenomenon. If tissue $P_{O_2}$ does not undergo an overshoot of $P_{O_2}$ at all locations, as observed in Devor et al. \[20\], what is the spatial variability of its response?

The metabolic rate of oxygen consumption is an essential physiological variable which is notoriously difficult to measure on a small scale. Its determination requires measurements of oxygen extraction from vessels or oxygen gradients in the tissue \[106\]. To relate oxygen fluxes to metabolic oxygen consumption, mathematical modeling is required. Which approach is best suited for small-scale measurements of metabolic oxygen consumption with moderate uncertainty?

Blood flow in the microcirculation generally exhibits a certain degree of heterogeneity. For instance, the transit times of RBCs through the mi-
crovasculature are known to be heterogeneous. How does the heterogeneity of blood flow affect hemoglobin saturation and tissue oxygenation in microvascular networks? Which impact do disturbed flow patterns that occur in small vessel disease or microstrokes have on oxygen transport?

The experimental methods outlined above measure either hemoglobin saturation or oxygen partial pressure. The former reflects the total blood oxygen content while the latter indicates the amount of oxygen available for energy metabolism. As both have a high physiological importance, the ability to convert either quantity to the other is desirable. This operation requires the knowledge of the oxygen dissociation curve as well as the oxygen distribution in capillaries. How can hemoglobin saturation be related to plasma $P_{O_2}$? Additionally, the application of two-photon phosphorescence lifetime microscopy with organic molecules in the medium photochemically consumes a certain amount oxygen molecules. Can the consumption of reactive oxygen species be quantified to ascertain under which experimental conditions it can safely be neglected?

1.6 Outline

In this thesis, we aim to develop a new computational modeling framework for oxygen transport in the microcirculation that satisfies the following requirements. First, it should harness the potential of two-photon phosphorescence lifetime microscopy for result comparison and validation. Second, it should provide new insights into the physics of oxygen supply from capillaries, rather than just consisting of a computing tool. With this combination, the framework should help to bridge the gap between theory and experiments and thus to elucidate some of the open questions listed above.

The following chapters, which are now outlined, introduce new models for oxygen transport that specifically target some of these issues.

- In Chapter 2, a computational model for oxygen transport from capillaries to tissue with moving RBCs is introduced. It was designed to allow easy result comparison with intra- and extravascular oxygen measurements. Results are compared with recent experimental data acquired in the rodent brain with two-photon phosphorescence lifetime microscopy. Particular attention is devoted to erythrocyte-associated transients. A model extension to realistic microvascular networks is presented and applied to quantify the influence of capillary dilations on tissue oxygenation in a simple network.
• Chapter 3 addresses the influence of the two main blood flow parameters on tissue oxygenation, namely hematocrit and RBC velocity. A theoretical model that builds upon previous works is presented and the available analytical formulas are employed to investigate this question. The predicted values of tissue $PO_2$ are compared to results from the computational model introduced in Chapter 2. Related features of the intravascular oxygen distribution are quantified in more detail and modeled.

• In Chapter 4, the theoretical tools introduced in Chapter 3 are extended to deal with the heterogeneity of blood oxygen content. The diffusive interaction between heterogeneously saturated RBCs, as well as between parallel capillaries with different oxygen supply rates, is modeled and validated using our computational model. The magnitude of these interactions is quantified and their relation to capillary transit time heterogeneity is discussed.

• Chapter 5 deals with numerical modeling of two-photon phosphorescence lifetime microscopy. A new model that includes oxygen diffusion and singlet oxygen removal by organic molecules is introduced. It is applied to quantify under which experimental conditions photochemical oxygen consumption may significantly reduce the observed phosphorescence decay times.

Chapter 2 is adapted from a previously published article by Lücker, Weber and Jenny [71]. Chapter 3 is largely based on a research paper by Lücker, Secomb, Weber and Jenny [70]. The improved advection scheme introduced in Ref. [70] is described in Chapter 2. Chapter 4 is based on the article “The heterogeneity of hemoglobin saturation in capillary networks and its relation to red blood cell transit time” by Lücker, Barrett, Secomb, Weber and Jenny, which will be submitted in the near future. The model extension to microvascular networks which was developed in that study is presented in Chapter 2.
In this chapter, a new computational model for oxygen transport from capillaries to tissue is introduced. Its aim is to facilitate the comparison of model predictions with experimental data acquired with two-photon phosphorescence lifetime microscopy (2PLM). On one hand, the single-file flow of RBCs in capillaries considerably affects the intravascular oxygen distribution \cite{51, 50, 86}, hence the presence of individual erythrocytes should be resolved. On the other hand, 2PLM measurements are carried out by focusing the laser at one or multiple fixed locations, which prompts to use a computational domain that is fixed with respect to the tissue. The key feature of the model presented here is the combined use of a fixed frame of reference and individual moving RBCs using overlapping meshes. This approach offers a relatively high degree of detail and considerable flexibility with respect to vessel geometry and blood flow. The oxygen tension values produced by this method are thoroughly compared to the experimental data by Parpaleix et al. \cite{86} and the influence of capillary dilations on tissue PO$_2$ in a simple network is investigated.

This chapter is largely based on the article “A dynamic model of oxygen transport from capillaries to tissue with moving red blood cells” by Lücker, Weber and Jenny \cite{71}. The numerical scheme with improved stability described in Subsection 2.2.4 was presented in the article “The relative influence of hematocrit and red blood velocity on oxygen transport from capillaries to tissue” by Lücker, Secomb, Weber and Jenny \cite{70}. Subsection 2.2.5 is based on the submitted manuscript “The heterogeneity of hemoglobin saturation in capillary networks and its relation to red blood cell transit time”.
2.1 Introduction

In order to justify the introduction of a new computational model for oxygen transport from capillaries to tissue, existing models are reviewed with a particular focus on the intravascular oxygen distribution. Current models for oxygen transport from capillaries to tissue generally employ two distinct approaches. The first class of models focuses on the tissue and does not represent individual RBCs. Instead, they employ a boundary condition at the capillary wall that accounts for oxygen transport from the capillary. While the original Krogh model assumed a constant oxygen tension at the capillary wall, more recent models often use a mass transfer coefficient (MTC) that relates the PO$_2$ drop from the RBC to the oxygen flux across the capillary wall ($j_t = k\Delta P$). Since these MTCs depend on hematocrit [50, 23], this approach can capture the influence of RBC flow on tissue oxygenation. Besides, these models have the advantage that they do not resolve the complex intravascular PO$_2$ field with individual RBCs, which makes them applicable to capillary networks [36, 115, 37, 133]. However, this first class of models depends on MTCs which are provided by other models.

The second approach models intravascular oxygen transport in more detail and can be used to compute MTCs. Accurate MTC estimates require discrete RBCs to be modeled [51, 26, 50] (as opposed to a continuous hemoglobin solution) and extracapillary oxygen transport to be included [23]. Most models with individual RBCs carry out computations in the frame of reference of the erythrocyte, which simplifies the numerical treatment of the reaction between oxygen and hemoglobin in RBCs. In this moving frame, the tissue has an apparent velocity opposite to the RBC velocity and appears to move backwards. This idea was first used by Hellums [51] who developed an analytical model with a cylindrical RBC and the adjacent tissue to compute MTCs. Eggleton et al. [23] built on this approach and using a model with concentric layers around the capillary for the endothelium, the interstitial fluid and the tissue. They investigated the dependence of MTCs on hematocrit, RBC velocity and capillary radius.

Although the models for intravascular oxygen transport described above are convenient for numerical computations and useful for estimating MTCs, they suffer from limitations that restrict their scope. In the RBC frame of reference, the boundary condition at the distal end of the tissue cylinder has a considerable effect on tissue PO$_2$ since the PO$_2$ value at that boundary is advected backwards by the apparent tissue motion. Therefore, models that use the RBC frame cannot fully capture the influence of RBC flow on tissue PO$_2$, which is essential in situations such as hypoxia. These models are also inflexible in terms of geometry, since the backward motion of
the tissue forces the computational domain to have the same radial cross section along the flow direction. For instance, local capillary dilations, as observed in vivo [47], cannot be simulated with this class of models. Furthermore, the simulation duration is limited to the time that RBCs spend in capillaries (100 to 300 ms in the cerebral cortex [56]). For applications that require a larger simulation time (e.g., functional hyperemia), it is also necessary to use the frame of reference of the tissue, as done by models based on MTCs. Unlike other studies, Groebe et al. [44] modeled individual RBCs in a fixed tissue region. However, their approach is limited to steady state situations and relies on multiple simplifying assumptions that allow an analytic treatment of the intra-erythrocyte PO$_2$ field.

Finally, Goldman [35] pointed out that thorough model validations have yet to be done. For intravascular PO$_2$, this task puts constraints on both the simulation method and the required experimental data. Since PO$_2$ is generally measured at one or more fixed locations, a convenient model validation should be performed in the fixed frame of reference of the tissue. Besides, a detailed comparison with measured intravascular PO$_2$ requires high spatial and temporal resolution. Pioneering work by Vanzetta et al. [137] has revealed PO$_2$ transients related to neuronal activation and oxygen metabolism with the use of phosphorescence lifetime microscopy. Using one-photon excitation with a lower excitation volume, Golub et al. [40] measured erythrocyte-associated transients (EATs) in the rat mesentery. However, until now, only two-photon phosphorescence lifetime microscopy achieved sufficiently high resolution to enable detailed in vivo measurements of plasma PO$_2$ between RBCs in depth. This technique was applied by Parpaleix et al. [86] in the olfactory glomerulus of the anesthetized rodent brain and subsequently by Lyons et al. [72] in the awake rodent brain. Sakadžić et al. [105] used it in the rat cerebral cortex, without reporting details of the intravascular PO$_2$ field. Due to the absence of other detailed experimental studies when this study was conducted, we compared our simulation results with the data from [86].

We propose a new model of oxygen transport in the microcirculation that is adapted for validation against experimental data. The main improvement over previous models is the use of overlapping meshes which simultaneously allows the frame of reference to be fixed and individual RBCs to be modeled. Hence, the coupling between intravascular oxygen transport and tissue PO$_2$ can be captured together with the details of the PO$_2$ field inside and around capillaries. Individual RBCs are followed by moving meshes that are used to compute hemoglobin diffusion and reaction with oxygen. These moving meshes are mapped onto a fixed mesh, where oxygen advection, diffusion and consumption in the tissue are computed.
The model is first presented in an axisymmetric computational domain and subsequently extended to capillary networks with arbitrary topology. This approach can capture the influence of heterogeneous RBC flow on tissue oxygenation in a time-dependent manner. Situations with unsteady blood flow such as functional hyperemia can be modeled by adapting blood velocity and hematocrit. Additionally, given the detailed treatment of the intravascular oxygen distribution, this method can be used as a benchmark for simplified models.

Model results are thoroughly compared with the experimental data from Parpaleix et al. [86], which shows that both intra- and extravascular oxygen transport are accurately simulated. For this comparison, an axisymmetric geometry based on Eggleton et al. [23] with concentric layers for the plasma, the capillary wall and the tissue was used. However, a cone-shaped geometry as in Hudetz [57] yields a better agreement with the measurements than a cylinder with constant radius. MTCs are also compared with results from previous models. As an application of the model extension to capillary networks, the influence of capillary dilations is investigated in a simple network. Our novel approach will enable the study of the effects of blood flow heterogeneity during physiologically relevant phenomena such as microstrokes or small vessel disease.

2.2 Finite-volume model with moving red blood cells

2.2.1 Governing equations

Oxygen transport and consumption was modeled in a domain that consists of four regions: tissue, capillary wall, plasma, and RBCs. Oxygen is consumed only in the tissue; the capillary endothelium does not consume oxygen and has a lower diffusion coefficient; in both plasma and RBCs, oxygen is convected by the blood flow. Finally, RBCs contain hemoglobin which carries oxygen in bound form. In fact, due to the low solubility of oxygen in plasma, most oxygen is bound to hemoglobin.

Dissolved oxygen can be quantified by its concentration $C$ [ml O$_2$ cm$^{-3}$] and partial pressure $P = P_{O_2}$ [mmHg], which are related by Henry’s law as

$$C = \alpha P,$$

where $\alpha$ is the solubility coefficient in ml O$_2$ mmHg$^{-1}$ cm$^{-3}$. The formulation of the conservation equation for oxygen in terms of $C = \alpha P$ is most
2.2. Model with moving red blood cells

convenient for our purposes. Hemoglobin is expressed using the saturation $S$, which is the concentration ratio of oxyhemoglobin to total hemoglobin.

The reaction between oxygen and hemoglobin in RBCs is most completely described by the Adair equation [17]. However, as in many previous studies, here we employ the Hill equation

$$S = \frac{P^n}{P_{50}^n + P^n}$$

(2.2)

to describe the equilibrium curve between $P$ and $S$, where $P_{50}$ is the oxygen partial pressure at hemoglobin half-saturation and $n$ is the Hill exponent. This results in a one-step reaction for the four heme groups of the hemoglobin molecule. To model the reaction rates when oxygen and hemoglobin are in nonequilibrium, we followed the approach of Clark et al. [17] and used the function

$$f(P, S) = \begin{cases} 
  k_- \left( S - (1 - S) \left( \frac{P}{P_{50}} \right)^n \right) & \text{inside RBCs,} \\
  0 & \text{outside RBCs,}
\end{cases}$$

(2.3)

where $k_-$ is the dissociation rate. This function satisfies $f = 0$ when oxygen and hemoglobin are in equilibrium (Eq. (2.2)). Since no hemoglobin is present in healthy blood plasma, the reaction term $f(P, S)$ was only used within RBCs.

Oxygen consumption was modeled using first-order Michaelis-Menten kinetics [35] and assumed to occur only in the tissue, which results in

$$M(P) = \begin{cases} 
  M_0 \frac{P}{P_{\text{crit}} + P} & \text{inside tissue,} \\
  0 & \text{outside tissue,}
\end{cases}$$

(2.4)

where $M_0$ is the maximal metabolic rate of oxygen consumption in units of $\mu$m$^3$O$_2$µm$^{-3}$s$^{-1}$ and $P_{\text{crit}}$ is the oxygen level at which consumption is half of $M_0$. Since we compared our results with measurements performed in the rodent brain where no muscles are present, we did not consider myoglobin-facilitated diffusion of oxygen inside the tissue.

Our model is based on a single equation for oxygen for all regions given by

$$\frac{\partial \alpha P}{\partial t} + \mathbf{v} \cdot \nabla (\alpha P) = \nabla \cdot (D \alpha \nabla P) + C_0 f(P, S) - M(P),$$

(2.5)

where $D$ is the diffusion coefficient and $\mathbf{v}$ the advection velocity. The factor $C_0$ is given by $C_0 = N_{\text{Hb}} V_{\text{mol}, \text{O}_2}$, where $N_{\text{Hb}}$ is the molar density of heme.
groups and \( V_{mol,O_2} \) is the molar volume of oxygen. Hemoglobin saturation is governed by the equation

\[
\frac{\partial S}{\partial t} + \mathbf{v} \cdot \nabla S = \nabla \cdot (D_{Hb} \nabla S) - f(P, S), \tag{2.6}
\]

where \( D_{Hb} \) is the diffusivity of hemoglobin in RBCs.

At interfaces between regions with different solubility or diffusion coefficients, the continuity of \( P_{O_2} \) and oxygen flux across the interface have to be satisfied \cite{140}. For example, at the wall-tissue interface, the latter condition is

\[
D_w \alpha_w \frac{\partial P}{\partial n} = D_t \alpha_t \frac{\partial P}{\partial n}, \tag{2.7}
\]

where the subscripts refer to the wall and the tissue, respectively.

Boundary conditions for the respective computational domains need to be modeled. We generally considered closed representative tissue domains with \( \partial P/\partial n = 0 \) at the tissue boundary. Thus, the simplest boundary condition for \( P_{O_2} \) in the domain \( \Omega \) is a homogeneous Neumann boundary condition at the whole boundary, including at the capillary inlet and outlet. This will be employed in the simulations performed in capillary networks. In the axisymmetric computational domain used to investigate EATs, the boundary condition at the capillary entrance is modeled in more detail. When a RBC overlaps with the domain boundary, the oxygen tension is interpolated from this RBC to the capillary entrance. When plasma is flowing in, a constant \( P_{O_2} \) value \( P_{p,a} \) was used. At the capillary outlet, the boundary condition \( \partial P/\partial n = 0 \) was applied.

Since RBC membranes are impermeable for hemoglobin, the boundary condition for hemoglobin saturation is \( \partial S/\partial n = 0 \). Unlike hemoglobin, oxygen is soluble in lipids and can diffuse through cell membranes. The different solubility and diffusion coefficients of oxygen in lipid bilayers was not taken into account since RBC membranes are generally less than 10 nm thick \cite{124}, which is negligible compared to the cell size.

The entry of RBCs into the capillary plays a crucial role, since it determines the amount of oxygen in bound form that enters the domain. The oxygen tension in entering erythrocytes was set to a constant value \( P_{c,a} \) and hemoglobin saturation was set to equilibrium with oxygen. The simplest model for capillary spacing is a constant distance between each RBC pair. However, Chaigneau et al. \cite{11} observed large instantaneous fluctuations of the RBC linear density. Moreover, they showed that variations of RBC flow were primarily caused by fluctuations of linear density, whereas instantaneous RBC velocity fluctuations were 2.5 times lower. Therefore, we treated RBC spacings as a random variable and modeled them using
2.2. Model with moving red blood cells

a log-normal random variable with independent values for each RBC pair. The parameters were chosen to match experimentally measured mean $\mu_{LD}$ and standard deviation $\sigma_{LD}$ of linear density.

At the beginning of the simulations, the $PO_2$ field in RBCs was set to $P_{c,a}$ and hemoglobin saturation was set to equilibrium with oxygen. The initial $PO_2$ was set to $P_{p,a}$ in the plasma and to 22 mmHg in the tissue.

2.2.2 Overlapping mesh method

The main objective of this chapter is to thoroughly compare simulation results with the experimental data acquired by Parpaleix et al. [86] with 2PLM. As pointed out in Section 2.1, an easy comparison with these data requires a model that focuses on a fixed region of interest. This approach also enables transient phenomena to be simulated, such as local changes in RBC flow or metabolism. A fixed frame of reference is problematic when solving Eq. (2.6). Hemoglobin is a large protein that cannot cross erythrocyte membranes. However, the discretization of the advection term would create numerical diffusion, which would in turn cause an unphysical leak of hemoglobin out of RBCs. These problems can be circumvented by solving Eq. (2.6) in a Lagrangian frame of reference that follows the moving RBC. This approach enables the no-flux boundary condition for hemoglobin at the RBC membrane to be exactly satisfied.

We therefore used a fixed computational domain for the capillaries and the tissue, denoted by $\Omega$, as well as a moving domain for each RBC, denoted by $\Omega_{rbc}$ (Fig. 2.1). Each domain is covered by its own computational mesh. This overlapping mesh approach was inspired by the overset grid method [96], which has been applied to aerodynamic problems with moving objects. Alternate names for this class of methods are composite grid schemes [125] and composite overlapping meshes [14]. The approach developed here will be referred to as overlapping mesh method. It differs from the previous techniques used in aerodynamics since two distinct equations for oxygen and hemoglobin are solved on the fixed and the moving mesh, respectively, and interpolation is required for the chemical reaction between these quantities. We will also refer to $\Omega$ as Eulerian domain and to $\Omega_{rbc}$ as Lagrangian domain. To simplify the notation, we will omit RBC indices. Since RBCs are entering and leaving $\Omega$, the Lagrangian domain $\Omega_{rbc}$ may be completely or partly inside $\Omega$.

Erythrocytes were assumed to have a fixed shape. While they actually deform, this assumption avoided the expensive treatment of fluid-structure interaction. Therefore, our modeled RBCs behaved similar to solid bodies
that follow the plasma flow. As a further simplification, we considered plasma flow to be uniform along radial cross sections of capillaries. Note that the detailed flow field around RBCs is not of importance here, since transport of oxygen is diffusion dominated (see [134] for a corresponding study about nitric oxide). Consequently, the blood velocity was given by $v = Q/A$, where $Q$ is the blood volume flow and $A$ the capillary cross section.

Eq. (2.5) for oxygen was solved in the Eulerian domain $\Omega$, whereas the hemoglobin equation (2.6) was solved in the Lagrangian domain $\Omega_{rbc}$. Since $\Omega_{rbc}$ moves with the velocity $v_{rbc}$, the coordinate transformation $\mathbf{x}' = \mathbf{x} + v_{rbc}t$ cancels the advection term and yields

$$\frac{\partial S}{\partial t} = \frac{\partial}{\partial x'_i} \left( D_{\text{Hb}} \frac{\partial S}{\partial x'_i} \right) - f(P, S). \quad (2.8)$$

Since this equation is discretized in $\Omega_{rbc}$, the oxygen partial pressure is also needed in that domain. This field, denoted by $P_{rbc}$, is obtained by interpolation from $\Omega$ to $\Omega_{rbc}$. Likewise, since Eq. (2.5) is solved in $\Omega$, values of $S$ in the Eulerian domain, denoted by $S_{\text{Euler}}$, have to be interpolated from $\Omega_{rbc}$ (Fig. 2.1). The interpolation method may considerably affect simulation results, since most oxygen in the blood is bound to hemoglobin. Thus, interpolation errors that cause inaccurate values of $S_{\text{Euler}}$ may have a large effect on the resulting $P_{O_2}$. A conservative interpolation scheme is therefore crucial.

To obtain $P_{rbc}$ and $S_{\text{Euler}}$, we used a volume-based interpolation scheme that is discretely conservative in the sense that the integral of the interpolated field on any subset of the target mesh is conserved. For grid cells $V_I$
2.2. Model with moving red blood cells

and $V_{rbc,J}$ in $\Omega$ and $\Omega_{rbc}$, respectively, interpolation weights were defined by

$$w_{I,J}^{rbc} = \frac{|V_{I} \cap V_{rbc,J}|}{|V_{rbc,J}|}$$ (2.9)

and

$$w_{I,J}^{Euler} = \frac{|V_{I} \cap V_{rbc,J}|}{|V_{I}|}.$$ (2.10)

The interpolation formulas for $P_{rbc}$ and $S_{Euler}$ are then given by

$$P_{rbc,J} = \sum_{I} w_{I,J}^{rbc} P_{I}$$ (2.11)

and

$$S_{Euler,I} = \sum_{J} w_{I,J}^{Euler} S_{J}.$$ (2.12)

The discrete conservation property for the interpolated field $S_{Euler}$ is shown as follows. Consider a subdomain $\Omega' = \bigcup_{k=1}^{m} V_{I_{k}}$ that consists of $m$ grid cells $V_{I_{k}}$. The integral of $S_{Euler}$ on $\Omega'$ is given by

$$\int_{\Omega'} S_{Euler} dV = \sum_{k=1}^{m} |V_{I_{k}}| S_{Euler,I_{k}}$$ (2.13)

$$= \sum_{k=1}^{m} \sum_{J} |V_{I_{k}}| |V_{I_{k}} \cap V_{rbc,J}| S_{J}$$ (2.14)

$$= \sum_{J} \sum_{k=1}^{m} |V_{I_{k}} \cap V_{rbc,J}| S_{J}$$ (2.15)

$$= \sum_{J} |\Omega' \cap V_{rbc,J}| S_{J}$$ (2.16)

$$= \int_{\Omega'} S dV.$$ (2.17)

The same argument can be used for the integral of $P_{rbc}$ on a subset of $\Omega_{rbc}$, which shows that the interpolation scheme given by Eqs. (2.11) and (2.12) is discretely conservative.

Grid cells in $\Omega$ that overlap with the RBC border require special care. If the intersection of a grid cell $V_{I}$ with $\Omega_{rbc}$ occupies a small volume, $S_{Euler,I}$ will be also small. This fact has to be accounted for in the discretization of the reaction term $f(P, S)$. We introduce the RBC volume fraction

$$\gamma_{I} = \frac{|V_{I} \cap \Omega_{rbc}|}{|V_{I}|}.$$ (2.18)
In $V_I$, we consider that the chemical reaction between hemoglobin and oxygen only occurs in a fraction of $V_I$ with volume $\gamma_I|V_I|$ where all the hemoglobin is contained. Since this volume fraction has hemoglobin saturation $S_{\text{Euler},I}/\gamma_I$, the discretized reaction term in $\Omega$ is given by

$$f(P_I, S_{\text{Euler},I}) = \gamma_I k_- \left( \frac{S_{\text{Euler},I}}{\gamma_I} - \left( 1 - \frac{S_{\text{Euler},I}}{\gamma_I} \right) \left( \frac{P_I}{P_{50}} \right)^n \right)$$

(2.19)

$$= k_- \left( S_{\text{Euler},I} - (\gamma_I - S_{\text{Euler},I}) \left( \frac{P_I}{P_{50}} \right)^n \right).$$

(2.20)

Continuity of the oxygen flux at interfaces between regions with different solubility or diffusion coefficient (Eq. (2.7)) is enforced by adequately interpolating the Krogh diffusion coefficient $D_\alpha$. At cell faces, mass conservation is enforced by using the harmonic average of $D_\alpha$ in both neighboring grid cells [87]. The boundary condition at the capillary inlets of $\Omega$ also requires interpolation. If a RBC overlaps a cell face at the capillary inlet, the PO$_2$ value at that face is obtained by bilinear interpolation of the RBC PO$_2$ at the corresponding location. Otherwise, the boundary PO$_2$ is set to the constant value $P_{p,a}$.

The results presented in this chapter were produced with the central scheme for the divergence operator. An alternate, more stable scheme will be presented in Subsection 2.2.4 and applied in the next chapter. For the Laplace operator, Gauss integration, centered differences for the surface normal gradient and harmonic interpolation for the diffusion coefficient were used. Time stepping and coupling between Eqs. (2.5) and (2.6) are addressed next. The algorithm was implemented in C++ using the open source software library OpenFOAM 2.1.1 [142].

### 2.2.3 Time integration

Generation of PO$_2$ maps in realistic capillary network may require simulations with at least hundreds of RBCs during several seconds. The ability to use large time steps is therefore crucial to keep the computational time sufficiently low. Special care is required to achieve this within our framework based on overlapping meshes. The nonlinear reaction term $f(P, S)$ (Eq. (2.3)) combined with RBC displacements prevents from using an explicit scheme. As observed by Clark et al. [17], the boundary layer inside erythrocytes is a region of chemical nonequilibrium, such that large explicit time steps inevitably cause overshooting. Another requirement is that the coupling between hemoglobin and oxygen equations conserves the total of free and bound oxygen.
2.2. Model with moving red blood cells

To achieve this, we use Godunov splitting for Eq. (2.5) and linearization of the reaction and consumption terms using Picard’s method. While the equation for oxygen can be integrated without Godunov splitting, this unsplit approach would severely limit the maximal stable time step, since the linearization of the reaction term requires $\text{PO}_2$ values in $\Omega$ to vary moderately. If RBCs undergo large displacements during one time step, the resulting large $\text{PO}_2$ variations would lead to instabilities.

Let the superscript $k$ indicate the current time $t^k$. To integrate Eqs. (2.5) and (2.6) from $t^k$ to $t^k + \Delta t$, an intermediate solution $P^*$ is obtained by integrating only the advection term:

$$\frac{\alpha^* P^* - \alpha^k P^k}{\Delta t} + \mathbf{v} \cdot \nabla (\alpha^* P^*) = 0. \quad (2.21)$$

Here, the solubility $\alpha^*$ corresponds to RBC positions after their displacement. The reaction term $f(P, S)$ and the consumption term $M(P)$ were both linearized and their linear part is treated implicitly as

$$\alpha^* \frac{P^{(\nu)} - P^*}{\Delta t} = \nabla \cdot (D \alpha^* \nabla P^{(\nu)}) + C_0 \left[ f(P^{(\nu-1)}_{\text{Euler}}, S^{(\nu-1)}_{\text{Euler}}) + (P^{(\nu)} - P^{(\nu-1)}) \frac{\partial f}{\partial P} (P^{(\nu-1)}_{\text{Euler}}, S^{(\nu-1)}_{\text{Euler}}) \right] - \left( M(P^{(\nu-1)}) + (P^{(\nu)} - P^{(\nu-1)}) \frac{\partial M}{\partial P} (P^{(\nu-1)}) \right) \quad (2.22)$$

and

$$\frac{S^{(\nu)} - S^k}{\Delta t} = \nabla \cdot (D_{\text{Hb}} \nabla S^{(\nu)}) - \left[ f(P^{(\nu-1)}_{\text{rbc}}, S^{(\nu-1)}_{\text{rbc}}) + (S^{(\nu)} - S^{(\nu-1)}) \frac{\partial f}{\partial S} (P^{(\nu-1)}_{\text{rbc}}, S^{(\nu-1)}_{\text{rbc}}) \right], \quad (2.23)$$

where $\nu$ is the iteration number and $P^{(0)} = P^*$. The coupling between both equations conserves the total oxygen amount if the integral of both terms in square brackets are equal. Although the volume-based interpolation method (Eqs. (2.11) and (2.12)) conserves $P$ and $S$, it does not exactly conserve the integral of $f(P, S)$ since the reaction term is nonlinear in $P$. However, this only causes a minimal amount of oxygen loss in the domain (less than 0.2% for a total RBC discharge).

The moving meshes $\Omega_{\text{rbc}}$ are displaced during each time step by the increment $\mathbf{v}_{\text{rbc}} \Delta t$. When a RBC leaves the domain $\Omega$ and no longer overlaps with it, the corresponding mesh is moved to the front of the RBC queue.
Numerical modeling of $O_2$ transport

and placed at a distance to the next RBC which is randomly generated based on a log-normal distribution. In the plasma, the coefficients $\alpha$ and $D$ have to be updated to reflect RBC motion. In a grid cell $V_I$, the discretized coefficients are given by

\begin{align}
D_I &= \gamma_I D_c + (1 - \gamma_I) D_p, \\
\alpha_I &= \gamma_I \alpha_c + (1 - \gamma_I) \alpha_p,
\end{align}

where the subscripts $c$ and $p$ refer to values in the RBCs and in the plasma. The algorithm is summarized in Algorithm 1.

**Algorithm 1** Time integration of the coupled oxygen and hemoglobin equations for one time step $\Delta t$

1: move all RBCs by $v_{rbc}\Delta t$
2: update interpolation coefficients (Eq. (2.9) and (2.10))
3: update $D$ and $\alpha$ (Eq. (2.24) and (2.25))
4: solve advection equation for $P^*$ (Eq. (2.21))
5: $P^{(0)} \leftarrow P^*$, $S^{(0)} \leftarrow S^k$
6: $R^{(0)} \leftarrow \infty$
7: $\nu \leftarrow 0$
8: while $R^{(\nu)} > \text{tol}$ do
9: \hspace{1em} for all RBCs that overlap $\Omega$ do
10: \hspace{2em} interpolate $P^{(\nu)}$ to $P^{(\nu)}_{rbc}$ using Eq. (2.11)
11: \hspace{2em} interpolate $S^{(\nu)}$ to $S^{(\nu)}_{\text{Euler}}$ using Eq. (2.12)
12: \hspace{1em} end for
13: \hspace{1em} solve for $P^{(\nu+1)}$ (Eq. (2.22))
14: \hspace{1em} $R^{(\nu+1)} \leftarrow$ initial residual of Eq. (2.22)
15: \hspace{1em} for all RBCs that overlap $\Omega$ do
16: \hspace{2em} solve for $S^{(\nu+1)}$ (Eq. (2.23))
17: \hspace{1em} end for
18: $\nu \leftarrow \nu + 1$
19: end while

The domain $\Omega$ was discretized using a Cartesian grid with constant grid spacing $\Delta x = 0.1\,\mu m$ in the axial direction. In the radial direction, the grid cell spacing in the capillary was constant ($\Delta y = 0.1\,\mu m$) and decreasing in the tissue region, since oxygen gradients decrease away from capillaries. The ratio between the height of the top-most grid cell to the bottom-most in the tissue was set to four. This results in a grid with $333 \times 29$ grid cells.
2.2. Model with moving red blood cells

The RBC domain \( \Omega_{\text{rbc}} \) consists of those Cartesian grid cells that lie entirely inside the RBC shape, which results in a “staircase” geometry (Fig. 2.1). A curvilinear shape-conforming mesh is not necessary for such an advection-diffusion problem. Besides, the computation of the interpolation coefficients defined in Eqs. (2.9) and (2.10) is easier for Cartesian grids.

The tolerance \( \text{tol} \) in Algorithm 1 was set to \( 10^{-4} \). A smaller tolerance affected results by less than 0.1 mmHg. Unless stated otherwise, the time step \( \Delta t \) was set to 0.5 ms. All our simulations were run for four seconds. After one second, the influence of the initial condition disappeared. The results were collected during the following three seconds.

The accuracy of the algorithm with a coarser Eulerian grid and larger time steps was also examined. Table 2.1 shows absolute and relative errors on the capillary centerline and in the tissue against a baseline case with \( \Delta t = 0.1 \) ms and \( \Delta x = \Delta y = 0.1 \mu m \) in the capillary. The relative error was normalized by the maximum \( P_{O_2} \) value in the considered longitudinal profile. When multiplying the grid spacing and the time step by three, the relative error stays below 2.5%. With a 50 times larger timestep (\( \Delta t = 5 \) ms), the absolute error in the tissue is still smaller than 1 mmHg, while the computational time is divided by 10. This is an indication that our numerical algorithm is very robust in terms of time step size and grid spacing. This property will allow for simulations of oxygen transport in larger capillary networks.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Centerline</th>
<th>Tissue (10 ( \mu m ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \Delta t )</td>
<td>( \Delta x )</td>
<td>abs. ( L^\infty )</td>
</tr>
<tr>
<td>0.3 ms</td>
<td>0.3 ( \mu m )</td>
<td>1.65</td>
</tr>
<tr>
<td>5 ms</td>
<td>0.3 ( \mu m )</td>
<td>3.51</td>
</tr>
</tbody>
</table>

Table 2.1: Algorithm accuracy with coarse time steps and grid cells. The grid cell size is given in the capillary, where \( \Delta x = \Delta y \). The errors were measured against longitudinal profiles computed with \( \Delta t = 0.1 \) ms and \( \Delta x = \Delta y = 0.1 \mu m \) in the capillary.

2.2.4 Stable advection scheme

Here we describe an improved discretization scheme for the advection term in Eq. (2.5). As explained in Subsection 2.2.3, the pure advection equation

\[
\frac{\partial (\alpha P)}{\partial t} + \mathbf{v} \cdot \nabla (\alpha P) = 0
\]  

(2.26)
Numerical modeling of $O_2$ transport is solved before dealing with the diffusion, reaction and consumption terms. Since the solubility coefficient $\alpha$ takes different values in the RBC and in the plasma, a discretization scheme that is monotone for $C = \alpha P$ may introduce spurious oscillations in $P$ in the capillary, since $P$ is then obtained by dividing $C$ by the variable coefficient $\alpha$. With the parameters used in this chapter, these oscillations were immediately damped by the diffusion term. However, at higher RBC linear densities or velocities, these spurious oscillations may not disappear after the diffusion-reaction step when Eq. (2.26) is discretized with a conventional method such as the upwind scheme. As simulations with high RBC flows will be performed in the next chapter, a stable numerical scheme for Eq. (2.26) is needed. The new scheme is particularly useful in simulations without axial diffusion, where spurious oscillations in axial direction would not be damped by diffusion. The improved method takes advantage from the fact that the exact position of the RBC interface is known at each time step. For simplicity, we describe it in a one-dimensional equidistant grid with a single RBC domain $\Omega_{\text{rbc}}$. The oxygen concentration in a grid cell $\Omega_I$ with width $\Delta x$ at time step $k+1$ is given by

$$C_{k+1}^I = \frac{1}{|\Omega_I|} \int_{\tilde{\Omega}_I} C_k dV,$$  \hspace{1cm} (2.27)

where $\tilde{\Omega}_I$ is the grid cell $\Omega_I$ shifted backwards by $v_{\text{rbc}} \Delta t$ (Fig. 2.2). The oxygen concentration $C_k$ at the previous time step is reconstructed based on the position of the RBC interface and the values of $P_k$ in the grid cells intersected by $\tilde{\Omega}_I$. Under the assumption that the RBC moves in positive direction and $v_{\text{rbc}} \Delta t \leq \Delta x$, Eq. (2.27) becomes

$$C_{k+1}^I = (\alpha_c \tilde{\gamma}_{c,I-1} + \alpha_p \tilde{\gamma}_{p,I-1}) P_{k-1}^I + (\alpha_c \tilde{\gamma}_{c,I} + \alpha_p \tilde{\gamma}_{p,I}) P_k^I,$$  \hspace{1cm} (2.28)

with $\tilde{\gamma}_{c,I} = |(\Omega_{\text{rbc}} \cap \tilde{\Omega}_I)/|\tilde{\Omega}_I|$ and $\tilde{\gamma}_{p,I} = |(\Omega \setminus \Omega_{\text{rbc}}) \cap \tilde{\Omega}_I)/|\tilde{\Omega}_I|$ as illustrated in Fig. 2.2. Finally, the oxygen partial pressure at the new time step is given by $P_{k+1}^I = C_{k+1}^I / \alpha_{I,k+1}^I$. The conservation property of this scheme is ensured by Eq. (2.27) which shows that the total oxygen concentration in the domain can only be changed by the boundary conditions. Finally, the observation that $\alpha_c \tilde{\gamma}_{c,I-1} + \alpha_p \tilde{\gamma}_{p,I-1} + \alpha_c \tilde{\gamma}_{c,I} + \alpha_p \tilde{\gamma}_{p,I} = \alpha_{I,k+1}^I$ implies that the scheme for $P$ can be expressed as $P_{k+1}^I = \lambda P_{k-1}^I + (1 - \lambda) P_k^I$ for some $\lambda \in [0, 1]$. In particular, the nonnegativity of the coefficients $\lambda$ and $1 - \lambda$ shows that the method is monotone for $P$, hence cannot introduce spurious oscillations \[130\], Chapter 13.

In OpenFOAM, numerical schemes need to be expressed in terms of fluxes over cell faces. Thus, the scheme (2.28) has to be recast in flux form.
2.2. Model with moving red blood cells

Figure 2.2: Schematic for the improved scheme for the advection term in Eq. (2.5). The RBC domain $\Omega_{\text{rbc}}$ in red is shifted by $v_{\text{rbc}} \Delta t$ between times $t^k$ and $t^{k+1}$. The volume fractions $\tilde{\gamma}_{c,I-1}$, $\tilde{\gamma}_{p,I-1}$ and $\tilde{\gamma}_{p,I}$ are defined in the main text.

\[
C_{I}^{k+1} = C_{I}^{k} + \frac{\Delta t}{\Delta x} (F_{I-1/2}^{k} - F_{I+1/2}^{k}),
\]

(2.29)

where the appropriate fluxes $F_{I-1/2}^{k}$ and $F_{I+1/2}^{k}$ need to be determined. In the sequel, we will assume that $v_{\text{rbc}} > 0$ and that the Courant-Friedrichs-Lewy number $c = v_{\text{rbc}} \Delta t / \Delta x$ satisfies $c \leq 1$. If there is no RBC interface in the cell $\Omega_{I-1}$ at time step $k$, the flux over the cell face $I-1/2$ reduces to $F_{I-1/2}^{k} = v_{\text{rbc}} C_{I-1}^{k}$. If $\Omega_{I-1}$ contains an RBC interface at time step $k$, the indices $l$ and $r$ are used to denote quantities at the left and right of this interface, respectively. In the situation illustrated in Fig. 2.2, the solubility on either side of the RBC interface are given by $\alpha_{I-1,l}^{k} = \alpha_c$ and $\alpha_{I-1,r}^{k} = \alpha_p$. Similarly, the volume fraction $\gamma_{I-1,r}^{k}$ denotes the plasma volume fraction in that case. With this notation, one can derive the oxygen flux from Eq. (2.28) to obtain

\[
F_{I-1/2}^{k} = v_{\text{rbc}} \frac{C_{I-1}^{k}}{\alpha_{I-1}^{k}} \left( \alpha_{I-1,l}^{k} \max \left( 0, 1 - \frac{\gamma_{I-1,r}^{k}}{c} \right) + \alpha_{I-1,r}^{k} \min \left( 1, \frac{\gamma_{I-1,r}^{k}}{c} \right) \right).
\]

(2.30)

In two-dimensional simulations, RBCs may cover only partly cells of the fixed grid in the radial direction. In this case, an area-weighted average of the scheme (2.30) and the simple upwind scheme is used. This is implemented in the file Po2AdvEqn.H used by our custom solver hbPOEulerFoam.
2.2.5 Reconstructed capillary networks

The oxygen transport model in axisymmetric domains presented in the above subsections was extended to capillary networks. The knowledge of the position and the velocity of individual RBCs is required by our algorithm. RBC trajectories through the capillary networks were obtained using the discrete RBC transport model by Schmid et al. [110] that takes into account the Fåhraeus and Fåhraeus-Lindqvist effects, as well as phase separation at divergent bifurcations.

Since the time steps in the oxygen transport model are smaller than in the RBC transport model [110], the computed RBC positions were linearly interpolated. RBCs were modeled as castellated rigid cylinders with the axis tangent to the capillary centerline. The RBC meshes were obtained from a Cartesian mesh by removing all cells that were not contained in a cylinder with the RBC diameter and length. Then, the mesh was scaled to exactly have the prescribed volume \( V_{rbc} \). The use of cylindrical RBC meshes was found to yield the same results as with castellated RBC meshes.

Since capillaries may have different cross sections, the RBC diameter was set to 80 percent of the lumen diameter. For capillary diameters between 4 and 8 \( \mu m \), this corresponds to an endothelial surface layer width between 0.4 and 0.8 \( \mu m \), which falls within the range of measured values in Pries et al. [98]. The discrete RBC transport model [110] enforces that RBC centers are separated by a minimal distance \( L_{rbc} = V_{rbc}/(\pi r^2_c) \), so the RBCs cannot overlap unless they are separated by a kink or a bifurcation.

Eq. (2.5) was solved in a rectangular box that contains multiple capillaries and oxygen-consuming tissue. Eq. (2.6) was solved in all moving RBCs that overlap with the box. In the axisymmetric domain shown in Fig. 2.4, a mesh can be generated so that each grid cell is either in the lumen, the capillary wall or the tissue. Thus, it is straightforward to set the diffusion and solubility coefficients in each grid cell. In capillary networks, these coefficients are computed using an approach based on volume fractions, since the generation of a mesh that conforms with tortuous, branching capillaries would be very challenging. Instead, a Cartesian mesh was used to discretize the fixed domain. For each capillary, a tortuous cylinder following the centerline was constructed (Fig. 2.3). The cylinder was composed of an inner region for the lumen and an outer annulus for the endothelium. For each grid cell in the fixed mesh, the lumen and endothelium volume fractions \( \gamma_p \) and \( \gamma_w \) were obtained by computing its intersection volume with the reconstructed cylinder. At branchings between capillaries, the reconstructed cylinders may overlap. In this case, the contributions of each cylinder were added and the volume fractions were clamped so that their sum is at most...
2.2. Model with moving red blood cells

The tissue volume fraction was then obtained by \( \gamma_t = 1 - \gamma_p - \gamma_w \).

At the beginning of each simulation, the diffusion and solubility coefficients for each grid cell without RBC influence were obtained as the weighted harmonic average of the respective coefficients in each region, where the weights are \( \gamma_p \), \( \gamma_w \) and \( \gamma_t \). Subsequently, at each time step, the obtained coefficient was corrected in grid cells that were overlapping with an RBC using the coefficient value in RBCs and the RBC volume fraction \( \gamma_c \).

Figure 2.3: Meshes used in the model extension to capillary networks. The parallelepiped is a clipped part of the computational domain and the colors show values of the plasma volume fraction \( \gamma_p \) (red: 1; blue: 0). A fraction of the submesh for the lumen used by the velocity correction scheme is also shown.

The velocity field in the blood vessels is required in Eq. (2.5). To obtain it, for each vessel between nodes \( i \) and \( j \), the RBC velocity \( v_{rbc,ij} \) from the discrete RBC transport model was mapped to the matching capillary. This was done by tagging each grid cell with \( \gamma_p > 0 \) with its corresponding vessel index and assigning to it the velocity vector with magnitude \( v_{rbc,ij} \) and direction tangent to the vessel centerline at the nearest point to the cell center. The resulting velocity field \( u_{\text{mapped}} \) was generally not discretely divergence free because of the tortuosity of the vessels and the branchings, which would result in a violation of mass conservation. Therefore, the velocity field was corrected based on the Helmholtz-Hodge decomposition theorem which states that a smooth vector field \( u \) can be decomposed as the sum of a divergence-free velocity field \( v \) and an irrotational component as \( u = v + \nabla \phi \). The velocity field \( v \) is obtained by applying the divergence
Numerical modeling of O$_2$ transport

operator, which results in the correction scheme

$$\nabla^2 \phi = \nabla \cdot \mathbf{u}, \quad (2.31)$$

$$\mathbf{v} = \mathbf{u} - \nabla \phi. \quad (2.32)$$

Eq. (2.31) was solved on the submesh of the fixed mesh that is composed of all grid cells with $\gamma_p > 0$ (Fig. 2.3). Before solving for $\phi$, the mapped velocity field $\mathbf{u}_{\text{mapped}}$ was corrected at the boundary of the submesh so that the influx exactly matches the outflux on each connected component, which is required to fulfill mass conservation. Finally, a homogeneous Neumann boundary condition was used for $\phi$. Eq. (2.31) was discretized using the central scheme and solved using the preconjugate gradient method.

The method described above was implemented in C++ with the open source software library OpenFOAM 2.3.0 [142]. The algorithm was parallelized to allow simulations in larger networks. The fixed mesh for oxygen transport was decomposed in rectangular parallelepipeds, each held by one processor. Eq. (2.5) was solved in parallel using the standard MPI-based capabilities of OpenFOAM. The RBCs meshes were distributed among processors as follows. A RBC mesh is held by the processor that contains its center. If no processor contains the RBC center, the RBC mesh is held by one of the processors that overlap with it, if any. With this decomposition, only two operations are parallel. The first parallel operation is field interpolation to and from RBCs that overlap multiple processor domains. Parallel interpolation between meshes is implemented in the OpenFOAM class meshToMesh and a derived class meshToMeshMoving that supports mesh motion was written. The second parallel operation is the communication of RBCs when their center crosses the boundary between two processors.

In our implementation, each processor has access to the position of all RBCs and to a reference RBC mesh. Thus, at each time step, each processor can individually compute the list of RBCs that it needs to send or receive, and the list of associated source and target processors. As each processor has access to a reference RBC mesh, the RBC meshes do not need to be communicated, which reduces the amount of interprocessor communication. All RBC meshes were contained in a single instance of fvMesh, hence we will now refer to RBC submeshes. The possibility to add and remove disconnected submeshes from an fvMesh was implemented in a custom class regionAddRemoveFvMesh deriving from topoChangerFvMesh. Each processor performs the following operations during mesh motion:

1. for each processor, compute the list of RBCs that have to be sent to or received from that processor;
2.2. Model with moving red blood cells

2. for each processor and for each RBC sent to that processor, send the RBC hemoglobin saturation field to that processor;

3. for each sent RBC, remove the associated RBC submesh from the local RBC mesh;

4. for each received RBC, create an associated RBC submesh by copying the reference RBC mesh, add this submesh to the local RBC mesh and transform it to the required position;

5. for each processor and for each RBC received from that processor, receive the RBC hemoglobin saturation field from that processor and assign it to associated RBC submesh;

6. for each received RBC, compute the RBC PO\textsubscript{2} in equilibrium with the hemoglobin saturation.

Only steps 2 and 5 involve parallel communication. These communication operations were performed using the classes IP\texttt{stream} and OP\texttt{stream} that wrap MPI calls. The serial steps 3 and 4 are required to prepare the mesh structure to store the received fields. Step 6 is required to prevent RBC PO\textsubscript{2} values from being uninitialized. The algorithm was successfully employed with 4.1 million grid cells on 48 processors.

2.2.6 Model parameters

Our main goal is the validation of the method explained above against the experimental data from Parpaleix et al. [86]. These data were acquired in the rodent olfactory glomerulus, which is an area with a high capillary density.

We used an axially symmetric geometry with a capillary at its center – similar to the classical Krogh model [63]. Instead of a cylinder, we employed a cone-shaped domain with different radii at the proximal (arteriolar) and distal (venular) ends. Due to symmetry, Ω can be represented by a two-dimensional domain. As shown in Fig. 2.4, Ω consists of three regions, namely the plasma, the capillary wall and the tissue.

In the olfactory glomerulus, the average distance from any point to the nearest capillary is 10.8 µm [64]. In a hexagonal array of Krogh cylinders with a capillary diameter of 4 µm this corresponds to a radius of 16 µm. Therefore, unless stated otherwise, the radii on the arteriolar and venular sides were set to \( r_{t,a} = 19 \) µm and \( r_{t,v} = 13 \) µm, respectively. The length of the capillary was set to 100 µm.
Figure 2.4: Computational domain with a fixed region containing plasma, the capillary wall and surrounding tissue. Moving RBCs enter and leave the capillary from the arteriolar and venular sides, respectively.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Value</th>
<th>Units</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha_c$</td>
<td>$O_2$ solubility in RBCs</td>
<td>$3.38 \times 10^{-5}$</td>
<td>ml $O_2$ mmHg$^{-1}$ cm$^{-3}$</td>
<td>[1]</td>
</tr>
<tr>
<td>$\alpha_p$</td>
<td>$O_2$ solubility in the plasma</td>
<td>$2.82 \times 10^{-5}$</td>
<td>ml $O_2$ mmHg$^{-1}$ cm$^{-3}$</td>
<td>[16]</td>
</tr>
<tr>
<td>$\alpha_w$</td>
<td>$O_2$ solubility in the capillary wall</td>
<td>$3.89 \times 10^{-5}$</td>
<td>ml $O_2$ mmHg$^{-1}$ cm$^{-3}$</td>
<td></td>
</tr>
<tr>
<td>$\alpha_t$</td>
<td>$O_2$ solubility in the tissue</td>
<td>$3.89 \times 10^{-5}$</td>
<td>ml $O_2$ mmHg$^{-1}$ cm$^{-3}$</td>
<td>[17]</td>
</tr>
<tr>
<td>$D_c$</td>
<td>$O_2$ diffusivity in RBCs</td>
<td>$9.5 \times 10^{-6}$</td>
<td>cm$^2$ s$^{-1}$</td>
<td>[17]</td>
</tr>
<tr>
<td>$D_p$</td>
<td>$O_2$ diffusivity in the plasma</td>
<td>$2.18 \times 10^{-5}$</td>
<td>cm$^2$ s$^{-1}$</td>
<td>[65]</td>
</tr>
<tr>
<td>$D_w$</td>
<td>$O_2$ diffusivity in the capillary wall</td>
<td>$8.73 \times 10^{-6}$</td>
<td>cm$^2$ s$^{-1}$</td>
<td>[65]</td>
</tr>
<tr>
<td>$D_t$</td>
<td>$O_2$ diffusivity in the tissue</td>
<td>$2.41 \times 10^{-5}$</td>
<td>cm$^2$ s$^{-1}$</td>
<td>[6]</td>
</tr>
<tr>
<td>$D_{Hb}$</td>
<td>Hemoglobin diffusivity in RBCs</td>
<td>$1.44 \times 10^{-7}$</td>
<td>cm$^2$ s$^{-1}$</td>
<td>[17]</td>
</tr>
<tr>
<td>$k_-$</td>
<td>Dissociation rate constant</td>
<td>44</td>
<td>s$^{-1}$</td>
<td>[17]</td>
</tr>
<tr>
<td>$n$</td>
<td>Hill exponent</td>
<td>2.64</td>
<td>–</td>
<td>fit from [138]</td>
</tr>
<tr>
<td>$N_{Hb}$</td>
<td>Total heme density</td>
<td>$2.03 \times 10^{-5}$</td>
<td>mol cm$^{-3}$</td>
<td>[17]</td>
</tr>
<tr>
<td>$P_{50}$</td>
<td>$O_2$ at hemoglobin half-saturation</td>
<td>47.9</td>
<td>mmHg</td>
<td>fit from [138]</td>
</tr>
<tr>
<td>$V_{mol,O_2}$</td>
<td>$O_2$ molar volume at 36.9°C</td>
<td>$2.54 \times 10^4$</td>
<td>ml $O_2$ mol$^{-1}$</td>
<td>ideal gas law</td>
</tr>
<tr>
<td>$V_{rbc}$</td>
<td>RBC volume</td>
<td>59.0</td>
<td>µm$^3$</td>
<td>[121]</td>
</tr>
</tbody>
</table>

Table 2.2: Parameter values

The RBC shape was taken from Secomb et al. [117] for a RBC velocity of 1 mm/s. This shape (computed for human RBCs) was scaled down to the size of mouse erythrocytes with volume $V_{rbc} = 59.0$ fl [121]. We used the mean RBC velocity $v_{rbc} = 0.57$ mm/s measured in the olfactory glomerulus by Chaigneau et al. [11].

The cerebral metabolic rate of oxygen consumption (CMRO$_2$) is an essential model parameter. To our knowledge, no measurement of CMRO$_2$ in the olfactory glomerulus has been performed. Therefore, we chose the value CMRO$_2 = 197$ µM s$^{-1}$ to obtain $P_{O_2}$ values in the tissue between 15 and 20 mmHg approximately (using the perfect gas law at 36.9°C, this corresponds to $M_0 = 0.005$ µm$^3$ O$_2$ µm$^{-3}$ s$^{-1}$). The resulting values of $P_{O_2}$ in the plasma agree well with the results of Parpaleix et al. [86]. The remaining
2.3. Results

2.3.1 Erythrocyte-associated transients

We now show simulated values of oxygen tension inside the sample capillary and the surrounding tissue region shown on Fig. 2.4. Whenever possible, we compare our results with the data measured by Parpaleix et al. [86] using two-photon phosphorescence lifetime microscopy in the rodent olfactory glomerulus. They characterized intracapillary oxygen tension with the three following quantities: RBC \(P_{\text{O}_2}\), mean \(P_{\text{O}_2}\) and inter-RBC \(P_{\text{O}_2}\). RBC \(P_{\text{O}_2}\) is the maximal oxygen tension in the plasma, which is attained at the erythrocyte membrane. Mean \(P_{\text{O}_2}\) is the average \(P_{\text{O}_2}\) value between two erythrocytes and inter-RBC \(P_{\text{O}_2}\) is the minimal \(P_{\text{O}_2}\) between two RBCs. The EAT amplitude is the difference between RBC \(P_{\text{O}_2}\) and inter-RBC \(P_{\text{O}_2}\) (Fig. 1.4). Throughout this section, the coordinate \(x\) denotes the axial direction. In this study, the upwind scheme was used to discretize the advection term in Eq. (2.21) as no numerical stability issues were observed with the employed linear density and RBC velocity.

Using the parameters listed in Tables 2.2 and 2.3, we obtained an averaged EAT amplitude of 29.7 mmHg (RBC \(P_{\text{O}_2}\) = 50.8 mmHg, inter-RBC \(P_{\text{O}_2}\) = 21.1 mmHg, mean \(P_{\text{O}_2}\) = 27.4 mmHg). These values were obtained by sampling \(P_{\text{O}_2}\) on the capillary centerline at nine evenly spaced longitudinal locations (between \(x = 10 \mu\text{m}\) and 90 \(\mu\text{m}\)). The maximal \(P_{\text{O}_2}\) in the plasma was attained on the rear side of the RBC membrane. Parpaleix et al. [86] also observed significant differences between these quantities (RBC \(P_{\text{O}_2}\)

Table 2.3: Specific parameters used in Subsection 2.3.1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Value</th>
<th>Units</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>(I_{\text{rbc}})</td>
<td>RBC length</td>
<td>7.27</td>
<td>(\mu\text{m})</td>
<td>based on [117], [121]</td>
</tr>
<tr>
<td>(M_0)</td>
<td>maximal (\text{O}_2) consumption rate</td>
<td>(5 \times 10^{-3})</td>
<td>(\text{mm}^3\text{O}_2 \text{m}^{-3}\text{s}^{-1})</td>
<td>fitted</td>
</tr>
<tr>
<td>(\mu_{LD})</td>
<td>mean linear density</td>
<td>0.36</td>
<td>–</td>
<td>[86]</td>
</tr>
<tr>
<td>(P_{\text{crit}})</td>
<td>critical (\text{P}_{\text{O}_2}) in the tissue</td>
<td>1.0</td>
<td>mmHg</td>
<td>[35]</td>
</tr>
<tr>
<td>(P_{c,a})</td>
<td>RBC (\text{P}_{\text{O}_2}) at the capillary entrance</td>
<td>90</td>
<td>mmHg</td>
<td>based on [86]</td>
</tr>
<tr>
<td>(P_{r,a})</td>
<td>plasma (\text{P}_{\text{O}_2}) at the capillary entrance</td>
<td>40</td>
<td>mmHg</td>
<td>based on [86]</td>
</tr>
<tr>
<td>(\sigma_{LD})</td>
<td>standard deviation of linear density</td>
<td>0.1</td>
<td>–</td>
<td>based on [11]</td>
</tr>
<tr>
<td>(r_p)</td>
<td>radius of capillary lumen</td>
<td>2.0</td>
<td>(\mu\text{m})</td>
<td>[132]</td>
</tr>
<tr>
<td>(r_{w} - r_p)</td>
<td>capillary wall thickness</td>
<td>0.6</td>
<td>(\mu\text{m})</td>
<td>[8]</td>
</tr>
<tr>
<td>(r_{t,a})</td>
<td>tissue radius on arteriolar side</td>
<td>19</td>
<td>(\mu\text{m})</td>
<td>based on [64]</td>
</tr>
<tr>
<td>(r_{t,v})</td>
<td>tissue radius on venular side</td>
<td>13</td>
<td>(\mu\text{m})</td>
<td>based on [64]</td>
</tr>
<tr>
<td>(v_{\text{rbc}})</td>
<td>RBC velocity</td>
<td>(5.7 \times 10^{-2})</td>
<td>cm s(^{-1})</td>
<td>[11]</td>
</tr>
</tbody>
</table>

model parameters are summarized in Tables 2.2 and 2.3.
= 57.1 ± 1.3 mmHg (mean ± s.e.m.), inter-RBC PO\textsubscript{2} = 23.6 ± 0.7 mmHg, mean PO\textsubscript{2} = 30.8 ± 0.9 mmHg). Since they performed 241 measurements, the results for our sample capillary differ from these average values by less than one third of a standard deviation.

Fig. 2.5 shows instantaneous longitudinal profiles on the capillary centerline and at various radial distances from the capillary wall. In RBCs close to the arteriolar end of the domain, the intracellular PO\textsubscript{2} variation exceeds 30 mmHg and decreases to 15 mmHg at the venular end. These strong intravascular oxygen variations extend to the nearby tissue. At 1 \(\mu\)m from the outer side of the wall, the amplitude of these fluctuations ranges from 12.7 mmHg to 4.2 mmHg. Away from the capillary entrance, these values agree well with the mean pulse amplitude of 5.0 mmHg reported by Parpaleix et al. [86] outside the vessel (< 2\(\mu\)m). At 5 \(\mu\)m from the endothelium, these pulses are almost entirely smeared out. The influence of instantaneous linear density fluctuations on inter-RBC PO\textsubscript{2} is clearly illustrated by the second and the third RBC spacings. Since short RBC spacings cause higher inter-RBC PO\textsubscript{2} values, the EAT amplitude drops when the instantaneous linear density increases.

We then investigated longitudinal variations of PO\textsubscript{2} along our sample capillary. Fig. 2.6 shows time-averaged oxygen partial pressures for the cone-shaped geometry (Fig. 2.4) and for a cylinder with equal tissue volume. Since RBC PO\textsubscript{2} declines faster than inter-RBC PO\textsubscript{2}, the EAT amplitudes
also decrease along the capillary. Parpaleix et al. [86] reported longitudinal variations of PO$_2$ in single capillaries over a mean distance of 49.7µm. Table 2.3.1 contains these values as well as our simulated PO$_2$ variations in the conical and cylindrical geometries. The maximal gradients in the cone-shaped geometry are a consequence of the high RBC PO$_2$ at the capillary entrance. However, the gradients away from the arteriolar end of the domain correspond very well to the experimental data, while in the cylinder geometry the gradients of mean PO$_2$ and inter-RBC PO$_2$ are significantly higher than in the reference data. A better match could not be obtained in a cylindrical geometry by changing CMRO$_2$, since this would considerably decrease the agreement of RBC PO$_2$ and inter-RBC PO$_2$ with experimental data. The chosen geometry with $r_{t,a} = 19$µm and $r_{t,v} = 13$µm had the smallest taper that yielded a good match with the measured longitudinal PO$_2$ variations. These results suggest that a cylindrical tissue geometry is not a suitable model for oxygen supply from capillaries, at least in the brain region considered in this study.

![Figure 2.6: Time-averaged PO$_2$ levels on the vessel centerline. A: Cone-shaped domain with $r_{t,a} = 19$µm and $r_{t,v} = 13$µm. B: Cylindrical domain with the same volume ($r_{t,a} = r_{t,v} = 16.1$µm). Circles: RBC PO$_2$; squares: mean PO$_2$; triangles: inter-RBC PO$_2$; dashed line: EAT amplitude.](image)

Our model includes instantaneous variations of linear density similar to those observed by Chaigneau et al. [11]. Fig. 2.7 shows values of RBC PO$_2$ and inter-RBC PO$_2$ that were collected during three seconds at 30µm from the capillary entrance. The linear density on the horizontal axis was quantified by the length occupied by RBCs over a given capillary segment divided by the segment length. As previously observed in Fig. 2.5, inter-
Table 2.4: Longitudinal variation of the time-averaged \( \text{PO}_2 \) over 50\(\mu m\) in the cone and cylinder geometries, compared with experimental data. The columns with the heading “art.” (“ven.”) show the averaged \( \text{PO}_2 \) variation between \( x = 10\mu m \) (\( x = 40\mu m \)) and \( x = 60\mu m \) (\( x = 90\mu m \)). Last column: mean \( \pm \) s.e.m.

\[
\begin{array}{cccc}
\Delta \text{RBC PO}_2 & 25.7 & 14.3 & 25.6 & 16.4 & 14.1 \pm 9.2 \\
\Delta \text{mean PO}_2 & 12.0 & 6.4 & 16.9 & 12.4 & 4.6 \pm 2.4 \\
\Delta \text{inter-RBC PO}_2 & 8.0 & 4.3 & 14.4 & 11.3 & 3.0 \pm 2.7 \\
\end{array}
\]

RBC \( \text{PO}_2 \) is correlated with the linear density. The dependency of inter-RBC \( \text{PO}_2 \) on linear density agrees very well with the experimental data, but the simulated RBC \( \text{PO}_2 \) is almost constant, while the reference data exhibit a positive correlation between linear density and RBC \( \text{PO}_2 \). Our simulations did not reproduce this trend since a single capillary with constant RBC \( \text{PO}_2 \) at its arteriolar end was used. However, Parpaleix et al. \[86\] measured EAT properties in 42 capillaries, which limits the scope of this comparison. This difference between the pooled experimental data and our computations in a single capillary indicates that capillaries with high average linear density also have a higher \( \text{PO}_2 \). Besides, Parpaleix et al. \[86\] have observed that inter-RBC \( \text{PO}_2 \) attains similar values as \( \text{PO}_2 \) in the neuropil. Fig. 2.7 also shows the difference between inter-RBC \( \text{PO}_2 \) and tissue \( \text{PO}_2 \) at 10\(\mu m\) from the capillary wall as a function of linear density. For linear densities lower than 0.25, this difference stays below 2.0 mmHg. For high hematocrit values, this gap exceeds 10 mmHg. Thus, our results indicate that inter-RBC \( \text{PO}_2 \) may significantly exceed tissue \( \text{PO}_2 \) for high linear densities.

Since linear density affects tissue \( \text{PO}_2 \), we investigated the influence of the standard deviation \( \sigma_{LD} \) of linear density on tissue \( \text{PO}_2 \). Fig. 2.8 shows tissue \( \text{PO}_2 \) at 10\(\mu m\) from the capillary wall and \( x = 50\mu m \) for two different values of \( \sigma_{LD} \). The same random numbers were used and the parameters of the log-normal distribution for RBC spacings were adjusted to obtain an average linear density of 0.28 over four seconds and the desired standard deviation. Only the last second of the simulation is shown. Random fluctuations of linear density led to large \( \text{PO}_2 \) oscillations. For \( \sigma_{LD} = 0.08 \), the difference between minimal and maximal \( \text{PO}_2 \) was 5.7 mmHg, and for higher fluctuations (\( \sigma_{LD} = 0.16 \)), it increased to 10.9 mmHg. This is a consequence of RBC groups that are close to or far away from each other. Occasionally, a large RBC spacing resulted in a sudden drop in tissue \( \text{PO}_2 \).
2.3. Results

Figure 2.7: Dependence of RBC PO$_2$ and inter-RBC PO$_2$ on linear density. A: Simulated PO$_2$ values collected during three seconds at 30 µm from the capillary entrance. Circles: RBC PO$_2$; triangles: inter-RBC PO$_2$; crosses: difference between inter-RBC PO$_2$ and tissue PO$_2$ at 10 µm from the capillary wall. B: Measurements [86]. Top bar: RBC PO$_2$; shaded bar: inter-RBC PO$_2$. Error bars: SD.

by several mmHg. Therefore, if linear density fluctuations as reported by Chaigneau et al. [11] are present, PO$_2$ in the tissue cannot be considered to be constant. The influence of the boundary condition for oxygen on these numerical values is discussed below.

Figure 2.8: Tissue PO$_2$ for different standard deviations of linear density at 10 µm from the capillary wall (x = 50 µm). The mean linear density is 0.28. Solid line: $\sigma_{LD} = 0.08$; dashed line: $\sigma_{LD} = 0.16$.

Finally, we compare our results with previous works by examining the
Numerical modeling of $O_2$ transport

intracapillary resistance to oxygen transport. MTCs were computed using a constant linear density and compared with previously published values. The MTC may be defined as $k = j/(P^* - P_w)$, where $j$ is the oxygen flux ($\text{ml} O_2 \text{ cm}^{-2} \text{s}^{-1}$), $P^*$ is the oxygen tension in equilibrium with the mean hemoglobin saturation in the RBC and $P_w$ is the average oxygen tension at the capillary wall around a RBC. For a tube hematocrit of 0.25, we obtained $k = 1.67 \times 10^{-6} \text{ ml} O_2 \text{ cm}^{-2} \text{s}^{-1}$, which exactly matches the results of Eggleton et al. \cite{23} for the same hematocrit and capillary radius ($r_p = 2.0 \mu m$). This consistency was expected, since the same equations as in Eggleton et al. \cite{23} were solved (except myoglobin-facilitated diffusion in the tissue) and similar diffusion and solubility coefficients were chosen.

Comparison with earlier works can also be performed using the Nusselt number which is defined by

$$Nu = \frac{j d_p}{D_p \alpha_p (P^* - P_w)},$$

where $d_p$ is the capillary diameter. For tube hematocrit values between 0.15 and 0.36, we obtained Nusselt numbers from 0.48 to 1.7. Hellums et al. \cite{50} summarized Nusselt numbers from various studies. For a diameter of 3.6 $\mu m$ and a tube hematocrit of 0.28, Secomb et al. \cite{114} calculated $Nu = 1.22$ using a solid cylinder model. Our computed value for this tube hematocrit is 1.17. Therefore, our model reproduces oxygen fluxes from previous studies in steady state situations.

### 2.3.2 Capillary dilations

The distribution of RBCs in capillaries affects the intraluminal oxygen field, gives rise to EATs, and alters the relation between $P_{O_2}$ in the plasma and in the tissue. We now take advantage from our model’s ability to capture the consequences of altered RBC distributions on oxygen transport. Based on a discrete RBC transport model, Schmid et al. \cite{109} showed that capillary dilations downstream of a diverging bifurcation can increase the number of RBCs in the dilated branch, while the flow rate in that branch stays constant and the RBC velocity decreases. In the undilated branch, no significant changes in RBC flow properties were found. This study was motivated by the presence of pericytes in the cerebral microvasculature. These contractile cells have the ability to dilate or constrict capillaries in the brain \cite{88} and were reported to actively participate in the regulation of cerebral blood flow \cite{47}. In view of the ongoing debate on the role of pericytes in neurovascular coupling \cite{52, 3}, it is important to elucidate how pericycle dilation affects oxygen transport. The consequences of the RBC
redistribution that follows a capillary dilation are now examined with our numerical model.

A simple capillary network with a divergent bifurcation followed by a convergent bifurcation was employed (Fig. 2.9). The domain size was $140 \times 80 \times 30 \mu m$ and due to the domain symmetry with respect to the $z$-plane, oxygen transport was only simulated in one half of the domain. The RBC trajectories were obtained using the RBC transport model by Schmid et al. [109] with an erythrocyte velocity of 1 mm/s, a tube hematocrit of 0.3 at the inlet and a standard deviation of linear density equal to 0.1. The baseline vessel diameter was set to 4.0 $\mu m$ and the diameter of the 30-µm-long upper branch was increased to 4.8 $\mu m$ in the capillary dilation scenario.

To compare the effect of the capillary dilation with that of upstream arteriolar dilations, a CBF increase was modeled by gradually raising the blood flow at the inlet by 50 percent over a time interval of 0.2 s. This increase is of the same order as measurements in the rodent olfactory bulb, where a RBC velocity increase of 31 ± 7% was measured after odor was applied [12]. The baseline CMRO$_2$ was set to $2.5 \times 10^{-3}$ mm$^3$ O2 μm$^{-3}$ s$^{-1}$. A local increase in oxygen metabolism was simulated by raising the peak CMRO$_2$ in the center of the domain by a factor four. In this activation scenario, the spatial profile of $M_0$ was given by a Gaussian distribution with a standard deviation of 10 $\mu m$. The capillary dilation and the increase in CMRO$_2$ were assumed to occur simultaneously and the CBF increase was started 0.8 s afterwards (Fig. 2.10). Simulations were run using the model extension to capillary networks presented in Subsection 2.2.5. A Neumann boundary condition was used for the oxygen partial pressure. For each scenario, the result of a simulation that reached a statistical steady state was used as an initial condition. Then, oxygen transport was simulated during six seconds after the CMRO$_2$ increase.

Four scenarios with a transient increase in oxygen metabolism are shown in Fig. 2.10. Without any vascular response, tissue PO$_2$ in the domain center drops by 9 mmHg. As a result of the capillary dilation, the oxygen tension in the domain center reaches its new value after approximately one second. The time-averaged tissue PO$_2$ difference over four seconds between the baseline case and the capillary dilation is 0.9 mmHg. After the gradual CBF increase, tissue PO$_2$ adapts within 0.6 s and becomes 4.8 mmHg higher than in the absence of any dilation. The additional capillary dilation further increases tissue PO$_2$ by 1.0 mmHg. Thus, the combined effect of the CBF increase and the capillary dilation turns out to be a linear superposition of their individual influence. The fluctuations in tissue PO$_2$ in the domain center reach a maximal amplitude of 3.3 mmHg over the six
seconds of simulation time. In the current activation scenario, the tissue oxygen tension prior to the CMRO$_2$ increase is not recovered, even with the 50 percent increase in CBF.

The spatial changes in tissue PO$_2$ that result from CMRO$_2$ and blood flow changes are shown in Fig. 2.11. The values at $y = 0$ coincide with the time-averaged PO$_2$ levels in Fig. 2.10. After dilation, the mean PO$_2$ in the dilated capillary rises by 4.2 mmHg, while it drops by 0.6 mmHg in the other branch. Tissue PO$_2$ at $y = 40$ µm increases more than in the domain center (2.7 mmHg instead of 0.9 mmHg). This difference is due to the Neumann boundary condition at the domain boundary that actually mirrors the dilated capillary. Thus, this higher increase expresses the effect of two identical capillary dilations downstream of distinct bifurcations. Finally, the tissue PO$_2$ responses to the CBF increase and the capillary dilation are again linearly superposed. These results suggest that capillary dilations have the ability to enhance the upregulation of tissue PO$_2$ induced by a
2.3. Results

Figure 2.10: Temporal profiles of PO$_2$ in the center of the domain shown in Fig. 2.9.

Figure 2.11: Oxygen profiles for the four different activation scenarios. The profiles are plotted along the dashed line in Fig. 2.9. The PO$_2$ values were averaged during four seconds, starting two seconds after the CMRO$_2$ increase. Left: baseline CMRO$_2$; right: increased CMRO$_2$ in the shaded area indicated in Fig. 2.9. Solid line: baseline; dashed line: capillary dilation; dotted line: cerebral blood flow increased by 50 percent; dash-dotted line: capillary dilation combined with cerebral blood flow increase.
CBF increase, but that a single dilation is too weak to elicit a substantial increase in tissue PO$_2$ away from capillaries.

### 2.4 Discussion

Oxygen transport from a capillary with moving RBCs to the surrounding tissue was simulated in an axisymmetric cone-shaped geometry. Oxygen partial pressure in the capillary and the tissue was compared with experimental data [86]. Longitudinal oxygen variations and the influence of linear density were investigated. As an application of the extension of our model to capillary networks, we investigated the impact of capillary dilations and cerebral blood flow increase on tissue oxygenation around a simple network. The computations in the axisymmetric computational domain are discussed first.

Our simulations reproduced a number of results from Parpaleix et al. [86]. Their average measured EAT amplitude was 33.5 mmHg, and similar amplitudes were obtained in the first section of our sample capillary (Fig. 2.6). At 30 µm from the capillary entrance, the simulated EAT amplitude was 33.6 mmHg. Close to the venular end, RBC PO$_2$ was lower due to oxygen consumption in the tissue, which gave rise to smaller EATs (< 25 mmHg). Therefore, our average EAT amplitude of 29.7 mmHg over the nine sampled positions is slightly lower than that from Parpaleix et al. [86]. Since the experimental data were collected independently of the measurement position in the vascular bed, it is difficult to further interpret these differences. However, the dependency of EAT values on the distance from the arteriolar side could be studied experimentally in the brain cortex.

The relationship between intracapillary oxygen tensions and tissue PO$_2$ was also examined. For linear densities lower than 0.25, simulated inter-RBC PO$_2$ exceeds tissue PO$_2$ at 10 µm from the capillary wall by less than 2.0 mmHg (Fig. 2.7), while this difference is larger than 10 mmHg for higher hematocrit values. These findings are only in partial agreement with the observation by Parpaleix et al. [86] that inter-RBC PO$_2$ attains similar values to those in the neuropil. However, measurements in capillaries and tissue were not performed simultaneously and results were averaged over several seconds, which filtered out PO$_2$ fluctuations, whereas we report instantaneous snapshots. Moreover, the influence of hematocrit fluctuations was not examined in this part of the experiment. Therefore, our simulations indicate that inter-RBC PO$_2$ is similar to tissue PO$_2$ only close to the capillary or at low linear densities. Since concentration gradients drive molecular diffusion, we suggest that inter-RBC PO$_2$ is on average higher
than tissue PO$_2$ far away from capillaries, provided they are not close to an arteriole or another capillary with substantially higher hemoglobin saturation. This hypothesis can be tested in vivo by measuring the dependency of tissue PO$_2$ on the distance to the nearest capillary.

Our simulation setup with RBCs moving through a fixed capillary allows the computation of longitudinal oxygen gradients. Motivated by the fact that capillary segments with high oxygen tensions can supply a correspondingly large tissue volume, we used a cone-shaped geometry (Fig. 2.4) similar to Hudetz [57]. We compared results obtained with this geometry and with a simple cylindrical domain to the data from [86], where longitudinal PO$_2$ variations were measured in individual capillaries. While gradients of mean PO$_2$ and inter-RBC PO$_2$ in the classical Krogh cylinder geometry are much higher than in the reference data (Table 2.3.1), the cone-shaped domain leads to a very good agreement. Although a conical geometry is idealized, it appears to be a suitable model to reproduce in vivo intracapillary oxygen gradients in the brain. This finding may imply that capillary density increases along RBC paths through capillary networks. In other words, we suggest that an evenly distributed tissue PO$_2$ requires denser capillary networks on venular side. However, one should examine whether these simulation results hold in realistic networks, where tortuosity and diffusive interaction between capillaries are present.

Instantaneous variations of hematocrit as observed by Chaigneau et al. [11] can be accounted for by our model, which overcomes a limitation of the models based on MTCs. We treated linear density as a random process governed by a log-normal RBC spacing distribution. The resulting dependency of inter-RBC PO$_2$ on linear density agrees very well with the data in Parpaleix et al. [86] (Fig. 2.7). On the other hand, RBC PO$_2$ stayed constant, which means that the drop in hemoglobin saturation along RBC paths was not influenced by instantaneous hematocrit fluctuations. Since our results were produced in one sample capillary and the data in Parpaleix et al. [86] were pooled from 42 capillaries, we propose the following interpretation of this discrepancy: while fast fluctuations of linear density do not influence RBC PO$_2$, capillaries with high average hematocrit have a higher RBC PO$_2$. This explanation should be investigated by measuring RBC PO$_2$ in capillaries that have different average linear densities.

Additionally, hematocrit fluctuations were also observed to affect tissue PO$_2$ (Fig. 2.8 and 2.10). With a RBC length of 7.27 $\mu$m, the standard deviation of linear density reported by Chaigneau et al. [11] is 0.12. Our results show that for this value, oscillations of oxygen tension in the tissue approach 10 mmHg. Results obtained in a cone-shaped domain exhibit fluctuations of oxygen tension in the tissue that are approaching 10 mmHg.
However, we suggest that the size of these fluctuations are an artefact of the no-flux boundary condition at the cylinder outer boundary that amplifies these fluctuations. The lower PO$_2$ fluctuations ($\leq 3.3$ mmHg) obtained in the center of the simple capillary network shown in Fig. 2.9 support this explanation, since the hematocrit fluctuations in the upper and lower capillaries are asynchronous. Moreover, the no-flux boundary condition at the domain boundary parallel to the z-plane likely still amplifies the PO$_2$ fluctuations, which indicates that the actual fluctuations of tissue PO$_2$ may be even lower. Therefore, we propose that substantial PO$_2$ fluctuations due to hematocrit fluctuations are only likely to occur at low RBC fluxes, when time intervals between RBCs approach the tissue response time to oxygen supply changes.

Although multiple experimental results from [86] could be reproduced, the simulation setup presented here has several limitations, in particular the axisymmetric geometry. While such a geometry is most relevant for parallel capillary arrays in muscles, Krogh cylinder models fail to capture the minimal tissue PO$_2$ in the capillary beds of the brain cortex [115]. Thus, the obtained values of tissue PO$_2$ may be fail to capture the actual minimal oxygen tension under the simulated flow conditions. Nevertheless, the simulated oxygen tensions in the plasma mainly depend on hemoglobin saturation in nearby erythrocytes and the local rate of oxygen extraction from the capillary. Hence it should not be directly affected by diffusive interaction between capillaries. This is confirmed by the good agreement between the simulated inter-RBC PO$_2$ and experimental data (Fig. 2.7).

Other limitations include constant blood velocity, the absence of shifts of the oxygen-hemoglobin dissociation curve and the uncertainty in the choice of parameters. While RBC velocity undergoes fluctuations, their amplitude is lower than that of linear density [11], hence we chose to keep it constant. However, RBC velocity is an important factor for tissue oxygenation and should be realistically modeled. Besides, variations of carbon dioxide concentration and pH are known to shift the equilibrium curve modeled by Eq. (2.2). This may be significant in regions with low PO$_2$ and high CO$_2$ concentration [19]. The inclusion of these shifts would require further modeling efforts. Finally, tissue oxygenation highly depends on CMRO$_2$, which is difficult to measure experimentally. Our chosen value (197 μM s$^{-1}$) is almost three times as high as the CMRO$_2$ in the cortex of awake rats (73.5 μM s$^{-1}$), which was obtained using the value 420 μmol (100 g)$^{-1}$ min$^{-1}$ [33] and a brain density of 1.05 g cm$^{-3}$ [78]. Based on estimates by Nawroth et al. [77], the neuron density in the olfactory glomerulus of the rat is $6.9 \times 10^6$ cells per mm$^3$, whereas this value is $1.17 \times 10^6$ in the mouse neocortex [132]. The high density of neural elements (possibly in combination
with a high steady state firing rate) in the olfactory glomerulus may explain why a high CMRO$_2$ value was needed to reproduce the tissue PO$_2$ observed by Parpaleix et al. \cite{86}. However, using a theoretical energy budget, Nawroth et al. \cite{77} obtained a CMRO$_2$ value of 75 µM s$^{-1}$ for the olfactory glomerulus, which is lower than our chosen value. Further interpretation of this discrepancy would require actual measurements of CMRO$_2$ in the olfactory bulb.

In addition to model limitations, the comparison with experimental data is also limited. When this study was conducted, only the data from \cite{86} allowed a detailed comparison of simulated intracapillary PO$_2$. Meanwhile, the measurements in the awake rodent cortex by Lyons et al. \cite{72} provide additional data. They obtained a slightly higher mean EAT value of 37.1 mmHg. The good agreement with the measurements in Parpaleix et al. \cite{86} was obtained by adapting CMRO$_2$ and the initial PO$_2$ in RBCs on the arteriolar side, and by choosing a tapered cylinder. Further data on intracapillary PO$_2$ and its relation to tissue PO$_2$ should be obtained and compared with \cite{86}. Measurements of intracapillary PO$_2$ and its relation to tissue PO$_2$ should be obtained with improved phosphorescent oxygen probes, such as PtT-CHP307 \cite{101} or PtT-G2p. The parameters mentioned above will most likely need to be modified to reproduce further experiments. The computational model presented in this study will be a useful tool to interpret possible differences between future experimental data.

The impact of capillary dilations on tissue oxygenation is now discussed. Using a two-branch capillary network, we simulated the response of tissue PO$_2$ to a capillary dilation and a cerebral blood flow increase after an elevation in CMRO$_2$. Capillary dilations are expected to locally upregulate tissue oxygenation due to an elevated number of RBCs in the dilated branch \cite{110}. The magnitude of the PO$_2$ changes yet had to be quantified. The impact of a 20 percent diameter increase of a single capillary on the minimal tissue PO$_2$ was considerably lower (+0.9 mmHg) than the effect of a CBF increase by 50 percent (+4.8 mmHg). Although the chosen CBF change was rather high, this indicates that an isolated capillary dilation does not have the ability to significantly raise tissue PO$_2$. However, the capillary hyperemia observed in numerous studies \cite{52} suggests that a substantial number of capillaries participate in this response. Although the mechanisms underlying the increase in total parenchymal hemoglobin concentration are still uncertain, capillary dilations could explain this phenomenon. Due to the no-flux boundary condition for oxygen, the larger tissue PO$_2$ increase (+2.7 mmHg) at the top of the computational domain (Fig. 2.11) is actually caused by two simultaneous capillary dilations. This indicates that an elevated number of RBCs in multiple capillaries may significantly upregulate tissue PO$_2$ away
from microvessels. This conjecture should be verified in larger capillary networks with a reduced influence of the boundary conditions.

Although tissue PO$_2$ responds more weakly to a single capillary dilation than to the CBF increase modeled here, the former is thought to occur before the latter in vivo. Chen et al. [13] showed that the onset of parenchymal hyperemia precedes arteriolar response by 0.211 $\pm$ 0.087 s. In Hall et al. [47], capillary dilation was reported to occur 1.38 $\pm$ 0.38 s before that of penetrating arterioles. Consistently with these data, Wei et al. [141] observed the RBC velocities to increase 1.66 s earlier in capillaries than in upstream arterioles. Therefore, capillary dilations could be a fast upregulation mechanism of tissue oxygenation that precedes the possibly more potent CBF increase induced by arterial dilation. Further, we hypothesize that capillary hyperemia contributes to reduce the initial dip in tissue PO$_2$ that should follow an increase in oxygen metabolism according to theoretical modeling.

Capillary dilations are one of several candidates for a local increase in the number of erythrocytes in the capillary network shown in Fig. 2.9. The conclusions reached by Schmid et al. [109] only hinge on the monotone dependence of the effective resistance to hematocrit and the employed bifurcation rule at capillary diverging bifurcations. Consequently, any mechanism able to reduce the effective resistance in capillaries could locally raise the number of RBCs under the appropriate conditions. Recently, erythrocytes were shown by Wei et al. [141] to enhance their deformability as a response to decreased PO$_2$. The higher RBC velocity obtained in their ex vivo experiments shows that resistance to flow drops when RBCs are more deformable. Besides, the width of the endothelial surface layer can be suggested as a candidate mechanism for blood flow regulation. Thus, a multitude of activation scenarios could be modeled with our simulation framework for oxygen transport from capillary networks.

The activation scenarios modeled in this study are extremely simplified. In particular, the instantaneous increase in capillary diameter as well as the fast linear increase in CBF do not capture the complexity of the vascular response to activation. Thus we suggest to perform further studies with temporal profiles of capillary dilation and CBF changes based on experimental data such as [47, 141]. The response to multiple capillary dilations should also be quantified. Besides, the conclusions reached in the two-branch capillary network employed here need to be verified in larger networks where the boundary condition for the oxygen partial pressure has a smaller influence.
2.5 Conclusion

In conclusion, we have developed a new model for oxygen transport from capillaries with moving RBCs based on overlapping meshes. This novel method was extended to reconstructed microvascular networks. We successfully validated it against experimental data acquired in the rodent brain. EATs and longitudinal gradients of $P_{O_2}$ could be reproduced using a cone-shaped geometry. The local increase in hematocrit produced by a single capillary dilation was found to increase the minimal tissue $P_{O_2}$ by 0.9 mmHg. However, our results suggest that multiple dilations or decreased resistance in several capillaries may be able to significantly upregulate tissue $P_{O_2}$ to compensate an increased metabolic consumption of oxygen. Additionally, as capillary hyperemia was reported to be the first vascular response to activation, these mechanisms likely act faster on oxygen transport than arteriolar dilations. The combined simulations of RBC dynamics with oxygen transport will eventually allow simulations of blood flow regulation mechanisms in health and disease with unprecedented detail.
Chapter 3

The relative influence of hematocrit and RBC velocity on tissue oxygenation

The numerical model presented in the previous chapter resolves the influence of individual RBCs on oxygen transport in a detailed manner. Its downside is the computational effort required for high-resolution, time-resolved simulations. This chapter deals with a simplified model for oxygen transport from capillaries which is based on an ordinary differential equation for the evolution of hemoglobin saturation. An analytical solution is available when convective transport of dissolved oxygen is neglected. The computational cost of this model is therefore negligible. Besides, it offers the possibility to analyze the effect of physiological variables with analytical tools. As an application, the influence of hematocrit and RBC velocity on tissue oxygenation is investigated in detail. The resulting insights can then be compared to the results from the computational model with moving RBCs. Additionally, the dependence of the intravascular PO$_2$ drop on hematocrit, RBC velocity, RBC diameter and capillary diameter is quantified, which has potential applications for the interpretation of intravascular oxygen measurements using two-photon phosphorescence lifetime microscopy (2PLM). In the next chapter, the equations derived here will serve as a basis for diffusive interaction models in capillary networks.

The content of this chapter is largely based on the article “The relative influence of hematocrit and red blood cell velocity on oxygen transport from capillaries to tissue” by Lücker, Secomb, Weber and Jenny [70].
3.1 Introduction

Since the energy requirements of parenchymal cells are fluctuating, oxygen transport to tissue is a dynamic process that needs to be regulated. For example, neurovascular coupling adapts oxygen supply to the varying energy consumption in different brain regions. In order to understand such regulation mechanisms, knowledge of physiological variables that determine tissue oxygenation is required. In this chapter, we investigate the influence of hematocrit and blood flow velocity, which are two of the most important variables pertaining to oxygen transport in the microcirculation.

In theoretical models, oxygen tension in the tissue is substantially affected by the intravascular resistance (IVR) to oxygen transport in capillaries, as Hellums [51] showed by taking into account individual erythrocytes. The IVR was then shown to largely depend on hematocrit in small vessels [25, 26, 50]. Later, Eggleton et al. [23] performed numerical simulations of oxygen transport in a capillary and the surrounding tissue to evaluate mass transfer coefficients (MTCs), which are an inverse resistance to oxygen transport, and fitted their dependence on hematocrit using a quadratic polynomial. In that study, these MTCs were found not to significantly depend on the erythrocyte velocity. The presence of IVR was experimentally confirmed by the measurements of high-amplitude EATs by Parpaleix et al. [86] using 2PLM. Also using 2PLM, Sakadžić et al. [104] directly observed radial $P_{O_2}$ gradients in arterioles, which are a reflection of IVR.

However, the knowledge of IVR is by itself not sufficient to determine tissue $P_{O_2}$. The unloading of oxygen by RBCs along their paths in the microcirculation also needs to be taken into account. Hence, tube hematocrit and related variables (discharge hematocrit, RBC linear density, cell spacing) are not the only determinants of tissue oxygenation. Blood velocity and RBC flow are also variables of paramount importance. Although much theoretical knowledge is available, there is to best of our knowledge no study that systemically quantifies the relative influence of hematocrit, RBC velocity and RBC flow on tissue oxygenation.

Here we use an analytical approach based on Hellums [51] and Roy et al. [102], as well as the numerical model presented in Chapter 2 to investigate how hematocrit and RBC velocity respectively affect tissue $P_{O_2}$. Our results show that the distance traveled by RBCs and oxygen consumption significantly affect the importance of the above variables. The discrepancies between analytical and numerical solutions are examined, providing a better understanding of the validity range of the analytical model. These results contribute to understanding the impact of blood flow regulation...
mechanisms on tissue oxygenation.

The intravascular oxygen distribution underpins tissue PO$_2$ via the IVR to oxygen transport, hence its dependence on flow parameters and vessel geometry will be quantified. A novel finding is that MTCs significantly depend on RBC velocity at low to medium hematocrit. IVR coefficients are evaluated using our computational model for a range of RBC and capillary diameters, which can be used in various models for tissue oxygenation [114, 34]. Finally, the relation between hemoglobin saturation and plasma PO$_2$ is described with a simple model, which may contribute to a more accurate interpretation of intravascular oxygen measurements in capillaries with 2PLM.

3.2 Methods

3.2.1 Ordinary differential equation model

To quantify the influence of blood velocity and hematocrit on tissue oxygenation, we performed computations in axisymmetric cone-shaped domains with four distinct regions: RBCs, plasma, capillary endothelium, and tissue, denoted by the indices c, p, w, and t, respectively. Cylindrical RBCs with radius $r_c$ and volume $V_{rbc}$ are employed. The domain geometry is described by its length $L$, the plasma radius $r_p$, the outer capillary wall radius $r_w$ and the tissue radius $r_t$ which may be a function of the axial position $x$. The arterial and venous capillary ends (also referred to proximal and distal ends) are denoted with the indices a and v. For simplicity, no interstitial space layer between the endothelium and the tissue was used. Additional computations have shown that the addition of an interstitial space layer as in Eggleton et al. [23] decreases tissue PO$_2$ by $\sim 1$ mmHg only.

In capillaries, RBCs flow in a single file and the RBC linear density (LD) is defined to be the ratio of the length occupied by the RBCs to the total vessel length. This choice is convenient since LD can be directly observed in capillaries in vivo (e.g., using two-photon laser scanning microscopy [1]) and naturally arises from the theoretical model described below. The tube hematocrit $H_T$ and LD are related by

$$
\mu_{LD} = H_T \left( \frac{r_p}{r_c} \right)^2.
$$

The equilibrium curve for hemoglobin and oxygen is modeled using the Hill
equation

\[ S_{eq}(P) = \frac{P^n}{P^n + P_{50}^n}, \tag{3.2} \]

where \( n \) is the Hill exponent and \( P_{50} \) the oxygen partial pressure at half-saturation. The inverse form of this equation

\[ P_{eq}(S) = P_{50} \left( \frac{S}{1 - S} \right)^{1/n} \tag{3.3} \]

will be often required.

The models developed here are based on the neglect of axial diffusion and the use of steady-state equations. These common assumptions \[51\][102] reduce the evolution of hemoglobin saturation to an ordinary differential equation. Based on the absence of axial diffusion, the oxygen outflux from the capillary at the axial position \( x \) is balanced by the metabolic oxygen consumption in the tissue slice normal to the capillary at \( x \), which is denoted by \( j_t(x) \). The mass balance between the capillary and the tissue is given by

\[ \frac{df(S)}{dx} = -j_t(x), \tag{3.4} \]

where \( f(S) \) is the convective oxygen flux through the capillary. Oxygen consumption is assumed to occur at a rate per unit volume \( M_0 \) which is independent from tissue \( P_{O_2} \). This simplification allows the existence of an analytical expression for \( P_{O_2} \) in the tissue. Based on this assumption, the oxygen consumption at \( x \) becomes

\[ j_t(x) = M_0\pi(r_t^2(x) - r_w^2). \tag{3.5} \]

Oxygen in the blood is present in bound form to hemoglobin in RBCs and in dissolved form in both plasma and RBCs. The total convective flux is given by

\[ f(S) = v_{rbc}(\pi r_p^3 H_D C_0 S + \pi r_p^2 \alpha_{eff} P_{IV}), \tag{3.6} \]

where \( C_0 = N_{Hb} V_{mol,O_2} \) is the product of the heme concentration and the molar volume of oxygen, and \( P_{IV} \) is the intravascular \( P_{O_2} \) which needs to be modeled. Here, the Fåhraeus effect is neglected, hence the discharge hematocrit \( H_D \) is set to \( H_T \). The effective oxygen solubility is defined by \( \alpha_{eff} = H_T \alpha_c + (1 - H_T) \alpha_p \). The average RBC oxygen partial pressure \( P_c \) is assumed to be in equilibrium with hemoglobin saturation, that is, \( P_c = P_{eq}(S) \); in the plasma, the oxygen partial pressure is set to be constant and equal to \( P_w \), the oxygen partial pressure at the capillary outer wall. To estimate it, we use the intravascular resistance coefficient \( K_{IV} \) defined by

\[ K_{IV} j_t = P_c - P_w. \tag{3.7} \]
As shown below, values of $K_{IV}$ can be fitted from numerical simulations or obtained using an analytical formula. This yields

$$P_{IV} = H_T P_{eq}(S) + (1 - H_T) P_w = P_{eq}(S) - (1 - H_T) K_{IV} j_t. \quad (3.8)$$

We can now summarize the above equations to obtain the evolution equation for hemoglobin saturation. For brevity, we define total oxygen convective capacity as

$$Q_{O_2}(S) = v_{rbc} \left( \mu_{LD} \pi r_c^2 C_0 + \pi r_p^2 \alpha_{\text{eff}} \frac{dP_{eq}}{dS} \right) \quad (3.9)$$

Eqs. (3.4), (3.6) and (3.8) result in

$$Q_{O_2}(S) \frac{dS}{dx} = -j_t(x) + v_{rbc} \pi r_p^2 \alpha_{\text{eff}} (1 - H_T) K_{IV} \frac{dj_t(x)}{dx}. \quad (3.10)$$

When the tissue domain is a straight cylinder, the last term vanishes. If the tissue radius is not constant, this term was found to be negligible. Therefore, it will be omitted in further derivations. However, this term was included for completeness in the numerical computations where convective transport of dissolved oxygen in plasma was taken into account. Alternatively, Eq. (3.10) can be simplified by neglecting the related terms, which amounts to set $\alpha_{\text{eff}}$ to zero and leads to

$$Q_{O_2,c} \frac{dS}{dx} = -j_t(x), \quad (3.11)$$

where $Q_{O_2,c} = v_{rbc} \mu_{LD} \pi r_c^2 C_0$ is the convective capacity of oxygen bound to hemoglobin and is independent of $S$. This equation can be integrated analytically in a cone-shaped domain, which yields

$$S(x) = S_a - \frac{M_0 \pi x}{Q_{O_2,c}} \left[ r_t^2 \left( 1 + \frac{x}{L} \left( \frac{r_t,v}{r_t,a} - 1 \right) + \frac{1}{3} \left( \frac{x}{L} \right)^2 \left( \frac{r_t,v}{r_t,a} - 1 \right)^2 \right) - r_w^2 \right]. \quad (3.12)$$

The average $P_{O_2}$ in RBCs at $x$ is obtained using Eq. (3.3). To determine the radial variation of $P_{O_2}$, equations for oxygen diffusion in the plasma, the endothelium and the tissue are solved. The steady-state equation for oxygen transport in the tissue is given by

$$D_t \alpha_t \frac{1}{r} \frac{d}{dr} \left( r \frac{dP}{dr} \right) = M_0. \quad (3.13)$$

In the plasma and the endothelium, oxygen consumption is neglected and the equation reduces to

$$D_i \alpha_i \frac{1}{r} \frac{d}{dr} \left( r \frac{dP}{dr} \right) = 0, \quad i = p, w. \quad (3.14)$$
Within the RBC, the oxygen partial pressure is governed by

\[ \frac{D_e \alpha_e}{r} \frac{d}{dr} \left( r \frac{dP}{dr} \right) = -T = C_0 \frac{dS(P)}{dt}, \quad (3.15) \]

where the rate of oxygen unloading from hemoglobin is assumed to be independent of \( r \). Eq. (3.15) yields

\[ P(r_c) = P_0 - \frac{1}{4} \frac{T r_c^2}{D_e \alpha_e}, \quad (3.16) \]

where \( P_0 \) is the \( P_{O_2} \) on the center-line. The mean \( P_{O_2} \) in the RBC is

\[ P_e = P_0 - \frac{1}{8} \frac{T r_c^2}{D_e \alpha_e}. \quad (3.17) \]

It follows from the continuity of fluxes and Eqs. (3.14) and (3.15) that

\[ P(r_w) = P_0 - \frac{1}{2} \frac{T r_c^2}{r_c^2} \left[ \frac{1}{2 D_e \alpha_e} + \frac{\log(r_p/r_c)}{D_p \alpha_p} + \frac{\log(r_w/r_p)}{D_w \alpha_w} \right]. \quad (3.18) \]

Mass conservation implies that \( j_t(x) = T \pi r_c^2 L_{rbc}/L_{tot} \). The combination of this with the definition of the IVR coefficient (Eq. (3.7)) and Eq. (3.18) yields

\[ K_{IV} = \frac{1}{2 \pi \mu_{LD}} \left[ \frac{1}{4 D_e \alpha_e} + \frac{\log(r_p/r_c)}{D_p \alpha_p} + \frac{\log(r_w/r_p)}{D_w \alpha_w} \right]. \quad (3.19) \]

This formulation includes the resistance of the capillary wall. According to this model, the IVR is inversely proportional to the RBC linear density.

Using this model, the oxygen partial pressure at any point in the tissue can be determined as follows. From the hemoglobin saturation \( S \) at an axial position \( x \), the average RBC oxygen partial pressure \( P_e \) is obtained using Eq. (3.3). The oxygen partial pressure at the capillary outer wall is then given by Eq. (3.7). Finally, the Krogh model for the oxygen partial pressure at a distance \( r \) from the capillary centerline reads

\[ P(x, r) = P(x, r_w) - \frac{M_0}{4 D_t \alpha_f} \left[ 2 r_t^2 \log \left( \frac{r}{r_w} \right) - r^2 + r_w^2 \right], \quad r \geq r_w. \quad (3.20) \]

Since we aim to quantify the effects of LD and RBC velocity, it is useful to reformulate tissue \( P_{O_2} \) as a function of these variables. For \( r \geq r_w \),

\[ P(x, r; \mu_{LD}, v_{rbc}) = P_{c,a} - \Delta P_{conv}(x; \mu_{LD}, v_{rbc}) - \Delta P_{IVR}(x; \mu_{LD}) - \Delta P_{EV}(x, r), \quad (3.21) \]
where the last three terms represent the PO$_2$ drops due to convection in the capillary, IVR and consumption in the tissue, respectively, and are defined below.

The influence of LD and RBC velocity can be assessed by differentiating Eq. (3.21) with respect to both variables. To this end, we define the non-dimensional variable

$$Z = \frac{\mu_{LD} \partial P}{v_{rbc} \partial v_{rbc}}.$$  

(3.22)

For example, the value $Z = 2$ means that an infinitesimal relative increase in $\mu_{LD}$ causes a PO$_2$ increase which is twice as large as that caused by the same relative increase in $v_{rbc}$.

We now give analytical expressions for the terms on the right-hand side of Eq. (3.21) under the assumption that there is no convective transport of dissolved oxygen. The convective PO$_2$ drop from the capillary inlet to the axial position $x$ is

$$\Delta P_{\text{conv}}(x) = P_{c,a} - P_{50} \left( \frac{S(x)}{1 - S(x)} \right)^{1/n},$$  

(3.23)

where $S(x)$ is given by Eq. (3.12). The intravascular drop $P_c - P(r_w)$ is given by

$$\Delta P_{IVR}(x) = \frac{K'_{IV} \hat{j}(x)}{\mu_{LD}},$$  

(3.24)

where the coefficient $K'_{IV} = K_{IV} \mu_{LD}$ is independent of $\mu_{LD}$. From Eq. (3.20), the extravascular drop

$$\Delta P_{EV}(x,r) = P(x,r_w) - P(r) = \frac{M_0}{4D_{1,\alpha}} \left[ 2r^2_t(x) \log \left( \frac{r}{r_w} \right) - r^2 + r^2_w \right], \quad r \geq r_w$$  

(3.26)

depends on neither $\mu_{LD}$ nor $v_{rbc}$. From Eq. (3.9), $\Delta P_{\text{conv}}$ depends on $\mu_{LD}$ and $v_{rbc}$ only through their product, and it follows that

$$\mu_{LD} \frac{\partial \Delta P_{\text{conv}}}{\partial \mu_{LD}} = v_{rbc} \frac{\partial \Delta P_{\text{conv}}}{\partial v_{rbc}}.$$  

(3.27)

The derivative of the IVR term with respect to $\mu_{LD}$ is given by

$$- \frac{\partial}{\partial \mu_{LD}} \Delta P_{IVR} = \frac{K'_{IV} \hat{j}(x)}{\mu^2_{LD}}.$$  

(3.28)
The right-hand side of Eq. (3.28) is positive, and this together with Eqs. (3.21) and (3.27) implies that
\[ \frac{\partial P}{\partial \mu_{LD}} < \frac{\partial P}{\partial v_{rbc}}, \] (3.29)
Therefore, the function \( Z \) defined in Eq. (3.22) is always larger than one.

### 3.2.2 Model parameters

We performed computations in two different cone-shaped domains (Fig. 3.1) which will be referred to as cortex domain and glomerulus domain. The results in these two domains will illustrate the influence of the metabolic oxygen consumption rate. The larger domain’s dimensions correspond to the rodent cerebral cortex. Its length was set to \( L = 300 \mu m \) according to Sakadžić et al. [104] who found an average capillary path length of \( \sim 343 \mu m \). The tissue radii were set to \( r_{t,a} = 26 \mu m \) and \( r_{t,v} = 20 \mu m \), so that the average tissue radius closely matches the measurements by Tsai et al. [132] and Sakadžić et al. [104]. The oxygen consumption was set to \( 0.001 \mu m^3 O_2 \mu m^{-3} s^{-1} \), which matches the value of \( 2.63 \mu mol g^{-1} min^{-1} \) measured by Zhu et al. [147] in the mouse cerebral cortex. The smaller domain has radii \( r_{t,a} = 19 \mu m \) and \( r_{t,v} = 13 \mu m \) that fit the high capillary density in the rodent olfactory glomerulus [65]. Its length was set to \( L = 100 \mu m \) and the taper was fitted to the longitudinal intravascular PO\(_2\) variations measured by Parpaleix et al. [86]. Due to the low tissue radius, we increased the oxygen consumption in this domain to \( 0.003 \mu m^3 O_2 \mu m^{-3} s^{-1} \) to obtain tissue PO\(_2\) values in the physiological range for most values of \( \mu_{LD} \) and \( v_{rbc} \). A RBC radius of 1.5 \( \mu m \) was employed [114]. As in the previous chapter, the radius of the capillary lumen \( r_p \) is set to 2.0 \( \mu m \) and the capillary endothelium thickness to 0.6 \( \mu m \). The oxygen tension in RBCs at the arterial inlet \( P_{c,a} \) was set to 90 mmHg, which is the highest RBC PO\(_2\) value measured by Parpaleix et al. [86] in rodent brain capillaries. The remaining model parameters are given in Table 2.2 The length \( L_{rbc} \) of the cylindrical RBCs was 8.35 \( \mu m \), which follows from the values of the RBC radius and volume.

For consistency with the analytical model, the numerical model used zero-order kinetics for metabolic oxygen consumption as
\[ M(P) = \begin{cases} M_0 & \text{inside the tissue and if } P > 0, \\ 0 & \text{otherwise}. \end{cases} \] (3.30)
This consumption model can cause negative values of tissue PO\(_2\) when \( v_{rbc} \) or \( \mu_{LD} \) are low. The PO\(_2\) level was set to zero in the grid cells where
this occurred. In the simulations presented in Subsection 3.3.1, the grid spacing was set to $\Delta x = 3\, \mu m$ in the capillary and the time step was chosen so that $\Delta t = \Delta x/v_{\text{rbc}}$, which ensures that the absolute numerical error in tissue $P_{O_2}$ at $10\, \mu m$ or more from the capillary is less than $0.5\, \text{mmHg}$ (see Subsection 2.2.3). In Subsection 3.3.2, the grid cell size was set to $\Delta x = 1\, \mu m$ for increased accuracy. The advection term in Eq. (2.21) was discretized using the stable scheme introduced in Subsection 2.2.4. The tolerance in the coupling between the oxygen and hemoglobin equations was set to $10^{-5}$.

Figure 3.1: Sketches of the computational domains. Top: “cortex geometry” has dimensions that represent the rodent cortex. Bottom: “glomerulus geometry” has dimensions that match the rodent olfactory glomerulus which has a very high capillary density.

3.3 Results

3.3.1 Tissue oxygenation

We computed tissue $P_{O_2}$ for a range of values of RBC linear density and velocity using both the analytical and the numerical model presented above. This allows us to assess the relative influence of LD, RBC velocity and RBC flow on tissue oxygenation. The analytical and numerical model results are compared, which sheds light on the validity range of the analytical model.

In both computational domains (Fig. 3.1), the $P_{O_2}$ was computed for values of LD between 0.1 and 0.6, with an increment of 0.02 for LDs between 0.1 and 0.3, and an increment of 0.05 for LDs between 0.3 and 0.6. In the cortex geometry, $v_{\text{rbc}}$ was varied between 0.2 and 2.4 mm/s. In the
glomerulus domain, values of $v_{\text{rbc}}$ between 0.2 and 1.6 mm/s were used. In both cases, an increment of 0.1 mm/s was employed. Therefore, in the cortex and glomerulus geometries, we ran $17 \times 23 = 391$ and $17 \times 15 = 255$ simulations, respectively. In the analytical model, convective transport of dissolved oxygen was neglected, that is, the effective solubility $\alpha_{\text{eff}}$ was set to zero. Thus, Eq. (3.12) was used to compute hemoglobin saturation along the capillary. The IVR coefficient $K_{IV}$ was fitted to the results of the numerical model using least squares, instead of using the value given in Eq. (3.19). We express the results using the coefficient $K_{IV,0.5}$, which is the IVR coefficient for $\mu_{LD} = 0.5$. According to Eq. (3.19), values of $K_{IV}$ for arbitrary LD are given by $K_{IV} = 0.5K_{IV,0.5}/\mu_{LD}$.

Fig. 3.2 shows tissue $P_{O_2}$ as a function of LD and RBC velocity, at 15 $\mu$m from the endothelium ($r = 17.6 \mu$m) in the cortex geometry and at 10 $\mu$m from the endothelium ($r = 12.6 \mu$m) in the glomerulus geometry. The fitted IVR coefficient $K_{IV,0.5}$ was 4.98 mmHg $\mu$m s/($\mu$m$^3$ $O_2$) in the cortex geometry and 5.43 mmHg $\mu$m s/($\mu$m$^3$ $O_2$) in the glomerulus geometry. These values are significantly lower (by 23% and 16%) than the analytical value $K_{IV,0.5} = 6.43$ mmHg $\mu$m s/($\mu$m$^3$ $O_2$) given by Eq. (3.19). The analytical model results approximately match the numerical calculations with these fitted IVR coefficients. However, the $P_{O_2}$ isolines from both models do not coincide. The deviations are largest at proximal locations. The causes of these discrepancies are discussed below.

In both geometries, tissue $P_{O_2}$ is an increasing function of LD and RBC velocity, as expected. Along any given isoline of RBC flux ($q_{\text{rbc}} = \mu_{LD}v_{\text{rbc}}/L_{\text{rbc}}$), $P_{O_2}$ increases with increasing LD, i.e., LD is a stronger determinant of tissue $P_{O_2}$ than RBC velocity. The strength of this variation is indicated by the angle between the $P_{O_2}$ and RBC flow isolines, which is larger at more proximal locations and also higher in the glomerulus domain than in the cortex domain.

The analytical model considers radial diffusion only of free oxygen within RBCs. In fact, radial oxygen transport in RBCs is facilitated by diffusion of oxyhemoglobin [50], which leads to a smaller $P_{O_2}$ drop for a given rate of delivery. With $\mu_{LD} = 0.3$ and $v_{\text{rbc}} = 1.2$ mm/s, the intra-RBC $P_{O_2}$ drop $P_c - P(r_c)$ at $x = 150 \mu$m is 6.8 mmHg with the analytical model. The numerical model yields $P_c - P(r_c) = 3.0$ mmHg, where $P(r_c)$ is the oxygen partial pressure averaged over the RBC membrane at $r = r_c$ (Fig. 3.3). Without diffusion of hemoglobin in the numerical model, the intra-RBC $P_{O_2}$ drop increases to 4.9 mmHg. This large difference is explained by the fact that most oxygen is released in a thin chemical boundary layer near the RBC membrane [17, 50] which almost disappears in the absence of
Figure 3.2: Tissue $P_O^2$ as a function of LD and RBC velocity. Solid isolines: numerical results; dashed isolines: analytical model with fitted IVR coefficient. Left: cortex geometry, at 15 µm from the capillary wall (top: $x = 40$ µm, middle: $x = 150$ µm, bottom: $x = 260$ µm). Right: glomerulus geometry, at 10 µm from the capillary wall (top: $x = 20$ µm, middle: $x = 50$ µm, bottom: $x = 80$ µm). The shaded area shows the region where $P_O^2 < 2$ mmHg. The dotted lines show isolines of RBC flux in cells/s.
Hematocrit and RBC velocity

The neglect of facilitated diffusion in the analytical model thus accounts for half of the discrepancy between the analytical and fitted IVR coefficients.

Figure 3.3: Radial $P_O_2$ profile with $\mu_{LD} = 0.3$ and $v_{rbc} = 1.2 \text{ mm/s}$ at $x_c = 150 \mu\text{m}$ in the cortex geometry. Solid line (in the RBC): $P_O_2$ from the numerical model, averaged over the RBC length when the RBC is centered at $x_c$; solid line (in the tissue): numerical time-averaged tissue $P_O_2$; dashed line: analytical tissue $P_O_2$ with fitted IVR coefficient; dotted line: analytical values based on Eqs. (3.16) to (3.20), without using an IVR coefficient. The shading indicates the frequency of the $P_O_2$ values from the numerical model. The black dot shows the averaged RBC $P_O_2$ value obtained from the analytical model.

Axial diffusion of oxygen is neglected by the analytical model. The consequences were examined by running the numerical model without axial diffusion. In this case, the best fit for $K_{IV,0.5}$ was $5.50 \text{ mmHg} \mu\text{m s/}(\mu\text{m}^3 \text{O}_2)$ in the cortex geometry and $5.93 \text{ mmHg} \mu\text{m s/}(\mu\text{m}^3 \text{O}_2)$ in the glomerulus geometry. These values are approximately 10% higher than those obtained with axial diffusion, yet still 14% and 8% lower than the analytical IVR coefficients, respectively. This can be explained by numerical axial diffusion introduced by the numerical scheme within the capillary, which has the effect of reducing the predicted IVR coefficient. Comparison with the analytical model in the absence of axial diffusion indicates that the reduction is less than 15%.

Although the neglect of axial diffusion has only a limited influence on the fitted value of the IVR coefficient, it significantly affects predicted longitudinal profiles of tissue $P_O_2$. Fig. 3.4 shows simulated $P_O_2$ profiles with and without axial diffusion, together with the respective fits from the analytical model (IVR coefficients set as above). The analytical model fits the simulation results without axial diffusion extremely well. The results with
and without axial diffusion differ most near the ends of the capillary. In simulations with axial diffusion, the no-flux boundary condition for $P_O_2$ at the domain boundary forces longitudinal $P_O_2$ profiles to be flat at $x = 0$ and $x = L$. Axial diffusion also smooths the longitudinal $P_O_2$ profiles, an effect which is most evident in the shorter glomerulus domain.

Figure 3.4: Longitudinal tissue $P_O_2$ profiles with and without axial diffusion ($\mu_{LD} = 0.35$, $v_{rbc} = 1.0$ mm/s). Top: cortex geometry with $P_O_2$ at 15$\mu$m from the capillary endothelium. Bottom: glomerulus geometry with $P_O_2$ at 10$\mu$m from the capillary endothelium. Solid line: numerical model with axial diffusion. Dashed line: analytical fit to the simulation with axial diffusion. Dash-dotted line: numerical results without axial diffusion. Dotted lines: analytical fit to the simulation without axial diffusion. The analytical fits use the IVR coefficients defined in the main text.

The results in the cortex and the glomerulus geometry display some qualitative differences, such as the higher angle between $P_O_2$ and flow isoclines at proximal locations and in the glomerulus geometry (Fig. 3.2). We now give a theoretical explanation of this fact. The function $Z$ defined in
Eq. \((3.22)\) is the normalized ratio of the \(P_o^2\) derivatives with respect to LD and RBC velocity. High values of \(Z\) imply that a relative change in LD has a stronger effect on tissue \(P_o^2\) than the same relative change in RBC velocity. Fig. 3.5 shows values of \(Z\) as a function of the axial position \(x\) and the metabolic rate of oxygen consumption \(M_0\), for several values of LD and RBC velocity. The contours of \(Z\) are masked for values of \(P_o^2\) below 2 mmHg. In all cases, \(Z\) is a decreasing function of \(x\). Therefore, the effect of LD changes is highest on the proximal side and \(Z\) tends to infinity when \(x\) approaches zero since the convective \(P_o^2\) drop vanishes in this case. The function \(Z\) always stays greater than one, even at distal positions (as shown at the end of Subsection 3.2.1), implying that a relative change in LD always has a larger effect on tissue \(P_o^2\) than the same relative change in RBC velocity. The dependency of \(Z\) on the oxygen consumption rate is more complex. In most cases, \(Z\) is an increasing function of \(M_0\). However, at distal locations and low tissue \(P_o^2\) values (close to hypoxia), values of \(Z\) decrease as \(M_0\) increases. Fig. 3.5 presents three cases: normal conditions; low LD with high RBC velocity; high LD with low RBC velocity (see the figure legend). The function \(Z\) attains its highest values when LD is low (Fig. 3.5B) since tissue \(P_o^2\) is limited by low hematocrit in this case. Conversely, when RBC velocity is low and LD is high (Fig. 3.5C), \(Z\) takes lower values since the low RBC velocity is the limiting factor for oxygen supply in that case.

### 3.3.2 Intravascular drop in oxygen partial pressure

After having focused on tissue oxygenation, the intravascular oxygen distribution is now addressed in more detail with a threefold objective. As a first application, it enables result comparison with previous works by means of MTCs. Secondly, coefficients that describe the intravascular \(P_o^2\) drop are required by certain oxygen transport models \[114, 34\]. Finally, the relation between hemoglobin saturation and plasma \(P_o^2\) should be known to relate oxygen measurements in the plasma to the actual blood oxygen content.

We first examine the dependence of the MTC and the related Nusselt number on LD and RBC velocity. The MTC is defined as

\[
k = \frac{j}{P_c - P_w} \tag{3.31}
\]

where \(j\) is the averaged oxygen flux through the capillary inner wall and \(P_w\) is the average oxygen tension at the capillary inner wall around a RBC. The Nusselt number is a nondimensional MTC that here represents the ratio of
3.3. Results

Figure 3.5: Values of the function $Z$ defined in Eq. (3.22) as a function of the axial position $x$ and the rate of oxygen consumption $M_0$. The computations were done in the cortex geometry. The shaded area shows hypoxic cases ($P_{O_2} < 2\text{ mmHg}$). The highest contour value is 6.0. Top: $\mu_{LD} = 0.35$, $v_{rbc} = 1.2\text{ mm/s}$. Middle: $\mu_{LD} = 0.2$, $v_{rbc} = 2.0\text{ mm/s}$. Bottom: $\mu_{LD} = 0.5$, $v_{rbc} = 0.6\text{ mm/s}$. 
the total oxygen flux to the diffusive oxygen flux based on the intravascular pressure gradient, and is defined following Hellums et al. [50] by

$$\text{Nu} = \frac{2r_p}{D_p \alpha_p (P_{eq}(S) - P_w)},$$

(3.32)

where $P_{eq}(S)$ is the oxygen tension in equilibrium with the average RBC hemoglobin saturation. The Nusselt number is approximately proportional to the MTC when $P_{eq}(S) - P_w$ and $P_c - P_w$ are close, which holds for medium to high LDs. To compute these quantities, the oxygen flux and wall PO$_2$ were averaged around a RBC centered at $x_c = L/2$. The averaged oxygen flux was given by

$$\bar{j} = \frac{\mu LD}{L_{rbc}} \int_{x_c - 0.5L_{rbc}/\mu LD}^{x_c + 0.5L_{rbc}/\mu LD} j(x) \, dx$$

(3.33)

and a similar formula was used for PO$_2$ at the capillary inner wall. A tissue cylinder with constant radius $r_t = 23 \mu$m and shorter length $L = 83.5 \mu$m ($= 10L_{rbc}$) was used to avoid zero PO$_2$ values in the tissue. Here, the use of a straight cylinder instead of a tapered domain facilitates result comparison with previous works, such as that by Eggleton et al. [23]. Additional computations showed that the cylinder shape changes MTCs and Nusselt numbers by less than one percent. RBC PO$_2$ at the capillary entrance was chosen using the analytical model to obtain $P_c(x_c) = 40$ mmHg. The oxygen consumption rate was set to $0.001 \mu$m$^3$O$_2$µm$^{-3}$s$^{-1}$ as in the cortex geometry. At low LDs and erythrocyte velocities, further changes were needed to avoid anoxic regions in the tissue. At $\mu LD = 0.1$, the tissue radius was reduced to 16 $\mu$m and the domain length was increased to $L = 125 \mu$m ($= 15L_{rbc}$) to prevent the integration bounds in Eqs. (24) from overlapping with the domain boundary. Additionally, the RBC PO$_2$ at the inlet was further increased to keep positive tissue PO$_2$ values in the tissue. At $v_{rbc} = 0.2$ mm/s, the oxygen consumption rate for $\mu LD \leq 0.2$ was lowered to prevent anoxic tissue regions from appearing.

Fig. 3.6 shows the calculated Nusselt numbers as a function of LD and RBC velocity. The obtained MTCs exhibit the same behaviour (Fig. 3.7). As found in previous studies (see Hellums et al. [50]), Nu strongly depends on hematocrit and exhibits a weaker dependency on blood velocity. Eggleton et al. [23] studied the MTC dependence on erythrocyte velocity and obtained a MTC increase of 2.7% when $v_{rbc}$ increases from 0.47 to 2.33 mm/s. However, when $v_{rbc}$ was raised from 0.2 to 2.4 mm/s, we found that the Nusselt number increased by $> 25\%$ for $\mu LD = 0.1$ and $< 2\%$ for $\mu LD = 0.9$ (Fig. 3.6B). The Nusselt number is an increasing function of $v_{rbc}$.
in the parameter range that was considered (Fig. 3.6C). Additional computations showed that the cone taper has no significant influence on Nu and MTCs (< 1%). Likewise, capillary length and spacing do not have a major influence (< 4% difference between MTCs in the cortex and glomerulus geometries). At last, the LD-dependent influence of RBC velocity on Nu is affected neither by the grid resolution nor by the time step size. Therefore, the MTC and the Nusselt number exhibit a significant dependence on RBC velocity that decreases with increasing LD.

The dependence of IVR coefficients on the capillary and RBC diameter is now computed. For each geometry, a parameter study was run with five values of LD from 0.2 to 0.6, and seven values for \( v_{\text{rbc}} \) from 0.4 to 1.6 mm/s. Here, a straight cylinder with length \( L = 100 \mu m \) and radius \( r_t = 23 \mu m \) was employed. The arterial value \( P_{o_2} \) in RBCs \( P_{c,a} \) was computed using Eqs. (3.10) and (3.3) to obtain \( P_c(x_c) = 50 \text{ mmHg} \) approximately, with \( x_c = L/2 \). From each simulation, the RBC oxygenation \( P_{eq}(S(x_c)) \) and the oxygen partial pressure at \( r = r_w + 15 \mu m \) were extracted after an approximate steady state was reached. The simulation time was set in such a way that tissue \( P_{o_2} \) varies by less than 0.2 mmHg during the last 0.5 s. The \( P_{o_2} \) drop between RBC and tissue was then fitted using least squares and the analytical model as \( P_{eq}(S(x_c)) - P(x_c, r) = K_{IV,t} + \Delta P_{EV}(r) \) (Eq. (3.26)). Here, the simulated RBC oxygenation was employed instead of computing it with Eqs. (3.12) and (3.3). Thus, the IVR computation is not affected by the error in hemoglobin saturation caused by the absence of axial diffusion in the analytical model.

The fitted and analytical values of \( K_{IV,0.5} \) are shown in Fig. 3.9 for a range of RBC and capillary diameters. The fitted values are also given in Table 3.1. The difference between the fitted and analytical IVR coefficients (Eq. (3.19)) observed in Subsection 3.3.1 increases with RBC and capillary size. For the highest capillary diameter \( (r_p = 3.0 \mu m) \), the fitted IVR coefficient is less than half of the analytical value, which highlights the necessity to use fitted coefficients instead of analytical ones. The increased surface area of the front and back sides of the RBC cylinder at higher RBC diameter most likely explains this large deviation, since Eq. (3.19) was derived by taking only the radial oxygen flux into account.

The knowledge of the \( P_{o_2} \) drop between RBCs and plasma is required to deduce hemoglobin saturation from measurements of plasma \( P_{o_2} \) as in 2PLM. Here, we consider a scenario where plasma \( P_{o_2} \) is averaged over the capillary cross section and subsequently time averaged. This roughly represents 2PLM data that would be obtained if the spatial resolution matches
Figure 3.6: Nusselt number as a function of LD and RBC velocity obtained in a straight cylinder with $L = 83.5 \, \mu m$ and $r_t = 23 \, \mu m$ for RBCs centered at $x_c = L/2$. The tube hematocrit $H_T$ was obtained using Eq. (3.1). Top: solid lines: $v_{rbc} = 0.2 \, \text{mm/s}$; dashed line: $v_{rbc} = 2.4 \, \text{mm/s}$. Middle: velocity dependence of the Nusselt number as a function of LD. Bottom: velocity dependence of the Nusselt number; the arrow indicates increasing values of LD.
the capillary diameter and phosphorescence decays are averaged irrespective of the distance to the RBC border. The averaged partial pressure in the plasma $P_p$ was computed using

$$P_p(x) = \frac{2}{r_p} \int_0^{r_p} \frac{\langle P(x, r; t) \gamma_p(x, r; t) \rangle}{\langle \gamma_p(x, r; t) \rangle} r \, dr,$$

(3.34)

where the angle brackets denote time averaging. The plasma volume fraction $\gamma_p(x, r; t)$ is zero when a RBC passes by, which accounts for the RBC shadows that occur in 2PLM.

We seek a model for a resistance coefficient $K_P$ that relates the oxygen
flux out of the capillary \( j_t \) and the \( \text{PO}_2 \) drop between RBC and plasma as

\[
P_{eq}(S) - P_p = K_P j_t. \tag{3.35}
\]

An approach that considers separately the plasma sleeve and the plasma region between two RBCs was adopted (Fig. 3.8). A first model with two degrees of freedom given by

\[
K_P = K_P' \left( \frac{1}{\mu_{LD}} - 1 \right) + \frac{K_{sl}}{\mu_{LD}} \tag{3.36}
\]

was employed, where the first term models the plasma region with \( r \leq r_c \) and the second accounts for the plasma sleeve \( (r_c < r \leq r_p) \). Our result analysis showed that the coefficient \( K_{sl} \) can be expressed as a multiple of \( K_P' \) as follows:

\[
K_P = K_P' \left( \frac{1}{\mu_{LD}} - 1 \right) + \frac{r_p^2 - r_c^2}{r_c^2} \frac{1}{2\mu_{LD}} \tag{3.37}
\]

This second model has only one degree of freedom and performs equally well as the first one, as will be seen below.

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Figure 3.8: Sketch of the plasma region between two cylindrical RBCs. The volumes of the plasma sleeve and the region between two RBCs are used to weight the terms in Eqs. (3.36) and (3.37).

The \( \text{PO}_2 \) drop between RBCs and plasma is now computed as a function of LD, oxygen outflux from the capillary and RBC radius. LD was varied between 0.2 and 0.9 with increments of 0.1, the oxygen metabolic oxygen consumption \( M_0 \) between 0.2 and 2 units of \( 10^{-3} \text{mm}^3 \text{O}_2 \text{mm}^{-3} \text{S}^{-1} \) with increments of 0.2, and \( r_c \) between 1.5 \( \mu \text{m} \) and 2.0 \( \mu \text{m} \) with steps of 0.1 \( \mu \text{m} \). Thus a total number of \( 8 \times 10 \times 6 = 480 \) simulations were performed. The lumen radius \( r_p \) was fixed to 2.0 \( \mu \text{m} \). For each value of the RBC radius, the model coefficient \( K_P' \) was fitted using least squares to the simulated values of \( S \) and \( P_p \) based on Eqs. (3.35) and (3.37). Fig. 3.9 shows the simulated and fitted \( \text{PO}_2 \) drops for \( r_c = 1.5 \mu \text{m} \) and 2.0 \( \mu \text{m} \). The fit from
the two-coefficient model (3.36) coincides almost perfectly with that of the one-coefficient model (3.37). Moreover, the values of $K'_p$ for the different RBC sizes (Table 3.1) are nearly constant. The fit in the whole parameter range is excellent, which demonstrates that the PO$_2$ drop between RBC and plasma is proportional to $j_t$ (Eq. (3.35)) and a function of both LD and the geometry (Eq. (3.37)). This description offers a solid theoretical basis for the determination of hemoglobin saturation from plasma PO$_2$ measurements in capillaries.

Figure 3.9: IVR coefficient for different RBC and capillary radii. The computations with varying RBC radius were run with $r_p = 2.0 \mu$m (black lines). When the plasma radius was varied, the RBC radius was set to $r_c = r_p - 0.5 \mu$m (blue lines). Solid lines: fitted IVR coefficient; dashed lines: analytical IVR coefficient (Eq. (3.19)).

3.4 Discussion

Oxygen partial pressure was computed in two different axisymmetric cone-shaped tissue domains around a capillary. We used a simple analytical model and a numerical model based on the full transport equations for oxygen and hemoglobin. The influences of RBC linear density (or equivalently, tube hematocrit) and RBC velocity were quantified. Results from the analytical and numerical models were compared and the origin of the discrepancies was identified. A theoretical analysis was conducted to explain the influence of the longitudinal position and the metabolic rate of
Hematocrit and RBC velocity

Figure 3.10: Difference between the averaged RBC and plasma $P_{O_2}$ as a function of linear density and metabolic oxygen consumption. Solid lines: numerical simulations; dashed lines: one-parameter model with fitted $K'_p$ (Eqs. (3.35) and (3.37)); dotted lines: two-parameter model with fitted $K'_p$ and $K_{sl}$ (Eqs. (3.35) and (3.36)). A: $r_c = 1.5\, \mu m$; B: $r_c = 2.0\, \mu m$.

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Table 3.1: IVR coefficient and resistance coefficient for the $P_{O_2}$ drop between RBCs and plasma (Eq. (3.37)) in mmHg\,\mu m s/(\mu m^3 O_2) for different RBC and capillary radii. The values in the first three columns were obtained with $r_p = 2.0\, \mu m$. For the last two columns, the RBC radius was set to $r_c = r_p - 0.5\, \mu m$.

oxygen consumption on our findings. Then, the intravascular oxygen distribution in capillaries was addressed in more detail. The dependence of MTCs on LD and RBC velocity was investigated and compared to previous works. The influence of RBC and capillary diameter on the IVR coefficient was quantified and a model for the $P_{O_2}$ drop between RBCs and plasma was developed and validated.

The results in Subsection 3.3.1 point out that RBC linear density is a very important determinant of tissue $P_{O_2}$. The RBC flux $q_{rbc}$, which is proportional to both LD and RBC velocity, is a variable of primary importance for tissue $P_{O_2}$. However, the RBC flux alone is not an accurate predictor of tissue $P_{O_2}$, as shown by the angle between $P_{O_2}$ and RBC flow.
3.4. Discussion

As shown in Eq. (3.21), tissue \( \text{PO}_2 \) is affected by two terms that depend on \( \text{LD} \) and \( v_{rbc} \): first, the convective \( \text{PO}_2 \) drop \( \Delta P_{\text{conv}} \), which is a function of the RBC flux \( q_{rbc} \); second, the intravascular resistance \( \text{PO}_2 \) drop \( \Delta P_{\text{IVR}} \), which is only a function of \( \text{LD} \). This second term causes the deviation between \( \text{PO}_2 \) and RBC flux contours. Therefore, \( \text{LD} \) is a more important determinant of tissue \( \text{PO}_2 \) than \( v_{rbc} \) in the following sense: a relative change in \( \text{LD} \) causes a higher variation of tissue \( \text{PO}_2 \) than the same relative change in \( v_{rbc} \), i.e. \( \mu_{LD} \partial P/\partial \mu_{LD} > v_{rbc} \partial P/\partial v_{rbc} \), as proved in Subsection 3.2.1.

Both the analytical and the numerical models show the circumstances under which the influence of \( \text{LD} \) is higher. As illustrated by the angles between \( \text{PO}_2 \) and RBC flow contours in the panels in Fig. 3.2, \( \text{LD} \) is most important for high values of oxygen consumption (glomerulus geometry) and on proximal side. Conversely, for lower values of oxygen consumption (cortex geometry) and on the distal side, \( \text{PO}_2 \) and RBC flow contours are more similar, which shows that the influence of \( \text{LD} \) is diminished under these conditions. These numerical results are supported by the analytical model. Indeed, the function \( Z \) defined in Eq. (3.22) decreases with the longitudinal position \( x \) and increases with the oxygen consumption (except for \( \text{PO}_2 \) levels near hypoxia for the latter). This means that the importance of \( \text{LD} \) for tissue \( \text{PO}_2 \) decreases with \( x \) and generally increases with \( M_0 \).

The good agreement between results from the theoretical and numerical models shows that the inverse dependence of the IVR coefficient on \( \text{LD} \) (Eq. (3.19)) holds for a large range of hematocrit and RBC velocity values. This agrees well with the findings of Eggleton et al. \[23\] and Vadapalli et al. \[136\], and highlights the necessity of using hematocrit-dependent MTCs for simulation methods that rely on these coefficients to couple intravascular and extravascular oxygen transport. The inverse dependence mentioned above means that a reduction of hematocrit by a factor of two causes the IVR to double. Therefore, in the presence of hematocrit heterogeneity, the assumption of hematocrit-independent IVR coefficient may yield large errors in the evaluation of oxygen fluxes through the vessel wall. We now review several oxygen modeling works by the standpoint of IVR coefficients or, equivalently, MTCs. Goldman et al. \[37\] studied the effect of capillary anastomoses and tortuosity on oxygen transport and used the MTCs computed by Eggleton et al. \[23\]. Similarly, Tsoukias et al. \[133\] simulated oxygen transport from a three-dimensional vascular network in the presence of hemoglobin-based oxygen carriers using MTCs computed in Vadapalli et al. \[136\]. Another body of works used the Green’s function method developed by Hsu et al. \[55\] to predict the \( \text{PO}_2 \) distribution in realistic microvascular networks. Some subsequent works \[114, 115, 116\] assumed constant hema-
Hematocrit and RBC velocity

tocrit values and used the constant IVR coefficients computed in Secomb et al. [114]. However, hematocrit-dependent IVR coefficients can be very easily added to this class of models. Recently, a dual-mesh approach was developed by Linneringer et al. [67] and enables fast \( \text{PO}_2 \) calculations in complex vascular networks. The coupling between intravascular and tissue \( \text{PO}_2 \) is based on the wall thickness and a hematocrit-independent oxygen permeability of epithelial tissue. Since this approach does not use \( \text{PO}_2 \) values at the vessel walls, the IVR coefficients computed here or in other works cannot be readily used in that model, but could be adapted based on the positions of the grid nodes.

The influence of LD on oxygen supply to tissue also has important physiological consequences. While blood velocity can be altered by arteriolar dilation in the cerebral cortex [129] or increased heart rate, mechanisms that influence hematocrit play a critical role. For example, during altitude adaptation, systemic hematocrit is increased over 24-48 h by plasma volume reduction and increased erythropoiesis [5]. This increases the oxygen carrying capacity of the blood and decreases the IVR to oxygen transport, hence reduces the risk of hypoxia. However, there may be RBC redistribution mechanisms that act on a faster scale in the microcirculation. Using a discrete RBC tracking model, Schmid et al. [110] hypothesized that pericyte-mediated capillary dilations are an efficient mechanism that can locally alter the distribution of RBCs in microvascular networks. Such a mechanism could reduce the IVR of the capillaries that contain an increased number of RBCs and therefore locally increase the oxygen availability. This could be important at distal locations since Devor et al. [20] suggested that functional hyperemia acts to maintain baseline tissue \( \text{PO}_2 \) at such locations. A local increase in LD could help to compensate for metabolism increases and ensure a safe margin of oxygen supply at the most critical locations.

While the analytical and numerical models used in this study clearly show the higher importance of LD compared to RBC velocity, they exhibit some discrepancies. First, the analytical IVR coefficients given by Eq. (3.19) overestimate by up to 23% the coefficients obtained by fitting the numerical results. The assumption of homogeneous release of oxygen from hemoglobin and the neglect of hemoglobin-facilitated radial diffusion were identified as the main causes for this, while the absence of axial diffusion in the analytical model only slightly affects the fitted IVR coefficients. However, the analytical model with fitted IVR coefficients agreed closely with the numerical model (Fig. 3.2) away from the proximal and distal domain boundaries. Therefore, numerical models for oxygen transport in microvascular networks that use appropriate IVR coefficients are expected to yield very good approximations of steady-state tissue \( \text{PO}_2 \) away from
domain boundaries and at > 5 µm from capillaries (Fig. 3.3). In particular, the oxygen transport models by Goldman et al. [34] and Hsu et al. [55] can directly use hematocrit-dependent IVR coefficients. Thus, they are very likely to yield similar tissue \( \text{PO}_2 \) values to those obtained from models with fewer assumptions, such as the one introduced in Chapter 2. Nevertheless, accurate simulations of the intravascular \( \text{PO}_2 \) field in capillaries require models with individual RBCs such as that by Eggleton et al. [23] or the one developed in this work.

The neglect of axial diffusion in the analytical model has a noticeable effect on longitudinal \( \text{PO}_2 \) profiles (Fig. 3.4). This assumption has been scrutinized in the context of the classical Krogh model [62, 29, 35]. Here, the largest deviations caused by this simplifying hypothesis were found in the glomerulus geometry, where the oxygen consumption per unit length in the capillary is highest. In such cases, alternative analytical methods that include axial diffusion could prove beneficial. For example, the approach based on spherical diffusion kernels suggested by Grimes et al. [43] could be extended to include the longitudinal oxygen gradient along the vessel. However, the boundary conditions for \( \text{PO}_2 \) at the domain boundary, or the domain geometry itself, would need to be adapted.

Our results can be compared to previous works by means of MTCs and the related Nusselt number (Eq. (3.32)). As in previous works reviewed by Hellums et al. [50], we obtained a strong dependence of \( \text{Nu} \) on hematocrit. Yet the effect of erythrocyte velocity that we observed differs from previous studies. We found that the increase in Nusselt number with increasing \( v_{\text{rbc}} \) was significantly greater than that obtained by Eggleton et al. [23] except at high LDs. To explain this discrepancy, we adapted our numerical method to simulate oxygen transport in the moving RBC frame with a relative backwards motion of the tissue, as in Eggleton et al. [23]. Computations with the same geometric and physiological parameters (with the exception of an interstitial fluid layer around the capillary and the presence of myoglobin) were performed. For the same RBC velocity increase, we found an increase in MTC of 7.5% at \( H_T = 0.43 \), which is higher than the 2.7% increase reported in Eggleton et al. [23]. Additional computations with fixed and moving RBCs produced no major difference in the computed MTCs. Two different boundary conditions at the proximal and distal tissue boundary (Krogh-type as in Eggleton et al. [23] and no-flux) were compared and did not show any significant influence. Therefore, this discrepancy might be explained by the numerical method since we observed that MTCs are slightly sensitive to the grid resolution. However, the absence of details on the numerical method used in Eggleton et al. [23] made further investigation difficult. The robustness of our results with respect to multiple parameters
(capillary length and spacing, cone taper, RBC frame of reference, grid resolution, time step size) provides strong evidence for a significant influence of RBC velocity on MTCs that decreases with increasing LD. This dependence may significantly affect the results of MTC-based oxygen transport simulations in realistic capillary networks as in Secomb et al. [117] since the erythrocyte velocity can be very heterogeneous. For example, in the mouse cortex, mean RBC velocities in the range of $2.03 \pm 1.42 \text{mm/s}$ have been measured [135].

In Subsection 3.3.2, IVR coefficients were fitted for various RBC and capillary diameters. An improved methodology was employed compared to the Subsection 3.3.1, namely: RBC oxygenation values from the computational model were employed, which removes the inaccuracy caused by the neglect of axial diffusion; the simulations were set up to enforce the same RBC oxygenation values at $x_c = L/2$, which avoids the potential influence of the absolute $P_{O_2}$ values on IVR coefficients; finally, a finer grid resolution was employed ($\Delta x = 1 \mu m$ instead of $3 \mu m$ in the capillary). As a result, the fitted IVR coefficient for $r_c = 1.5 \mu m$ and $r_p = 2.0 \mu m$ was $5.59 \text{mmHg} \mu m s/\mu m^3 O_2$ instead of the previously obtained values of 4.98 and 5.43 in the cortex and glomerulus geometries, respectively. The higher resistance is consistent with the finer grid that reduces the amount of numerical diffusion. The IVR coefficients display a very limited dependence on the width of the plasma sleeve (Fig. 3.9), unlike what the analytical formula (3.19) suggests. The values obtained here can be compared to the MTCs obtained in Eggleton et al. [23]. For an increase in $r_p$ from 1.8 $\mu m$ to 2.15 $\mu m$ (respectively, an increase of $r_c$ from 1.64 $\mu m$ to 1.99 $\mu m$), they reported an MTC increase of 14%. Although we did not use the same range of capillary diameters, we can compare the coefficient dependence on the RBC radius. Here, with the same increase in $r_c$, the IVR coefficient decreases by 23%. Since both quantities are inversely proportional, the result agreement is qualitative yet not quantitative. The differing width of the plasma sleeve is not expected to explain this discrepancy. A comparison with the exact same set of parameters should be performed to elucidate the origin of this difference.

The EATs addressed in the previous chapter were analyzed with a different approach. To the best of our knowledge, the intravascular $P_{O_2}$ drop between RBCs and plasma in capillaries has not yet been quantitatively dealt with by theoretical studies. However, the interpretation of plasma $P_{O_2}$ measurements in terms of blood oxygen content crucially depends on this relation. For instance, Sakadžić et al. [104] measured $P_{O_2}$ “in the plasma adjacent to the red blood cells” using 2PLM and directly converted these values to hemoglobin saturation using the Hill equation. Here, we
modeled the PO₂ drop between RBCs and plasma using a resistance coefficient \( K_P \) (Eq. (3.35)). A one-parameter description of \( K_P \) (Eq. (3.37)) was validated across a range of oxygen extraction rates, linear densities and RBC radii. In this parameter range, the drop from RBC to plasma takes values below 2 mmHg at high LD and low oxygen consumption rates, and above 20 mmHg at low LD and high oxygen consumption rates. Therefore, estimating hemoglobin saturation by applying the Hill equation to plasma PO₂ may cause substantial underestimations at low LD and high oxygen outflux from the capillary. Although the averaged plasma PO₂ was considered instead of PO₂ near the RBC membrane (RBC PO₂ in Fig. 1.4), our results suggest that using the latter PO₂ value would also introduce a significant bias due to the neglect of the intra-erythrocyte PO₂ gradient.

The characterization of the PO₂ drop between RBCs and plasma provides a theoretical basis for estimating hemoglobin saturation in capillaries based on plasma PO₂ measurements. Nevertheless, the formulation given by Eq. (3.35) is based on the oxygen flux out of the capillary \( j_t \) which is a priori unknown. To make this method applicable to 2PLM experiments, we suggest to model \( j_t \) as a function of the EAT amplitude, LD and the vessel size, each of which is measurable. If this succeeds, accurate measurements of EATs could yield estimates of the intra-erythrocyte oxygen distribution and the oxygen extraction from capillaries. This could in turn be employed to locally estimate the metabolic oxygen consumption rate.

Here, the influence of certain parameters was studied while keeping the others unchanged. This theoretically useful uncoupling does not take into account the relation between some of these parameters. For instance, the oxygen partial pressure \( P_{c,a} \) in RBCs at the capillary entrance was kept constant. However, PO₂ in proximal capillaries is expected to rise during blood flow increase due to decreased transit times and/or IVR in arterioles, since they also participate in oxygen supply to tissue \[22, 104\]. The effects of precapillary oxygen supply could be taken into account by parameterizing \( P_{c,a} \) as a function of LD and \( v_{rbc} \). Similarly, the no-flux boundary condition for PO₂ means that our results only pertain to cases where no diffusive interaction between the capillary and a nearby arteriole occurs.

The model for metabolic oxygen consumption may have an important influence on the reported tissue PO₂ values. Since an oxygen-independent consumption rate yields an analytical solution, we used Eq. (3.30) in both the analytical and the numerical model. The nonlinear Michaelis-Menten model given by \( M(P) = M_0 P/(P_{crit} + P) \) has been often used in modeling \[35\] and there is evidence that the constant \( P_{crit} \) may be > 1 mmHg \[41\]. Therefore, the low tissue PO₂ values are subject to this model uncertainty. Nevertheless, the key findings of this study are not expected
to be influenced by the assumed oxygen consumption kinetics. Similarly, changes in pH, carbon dioxide tension or temperature can affect the oxygen dissociation curve, as modeled in Dash et al. [19]. Despite that, our main conclusions are insensitive to the Hill equation parameters (Eq. (3.2)), as can be seen using the analytical function defined by Eq. (3.22). Finally, high-frequency fluctuations in LD and RBC velocity are known to occur [12] but their effect was not investigated here.

3.5 Conclusion

In this chapter, we have quantified the relative influence of hematocrit and RBC velocity on tissue oxygenation around capillaries using an analytical and a numerical model. The analytical model relies on the neglect of axial oxygen diffusion and the simplification of kinetics between hemoglobin and oxygen. The resulting tissue $P_{O_2}$ values were found to agree very well with the results from the computational model when using a fitted IVR coefficient that is inversely proportional to LD. Therefore, further modeling tasks that require reliable estimations of tissue $P_{O_2}$ can use this analytical model as a foundation, as will be done in the following chapter.

Our main finding is that hematocrit is a stronger determinant of oxygen partial pressure in the tissue than RBC velocity, mostly on the arterial side of the vasculature and at high oxygen metabolic consumption. These conclusions were not only drawn from numerical simulations, but could also be mathematically proved based on the analytical model. The proof essentially relies on the decrease of the IVR to oxygen transport with increasing hematocrit. Additionally, the intravascular $P_{O_2}$ drop was quantified across a large parameter range. The dependence of the related MTCs on RBC velocity at low and medium hematocrit is a novel finding. The successful characterization of the $P_{O_2}$ drop between RBCs and plasma offers a new tool to interpret measurements of plasma $P_{O_2}$ in terms of hemoglobin saturation.

The numerical model developed in Chapter 2 provides a solid foundation to the simplified model for oxygen transport developed here and thus is the cornerstone of our theoretical approach. This strategy will be pursued in the next chapter where the ordinary differential equation model will be extended to situations with heterogeneous blood oxygen content and validated by comparison with the numerical model.
Chapter 4

The heterogeneity of hemoglobin saturation in capillary networks

The model for hemoglobin saturation based on an ordinary differential equation introduced in the previous chapter employs an axisymmetric tissue region with a single capillary and constant blood flow properties. This idealized setting does not reflect features of microvascular blood flow such as flow heterogeneity and the presence of branchings. While simulations in realistic geometries can be performed with the computational model from Chapter 2, a theoretical analysis of transport phenomena that occur in capillary networks has the potential to improve our physical understanding of oxygen supply.

In the microcirculation, the transit times of RBCs through capillaries are heterogeneous and excessive variability has been associated to numerous diseases and conditions. The heterogeneity of transit times directly affects blood oxygenation. In this chapter, theoretical models for the heterogeneity of blood oxygen content in capillary networks are developed. The diffusive interaction between RBCs and capillaries with heterogeneous hemoglobin saturation is described by extending the approach used in Chapter 3. The resulting models are validated using our computational model with moving RBCs. The predicted RBC diffusive interaction in capillaries is shown to occur in reconstructed microvascular networks from the mouse cerebral cortex.

4.1 Introduction

Malfunctions in the microvasculature occur in many diseases and conditions, as shown by the following examples. Cerebral small vessel disease plays a crucial role in stroke, dementia and aging [85]. Cerebral pericytes, which were reported to regulate capillary diameter during functional activation
are susceptible to damage in ischemia [146] and to loss or degeneration in conditions such as aging, hypertension and diabetes [82]. Exposure to β-amyloid is toxic to pericytes and amyloid accumulation often occurs in relation to Alzheimer’s disease [49]. These deposits characterize cerebral amyloid angiopathy which is associated with cerebral blood flow disturbance [128]. Furthermore, damage to the endothelial glycocalyx causes increased capillary hematocrit [18] with potentially detrimental effects on the distribution of RBCs in the microcirculation. The disruption of endothelial signaling was linked to excessive functional shunting which causes dysfunctional microcirculation in tumors [99]. Finally, erythrocyte deformability, which is involved in the regulation of the microcirculation [141], was found to be lower in diabetic patients with the most extensive microangiopathy [4]. Therefore, the study of these conditions requires a proper understanding of the consequences of capillary dysfunction on oxygen transport.

Capillary dysfunction can be quantified by the capillary transit time heterogeneity (CTH), which is the standard deviation of the transit time distribution. In their seminal study, Jespersen and Østergaard [58] showed using a theoretical model that the efficacy of oxygen extraction decreases with CTH. In particular, disturbed capillary flow patterns can decrease oxygen extraction even in the absence of changes in mean flow. CTH was linked to a number of diseases and conditions such as Alzheimer’s disease [80], stroke-like symptoms [83], traumatic brain injury [81] and ischemic heart disease [84]. Measurements of CTH can be achieved using dynamic susceptibility contrast magnetic resonance imaging [76] and bolus tracking with two-photon microscopy [45]. The latter study showed that CTH decreases more than mean transit time during stimulation, consistent with the capillary flux homogenization in response to neural activation observed using optical coherence tomography [66].

In Jespersen et al. [58], CTH was related to the oxygen extraction fraction by extending the Bohr-Kety-Crone-Renkin (BKCR) model which assumes homogeneous oxygen partial pressure (PO2) in the extravascular compartment. This flow diffusion equation relates the decrease in blood oxygen concentration to the capillary transit time and the oxygen partial pressure drop across the capillary wall by means of a single rate constant k. This constant was fitted to yield a resting oxygen extraction fraction value of 0.3 and the tissue PO2 was required as a model input. Angleys et al. [2] refined this model to determine tissue PO2 values that match metabolic oxygen consumption and the oxygen extraction fraction values predicted by the BKCR model. They found that the maximal cerebral metabolic rate of oxygen consumption (CMRO2) that can be sustained for a given increase in cerebral blood flow was lower than with the original model. Thus, model
predictions are sensitive to the flow-diffusion equation for the evolution of blood oxygen content.

The distribution of PO$_2$ levels at the distal ends of capillaries is a key determinant of tissue oxygenation. Even if blood flow to a given region is adequate to meet the oxygen needs of the tissue, a maldistribution of flow can lead to wide variations of end-capillary PO$_2$. If the PO$_2$ distribution is highly heterogeneous, low values in some capillaries can cause tissue hypoxia, whereas a uniform distribution of end-capillary PO$_2$ tends to minimize tissue hypoxia, for a given overall oxygen supply. The heterogeneity of end-capillary PO$_2$ can equivalently be expressed in terms of the heterogeneity of hemoglobin saturation, which is functionally dependent on PO$_2$ according to the oxy-hemoglobin saturation curve. This study therefore focuses on capillary outflow saturation heterogeneity (COSH), a measure of the variability of blood oxygen content at the distal end of capillaries where blood flows into venules. Elevated COSH may imply that a fraction of microvessels cannot supply oxygen to their surrounding tissue even if the average saturation is sufficiently high.

In previous modeling works [58, 2], the effects of CTH on brain oxygenation were quantified using blood oxygen levels. Both tissue PO$_2$ and capillary hemoglobin saturation were shown to depend on transit times through the erythrocyte velocity. Like other microvascular beds, the brain microvasculature has a heterogeneous geometric structure and hemodynamics, with substantial variability in path lengths, vessel diameters, flow rates, hematocrits, and tissue volumes supplied by individual capillaries. In parallel capillary arrays, the interaction among vessels reduces the heterogeneity of PO$_2$ when RBC velocity and inlet oxygen concentration take different values in each capillary [95, 107]. Besides, in the absence of CTH, irregular capillary spacings lead to a more heterogeneous oxygenation compared to regularly spaced arrays [54], which also affects the distribution of hemoglobin saturation. Therefore, multiple factors beside CTH contribute to COSH, and CTH by itself does not provide a sufficient basis for understanding and predicting tissue oxygenation. The present study addresses the factors determining COSH and its relation to CTH, using theoretical models.

To compute COSH, models that describe the evolution of hemoglobin saturation heterogeneity in single and multiple capillaries are developed. Diffusive oxygen transfer is shown to be the main physical mechanism that underpins the reduction of hemoglobin saturation heterogeneity. The diffusive interaction between RBCs in single capillaries and between parallel capillaries is modeled based on ordinary differential equations. These interaction models are validated for a large range of physiological parameters.
using a computational model with individual moving RBCs [71]. Explicit formulas for the associated length and time scales are given and the resulting values are compared to RBC transit times and path lengths in capillaries. Model predictions are compared to oxygen transport simulations in two microvascular networks (MVNs) acquired from the mouse cerebral cortex. To this end, a novel flow reconstruction algorithm based on sparse RBC flow measurements is developed. The modeling of COSH is an essential step towards an actual understanding of CTH and its relation to oxygen transport in MVNs. The resulting insights have potentially broad implications in the study of capillary dysfunction and related conditions.

4.2 Methods

4.2.1 Diffusive interaction models

Models for the diffusive interaction between RBCs in single capillaries and between multiple capillaries were developed based on the differential equations introduced in Section 3.2. The interaction models were compared to the oxygen transport model with individual moving RBCs presented in Section 2.2.

Based on the observation that the RBC transit time is only one of multiple parameters that determine hemoglobin saturation [70], we asked the question: “Which phenomena do cause a difference between CTH and COSH?” Two representative situations that contribute to a reduction of hemoglobin saturation heterogeneity were identified. The first one pertains to branchings where two capillaries with different hemoglobin saturation levels are converging. This heterogeneity, which may result from different transit times, causes the rates of oxygen unloading from individual RBCs to differ. The second situation concerns parallel capillaries with different hemoglobin saturations. In this case, the tissue volume supplied by each capillary can differ. Diffusive interaction models are derived for these two representative situations.

As in Chapter 3, cylindrical RBCs and an axisymmetric geometry with concentric regions for the plasma, the capillary endothelium, and the tissue are considered. Our starting point is the differential equation (3.10). The interaction models for hemoglobin saturation heterogeneity hinge on appropriate modifications of the oxygen flux out of the capillary $j_t$. The second term on the right-hand side of Eq. (3.10) was found to be negligible and will be omitted from further derivations. However, this term was included in all numerical computations for completeness.
4.2. Methods

Eq. (3.10) can be recast in terms of the capillary transit time \( \tau \) and integrated as

\[
S(\tau) = S_a - \int_0^\tau \frac{j_t(v_{rbc})}{\mu LD\pi r^2 C_0 + \pi r^2 \alpha_{eff} \frac{dP_{pa}}{dS} \mu L} dt.
\]  

(4.1)

Thus, hemoglobin saturation on distal side is influenced by the RBC transit time, the oxygen consumption per unit length \( j_t \), hematocrit and vessel diameter. This description of distal blood oxygen concentration is more complete than the BKCR equation used in Refs. [58, 2]. The BKCR model uses the normalized coordinate \( \tilde{x} = x/L \) and reads

\[
\frac{dC}{dx} = -k \tau \left( \alpha_c P_{50} \left( \frac{C}{B-C} \right)^{1/n} - C_t \right),
\]

(4.2)

where \( C \) is the bound oxygen concentration in RBCs, \( B \) is the maximal amount of oxygen bound to hemoglobin and \( C_t \) the oxygen concentration in the tissue. The model constant \( k \) was adjusted to obtain an oxygen extraction fraction of 0.3 in Refs. [58, 2]. In Eq. (3.10), the counterpart of \( k \) is the inverse of \( Q_{O_2}(S) \) (Eq. (3.9)) which is an explicit function of hematocrit and the vessel geometry.

Given the sink term \( j_t(x) \), Eq. (3.10) can be integrated numerically using a standard differential equation solver. The implementation in SciPy [59] of an explicit Runge-Kutta method of order 4(5) was used [46].

4.2.1.1 Diffusive interaction between RBCs in a single capillary

Capillary networks in the cerebral microvasculature form a mesh-like structure [69] with both diverging and converging bifurcations. At converging bifurcations, RBCs from either inflow branch may have different hemoglobin saturations, for instance due to different transit times or hematocrit values. Here, we derive an interaction model for the evolution of hemoglobin saturation in a single capillary where the saturation at the capillary inflow is fluctuating.

The inlet hemoglobin saturation is expressed as a random variable \( S_a \) (here, the variable \( S \) is to be understood as a random variable). The RBC interaction model is based on Fick’s law as follows: the oxygen flux out of the capillary is assumed to be proportional to the oxygen partial pressure difference between the RBC and the tissue. In other words, we assume that

\[
Q_{O_2}(S) \frac{dS}{dx} = -C(P_{eq}(S) - P(r_s)),
\]

(4.3)
Figure 4.1: Schematics for RBC diffusive interaction. A: cylindrical domain used for the study of RBC diffusive interaction. In the modeled scenario, RBCs are flowing from a converging bifurcation into the domain with different hemoglobin saturation values. B: sketch for the definition of the integral spreading distance of $P_{O_2}$ oscillations (Eq. (4.10)). The top and bottom radial profiles indicate the maximal and minimal values of $P_{O_2}$ as RBCs are passing. The integral in cylindrical coordinates of the blue rectangle with width $\Delta r_{osc}$ is equal to that of the black hatched area.

where $r_s$ is a radial position which is independent from the fluctuations of $S$ and where the $P_{O_2}$ fluctuations in the tissue are small (Fig. 4.1B), and $C$ is a proportionality factor that will be derived. From now on, averaged quantities will be denoted with an overline. In the next steps, the nonlinearity in $S$ of the total convective oxygen capacity $Q_{O_2}(S)$ (Eq. (3.9)) will be ignored, which allows the simplification $Q_{O_2}(S) = Q_{O_2}(\overline{S})$. Based on this, the averaged mass balance is given by

$$Q_{O_2}(\overline{S}) \frac{d\overline{S}}{dx} = -j_t(x).$$

(4.4)

The averaging of Eq. (4.3) combined with Eq. (4.4) yields an expression for $C$ which can be inserted into Eq. (4.3). The terms can be rearranged as

$$Q_{O_2}(S) \frac{dS}{dx} = -j_t(x) \left(1 + \frac{P_{eq}(S) - P_{eq}(\overline{S})}{P_{eq}(\overline{S}) - P(r_s)} \right).$$

(4.5)

The oxygen partial pressure $P(r_s)$ still needs to be modeled. This is achieved by introducing the resistance coefficient for RBC diffusive interaction $K_{RI} = (\overline{P_{eq}(S)} - P(r_s))/j_t(x)$, which is the only parameter of this model. Thus, the model equation for randomly distributed hemoglobin saturation in a
single capillary is given by

\[ Q_{O_2}(S) \frac{dS}{dx} = -j_t(x) - \frac{1}{K_{RI}} (P_{eq}(S) - \overline{P_{eq}(S)}). \]  (4.6)

The second term on the right-hand side is a return-to-mean term that makes the difference between the average and instantaneous hemoglobin saturation decay towards zero.

By a suitable linearization, the nonlinear Eq. (4.6) can be further simplified to an evolution equation for the standard deviation of \( S \). Here, we again assume that the function \( P_{eq} \) (Eq. (3.3)) is linear around \( \overline{S} \) and that the derivative \( \frac{d}{dx} \frac{dP_{eq}}{dS}(\overline{S}) \) can be neglected. It follows that

\[ Q_{O_2}(\overline{S}) \frac{d}{dx} (S - \overline{S}) = -\frac{1}{K_{RI}} \left. \frac{dP_{eq}}{dS} \right|_{\overline{S}} (S - \overline{S}). \]  (4.7)

Using the above assumptions, the standard deviation of hemoglobin saturation \( \sigma_S \) satisfies the differential equation

\[ Q_{O_2}(\overline{S}) \frac{d\sigma_S}{dx} = -\sigma_S \left. \frac{dP_{eq}}{dS} \right|_{\overline{S}}. \]  (4.8)

This equation can be solved numerically given the average hemoglobin saturation \( \overline{S} \) which is itself obtained by integrating Eq. (3.10).

The resistance coefficient \( K_{RI} \) describes the resistance to a \( P_{O_2} \) drop between the RBC and a point in the tissue at a certain distance from the capillary. Therefore, we decompose this coefficient as \( K_{RI} = K_{IV} + K_{OS} \) where \( K_{OS} \) accounts for the resistance to the \( P_{O_2} \) drop between \( r_w \) and \( r_s \). Since \( r_s \) was defined to be at a distance where \( P_{O_2} \) oscillations in the tissue become small, we introduce an integral quantity related to the \( P_{O_2} \) oscillations in the tissue. The integral oscillation radius \( r_{osc} \) is implicitly defined as

\[ \pi (r_{osc}^2 - r_w^2) \Delta P_{max} = \int_{r_w}^{r_s} r \Delta P(r) dr. \]  (4.9)

The terms in this definition are illustrated in Fig. 4.1B. The maximal amplitude \( \Delta P_{max} \) is reached at the capillary outer wall. Finally, the integral oscillation spreading distance \( \Delta r_{osc} \) is defined as

\[ \Delta r_{osc} = r_{osc} - r_w. \]  (4.10)

Our numerical results will show that \( \Delta r_{osc} \) is an accurate predictor for the model coefficient \( K_{OS} \).
Figure 4.2: Schematics for capillary diffusive interaction. A: sketch of the geometry with four parallel capillaries. B: transverse view of the computational domain with four parallel capillaries. The shaded areas represent the tissue regions supplied by each capillary. Solid lines: boundary of the representative domain with periodic boundary condition; dash-dotted line: actual computational domain with symmetry boundary condition; dotted line: boundary of the tissue region supplied by each capillary. C: sketch of the tissue Po2 between two capillaries with different RBC Po2 values $P_{c,\phi}$ (red dot) and $P_{c,\psi}$ (blue dot). The colored circles indicate the locations of the capillaries $\phi$ and $\psi$. Solid line: continuous Po2 profile with adapted tissue radii $r_{t,\phi}$ and $r_{t,\psi}$; dashed line: discontinuous Po2 profile under the assumption of equal oxygen fluxes out of the capillaries.

### 4.2.1.2 Diffusive interaction between parallel capillaries

Having examined the diffusive interaction between heterogeneously saturated RBCs in the same capillary, we now consider the diffusive interaction between capillaries with different saturation levels. For our analysis, four parallel capillaries are considered where both pairs of diagonally opposed capillaries will be denoted by the indices $\phi$ and $\psi$, respectively (Fig. 4.2).

Given different oxygen saturations $S_{\phi,a}$, $S_{\psi,a}$ at the proximal inlets, we aim to derive the evolution of $S$ in both capillaries. To do this, the tissue region supplied by each capillary is approximated by a cylinder with varying radius which will be determined using the continuity of tissue Po2. As above, the neglect of axial diffusion allows tissue slices that are orthogonal to the capillary to be decoupled. Let $A$ be the area of the normal domain slice supplied by the two model capillaries $\phi$ and $\psi$. Mass conservation implies that the oxygen flux at $x$ out of both model capillaries balances the metabolic oxygen consumption in the tissue slice normal to $x$:

$$j_{t,\phi}(x) + j_{t,\psi}(x) = M_0 (A - 2\pi r_w^2).$$  \hspace{1cm} (4.11)
4.2. Methods

Using the IVR coefficient and the Krogh model (Eq. (3.20)), the continuity of tissue PO$_2$ at the interface between the Krogh cylinders is given by

\[ P_{c,\phi}(x) - K_{IV,\phi}j_{t,\phi}(x) - \Delta P_{EV}(j_{t,\phi}(x)) = P_{c,\psi}(x) - K_{IV,\psi}j_{t,\psi}(x) - \Delta P_{EV}(j_{t,\psi}(x)), \] (4.12)

where \( \Delta P_{EV}(j_{t}) \) is the extravascular PO$_2$ drop associated to the local oxygen outflux \( j_{t} \). Based on the right-hand side of Eq. (3.20), it is given by

\[ \Delta P_{EV}(j_{t}) = \begin{cases} \frac{M_{0}}{4D_{t}\alpha_{t}} \left[ 2r_{t}^{2} \log \left( \frac{r_{t}}{r_{w}} \right) - r_{t}^{2} + r_{w}^{2} \right], & j_{t} \geq 0, \quad r_{t} = \sqrt{\frac{j_{t}M_{0}}{\pi} + r_{w}^{2}} \\ 0, & j_{t} < 0. \end{cases} \] (4.13)

Given \( P_{c,\phi} \) and \( P_{c,\psi} \), the nonlinear equation system formed by Eq. (4.11) and (4.12) can be solved numerically for \( j_{t,\phi} \) and \( j_{t,\psi} \). Then, Eq. (3.10) is solved in both model capillaries one step forward using an explicit differential equation integrator. This model will be referred to as nonlinear Krogh-based model and can be applied to capillaries with different flows and radii. Cases where one capillary is supplied with oxygen by the other (for instance, \( j_{t,\psi} < 0) \) are captured by this formulation.

A slight simplification in Eq. (4.12) provides an explicit expression for the oxygen flux out of both model capillaries. Under the assumption that both capillaries have the same geometry and linear density, the IVR coefficient \( K_{IV} \) takes the same value in both capillaries, so Eq. (4.12) can be rearranged as

\[ \frac{P_{c,\phi}(x) - P_{c,\psi}(x)}{M_{0}} = \left( K_{IV}\pi - \frac{1}{4D_{t}\alpha_{t}} \right) \left( r_{t,\phi}^{2} - r_{t,\psi}^{2} \right) + \frac{1}{2D_{t}\alpha_{t}} \left( r_{t,\phi}^{2} \log \left( \frac{r_{t,\phi}}{r_{w}} \right) - r_{t,\psi}^{2} \log \left( \frac{r_{t,\psi}}{r_{w}} \right) \right). \] (4.14)

The assumption that

\[ \log \left( \frac{r_{t,\phi}}{r_{w}} \right) \simeq \log \left( \frac{r_{t,\psi}}{r_{w}} \right) \simeq \log \left( \frac{r_{t,\text{mean}}}{r_{w}} \right), \] (4.16)

where \( r_{t,\text{mean}} = \sqrt{\frac{1}{2}(r_{t,\phi}^{2} + r_{t,\psi}^{2})} \), yields an explicit expression for the oxygen outflux

\[ j_{t,\phi}(x) = M_{0}\pi(r_{t,\text{mean}}^{2} - r_{w}^{2}) + \frac{P_{c,\phi}(x) - P_{c,\psi}(x)}{2K_{IV} + \frac{1}{\pi D_{t}\alpha_{t}} \left( \log \left( \frac{r_{t,\text{mean}}}{r_{w}} \right) - \frac{1}{2} \right)}. \] (4.17)
86 Hemoglobin saturation heterogeneity

and a similar expression for \( j_{t,\psi}(x) \). We now define the resistance coefficient \( K_{CI} \) for diffusive interaction between capillaries as

\[
K_{CI} = K_{IV} + \frac{1}{2\pi D_t \alpha_t} \left( \log \left( \frac{r_{t,\text{mean}}}{r_w} \right) - \frac{1}{2} \right). \tag{4.18}
\]

By using the average oxygen outflux \( j_t = M_0 \pi (r_{t,\text{mean}}^2 - r_w^2) \), the evolution equations for hemoglobin saturation in both model capillaries become

\[
Q_{O_2,\phi}(S_\phi) \frac{dS_\phi}{dx} = -j_t - \frac{1}{2K_{CI}} (P_{c,\phi}(x) - P_{c,\psi}(x)) \tag{4.19}
\]

\[
Q_{O_2,\psi}(S_\psi) \frac{dS_\psi}{dx} = -j_t - \frac{1}{2K_{CI}} (P_{c,\psi}(x) - P_{c,\phi}(x)). \tag{4.20}
\]

This model will be referred to as explicit Krogh-based model. To derive this equation, the respective linear densities in both capillaries were assumed to be equal. However, the respective RBC velocities were still allowed to be different. Under the assumption that \( v_{rbc} \) is equal in both capillaries, the linearization of \( P_{eq} \) around the average hemoglobin saturation \( \overline{S} = \frac{1}{2}(S_\phi + S_\psi) \) yields the following evolution equation for the saturation difference \( \Delta S = S_\phi - S_\psi \) between both model capillaries:

\[
Q_{O_2}(\overline{S}) \frac{d\Delta S}{dx} = -\Delta S \frac{dP_{eq}}{dS} \bigg|_{\overline{S}}. \tag{4.21}
\]

This third model will be referred to as linearized capillary interaction model. This equation leads to the definition of the characteristic length scale \( L_{CI} \) for diffusive interaction between parallel capillaries

\[
L_{CI} = K_{CI} Q_{O_2}(\overline{S}) \left( \frac{dP_{eq}}{dS} \bigg|_{\overline{S}} \right)^{-1}. \tag{4.22}
\]

Similarly, the characteristic time scale \( \tau_{CI} \) is defined as

\[
\tau_{CI} = \frac{L_{CI}}{v_{rbc}} = K_{CI} \left( \mu_{LD} \pi r_c^2 C_0 \left( \frac{dP_{eq}}{dS} \bigg|_{\overline{S}} \right)^{-1} + \pi r_p^2 \alpha_{eff} \right). \tag{4.23}
\]

Thus it is independent from the RBC velocity and depends on linear density, the average hemoglobin saturation \( \overline{S} \) and the geometry. These characteristic quantities will be compared to fits obtained using the computational model.
4.2. Methods

4.2.2 Flow reconstruction algorithm based on sparse measurements

Given a sparse set of RBC velocity measurements in a reconstructed MVN, a matching blood flow field needs to be reconstructed for oxygen transport simulations to be run. In the networks used here, most boundary vessels are capillaries where the pressure is unknown. Therefore, the flow reconstruction method introduced by Gagnon et al. [32] cannot be directly employed. Since RBC velocity measurements from longitudinal line scans are restricted to vessels in the microscope focal plane, the resulting flow reconstruction problem is generally underdetermined. To solve this issue, we introduce a cost function based on energy minimization and determine a pressure solution using optimization under constraints. The resulting pressure boundary conditions can subsequently be used in the discrete RBC transport model [109] which provides RBC trajectories to our oxygen transport model with moving RBCs.

Consider a graph representation of a MVN with a set of $n_v$ vertices $V$ and edges $(i,j) \in E$ that represent blood vessels with associated lengths $L_{ij}$ and diameters $d_{ij}$. The flow $q_{ij}$ through a vessel $(i,j)$ and the pressure difference between nodes $i$ and $j$ are related by the hydraulic resistance as

$$q_{ij} = \frac{p_i - p_j}{R_{ij}^e}.$$  \hspace{1cm} (4.24)

The effective resistance $R_{ij}^e$ is related to the resistance obtained by Poiseuille's law by the in-vitro apparent viscosity $\mu_{\text{vitro},ij}$ as

$$R_{ij}^e = \mu_{\text{vitro},ij} \frac{128\mu_p}{\pi d_{ij}^4} L_{ij},$$  \hspace{1cm} (4.25)

where $\mu_p$ is the plasma viscosity and was set to 1.2 mPa s [60]. The expression for $\mu_{\text{vitro},ij}$ is given in Pries et al. [97] and depends on the vessel diameter and hematocrit. The determination of hematocrit is discussed below and its values can be assumed to be given for now.

The flow balance at each internal node $j$ is enforced by

$$\sum_{i|(i,j) \in E} \frac{p_i - p_j}{R_{ij}^e} = 0.$$  \hspace{1cm} (4.26)

Measurements of the RBC velocity $v_{\text{rbc},ij}^{\text{meas}}$ provide additional constraints. The corresponding flow is obtained by taking the Fåhraeus effect into account as follows:

$$q_{ij}^{\text{meas}} = v_{\text{rbc},ij}^{\text{meas}} \frac{\pi d_{ij}^2}{4} H_{T,ij} H_{D,ij}.$$  \hspace{1cm} (4.27)
where $H_T$ and $H_D$ are the tube and discharge hematocrit, respectively. The discharge hematocrit was computed from the tube hematocrit using the empirical relations in Pries et al. [98]. Since some combinations of measurements can lead to a violation of flow balance, the vessels with associated measurements need to be split in two categories. For instance, if all edges that are adjacent to a certain vertex have prescribed RBC velocity values, the flows obtained with Eq. (4.27) will not sum to zero in general. Denote by $E_{\text{comp}}$ the set of edges with compatible measurements that do not cause a violation of flow balance. A measurement between nodes $i$ and $j$ yields the equation

$$\frac{p_i - p_j}{R_{ij}^c} = q_{ij}^{\text{meas}} \quad \text{for } (i, j) \in E_{\text{comp}}. \quad (4.28)$$

The treatment of incompatible measurements is discussed below.

The unknowns in the flow reconstruction problem are the pressure values at the $n_v$ vertices in the network. Vertices can be split into $n_{v,\text{int}}$ internal nodes and $n_{v,b}$ boundary nodes. Flow conservation yields an equation for each internal node and each compatible measurement adds one more equation ($n_{\text{meas,comp}}$ in total). Therefore, if $n_{\text{meas,comp}} < n_v - n_{v,\text{int}} = n_{v,b}$, the problem is underdetermined, which occurred in both MVNs employed here.

The following cost function is introduced to solve the underdetermination issue:

$$J(p) = \omega_{\text{pow}} J_{\text{pow}}(p) + \omega_{v,\text{avg}} J_{v,\text{avg}}(p) + \omega_{\text{meas}} J_{\text{meas}}(p), \quad (4.29)$$

where $p$ is the vector of pressure values at the nodes and the $\omega$-coefficients have implied units. The first term represents the hydraulic power through the MVN, namely

$$J_{\text{pow}}(p) = \sum_{(i,j) \in E} \frac{(p_i - p_j)^2}{R_{ij}^c}. \quad (4.30)$$

This ensures that the pressure solution uses low hydraulic power, which expresses energy minimization. However, the use of this single term may cause vessels away from measurements to have zero or very low flow. To compensate for this effect, an additional term is introduced to guarantee an average RBC velocity in accordance with experimental data:

$$J_{v,\text{avg}}(p) = (v_{\text{rbc,avg}}(p) - v_{\text{rbc,ref}})^2, \quad (4.31)$$

where $v_{\text{rbc,avg}}(p)$ is the length-weighted RBC velocity in the network for the pressure values $p$ and $v_{\text{rbc,ref}}$ is a reference RBC velocity that can be chosen. The combination of the first two terms in Eq. (4.29) with appropriate
weights ensures that the obtained RBC velocities are neither too high nor too low (Fig. 4.15).

The last term in Eq. (4.29) addresses RBC velocity measurements that lead to a violation of flow balance. Let $E_{\text{inc}}$ be the set of edges with measurements that are incompatible with flow balance. On these edges, the squared deviation between measurements and reconstructed flow is minimized using

$$J_{\text{meas}}(p) = \sum_{(i,j) \in E_{\text{inc}}} \left( q_{\text{meas}}^{ij} - \frac{p_i - p_j}{R_{ij}} \right)^2.$$  \hspace{1cm} (4.32)

The weights in Eq. (4.29) were chosen to provide a satisfactory balance between the terms and set to $\omega_{\text{pow}} = 1$, $\omega_{v,\text{avg}} = 200$ and $\omega_{v,\text{avg}} = 50$ (there are only two actual degrees of freedom since the cost function can be arbitrarily rescaled by a multiplicative constant). The reference velocity $v_{\text{rbc,ref}}$ was set to 1.5 mm/s. Due to minimization of hydraulic power, the mean RBC velocities obtained with this method were close to 1.0 mm/s (Table 4.1). This value can be adapted to obtain average RBC velocity values that match experimental observations. The minimization of $J(p)$ under the constraints given by Eqs. (4.26) and (4.28) was performed using the implementation of sequential least squares programming in the scientific computing library SciPy [59].

There remains to discuss the distribution of hematocrit in the MVNs. An inlet tube hematocrit value $H_{T,\text{in}}$ of 0.25 was chosen as an initial condition. However, due to phase separation, the hematocrit distribution in MVNs is not constant in general. When using the discrete RBC tracking model [109] with the boundary pressure values $p_b$ obtained from the minimization problem described above, the resulting steady-state hematocrit may differ from the initial hematocrit value. In turn, this affects the effective resistances $R_{ij}^e$ (Eq. (4.25)) and the agreement between the simulated flow and the measurements. Therefore, an iterative procedure is used where the steady-state hematocrit from the RBC transport model is fed back to the optimization procedure, which yields updated effective resistances. The flow reconstruction algorithm then provides a new pressure solution which is used as a boundary condition for the RBC transport model. The procedure is repeated until the resistances vary by less than 1%. This method is summarized in Algorithm 2 and was found to converge in both MVNs. At each iteration, the RBC transport model was run for 1 s and the RBC positions from the previous iteration were used as an initial condition.
Algorithm 2: Flow reconstruction based on sparse measurements

1: compute $R_{ij}^{e,0}$ using $H_{T,in}$
2: $\epsilon_{rel} \leftarrow \infty$
3: $k \leftarrow 0$
4: while $\epsilon_{rel} < \text{rel.tol}$ do
5:   $k \leftarrow k + 1$
6:   find $p$ by minimizing $J(p)$ (4.29) under the constraints (4.26), (4.28)
7:   run a discrete RBC transport simulation with $p_b$ and $H_{T,in}$ at the boundary nodes over a given time interval
8:   compute $R_{ij}^{e,k}$ from the time-averaged tube hematocrit
9:   $\epsilon_{rel} \leftarrow \max_{(i,j) \in E} \left| \frac{R_{ij}^{e,k}}{R_{ij}^{e,0}} - 1 \right|$
10: end while

4.2.3 Model parameters

The heterogeneity of hemoglobin saturation was investigated in different computational domains. The diffusive interaction between RBCs was studied in a two-dimensional cylindrical domain with radius $r_t = 23 \mu m$, which corresponds to the distances between nuclei of neurons and capillaries measured in Tsai et al. [132]. Unless stated otherwise, a domain length $L = 100 \mu m$ was chosen. Cylindrical RBCs with volume $V_{rbc} = 59 \mu m^3$ and radius $r_c = 1.5 \mu m$ were employed. The capillary lumen diameter was set to $r_p = 2.0 \mu m$ and the endothelium thickness to $0.6 \mu m$ [8], so that the endothelium radius was $r_w = 2.6 \mu m$. At the tissue boundary, the gradient of the PO$_2$ field was set to zero. In this domain, the grid cell size was set to $\Delta x = \Delta y = 0.3 \mu m$ in the capillary. The radial grid spacing in the tissue was smoothly increased to save computational effort, so that $\Delta y$ was four times higher at the tissue boundary than in the capillary. The grid spacing in the RBC meshes was set to $\Delta x_{rbc} = \Delta y_{rbc} = 0.1 \mu m$. The time step size was set to $\Delta t = \Delta x/v_{rbc}$.

The diffusive interaction between capillaries was investigated in an array with four parallel capillaries with radius $r_p = 2.0 \mu m$. The symmetry of the domain allowed that only one quarter of each capillary had to be simulated (Fig. 4.2). The normal PO$_2$ gradient was set to zero at each boundary plane. A spacing of 40$\mu m$ between the capillaries was chosen, which yields an averaged supplied tissue volume per capillary very close to that of a cylinder with radius $r_t = 23 \mu m$. In all simulations with multiple capillaries, the RBC radius was set to $r_c = 0.8r_p$ and the endothelium radius to $r_w = 1.25r_p$. For this three-dimensional domain, a coarser grid spacing than in the two-
4.2. Methods

Table 4.1: Topology and flow characteristics of the two reconstructed MVNs (mean ± SD).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>whole sub-network</th>
<th>MVN 1</th>
<th>whole sub-network</th>
<th>MVN 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>bounding box [µm]</td>
<td>219 × 220 × 168</td>
<td>126 × 216 × 152</td>
<td>194 × 193 × 149</td>
<td>108 × 148 × 116</td>
</tr>
<tr>
<td>vessels</td>
<td>92</td>
<td>60</td>
<td>89</td>
<td>45</td>
</tr>
<tr>
<td>converging bifurc.</td>
<td>22</td>
<td>14</td>
<td>23</td>
<td>14</td>
</tr>
<tr>
<td>diameter [µm]</td>
<td>5.03 ± 1.45</td>
<td>4.91 ± 1.11</td>
<td>5.01 ± 1.47</td>
<td>5.26 ± 1.50</td>
</tr>
<tr>
<td>vessel length [µm]</td>
<td>57.3 ± 41.1</td>
<td>58.1 ± 39.9</td>
<td>47.0 ± 34.5</td>
<td>52.5 ± 37.9</td>
</tr>
<tr>
<td>v_rbc [mm/s]</td>
<td>0.879 ± 0.475</td>
<td>0.899 ± 0.518</td>
<td>0.997 ± 0.564</td>
<td>1.095 ± 0.592</td>
</tr>
<tr>
<td>tube hematocrit</td>
<td>0.221 ± 0.087</td>
<td>0.235 ± 0.085</td>
<td>0.265 ± 0.09</td>
<td>0.254 ± 0.082</td>
</tr>
</tbody>
</table>

dimensional cylinder was chosen. The grid cell size in the tissue away from the capillaries was set to 1 µm. At ≤ 8 µm from the capillaries, the grid was refined by a factor two to better resolve the high oxygen gradients in and close to the capillaries. The grid cell size in the RBCs was set to Δx_rbc = 0.25 µm and the time step to Δt = Δx/v_rbc. Since the hemoglobin saturation difference ΔS_v between the venous ends of the capillaries is our main quantity of interest here, the grid spacing needs to be sufficiently high to accurately resolve this quantity. A grid convergence study showed that doubling the spatial resolution in each dimension and reducing the time step correspondingly increases ΔS_v by < 2.2%. Therefore, all simulations were run with the grid resolution described above, since it provides a good compromise between accuracy and run time (~20 hours per simulation on a single core). The coarser grid resolution in the tissue was found not to affect the values of ΔS_v.

To verify whether the conclusions drawn from our interaction models also hold true in a more realistic setting, we performed simulations in two reconstructed MVNs from the mouse somatosensory cortex. The details of the in vivo experiments are given below. Two in vivo two-photon microscopy datasets were analyzed according to Schneider et al. [112]. Segmentation and centerline extraction was used to reconstruct the MVNs. The vessel diameter distribution was scaled to a mean of 5 µm ± 1.5 µm (SD) and a lowest bound of 3 µm was enforced. Since our computational model for oxygen transport currently does not handle non-capillary vessels, a single file flow was assumed in all vessels. Additionally, the RBC diameters were clamped between 3 and 8 µm to avoid unrealistic shapes. The grid spacing was set to 1 µm in the tissue domain and to 0.67 µm for RBCs with a diameter of 4 µm. The topology and flow characteristics in both MVNs are summarized in Table 4.1.
Figure 4.3: Reconstructed MVNs from the mouse cerebral cortex. The colors show the RBC velocities obtained from the flow reconstruction algorithm. The vessel diameters are to scale and the top of the domain is closest to the cortical surface. The boxes show the computational domains used in the oxygen transport simulations. A: MVN 1. B: MVN 2. Scale bar: 20 µm. See Table 4.1 and Fig. 4.15 for further information about the network topology and the reconstructed flow.

The oxygen consumption rate was set to 0.001 µm³ O₂ µm⁻³ s⁻¹, which is within the range of values measured in the anesthetized rodent cerebral cortex in Zhu et al. [147], using a brain density of 1.05 g cm⁻³ and the ideal gas law at body temperature for the molar volume of oxygen (2.544 × 10⁴ ml O₂/(mol O₂)). The IVR coefficient, which is used for the model coefficients $K_{Rl}$ and $K_{CI}$ (Eq. (4.18)), was determined as in Lücker et al. [70] using the formula $K_{IV} = 0.5K_{IV,0.5}/\mu_{LD}$, with the difference that dissolved oxygen content was included in Eq. (3.10). For $r_c = 1.5$ and 1.6 µm, the values of $K_{IV,0.5}$ were 5.15 and 4.75, both in units of mmHg µm s/(µm³ O₂). The rest of the physiological parameters is given in Table 2.2.

4.2.4 Experimental procedures

All procedures involving animals were approved by the Canton of Zurich Veterinary Office, in accordance with Swiss law (Federal Act of Animal Protection 2005 and Animal Protection Ordinance 2008). Two adult, female C57BL/6J mice (Charles River, Germany) were used for these experiments. The mice were prepared for chronic imaging as described previously [126, 73].
4.3 Results

Imaging data were acquired using a custom built two-photon microscope \[75\]. For imaging sessions, the mice were anesthetized subcutaneously with a mixture of fentanyl (0.05 mg/kg, Sintenyl, Sintetica), midazolam (5 mg/kg, Dormicum, Roche), and medetomidine (0.5 mg/kg, Domitor, Orion Pharma), and anaesthesia was maintained with midazolam (5 mg/kg) after 50 min. The vasculature was labelled using FITC dextran (5%, 59-77 kDa, Sigma), injected via the tail vein.

High resolution structural images of microvasculature networks within the somatosensory cortex were obtained at an in-plane resolution of \( \sim 0.4 \times 0.4 \, \mu m \), and spaced \( \sim 2 \, \mu m \) apart in depth. Both networks were located in the upper 250 \( \mu m \) of the cortex.

RBC velocities in individual vessels were measured using line scans along the axis of the vessel \[61\]. Briefly, the RBCs appear as dark shadows compared to the fluorescently-labelled plasma, and their motion along the vessel leads to streaks on a space-time image. The angle of these streaks can be used to calculate RBC velocity, which was done here using custom-built software based on an algorithm using the radon transform \[21\].

4.3 Results

The evolution of hemoglobin saturation heterogeneity was simulated in the geometries shown in Fig. 4.1A, 4.2A and 4.3, and compared whenever possible to the RBC and capillary interaction models.

4.3.1 Diffusive interaction between RBCs

The diffusive interaction between RBCs with different hemoglobin saturations was investigated in a cylindrical tissue domain (Fig. 4.1A). This single-capillary setup with differently saturated RBCs aims to represent a capillary after a converging bifurcation where RBCs with different transit times are flowing in. The simplest model for the inlet hemoglobin saturation of RBCs is when erythrocytes alternatingly take two fixed saturation values (one value per upstream branch). Fig. 4.4 shows the evolution of hemoglobin saturation in a capillary with length \( L = 300 \, \mu m \) with inlet values \( S = 0.8 \) and 0.6. The standard deviation \( \sigma_{S,v} \) of the hemoglobin saturation from the numerical model at the venous end is approximately seven times lower than at the inlet. The values of \( \sigma_S \) from the RBC interaction model is almost indistinguishable from the numerical results (Fig. 4.4B) when the model coefficient \( K_{RI} \) is fitted to match the standard deviation from the computational model (here, \( K_{RI} = 11.1 \, \text{mmHg} \, \mu m \, s / (\mu m^3 O_2) \)). The coeffi-
The linearized RBC diffusive interaction model with the same value of $K_{RI}$ also agrees very well with the numerical results, although it slightly underestimates $\sigma_S$. The simulated values of $\sigma_S$ were also fitted with a single exponential function of the form $f(x) = a \exp(x/L_{RI})$. Since this fit is also very good, our results can be expressed in terms of the characteristic decay length $L_{RI}$ and the related decay time $\tau_{RI} = L_{RI}/v_{rbc}$. To reduce the computational effort in further parameter studies, we compared the results obtained with domain lengths of 100 $\mu$m ($S_a = 0.6$ and 0.4) and 300 $\mu$m ($S_a = 0.8$ and 0.6, respectively). The fitted value of $K_{RI}$ in the short domain was only 4.6% lower than in the long domain. Therefore, the domain length does not have a major influence on the results and from now on we will use $L = 100$ $\mu$m. These first results suggest that hemoglobin saturation heterogeneity can be considerably reduced by diffusive interaction between RBCs within a single capillary.

The reduction of hemoglobin saturation heterogeneity observed in Fig. 4.4 is also observed when some previously made assumptions are dropped. Our models for RBC diffusive interaction were formulated for arbitrary random distributions of $S$ at the capillary inlet. Additionally, our conclusions should not be affected by the cylindrical geometry that was employed. Indeed, the no-flux boundary condition for oxygen artificially increases fluctuations of tissue $P_{O_2}$ by mirroring them and might affect the results. To
4.3. Results

Figure 4.5: Hemoglobin saturation profiles with a uniform distribution of $S_a$. The simulation was run in the geometry with four parallel capillaries (Fig. 4.2B). The mean and the standard deviation of $S_a$ were set to 0.5 and 0.1 in all capillaries, respectively. $\mu_{LD} = 0.3$; $v_{rbc} = 1.0$ mm/s. A: thin green lines: individual RBCs from the numerical simulation; thick solid lines: mean $S \pm \sigma_S$; dash-dotted lines: RBC interaction model; dotted lines: assumption of equal oxygen flux out of the RBCs. B: see legend of Fig. 4.4.

We now examine the influence of model parameters such as linear density, RBC velocity, oxygen consumption rate and hemoglobin saturation difference at the inlet on the results. Fig. 4.6 shows that the RBC interaction models with fitted $K_{RI}$ perform very well across a wide range of parameters. The relative model error in $\sigma_{S,v}$ normalized by the standard deviation drop from the numerical model $\sigma_{S,a} - \sigma_{S,v}$ is $\leq 2\%$ for the initial model and $\leq 4\%$ for the linearized model across the whole parameter range. Additionally, the exponential fit to the numerical results also matches very well the numerical results ($< 2\%$ error), which confirms that the decay
length $L_{RI}$ and decay time $\tau_{RI}$ introduced above can be used to compare results. The decay time $\tau_{RI}$ decreases from 206 to 157 ms when $v_{rbc}$ increases from 0.4 to 2.0 mm/s, but is rather insensitive to the linear density (10.2% decrease when $\mu_{LD}$ increases from 0.2 to 0.6, Fig. 4.7). The oxygen consumption rate has an even smaller influence on $\tau_{RI}$ (6.3% variation), while the inlet standard deviation of hemoglobin saturation almost does not affect it (1.2% variation).

The fitted decay length was found to be quite insensitive to the oxygen consumption rate (6.3% variation) and linear density (10.2% variation), and almost unaffected by the inlet hemoglobin saturation difference (1.2% variation). The decay length almost quadruples when the RBC velocity increases for 0.4 to 2.0 mm/s. Similarly to $L_{RI}$, a decay time $\tau_{RI}$ can be defined by $\tau_{RI} = L_{RI}/v_{rbc}$. This decay time decreases from 206 to 157 ms across the same range of erythrocyte velocities.

![Figure 4.6: Relative errors from the RBC diffusive interaction models compared to the numerical simulations. The relative error in the drop in $\sigma_S$ across the capillary is defined as $(\sigma_{S,v,simul} - \sigma_{S,v,model})/(\sigma_{S,a,simul} - \sigma_{S,v,simul})$. Dash-dotted lines: RBC interaction model; dotted lines: linearized model; solid lines: exponential fit to the numerical results. A: oxygen consumption rate; B: standard deviation of hemoglobin saturation at the inlet; C: RBC velocity; D: linear density.](image.png)
4.3. Results

Figure 4.7: Decay time scale $\tau_{RI}$ for RBC diffusive interaction. The time scale $\tau_{RI}$ was obtained with an exponential fit of the standard deviation of hemoglobin saturation from simulations across a range of parameters. A: linear density and RBC velocity; B: oxygen consumption rate and standard deviation of hemoglobin saturation at the inlet.

The above results show that the RBC interaction models agree closely with numerical simulations when using fitted values of $K_{RI}$. To show the models’ predictive power, it is necessary to characterize this model coefficient. Recall that $K_{RI}$ was decomposed as $K_{IV} + K_{OS}$, where $K_{OS}$ was related to the spreading distance of $P_{O_2}$ oscillations in the tissue due to individual passing erythrocytes. Fig. 4.8A shows the dependence of $K_{OS}$ on linear density and RBC velocity. Here, the coefficient $K_{OS}$ appears to decrease with the sum of both parameters. The plot of $\Delta r_{osc}$ against $K_{OS}$ for all the simulated values of linear density and RBC velocity (Fig. 4.8B) shows a strong correlation between these two quantities ($R^2 = 0.978$). The linear fit in Fig. 4.8 has the form $K_{OS} = 1.49\Delta r_{osc} - 3.58$, where the coefficient units are implied. Therefore, consistently with our initial assumption in Eq. (4.3), the model coefficient for RBC diffusive interaction is closely related to the $P_{O_2}$ oscillations in the tissue.

4.3.2 Diffusive interaction between parallel capillaries

The capillary diffusive interaction models are now compared to our computational model for oxygen transport. Numerical simulations were run in an array of four straight, parallel capillaries (Fig. 4.2A). In the two pairs of diagonally opposed capillaries, two different inlet values of hemoglobin sat-
Hemoglobin saturation heterogeneity

Figure 4.8: Model coefficient $K_{OS}$ for RBC diffusive interaction. A: $K_{OS}$ in mmHg $\mu$m s/($\mu$m$^3$ O$_2$) as a function of linear density and RBC velocity. B: $K_{OS}$ as a function of the integral spreading distance of $P_{O_2}$ oscillations defined in Eq. (4.10); solid line: linear fit.

The evolution of $S_{a,\phi}$, $S_{a,\psi}$ and the hemoglobin saturation difference $\Delta S = |S_{a,\phi} - S_{a,\psi}|$ were computed with the numerical model and compared to predictions from the three interaction models for the oxygen flux out of the capillaries (nonlinear Krogh-based model, explicit model and linearized model for $\Delta S$). Additionally, theoretical results based on the assumption of equal oxygen outflux will be shown to highlight the effects of capillary diffusive interaction. Unless otherwise stated, the average value of $S_a$ over all capillaries was 0.7 and the capillaries were 40 $\mu$m apart. The linear density and the erythrocyte velocity were set to 0.3 and 1.0 mm/s, respectively.

Fig. 4.9 shows hemoglobin saturation profiles along both capillary pairs from the numerical model and the interaction models. Mean hemoglobin saturations from the models correspond very well to the simulated results, which shows that mass conservation is fulfilled (the nonlinear and explicit Krogh-based models yield the same mean $S_a$ hence only the former is shown). In this setup, the hemoglobin saturation difference between both capillary pairs drops by $\sim$ 50% over 100 $\mu$m. This decrease is captured well by each interaction model, albeit slightly underestimated by the explicit Krogh-based model and the linearized model. Fig. 4.9 also illustrates that the assumption of equal oxygen outfluxes cannot be used in the present context. As above, the underestimation of $S_a$ by the interaction models away from the domain ends is caused by the absence of axial diffusion [70]. These first results suggest a strong reduction of hemoglobin saturation het-
4.3. Results

Figure 4.9: Hemoglobin saturation profiles in parallel capillaries. $\mu_{LD} = 0.3$; $v_{rbc} = 1.0 \text{ mm/s}$. Solid lines: numerical simulation; dash-dotted lines: nonlinear Krogh-based model; dashed lines: explicit Krogh-based model; dotted line: linearized model for $\Delta S$; pale dotted line: equal outflux assumption. A: hemoglobin saturation profile in both model capillaries (thin lines: average over all capillaries). B: hemoglobin saturation difference between both capillaries.

erogeneity between parallel capillaries.

To show model robustness, several input parameters were varied and the predicted drop in hemoglobin saturation difference $\Delta S_a - \Delta S_v$ was compared to numerical simulation results. The spacing between capillaries, the oxygen consumption rate, the RBC velocity and linear density were investigated (Fig. 4.10). In almost all cases, the nonlinear Krogh-based model shows the best agreement with numerical results (relative error $\leq 4\%$ except at very low oxygen consumption rates). The explicit Krogh-based model and the linearized model perform almost equally well, with relative errors $\leq 8\%$. These parameter studies show that capillary diffusive interaction models perform well over a large range of physiological parameters.

The capillary interaction models rely on a single model parameter $K_{CI}$ defined in Eq. (4.18). Unlike the coefficient $K_{RI}$ for RBC diffusive interaction, the expression for $K_{CI}$ only depends on the intravascular resistance coefficient $K_{IV}$ which can be computed as in Lücker et al.\cite{luecker2011}. Therefore, given a suitable value of $K_{IV}$ and mean hemoglobin saturation $S$, the decay length and time scales $L_{CI}$ and $\tau_{CI}$ (Eqs. (4.22) and (4.23), respectively) can be computed analytically. Fig. 4.11A shows values of $\tau_{CI}$ for a range of linear densities and mean hemoglobin saturation values. The decay time scale increases with linear density and attains its highest values at $S \simeq 0.3$, where $dP_a/dS$ attains its minimum with the employed parameters for the Hill equation (Eq. (3.2)). Similarly to RBC diffusive interaction, the sim-
Figure 4.10: Relative errors from the capillary diffusive interaction models compared to the numerical simulations. The relative error of the drop in hemoglobin saturation difference across the capillary is defined as $(\Delta S_{v,\text{simul}} - \Delta S_{v,\text{model}})/(\Delta S_{a,\text{simul}} - \Delta S_{v,\text{simul}})$. Dash-dotted lines: nonlinear Krogh-based model; dashed lines: explicit Krogh-based model; dotted lines: linearized model for $\Delta S$ (Eq. (4.21)). A: distance between capillaries; B: oxygen consumption rate; C: RBC velocity; D: linear density.

Simulated values of $\Delta S$ are very well fitted by exponential decays. Fig. 4.11B and 4.11C show a comparison of the analytical and the fitted decay time scales $\tau_{CI}$ for different values of capillary spacing, oxygen consumption rate, RBC velocity and linear density. For the analytical time scale, the simulated value of $\mathcal{S}$ at $x = L/2$ was employed. Over the investigated range of parameters, the analytical estimates of $\tau_{CI}$ overestimate the fitted values by at most 12 ms (relative error of $\leq 7.9\%$). This shows that the variations in $\tau_{CI}$ that occur in the investigated parameter range can be entirely explained by the dependency of the analytical $\tau_{CI}$ on $\mu_{LD}$ and $\mathcal{S}$. The decay length scale $L_{CI}$ is equally well predicted by the analytical formulation. Therefore, our interaction models provide an accurate quantification of the time and length scales involved in the reduction of hemoglobin saturation heterogeneity.
The previous results all assumed the same RBC velocity, flow direction and hematocrit in each capillary. These assumptions are now dropped to further examine model robustness. First, simulations with countercurrent flow instead of concurrent flow were run. Namely, the flows in both pairs of diagonally opposite capillary were set to opposite directions with the same RBC velocity. The inlet hemoglobin saturation difference $\Delta S_a$ was varied and the relative drop $\Delta S_v/\Delta S_a$ with countercurrent flow was compared to the concurrent flow setup used previously. Fig. 4.12A shows profiles of $S$ from the numerical model and the interaction models. Due to the countercurrent flow, the hemoglobin saturation difference at $x = 0 \mu m$ is larger than with concurrent flow (Fig. 4.9A) and smaller at $x = 100 \mu m$. The hemoglobin saturation difference between the venous capillary ends turns out to be practically the same as with concurrent flow (Fig. 4.12B). This indicates that the scope of the models introduced here is not limited to parallel capillaries with concurrent flow.

The motivation for the models developed here originate in the relationship between CTH and COSH. So far, the effects of CTH were only modeled using different values of $S_a$ at the capillary inlets, while the transit times of RBCs in the computational domain were constant. Now, actual CTH is introduced by setting different RBC velocities in the pairs of diagonally opposite capillaries. In this setting, the linearized capillary interaction model (Eq. (4.21)) cannot be used since it assumes the same $v_{rbc}$ in all capillaries.
Hemoglobin saturation heterogeneity

Figure 4.12: Capillary diffusive interaction with concurrent and countercurrent flow $\mu_{LD} = 0.3$; $v_{rbc} = 1.0 \text{mm/s}$. A: hemoglobin saturation profiles with countercurrent flow and inlet values $S = 0.8$ and 0.6, respectively. Solid lines: numerical model; dashed lines: Krogh-based model; dotted lines: equal oxygen flux assumption. Thick lines: values in individual capillaries; thin lines: averaged value over all capillaries. The arrows indicate the flow direction. B: percentual drop in $\Delta S$ from the arterial to the venous side of the capillaries. Solid line: concurrent flow; dashed line: countercurrent flow.

As in all previous cases, the simulated final hemoglobin saturation difference $\Delta S_v$ with $v_{rbc} = 1.5 \text{mm/s}$ and 0.5 mm/s, respectively, is significantly lower (42%) than if the oxygen fluxes out of the capillaries were assumed to be equal (Fig. 4.13). Here, the discrepancy between the interaction and the numerical models is higher than before. The overestimation of $\Delta S_v$ by the nonlinear and explicit Krogh-based models is 18.8% and 15.8%, respectively. For smaller differences in $v_{rbc}$, the model errors were in the same range. Conversely, differences in hemoglobin saturation can arise due to different values of linear density, even with equal transit times (Eq. 4.1). With $\mu_{LD} = 0.5$ and 0.3, respectively, the simulated distal hemoglobin saturation difference is 82% lower than under the assumption of equal oxygen fluxes (Fig. 4.14). Here, the nonlinear Krogh-based model is more inaccurate and overestimates $\Delta S_v$ by 45.0 % (the explicit Krogh-based model cannot be applied to heterogeneous linear densities). Although the interaction models are not as accurate as before with heterogeneous RBC velocities and linear densities, our conclusions about the reduction of hemoglobin saturation heterogeneity still hold in the presence of CTH or hematocrit heterogeneity.
4.3. Results

Figure 4.13: Capillary diffusive interaction with different RBC velocities ($v_{rbc} = 1.5$ and $0.5 \text{mm/s}$). Solid lines: numerical model; dash-dotted lines: nonlinear Krogh-based model; dashed lines: explicit Krogh-based model; dotted lines: equal oxygen flux assumption. A: hemoglobin saturation profiles (thin lines: mean $S$); B: hemoglobin saturation difference between both capillary pairs.

Figure 4.14: Capillary diffusive interaction with different linear densities ($\mu_{LD} = 0.5$ and $0.3$). Solid lines: numerical model; dash-dotted lines: nonlinear Krogh-based model; dotted lines: equal oxygen flux assumption. A: hemoglobin saturation profiles (thin lines: mean $S$); B: hemoglobin saturation difference between both capillary groups.
4.3.3 Reconstructed microvascular networks

In the previous sections, the evolution of hemoglobin saturation heterogeneity in idealized geometries was investigated using the developed interaction models and our computational model for oxygen transport from capillaries to tissue. Diffusive interaction between RBCs and capillaries was consistently observed to reduce hemoglobin saturation heterogeneity that may be produced by CTH. We now investigate whether the previous findings still hold in MVNs reconstructed from the mouse cerebral cortex. Here, we focus on the RBC diffusive interaction within single capillaries.

The blood flow in both MVNs was reconstructed based on flow measurements as explained in Subsection 4.2.2. Fig. 4.15 shows the diameters and the RBC velocities obtained using the flow reconstruction algorithm. Since there were no available \( \text{Po}_2 \) measurements, we set \( S = 0.6 \) for all RBCs entering the computational domain. With this choice, hemoglobin saturation heterogeneity develops only inside the MVNs, among others due to heterogeneous transit times of RBCs. The simulations were run for 10 s to allow the system to reach a steady state. In the following, results are reported only for vessels that were traversed by at least 100 RBCs.

The vessels in both MVNs can be split in two categories based on the hemoglobin saturation values at the vessel entrance. The first one consists of vessels that are downstream of a converging capillary bifurcation and may have a comparatively high hemoglobin saturation heterogeneity at the inlet. The second category is composed of vessels with low initial hemoglobin saturation heterogeneity such as occur at the inlet boundary or in the absence of upstream converging bifurcations in the employed MVNs. Fig. 4.16 shows hemoglobin saturation profiles in four selected capillaries in MVN 1 that follow a converging bifurcation. In these vessels, the hemoglobin saturation heterogeneity \( \sigma_S \) significantly drops, similarly to the idealized scenario presented in Fig. 4.4. In three of these four capillaries (panels A, B, and D in Fig. 4.16), many RBCs with low initial hemoglobin saturation almost do not release any oxygen, or even undergo an increase in \( S \). In Fig. 4.16A, the increase in \( S \) at the distal end of the capillary is caused by a converging bifurcation with a capillary with more highly saturated RBCs. The fact that RBCs from different vessels may overlap near bifurcations is likely contributing to this effect. However, these selected vessels display an evolution of hemoglobin saturation which is very similar to that observed in Fig. 4.4A and Fig. 4.5A.

Microvessels with very low initial hemoglobin saturation heterogeneity
display a different behavior. As shown in Fig. 4.17, the standard deviation $\sigma_S$ increases in these vessels. This phenomenon, which was not observed in the idealized setups presented above, can be explained by the rapid fluctuations of hematocrit that occur both in the employed discrete RBC simulations and in experiments [11]. The drop in hemoglobin saturation in individual RBCs was correlated to the time interval to the previous RBC that passed through the vessel. In the three vessels, the standard deviation of $S$ was increasing along the vessel, albeit at different rates. A significant positive correlation was found in the first two selected vessels (Fig. 4.17B, D), with the Pearson’s coefficient of determination as high as 0.949 in one case. In one of the selected vessels (Fig. 4.17F), the correlation was close to zero when using all RBCs. However, restriction to RBCs with a time difference to the previous RBC $\leq$ 20 ms increased the coefficient of determination to 0.447. This indicates that the observed correlation between hemoglobin saturation drop and time difference between RBCs mainly occurs at RBC flow values $\geq$ 50 cells/s. Additionally, the correlation was found to be higher
Figure 4.16: Profiles of hemoglobin saturation in selected vessels downstream of a converging bifurcation. Each vessel was selected in MVN 1. Thin green lines: individual RBCs; thick black line: mean hemoglobin saturation $\bar{S}$; thick black lines: $\bar{S} \pm \sigma_S$.

The observed correlation for RBCs with small time differences can be explained by transient oscillations in tissue $P_{O_2}$ caused by linear density fluctuations. For high spacings between RBCs, tissue $P_{O_2}$ undergoes a slight transient drop which causes more oxygen to be extracted from the next RBC. Conversely, when two RBCs are close to each other, the second RBC is surrounded by tissue with elevated $P_{O_2}$ and releases less oxygen due to the lower $P_{O_2}$ gradient. Unlike our previous findings, these results suggest a mechanism that causes the hemoglobin saturation heterogeneity within single vessels to slightly increase.

The microvessels that were selected so far exhibited opposing evolutions of hemoglobin saturation heterogeneity, namely a reduction due to RBC diffusive interaction and an increase caused by hematocrit fluctuations. This raises the question whether one of these effects dominates the other. Fig. 4.18 provides an overview of the evolution of $\sigma_S$ in all vessels in both MVNs that were traversed by more than 100 RBCs during the 10-s simulation time. Fig. 4.18A displays the rate of change of $\sigma_S$ per 100-µm
Figure 4.17: Profiles of hemoglobin saturation in three selected inlet vessels in MVN 1. A, C, E: see legend of Fig. 4.16. B, D, F: drop in hemoglobin saturation for individual RBCs as a function of the time difference to the previous RBC that passed through the vessel; solid line: linear fit. In panel F, the linear fit was only based on RBCs with a time difference \( \leq 20 \text{ ms} \) (see main text).
Figure 4.18: Evolution of the standard deviation of hemoglobin saturation $\sigma_S$ in both reconstructed MVNs. A: Rate of change of $\sigma_S$ as a function of the inlet value of $\sigma_S$ and the vessel type. Each symbol corresponds to a vessel traversed by at least 100 RBCs. Dark blue symbols: MVN 1; purple symbols: MVN 2. Diamonds: inlet vessels; triangles: vessels after a converging bifurcation; crosses: remaining vessels. B: explanatory sketch for the symbols used in panel A. The symbols show the three different vessel types. The bars illustrate the typical evolution of hemoglobin saturation along a vessel located downstream of a converging bifurcation (mean ± SD). Green lines: representative profiles of hemoglobin saturation in individual RBCs.

length in each retained vessel. Most vessels with $\sigma_{S,a} \leq 0.05$ undergo a slight increase in $\sigma_S$. However, the standard deviation of hemoglobin saturation decreases in all vessels with $\sigma_{S,a} \geq 0.1$, many of which directly follow a converging bifurcation. Additionally, the rate of change of $\sigma_S$ along microvessels decreases with $\sigma_{S,a}$. This relation is a direct reflection of Eq. (4.8) which states that $d\sigma_S/dx$ is negative and proportional to $\sigma_S$. Therefore, although rapid fluctuations in hematocrit cause a non-negligible increase in hemoglobin saturation heterogeneity in some microvessels, the large heterogeneities are damped at a faster rate due to RBC diffusive interaction. This shows that the model predictions for RBC interaction also hold in realistic MVNs.

4.4 Discussion

We identified two diffusive interaction mechanisms that cause a large reduction of hemoglobin saturation heterogeneity in capillary networks, developed associated interaction models and validated them using a detailed
4.4. Discussion

computational model with individual moving RBCs. Additional oxygen transport simulations in reconstructed MVNs showed that diffusive interaction between RBCs significantly decreases the heterogeneity of blood oxygen content in realistic topologies. Our analysis also provides analytical and fitted estimates of the length and time scales that underpin the reduction of hemoglobin saturation heterogeneity. This work shows that CTH should be considered as a proxy for the actual heterogeneity of blood oxygen content and that estimating hemoglobin saturation heterogeneity only based on CTH may lead to considerable overestimations.

Diffusive interaction between RBCs in a single capillary occurs when two branches with different hemoglobin saturation levels converge. This phenomenon is therefore more prevalent in the presence of multiple converging bifurcations along RBC paths. In the mouse cerebral cortex, Sakadžić et al. [104] estimated the number of capillary branches between arterioles and venules to be $5.9 \pm 2.1$, with mean segment lengths between 65.6 and 81.4 µm. Since cortical capillary beds have a mesh-like structure [69], each RBC will on average travel through several converging bifurcations. According to the interaction models developed here and the numerical simulations, the standard deviation of hemoglobin saturation decays exponentially (Eq. (4.8)) with a time scale between 0.15 and 0.21 s (Fig. 4.7). This is considerably lower than the mean capillary transit times measured in Gutiérrez-Jiménez et al. [45] using bolus tracking (0.81 ± 0.27 s at baseline, 0.69 ± 0.18 s during activation). These obtained time scales are also smaller than the transit times computed in Schmid et al. [109] in five analysis layers at different depths in the mouse parietal cortex (0.19 to 0.79 s). Therefore, in the presence of converging bifurcations, this analysis indicates that RBCs spend sufficient time in capillary branches for the hemoglobin saturation heterogeneity to significantly drop.

The oxygen transport simulations in reconstructed MVNs showed that rapid hematocrit fluctuations can cause the standard deviation of hemoglobin saturation to slightly increase along vessels with very low initial $\sigma_S$ (Fig. 4.17). The relation of the time difference between consecutive RBCs and the hemoglobin saturation drop is suggested to be stronger for vessels with high RBC flow. However, the magnitude of the increase in $\sigma_S$ in the concerned vessels varied considerably. The reasons for this variation still need to be elucidated. Our simulation results lead to the hypothesis that most capillaries display a small degree of heterogeneity in hemoglobin saturation. Thus, setting the standard deviation $\sigma_{S,a}$ to zero in inlet capillaries could be considered as a model artefact that artificially increases the number of vessels with low $\sigma_{S,a}$ (Fig. 4.18). However, despite these fluctuations, the heterogeneity of hemoglobin saturation in individual cap-
illaries was found to decrease in all vessels with a medium-to-large initial value of $\sigma_S$. This leads to the conclusion that the reduction of hemoglobin saturation heterogeneity caused by diffusive interaction is stronger than its increase due to rapid hematocrit fluctuations.

While the standard deviation of hemoglobin saturation in a single capillary generally decreases, our results show that the average value of $S$ in a single capillary is not affected by fluctuations of $S$. Since the $P_o_2$ oscillations caused by the individual erythrocytes do not spread far into the tissue (see values of $\Delta r_{osc}$ in Fig. 4.8B), tissue oxygenation is likely not adversely affected by the fluctuations in hemoglobin saturation observed here. This provides a justification for the oxygen transport models based on continuum approach for $S$ if the hemoglobin saturation downstream of a converging bifurcation is set to the RBC flow-weighted average of $S$ in the upstream branches. Nevertheless, we postulate that the homogenization of $S$ in individual vessels is beneficial for oxygen transport since it reduces the probability of RBCs with very low saturation. Indeed, hypoxia as well as large tissue $P_o_2$ fluctuations are most likely to occur near vessels with low RBC flow. The homogenization of hemoglobin saturation makes it less probable that RBCs with low oxygen content enter such vessels.

Diffusive interaction between capillaries is the second reduction mechanism of hemoglobin saturation heterogeneity that was investigated here. While RBC diffusive interaction primarily occurs downstream of converging bifurcations, capillary diffusive interaction is a more general phenomenon since it does not require the presence of branchings. This is for instance relevant in skeletal muscles where capillary networks have fewer branchings than in the cerebral cortex (Fig. 1 in Fraser et al. [31]). Our results qualitatively agree with the computations by Popel et al. [95] in parallel capillary arrays with heterogeneous inlet $P_o_2$ and erythrocyte velocities. They used tissue units with different number of capillaries to model the interaction between capillary domains and found that the coefficient of variation of tissue $P_o_2$ and end-capillary $P_o_2$ decreases with the number of interacting capillaries per tissue unit. Salathe [107] performed similar computations in a $5 \times 5$ capillary array and reported that modeling interaction between functional units smooths out the oxygen concentration differences between capillaries and delays the onset of anoxia. Our results confirm the trends observed in these studies and shed further light on the physiological parameters involved in capillary diffusive interaction.

The range of distances between capillaries (20 to 60 $\mu$m) that was examined in our simulations in parallel capillary arrays corresponds to Krogh cylinder radii between 11.3 and 33.8 $\mu$m. This includes the mean Krogh radii of the reconstructed MVNs in Fraser et al. [31] (21.3 ± 2.1 $\mu$m to
25.6 ± 3.9 μm) and in Sakadžić et al. [104] (22.3 ± 1.2 μm to 24.2 ± 2.2 μm) which were obtained by approximating the tissue volume closest to each capillary segment by a cylinder. In our simulations, although the intercapillary distance was tripled, the decay time of the hemoglobin saturation difference between parallel capillaries only increased from 0.115 to 0.188 s. This weak dependence on capillary spacing is explained by the formulas for the decay time scale $\tau_{CI}$ (Eq. (4.23)) and the model coefficient $K_{CI}$ (Eq. (4.18)) which only depend on the logarithm of the ratio between the mean Krogh radius and the capillary endothelium radius. These time scales values are lower than the decay time scale $\tau_{RI}$ for RBC diffusive interaction and also significantly smaller than the capillary transit times reported above. Additionally, our theoretical analysis showed that the hemoglobin saturation difference between parallel capillaries decays exponentially (Eq. (4.21)). This provides compelling evidence that diffusive interaction between capillaries is a strong mechanism for the reduction of hemoglobin saturation heterogeneity at the scale of neighboring capillaries. Its occurrence regardless of the presence of converging bifurcations suggests that this is a more general phenomenon than RBC diffusive interaction. Finally, unlike the latter mechanism, capillary diffusive interaction strongly influences the mean hemoglobin saturation drop along microvessels and thus affects more significantly tissue oxygenation.

After having shown the importance of diffusive interaction mechanisms, it is natural to ask up to which length scale they can act. While RBC interaction is confined to single capillaries, hence very local, it is not evident how far reaching capillary interaction can be. The weak dependence of the decay time scale $\tau_{CI}$ on capillary spacing (Eq. (4.23) and Fig. 4.11C) suggests that this oxygen transfer mechanism can be relevant for capillary distances above 50 μm, which is higher than typical inter-capillary spacings in the cerebral cortex [132] or muscles [24]. To determine the maximal length scale of capillary diffusive interaction, it will be necessary to understand how capillaries with irregular spacings, as modeled in Hoofd et al. [54], influence each other’s supplied tissue regions. We propose the concept of diffusive interaction length scale as a tool to compare CTH and COSH. Our results provide strong evidence that hemoglobin saturation heterogeneity on the scale of the inter-capillary distance is efficiently damped by diffusive interaction. Whether this still holds for medium or large-scale hemoglobin saturation heterogeneity is an open question. Its answer is essential to assess the consequences of disturbed capillary flow patterns on oxygen transport based on their spatial scale.

The models for RBC and capillary diffusive interaction enable the computation of mean hemoglobin saturation and its heterogeneity in single and
parallel vessels, respectively. Previously, the relation between CTH and oxygen extraction fraction was first studied by Jespersen et al. [58] using the BKCR equation (Eq. (4.2)) under the assumption of a given constant tissue Po2. This model was extended by Angleys et al. [2] to include the computation of tissue Po2 based on Michaelis-Menten kinetics for oxygen consumption. This approach uses a calibrated constant \( k \) and does not account for the heterogeneity of hematocrit, vessel size and spacing which occurs in capillary networks. Our approach takes into account each of these parameters and shows that distal hemoglobin saturation is not only a function of the transit time (Eq. (4.1)). In particular, it includes the influence of hematocrit which was shown to have a paramount influence of tissue Po2 [70]. Another major advance in this work is the modeling of interaction between capillaries through the presence of converging bifurcations and diffusive oxygen transfer. Instead of dealing with idealized distributions of capillaries with independent supplied tissue regions, the models developed here lay the ground for a refined analysis of hemoglobin saturation heterogeneity in realistic MVNs. This is an essential step to assess the actual consequences of CTH on oxygen transport and availability in the microcirculation.

The limitations to our diffusive interaction models include the oxygen-independent metabolic consumption term \( M_0 \), which provides an analytical solution to the radial oxygen transport equation in the tissue. At low tissue Po2 values, the Michaelis-Menten kinetics model \( M(P) = M_0 P/(P + P_{\text{crit}}) \), where \( P_{\text{crit}} \) is the Po2 at half consumption) may yield slightly different Po2 radial profiles in the tissue. This can affect the actual supplied tissue cylinder radii in capillary diffusive interaction. Therefore, the influence of the consumption term should be assessed using the computational model. Likewise, shifts of the oxygen-hemoglobin dissociation curve caused by variations in \( CO_2 \), \( pH \) and temperature (Bohr effect) were not modeled. Since the derived equations for the evolution of hemoglobin saturation heterogeneity (Eqs. (4.8) and (4.21)) and the characteristic scales \( L_{CI} \) and \( \tau_{CI} \) (Eqs. (4.22) and (4.23)) explicitly depend on the derivative of the equilibrium curve \( P_{eq}(S) \), such shifts may have a significant influence on the reduction mechanisms of hemoglobin saturation heterogeneity. Additionally, the large inter-species variations in the dissociation curve [111] may lead to different rates of hemoglobin saturation heterogeneity reduction across species.

Both the interaction and computational models are currently limited to capillaries. This restricts our analysis to capillaries and limits the size of reconstructed MVNs that can be used, since larger vessels are not supported by our computational model. Distances between pairs of nearest arterioles
or venules are typically between 100 and 200 µm (Fig 5c in Blinder et al. [9]) with a mean ± SD of 130 ± 60 µm for arterioles [79]. Therefore, oxygen transport simulations in reconstructed MVNs with domain size > 200 µm generally require the ability to model vessels larger than capillaries. The presence of arterioles and venules would enable full RBC paths through capillaries to be simulated. Thus, actual comparisons between CTH and COSH could be performed, which will shed further light on the reduction of hemoglobin saturation heterogeneity. Additionally, this will enlarge the tissue volume which is not directly affected by the boundary condition for oxygen at the tissue domain boundary, and thus yield more robust results.

Future work will include a more elaborate analysis of the tissue regions supplied by each microvessel. As done by Sakadžić et al. [104] based on PO$_2$ measurements, a mean Krogh radius based on the tissue volume nearest to each vessel can be compared to the radius of the tissue region actually supplied by each vessel. This is a necessary step to evaluate our models for capillary diffusive interaction in a realistic setting and will provide insights to generalize models to arbitrary network topologies. These simulations could be performed with different models without moving RBCs, such as [115, 34]. However, the sensitivity of the results to the grid resolution that was observed shows that the intravascular drop in oxygen tension needs to be correctly captured. Therefore, if applied to this problem, the models [115, 34] should employ accurate IVR coefficients that are dependent on the vessel radius and hematocrit, as computed in Lücker et al. [70]. To further bridge the gap between theory and experiments, combined RBC flow and PO$_2$ measurements as in Parpaleix et al. [86] and [104] could be used to reconstruct a realistic blood flow using the algorithm described in Subsection 4.2.2 and determine the hemoglobin saturation values at the vessel inlets to match the measurements. Such measurements would enable a high-resolution reconstruction of the PO$_2$ field directly based on data and open possibilities for model validation. Finally, model predictions about the relation between CTH and hemoglobin saturation heterogeneity could be assessed by combined measurements of CTH (as in Gutiérrez-Jiménez et al. [45]) and PO$_2$ measurements at the distal end of the microvasculature. Recent improvements in oxygen-sensitive two-photon phosphorescent probes [101] enable faster in vivo PO$_2$ measurements, which increases the feasibility of such an experiment.
4.5 Conclusion

This study lays the theoretical basis for the analysis of hemoglobin saturation heterogeneity in MVNs. It is a substantial improvement over previous approaches that are currently limited to independent, identical capillaries without branchings. Models for RBC and capillary diffusive interaction were been developed and successfully validated using a detailed computational model in both idealized geometries and realistic MVNs. The following conclusions can be drawn: (1) diffusive interaction leads to a strong reduction of small-scale hemoglobin saturation heterogeneity caused by CTH or other factors; (2) hemoglobin saturation heterogeneity can arise in the absence of CTH, for instance due to differences in hematocrit or supplied tissue volume, and to a lesser degree because of fast hematocrit fluctuations; (3) CTH influences COSH, but does not determine it. Thus, this modeling work is a major step to better understand the actual effects of CTH on blood oxygen content. This has potential implications in the study of all conditions where capillary dysfunction and CTH are thought to be involved, such as Alzheimer’s disease [80], stroke [82], traumatic brain injury [81] and ischemic heart disease [84].
5.1 Introduction

The modeling of oxygen transport in the microcirculation requires experimental data for parameter values, boundary conditions and result comparison. Due to the high intracapillary oxygen gradients associated with EATs, the employed measurement technique should achieve micrometric resolution to allow the validation of the intravascular oxygen distribution predicted by models. Currently, the most suitable method for noninvasive high-resolution oxygen measurements in depth is two-photon phosphorescence lifetime microscopy (2PLM) [28, 105, 86, 104, 72]. While this technique has dramatically improved the ability to measure oxygen partial pressure in vivo, a thorough uncertainty quantification in 2PLM oxygen measurements has yet to be done. While it is known that local oxygen depletion can affect experimental results, there is a lack of quantitative understanding of these processes. Using numerical methods similar to those in Chapter 2 we investigated certain potential limitations of 2PLM with a computational model. The main improvements over previous approaches are the presence of oxygen diffusion and singlet oxygen removal by organic molecules. The results presented here aim to help experimenters to choose laser parameters and probe concentration in such a way that local oxygen depletion does not significantly affect measured Po2 values.

Several experimental methods for in vivo oxygen tension measurements with a resolution on the order of the capillary diameter are available. Before presenting phosphorescence quenching microscopy, we give a short review
116 Modeling O\textsubscript{2} measurements with 2PLM

of alternative techniques. Polarographic microelectrodes generate a current which is proportional to the oxygen concentration around the electrode tip \cite{10}. Recessed tip microelectrodes, also called Whalen electrodes \cite{143}, can have a diameter smaller than 2 \textmu m. Although their spatial and temporal resolution is very high, they are invasive and make synchronous measurements at different locations complicated to set up. Micro-spectrophotometry is based on the different light absorption spectra of oxy- and deoxyhemoglobin. Oxygen saturation is evaluated by measuring the optical density of hemoglobin at two or three different wavelengths. Oxygen tension can then be estimated using the oxygen saturation curve which depends on carbon dioxide, pH and temperature. These dependences introduce uncertainty in the resulting PO\textsubscript{2} values. Micro-spectrophotometry requires transillumination of the region of interest and was applied to muscles that can be extended on the microscope stage such as the hamster cheek pouch retractor \cite{24}. As this technique is usually limited to thin sections \cite{127}, it cannot be applied to organs such as the brain. Both techniques are briefly reviewed in Refs. \cite{131,90}. Recently, two alternate techniques were introduced, as reviewed by Gagnon et al. \cite{32}. High-speed photoacoustic microscopy \cite{145} can simultaneously acquire the microvasculature, blood flow and the concentration of oxy- and deoxyhemoglobin in depth. Large volumes can be scanned with a frame rate of 1 Hz and no exogenous contrast agent is needed. However, its resolution and imaging quality at higher depth are currently inferior to those of 2PLM. Visible light spectroscopic optical coherence tomography can also provide fast measurements of blood oxygen content \cite{15}, but its current spatial resolution is one order of magnitude lower than that of 2PLM.

Phosphorescence quenching microscopy, introduced by Wilson and co-workers \cite{103}, relies on the quenching of phosphorescent dyes such as metalloporphyrins by oxygen and enables the measurement of oxygen distribution in the vasculature and intact tissue. However, since excited phosphorescent dyes produce singlet oxygen that may react with organic molecules, there is a risk of photodamage and oxygen depletion in the excited region. This can critically influence the measured PO\textsubscript{2} in tissue if the rate of singlet oxygen consumption exceeds that of replenishing by diffusion. These issues were alleviated by the development of two-photon-excited oxygen sensors by Vino-gradov and coworkers \cite{28}. Since the probability of a two-photon excitation event is proportional to the squared photon flux, significant excitation of the phosphorescent probe occurs only near the microscope focal point. These molecules enabled the first noninvasive, micrometric-resolution in vivo measurements of oxygen tension in depth. In particular, 2PLM provides an unprecedented resolution in the microscope axial direction.
5.1. Introduction

The experimental method is now briefly described. For more details on two-photon microscopy and the related oxygen sensors, the reader is referred to Refs. [123, 28]. The technique is based on the oxygen-dependent quenching of a phosphorescent dye. The phosphorescence decay times at oxygen concentration \([O_2]\) and zero oxygen are denoted by \(\tau\) and \(\tau_0\), respectively. Under the assumption that no variation in oxygen concentration occurs in the process, the Stern-Vollmer equation

\[
\frac{1}{\tau} = \frac{1}{\tau_0} + k_q[O_2]
\] (5.1)

can be derived, where \(k_q\) is the quenching rate by oxygen. 2PLM uses cycles with an on-phase followed by an off-phase, also called collection period. During the on-phase, the phosphorescent molecules are excited by pulse trains produced by a laser (typically a Ti:Sapphire laser with near-infrared wavelength); during the off-phase, the laser beam is deflected by an acousto-optic or electro-optic modulator and the photons produced by the phosphorescent dye are collected. The focal point can be rapidly moved within the focal plane using a galvanometric scanner (Fig. 5.1). The collected photons traverse the microscope lens and are reflected by a dichroic beam splitter. Green and red channels are split using a further dichroic beam splitter and filtered, until the photons reach a photomultiplier tube or an avalanche photodiode that records the signal. If fluorescent dyes are simultaneously used, their emission is detected in the green channel; the phosphorescence signal is detected in the red channel. Finally, the number of photons averaged over a given number of collection periods is fitted with a single exponential, which yields the decay time. The corresponding \(P_{O_2}\) value is then obtained from a given oxygen calibration curve.

The main developments of 2PLM are now briefly reviewed (see Ref. [32] for a longer review). Phosphorescence lifetime microscopy was introduced by Wilson and coworkers [103]. In their seminal study, Vanzetta et al. [137] measured \(P_{O_2}\) changes during sensory stimulation using phosphorescence lifetime changes under one-photon excitation. The first two-photon-excited phosphorescent nanoprobe developed by Finikova et al. [28], PtP-C343, was used to provide high-resolution \(P_{O_2}\) maps in the rodent cerebral cortex [105]. Simultaneous measurements of capillary blood flow and oxygen tension were performed by Lecoq et al. [65] and provided evidence for the presence of EATs. The relation between EAT amplitude and blood flow properties at rest and during sensory stimulation was then investigated by Parpaleix et al. [86]. Oxygen delivery from arterioles and capillaries was computed by Sakadžić et al. [104] from estimations of hemoglobin saturation based on
Figure 5.1: Optical path of the scan optics and detection path (inset). Galvano-
metric scanner (SCAN), scan lens (SL), tube lenses (TL1 and TL2), dichroic
beam splitter for camera path (DBS3), camera path mirror (CPM), objective
(OBJ). Inset: condenser lens (CL), dichroic beam-splitter for emission (DBS2),
and photomultiplier modules (PMM1 and PMM2). Image and caption adapted
from Mayrhofer et al. [75].

PO2 measurements. A new oxygen sensor, PtTCHP-C307, with improved
antenna-core energy transfer, phosphorescence quantum yield and oxygen
sensitivity was introduced by Roussakis et al. [101] and its performance was
illustrated in the bone marrow. More recently, an oxygen nanoprobe with
improved two-photon absorption cross section (PtG2p) was developed by
Vinogradov and coworkers. Transient PO2 changes upon nitrogen inhala-
tion were recently measured with it and its improved temporal resolution
was compared to that of PtP-C343 by the group of B. Weber (private com-
munication).

Although the high resolution of 2PLM greatly diminishes the risks asso-
ciated to photodamage by reactive oxygen species, local depletion of triplet
oxygen in the excitation volume can lead to a decrease in phosphorescence
quenching and thus to longer phosphorescence lifetimes, compared to the
calibration curves measured under ideal conditions. This is most likely to
happen when a larger number of probe molecules are excited, which can
occur when the magnification, the laser power or the probe concentration
are high. Permanent oxygen removal by reactions between singlet oxygen
and organic compounds, such as albumin in plasma, can increase this effect.
Our goal is to provide quantitative insight on the influence of local oxygen depletion on measured phosphorescence lifetimes. This will help scientists to choose experimental parameters in 2PLM in such a way that the provided calibration curve can be employed with a minimal risk of systematic bias.

5.2 Methods

5.2.1 Mathematical model

The spatio-temporal evolution of the excited phosphorescent nanoprobe and oxygen concentrations in an immobile medium during consecutive laser cycles will be simulated numerically. The model developed here extends that in Refs. [28, 122] where the oxygen concentration was assumed to be zero and diffusion was absent. The phosphorescent probe is only modeled in its ground state and triplet (i.e., excited) state. Ultrafast processes within the probe, such as Förster-type resonance energy transfer (FRET) and intersystem crossing, are not described. FRET is assumed to be efficient, i.e., no fluorescence is observed and each excited antenna transfers its energy to the phosphorescent core of the probe. Oxygen is modeled in both ground state (also referred to as triplet state) and singlet state. Singlet oxygen is a highly reactive species that can decay back to triplet oxygen or oxidize organic molecules. In the latter case, the singlet oxygen molecule is permanently removed from the model system. Oxygen diffusion is modeled using Fick’s law, while the diffusion of the phosphorescent probe is neglected due to its large molecular mass.

Figure 5.2: Chemical reaction model for oxygen measurements with 2PLM in the presence of organic molecules, denoted by “org”.

\[
P \xrightarrow{k_p} P^T \xrightarrow{k_q} \text{O}_1 \xrightarrow{k_d} \text{O}_2 \xrightarrow{k_{q,\text{org}}} \text{org} \xrightarrow{k_r} \text{org}
\]
The probe in ground state and triplet state is denoted by $P$ and $P^T$, respectively, and the symbol $O_2^1$ is used for singlet oxygen. An overview of the modeled chemical reactions is given by Fig. 5.2. Rate equations up to second order are used to describe the reactions. Quantities in brackets denote molar concentrations which will be expressed in $M = \text{mol L}^{-1}$. Let $k_p$ be the phosphorescence decay rate of the oxygen probe and $k_q$ the oxygen quenching rate in $\text{M}^{-1}\text{s}^{-1}$. The singlet oxygen produced by phosphorescence quenching decays to triplet oxygen with a rate $k_d$. In the presence of organic compounds, there is additional quenching of singlet oxygen with rate $k_{q,\text{org}}$ in $\text{M}^{-1}\text{s}^{-1}$ and irreversible removal of singlet oxygen with rate $k_r$. The concentration of organic molecules in the medium, $[A]$, is assumed to be constant. The diffusivity of oxygen is denoted by $D_{O_2}$ and the excitation rate of the probe by $\alpha$. The equations for the probe concentration in ground state and triplet state and the oxygen concentration in triplet and singlet state are given by

$$\frac{\partial [P]}{\partial t} = -\alpha [P] + (k_p + k_q [O_2]) [P^T], \quad (5.2)$$

$$\frac{\partial [P^T]}{\partial t} = \alpha [P] - (k_p + k_q [O_2]) [P^T], \quad (5.3)$$

$$\frac{\partial [O_2]}{\partial t} = D_{O_2} \nabla^2 [O_2] - k_q [O_2] [P^T] + (k_d + k_{q,\text{org}} [A]) [O_2^1], \quad (5.4)$$

$$\frac{\partial [O_2^1]}{\partial t} = D_{O_2} \nabla^2 [O_2^1] + k_q [O_2] [P^T] - (k_d + (k_{q,\text{org}} + k_r) [A]) [O_2^1]. \quad (5.5)$$

Since the total probe concentration $[P]_0 = [P] + [P^T]$ is constant, Eqs. (5.2) and (5.3) reduce to

$$\frac{\partial [P^T]}{\partial t} = \alpha ([P]_0 - [P^T]) - (k_p + k_q [O_2]) [P^T]. \quad (5.6)$$

A boundary condition at the domain boundary is required for $[O_2]$ and $[O_2^1]$. Here we assume that the excitation volume is surrounded by an immobile medium that can replenish the computational domain with triplet oxygen. To this end, we choose a computational domain which is assumed to be significantly larger than the excitation volume and set a Dirichlet boundary condition with $[O_2] = [O_2]_0$ and $[O_2^1] = 0$ at the domain boundary. The effect of the boundary condition will be discussed below. At time $t = 0$, we set $[P^T] = 0$, $[O_2] = [O_2]_0$ and $[O_2^1] = 0$.

The modeling of the excitation rate $\alpha$ is now addressed. The laser on-phase consists of a train of $N_p$ pulses with repetition rate $f$ and pulse length $\delta t$ that excite the probe molecules in ground state. The laser uses a fixed
wavelength $\lambda$ and its mean power is denoted by $P_m$. Under two-photon excitation, the phosphorescent probe is excited with a rate proportional to the square of the photon flux. More precisely, the excitation rate $\alpha$ is given by $\sigma_2 \Phi^2$, where $\sigma_2$ is the two-photon absorption cross section and $\Phi$ is the photon flux which is computed as follows. Let $c$ be the speed of light and $h$ Planck’s constant. The number of photons per pulse is

$$m = \frac{P_m \lambda}{hc f}. \quad (5.7)$$

To obtain the photon flux, the intensity profile of the focused laser beam is required. The numerical aperture of the objective is $NA = n_0 \sin(\theta)$, where $n_0$ is the refractive index of the immersion medium of the lens and $\theta$ is the half-angle of the light cone formed by the objective lens. We use the approximation for the illumination intensity profile $I(u, v)$ given by Sheppard et al. [120] as

$$I(u, v) = \left| 2 \int_0^1 J_0(v \rho) e^{-\frac{i}{2} u \rho^2} \rho \, d\rho \right|^2, \quad (5.8)$$

where $J_0$ is the zero-th order Bessel function, $u = 4k \sin^2(\theta/2) z$, $v = kr \sin(\theta)$ are the dimensionless axial and radial coordinates and $k = 2\pi/\lambda$ is the wave number. In the sequel, we normalize $I$ as

$$\bar{I}(z, r) = \frac{I(4k \sin^2(\theta/2) z, kr \sin(\theta))}{\int_0^\infty I(4k \sin^2(\theta/2) z, kr \sin(\theta))r \, dr}. \quad (5.9)$$

Then, the photon flux through an annular surface element with inner and outer radii $r_1$ and $r_2$, respectively, and parallel to the focal plane is given by

$$\Phi(r_1, r_2; z) = \frac{m}{(\delta t) \pi (r_2^2 - r_1^2)} \int_{r_1}^{r_2} \bar{I}(z, r) \, r \, dr. \quad (5.10)$$

Thus, the excitation rate at that surface element is given by

$$\alpha(r_1, r_2; z) = \sigma_2 \Phi(r_1, r_2; z)^2. \quad (5.11)$$

The number of photons that reach the collecting device can be estimated from the computed evolution of $[P^T]$. The collected phosphorescence signal per unit of time in a domain $V$ is given by

$$J(t) = \chi N_A \phi_p k_p \int_V [P^T](x, t) \, dV, \quad (5.12)$$

where $\chi$ is the collection efficiency, $N_A$ the Avogadro number and $\phi_p$ the phosphorescence quantum yield of the probe. The factor that multiplies the integral in Eq. (5.12) does not influence the simulated phosphorescence decays times. However, it affects the predicted number of acquired photons.
5.2.2 Numerical method

Eqs. (5.4) through (5.6) are solved using the finite-difference method on a Cartesian grid in cylinder coordinates. The coordinate $r$ is used for the focal plane and $z$ for the microscope $z$-axis. A non-equidistant grid with radius $r_{\text{max}}$ and half-height $z_{\text{max}}$ is employed. The number of grid cells in the respective directions is denoted by $N_r$ and $N_z$. Each grid cell is associated to a pair of indices $(I, J)$, where $I$ and $J$ are the cell indices in $r$-direction and $z$-direction, respectively. Lexicographic order is used to build the matrix, i.e., grid cells have linear indices $k = I + (J - 1)N_r$.

We now describe the spatial discretization of the Laplace operator which is used in the diffusion term for ground state and singlet oxygen. The notation for the grid cell coordinates and spacings is explained by Fig. 5.3. For a grid cell with index $k$ in the domain interior, the discretized Laplacian coefficients in cylinder coordinates are given by

\begin{align*}
A_{k,k} & = \frac{1}{r\Delta r} \left( \frac{r_E}{\Delta r_E} + \frac{r_W}{\Delta r_W} \right) - \frac{1}{\Delta z} \left( \frac{1}{\Delta z_N} + \frac{1}{\Delta z_S} \right), \\
A_{k,kE} & = \frac{1}{r\Delta r} \left( \frac{r_E}{\Delta r_E} \right), \\
A_{k,kW} & = \frac{1}{r\Delta r} \left( \frac{r_W}{\Delta r_W} \right), \\
A_{k,kN} & = \frac{1}{z\Delta z_N}, \\
A_{k,kS} & = \frac{1}{z\Delta z_S}.
\end{align*}

For boundary cells, the terms for the diffusive flux at the domain boundary are removed. The treatment of the boundary condition is explained below. The remaining non-zero matrix entries are obtained using the symmetry of $A$.

For oxygen in triplet and singlet state, we use a Dirichlet boundary condition at the domain boundary. For a prescribed boundary value $a$ and a grid cell $k$ at the boundary with $r = r_{\text{max}}$, the matrix and load vector entries are set to

\begin{align*}
A_{k,k} & = A_{0,k,k} - 2 \frac{r_E}{r\Delta r^2}, \\
q_k & = 2a \frac{r_E}{r\Delta r^2},
\end{align*}

where $A_{0,k,k}$ is the matrix coefficient without contribution of the boundary condition. For a cell $k$ at the boundary $z = z_{\text{max}}$, the respective entries are
5.2. Methods

Figure 5.3: Notation for the grid cell coordinates and spacings

given by

\[ A_{k,k} = A_{0,k,k} - \frac{2}{\Delta z^2}, \quad (5.20) \]
\[ q_k = \frac{2a}{\Delta z^2}. \quad (5.21) \]

The cells at the domain corners contain both contributions and the remaining values of \( q \) are zero.

To compute the excitation rate, the normalized intensity profile \( \bar{I} \) (Eq. (5.9)) is evaluated at cell corners using MATLAB’s function \texttt{integral} described in Shampine [119]. For a grid cell \( k \) with center \((r_c, z_c)\) and spacings \( \Delta r \) and \( \Delta z \), the integral in Eq. (5.10) is approximated by

\[
\int_{r_c - \Delta r/2}^{r_c + \Delta r/2} \bar{I}(z_c, r) r \, dr \simeq \frac{\Delta r}{4} \left( \frac{r_c + \Delta r}{2} \right) \bar{I} \left( z_c + \frac{\Delta z}{2}, r_c + \frac{\Delta r}{2} \right) \\
+ \left( \frac{r_c + \Delta r}{2} \right) \bar{I} \left( z_c - \frac{\Delta z}{2}, r_c + \frac{\Delta r}{2} \right) \\
+ \left( \frac{r_c - \Delta r}{2} \right) \bar{I} \left( z_c + \frac{\Delta z}{2}, r_c - \frac{\Delta r}{2} \right) \\
+ \left( \frac{r_c - \Delta r}{2} \right) \bar{I} \left( z_c - \frac{\Delta z}{2}, r_c - \frac{\Delta r}{2} \right). \\
(5.22)
\]

This enables the computation of the photon flux through the cell \( k \) as

\[
\Phi_k = \Phi \left( r_c - \frac{\Delta r}{2}, r_c + \frac{\Delta r}{2}; z_c \right) \quad (5.23)
\]
using Eqs. (5.10) and (5.22). Finally, the excitation rate $\alpha$ in cell $k$ is given by $\alpha_k = \sigma_2 \Phi_k^2$.

We now describe the time integration of the governing equations. For the values given in Table 5.1, the summed pulse length $N_p \delta t$ is approximately $10^{-10}$ s. Based on the values of $D_{O_2}$, $k_p$, and $k_q$ in Tables 5.1 and 5.2, both radiative decay and quenching of the probe can be neglected during excitation pulses. Therefore, only the term with $\alpha$ on the right-hand side of Eq. (5.6) is used during the laser pulses. The resulting ordinary differential equation is solved analytically from $t$ to $t + \delta t$ by

$$[P^T](t + \delta t) = [P]_0 - ([P]_0 - [P^T](t))e^{-\alpha \delta t}. \quad (5.24)$$

After each laser pulse, Eqs. (5.4) through (5.6) are integrated over a time interval $1/f$ with $\alpha = 0$. Subsequently, these equations are integrated during the collection period with $\alpha = 0$. The three spatially discretized partial differential equations can be considered as a system of ordinary differential equations with $3N_r N_z$ unknowns which is integrated using MATLAB’s fourth-order differential equation integrator ode45 with a relative tolerance of $10^{-6}$ and an absolute tolerance of $10^{-10}$.

In the algorithm described above, the coupled system of ordinary differential equations needs to be successively integrated after each of the $N_p$ laser pulses. With the parameters used below, a significant amount of computational time is spent on this operation, so this part of the algorithm was optimized as follows. Instead of computing separately each laser pulse, a consecutive number $n_{opt}$ of pulses is aggregated and Eq. (5.24) is integrated from $t$ to $t + n_{opt} \delta t$. Then, the equation system (5.4) – (5.6) is solved over a time interval $n_{opt}/f$. For each on-phase, the operation is repeated for $N_p/n_{opt}$ aggregated pulses. In all computations, we used $n_{opt} = 100$ and found that the computational time was reduced almost by the same factor, while producing an error in the increase in $\tau$ of only 0.22% with the simulation parameters used below.

Finally, the decay times were obtained by fitting the collected intensity (Eq. (5.12)) during the off-phase with a single exponential function using least squares and the Levenberg-Marquardt algorithm. The complete method is shown in Algorithm 3.

5.2.3 Model parameters

Laser properties such as the duration of the pulse train and the collection period are parameters that can be set by the experimenter. The values shown
5.2. Methods

Algorithm 3 Simulation of consecutive laser cycles in 2PLM

1: construct the grid, assemble the matrix $A$ and the load vector $q$.
2: $[O_2] \leftarrow [O_2]_0$, $[O_2^+] \leftarrow 0$, $[P^+] \leftarrow 0$
3: compute $\Phi$ and $\alpha$ in each grid cell (Eq. (5.23)).
4: for all cycles do
5: for all aggregated laser pulses (1 to $N_p/n_{opt}$) do
6: integrate Eq. (5.6) using Eq. (5.24) over $n_{opt} \delta t$ s.
7: integrate Eqs. (5.4)–(5.6) with $\alpha = 0$ over $n_{opt}/f$ s.
8: end for
9: integrate Eqs. (5.4)–(5.6) with $\alpha = 0$ over the collection period.
10: integrate the phosphorescence signal at each integration step (Eq. (5.12)).
11: fit the phosphorescence signal with a single exponential function.
12: end for

in Table 5.1 are typical for in vivo 2PLM experiments. If the laser wavelength is tunable, it is chosen to maximize the two-photon absorption cross section $\sigma_2$. For the latest nanoprobe PtG2p, the maximum of $\sigma_2$ is reached at 950 nm approximately, hence this wavelength will be used in all computations. The physical properties of the nanoprobes (Table 5.2) were obtained during titration experiments performed by the group of S. Vinogradov at the University of Pennsylvania. The values used here were acquired at body temperature (generally 36.5°C).

The reaction rates of singlet oxygen with organic molecules need to be set. As there are only sparse literature data, the parameters $k_r$ and $k_{q,org}$ are the most uncertain in the model. The overall consumption rate of singlet oxygen by organic compounds $k_{rem} = k_r + k_{q,org}$ was measured by Pimenta et al. [89] in the serum albumin of different species. The values $k_{rem} = 0.40 \times 10^9$, $2.6 \times 10^8$ and $4.1 \times 10^8$ M$^{-1}$ s$^{-1}$ were reported in human, bovine and rat serum albumin, respectively. Since experiments with rodents are our primary source of in vivo data, the last value was chosen. To determine the rate constants $k_r$ and $k_{q,org}$, the ratio $k_r/k_{rem} = 0.16$ measured during photooxidation of bilirubin was used [30]. The protein concentration in the plasma was set to 42.5 g L$^{-1}$, based on the reference category in Goldwasser et al. [39], and the albumin molecular mass to 66 000 Da. This yields a concentration of organic molecules of 644 µM. Due to the uncertainties in these estimations, a sensitivity study for the ratio $k_r/k_{rem}$ will be presented.

The computational domain needs to be large enough to contain the entire excitation volume, even at high saturation, and to minimize the effect of the boundary. Computations were performed in a cylindrical domain with
radius \( r_{\text{max}} = 16 \mu m \) and half-height \( z_{\text{max}} = 20 \mu m \). Due to symmetry with respect to the microscope focal plane, only the domain part with \( z \geq 0 \) was simulated. The influence of the domain size will be examined below. A non-equidistant Cartesian mesh was built as follows. Given a grading function \( g(x) \) and a reference spacing \( \Delta x_0 \), the grid spacings were successively defined by \( \Delta x_{I+1} = g(x_I + \Delta x_I/2) \Delta x_0 \). For both radial and axial coordinates, the grading function was given by

\[
g(x) = \begin{cases} 
1 & 0 \leq x \leq 3 \mu m, \\
1 + \frac{x-3\mu m}{2.5\mu m} & x > 3 \mu m.
\end{cases}
\] (5.25)

and the reference spacings were set to \( \Delta r_0 = \Delta z_0 = 0.1 \mu m \). At \( r = 8 \mu m \) and \( z = 8 \mu m \), the grid spacing was 0.3 \mu m. This produced a grid with 77 \times 82 grid cells, which is five times less than the equidistant grid with the same minimal grid spacing. The adequacy of the grid was tested in a convergence study. The increase in decay time with the nonequidistant grid described above was 0.12% different from that obtained with the corresponding equidistant grid. Similarly, quadrupling the grid resolution in both directions changed the increase in decay time by less than 0.38%. Therefore, the chosen grid provides sufficiently robust results.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Unit</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>( c )</td>
<td>( 3 \times 10^8 )</td>
<td>m s(^{-1} )</td>
<td>speed of light</td>
</tr>
<tr>
<td>( D_{O_2} )</td>
<td>( 2.1 \times 10^{-5} )</td>
<td>cm(^2) s(^{-1} )</td>
<td>plasma at 37°C [38]</td>
</tr>
<tr>
<td>( \delta t )</td>
<td>( 110 \times 10^{-15} )</td>
<td>s</td>
<td>pulse duration</td>
</tr>
<tr>
<td>( f )</td>
<td>( 80 \times 10^6 )</td>
<td>s(^{-1} )</td>
<td>pulse repetition rate</td>
</tr>
<tr>
<td>( h )</td>
<td>( 6.626 \times 10^{-34} )</td>
<td>J s</td>
<td>Planck’s constant</td>
</tr>
<tr>
<td>( k_d )</td>
<td>( 2.4 \times 10^5 )</td>
<td>s(^{-1} )</td>
<td>[144, Table 1]</td>
</tr>
<tr>
<td>( \lambda )</td>
<td>950</td>
<td>nm</td>
<td>laser wavelength</td>
</tr>
<tr>
<td>NA</td>
<td>0.95</td>
<td>-</td>
<td>numerical aperture</td>
</tr>
<tr>
<td>( N_p )</td>
<td>2000</td>
<td>-</td>
<td>pulses per excitation gate</td>
</tr>
</tbody>
</table>

Table 5.1: Physical properties of the medium and laser parameters

### 5.3 Results

The effect of singlet oxygen production and consumption on phosphorescence decay times was investigated with the reaction-diffusion model pre-
5.3. Results

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>PtP-C343</th>
<th>PtTCHP-C307</th>
<th>PtG2p</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_p$</td>
<td>s$^{-1}$</td>
<td>$2.17 \times 10^4$</td>
<td>$1.4 \times 10^4$</td>
<td>$2.44 \times 10^4$</td>
</tr>
<tr>
<td>$k_q$</td>
<td>mmHg$^{-1}$ s$^{-1}$</td>
<td>341</td>
<td>1200</td>
<td>532.6</td>
</tr>
<tr>
<td>$\sigma_2$</td>
<td>GM</td>
<td>65</td>
<td>100</td>
<td>320</td>
</tr>
<tr>
<td>$\phi_p$</td>
<td>–</td>
<td>0.10</td>
<td>0.20</td>
<td>0.18</td>
</tr>
</tbody>
</table>

Table 5.2: Phosphorescent nanoprobe characteristics. 1 GM = $10^{-50}$ cm$^4$ s $\text{photon}^{-1}$. The characteristics of PtP-C343 and PtG2p were given by Sergei Vinogradov (private communication). The data on PtTCHP-C307 were taken from Ref. [101].

Presented above for three oxygen-sensitive nanoprobes under two-photon excitation. The influence of probe concentration, laser power and collection period duration was quantified. Oxygen calibration curves were simulated with and without singlet oxygen removal by organic molecules. Due to the uncertainty in the rate of permanent singlet oxygen removal, this parameter was also varied. Finally, we show that diffusion has a paramount influence on the obtained decay times. Frequently, the simulated decay times will be expressed using the PO$_2$ value obtained from the Stern-Vollmer equation (Eq. (5.1)). This value will be referred to as apparent PO$_2$.

Unless stated otherwise, the following parameters were used. The initial partial pressure of ground state oxygen was set to 50 mmHg and the initial probe concentration to 50 µM. Although this value can be considered as high, it is smaller than the concentration of 200 µM used in Parpaleix et al. [86]. A mean laser power of 50 mW was employed. Simulation results were obtained using the nanoprobe PtG2p with singlet oxygen removal by organic molecules. The off-phase duration was set to 225 µs and the results were reported after 240 consecutive gates, so the total acquisition time was 0.06 s. The influence of these parameters is discussed below.

The spatial distribution of the excited probe and ground state oxygen is shown in Fig. 5.4. With the chosen laser power and probe concentration, the probe is saturated in a volume which has a half-width of 2.5 µm and a half-height of 5 µm approximately. This is much larger than the diffraction-limited excitation volume that has half-width and half-height of 0.6 µm and 2 µm with the employed laser parameters. The oscillations caused by the Bessel function in the formula for the illumination intensity (Eq. (5.8)) appear very clearly. The ground state oxygen concentration after the on-phase attains a minimum value of 30 mmHg in the excitation volume with the chosen set of parameters. During the off-phase, diffusion smooths the oxygen distribution but does not have sufficient time to replenish the ex-
citation volume. The nonlinear increase in collected intensity (bottom left panel) during the on-phase reflects the probe saturation. The inset shows the individual laser pulses as well as a slight decrease in signal intensity between pulses which is caused by radiative decay and quenching by oxygen. During the off-phase, the collected intensity decreases exponentially. In the parameter range investigated in this study, single exponential functions were consistently found to provide excellent fits to the simulated signal.

Figure 5.4: Top left: triplet probe concentration in µM after the on-phase; top right: triplet oxygen partial pressure in mmHg after the on-phase; bottom left: temporal profile of the collected intensity (inset: detailed profile during the on-phase); bottom right: triplet oxygen partial pressure in mmHg after the off-phase.

The influence of singlet oxygen removal on the oxygen calibration curves is shown in Fig. 5.5. For the three simulated probes, decay times were computed in a range of PO2 values with and without organic molecules. In the latter case, all generated singlet oxygen molecules decay back to triplet oxy-
gen with the rate $k_d$. Additionally, the simulated calibration curves were compared to measured calibration curves. In all cases, the simulated curves with organic compounds were shifted to the right, which indicates a significant oxygen depletion in the excitation volume. The red dotted lines show the $\text{PO}_2$ difference between calibration curves simulated with and without organic molecules. This deviation is the potential underestimation of $\text{PO}_2$ when a calibration curve obtained without singlet oxygen removal is used while a fraction of it is actually consumed. According to our results, the difference is largest at low decay times, hence at high $\text{PO}_2$ values. For the three probes, the Stern-Vollmer curve (Eq. (5.1)) almost perfectly overlaps with the simulated calibration curve without organic molecules. This shows that singlet oxygen generation without consumption is not sufficient to significantly affect phosphorescence decay times. However, for PtP-C343, the measured calibration curve significantly deviates from the Stern-Vollmer curve, as discussed in Finikova et al. [28]. The Stern-Vollmer plots (bottom panels) show a slight deviation from the Stern-Vollmer regime in the presence of singlet oxygen consumption. The curve is not entirely linear, has its lowest slope at low $\text{PO}_2$ and becomes almost parallel to the Stern-Vollmer curve at high $\text{PO}_2$ values. However, this deviation from the Stern-Vollmer equation is smaller than that of the measured oxygen calibration curves.
In Fig. 5.5, the shifts in the oxygen calibration curves were shown for a particular choice of probe concentration and laser power. These parameters can be chosen by the experimenter and their influence on simulated decay times and measured \( P_O \) is shown in Fig. 5.6. In all cases, the decay times significantly increase with both parameters. The apparent \( P_O \) values obtained with the Stern-Vollmer equation are close to the prescribed value of 50 mmHg at low probe concentration and laser power, but considerably rise with both parameters. However, the magnitude of this increase differs for each probe. The variation in \( \tau \) reflects the probe sensitivity to \( P_O \) changes which is quantified by the oxygen quenching rate \( k_q \) and is highest for PtTCHP-C307 and lowest for PtP-C343. Additionally, the differences in two-photon absorption cross section also manifest themselves in the apparent \( P_O \) values. At equal photon flux and probe concentration, PtP-C343 causes the least decrease in apparent \( P_O \) as it has the lowest cross section, hence produces a lesser amount of singlet oxygen. In spite of these differences, these results show that singlet oxygen removal can have dramatic consequences on the apparent \( P_O \) with the assumed reaction rates.

The apparent \( P_O \) values shown in Fig. 5.6 suggest that the number of excited probe molecules per unit time plays a key role. This quantity...
5.3. Results

Figure 5.6: Top: simulated decay times in $\mu$s as a function of probe concentration and mean laser power; bottom: apparent $P_{O_2}$ in mmHg obtained using the simulated decay times and the Stern-Vollmer equation. $P_{O_2} = 50$ mmHg.

is also affected by the respective durations of the excitation phase and the collection period. In Fig. 5.7, the decay times are shown as a function of the acquisition time for different cycle lengths. The values of $\tau$ increase with the acquisition time until they reach an asymptotic value. For the three tested cycle lengths, the decay time after 0.06 s is $< 1\%$ smaller than the final simulated value. With a 225-µs-long collection period, this corresponds to 240 gates, which justifies the choice made previously. Besides, the increase in $\tau$ due to singlet oxygen consumption decreases with the collection period, as diffusion replenishes the excitation volume during a longer time. Therefore, the duration of the collection period can be increased to mitigate the drop in apparent $P_{O_2}$.

Since there are only sparse literature data on singlet oxygen removal by organic compounds in the plasma, the rate of singlet oxygen consumption $k_r$ was varied between 0 and $0.5k_{rem}$. In these computations, the quenching rate $k_{q,org}$ of singlet oxygen decay to triplet oxygen was set in such a way that $k_r + k_{q,org} = k_{rem}$. Fig. 5.8 shows that the decay time increases in a sublinear fashion with the oxidation-to-removal ratio of singlet oxygen. When this ratio is small, its influence on $\tau$ can be assumed to be linear. This relation can be used for quick estimates of the influence of $k_r$ on $\tau$.

Now we quantify the dependence of simulated decay times on oxygen
Figure 5.7: Influence of the acquisition time and cycle length on the decay time in the presence of singlet oxygen removal. Legend: cycle length. Each cycle is composed of an on-phase (25 µs) followed by an off-phase (225, 475 and 975 µs, respectively). The number of gates was 400, 200 and 100, respectively. The right $y$-axis shows the apparent PO$_2$ obtained from $\tau$ using the Stern-Vollmer equation.

Figure 5.8: Influence of the ratio $k_r/(k_r + k_{q,org})$ on the simulated decay time. The total removal rate $k_r + k_{q,org}$ was kept constant.

diffusion which is a novel feature of this model. Fig. 5.9 shows decay times as a function of oxygen diffusivity values between 0 and $2.5 \times 10^{-5}$ cm$^2$s$^{-1}$, which is slightly above its value in water. When diffusivity is low, the simulated decay times do not reach a plateau during the 0.06 seconds of acquisition time. This occurs since diffusion is too weak to compensate singlet oxygen removal. For values of $D_{O_2}$ closer to oxygen diffusivity in water, the values of $\tau$ reach an asymptotic value within the simulated acquisition time and exhibit a moderate dependence on $D_{O_2}$. Therefore, the modeling of oxygen diffusion is indispensable in the presence of singlet oxygen removal by organic molecules. However, small uncertainties in $D_{O_2}$ are unlikely to have a major influence on the apparent value of PO$_2$. 
5.4 Discussion

The phosphorescence decays arising in 2PLM in the presence of singlet oxygen consumption by organic molecules were simulated. To this end, we developed a reaction-diffusion model for ground state and singlet oxygen. Finally, we address the influence of the domain size and the boundary condition for oxygen. Simulations were run in four domains of different size ($r_{\text{max}} =$ 12, 16, 20 and 32 µm, and $z_{\text{max}} =$ 1.25$r_{\text{max}}$) with either a Dirichlet or a homogeneous Neumann boundary condition. The former is an approximate model for an infinite medium, while the latter represents a closed domain with no oxygen influx. With the Dirichlet boundary condition the asymptotic value of $\tau$ and the time required to reach this plateau increase with domain size, as shown in Fig. 5.10. For $r_{\text{max}} =$ 16 µm, the asymptotic increase in $\tau$ is 7.3% lower than for $r_{\text{max}} =$ 32 µm. When using the Neumann boundary condition, the decay time does not reach a constant value since the domain is continuously depleted without being replenished with oxygen. However, in the largest simulated domain, the values of $\tau$ for both boundary conditions stay very close. This indicates that the largest domain can be considered as an infinite medium in this context. These results show that the domain size and the boundary condition affect results substantially more than numerical parameters such as the grid spacing, hence require special care.

Figure 5.9: Influence of the oxygen diffusion coefficient on the decay time. Left: simulated decay times after 0.06 s of acquisition time (240 gates). Right: simulated decay times during acquisition for all values of $D_O_2$ shown in the left panel.

Finally, we address the influence of the domain size and the boundary condition for oxygen. Simulations were run in four domains of different size ($r_{\text{max}} =$ 12, 16, 20 and 32 µm, and $z_{\text{max}} =$ 1.25$r_{\text{max}}$) with either a Dirichlet or a homogeneous Neumann boundary condition. The former is an approximate model for an infinite medium, while the latter represents a closed domain with no oxygen influx. With the Dirichlet boundary condition the asymptotic value of $\tau$ and the time required to reach this plateau increase with domain size, as shown in Fig. 5.10. For $r_{\text{max}} =$ 16 µm, the asymptotic increase in $\tau$ is 7.3% lower than for $r_{\text{max}} =$ 32 µm. When using the Neumann boundary condition, the decay time does not reach a constant value since the domain is continuously depleted without being replenished with oxygen. However, in the largest simulated domain, the values of $\tau$ for both boundary conditions stay very close. This indicates that the largest domain can be considered as an infinite medium in this context. These results show that the domain size and the boundary condition affect results substantially more than numerical parameters such as the grid spacing, hence require special care.

5.4 Discussion

The phosphorescence decays arising in 2PLM in the presence of singlet oxygen consumption by organic molecules were simulated. To this end, we developed a reaction-diffusion model for ground state and singlet oxygen,
and a phosphorescent probe under two-photon excitation. The presence of oxygen diffusion and irreversible oxygen consumption are novel features, as well as the ability to simulate consecutive decays. Our main goal was to quantify to what extent phosphorescence decays are affected by singlet oxygen consumption. To achieve this, the influence of laser settings and probe concentration was assessed for three oxygen-sensitive nanoprobes. We show that permanent singlet oxygen removal shifts the oxygen calibration curves and can cause considerable underestimations of \( \text{PO}_2 \) when using the Stern-Vollmer equation to convert measured decay times to \( \text{PO}_2 \) values.

Our results aim to help experimenters to choose parameters in 2PLM so as to minimize errors due to photochemical oxygen consumption. At the assumed reaction rates of singlet oxygen consumption, experiments with low probe concentration (\( \leq 10 \mu\text{M} \)) and laser power (\( \leq 10 \text{mW} \)) are not expected to significantly affect the measured decay times (Fig. 5.6). However, when signal strength is insufficient, as may occur in measurements at high depth, it might be necessary to increase laser power and/or probe concentration to obtain a satisfying signal-to-noise ratio. With the probe PtP-C343, probe concentration up to \( 200 \mu\text{M} \) have been reported [86]. Unfortunately, the laser power is often unspecified, which makes the assessment of previous 2PLM measurements difficult. Even at high laser power and probe concentration, the potential decrease in phosphorescence decay times can be mitigated by lowering the laser duty cycle (Fig. 5.7). However, this countermeasure proportionally reduces the temporal resolution of the method. Therefore, despite uncertainties in several model parameters, our results enable the formulation of concrete guidelines for experimental parameters.
5.4. Discussion

The phosphorescence decays simulated in this study provide additional theoretical insight. Before this work, it was unclear whether the temporal variations in ground state oxygen due to diffusion could result in phosphorescence decays of multi-exponential type. However, in the parameter range investigated here, single exponential fits were virtually indistinguishable from the simulated decays. Therefore, the variation in oxygen concentration during the off-phase is too small to produce multi-exponential decays. Further, the question whether singlet oxygen consumption could result in significant deviations from the Stern-Vollmer regime was open. Our computations produced Stern-Vollmer plots with permanent oxygen removal that are slightly nonlinear (Fig. 5.5, bottom panels), which means that the Stern-Vollmer curve cannot be entirely corrected by adapting the quenching rate $k_q$. However, this deviation from the Stern-Vollmer regime is lower than that of the measured calibration curves. Thus, our results show that, given the required reaction rates, oxygen calibration curves can be in principle corrected for singlet oxygen consumption, but further research is needed to perform this correction when the calibration curve departs from the Stern-Vollmer regime.

Two-photon phosphorescence decays were simulated under the assumption of an immobile medium. While the probe solution can be assumed not to move during measurements in tissue or cuvette experiments, different conclusions may be reached during measurements of intravascular PO$_2$. Although convective transport of the medium was not modeled, we now comment on its possible influence. Under saturation conditions, the size of the excitation volume may reach 5 $\mu$m in the focal plane and 10 $\mu$m in the microscope axial direction (Fig. 5.4). At the typical capillary blood velocity of 1 mm/s, a fluid element takes 5 ms to cross the excitation volume if the capillary is in the focal plane and 10 ms if blood flows along the laser beam. According to Fig. 5.7, the simulated decay time undergoes almost half of its total increase after 5 ms. Thus, the shift in decay times due to oxygen consumption is decreased when plasma is moving. However, based on these estimates, the shifts in apparent PO$_2$ might still be significant in intravascular measurements, especially at low blood velocity.

In terms of numerical modeling, the main limitation of the reaction-diffusion model developed here is the boundary condition for oxygen. While the assumption of an infinite medium with constant oxygen concentration at infinity adequately models cuvette experiments, the Dirichlet boundary condition employed at the boundary of the finite cylindrical computational domain is not satisfactory. The result dependence on the domain size (Fig. 5.10) shows that this is not a robust choice. A further increase
in domain size is expected to yield converged results, but at higher computational costs. The homogeneous Neumann boundary condition which represents a closed domain without oxygen influx was also shown to be inappropriate. To solve this issue while keeping a reasonable domain size, we suggest to approximate the far-field oxygen distribution using a Green’s function with a source strength given by the rate of singlet oxygen removal. Thus, an inhomogeneous Neumann boundary condition with the gradient of the Green’s function as a boundary value could be used to model an infinite medium.

The physical model for the phosphorescent probe used here is elementary, as it is only modeled in ground state and triplet state. Each excitation event is assumed to give rise to a probe molecule in triplet state that either produces a photon by phosphorescence or is quenched by oxygen. For probes composed of a metalloporphyrin core and harvesting antennas, a wider range of mechanisms is potentially involved, such as fluorescent emission, Förster-type resonance energy transfer, inter-system crossing and quenching by electron transfer. Additionally, a heterogeneous probe population can lead to a distribution of phosphorescence lifetimes, rather than a single value. This can in turn cause non-linear Stern-Vollmer plots [28]. As a next step, we suggest to identify the mechanisms that need to be included so that model results reproduce measured oxygen calibration curves (see Fig. 5.5).

The primary goal of this study was to provide experimenters who use 2PLM for oxygen measurements with estimates of the measurement errors that can arise due to singlet oxygen consumption. The model results presented here are a first step in this direction. However, model validation with experimental data is indispensable to ensure that the modeling assumptions are correct and the experiments behave according to theory. Our first experiment suggestion is to perform a reference titration in a saline solution at low laser power and probe concentration, then another titration in a solution with high protein content and increasing laser power. Although the insensitivity to organic molecules of the phosphorescent probes modeled here was demonstrated [28, 101], these experiments were conducted under one-photon excitation with an unfocused laser beam and shorter excitation durations. Thus, the photon fluxes during these titrations were much lower than in typical in vivo experiments and the influence of organic compounds on decay times cannot be ruled out.

To gain further understanding of the influence of oxygen consumption on phosphorescence decay times, an analytical (or semi-empirical) model for the prediction of decay times could be developed and validated with the computational model used here. The dependence of $\tau$ on the laser power and
probe concentration (Fig. 5.6) as well as on the collection period duration (Fig. 5.7) suggests that the average rate of singlet oxygen removal is a plausible predictor for the decay times. Oxygen diffusion could be accounted for by a mass balance between singlet oxygen removal and excitation volume replenishing. If the development of an analytical model succeeds, it will greatly facilitate the estimation of the shifts in apparent PO$_2$ due to singlet oxygen consumption.

5.5 Conclusion

Two-photon phosphorescence lifetime microscopy is an invaluable source of data for computational models for oxygen transport. The highly reactive oxygen species produced by this technique can however interfere with chemical processes and, in principle, cause local oxygen depletion that lead to measurement errors. Here we developed a reaction-diffusion model for a two-photon phosphorescent probe and oxygen in ground state and singlet state with consumption by organic molecules. Our results show that considerable PO$_2$ underestimations may arise at high laser power and probe concentration, provided the rate of photochemical consumption is significant. However, since singlet oxygen rapidly decays back to the ground state, the model does not predict significant deviations from the Stern-Vollmer regime in the presence of moderate amounts of excited probe molecules.
Chapter 6

Conclusion and outlook

6.1 Conclusion

The study of oxygen transport to tissue is fundamental in biology and medicine since aerobic cell metabolism depends on a continuous oxygen supply. Recent progress in experimental techniques has considerably improved our grasp of microvascular blood flow and oxygenation. Due to the limited resolution and parameter control in experimental studies in vivo, computational modeling offers an invaluable complement from which insight into the physics of oxygen transport can be gained. In this thesis, we developed a model for oxygen transport from capillaries to tissue that lends itself to validation with experimental data and successfully compared results to recent in vivo measurements. Building on this basis, theoretical analyses of oxygen transport were performed and validated.

Based on a novel modeling approach with moving RBCs, the erythrocyte-associated transients (EATs) observed by Parpaleix et al. [86] using 2PLM could be reproduced in the presence of a high oxygen extraction rate from capillaries. This theoretical confirmation is relevant since several research groups obtained considerably different EAT amplitudes with this technique. Moreover, the comparison of longitudinal plasma PO2 gradients led to the hypothesis that capillary networks are denser on venous side. A preliminary topological analysis of cerebral microvascular networks performed by Dr. M. Schneider in the group of Prof. B. Weber indicated this trend.

Regulation mechanisms of oxygen supply target two physiological variables in particular: blood velocity and hematocrit. As their effects are difficult to isolate in vivo, an analysis of their relative influence on tissue oxygenation was conducted. A simplified differential equation model and the computational model showed that hematocrit is a stronger determinant of tissue oxygenation than erythrocyte velocity. The influence of these flow variables is itself modulated by the distance from arterioles and metabolic consumption of oxygen. The interplay between these variables could be
described by an analytical function, which illustrates the advantages of simplified models.

The differential equation model was extended to take the heterogeneity of blood oxygen content into account. The concept of capillary outflow saturation heterogeneity (COSH) was introduced as a measure of the heterogeneity of blood oxygen content at the venous end of capillaries, where an insufficient oxygen supply is most likely to occur. This quantity is related to capillary transit time heterogeneity (CTH) which has attracted considerable attention lately. Our theoretical analysis demonstrated that, on a small spatial scale, COSH is significantly reduced by the diffusive interaction within and between capillaries. Thus, although CTH influences COSH, the topology of realistic capillary networks significantly damps the small-scale heterogeneity of blood oxygen content. These findings propose a new way of analyzing the effects of disturbed flow patterns that occur in numerous diseases \[82\].

Our computational model was validated with data acquired using 2PLM. Potential limitations of this technique due to photochemistry and oxygen depletion were investigated quantitatively using a new reaction-diffusion model with singlet oxygen consumption by organic molecules. At sufficiently high rates of permanent oxygen removal, phosphorescence decay times were found to be significantly reduced at high laser power and probe concentrations, which can lead to underestimations of measured $P_{O_2}$ values. The model can be used to compute bounds on experimental parameters to avoid such inaccuracies.

### 6.2 Outlook

As a general remark, oxygen transport simulations in realistic microvascular networks are needed to make further progress. However, they are not self sufficient due to the uncertainties in model parameters and boundary conditions. This issue can be alleviated in two different ways. First, such simulations can be used to examine hypotheses that follow from the physics of oxygen transport. Thus, even though the quantitative results may not accurately reflect in vivo situations, further understanding of physical processes in realistic networks can be gained. Second, the direct combination of experimental data with simulations can be used to provide truly quantitative results.

The diffusive interaction mechanisms involved in COSH should be further analyzed in realistic topologies. In particular, capillary diffusive interaction should be quantified in microvascular networks and its modeling
extended to irregularly spaced capillaries. Additionally, computations in larger networks with full RBC paths through capillaries would enable the direct comparison of CTH to COSH.

The preferred locations in the microvasculature for oxygen delivery during rest and activation have not been fully elucidated yet. The significant oxygen extraction from arterioles observed recently \(^\text{[104]}\) suggests to employ computational models that can handle non-capillaries. Then, the robustness of the microvasculature at different levels could be investigated. While the occlusion of penetrating arterioles near the cortical surface causes severe local ischemia \(^\text{[79]}\), capillary occlusions should also be studied. They occur for instance in the human cortex affected by capillary cerebral amyloid angiopathy \(^\text{[128]}\), which is observed in most Alzheimer’s disease patients. Computational modeling could help identify to what degree the cortical microvascular can undergo microvessel occlusions without the onset of hypoxia.

Finally, the setup of oxygen transport simulations that replicate in vivo measurements of blood flow and oxygenation is a challenging yet worthwhile undertaking. Multimodal imaging, for instance with combined Doppler optical coherence tomography and 2PLM \(^\text{[104, 32]}\), could provide sufficient data for the reconstruction of blood flow and the oxygen distribution. With a suitable optimization algorithm, an in silico setup that closely matches experimental observations could be achieved. Then, numerous scenarios could be quantitatively studied, such as activation patterns observed in neurovascular coupling or malfunctions of microvessels.

Addressing these questions entails considerable computational challenges as it requires an efficient and accurate oxygen transport model that can handle arterioles and venules, and simulate transient problems. The computational model with moving RBCs developed here is currently too expensive for these applications, although it can be used to validate further models. Instead, two strategies can be pursued. The first one involves the solution of the diffusion equation on a three-dimensional grid, as done here or in the model by Goldman et al. \(^\text{[37]}\). The required domain sizes would require high-performance computing. Alternatively, a three-dimensional grid can be avoided as with the Green’s function methods by Secomb and coworkers \(^\text{[116, 118]}\). As a novel approach, the differential equation models used in this thesis could be combined with modeling of the diffusive interaction between capillaries in order to avoid the detailed computation of tissue oxygenation. The involved modeling effort would be offset by considerable gains in computational time.

This thesis contributed to strengthening the interaction between in silico and in vivo studies of microvascular oxygen transport. Meeting the chal-
lenges proposed here will surely lead to new insights into this fascinating biological system.


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List of Publications

Journal papers


Peer-reviewed abstracts


A Lücker, TW Secomb, B Weber and P Jenny. “The relative influence of hematocrit and red blood cell velocity on oxygen transport from capillaries to tissue”, ISOTT 2016, Chicago, USA.

Oxygen supply to cells by the cardiovascular system involves multiple physical and chemical processes that aim to satisfy fluctuating metabolic demand. Regulation mechanisms range from increased heart rate to minute adaptations in the microvasculature. The challenges and limitations of experimental studies in vivo make computational models an invaluable complement. In this thesis, oxygen transport from capillaries to tissue is investigated using a new numerical model that is tailored for validation with experimental data. On this basis, theoretical analyses of tissue oxygenation and intravascular oxygen distribution are conducted with applications to blood flow regulation mechanisms and heterogeneity in microvascular networks.

The computational model developed here employs moving red blood cells (RBCs) in the frame of reference of the tissue. This key feature enables direct result comparison to oxygen measurements in capillaries and tissue. Thus, the first model validation with micrometric-resolution measurements of oxygen partial pressure (PO₂) in vivo could be performed. This novel technique is used to describe the complex relation between hemoglobin saturation and plasma PO₂ which manifests itself by the presence of erythrocyte-associated transients.

Dynamic regulation mechanisms of microvascular blood flow in the brain were modeled. The effect of individual pericyte-induced capillary dilations was investigated in a simple capillary network. Although a slight increase in tissue PO₂ was observed, multiple dilations need to simultaneously occur to produce a response comparable to the effect of arteriolar dilations. The dependence of tissue oxygenation on hematocrit and blood velocity was also described. Based on analytical tools supported by the computational model, the relative influence of hematocrit was demonstrated to be stronger.

The complex topology of microvascular networks entails a certain degree of heterogeneity in oxygen supply. Excessive variations in RBC transit times through the microvasculature have been associated to conditions such as diabetes, Alzheimer's disease and hypertension. Their relation with hemoglobin saturation heterogeneity was modeled based on the computational and analytical tools developed here. Diffusive interaction within and between capillaries was shown to lead to a considerable reduction of hemoglobin saturation heterogeneity at the scale of neighboring capillaries. These findings may substantially affect the interpretation of RBC transit time measurements in health and disease.