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INSTRUMENTED INDENTATION OF
SOFT MATERIALS AND BIOLOGICAL
TISSUES

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Abstract

The enthusiasm of human beings to understand the fascinating universe of living organism is ancestral. Over the past 40 years, a growing interest has formed around the use of engineering principles to explain biological processes taking place at the micro/nano-scale. As the behavior of soft biological tissues are closely related to their physiological functions, local techniques allowing the mechanical characterization of soft materials at the micro/nano-scale provide very helpful information for a better understanding of biological materials complexity.

Instrumented indentation is such a local technique and consists of poking specimens of interest with tips of known shape monitoring in real time both the tip penetration depth and the sample reaction forces. Over the past 10 years, it has been shown to have a promising potential for characterizing biological tissues at the microscale. Indeed, nanoindenter machines that were originally developed for hard material characterization have been used to characterize relatively stiff biological materials such as bones, dentin, cartilage and skin among others. However, very few attempts only have been conducted on characterizing very soft materials at the microscale with such a technique, mainly due to machines working range limitations and problems associated with the meaningfulness of mechanical properties extracted from experimental data.

The present work addresses the experimental challenges related to the indentation of very soft materials and the associated extraction of meaningful mechanical properties. For this purpose, a very sensible nanoindenter allowing low force sensing and the application of large displacement was used to characterize synthetic materials exhibiting similar proper-
Abstract

ties as biological tissues in terms of compliance and time-dependence. Spherical indentation was conducted at the microscale on three materials exhibiting gradual challenges to overcome, namely a soft silicone behaving hyperelastically, a soft elastomer exhibiting hyperelastic-viscoelastic properties and finally agarose gels expected to behave poroelastically.

The capabilities of a force-controlled instrumented indentation machine to characterize very compliant materials exhibiting time-dependent behaviors have been demonstrated. The drift stability provided by the UNHT nanoindenter allowed acquiring a large number of measurement data. The pull-in forces sensed when approaching surface sample were shown to be due to the formation of a capillary neck at the interface, decreasing in magnitude for lower humidity. The influence of sample surface topography on measurement data and the importance of accounting for it in the post-processing phase were investigated through Finite Element (FE) analysis. A fully automated FE based tool was developed in order to solve the inverse problem, i.e. to extract mechanical properties from experimental data; non-linear inverse analysis has been shown to provide a valuable alternative to analytical solution when large strains (> 10%) are achieved. Extensive data were acquired and analyzed to characterize the time dependent mechanical behavior of a viscoelastic acrylic elastomer and agarose gels. Finally, initial experiments were conducted on soft biological tissues, such as tissue engineering heart leaflet and mice liver.
RÉSUMÉ

L’enthousiasme des êtres humains à comprendre l’univers fascinant des organismes vivants qui nous entourent est ancestrale. Au cours des quarante dernières années, un intérêt grandissant s’est développé autour de l’utilisation de principes d’ingénierie pour comprendre et expliquer les processus biologiques qui ont lieu à l’échelle micro/nano. Les comportements mécaniques des tissus biologiques étant étroitement liés à leur fonctions physiologiques, des techniques expérimentales permettant de caractériser localement des matériaux dits mous à l’échelle micro/nano fournissent des informations très utiles pour une meilleure compréhension de leurs complexités.

L’indentation instrumentée est un exemple de technique locale consistant à enfoncer des pointes dures de formes connues dans des spécimens d’intérêts, mesurant en temps réel à la fois la profondeur de pénétration et les forces de réaction de l’échantillon. Au cours des dix dernières années, il a été démontré qu’une telle technique a un potentiel prometteur pour la caractérisation de tissus biologiques à l’échelle microscopique. En effet, des nano-indenteurs initialement développés pour la caractérisation de matériaux durs ont été utilisés pour caractériser des matériaux biologiques relativement rigides tels que des os, de la dentine, du cartilage et de la peau entre autres. Cependant, seulement un nombre très faible de tentatives ont été menées afin d’utiliser une telle technique pour caractériser des matériaux très mous à l’échelle microscopique, principalement en raison des limitations techniques liées à la gamme de force des machines et de la signification des paramètres extraits des données expérimentales acquises.
Résumé

Le travail présenté a pour but de relever les défis expérimentaux liés à l’indentation de matériaux très mous, ainsi que d’en extraire des propriétés mécaniques significatives. Dans ce but, un nano-indenteur très sensible permettant la mesure de forces très faibles et l’application de grands déplacements a été utilisé pour caractériser des matériaux synthétiques présentant des propriétés similaires à celles des tissus biologiques en termes de dureté et de dépendance temporelle. Des indentations sphériques ont été menées à l’échelle microscopique sur trois matériaux présentant des difficultés progressives à surmonter, à savoir; un silicone souple se comportant de manière hyper-élastique, un élastomère souple présentant des propriétés viscoélastiques hyper-élastiques et enfin, des gels d’agarose censés se comporter poroélastiquement.

Les capacités d’une machine de pénétration instrumentée contrôlée en force à caractériser des matériaux mous dont le comportement est dépendant du temps ont été démontrées. La stabilité de dérive fournie par le nano-indenteur UNHT a permis l’acquisition d’un grand nombre de mesures. Les forces de traction détectées à l’approche de la surface de l’échantillon ont été démontrées comme étant dues à la formation d’un col capillaire à l’interface, son ampleur diminuant pour des taux d’humidité inférieurs. L’influence de la topographie de l’échantillon sur l’acquisition de mesures ainsi que l’importance d’en tenir compte dans la phase de post-traitement ont été étudiées grâce à des analyses par éléments finis. Un outil automatisé basé sur des analyses par éléments finis a été développé afin de résoudre le problème inverse, c’est-à-dire pour extraire des propriétés mécaniques à partir de données expérimentales. L’analyse inverse non-linéaire a démontré fournir une alternative intéressante à la solution analytique lorsque de grandes déformations (> 10%) sont atteintes. De nombreuses données ont été acquises et analysées afin de caractériser le comportement mécanique dépendant du temps d’un élastomère acrylique viscoélastique et de gels d’agarose. Enfin, des expériences initiales ont été menées sur des tissus biologiques mous, tels que des valves cardiaques et des foies de souris.
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XIII
Introduction

Instrumented indentation is a technique consisting of poking specimens of interest with tips of known shape made of very stiff materials such as diamond, monitoring in real time both the tip penetration depth and the sample reaction forces. Mechanical characteristics related to samples are then extracted from force-displacement curves using well-established equations based on elastic contact theory, or performing an inverse analysis based on the Finite Element (FE) method.

1.1 Instrumented Indentation

1.1.1 The evolution through the years

The following chapter aims at summarizing the theoretical and technological breakthroughs that lead to the realization of the current commercially available instrumented indentation machines using reduced size tips and improved depth/load resolutions.

*Depth-sensing machines originate from Hardness scratch testing (1750), a technique developed by mineralogists in the mid-18th century and consisting of scratching a materials on a bar with decreasing hardness*
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from end to end (bar scratch testing); the level at which the material could form a scratch determined the hardness of the tested material (Tabor, 1951).

Carl Friedrich Christian Mohs (1822) developed the Mohs scale method, which consists of ten minerals (i.e. 1 = Talc, 10 = Diamond) and the ability of the material of interest to scratch them. He additionally created a hardness scratch testing method involving the drawing of a diamond stylus on surfaces of interest under finite loads; the residual scratch imprint, characterized by its width and depth, was further used as a measure to determine the hardness of the materials. Note the inappropriate application of such a method for material exhibiting pronounced frictional properties (i.e. metals) (Tabor, 1951).

Heinrich Rudolf Hertz (1880) made a major contribution for the indentation field in developing the first theory for indentation of materials, namely describing the linear elastic contact between two spheres through the analysis of Newton’s interference rings. This simple analytical solution is still widely used for elastic contact analysis of spherical indentation (Johnson, 1985).

Joseph Valentin Boussinesq (1885) used the methods of potential theory to solve the contact problem between two linearly elastic isotropic solids; this step was of significant importance as it served as a basis for the development of the contact problem for a rigid conical indenter by Sneddon half a century later (Sneddon, 1948).

Nearly 50 years of development were needed to give birth to the first accepted and standardized indentation-hardness test: the Brinell hardness test.

Johan August Brinell (1900) introduced the Brinell test which consists on pushing a hard ball (1-10 [mm]) made of either hard steel, tungsten carbide, or diamond into a material of choice at heavy normal loads (up to 3000 [kg]); the imprint is further measured with a low-power microscope after removal of the load, and the Brinell hard-
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iness number (BHN) is then expressed as the ratio of the load P to the curved area of indentation (Tabor, 1951).

Eugene Meyer (1908) proposed a new measure of hardness being the ratio of the load to the so-called projected area of indentation (Fig. 2.10), which is still the currently accepted and standard measure of hardness (Tabor, 1951).

The blossoming of the industrial revolution, the increased manufacturing requirements and the global industrialization called for refined and efficient mechanical characterization methods.

Due to the limited hardness of steel balls and to the fact that spherical indenter could hardly be manufactured from diamond, Smith and Sandland (1922) introduced a new testing procedure based on a 4-sided diamond pyramid (Vickers), resulting in a more versatile and consistent hardness test: the Vickers hardness test was born.

In the case of spherical indentation of soft metal, an indent is formed due to plastic flow of the specimen. Once the indenter is removed, the permanent indent formed has a radius of curvature larger than that of the indenter; David Tabor (1948) experimentally showed that the relaxation along the indentation depth is due to the release of elastic stresses in the material. Indeed, he observed that a second application and removal of the indenter under the original load did not change the size and shape of the indent, confirming the unloading process to be purely elastic; when reloaded, the deformation of the indenter (loading, unloading) is elastic and should conform with Hertz theory. Those observations set the basis for today’s nanoindentation elasticity analysis.

Starting from Boussinesq problem formulation, Ian Naismith Sneddon (1948) developed the contact problem for a rigid conical indenter and a semi-infinite half plane; few years later (Sneddon, 1965), he extended his solution to the contact between any axisymmetric indenters and a semi-infinite half plane.
In the mid-20th century, researchers diversified their use of hardness tester to materials behaving viscoelastically, poroelastically or exhibiting adhesive properties.

Based on Boltzmann operators, Lee and Radok (1960) extended the Hertzian contact to viscoelastic bodies; the derived model predicts the viscoelastic material response to an applied step load through the creep compliance function.

Dumas and Baronet (1971) developed the first finite element model describing the elasto-plastic indentation of a half space by a cylindrical indenter; using a linear strain-hardening law, they were able to determine the residual depth for different indentations.

Johnson et al. (1971) extended the Hertzian contact for adhesive bodies, accounting for attractive forces between the surfaces of the two spheres.

Depth-sensing indentation machine were considerably improved in the 70’s, where the technological advances allowed for larger indentation depth and more accurate load control.

Based on the observation made by Tabor stating that the unloading indentation phase of materials undergoing elasto-plastic deformation can be considered as purely elastic and on the derivation of the contact law proposed by Sneddon, Bulychev et al. (1975) proposed a solution for the calculation of the reduced Young’s modulus (Eq. 2.18); this solution is commonly referred as the contact stiffness equation where the stiffness is a function of the reduced modulus and of the projected area (Eq. 2.39).

However, due to the impossibility of determining accurately the projected area, Bulychev et al. could not relate the measured stiffness to the reduced Young’s modulus.

Doerner and Nix (1986) provided the first complete nanoindentation data analysis using the stiffness equation introduced by Bulychev.
et al; they argued that if the change in contact area is small during unloading, the indenter can be treated as a flat punch; the contact stiffness equation was for the first time completed.

**Oliver and Pharr (1992)** demonstrated at first that the contact stiffness equation proposed by Bulychev et al. (1975) is valid for any axisymmetric indenters. They further observed that the initial part of the unloading data would rather be fitted by a power law as compared to linear fitting used by Doerner and Nix (1986). Finally, they defined new area functions better predicting the projected area. These improvements became popular in the nanoindentation field and the Oliver and Pharr model is currently seen as the standardized model for extracting mechanical properties from instrumented indentation experiments; indeed, the method is still widely implemented into commercially available nanoindenter machines.

Though all the previously defined analytical solutions were consistently developed and are easily applied to measurements data in order to extract meaningful mechanical properties, they are limited to the indentation of an infinite half-space specimen undergoing small deformations (linear elasticity) by an indenter with ideal geometry; the discussed assumptions are thus limiting the range of application of such solutions and make of the advent of finite element (FE) modeling a reliable alternative for data analysis (Giannakopoulos and Larsson, 1997).

### 1.1.2 TOWARDS BIOLOGICAL TISSUES

The application of mechanical principles to biology gave rise to biomechanics in the early 1970’s, a field aiming at understanding the mechanical functioning of living organisms (Oyen, 2011); indeed, mechanical characteristics of biological materials are very closely related to their functionalities. The difference in behavior between malignant cancerous prostatic and benign tissues was for instance shown to correlate with the structural changes in morphology observed in the microstructure of the tissues (Phipps et al., 2005). A good understanding of the mechanical behavior
of such soft materials is further of great help for the development of new medical practices for diagnosis (Paszek et al., 2005), tissue replacement engineering (Ayache et al., 1998), trauma research (Snedeker et al., 2002), as well as for a better understanding of cell-biology (Wells, 2008).

Soft biological tissues are composed of different microstructures and exhibit nonlinear stress-strain and time-dependent mechanical behaviors. Depth-sensing indentation testing devices have been shown to have a promising potential for characterizing mechanically such very soft materials; indeed, as biological materials are inhomogeneous, they call for a local technique (Oyen, 2011) capable of easy specimens handling. Moreover, indentation techniques leave small imprints and are perceived as non-destructive experiments. Please note that even though biological tissues can be considered as anisotropic materials, the potential anisotropy of soft tissues will not be further illustrated since all the soft materials tested within the scope of this thesis have been assumed to be isotropic. Over the last decade, a growing interest has formed around the characterization of biological tissues at different length-scale using commercially available techniques. Atomic Force Microscopes (AFM) (Engler et al., 2004; Lekka et al., 1999) and classic micro/macro-indenter machines (Barnes et al., 2007; Kendall et al., 2007) have been shown to have reliable capabilities for acquiring meaningful experimental data for such very soft materials, respectively at the nano and micro/macro-scales. The use of nanoindenter machines for characterization of biological tissues has been mainly limited to relatively stiff materials such as dentine (Cuy et al., 2002; Finke et al., 2001; Ge et al., 2005; Habelitz et al., 2002) bone (Bembey et al., 2006; Haque, 2003; Rho et al., 1997; Roy et al., 1999) or cartilage (Ebenstein et al., 2004; Franke et al., 2011; Li et al., 2006; Lu and Mow, 2008) due to machine limitations related to both measurable force range and indentation depth; very few studies aimed at investigating the mechanical response of very soft biological tissues (Constantinides et al., 2008; Ebenstein et al., 2004; Yuan and Verma, 2006) to nano/micro-indentation. Ebenstein and Pruitt (2004) and Wahl et al. (2006) have investigated very soft isotropic polymers exhibiting similar properties as biological tissues. Technological limitations lead to inaccurate contact point detections (Ebenstein et al., 2004; Ebenstein and Pruitt, 2004), and further to mod-
Instrumented indentation

ulus overestimation (Kaufman and Klapperich, 2009).

1.1.3 Current Indentation Devices

Existing machines on the market

Hysitron was among the first companies to build a nanoindenter allowing the characterization of biological materials such as bone, dentine, or cartilage. Although the high resolution of Hysitron measurement head allows the application of precise low loads (few [nm]-depth), problems related to thermal drift make the indentation of very soft biological tissues such as skin or liver a very challenging task to accomplish with such a machine. Such a thermal drift (temperature stabilization problem) is present on all commercially available nanoindenters (Agilent, Micro Materials, IBIS) and is a major issue when performing creep experiments or long time measurements on very compliant materials.

The unique design of CSM UNHT nanoindenter head (referencing, see Chapter 3.1.1) allows the reductions of the influence of both thermal expansion and compliance of the sample/instrumentation. This unique feature makes of the CSM UNHT an ideal candidate for application to soft materials and biological tissues; the UNHT used in the present work is further compared to its competitors (Table 1.1). Among the parameters listed for each nanoindenter manufactured, the minimum contact load ($P_{\text{min}}$) is of great concern. Indeed, as the forces sensed during the indentation process of very compliant materials are very low, it is of major importance to have the capabilities of sensing such very low forces with the available force sensors. Please note that personal confidence about the values of $P_{\text{min}}$ is high only for the UNHT machine, which capabilities have been experimentally tested in the present work.

Positioning among scientific papers

The current section aims at positioning the present work among findings reported in the literature as well as clarifying the terminologies used later on in the thesis.

The field of indentation is wide and scientific papers treating the characterization of relatively soft ($E < \text{[MPa]}$) materials by means of in-
1. Introduction

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<tr>
<th>Nanoindenter (Constructor)</th>
<th>P-<a href="P_%7Bmin%7D"> </a></th>
<th>h-[ ]</th>
<th>Remarks</th>
</tr>
</thead>
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<tr>
<td>UNHT CSM Instruments</td>
<td>0-50 (2)</td>
<td>0-100</td>
<td>“Referencing” unique feature allowing a reduction of the influences of both thermal expansion and compliance of the sample instrumentation.</td>
</tr>
<tr>
<td>Hysitron TI 950 TriboIndenter</td>
<td>0-10'000 (-)</td>
<td>0-10</td>
<td>Fast indentation time are required in order to avoid thermal drift; as a results, long time of thermal stabilization are required prior to measurements.</td>
</tr>
<tr>
<td>Nano Indenter G200 DCM II head Agilent</td>
<td>0-30 (1)</td>
<td>0-70</td>
<td>Thermal drift is high resulting in a large time required for thermal stabilization; post-processing corrections of the thermal drift are necessary.</td>
</tr>
<tr>
<td>NanoTest Vantage Micro Materials</td>
<td>0-500 (10)</td>
<td>0-100</td>
<td>Possibility to control humidity, liquid cell available. Sample holder in vertical position. Time required for thermal stabilization.</td>
</tr>
<tr>
<td>Nanoindentation System Model B IBIS</td>
<td>0-50 (5)</td>
<td>0-2</td>
<td>The nanoindenter can be coupled to an AFM for a purpose of indents visualization. FE automated interface is proposed users.</td>
</tr>
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* P-range \([mN]\), h-range \([\mu m]\), P_{min} \([\mu N]\)

Table 1.1 Nanoindenter competitor analysis.

Indentation can be classified in multiple ways; here, an attempt is made in representing the field in four dimensions (Fig. 1.1), namely with the maximum force \((P_{max})\) and displacement \((h_{max})\) applied during the measurement procedure being the 2D grid serving as a basis for measurement differentiation. Additional useful information are provided by exhibiting the size of the indenter used (size of circles) during indentation as well as the relative stiffness of the material tested (Young’s moduli) represented by the different colors of the circles. Note that very soft \((E < 100 [kPa])\) materials have mainly been tested with AFM and macro indenter machines; as a result, the determination of the mechanical properties of such materials at an intermediate lengthscale is of major interest. The present work aims at using a nanoindenter machine allowing for indentation up to 50 \([\mu m]\) depth and force resolution down to few \([\mu N]\) using spherical tips in the order of few hundreds of \([\mu m]\); as such, the method can be
Instrumented indentation

Figure 1.1 Four-dimensional representation of literature findings exhibits the need for using nanoindenter machines to determine the mechanical properties of soft materials at the micro lengthscale. The range of Young’s modulus reported for liver is thought to be due to variability in material properties among species and pathologies measured through different characterization method (Constantinides et al., 2008).
addressed as a "micro-indentation" procedure.

1.2 **THESIS THREAD**

1.2.1 **MOTIVATIONS**

The present work aims at stepping towards the characterization of soft materials and biological tissues by means of micro-indentation. For this purpose, theoretical and technological limitations related to the indentation of very soft isotropic materials have been investigated with the help of a modified nanoindenter machine (UNHT, CSM Instruments) commonly used for indentation of hard materials; besides difficulties related to the experimental procedure, the analysis of experimental data by reliable models is the main prerequisite to the accurate characterization of soft time-dependent material response. These limitations became as many challenges to overcome and led to:

- The creation of a setup allowing for reliable indentation measurements of very soft isotropic materials comparably challenging as biological tissues in terms of compliance, roughness and surface forces (Chapter 6).

- The validation of micro-indentation procedures for analyzing the time-dependence of soft viscous and porous materials (Chapters 7, 8).

- The creation of a new Bioindenter machine commercialized by CSM Instruments mid 2013.

In order to investigate the capabilities of nanoindenter machines, a soft silicon (Ecoflex0030) expected to behave hyper-elastically was initially picked for micro-indentation experiments in the air (40% humidity) by a spherical indenter (R = 100 [$\mu$m]). The tip geometry was selected from an optimization analysis involving 2D and 3D FE modeling (Chapters 4, 5). Due to the low forces sensed during the indentation of very soft materials at the microscale, surface forces were shown to become prominent and to have a significant influence on measurement data. Comparably,
these forces are below the resolution limit in the case of hard material nano-indentation and are thus disregarded and further neglected. Negative forces pulling down the indenter were indeed observed during the approach phase and were shown to be due to meniscus forces induced by the presence of a water film at the interface between the indenter and the surface (Chapter 6). A liquid cell allowing for full sample hydration was consequently built in order to displace the meniscus forces acting at the interface to a more stable region, namely to the upper parts of the extender shafts, where they can be assumed to be constant.

As biological tissues cannot be considered as nearly flat, the influence of their surface roughness on measurement data cannot be disregarded. In order to analyze the importance of surface roughness on soft materials indentation, two silicon specimens with different roughness (i.e. flat, rough) were synthetized and further tested; the lower forces sensed during the indentation process of rough samples demonstrated the importance of roughness and its influence on measurements; its impact on experimental data was additionally analyzed through FE modeling analysis, where the material nonlinearity was taken into account. The complete procedure was finally validated through a comparison of meaningful hyperelastic properties extracted from both micro-indentation and uniaxial tensile experimental data based on FE inverse analysis.

One of the main properties of biological tissues is their time-dependence when subjected to deformation, which are commonly explained by viscoelasticity or poroelasticity. It is thus of major importance, when aiming at characterizing such soft materials through micro-indentation experiments, to investigate dissipative behavior through specialized protocols including relaxation or creep phases (our case), and further to extract meaningful properties from the acquired data using reliable time-dependent models. In an attempt to understand the differences between visco and poro-like behaviors, two isotropic materials exhibiting one or the other property were subsequently chosen and tested, namely a soft viscous elastomer (VHB4910) and agarose gels at different agarose concentration (0.5, 1, 2 and 4 % w/w).

A soft viscous elastomer (VHB4910) that had been extensively characterized and shown to behave hyperelastic-viscoelastic was picked to un-
1. Introduction

dergo micro-indentation for different maximal applied loads and at different deformation rate. A creep phase of 60 [s] at the end of the loading phase served as a basis for time-dependence analysis through both an extension of the Hertzian formulation for viscoelastic bodies valid for small strains and a Quasi-Linear Viscoelastic (QLV) model accounting for both stress-strain non-linearities and time-dependence. The capabilities of both models to predict the time-dependent behavior of the material undergoing micro-indentation were further investigated; the Hertzian contact extended for viscoelastic bodies was shown to deliver good predictions for a wide range of deformations in the linear regime whereas the non-linear model formulation obtained through finite element modeling allow for accurate predictions of larger non-linear deformations, such as seen also in corresponding uniaxial tensile testing experiments. The relaxation function obtained from FE inverse analysis showed good agreement with the literature, validating the whole data analysis procedure.

As previously mentioned, the time-dependent behavior of soft biological tissues is, in some cases, better described by means of poroelasticity, where the time-dependence is due to the flow of a fluid inside a poroelastic structure. In order to investigate the capability of such a model to describe the indentation response, agarose gels were synthetized and indented to different depth with spherical indenters of different size at several loading rates. The previously defined QLV model and the Linear Poroelastic (LPE) material model describing the non-linear, time-dependent behavior of the gels were determined by means of FE inverse analysis. Predictions for different maximal loading and loading rates were evaluated with both models. The determined material formulations for the three gels using the LPE model were in line with the literature in terms of instantaneous Young’s moduli. A procedure for direct determination whether a material behaves visco or poro-like from measurements data was further proposed.

Based on the experience acquired during the defined investigations, CSM Instruments developed a new machine named CSM Bioindenter allowing for displacement-controlled indent up to 100 [µm] depth using a single spherical tip mounted on a long shaft.

Chapter 6-8 are organized similar to scientific papers, gradually tackling challenges encountered attempting to indent soft materials at the
microscale, namely challenges related to material non-linearities, surface forces and sample roughness (Chapter 6), challenges related to the analysis of time-dependent material indentation data (Chapter 7), and finally experimental challenges related to the indentation of materials exhibiting poroelastic behavior similar to some biological tissues (Chapter 8). Each of the discussed chapters are completed by an Appendix reporting corresponding indentation experimental data in greater details. These chapters are complemented with a brief introduction to the mechanical analysis of indentation (Chapter 2), a description of the setups used (Chapter 3) and an introduction to the FE automated tool used to run the simulations and perform the inverse analysis (Chapter 5). Chapter 9 concludes the present thesis recapitulating the main findings and giving recommendations for future research.
2.1 Contact Theory

A key influent factor in the indentation process is the interaction of the indenter tip with the material tested. Contact theories have been extensively documented and an introduction is given here; the following chapters aim at making the reader familiar with surface forces acting at the interface between the indenter and the sample as well as giving him insights about the analytical models and their assumptions that were developed over the years to describe such a contact.

2.1.1 Surface forces

The interaction between two macroscopic bodies is influenced by so-called surface forces acting in a third medium (e.g. vacuum or vapor) at the interface of their surfaces; the medium in which those forces raise is of major importance. Indeed, the main interactions present in vacuum are due to electromagnetic forces between ions (electrostatic forces) and between permanent or induced dipoles (van der Waals forces). In a vapor environ-
ment (i.e. atmospheric air containing water), the main contributor are the strong attractive capillary forces. These surface forces can be categorized over three aspects, namely their magnitude, the lengthscale at which they act, and the environment through which they act (Israelachvili, 2011), see Table 2.1.

**Electrostatic**

-La force répulsive de deux petits globes électrifiés de la même nature d’électricité, est en raison inverse du carré de la distance du centre des deux globes. Coulomb, 1785

Charles-Augustin de Coulomb (1785) was the first to quantitatively measure the attraction and the repulsion between two charged objects. He namely observed that the force between two charges is proportional to the product of the charges and inversely proportional to the square of the distance between them

\[
P_e = \frac{1}{4\pi\epsilon_0} \frac{q_1 q_2}{r^2}
\]  

(2.1)

where \( r \) is the straight distance between the charges, \( P_e \) represents the force acting in the direction along \( r \), \( q_1 \) and \( q_2 \) are the two charges, and \( \epsilon_0 \) is the permittivity constant.

Electrostatic interactions are highly attenuated in vapor environments such as fully immersion in water. Indeed, as the attractive force between two oppositely charged ions in a solution is inversely proportional to the dielectric constant of the solvent, the electrostatic force acting in water (\( \epsilon_0 = 80 \)) is attenuated as compared to vacuum environment (\( \epsilon_0 = 1 \)).

**Van der Waals**

Every object is made of thousands of atoms from which transient electric and magnetic fields arise spontaneously. The electric currents created through movement of charges interact with other charges and their fields; the coordinated interaction of these moving electric charges, currents and fields give rise to the so-called van der Walls forces.

The main difference between electrostatic and van der Waals forces is that electrostatic forces depend on the response of stationary charges to
constant electric fields, whereas electrodynamic van der Waals forces depend on all possible electromagnetic fields surrounding charges in motion.

Van der Waals forces existing between neutral atoms and molecules include three kinds of dipole-dipole interactions, namely the Keesom, Debye and London interactions. Their free energies, which represent the work needed to bring particles together, vary with the inverse-sixth power of distance \((-C/r^6)\), with the different coefficient \(C_{Keesom}\), \(C_{Debye}\) and \(C_{London}\) (Parsegian, 2005).

The Keesom interactions consist of dipole-dipole forces, in which the molecules involved have permanent dipoles that can interact by dipole-dipole interaction. In the case of Debye interaction, a permanent dipole induces a dipole in another non-polar atom or molecule. Finally, the so-called London dispersion are due to a fluctuations of the atoms that lead to an instantaneous displacement of the center of the positive charge against the center of the negative charge; a dipole is eventually created which induces a dipole in another atom.

In the 1930’s, Hamaker investigated the influence of van der Waals forces on large bodies interaction as opposed to small molecules interaction we discussed previously (Hamaker, 1937). Van der Waals forces can be significant between macroscopic bodies interacting closely (1-100 [Å]); the forces involved for the macroscopic interaction of a sphere and a flat surface are given by

\[
P_{vdW} = -\frac{A_H R}{6D}
\]

where \(R\) corresponds to the radius of the sphere, \(D\) the distance separating the sphere and the flat surface, and \(A_H\) is the interaction Hamaker constant defined as

\[
A_H = \pi^2 C \rho_1 \rho_2
\]

where \(C\) is the attractive interaction strength and \(\rho_i\) is the number density of the molecules in the solids. Van der Waals interaction thus highly depends on the dielectric properties of the materials that interact and of the medium that separates them. For instance, the Hamaker constant when interacting in a medium is one order of magnitude lower than in vacuum. As such, van der Waals forces are attenuated in solution though remaining ubiquitous forces acting both in vacuum and in liquids.
Figure 2.1 Water meniscus formation when approaching a sample with a spherical tip. Capillary forces pull the indenter towards the sample surface; as a result, the nanoindenter machine senses attractive forces.

CAPILLARITY

In air (i.e. typically 45% Relative Humidity (RH)), water vapor induces the formation of a thin water film at the surface of macroscopic bodies. In the special case of indentation, it is hypothesized that if the film reaches a specific minimum thickness (Beaglehole, 1992), a water meniscus can be formed at the interface between the sample’s surface and the indenter tip (Fig. 2.1); in air, forces due to water vapor are thought to play the dominant role. The formation of a water meniscus at the interface between the sample and the spherical tip leads to the detection of negative forces named meniscus forces or capillary forces ($P_{\text{cap}}$) by the nanoindenter machine. A representative maximum force at contact ($D = 0$) is given by

$$P_{\text{cap}} = 4\pi R\gamma_c \cos(\theta)$$  \hspace{1cm} (2.4)

where $R$ is the radius or the spherical tip, $\gamma_c$ is the surface tension of water (0.074 [N/m] at $T = 20^\circ C$) and $\theta$ is the meniscus contact angle (Fig. 2.1) (Israelachvili, 1991). Note that the previously introduced Eq. 2.4 is independent of the amount of water present at the interface. However, the capillary forces sensed are highly dependent of the thickness of the water
layer; indeed, an increase of the relative humidity leads to an increase of the thickness of the water layer and as a consequence of the capillary forces (Beaglehole, 1992), which underlined the limitation of the application of Eq. 2.4 to determine accurate capillary forces fully representative of the testing environment.

Studies investigating the influence of relative humidity on the magnitude of capillary forces have mainly been conducted through pull-off experiments with the help of AFM machines allowing for very low forces measurements (Thundat et al., 1993; Xu et al., 1998; Yang et al., 1996). It has been shown that the formation of the capillary neck requires a minimum height of the water film corresponding to a relative humidity of 40% (Beaglehole, 1992). As soon as the water film thickness reaches the minimum thickness requirement at 40% RH, a capillary neck forms between the tip and the sample surface, leading to an immediate increase of the pull-off forces Fig. 2.2). Under very low relative humidity levels (< 40% RH), which corresponds to an indentation in vacuum environment, the principal forces expected are the van der Waals forces. If the relative humidity is raised above 40% humidity, the forces due to meniscus formation might become dominant.

By deduction, if attractive forces would be sensed during the approach of the indenter in water (100% humidity), they would be assumed to be due to van der Walls forces as both capillarity and electrostatic effects are negligible in such environmental conditions. In the special case of spherical ($R = 100 \, [\mu m]$) indentation of a flat sample in air (45% Relative Humidity), the capillary forces are estimated through Eq. 2.4 in the order of tenth of $[\mu N]$.

### ADHESION

The work of adhesion ($\gamma$), which corresponds to the energy per unit area necessary to separate two bodies (1 and 2) for the special case of a spherical indenter being in contact with a flat surface is given by

$$\gamma = -\frac{A_H}{12\pi D_0^2}$$  \hspace{1cm} (2.5)

with $D_0$ corresponding to the cutoff distance and $A_H$ the previously defined Hamaker constant (Ebenstein and Wahl, 2006; Lomboy et al., 2011).
2. Mechanical Analysis of Indentation

Figure 2.2  Capillary and van der Waals forces for different relative humidity during the pull-off phase of indentation. These hypothesis have been confirmed analyzing surface forces under different relative humidity conditions with atomic force microscope (Thundat et al., 1993; Xu et al., 1998; Yang et al., 1996).
### Contact Theory

<table>
<thead>
<tr>
<th>Force type</th>
<th>Subclasses (Attractive)</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrostatic</td>
<td>Ionic bond (v)</td>
<td>Long-ranges force arising in polar solvents. Requires surface charging or charge-separation mechanism.</td>
</tr>
<tr>
<td></td>
<td>Coulombic force (v &amp; s)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hydrogen bond (v)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Charge exchange interaction (v &amp; s)</td>
<td></td>
</tr>
<tr>
<td>Van der Waals</td>
<td>Debye induced dipole force (v &amp; s)</td>
<td>Ubiquitous force occurring both in vacuum and in liquids. They are significant in the range of a few Å to hundreds of Å.</td>
</tr>
<tr>
<td></td>
<td>London dispersion force (v &amp; s)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Keesom force (v &amp; s)</td>
<td></td>
</tr>
<tr>
<td>Capillary</td>
<td>Meniscus force (40% &lt; RH &lt; 80%)</td>
<td>Formation of a meniscus if the thickness of the water film is sufficiently large. They act over large length-scale (tenth of microns).</td>
</tr>
</tbody>
</table>

Table 2.1 Surface forces summary occurring in vacuum (v) or in solution (s) (Israelachvili, 1991, 2011).

The pull-off force \( P_{adh} \) at maximum adhesion is given by

\[
P_{adh} = -\frac{3}{2} \pi \gamma R \tag{2.6}
\]

The work of adhesion serves as a basis for the introduction of the JKR model (Section 2.1.3).

#### 2.1.2 Hertzian Contact

In 1880, Hertz developed the first complete contact theory when studying Newton’s optical interference fringes in the gap between two glass lenses; he was particularly interested by the possible influence of elastic deformation of lenses surfaces due to the contact pressure between them. The following text is based on the Hertzian contact theory reported by Johnson (1985).

The Hertzian contact theory aims at predicting the contact between two non-conforming solids in the vicinity of the contact region, namely helping describing the shape of the area of contact, the magnitude and distribution of the surface tractions, and finally the components of deformation and stress in both bodies. Prior to the description of the elastic formulation of contact stated by Hertz, an introduction to the geometry
of contacting surfaces will be given.

Considering the surfaces of two bodies being smooth (i.e. surface irregularities disregarded), we can express their profile in the region close to the origin by homogeneous functions which can be seen as ellipses (Hertz first assumption)

\[
\begin{align*}
  z_1 &= A_1 x_1^2 + B_1 y_1^2 \\
  z_2 &= A_2 x_2^2 + B_2 y_2^2
\end{align*}
\] (2.7)

The terms \( A_1, A_2, B_1 \) and \( B_2 \) can be expressed as functions of \( R'_1, R''_1, R'_2 \) and \( R''_2 \) which are the principal radii of curvature of the surface at the origin; they correspond to the maximum and minimum values of the radius of curvature of all possible cross-sections of the profile (Fig. 2.3). One can thus rewrite the profile equations as

\[
\begin{align*}
  z_1 &= \left( \frac{1}{2R'_1} \right) x_1^2 + \left( \frac{1}{2R''_1} \right) y_1^2 \\
  z_2 &= \left( \frac{1}{2R'_2} \right) x_2^2 + \left( \frac{1}{2R''_2} \right) y_2^2
\end{align*}
\] (2.8)

Note that assuming the simple case of solids of revolution, the radii of curvature previously defined simplify as

\[
\begin{align*}
  R'_1 &= R''_1 = R_1 \\
  R'_2 &= R''_2 = R_2
\end{align*}
\] (2.9)

The separation (Fig. 2.4) between the surfaces is further given by

\[
\begin{align*}
  h_{\text{initial}} &= z_1 - z_2 = Ax^2 + By^2 \\
  &= \frac{1}{2} \left( \frac{1}{R_1} + \frac{1}{R_2} \right) (x^2 + y^2) = \frac{1}{2R} r^2
\end{align*}
\] (2.10)

\[
\text{with } A = B = \frac{1}{2} \left( \frac{1}{R_1} + \frac{1}{R_2} \right) = \frac{1}{2}
\]

One can further consider the deformation of the two bodies as a compressive normal load \( P \) is applied. Prior to deformation, the separation between two corresponding surface points \( S_1(x, y, z_1) \) and \( S_2(x, y, z_2) \) is given by \( h_{\text{initial}} \) (Eq. 2.10). During the compression phase, two points \( T_1 \) and \( T_2 \) belonging to bodies 1 and 2 respectively, move straight towards each other by displacements corresponding to \( \delta_1 \) and \( \delta_2 \); please note that
Figure 2.3 Principal planes of curvature for two Bodies 1 and 2 allow the description of $R_1$, $R'_1$, $R''_1$, $R_2$, $R'_2$, and $R''_2$. 
Figure 2.4 Surface profile of bodies 1 and 2 in the vicinity of contact exhibiting the separation $h$ between them.

these two points are assumed to be far enough from the zone of interaction in order for their vicinity not to be deformed by the contact. The dotted lines and the points $S_1$ and $S_2$ represent the surface profiles if the bodies would not deform (Fig. 2.5). However, due to contact pressure, the surfaces of each body deform and the actual location of $S_1$ and $S_2$ is shifted parallel to the $z$-axis by an amount $u_{z1}$ and $u_{z2}$. Assuming the points $S_1$ and $S_2$ coincident with the contact surface after deformation, the separation between the surfaces is given by

$$h_{\text{deformed}} = h_{\text{initial}} - \delta + u_z$$

$$= \frac{1}{2} \left( \frac{1}{R_1} + \frac{1}{R_2} \right) (x^2 + y^2) - (\delta_1 + \delta_2) + (u_{z1} + u_{z2})$$

(2.11)

where $\delta$ corresponds to the applied displacement and $u_z$ to the elastic displacement. The elastic vertical displacement is finally given in the contact zone as

$$(u_{z1} + u_{z2}) = \delta - h_{\text{initial}}$$

(2.12)

and outside the contact area as

$$(u_{z1} + u_{z2}) < \delta - h_{\text{initial}}$$

(2.13)
In order to solve the problem, one needs to find the distribution of stress that is transmitted through the two bodies in the contact region such that the resulting displacement satisfies Eq. 2.12 and Eq. 2.13. Recalling Eq. 2.10, Eq. 2.12 can be rewritten as

\[(u_{z1} + u_{z2}) = \delta - \left( \frac{1}{2R^2} r^2 \right) \] (2.14)

The contact area is further assumed to be circular and its radius (Fig. 2.6) is defined as

\[r_{contact\ circle} = \sqrt{x_1^2 + y_1^2} = r_1 = a \] (2.15)

The pressure distribution proposed by Hertz in the contact region (Fig. 2.7)

\[r_{contact\ region} \leq r_1 \]

\[p(r) = p_0 \left\{ 1 - \left( \frac{r}{a} \right)^2 \right\}^{1/2} \] (2.16)

induces normal displacements given by

\[u_z = \frac{1 - \nu^2}{E} \frac{\pi p_0}{4a} (2a^2 - r^2) \] (2.17)

As the pressure acting on the first body is equal to the pressure acting
2. Mechanical Analysis of Indentation

Figure 2.6 Representation of the contact radius \( a \) \((r_1)\).

Figure 2.7 Pressure distribution introduced by Hertz (Johnson, 1985).

on the second body and knowing that the reduced modulus is defined as

\[
\frac{1}{E_r} = \frac{1 - v_1^2}{E_1} + \frac{1 - v_2^2}{E_2}
\]

(2.18)

One can rewrite Eq. 2.17 as

\[
(u_{z1} + u_{z2}) = \frac{\pi p_0}{4a E_r} (2a^2 - r^2) = \delta - \left( \frac{1}{2R} r^2 \right)
\]

(2.19)

The contact radius can thus be computed from

\[
a = \frac{\pi p_0 R}{2E_r}
\]

\[
p_0 = \frac{2a E_r}{\pi R}
\]

(2.20)
and the mutual approach between 2 points in the two bodies is given by

$$\delta = \frac{\pi ap_0}{2E_r}$$  \hfill (2.21)\\

In the contact region, one can express the total compressive load by integrating the pressure distribution over a contact region \((a)\) by

$$r_{contact\ region} = [0, a]$$

$$P = \int_0^a p(r)2\pi r \, dr = \frac{2}{3} p_0 \pi a^2$$  \hfill (2.22)\\

The load \(P\) can be expressed as a function of the contact radius, the displacement of the spheres \(\delta\) and the pressure \(p_0\)

$$a = \left(\frac{3PR}{4E_r}\right)^{1/3}$$  \hfill (2.23)\\

$$\delta = \frac{a^2}{R} = \left(\frac{9P^2}{16RE_r^2}\right)^{1/3}$$  \hfill (2.24)\\

$$p_0 = \frac{3P}{2\pi a^2} = \left(\frac{6PE_r}{\pi^3 R^2}\right)^{1/3}$$  \hfill (2.25)\\

Note that the present model is valid for spherical bodies and only if the assumptions listed below are respected, namely

- The surface are continuous and non-conforming (concentrated contact)
- Smooth surface considered: \(\text{roughness} \ll R\)
- The strains are small: \(a \ll R\)
- Each solid can be considered as an elastic half-space
- The surfaces are frictionless

As underlined by Johnson (1985), the use of such an analytical solution for analyzing nanoindentation experiments conducted on metallic solids complies with those restrictions. However, indenting much softer materials like biological tissues for which the radius of contact can reach half the size of the indenter tip radius clearly exceed the restriction to small strains, and as a consequence question the use of such a model for data analysis.
2. Mechanical Analysis of Indentation

EXTENSION TO VISCOELASTIC BODIES

Lee and Radok (1960) were the first to extend the Hertzian contact to materials that exhibit viscoelastic properties; the assumptions of the previously defined Hertz model are maintained and the model extension described further is limited to small strain elastic deformations. In the case of a rigid indenter tip entering a deformable material, Eq. 2.24 can be rewritten as

\[
\frac{4\sqrt{R}}{3} h^{3/2} = \frac{(1 - v^2)}{E} P
\]

(2.26)

where \( h \) represents the displacement of the indenter previously introduced as \( \delta \). Based on linear viscoelasticity, assuming that the material tested is an incompressible solid \( (v = 0.5) \), and replacing the elastic constant \( (3/2E) \) by a corresponding linear viscoelastic integral operator (Lee and Radok, 1960), one can rewrite the contact equation as

\[
\frac{16\sqrt{R}}{9} h(t)^{3/2} = \int_0^t J_{AS}(t-u) \frac{dP}{du} du
\]

(2.27)

where \( u \) represents the variable of integration and \( J_{AS}(t) \) is the creep compliance function, representing the time function of strain increase for a constant applied stress; in the present work, the particular form of creep compliance

\[
J_{AS}(t) = C_0 - \sum C_i \exp(-t/\tau_i)
\]

(2.28)

for which \( i = 1,2 \) was chosen. In the case of a step load experiment (Heaviside step function), the relationship between the displacement \( (h) \) and the maximal load \( (P_{max}) \) becomes

\[
t \geq 0, \quad P(t) = P_{max} = \text{const.}
\]

\[
h(t) = \left( \frac{9P_{max}}{16\sqrt{R}} \left\{ C_0 - \sum C_i \exp(-t/\tau_i) \right\} \right)^{2/3}
\]

(2.29)

where the displacement \( h \) is, additionally to the maximum load \( P_{max} \), a function of amplitude coefficients \( (C_0, C_i) \) and time constants \( (\tau_1, \tau_2) \).

Oyen (2005) introduced a more realistic version of the extension proposed by Lee and Radok (1960), in that sense that it incorporate a ramp
function (instead of the step function) more appropriate to analyze experiments. For the special case of indentation ramp loading, the load is split into a ramp phase \(0 \leq t \leq t_R\) and an hold phase \(t \geq t_R\) giving rise to the relationship between the displacement and the creep function parameters

\[
0 \leq t \leq t_R, \quad P(t) = kt
\]

\[
h(t) = \left(\frac{9k}{16\sqrt{R}} \left\{C_0 t - \sum C_i \tau_i [1 - \exp(-t/\tau_i)]\right\}\right)^{2/3} \tag{2.30}
\]

\[
t \geq t_R, \quad P(t) = P_{max} = kt_R = \text{const.}
\]

\[
h(t) = \left(\frac{9k}{16\sqrt{R}} \left\{C_0 t - \sum C_i \tau_i \exp(-t/\tau_i) \left[\exp(t_R/\tau_i) - 1\right]\right\}\right)^{2/3} \tag{2.31}
\]

### 2.1.3 Adhesion

Johnson, Kendall and Roberts (JKR) modeled the adhesion between two smooth surfaces being in contact, extending the analytical solution introduced by Hertz to adhesive surfaces. Defining the separation between two surfaces as

\[
(u_z^1 + u_z^2) = \delta - \frac{1}{2} \left(\frac{1}{R_1} + \frac{1}{R_2}\right)(x^2 + y^2)
\]

\[
\text{with} \quad \delta = \frac{\pi a (p_0 + 2p'_0)}{2E_r} \tag{2.32}
\]

and a pressure distribution as

\[
p(r) = p_0 \left\{1 - \left(\frac{r}{a}\right)^2\right\}^{1/2} + p'_0 \left\{1 - \left(\frac{r}{a}\right)^2\right\}^{-1/2}
\]

\[
\text{Hertz} \quad \text{Adhesion} \tag{2.33}
\]

The total compressive load can be calculate as

\[
P = \int_0^a p(r)2\pi r \, dr = \left(\frac{2}{3}p_0 + 2p'_0\right) \pi a^2 \tag{2.34}
\]

with

\[
p_0 = \frac{2aE_r}{\pi R}
\]

\[
p'_0 = -\left(\frac{4\gamma E_r}{\pi a}\right) \tag{2.35}
\]
2. Mechanical Analysis of Indentation

The contact radius is finally related to the load by

\[ a^3 = \left( \frac{3R}{4E_r} \right) \left[ P_{\text{Hertz}} + 6\pi\gamma E_r R + \sqrt{12\pi\gamma E_r R P + 6(\pi\gamma E_r R)^2} \right] \]

(2.36)

where \( \gamma \) represents the work of adhesion previously introduced (Eq. 2.5). Comparison with Eq. 2.23 allows for evaluation of the differences between the two models. Note that the added term account for the tensile force required to separate both surfaces from each other.

2.1.4 **Sneddon**

In 1948 and further on in 1965, Sneddon proposed that the elastic load-displacement relationships for many simple indenter tip geometries can conveniently be written as

\[ P = \alpha h^m \]

(2.37)

where \( P \) is the indenter load, \( h \) is the elastic displacement of the indenter, and \( \alpha \) and \( m \) are constants. Values of the exponent \( m \) for different indenter tip geometries can be reported namely \( m = 1 \) for flat cylinders, \( m = 1.5 \) for spheres and paraboloids of revolution, \( m = 2 \) for cones in the limit of small displacement (i.e. linear deformation).

2.1.5 **Indentation data analysis**

**Introduction**

A common procedure widely used for nanoindentation data analysis is the well-known Oliver and Pharr method (Oliver and Pharr, 1992). The following chapter aims at underlying the different contributions that lead to the development of this analysis method for the determination of elastic properties from materials exhibiting elasto-plastic behaviors.

**Tabor (1948)**

Initial experiments using depth-sensing machine were conducted by Tabor on metals; the main observation he made analyzing the residual imprints
using a profilometer was that its radius was bigger than the radius of the spherical indenter tips mounted for the indentation tests (Fig. 2.8). He concluded that the diameter of the contact impression did not recover during the unloading phase, as opposed to the indentation depth which reduced significantly. He further observed that a second application and removal of the indenter under the original load did not change the size and shape of the indent, confirming the unloading process to be purely elastic and possibly described by Hertz theory. Tabor was thus able to extract elastic properties from materials exhibiting elasto-plastic behavior under deformation. He additionally introduced the relation to obtain representative strains as

$$\epsilon \approx 0.2 \frac{a_c}{R}$$  \hspace{1cm} (2.38)

where $a_c$ stands for the contact radius (see Fig. 2.10) and $R$ for the radius of the sphere (Hutchings, 2009).

**Bulychev et al. (1975)**

In the early 1970’s, researchers from the Baikov Institute of Metallurgy used instrumented micro-hardness instruments to obtain load-displacement...
data as shown in Fig. 2.9. Based on the previously described observations made by Tabor and on the derivations of the contact law proposed by Sneddon (1948, 1965), Bulychev et al. (1975) introduced an experimental procedure allowing the calculation of the reduced Young’s modulus (Eq. 2.39). This solution is based on the so-called contact stiffness equation where the stiffness \( S \) is a function of the reduced modulus \( E_r \) and of the projected contact area \( A \) given as

\[
S = \frac{dP}{dh} = \frac{2}{\sqrt{\pi}} E_r \sqrt{A}
\]

where \( S = \frac{dP}{dh} \) corresponds to the experimentally measured stiffness of the upper portion of the unloading data, \( E_r \) being the reduced modulus defined by Tabor (1948), and \( A \) is the projected area of the elastic contact (Fig. 2.10). Measuring the initial unloading stiffness and assuming that the contact area is equal to the optically measured area of the hardness impression, the reduced modulus can be derived. Bulychev and al. showed that Eq. 2.39 is not only valid for a conical indenter, but also for spherical and cylindrical indenters. Although Bulychev and al. were able to successfully measure the experimental stiffness of tested materials, they were able to relate the determined stiffness to the reduced modulus only approximately due to the technical limitations preventing them to accurately determine the projected area. Indeed, they approximated the contact area at maximum depth to be equal to the optically measured area of the hardness impression (Oliver and Pharr, 1992).

DOERNER AND NIX (1986)

Based on the stiffness equation introduced by Bulychev et al. (1975) and their observations that limited changes in contact area occur only during the early phase of unloading, Doerner and Nix (1986) argued that the indentation contact behaves similarly to a flat cylindrical punch indentation in the early phase of unloading (i.e. linear behavior of the initial portions of unloading curves). As previously stated, the contact stiffness equation requires the accurate determination of the indentation area; knowing the accurate shape of their tips, Doerner and Nix proposed to express the projected contact area \( A \) as a function of the contact
Figure 2.9 Bulychev and al. proposed a simple experimental method for extracting elastic properties from elasto-plastic material behavior based on the retraction stiffness $dP/dh$. The method was further extended by Doerner and Nix using $h_c$ for determination of the area of contact later used by Oliver and Pharr.

depth ($h_c$), which is defined as the contact distance along which contact is made (Figs. 2.9, 2.10) (Oliver and Pharr, 1992)

$$A = f(h_c) \quad (2.40)$$

The method developed to determine the contact area function is based on the extrapolation of the initial linear portion of the unloading curve to the zero load (displacement) axis; the corresponding extrapolated depth is used together with the indenter shape function to determine the contact area. Doerner and Nix further showed that the extrapolated depth allows for a better estimation of the contact area than the final depth ($h_f$) or the depth at peak load ($h_{max}$) (Fig. 2.10). The contact stiffness equation was for the first time completed and elastic properties of materials for the first time extracted from nanoindentation experiments.

OLIVER AND PHARR (1992)

As an initial step, Oliver and Pharr (1992) demonstrated that the contact stiffness equation proposed by Bulychev et al. (1975) is valid for
2. Mechanical Analysis of Indentation

any axisymmetric indenters. They further observed that the initial part, originally considered as behaving linearly (flat punch assumption) by the Doerner-Nix method, would rather be fitted by a power law written as

\[ P = \alpha h^m \]  

(2.41)

where \( P \) corresponds to the indenter load, \( h \) to the elastic displacement, \( \alpha \) and \( m \) (1.2 to 1.6) being constants relative to the indenter tip shape. The slope of this function at maximum load is further defined as the contact stiffness. Note that this power law account for changes in contact area expected to occur during the unloading process. As a second step, Oliver and Pharr defined new area functions that better predict the projected area; the contact depth (Fig. 2.10) for different indenter tips was defined as

\[ h_c = h_f - \lambda \frac{P_{max}}{S} \]  

(2.42)

where \( \lambda \) corresponds to a geometric constant specific to indenter tip shapes (0.72 for conical, 0.75 for spherical, and 1 for flat punch). Both the improved contact stiffness and the improved projected area function are further used for the determination of the indentation modulus through the use of the contact stiffness equation. Please note that the method assumes a purely elastic contact (Tabor assumption), an ideal geometry of the indenter tips, and that no surface forces take place at the contact interface during the unloading phase of indentation.

The improved Doerner and Nix (1986) method, commonly referred to as the Oliver and Pharr model, is still widely used as the standard model for extracting elastic properties of materials from indentation experiments.

2.2 CONSTITUTIVE MODELING

Physical objects are assemblies of many molecules and particles; the method of continuum mechanics used in the present work consists of considering these assemblies of discrete particles as continuum bodies. The field of continuum mechanics namely comprises the study of motion and deformation, the study of stress, and the description of fundamental laws of
Constitutive modeling

Figure 2.10 Representation of the area of contact and its related contact depth during the indentation process.

physics that govern the motion of continuums (Holzapfel, 2006). Note that the solution extracted from continuum studies are not exact but are intended to provide sufficient approximation to engineering problems.

When aiming at describing the motion or deformation of solids, it is conventional to track the evolution of their configurations as time progresses; it can be done using either the Lagrangian (Fig. 2.11) or the Euler description. In the first one, the motion is described in terms of a fixed referential coordinates whereas the second one describes the motion through a spatial coordinates corresponding to the current configuration. The theory further introduced (Banks et al., 2011; Holzapfel, 2006) is based on the Lagrangian description and focuses on deformation (as opposed to rigid body motion). In the Lagrangian description, one observes and describes the motion or deformation of an object from a fixed point; the motion of
2. Mechanical Analysis of Indentation

![Reference configuration](image1) ![Current configuration](image2)

**Figure 2.11** Lagrangian description exhibiting undeformed \(dX\) and deformed \(dx\) differential elements.

A continuum is described by the mapping relation between its reference \((K)\) and current \((k)\) configurations and expressed by a function \(h\), which relates how a particle moves as time goes by

\[
x = h(X, t)
\]  

(2.43)

where the position of a particle \(P\) in the reference configuration is given by the position vector \(X = (X_1, X_2, X_3)^T\); the same particle in the current configuration is denominated \(p\) and is described by the position vector \(x = (x_1, x_2, x_3)^T\). In the following section, the vectors are singly underlined and the tensors doubly underlined.

**Kinematics**

The deformation gradient \(A\), embodying the local nature of the deformation, is defined as

\[
A = \frac{dx}{dX} = \begin{bmatrix}
\frac{\partial x_1}{\partial X_1} & \frac{\partial x_1}{\partial X_2} & \frac{\partial x_1}{\partial X_3} \\
\frac{\partial x_2}{\partial X_1} & \frac{\partial x_2}{\partial X_2} & \frac{\partial x_2}{\partial X_3} \\
\frac{\partial x_3}{\partial X_1} & \frac{\partial x_3}{\partial X_2} & \frac{\partial x_3}{\partial X_3}
\end{bmatrix}
\]  

(2.44)

It describes the local deformation of the body \(B\) through the mapping of a differential line element \(dX\) at position \(X\) from the reference configuration.
Constitutive modeling

\( K \) to its respective differential element \( d\mathbf{x} \) at position \( \mathbf{x} \) in the current configuration \( (k) \) (Fig. 2.11), i.e.

\[
\mathbf{d} \mathbf{x} = \mathbf{A} d\mathbf{X}
\]  

(2.45)

In order to obtain measures of strain, it is sufficient to additionally know about the change in length of any arbitrary differential line element (e.g. \( PQ - pq \) in our case) present in the continuum for two different configurations. One can easily calculate the square of the infinitesimal line segments connecting \( PQ \ (d\mathbf{X}) \) and \( pq \ (d\mathbf{x}) \) through

\[
|\mathbf{d} S|^2 = (d\mathbf{X})^T d\mathbf{X} = (dX_1)^2 + (dX_2)^2 + (dX_3)^2
\]

(2.46)

\[
|\mathbf{d} s|^2 = d\mathbf{x}^T d\mathbf{x} = (dx_1)^2 + (dx_2)^2 + (dx_3)^2
\]

and define the stretch ratio \( (\lambda) \) of a material line element in terms of the ratio of the lengths \( |\mathbf{d} s| \) and \( |\mathbf{d} S| \), namely

\[
\lambda = \frac{|\mathbf{d} s|}{|\mathbf{d} S|}
\]  

(2.47)

Subtracting the reference squared length \( |\mathbf{d} S|^2 \) to the current squared length \( |\mathbf{d} s|^2 \) and recalling Eq. 2.45, one can get

\[
|\mathbf{d} s|^2 - |\mathbf{d} S|^2 = (d\mathbf{x})^T d\mathbf{x} - (d\mathbf{X})^T d\mathbf{X} = (d\mathbf{X})^T (\mathbf{A}^T \mathbf{A} - I) d\mathbf{X}
\]

(2.48)

The Lagrangian (Green) strain tensor \( \mathbf{E} \) is finally defined as

\[
\mathbf{E} = \frac{1}{2} (\mathbf{A}^T \mathbf{A} - I)
\]  

(2.49)

and the relationship between the Lagrangian strain and the displacement \( (U) \) is given by

\[
E_{ij} = \frac{1}{2} \left[ \frac{\partial U_i}{\partial X_j} + \frac{\partial U_j}{\partial X_i} + \frac{\partial U_k}{\partial X_i} \frac{\partial U_k}{\partial X_j} \right]
\]  

(2.50)

where \( E_{ij} \) is the \( (i, j) \) component (see Appendix A) of the Lagrangian strain tensor \( \mathbf{E} \) (Eq. 2.49). Additional tensors often encountered in the finite strain theory are the right Cauchy-Green configuration tensor defined as

\[
\mathbf{C} = \mathbf{A}^T \mathbf{A}
\]  

(2.51)
and the left Cauchy-Green configuration tensor given by

\[ B = AA^T \quad (2.52) \]

Scalar terms such as the invariants of the right and left Cauchy-Green configuration tensors are often used to define expressions for strain energy density functions; the frequently encountered equivalent main invariants of \( C \) and \( B \) are

\[
\begin{align*}
I_1 &= tr(C) = \lambda_1^2 + \lambda_2^2 + \lambda_3^2 \\
I_2 &= \frac{1}{2} \left[ (tr(C))^2 - tr(C^2) \right] = \lambda_1^2\lambda_2^2 + \lambda_2^2\lambda_3^2 + \lambda_3^2\lambda_1^2 \\
I_3 &= det(C) = \lambda_1^2\lambda_2^2\lambda_3^2 = J^2
\end{align*}
\]  

(2.53)

where \( \lambda_i \) represent the eigenvalues of \( A \) corresponding to the principal stretches, and \( J \) corresponds to the ratio between a volume element in the current \((dv)\) and the reference configuration \((dV)\)

\[
J = det(A) = \frac{dv}{dV} \quad (2.54)
\]

with \( det(A) \) being a pure measure of dilatation; note that for an incompressible material, \( J \) is equal to 1.

**THE CONCEPT OF STRESS**

The stress, which concept was first introduced by Cauchy (1882), is a measure of the average amount of force exerted per unit area \([(N/m^2) = [Pa]]\). The principal idea introduced by Cauchy, most often referred to as the free body principle, consists of the assumption that an arbitrary subsystem of a body \( B \) can be freed and internal contacting forces keeping it shape-consistent imagined; indeed, upon any arbitrary surface element \((da)\) present within the body \( B \), one can imagine an existing integrable field of traction vectors \( t \) equipollent to the action exerted by the matter exterior to the surface element (Fig. 2.12). Note that an infinite number of traction vectors act simultaneously at a point through different normal vectors on different surfaces. Additionally, as a direct consequence of Newton’s law of action and reaction, the Cauchy’s lemma states that
traction vectors acting on opposite sides of a surface element are equal and opposite in direction, i.e.

\[
\mathbf{t}(\mathbf{n}) = -\mathbf{t}(-\mathbf{n}) \tag{2.55}
\]

Cauchy’s law states that there exists a unique second order tensor field \( \mathbf{\sigma} \), known as the Cauchy stress tensor, which allow mapping the normal surface vector \( \mathbf{n} \) to the traction vector \( \mathbf{t} \) as

\[
\mathbf{t} = \mathbf{\sigma} \mathbf{n} \tag{2.56}
\]

The local load \( dp \) exerted on an element of surface \( da \) may finally be written as

\[
dp = \mathbf{t}da = \mathbf{\sigma} \mathbf{n}da \tag{2.57}
\]

where \( \mathbf{t} \) physically represents the force per unit area exerted on a surface element \( da \) oriented with the normal \( \mathbf{n} \). Note that the Cauchy stress tensor is symmetric due to the fact that equilibrium requires the resultant moments to vanish. Additional stress tensors encountered in practice are further introduced, namely the two Piola-Kirchhoff stress tensors. The first Piola-Kirchhoff stress tensor \( \mathbf{P} \) is defined as

\[
\mathbf{P} = J \mathbf{\sigma} \mathbf{A}^{-T} \tag{2.58}
\]

and relates forces in the present configuration to areas in the initial configuration; the first Piola-Kirchhoff tensor is in general not symmetric.
2. Mechanical Analysis of Indentation

The second Piola-Kirchhoff stress tensor \( \mathbf{\mathcal{S}} \) is symmetric and is given by

\[
\mathbf{\mathcal{S}} = JA^{-1} \alpha A^{-T} = A^{-1} P
\]

(2.59)

It relates forces to areas in the reference configuration.

**Constitutive relationship**

Once kinematic relations and the concept of stress have been defined, constitutive laws describing the relationship between stress and strain should be introduced.

2.2.1 **Elastic materials**

Elasticity can be defined as the property of a material that when deformed under specific stress, it can fully recover its original shape when the stress is removed. It thus means that the stress-strain curve follows the same path for its loading and unloading process. Note that the stress is only dependent of the current strain and not on its history (as it would be for viscoelasticity).

**Linear elasticity**

In the infinitesimal theory, the necessity of specifying whether the strains are measured with respect to the current (Eulerian description) or reference (Lagrangian description) configurations is not encountered as it is irrelevant whether the derivatives of the displacement are calculated at the position of a point before or after deformation; as such, the distinction between the Lagrangian and Eulerian strain tensors disappears \( (\epsilon_{ij} \approx E_{ij} \approx e_{ij}) \). Additionally, the first derivatives of the displacement \( u_i \) are so small that higher order terms (see Eq. 2.50) such as the products of their partial derivatives are negligible; as such, the strains \( \epsilon_{ij} \) reduces to the Cauchy’s infinitesimal symmetric strain tensor given by

\[
\epsilon_{ij} = \frac{1}{2} \left[ \frac{\partial u_i}{\partial x_j} + \frac{\partial u_j}{\partial x_i} \right]
\]

(2.60)

Recalling that no distinction exists between the Lagrangian and Eulerian descriptions in the infinitesimal theory, the Cauchy stress tensor, the first
Piola-Kirchhoff stress tensor and the second Piola-Kirchhoff tensor are identical; indeed, the necessity of specifying whether the stresses are measured with respect to the initial or deformed configurations arise only in finite strain theory. Most materials behave elastically under small strains and follow a linear constitutive law known as Hooke’s law given by

$$\sigma_{ij} = C_{ijkl}\epsilon_{kl}$$  \hspace{1cm} (2.61)

where the stress $\sigma_{ij}$ is related to the strain $\epsilon_{ij}$ by a $4^{th}$-order elasticity tensor $C_{ijkl}$. For isotropic materials, the following relationship holds

$$\sigma_{ij} = 2\mu\epsilon_{ij} + \lambda\delta_{ij}\epsilon_{kk}$$  \hspace{1cm} (2.62)

where $\mu$ and $\lambda$ are the Lamé constants, and $\delta$ the Kronecker delta. Additionally, the von Mises stresses can be defined as

$$\sigma_{\text{von Mises}} = \sqrt{\frac{3}{2} \sigma_{ij}\sigma_{ij} - \frac{1}{2}(\sigma_{kk})^2}$$  \hspace{1cm} (2.63)

**NONLINEAR ELASTICITY**

Hyperelastic (i.e. Green elastic) materials are ideal elastic (i.e. non-dissipative) materials for which a strain energy density function exists. The strain energy density function ($W$) provides a measure of the energy stored in the material as the material deforms. Note that soft elastomers and biological tissues are considered as incompressible materials that most often exhibit hyperelastic, time-dependent behaviors.

Denoting $W$ the strain energy function and defining it as a scalar function of the deformation gradient $A$ (Eq. 2.45), the first Piola-Kirchhoff stress tensor ($P$) is given by

$$P = \frac{\partial W}{\partial A}$$  \hspace{1cm} (2.64)

or in terms of the Lagrangian strain tensor ($E$) as

$$P = A\frac{\partial W}{\partial E}$$  \hspace{1cm} (2.65)
2. Mechanical Analysis of Indentation

Please note that the stress state is independent of time (i.e. no time-dependence) and only depends of the deformation state of the material itself. The second Piola-Kirchhoff stress tensor \( \tilde{\mathbf{S}} \) is defined as

\[
\tilde{\mathbf{S}} = \frac{\partial W}{\partial \mathbf{E}}
\]  

(2.66)

Finally, the Cauchy stress tensor \( \mathbf{\sigma} \) is given by

\[
\mathbf{\sigma} = \frac{1}{J} \mathbf{A} \frac{\partial W}{\partial \mathbf{E}} \mathbf{A}^T
\]  

(2.67)

For the special case of isotropic materials, the deformation gradient \( \mathbf{A} \) can be expressed in terms of the main invariants \( I_1, I_2, I_3 \) of the left Cauchy-Green deformation tensor, thus a general form of strain energy function can be written as

\[
W(I_1, I_2, I_3) = \sum_{i,j,k=0}^{\infty} C_{ijk} (I_1 - 3)^i (I_2 - 3)^j (I_3 - 1)^k
\]  

(2.68)

Note that although the formulation of the strain energy functions must be carefully chosen, tuning of the corresponding parameters is based on empirical observations and is material specific. Assuming the incompressibility of materials, the determinant of the deformation gradient \( \mathbf{A} \) equals 1 (i.e. \( \det(\mathbf{A}) = 1, \lambda_1 \lambda_2 \lambda_3 = 1 \) and \( I_3 = 1 \)), so Eq. 2.68 reduces to

\[
W(I_1, I_2) = \sum_{i,j=0}^{\infty} C_{ij} (I_1 - 3)^i (I_2 - 3)^j
\]  

(2.69)

The present work focuses on the use of two strain energy density functions, namely the Neo-Hookean (Chapter 6) and the Yeoh formulations (Chapters 7, 8). The simplest form of the reduced polynomial functions is known as the Neo-Hookean formulation and is defined as

\[
W = C_{10} (I_1 - 3)
\]  

(2.70)

where \( C_{10} \) is the single material parameter. The reduced Yeoh formulation is further defined as

\[
W = \sum_{i=1}^{3} C_{i0} (I_1 - 3)^i
\]  

(2.71)
where $C_{10}$, $C_{20}$ and $C_{30}$ are the material parameters. Some materials behave differently in spherical and shear deformations, and as such, it is most convenient to split the deformation locally into a volumetric (dilational) and an isochoric (distortional) parts. This can be achieved by a multiplicative decomposition of $\mathbf{A}$ and $\mathbf{C}$ into a volume-changing (dilational) and a volume-preserving (distortional) parts, namely according to

$$
\mathbf{A} = (J^{1/3}I)\mathbf{A}
$$
$$
\mathbf{C} = (J^{2/3}I)\mathbf{C}
$$

(2.72)

where the terms $J^{1/3}I$ and $J^{2/3}I$ are associated with the volume-changing deformations, whereas $\mathbf{A}$ and $\mathbf{C}$ correspond to volume-preserving deformations of the material (Holzapfel, 2006) and are referred to as the modified deformation gradient and modified right Cauchy-Green tensor, respectively. Similarly, the strain energy density function $W$ can be decomposed into a volumetric elastic ($W_{vol}$) and an isochoric elastic ($W_{iso}$) parts, i.e.

$$
W = W_{vol}(J) + W_{iso}(\mathbf{C})
$$

(2.73)

which can be expressed in terms of the invariants of $\mathbf{C}$, i.e.

$$
W = W_{vol}(J) + W_{iso}(I_1, I_2)
$$

(2.74)

that are functions of the invariants of $\mathbf{C}$

$$
\mathbf{I}_1 = J^{-2/3}I_1
$$
$$
\mathbf{I}_2 = J^{-4/3}I_2
$$
$$
\mathbf{I}_3 = 1
$$

(2.75)

The Yeoh strain energy formulation can finally be adapted as

$$
W = \sum_{i=1}^{3} C_{i0}(\mathbf{I}_1 - 3)^i + \frac{1}{D_i} (J - 1)^{2i}
$$

(2.76)

where $C_{i0}$ characterize the distortional response of the material whereas $D_i$ are the material parameters accounting for its dilatation.
2. Mechanical Analysis of Indentation

2.2.2 Viscoelastic materials

The term viscoelasticity applies usually to materials displaying both elastic and viscous (time-dependent) features and exhibiting a stress-strain phase lag (hysteresis); soft biological tissues most often exhibit viscoelastic behaviors. Linear viscoelastic properties of materials can be determined mainly through stress relaxation and creep experiments (Fig. 2.13). During a relaxation test, a constant strain is instantaneously applied to the material and its stress relaxation is observed; the time function $E(t) = \sigma(t)/\epsilon_0$ is referred to as the relaxation modulus. On the other hand, creep experiments involve the instantaneous application of a constant stress and the corresponding observation of time-dependent strain, leading to creep compliance $J(t) = \epsilon(t)/\sigma_0$.

Two main approaches based on infinitesimal strain theory have been used to develop constitutive equations for linear viscoelastic materials, namely mechanical analogs and the Boltzmann superposition principle.

Mechanical analogs

The viscoelastic behavior of materials can be represented as a combination of springs (elastic) and dashpot (viscous) in series and parallel. As such,
Figure 2.14 The standard linear solid model is an example of viscoelastic models built from springs and dashpots.

the "spring" component can be described as

\[ \sigma = \kappa \varepsilon \]  \hspace{1cm} (2.77)

or

\[ \frac{d \varepsilon}{dt} = \frac{1}{\kappa} \frac{d \sigma}{dt} \]  \hspace{1cm} (2.78)

with the stress-strain (\(\sigma - \varepsilon\)) relationship depending of the elastic constant \(\kappa\) [N/m\(^2\)], the viscous component is given by

\[ \sigma = \eta \frac{d \varepsilon}{dt} \]  \hspace{1cm} (2.79)

The stress-strain relationship for the standard linear solid model (Fig. 2.14) is, for example, given by

\[ \sigma + \tau_\varepsilon \frac{d \sigma}{dt} = \kappa_r \left( \varepsilon + \tau_\sigma \frac{d \varepsilon}{dt} \right) \]  \hspace{1cm} (2.80)

with

\[ \tau_\varepsilon = \frac{\eta_1}{\kappa_1} \quad \tau_\sigma = \eta_1 \frac{\kappa_2 + \kappa_1}{\kappa_2 \kappa_1} \]  \hspace{1cm} (2.81)
2. Mechanical Analysis of Indentation

THE BOLTZMANN SUPERPOSITION MODEL

The behavior of linear viscoelastic materials is often described using the Boltzmann superposition approach relating the stress \( \sigma(0) = 0 \) to the strain \( \epsilon(0) = 0 \) by

\[
\sigma(t) = \int_0^t E(t-s) \frac{d\epsilon(s)}{ds} ds
\]

(2.82)

where \( s \) represents the dummy variable of integration and \( E(t) \) the relaxation modulus function; it results in a decaying stress for constant strain applied.

This approach was used in the present work to derive the Hertzian contact for viscoelastic bodies. A Quasi-linear viscoelastic material model coupling an hyperelastic material law (Yeoh) to a linear viscoelastic constitutive law was additionally used in Chapter 7.

2.2.3 LINEAR POROELASTIC MODEL

The linear poroelastic model aims at predicting the behavior of an elastic porous medium partially or fully saturated with some liquid. Note that the following section is based on the theory of 3D consolidation reported by Biot (1941); the model described namely assumes the material to be isotropic, a reversible and linear stress-strain relations, deformations leading to small strains, that the liquid contained in the pores is incompressible with no air bubbles and that the flow of liquid through the porous skeleton is described by Darcy’s law.

The well-known constitutive equations for an isotropic, linear elastic material

\[
\epsilon_{ij} = \frac{1+\nu}{E} \sigma_{ij} - \nu \delta_{ij} \frac{\sigma_{kk}}{E}
\]

(2.83)

\[
\sigma_{ij} = 2\mu \epsilon_{ij} + \lambda \delta_{ij} \epsilon_{kk}
\]

(2.84)

(where \( E \) and \( \nu \) are the Young’s modulus and Poisson’s ratio respectively, \( \mu \) and \( \lambda \) the Lamé constants, and \( \delta \) the Kronecker delta) can be extended by a term accounting for pore pressure \( p \) leading to

\[
\epsilon_{ij} = \frac{1+\nu}{E} \sigma_{ij} - \nu \delta_{ij} \frac{\sigma_{kk}}{E} + \delta_{ij} \frac{p}{3H}
\]

(2.85)
σ_{ij} = 2\mu\epsilon_{ij} + \lambda\delta_{ij}\epsilon_{kk} - \delta_{ij}\alpha p \quad (2.86)

where α measures the ratio of the liquid volume that leaves a considered volume of material to the volume change of the material assuming a pore pressure of 0, and is related to H by

$$\alpha = \frac{2(1 + v)}{3(1 - 2v)} \frac{E}{2(1 + v)H} \quad (2.87)$$

Note that assuming the material to be isotropic, the pore pressure cannot produce any shear strain and that all the normal strains are affected likewise by \( p \). The variation in liquid content \( \theta \), which corresponds to the increment of liquid volume per unit volume of material is further introduced in order to account for the amount of liquid that is present in the pores of the material, namely by

$$\theta = \frac{1}{3H} \sigma_{kk} + \frac{p}{R} \quad (2.88)$$

where \( R \) represents an additional physical constant. Note that the variation in liquid content is directly dependent of the stress state of the material. One can also express it as a function of the trace of the strain tensor as

$$\theta = \alpha\epsilon_{kk} + \frac{p}{Q} \quad (2.89)$$

where the constant \( 1/Q \) is given by

$$\frac{1}{Q} = \frac{1}{R} - \frac{\alpha}{H} \quad (2.90)$$

The two constants characterizing an isotropic, linear elastic material \( E \) and \( v \) have been complemented by two more constants (\( \alpha \) and \( Q \) or \( H \) and \( R \)) that account for pore pressure and flow of liquid within the material. The static equilibrium conditions, the kinematic relations and the constitutive equations that account for the effect of a pore pressure can be merged to a set of three differential equations (see Appendix A.i)

$$G\nabla^2 u_j + \frac{G}{1 - 2v} u_{k,kj} - \alpha p_j = 0 \quad (2.91)$$

containing four unknowns, namely the three entries of the displacement vector \( u_i \) and the pore pressure \( p \). Darcy’s law, which describes the flow
of liquid in a porous medium, allows completing the system of equations, namely through

\[ q_i = -\frac{\kappa}{\mu_f} p_{i,i} \]  

(2.92)

where \( q_i \) represents the volume of liquid flowing per second and unit area in a certain direction, \( \kappa \) is the permeability parameter accounting for the resistance of the material to the flow of liquid, and \( \mu_f \) defined as the interaction properties of the liquid named as dynamic viscosity (Chen et al., 2006). As the liquid was assumed to be incompressible, the rate of variation in liquid content for a considered material element has to equal the volume of liquid that enters or leaves the material element per second, thus

\[ \theta_{,t} = -q_{i,i} \]  

(2.93)

A fourth differential equation rises from Eq. 8.5, 2.91 and 2.93

\[ \frac{\kappa}{\mu_f} \nabla^2 p = \alpha u_{k,kt} + \frac{1}{Q} p_{,t} \]  

(2.94)

Accounting for boundary and initial conditions, the four introduced differential equations (Eq. 2.91, 2.94) can be solved for the three unknowns displacements \( u_i \) and the pore pressure \( p \). Strains and finally stresses can further be calculated thanks to kinematic relations (Eq. 8.5) and constitutive equations (Eq. 8.6). Assuming the material to be fully saturated and the volume change to be due to the flow of liquid out of the material uniquely (\( Q \to \infty \) and \( \alpha = 1 \)), the governing equations become

\[ G \nabla^2 u_j + \frac{G}{1 - 2\nu} u_{k,kj} - p_{,j} = 0 \]  

(2.95)

\[ \frac{\kappa}{\mu_f} \nabla^2 p = u_{k,kt} \]  

(2.96)

In the FE code ABAQUS 6.10, the hydraulic conductivity \( \bar{k} \) is related to the permeability \( \kappa \) accounting for the porosity of the material according to

\[ \kappa = \frac{\mu_f}{\gamma_f} \bar{k} \]  

(2.97)
where $\gamma_f$ denotes the specific weight of the wetting liquid (water = 9807 [N/m$^3$]) and is defined in terms of the density of the liquid $\rho_f$ (water = 1000 [kg/m$^3$]) and the magnitude of the gravitational acceleration $g$ (9.807 [m/s$^2$])

$$\gamma_f = \rho_f g$$  \hspace{1cm} (2.98)

If the expression of the permeability according to ABAQUS is used, Eq. 2.96 transforms to

$$\frac{k}{\gamma_f} \nabla^2 p = u_{k,kt}$$  \hspace{1cm} (2.99)

### 2.3 FE MODELING OF INDENTATION

Depth-sensing indentation machines have been developed and initially mainly used to characterize the mechanical properties of materials exhibiting elasto-plastic behaviors. As such, it is no surprise that the first finite element model developed for indentation described the elasto-plastic response of a material through a linear strain-hardening law (Dumas and Baronet, 1971). Indeed, many research groups around the world have been trying to understand plasticity phenomenon occurring in materials through nanoindentation experiments (Gao et al., 1999).

Indentation methods were further intensively used to determine the mechanical properties of thin films; particular attention was brought to the understanding of the influence of the substrate on the spherical indentation process (Strojny et al., 1998; Suresh et al., 1997). Correction methods were required and further developed to separate the substrate and film properties from the force-displacement curves; calculations are additionally complicated if plastic deformation occur in the materials. The use of spherical indenters allowed for smaller indentation depth ($h_f < 200$ [nm]), indirectly allowing the material to remain in the elastic range (i.e. plastic deformation avoided) and limiting the influence of the substrate on measurements data (Chudoba et al., 2000). Moreover, Field and Swain (1995) observed that the distribution of stress under a spherical indenter is constant regardless of indentation size. A concept similar to the minimization of plastic deformation was used in the present work using spherical indenter tips for indentation of soft polymers and biological
tissues (Larsson1998, Klapperich2001, Ebenstein2004, Ebenstein2004a, Ebenstein2006). Aiming at characterizing soft biological tissues at the microscale, it is of major concern to use a technique avoiding sample damaging (i.e. stress concentration); as such, the requirements for indenting soft biological tissues with nanoindenter machines can be formulated as the minimization of stress concentrations and the correspondence of the measured forces with the working range of the machine (i.e. to sense large forces).

The following chapters aim at summarizing the existing literature treating the different problems that have been tackled in the present work.

**Indenter geometries**

Pyramidal indenter tips (i.e. three-sided Berkovich) are commonly used in indentation testing and most often considered as conical indenter in order to ease data analysis; indeed, their area to depth relationship are identical for small indentation depth. Thanks to FE modeling, Lichinchi et al. (1998) investigated the stress-strain field (i.e. plastic deformation history) of thin hard coatings (titanium nitride on high speed steel) subjected to Berkovich nanoindentation; the suitability of using a simplified 2D conical axisymmetric model instead of a six-fold symmetric 3D Berkovich model was validated for small displacements. They further showed that the substrate influenced the hardness measurement for relative indentation depth greater than 15% of the film thickness.

Sakharova et al. (2009) performed 3D simulations of Berkovich, Vickers and conical indenter tips to clarify the influence of their shapes on the indentation data acquired; indeed, comparing the indentation simulation results for the three indenter tips, they could show that the load-indentation depth curves are highest (slightly only for corresponding displacement of few tenth of [$\mu m$]) for the Berkovich tip and lowest for the conical tip, the results for Vickers tips lying between those two curves. The maximum value of the equivalent plastic strain was shown to be higher for Berkovich than for Vickers and conical indenter tips.
MODELING OF ROUGHNESS

Unlike hard materials that can be polished or most often show small-scale roughness in the order of few [nm], biological tissues exhibit relatively large-scale [$\mu$m] untreatable roughness that needs to be taken into account when analyzing indentation measurement data. There is thus a need to account for such in-homogeneities during the measurement data post-processing phase rather than having the possibility to experimentally modify their surface topography.

Chen and Diebels (2012a) investigated the effects of surface roughness on the characterization of soft polymers (i.e. PDMS and silicone rubber) by means of AFM spherical ($R = 100$ [nm]) nanoindentation ($h_f = 50$ [nm]); indeed, roughness profiles in the form of sine functions and further protuberance-on-protuberance sinusoidal roughness were incorporated into FE models in order to investigate the effects of surface roughness on force-displacement curves. Virtual experimental data exhibited lower stiffness response for rough than for flat sample indentation.

HYPERELASTICITY AND VISCOELASTICITY

The research on the hyperelastic behavior of very soft materials undergoing indentation experiments is still at its first stage and experiments related to such behaviors have been mainly conducted at the macroscopic scale on relatively stiff elastic materials (Giannakopoulos and Papanicolaou, 2009; Giannakopoulos and Triantafyllou, 2007; Zhang et al., 2013).

Chen and Diebels (2012b) recently investigated the hyperelastic behavior (virtual experimental data) of thin polymer layers by means of FE analysis undergoing spherical micro-indentation testing; the behavior of three hyperelastic models (neo-Hookean, Mooney-Rivling and Yeoh) were investigated in reference to their load-displacement curves and their predictive capabilities were discussed as a function of the indentation depth.

Through FE analysis, Zhang et al. (2013) analyzed the capabilities of four commonly used hyperelastic constitutive models (neo-Hookean, Mooney-Rivlin, Fung, and Arruda-Boyce) to extract meaningful parameters from spherical macro-indentation experiments on polydimethylsilox-
ane (PDMS). Based on the obtained results, the applicability of the Hertzian contact to determine the initial shear modulus of a hyperelastic solid was shown to be reliable, for an indentation depth \((h)\) to indenter radius \((R)\) smaller than 20%.

Very few studies (Briody et al., 2012; Crichton et al., 2011; Huang et al., 2005) aimed at incorporating the influence of material time-dependence into hyperelastic FE analysis of indentation (nano, macro), namely forming quasi-linear viscoelastic models. Both hyperelasticity and quasi-linear viscoelasticity are further considered for the analysis of the experiments carried out in the present work.
Nanoindenter machines were originally developed for the mechanical characterization of hard materials allowing the application of [mN] forces corresponding to displacement in the [nm] range over a short experimental period (typically within a few seconds). With the growing interest driven towards the characterization of soft materials exhibiting time-dependent behavior, instrumented indentation technique call for new protocols including holding periods for either constant maximum load or maximum indentation depth and requiring substantially longer experimental times more likely to be influenced by their testing environment. In order to limit the effects of such environmental perturbations, most nanoindenter machines are placed on an antivibrgation table in a soundproof enclosure to reduce to a minimum both the effects of vibration and noise on the acquired measurement data.

However, most attempts for attenuating the effect of thermal variation on the acquisition of experimental data over long-term indentation times have failed. This effect, leading to thermal drift, is attributed to dimensional variations of both the sample and the frame (Chudoba and
3. Setup description

Richter, 2001; Feng and Ngan, 2002). Measurement data incorporating the effect of thermal drift are sometimes post-processed for removing the undesired effect and extracting corrected data reflecting the actual indentation performed.

Dealing with very small forces and displacement, additional variations due to the overall deformation of the system (frame and sample) may arise during the indentation process; this effect, known as frame compliance, influence directly the displacement acquired and further the outputted force-displacement curves (Nohava et al., 2009; Van Vliet et al., 2004). Indeed, although the stiffness of the whole frame is usually high and the loads applied very low, the deformation of the frame can sensibly affect the measurement; such an influence is usually dealt with a post-processing correction factor accounting for the frame stiffness and allowing a correction of the measured displacement.

3.1.1 Ultra Nanoindentation Tester

The principle

The Ultra Nanoindentation Tester (UNHT) developed and manufactured by CSM Instruments SA tends to reduce the problems associated with thermal variations and deformation of the overall system, thanks to its active top referencing system that is further introduced (Nohava et al., 2009).

The principle of the UNHT is based on the idea of using two independent axes dedicated to indentation measurement (indenter axis) and active top referencing (reference axis), that are conjointly assemble in a feedback loop allowing for a continuous control of the applied load. Indeed, each axis possesses its own actuator empowering the machine of accurate displacement application, and its own displacement and load sensors providing real measurements of depth and load.

Instrument frame compliance

An independent control of the reference axis allows for the elimination of sample surface displacements consequent to deformations of both the frame and the sample. When testing relatively hard materials, the refer-
ence is indeed maintained in contact with the sample surface at a specific load during the whole process of indentation and therefore follows all the movements of the sample. Note that for the special case of very soft materials indentation, the reference is generally placed on the sample holder in order to avoid the instabilities due to their very low stiffness and/or time-dependent behaviors; although the small displacement [nm] of the sample due to the deformation of the system cannot be assess with such a configuration, its impact on data acquisition is expected to be negligible due to the low force responses that these small displacements imply. In other words, the frame compliance is expected to have no critical influence on the acquisition of soft materials data by means of micro-indentation and is thus disregarded.

**Temperature drift**

The technical components forming the UNHT measurement head are made of materials with extremely low coefficient of thermal expansion such as Zerodur materials allowing for extremely low thermal dilatation only (Bach, 2005). The combined use of such materials with the active top referencing system leads to an excellent thermal stability and thus very limited (close to zero) displacement drift, once temperature stabilization is achieved. The UNHT nanoindenter brings a technological solution to the problems linked to thermal drift and frame compliance; as such, there is no need to post-process measurement data. As a consequence, all indentation experimental data acquired for the present work with the help of an UNHT are shown as raw data.

3.1.2 Experiments

**Setup specifications**

The specimens to be tested were placed on a motorized table allowing for translation in the x, y, and z-direction with a positioning resolution up to 1 [µm]. Note that the UNHT was placed on a antivibration table in a sound-proof enclosure. Due to its drift minimization, the UNHT nanoindenter is quite at ease for indenting very soft materials exhibiting time-dependent behavior (creep, relaxation) over long-term periods for relative humidity
3. Setup description

![Figure 3.1](image)

**Figure 3.1** Liquid cell allowing for soft material indentation in water.

of 40%. However, testing fully immersed in water, thanks to a transparent liquid cell allowing for sample hydration and surface forces suppression (Fig. 3.1), makes the thermal drift increases to a proportion that is significant for the first few indents performed in solution; its influence vanish indeed after the temperature of the indenter shafts have stabilized and measurement cleaned of drift can be acquired. The measurement data shown in the present work were mainly acquired using spherical indenter tips (R=100, 200, 500, 1500 [µm]) that allow a good compromise between minimization of stress concentration and maximization of force sensed in order to fit in the working range of the nanoindenter machine (see Chapter 4). A ruby spherical tip of radius 1.5 [mm] was used, on the other hand, as the reference tip for all the experiments conducted. Please note that the maximal vertical distance between the two tips is of 50 [µm]. The fine force range of the machine (P < 10 [mN]) and the large displacement range (h < 50 [µm]) were used to accommodate the UNHT to the low stiffness of the materials tested.

**Contact detection**

As a full immersion of the samples into water allows to get rid of surface forces (Chapter 6) and since no particular interest was formulated towards the analysis of adhesive properties of the materials tested, simple linear force-controlled loading (loading-creep) experiments were con-
Nanoindenter setup

Conducted on the different materials tested in the present study. The UNHT is driven in displacement-controlled mode until it reaches the surface of the sample; the stiffness contact method (change in slope of 30%) or the threshold method ($P_{min} = 5 [\mu N]$) allow the detection of contact and the force-controlled mode leading to the indentation of the material is further initiated. The complete method for accurate contact point detection is further introduced in Chapter 6. Note that the contact point can be adjusted manually by the user during post-processing manipulations in order to ensure the good interpretation of experimental data.

**FORCE-CONTROLLED VS. DISPLACEMENT-CONTROLLED MODE**

All experiments were conducted in a force-controlled mode allowing the application of a constant loading rate during the indentation process. Although this approach has been proven to provide reliable acquisitioned data, force and loading rate are difficult to link to stress or strain and a displacement-controlled system enabling the application of constant displacement rate would allow a direct control of indentation strain and strain rate (VanLandingham, 2003). Indeed, for the special case of paraboloidal tip, the Tabor relationship previously introduced (Eq. 2.38) relates the indentation strain to the ratio of the contact radius to the tip radius, with the contact radius ($a_c$) being directly related to the indentation depth $h$ (Eq. 2.24).

Another concern related to force-controlled mode rises when attempting to analyze the time-dependence of soft materials through so-called indentation creep experiments. Indeed, in such a procedure involving the application of a constant maximum force, the monitored displacement of the indenter tip into the specimen induces a substantial change in the contact area and further evolutions of the stress and strain fields as the test procedure goes on. On the contrary, the displacement is held constant during a stress relaxation indentation test, and a decreasing force is recorded; as the indentation displacement is directly related to the indentation strains, data extracted from such a measurement method are more easily analyzed.
3. Setup description

3.2 MATERIALS INDENTED

The different materials tested in the present work by means of indentation at the microscale were specifically chosen to gradually tackle the different challenges that are associated with indenting soft materials and biological tissues.

A two-part incompressible silicon rubber material called Ecoflex0030 (Smooth-On) that was previously shown to behave hyperelastically under large deformations (Hollenstein2004) was initially picked to test the capabilities of the nanoindenter machine to characterize such a very soft material at the microscale. A substantially stiffer material (Fused silica) was subsequently selected for analyzing the surface forces present at the interface between hard materials and indenter tips. As part of the second step of the investigation campaign, VHB4910, a soft acrylic elastomer known for its hyperelastic viscoelastic behavior (Schmidt et al., 2011; Wissler and Mazza, 2007), was selected in order to investigate the possibilities to characterize the time-dependent behavior of soft materials. Soft porous hydrogels (agarose 0.5, 1, 2 and 4%) hypothesized to behave poroelastically were further synthetized in order to analyze the capability of the nanoindenter setup to differentiate between the visco or poro-like behavior of the material being indented.

3.2.1 FUSED SILICA

Fused silica is often used as a reference material for nanoindenter machine calibration, thanks to its well-known Young’s modulus ($E = 72 \text{ GPa}$) and isotropic properties at room temperature. In the present work, a polished fused silica (Saint Gobain Glass AG, Switzerland) cylindrical sample ($R = 12.5 \text{ mm}$, thickness $= 5 \text{ mm}$) was selected as a good candidate for investigating the surface forces present at the interface of hard materials.

3.2.2 ECOFLEX0030

Ecoflex0030 is a platinum-catalyzed silicone rubber that was synthetized mixing its two parts constituents (2/3), composed of polyorganosiloxanes
and amorphous silica, with a silicone thinner (1/3) used to soften the material. Prior to solidification, the mixture was put into a vacuum chamber to remove air bubbles from it. The liquid solution was then poured into Teflon molds containing flat wells of 1 and 3 [mm] depths and the samples were covered for solidification overnight. The samples were finally cut and their dimensions accurately measured using a slider caliper with precision of 0.1 of [µm]. The chemical compositions of the parts are not fully declared by the manufacturer.

3.2.3 VHB4910

VHB4910 produced by 3M is an acrylic elastomer recently used in electro active polymer (EAP) actuators; it can indeed transform electrical energy into mechanical deformations with up to 100% active strains (Pelrine et al., 2000). In the present work, only the passive mechanical response of this acrylic elastomer commercially available in double sided tape roller of thickness 1 [mm] is investigated by means of indentation; in order to reach a 3 [mm] sample thickness, three layers of material were brought together thanks to the relative stickiness of their surfaces. The chemical composition of the material is not declared by the manufacturer. EAP materials can be applied to the biomedical field, namely serving to the creation of micro-pumps, micro-valves and prosthetic devices (Pelrine et al., 2000).

3.2.4 Paraffin wax

Small grains of solid commercially available paraffin wax were heated up to their melting point and further poured into a well characterized by a diameter of 2 [mm] and a depth of 3 [mm]; the samples were covered for solidification overnight. Paraffin wax is well-known for its hydrophobic properties. Paraffin wax is commonly known for being used in candle-making.

3.2.5 Agarose gels

The agarose is a linear polysaccharide which is extracted from marine red algae (Normand et al., 2000); it is composed of 1,3-linked-β-D-galactose
and 1,4-linked 3,6-anhydro-α-L-galactose residues called agarobiose that form polymeric chains. Four types of agarose gels were prepared by dissolving powdered agarose (Life Technologies) in water with varying agarose concentrations, respectively of 0.5%, 1%, 2% and 4% w/w. The mixtures were subsequently microwaved up to a temperature of 99°C (randomly coiled polysaccharide chains) and the transparent warm solutions were poured into petri dishes and left half covered for cooling (gel point = 36°C) until they reached room temperature and their final well-ordered helical structures. After the conformational transition has taken place, a gel is generated in which the agarose chains build a percolating network that contains water-filled cavities (Zhou et al., 2006). The gel thickness was consistently chosen to be 3 [mm] thick in order to avoid any influence of the substrate on subsequent indentation experiments. Agarose gels are commonly used as networks of specific pore size allowing the separation of proteins larger than 200 [kDa] through electrophoresis.

### 3.3 Surface Topography

Surface roughness has been shown to have a significant influence on the mechanical properties of hard materials extracted from indentation experimental data (Donnelly et al., 2006; Gerberich et al., 1998; Klapperich et al., 2001); one can thus either try to reduce its influence experimentally by increasing the ratio of the indenter tip radius to roughness, or one can account for it to some extent in a structural FE model or in a wisely formulated analytical solution.

Biological tissues exhibit often significant roughness at the microscale, and as such, there is a need to understand and incorporate physiological structure of biological tissues into data analysis.

In order to account, if necessary, for surface roughness in the extraction of meaningful mechanical parameters from measurement data, microscopic images of the self-synthesized materials were obtained using both a laser scan microscope (air) and an Atomic Force Microscope (AFM) in sliding mode (water).
3.3.1 Laser Scan Microscopy

A laser scanning microscope (LSM) of Fifth Generation (Pascal) coupling both confocal technique and the point scanning method was used to acquire 3D images of Ecoflex0030 surfaces. The main advantage of such a confocal microscope is that it can collect the light reflected by a single plane in the sample; a laser beam (Argon ion) scans the specimen pixel by pixel and these information are further assembled into a high contrast image that is an optical section of the sample. The combination of several shifted images generated with the focal plane lead to the creation of a 3D stack representative of the sample surface roughness.

Ecoflex0030

All the information related to Ecoflex0030 surface determination are reported in Chapter 6.

3.3.2 AFM Sliding Mode Microscopy

Due to the inability of the Laser Scanning Microscope to characterize sample surface in an aqueous environment, the surfaces of both VHB4910 and 2% agarose gels were characterized using an AFM (Nanowizard I, JPK instruments) driven in contact (i.e. sliding) mode with a triangular DNP-S10 D tip (Bruker AFM Probes) allowing for testing fully immersed in water. Postprocessing was conducted with the freeware Gwyddion.

Atomic Force Microscopy consists of moving a cantilever with a tip of known nanometer radius towards a specimen surface thanks to a piezo motor allowing for displacement in a range of few microns; once in contact, the resistance of the sample to tip penetration causes the cantilever to bend. The cantilever bending is quantified, thanks to a laser beam that is reflected on the cantilever and captured by a photo-sensitive diode, and related to force quantities through its known spring constant.

When performing topographic measurements with an AFM driven in sliding mode, the cantilever is moved laterally along the x- (slow) and y-axis (fast) while a constant cantilever bending (and hence a force) is maintained using a feed-back control system. Each fast axis is measured in both directions, as high hills and steep valleys can cause the feed-back
3. Setup description

system to fail in one direction (i.e. loss of contact). Note that if the control system is well enough, the topography corresponds to the movement of the piezo actuator.

VHB4910

The surface roughness of VHB4910 was determined on two distinct spots (20x20 [µm]) in order to insure measurements repeatability (Fig. 3.2); recalling that the root-mean-squared roughness ($R_q$) is defined as the standard deviation of the elevation ($z$-values) within a given area (Miller et al., 1996) calculated as

$$R_q = \sqrt{\frac{1}{N} \sum_{i=1}^{N} (z_i - z_{ave})} \quad (3.1)$$

where $z_{ave}$ represents the average of the $z$ values within the given area, $z_i$ is the elevation value $z$ for a given point, and $N$ corresponds to the number of points within the given area, the VHB4910 specimens exhibited RMS roughnesses of 67.4 and 79.9 [nm], respectively with maximum peak values up to 400 [nm].

Agarose gels

The surface of 2% agarose gels was characterized through a 20x20 [µm] zone scan exhibiting [nm]-scale roughness (Fig. 3.3). The present data support assumptions considering both VHB4910 and agarose gels as flat specimens with maximum peaks of 100 [nm].

3.3.3 AFM force spectroscopy

AFM machines also grant possibilities for force spectroscopic measurements; similarly as during the indentation procedure, the AFM tip is moved towards sample surface through piezoelectric displacement, monitoring the deflection of the cantilever as the tip indent (loading) and retract (unloading) from the material. Testing fully immersed in water allows measurement of nanoscale contacts such as Van der Waals forces; in the present work, AFM spectroscopic measurements were conducted
on VHB4910 samples in order to evaluate the presence of surface forces in water. As observed in Fig. 3.4, no pull-in forces were sensed during the approach towards the sample; on the other hand, adhesive forces between the tip and the specimen were recorded during the unloading phase.
3. Setup description

**Figure 3.3** 2% Agarose exhibiting a RMS roughness of 40.1 [nm].

**Figure 3.4** AFM force spectroscopy on VHB4910 immersed in water exhibiting no pull-in effect.
Nanoindenter Tip Choice

4.1 Tip Geometries

Commercially available nanoindenter machines are compatible with tips exhibiting pyramidal, cylindrical and spherical shapes among others. In order to minimize their influence on measurement data, tips are usually made of very stiff materials such as pure diamond or ruby. For small displacements, a conventional method for extracting the elastic properties from indentation data relies on the solution derived by Sneddon (1948, 1965) that complete the Hertzian formulation valid for spheres to any shape of revolution in contact with a plane. Sneddon showed that the load-displacement relationship for different tip geometry can be written as

\[ P = C_T E_r h^m \]  (4.1)

where \( P \) is the indenter load, \( h \) is the elastic displacement of the indenter, \( C_T \) contains the indenter geometry constants, \( E_r \) refers to the reduced modulus containing the material properties and \( m \) is a constant related to tip geometry, taking namely the value 1 for flat punch, 1.5 for spheres and 2 for cones.
4. Nanoindenter Tip Choice

Pyramidal indenters such as Berkovich (3-sided) tips are commonly treated as conical indenters defined by a sphere in the order of hundreds of nanometers and by an angle $\alpha$ (70.3°) allowing for equivalent area-to-depth relationship for small displacements (Dao et al., 2001; Lichinchi et al., 1998; Min et al., 2004; Wang et al., 2007; Warren and Guo, 2006; Yu et al., 2004) (Fig. 4.1). Indeed, using the rule of volume equivalence (Min et al., 2004) stipulating that a conic indenter is equivalent to a non-conic but self-similar indenter when their base area/(height)$^2$ ratio are equal for the same indentation depth ($h$), the projected areas ($A$) for both tips can be approximated by the expressions (see Fig. 4.1) (Fischer-Cripps, 2005):

$$A_{Berkovich} = 3\sqrt{3}h_c^2 \tan^2(\theta) = 24.56h_c^2 \text{ [\mu m}^2\text{]}$$
$$A_{Conical} = \pi h_c^2 \tan^2(\alpha) = 24.51h_c^2 \text{ [\mu m}^2\text{]}$$

(4.2)

Figure 4.1 The geometry of Berkovich indenter tips is commonly simplified by a conical shape for an ease of data analysis.

In the following chapters, the capabilities of three commonly used indenter tips, namely Berkovich, Flat punch, and Spherical indenter tips, to characterize very soft biological tissues at the microscale are investigated by means of large displacement FE analysis; the influence of large deformations on indentation data analysis is introduced in the present Chapter and further discussed in Chapters 6, 7 and 8.

4.1.1 BERKOVICH

Pyramidal indenters such as Berkovich (3-sided) tips are commonly treated as conical indenters defined by a sphere in the order of hundreds of nanometers and by an angle $\alpha$ (70.3°) allowing for equivalent area-to-depth relationship for small displacements (Dao et al., 2001; Lichinchi et al., 1998; Min et al., 2004; Wang et al., 2007; Warren and Guo, 2006; Yu et al., 2004) (Fig. 4.1). Indeed, using the rule of volume equivalence (Min et al., 2004) stipulating that a conic indenter is equivalent to a non-conic but self-similar indenter when their base area/(height)$^2$ ratio are equal for the same indentation depth ($h$), the projected areas ($A$) for both tips can be approximated by the expressions (see Fig. 4.1) (Fischer-Cripps, 2005):

$$A_{Berkovich} = 3\sqrt{3}h_c^2 \tan^2(\theta) = 24.56h_c^2 \text{ [\mu m}^2\text{]}$$
$$A_{Conical} = \pi h_c^2 \tan^2(\alpha) = 24.51h_c^2 \text{ [\mu m}^2\text{]}$$

(4.2)
with $h_c$ referring to the contact depth (see Fig. 2.10), $\theta$ and $\alpha$ being the representative angles for Berkovich and Conical indenter tips, respectively.

According to Sneddon, the quadratic elastic load-displacement ($P$-$h$) relationship for conical indenters ($m = 2$) is given by:

$$ P = \frac{2\tan(\alpha)}{\pi} \frac{E}{(1 - v^2)} h^2 $$

(4.3)

where $\alpha$ is the indenter included half-angle, $E$ and $v$ are the Young’s modulus and Poisson’s ratio, respectively.

### 4.1.2 Spherical

For the special case of spherical indenter tips ($m = 1.5$), the expression given by Sneddon becomes

$$ P = \frac{4\sqrt{R_S}}{3} \frac{E}{(1 - v^2)} h^{3/2} $$

(4.4)

As expected, the elastic load-displacement relationship corresponds to the Hertzian contact (Johnson, 1985) in which $R_S$ represents the radius of the indenter (Fig. 4.2).

### 4.1.3 Flat punch

In the case of cylindrical indenters commonly called flat punch, the contact area is constant through the whole indentation process and the elastic
4. Nanoindenter Tip Choice

load-displacement relationship is linearly given by

\[ P = 2R_F \frac{E}{(1 - v^2)} h \]  \hspace{1cm} (4.5)

with \( R_F \) being the radius of the cylinder (Fig. 4.2).

4.2 Towards biological tissues

Aiming at stepping towards the indentation of soft biological tissues using nanoindenter machines, two main considerations must be taken into account, namely that the working range of nanoindenter machines is limited in force (resolution) and displacement (maximum indentation depth) and that stresses/maximal stresses applied to the specimens should be homogenized/minimized in order to avoid damaging of the samples. With them in mind, the capability of the previously introduced tips to indent soft biological tissues was investigated using FE modeling accounting for material and geometrical non-linearities occurring for large deformations.

4.2.1 Indenter working range

Nanoindenter machines have originally been developed for stiff materials characterization with displacements ranging from few nanometers up to few micrometers and forces sensed from tenth of micro-newton up to few milli-newton (Oyen, 2011). As a result, most nanoindenter devices are not suited for indentation of soft biological tissues, requiring low force sensing and demanding large displacement ranges. The following chapter aims at understanding the impact of the different indenter shapes on the experimental working range needed for indentation of soft tissues; the investigations were conducted using the material formulation obtained for Ecoflex0030 \((E = 27.6 \text{ [kPa]})\) exhibiting similar properties as biological tissues in terms of compliance.

Berkovich indenter tips are most often considered as conical indenter for shallow indentation data analysis (Lichinchi et al., 1998). However, for larger indentation depth, this assumption is unlikely to stand in terms of maximum force sensed as well as maximum stress applied to the specimen;
2D axisymmetric model displaying the different mesh regions; the bottom of the specimen was clamped to the substrate and the left part of the model (the center of the specimen) was let free to move along the direction of indentation.

The Load-Displacement ($P - h$) curves obtained for simulations of conical indentation through 2D and 3D FE modeling overlap perfectly (Fig. 4.4); the 3D model provides identical prediction as the 2D model, though the computation time needed for such an analysis is tremendously larger (100x). The validity of the assumption stating that the Berkovich indenter can be approximated by a Conical indenter was further challenged through a 3D analysis involving 1/6th of a Berkovich indenter (Fig. 4.5); the forces involved in the indentation of a specimen by a Berkovich indenter were shown to be significantly higher than the one involved with a conical indenter. The divergence of the load-displacement curves during the loading process can be explained by the difference in projected area between the two types of indenter (i.e. $A_{Berkovich} =$
4. Nanoindenter Tip Choice

![Graph showing force vs contact depth for different indenter tips.]

**Figure 4.4** Berkovich indenters allow for higher force sensing than what conical approximation predicts. Sneddon formulation additionally underestimates FE modeling predictions.

5526 $[\mu m^2]$, $A_{Conical} = 5514 [\mu m^2]$ for a maximal contact depth of 15 $[\mu m])$, as well as the presence of elastic singularities that are likely to occur at the edges of the Berkovich tip and which affect the stress-strain response of elements adjacent to them (Sun et al., 1995). The assumptions formulated by Sneddon (1948), namely that the specimen is considered as infinitely large (Poon et al., 2008) and that the indenter tip is assumed to be infinitely sharp (Shih et al., 1991; Wang et al., 2007; Yu et al., 2004), are most often violated while conducting indentation experiments with Berkovich indenter tips. As observed by Poon et al. (2008), the predictions of conical indentation load-displacement curves obtained through Sneddon formulation underestimate FE modeling which attempts to account for the finite geometries of both the specimen and the radius of curvature of the indenter tip (Fig. 4.4). As previously seen in the comparison between Berkovich and conical indenters, an increase in contact area leads to an increase of the forces sensed by the nanoindenter machine: in other words, an increase of the size of the indenter leads to an increase of the forces sensed. Such a mechanism is illustrated in (Fig. 4.6), where
indentation load-displacement curves are represented for different size of spherical indenter \((R = 50\text{ to } 500 \text{ [}\mu\text{m}]\)) reaching a maximal depth of 20 [\mu m]; an increase of radius of one order of magnitude leads to a multiplication of the forces sensed by 3, a result which is in line with small strain theory (Johnson, 1985) (Eq. 4.4). It should be noted that an increase of the indenter size changes the lengthscale at which specimens are indented and thus characterized. For instance, in order to characterize single cells, a spherical tip with a radius of 50 [\mu m] would be preferred to
4. Nanoindenter Tip Choice

The mechanism influencing the forces sensed by nanoindenter machines is clear: an increase in contact area (size of the indenter) lead to higher force sensing.

A 500 [µm] spherical tip that would on the other hand particularly well fit for indentation of cell aggregates such as muscles. Due to their large area of contact, flat punch indenter tips allow for large force sensing from the start, leading to the linear load-displacement relationship (Fig. 4.7).

The Hertzian contact overestimates the forces calculated by FE modeling, as observed by (Lu et al., 2008). In order to ease the convergence of FE modeling of flat punch indentation, a 5 [µm] radius rounded edge was added to the original cylinder geometry; this modification explains partially the overestimation of the analytical solution as compared to simulation results.

4.2.2 Stress concentration

For easier data analysis, a particular care should be brought on avoiding sample damaging which is most often a consequence of stress concentration. An FE analysis conducted for different indenters brought to light the evidence that spherical indenters make a good compromise between large force sensing and low maximal stresses applied to the samples (Fig. 4.8).
Figure 4.7 Flat punch exhibits a linear force-displacement relationship whereas Spherical indenter tips lead to a non-linear curve due to the increase of contact area.

Figure 4.8 Stress to force mapping for different indenter tips for a maximum indentation depth ($h_{max}$) of 20 [$\mu m$].
Figure 4.9 von Mises stress representation for Spherical and Flat punch indentation.

Indeed, Berkovich and Flat punch indenters possess sharp angles that allow stresses concentration and may then damage more easily the sample for a same range of forces sensed (Fig. 4.9).

Note that even though spherical indentation allows for homogenized stress distribution, the choice of reliable indenter for indentation of soft biological tissues remains case specific and is mainly dependent of the lengthscale at which one wish to characterize the material of interest.
5.1 FE MODELING

Large deformations might occur when compliant materials are subjected to micro-indentation testing; both material and geometrical nonlinearities occur as the specimen is indented. The commercially available FE software ABAQUS 6.10 (ABAQUS Inc., Providence, RI) accounting for these nonlinearities was used to extract mechanical properties from measurements data.

5.1.1 Procedure

Finite element analysis conducted through commercial finite element software can be subdivided into three steps commonly referred as pre-processing, solution acquisition and post-processing (Hutton, 2003). The following section will detail each of these crucial steps towards obtaining meaningful solution to physical problems.
5. FE Automated Tool

PRE-PROCESSING

The pre-processing consists of defining the properties related to the finite element model prior to analysis, namely the definition of the geometric domain of the problem, the geometric properties of the element, the element types and the material formulation associated with them, the element connectivity, and finally the boundary conditions.

SOLUTION ACQUISITION

The finite element software assembles then the governing equations into matrix forms in order to compute the unknown values of the field variables; additional derived variables such as the reaction forces and the element stresses are calculated. Due to the large number of equations that need to be solved, it is of major importance to optimize the FE mesh in order to reduce the computation time.

POST-PROCESSING

The analysis of the solution results is further conducted through post-processor software allowing for sorting, printing and plotting results of interest from the finite element solution. Among all the operations enabled by the software, extracting element stresses by order of magnitude, plotting deformed structural shape, and creating animate dynamic model behavior are of major interest.

NON-LINEAR ANALYSIS

In most cases, linear analysis provides an acceptable approximation to engineered problem encountered; however, from time to time, more complicated problems that call for nonlinear approaches arise.

Linear analysis assumes that neither the shape nor the material properties change during the deformation process; in other words, it means that the initial stiffness is retained during the entire process of deformation. Under the linear assumption that the element stiffness matrix does not change over the deformation, the equation constituting the stiffness matrix must be solved once throughout the whole process. On the other
hand, non-linear analysis calls for an update of the stiffness matrix as the solver progresses, resulting in a substantial increase of the amount of time needed for convergence. Non-linearities mainly arise from two sources, namely from changes in geometry and/or in material properties; they cannot be separated in a consistent representation, thus, analysis software account for both type of non-linearities simultaneously. Fig. 5.1 includes results of a calculation using non-linear geometry (nlGeom) in combination with a linear elastic model; such a calculation is thermodynamically inconsistent.

5.2 FE BASED ANALYSIS OF INDENTATION

5.2.1 3D AND 2D AXI symmetric MODELS

In order to fully analyze the influence of indenter tip shapes and sizes on the indentation process of soft materials, both 3D and 2D axisymmetric models incorporating geometrical and material non-linearities were created with the help of the commercially available FE software ABAQUS.
As the use of 3D modeling requires a substantial amount of computational time, a corresponding 2D axisymmetric model was developed; a revolution of the 2D slice around the Y-axis of the reference coordinate system allows for the reconstruction of the original 3D structure. Please note that in the present work, the 2D axisymmetric slice was meshed identically as the faces serving as basis for the 3D model. The limitation of application of such an axisymmetric model to the modeling of indentation experiments was shown for the special case of Berkovich indenter; indeed, the approximation of a pyramidal (3D) by a conical tip (2D axisymmetric) lead to substantially different solutions for large deformation. Please note that all simulations were conducted with the assumption of frictionless contact between the indenter tip and the surface sample; although this assumption may be questionable for indentation in air, the lubricant properties of water may reduce the effects of friction to a great extent.

In the present chapter, non-linear (both geometrical and material) FE analysis have been conducted using a hyperelastic material formulation (Neo-Hookean, $C_{10} = 0.0046$ [MPa]) characterizing an incompressible soft silicone (Ecoflex0030). Note that all simulations were conducted in a quasi-static displacement-controlled mode and that no time-dependence of the material response was taken into account for these calculations.

3D MODEL

Indenter tips were models as rigid bodies representing 1/6th of their complete geometries (Lichinchi et al., 1998). The 3D model was originally created in order to investigate the response of soft materials to indentation by Berkovich indenter tip, which corresponds to a three-sided pyramid with inner angle of 65.27°; as such, the triangular-based pyramidal tip having a six-fold symmetry calling for 3D analysis was the limiting factor to any further reduction of the model (i.e. 2D axisymmetric model). Note that Flat punch tips were modeled incorporating rounded edges ($R = 5$ [$\mu$m]) in order to avoid sharp edge contact.

A 3D mesh representing 1/6th of the sample cylindrical shape (radius = 9 [mm], thickness = 3 [mm]) was created with 78840 nodes assembled in 68'424 C3D8RH elements (Fig. 5.2). Reducing the size of the 3D model to 1/6th of its real volume allows a substantial reduction of the number
of nodes and thus a tremendous decrease of the computational time. In order to insure the newly defined volume to behave as being part of a whole volume, corresponding boundary conditions (in-planes constraints) were applied to the two surfaces defined by their two perpendicular vectors \( n_1 \) and \( n_2 \) (Fig. 5.2). Additionally, the line in the middle of the model was constrained and allowed for movement along the \( n_3 \)-direction only.

2D AXISYMMETRIC MODEL

The Berkovich indenter tip was approximated in the 2D axisymmetric model by a conical shape characterized by its inner angle of 70.3° and its spherical end-tip of radius 150 [nm] (Fig. 5.3). The 2D slice serving as a basis for the axisymmetric model was identically meshed as the side surfaces of the 3D model, namely reaching dimensions of 9 [mm] (radius) by 3 [mm] (thickness). The 8760 nodes assembled in 8553 CAX4RH elements provided indeed a substantial decrease of the computational time.
Figure 5.3  Optimized 2D axisymmetric model (3000x3000 [µm]) exhibiting the master slice with its associated boundary conditions, namely its bottom surface clamped to the ground and its center line constraints in x and y-directions.

OPTIMIZATION

A sensitivity study was further conducted on the 2D mesh in order to analyze the influence of parameters such as the smallest element size and the overall dimensions of the specimen (thickness and width) on the accuracy of the final solution; the maximal force sensed for a maximal indentation depth of 40 [µm] corresponding to the maximal depth enabled by the UNHT nanoindenter was subsequently calculated for spherical ($R = 100$ [µm]) indentation and compared for different model configurations.
In the current work, the impact zone is defined as the principal zone of contact (100x100 [µm]) and is constituted of the smallest elements present in the model. As an initial optimization step, the size of the whole specimen was kept constant (9000x3000 [µm]) and the smallest element size (length of one side of a squared element) was varied from 0.26625 to 68.16 [µm] (Fig. 5.4); the stabilization of the maximal force around 115.8 [µN] was noticed for elements smaller than 3 [µm]. As a result, the smallest element size was chosen as 2.13 [µm] for further investigations.

The influence of the substrate on indentation measurements was further investigated varying the specimen thickness (250 to 3500 [µm]) for a given smallest element size of 2.13 [µm] and a fixed sample width of 9000 [µm] (Fig. 5.5). The maximal forces sensed stabilized around 115.8 [µN] for thickness larger than 3000 [µm], exhibiting the limit of substrate influence. Specimens were thus all synthetized/designed cylindrically shaped with a thickness of 3 [mm].

The influence of sample width on indentation simulations was further investigated through samples of constant thickness (3 [mm]) and different width (250 to 9000 [µm]) for a smallest size element of 2.13 [µm]. A stable
5. FE Automated Tool

Figure 5.5  Modeling of the influence of sample thickness on the forces sensed by the indenter.

maximum force was reached for samples with a width of 3000 [µm]; the original size of the specimen (9000x3000 [µm]) were thus approximated for all further investigations by a mesh of 3000x3000 [µm] (Fig. 5.6) allowing a sensible decrease of the computational time.

The conditions for building an optimized specimen geometry for the special purpose of FE spherical ($R = 100[µm]$) indentation can be summarized as:

$$\frac{\text{Smallest element size}}{R} \leq 2.13\%$$

$$\frac{\text{Thickness}}{h_{\text{max}}} \geq 75$$

$$\frac{\text{Width}}{h_{\text{max}}} \geq 75$$

(5.1)

These observations are consistent with results reported for conical indentation by Poon et al. (2008).

5.2.2 MOTIVATIONS FOR AUTOMATION

Indentation of soft materials at the microscale requires deep displacement into the materials leading to relatively large deformations; in this way, their mechanical behavior can be characterized in a relevant deformation
As previously mentioned, large deformations induce geometric and material non-linearities that can be accounted for in FE software. However, significant effort is typically required to create an accurate FE model.

The main idea behind the creation of the present automated algorithm was to enable the users to easily extract non-linear time-dependent mechanical characteristics out of indentation measurement data (Fig. 5.7). The analysis is conducted through three steps, namely the creation of a FE model from experimental data (input file), the calculations through an FE solver (modeling), and finally an iterative optimization of the simulation to fit the experimental data (inverse analysis).

### 5.2.3 FE AUTOMATED TOOL ALGORITHMS

**Input file**

Input files (.inp) are text files specific to the commercially available FE software ABAQUS that contain all necessary information required to assemble and execute an FE simulation; the FE tool introduced in the
present work allows, as a first step, for a fully automated creation of indentation FE input files (Matlab, The MathWorks Inc., Natick, MA, USA), namely empowering the user (Fig. 5.8 (a)):

- to choose between three different kind of indenters, namely spherical \((R = 100, 200 \, [\mu m])\), conical and flat punch (with rounded edge of \(R = 5 \, [\mu m]\)).

- to input the exact dimensions of the specimen to be indented by controlling the meshing process while optimizing the computational time needed for simulation completion. Indeed, the algorithm allows for automated mesh creation based on sample dimensions, smallest element size, impact zone size and number of refined mesh domains.

- to select the right material properties among elasticity theory (elastic or hyperelastic formulations); the possibility is also given to the user to use quasi-linear viscoelastic and poroelastic formulations.

- to apply displacements (displacement-controlled) or forces (force-controlled) corresponding to the actual indentation experiments; this last step is
eased by the direct transmission to the input file creator of boundary conditions related to measurement data. Indentation creep and relaxation experiments are additionally enabled by the selection of holding time following the loading phase.

Note that the assumptions of frictionless contact, fixed bottom, and axisymmetry in the center of the model are fixed conditions that cannot be changed.

**Modeling**

The input file can be transmitted to the FE solver of ABAQUS through the execution of a job file; the modeling is further initiated and a so-called .odb-file containing the information related to FE calculation is created and iteratively modified as the simulation progresses. A python script is used to extract force-displacement curves (entire curve, loading and holding phases) from the .odb-file and to write these specific information into text files (.rpt-files). Note that the two initial steps (input file creation, modeling execution) can be conducted independently of the third step (inverse analysis) (Fig. 5.8 (b)).

**Inverse Analysis**

As previously stated, the main objective of the FE automated tool is to ease the extraction of meaningful mechanical parameters from simple indentation experiments; the determination of such parameters is conducted through a so-called inverse analysis involving an initial guess of model parameters that is used as initial value for the solution algorithm. The inverse analysis consists of a fitting of FE simulation to experimental data by an iterative process involving the change of material model parameters; the previously introduced input file was created based on specific boundary conditions of the measurement. The parameters of the constitutive model were adjusted by means of the nonlinear simplex method (Nelder and Mead, 1965) (fminsearch in Matlab) such that the measured and calculated displacements matched in a least squares sense.

Note that the previously introduced input file was optimized in order to reach a good compromise between solution accuracy and computational
efficiency.

In order to analyze the quality of the parameter set, an objective function \( f(x) \) defined as the root mean square error of the displacement measurements is minimized according to

\[
f(x) := \sqrt{\frac{\sum_{i=1}^{n} r_i(x)^2}{n}}
\]

where \( n \) corresponds to the number of response values and \( r_i \) represents the residual at the \( i \)-th temporal sampling point (Fig. 5.8 (c)) given by

\[
r_i(x) := \frac{h_i - \hat{h}_i(x)}{h_i}
\]

with \( h_i \) corresponding to the simulated displacements and \( \hat{h}_i(x) \) to the experimental displacements. Please note that the predicted displacement responses were linearly interpolated at the sampling points. The parameters are further iteratively refined until the objective function satisfies a convergence criterion (\( \text{tol } f, \text{tol } x \)). As previously mentioned, the discussed algorithm requires the specification of initial values for the mechanical parameters to be optimized. The results of such an optimization are typically dependent of such initial terms and problems related to multiple convergent solutions (minima) are often associated with them.

**Uniqueness of ill-posed problem solutions**

The inverse analysis method consists of identifying parameters of physical meaning based on acquired experimental data; such inverse problems are typically ill-posed (“questions mal posées” as opposed to “problèmes bien posés”), a notion introduced by Hadamard at the beginning of the 20\(^{th}\) century arguing that such problems have no physical meaning (“sont dépourvus de signification physique”) and that the existence, the uniqueness and the stability of their solutions cannot be guaranteed. In general, a finite number of data points serves the reconstruction of a model made of a large amount of degrees of freedom leading to the non-uniqueness of the inverse problem, meaning that many different models can explain the data equally well (Snieder, 1998). Moreover, the experimental data are always contaminated with errors that are further affecting the inverse
FE based analysis of indentation

Figure 5.8 Optimization procedure for evaluation of the material parameters: (a) input file creation, (b) execution of the simulation, and (c) the inverse algorithm allowing for measurement fitting.
The objective function $f(x)$ described in Eq. 5.2 is defined in order to quantify the performance and measure the quality of the parameter sets iteratively obtained; indeed, the estimation of the accuracy of the model is conducted through the minimization of such an objective function. If the forward problem is linear and the objective function is formulated in a least squares sense, the problem is usually referred to as linear least squares regression and can be solved analytically with no need of iterative algorithm, giving rise to a unique solution (Fig. 5.10 (a)) providing that the number of data points is equal or exceeds the number of unknown parameters (Hollenstein, 2011). If, on the other hand, the forward problem is non-linear, no closed-form solution exists to the non-linear least squares problem; iterative algorithms are thus used to determine the parameters $x$ by minimizing the objective function until the objective function satis-
fies a convergence criterion. Problems related to non-linear least squares optimization are mainly due to the existence of multiple local minima misidentified as global minimum; in such cases, the model estimated is not the model that best fit experimental data (Fig. 5.10 (b)).

Among the strategies granting the estimation of minima of objective functions, the descent method allowing the minimization of the objective functions by moving downhill is generally used. However, as previously mentioned, the objective functions of non-linear inverse problems may exhibit several minima and such a minimization method would lead to model estimate corresponding to local minimum of the objective functions. As a direct consequence, techniques that sample model space have been of increasing interest despite their high computational cost (Sambridge and Mosegaard, 2002).

The non-linear simplex method (fminsearch in Matlab) introduced by Nelder and Mead (1965) was used in the present work to minimize the objective function (Eq. 5.2). A simplex is a generalization of the notion of triangle (two-space) to any arbitrary dimension k; for example, in three-space, a simplex is a pyramid made of $4(k + 1)$ vertices. A new point (i.e. a new set of candidate parameters) is generated in or near the current
Figure 5.11  Nelder-Mead possible operations that can be conducted on the initial simplex (a) at each step, namely (b) reflection, (b) expansion, (c) outside construction, (d) inside contraction and (e) shrink of the simplices (Lagarias et al., 1998).

simplex following specific rules (Fig. 5.11) at each step of the searching process, its function value is compared to the function values of the vertices of the simplex; one of the vertices (the one with largest function value) is then replaced by the new point, giving rise to a new simplex. Such steps are repeated until the convergence criterion are fulfilled, i.e. the function value is less than the specified tolerance; this procedure allows exploring a large solution space, moving towards a local optimum and contracting around it. Such a procedure generates a sequence of triangles for which the function values at the vertices decrease; as the size of the triangles is reduces, the algorithm finds the minimum point (Fig. 5.12).

VALIDATION Procedure

In order to ensure that the estimated models determined through inverse analysis tend to approximate true models as close as possible, i.e. evidences that Inverse Analysis provide good results, initial parameters describing the models were chosen (when available) consistently with existing literature (e.g. Schmidt et al. (2012) for VHB4910, see Chapter 7). A three-step procedure is presented here to validate the accuracy of the present inverse analysis algorithm:

A nanoindentation ($R = 100 \, [\mu m]$, $P_{max} = 50 \, [\mu N]$, $k = 130 \, [\mu N/min]$) forward analysis was conducted using the pre-determined material for-
Figure 5.12  An example of sequence of triangles ($T_k$) converging to an optimum solution (e.g. (3,2)).

Different intensities of noise were added to the simulated data in order to account for instabilities during experiments (Fig. 5.13).

Inverse analysis was performed on each set of simulated data exhibiting different noise intensities, using the same initial parameters (Schmidt et al., 2012).

Model parameters obtained through inverse analysis of different forward analysis data incorporating noises were compared to the initial material formulation in order to investigate the robustness of the procedure. As reported in Table 5.1, the inverse analysis procedures gave rise to material parameters close to the true model, with high accuracy for the most relevant parameters ($C_{10}, g_1, g_2, t_1, t_2$) and increasing deviations for the others. Fig. 5.14 compares the predictions of the models with the reference response, for the case of a uniaxial tension test including a relaxation phase. Limitations of application of the inverse analysis procedure can be observed for an increase of noise in the experimental data. As described in
Figure 5.13  Data obtained through forward analysis with increasing noise input.

<table>
<thead>
<tr>
<th>QLV model (true) (Yeoh, longterm)</th>
<th>$C_{10}$, $C_{20}$, $C_{30}$ [MPa]</th>
<th>Initial parameters</th>
<th>Estimated parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>$g_1$, $g_2$</td>
<td>$t_1$, $t_2$ [s]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.2E-2, -8.7E-8, 1.2E-7</td>
<td>8.2E-3, -6.3E-5, 5.9E-7</td>
<td>8.2E-3, -6.3E-5, 5.9E-7</td>
<td></td>
</tr>
<tr>
<td>1.0E-2, -8.3E-5, 5.5E-7</td>
<td>1.0E-2, -7.9E-5, 5.0E-7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5.1  Estimated model parameters by IA starting from the same initial parameters.

Chapters 6, 7 and 8, the solutions obtained with the new inverse analysis were shown to be consistent with the results of data analysis procedures based on existing analytical models.
Figure 5.14 Predicted responses to an instantaneous strain (0 to 18%) and hold, in uniaxial stress configuration.
CHAPTER SIX

SPHERICAL INDENTATION OF VERY SOFT MATERIALS

The work presented in the following Chapter has been written in a manuscript format for further submission to a scientific journal. All experimental data related to it are reported in Appendix B.

6.1 Introduction

Instrumented indentation conducted with nanoindenter machines is established as a useful technique for highly localized mechanical characterization of hard materials. It consists of poking samples of choice with tips of known shape made of very stiff materials, such as diamond, monitoring in real time both the tip penetration depth, typically in the range of a few nanometers, and sample reaction forces, typically up to few milli-Newton. Mechanical characteristics related to samples are then extracted from force-displacement ($P-h$) curves using well-established equations based on elastic contact theory (Johnson, 1985; Oliver and Pharr, 1992) or using Finite Element (FE) modeling (Bolshakov et al., 1996; Ovaert et al., 2003).
Over the last decade, a growing interest has formed around the characterization of biological tissues or soft polymers at different length-scale using commercially available depth-sensing indentation testing devices; Atomic Force Microscopes (AFM) and classic micro/macro-indenter machines have been shown to have reliable capabilities for acquiring meaningful experimental data for such very soft materials (Notbohm et al., 2012). Indeed, AFM enabled the description at the nanoscale of the mechanical behavior of biological tissues such as cells (Lee, 2007; Lekka et al., 2005; Lekka and Laidler, 2009; Lekka et al., 1999; Zhou et al., 2012), cornea tissues (Last et al., 2009) and muscle fibers of the artery wall (Engler et al., 2004); micro/macro-indenter devices were used for mechanical characterization of breast tissue (Paszek et al., 2005; Samani et al., 2003), liver (Barnes et al., 2007; Carter et al., 2001; Chen et al., 1996; Tay et al., 2006) and skin (Kendall et al., 2007; Leung et al., 2002; Ling et al., 2007; Mak et al., 1994) among other tissues at larger length scales (Fig. 6.1). The use of nanoindenter machines for characterization of biological tissues has been mainly limited to relatively stiff materials such as dentine (Cuy et al., 2002; Finke et al., 2001; Ge et al., 2005; Habelitz et al., 2002) bone (Bembey et al., 2006; Haque, 2003; Rho et al., 1997; Roy et al., 1999) or cartilage (Ebenstein et al., 2004; Franke et al., 2011; Li et al., 2006; Lu and Mow, 2008) due to machine limitations related to both measurable force range and indentation depth; very few studies aimed at investigating the mechanical response of soft biological tissues (Constantinides et al., 2008; Ebenstein and Pruitt, 2004; Yuan and Verma, 2006) to nano-indentation. Ebenstein and Pruitt (2004) and Wahl et al. (2006) have investigated soft isotropic polymers exhibiting similar properties as biological tissues. Technical limitations lead to inaccurate contact point detections (Ebenstein et al., 2004; Ebenstein and Pruitt, 2004), and further to modulus overestimation (Kaufman and Klapperich, 2009).

Researchers commonly use the Oliver and Pharr approach to extract mechanical properties from nanoindentation data with no consideration for the influence of adhesion that most likely occur when indenting very soft materials. However, Ebenstein and Wahl (2006) and Wahl et al. (2006) made a special effort in extracting meaningful mechanical properties from unloading nanoindentation curves using the Hertzian contact
theory extended by adhesive contact (Johnson et al., 1971); as such, their focus has been driven towards the understanding of the adhesive effects. In the present work, the term pull-in (i.e. snap-on) refers to the attraction of the indenter tip observed when approaching the sample surface as opposed to adhesion which refers exclusively to the interaction of the indenter tip with the surface of the specimen during the unloading phase. Recently, nanoindentation experiments conducted on soft materials fully immersed into liquids such as water and surfactant solutions have been shown to reduce the effects of meniscus on the long indenter shaft as well as diminishing/eliminating both pull-in and adhesive effects (Ebenstein, 2011; Kohn and Ebenstein, 2013). The present study is based on the use of a commercially available nanoindenter and develops a new set-up and procedure in order to address the following challenges related to the characterization of very soft materials at the microscale:

Attraction forces arises when the indenter approaches the surface. For
hard material indentation these forces are below the resolution limit and thus disregarded. Here, their influence cannot be neglected. The pull-in effect is analyzed in order to understand its cause.

When testing soft biological tissues, the surface topography cannot be selected as nearly flat. The influence of surface roughness on force-indentation curves has to be analyzed.

Large deformations have to be applied for a meaningful mechanical characterization of soft materials. The validity of linear theories (such as Hertz contact modified analytic solution) has to be evaluated. This is done here with comparison to non-linear finite element based inverse analysis.

6.2 METHODS

6.2.1 MICRO-INDENTATION EXPERIMENTS

Experimental data were acquired with the help of a modified Ultra Nanoin dentation Tester (UNHT, CSM Instruments SA) (displacement range = 50 [µm], normal force range = 1-50000 [µN]) driven in force-controlled mode. Tests were performed on the following materials: (i) Ecoflex0030, a two-part, platinum-catalyzed silicone rubber material manufactured with two types of surface roughness (rough and flat), (ii) fused silica, a well-characterized, hard material often used as a certified reference material to calibrate nanoindenter machines, and (iii) paraffin wax, a material known for its hydrophobic properties (Quere and Reyssat, 2008). The existing reference/indenter (ruby sphere with radii of 1500/100 [µm]) sensor-actuator loop allowed minimization of both thermal drift and instrument frame compliance (Nohava et al., 2009). The UNHT machine has been modified in order to test specimens at 40% and 80% humidity (water evaporative source coupled to a relative humidity meter) in a sealed chamber or immersed in water (100% humidity) in a liquid cell (Mann and Pethica, 1996). For the set-up allowing a total immersion of the specimen in water, both reference and indenter tip lengths have been extended to a total length of 12 [mm] (Fig. 6.2). Dimensions of specimen (Diameter (D) = 18
Methods

Sensor - Actuator Loop
Reference
Holder
Specimen
Tip 200 [µm]
Retraction
Specimen
D
H
Water

Figure 6.2  Modified setup for nanoindentation immersed in water (i.e. 100% humidity) with a zoom on the spherical tip (Radius: 100 [µm]).

[mm], Height (H) = 3 [mm]) were chosen in order to avoid influence of the substrate on nanoindentation measurements. The following indentation procedure has been applied for measurements performed on Ecoflex0030 immersed in water, a standard process for hard materials being used to indent fused silica and paraffin wax. The surface position relative to the specimen holder was first estimated through a standard surface detection procedure. A two-steps loading procedure was then initiated, namely starting by a pre-approach involving movement of the indenter tip towards sample surface and landing of the reference on the sample holder; a retraction of 25 [µm] of the indenter tip was prescribed (Fig. 6.2) in order to avoid any contact with the specimen, allowing further monitoring of the contact interaction between specimens and the indenter in the approach phase. Note that at this point of the procedure, both the indenter and the reference were immersed in water and maintained at this position for 2 hours in order to stabilize the influence of water on measurements data. As a second step, the indenter was driven down towards the specimen surface at a speed of 18 [µm/min], recording measurements data at a rate of 10 [Hz] until reaching a contact force of 2 [µN]. Then, a linear ramp load (130 [µN/min]) was applied to the specimen until reaching a specific deformation (corresponding to strains up to 5%).
6. Spherical Indentation of very soft Materials

6.2.2 Analytical solution

For spherical indenter, the small strain elastic deformation of specimens can be described by the Hertzian contact modified relation (Johnson, 1985)

\[ P = \frac{4\sqrt{R}}{3} \frac{E}{(1 - \nu^2)} h^{3/2} \]

(6.1)

where the relationship between force \((P)\) and displacement \((h)\) is nonlinear due to the increasing contact area between the sphere and the specimen. The material characteristics are expressed through Young’s modulus \((E)\) and Poisson’s ratio \((\nu)\). Geometrical nonlinearities that occur for deformations induced by deep indentations (large strains) are not taken into account in this solution.

6.2.3 FE modeling

In the case of large deformations, the strains are no longer infinitesimal so that material and geometrical nonlinearities occur as the specimen is indented. The FE Software ABAQUS 6.10 (ABAQUS Inc., Providence, RI) was used to extract mechanical properties from measured data. Calculations have been performed by means of axisymmetric models consisting of a perfectly rigid spherical indenter \((R = 100 [\mu m])\), which penetrates a specimen (width \((W_1) = 3 [mm]\), height \(H_1 = 3 [mm]\)) covered by an alterable roughness layer \((W_2 = 3 [mm], H_2 = 40 [\mu m])\) (Fig. 6.3). The main model included 3227 nodes assembled in 3135 quadratic elements (CAX8RH) and the roughness layer was made of 11668 nodes forming 22055 CAX6-elements. All simulations were conducted in a quasi-static displacement-controlled mode; no time-dependence of the material response was taken into account for the present case. FE sensitivity studies have been conducted for optimizing the specimen size and analyzing the impact of the ratio indenter radius/indentation depth on simulations. The size of the smallest element \((2.13 [\mu m])\) also rose from an optimization procedure. Frictionless conditions were assumed as interaction between the indenter and the specimen for all simulations. An inverse iterative FE algorithm involving minimization of least squared errors between experimental and simulated loads has been programmed with the help of Matlab.
Methods

\[ W_i = W_z \]

Figure 6.3  FE model with (a) the axisymmetric structured mesh representing the whole specimen, (b) the adjustable roughness layer with an example of roughness mesh (wavelength \( w = 35 \text{ [\( \mu m \)]}, \text{dent} \ d = 20 \text{ [\( \mu m \)])}, \) and (c) the visualization of von Mises stresses in a specimen displaying rough surface at maximum indentation depth \( (h) \).

(The MathWorks Inc., Natick, MA, USA) in order to identify parameters of the constitutive model.

6.2.4 CONSTITUTIVE MODEL

The Neo-Hookean formulation for incompressible material, which is a one-term reduced-polynomial strain-energy function, was used as a constitutive model in order to represent the mechanical properties of Ecoflex0030. The strain energy density is given as

\[ W = C_{10}(I_1 - 3) \]  

(6.2)

where \( C_{10} \) is the only material constant, which can be related to both the initial shear modulus \( (\mu_0) \) and Young’s modulus \( (E_0) \) in case of incompressible material \( (\nu = 0.5) \)

\[ \mu_0 = 2C_{10} \]

\[ E_0 = 6C_{10} \]  

(6.3)

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6. Spherical Indentation of very soft Materials

The first invariant $I_1$ of the Cauchy-Green deformation tensor $C$ is defined as

$$I_1 = tr(C) = \lambda_1^2 + \lambda_2^2 + \lambda_3^2$$

$$C = A^T A$$

(6.4)

where $A$ represents the deformation gradient.

6.3 EXPERIMENTAL OBSERVATIONS

6.3.1 THE INFLUENCE OF HUMIDITY

Experimental load-displacement ($P-h$, loading path only) curves for flat Ecoflex0030 samples are shown in Fig. 6.4 for nanoindentation tests performed at 40% humidity ("normal" environmental conditions in the laboratory) and immersed in water (100% humidity), with a maximum displacement of 20 [$\mu$m]. Negative forces (down to -17 [$\mu$N]) were observed when approaching closely the sample surface in the air, forces that vanished indenting immersed in water. An FE inverse analysis was conducted on measurements data acquired indenting fully immersed in water, leading to $C_{10}=0.0046$ [MPa] for Ecoflex0030 (Eq. 6.2). Nanoindentation measurements have further been conducted on fused silica for different humidity levels (40%, 80%, 100%) (Fig. 6.5). Attractive forces similar to those which had been previously observed for Ecoflex0030 were sensed when approaching the surface at 40% humidity (12 [$\mu$N]), with an increase of their magnitudes at 80% humidity (28 [$\mu$N]). Negative forces similarly vanished when indenting with indenter and sample totally immersed in water. Nanoindentation measurements were finally performed at 40% humidity on paraffin wax (Fig. 6.6). No negative forces were observed.

6.3.2 THE INFLUENCE OF SURFACE TOPOGRAPHY

Surface topographies of two Ecoflex0030 specimens exhibiting distinct roughness (rough, flat) were acquired with the help of a LSM 5 PAS-CAL microscope (Carl Zeiss Microscopy, Laser Scanning Microscope) in a
Experimental observations

Figure 6.4  Ecoflex0030 tested at 40% humidity (air) and immersed in water (100% humidity).

Figure 6.5  Fused Silica tested at 40%, 80% and 100% humidity.

z-stack laser mode (Fig. 6.7). 3D topographic data were then smoothened using a Gauss filter prior to analysis. Surface geometry analysis (Zeiss LSM Image Examiner) provided characteristic values of amplitude and
wavelength. The surface of the rough specimen showed the presence of significant corrugation with maximum amplitude and wavelength of 20 $\mu$m and 50 $\mu$m as compared to small dent size present at the surface of flat sample reaching 5 $\mu$m depth. Nanoindentation experiments conducted on each specimen type immersed in water are shown in Fig. 6.8; the responses are quite different. Different surface roughnesses were simulated by FE (Fig. 6.3) respectively for constant dent amplitude ($d = 20 \mu$m) or wavelength ($w = 35 \mu$m) (Fig. 6.9). At constant amplitude, the normal force decreases with decreasing wavelength. For constant wavelength, the force sensed increases with decreasing amplitude. A comparison between
6.3.3 Uniaxial Tensile Tests

Uniaxial tensile tests were conducted on 4x1.5x0.1 [mm] Ecoflex0030 samples stretched with a strain rate of 30%/min (Fig. 6.11), similar to the rate of deformation in nanoindentation experiments; experimental data are shown in Fig. 6.12. Corresponding FE modeling using Neo-Hookean material formulation (C10 = 0.0046 [MPa]) and linear elastic model results ($E = 6C_{10} = 0.0276$ [MPa]) are shown.

6.4 Discussion

Indenting very soft materials at the micro-level, the forces sensed by the UNHT are in the range of few $\mu$-Newton, thus in the range of capillary forces (Chen and Soh, 2008). Surface roughness has also been shown to
Figure 6.9  FE modeling for different surface roughness; $d$ and $w$ are defined in Fig. 6.3.

play a critical role in the response of soft materials to nanoindentation experiments.
Figure 6.10 Hertzian contact and FE modeling vs. measurements made on rough (M. Rough) and flat samples (M. Flat). The analytical and FE predictions for flat surface (FE Flat) almost perfectly coincide with measured data (M. Flat). The FE model with d = 20 [µm] and w = 35 [µm] provides excellent predictions of the M. Rough data.

6.4.1 The Pull-In Effect

In vacuum, the leading interactions acting at the micro-level are van der Waals, electrostatic and electromagnetic forces (Israelachvili, 2011). In air (40% humidity), water vapor plays the dominant role, creating condensation (water film) at the interface between the specimen surface and the indenter, leading to the formation of meniscus forces, also called capillary forces. Capillary effects have been studied at the nanoscale for various relative humidity (RH) with the help of AFM, during the so-called snap-on and pull-off (retraction) phases (Eastman and Zhu, 1996; Grobelny et al., 2006; Ouyang et al., 2001). There is no consensus in the AFM literature on the effect of RH on pull-off forces; as for the snap-on effect, it has not been systematically investigated in previous studies.

In the present work, negative forces, named as pull-in forces, were observed when approaching Ecoflex0030 surface in the air (40% humidity). Nanoindentation experiments conducted on the same sample immersed in
water allowed to eliminate the attraction forces exerted on the indenter. An inverse analysis for flat sample nanoindentation measurements in water allowed determining the material parameters of Ecoflex0030 (Eq. 6.2, $C_{10}=0.0046$).

Further tests on Fused Silica have confirmed that capillary forces were not only present in the indentation process of very soft materials, but also that they were of the same magnitude when indenting hard materials at 40% humidity; these surface forces have not been previously identified mainly because of two reasons, (i) Berkovich indenters that are usually chosen to indent hard materials form limited meniscus with water due to the small area which interacts with the specimen, and more importantly
(ii) the forces sensed during loading are typically in the order of milli-Newton, surface forces being far below this-range. An increase of pull-in forces with rise of relative humidity (40% to 80%) was further observed (Fig. 6.5). As expected, pull-in effects vanished when indenting totally immersed in water.

In order to further validate the proposed interpretation, paraffin wax, a material displaying repulsion property, was tested in air. No pull-in effects were observed in these tests (Fig. 6.6), confirming the hypothesis and in line with previous observations made with AFM (Eastman and Zhu, 1996).

6.4.2 THE INFLUENCE OF ROUGHNESS

Further insights about the impact of surface geometry on nanoindentation measurements of very soft materials are provided through the measurements on specimens with same material properties (Ecoflex0030) but different surface roughness (Rough, Flat) (Fig. 6.8). The discrepancy between the two force-displacement curves was rationalized using simplified FE modeling of roughness; an analysis was conducted in order to estimate the influence of dent amplitude (d) and wavelength (w) on measurements.
outcomes (Figs. 6.8, 6.9). Calculations demonstrate that this model can explain the difference observed between rough and flat specimen. Moreover, the parameters of the simulation closest to reality ($d = 20 \, \mu m$, $w = 35 \, \mu m$) are in line with the corresponding laser-scan roughness measurements for the rough samples.

The Hertzian contact formulation (Eq. 6.1) was compared to the results obtained with FE modeling when analyzing the impact of roughness on measurement prediction (Fig. 6.10). Obviously, the Hertz modified model does not predict the response of the rough surface and slightly over-predicts forces for the flat sample (Lu et al., 2008). The flat and rough FE models are able to predict the behavior of the corresponding samples.

### 6.4.3 Model validation

Uniaxial tensile tests data were used to validate the elastomer model obtained using nanoindentation for (multi-axial) strains up to 20% (Fig 6.12). Uniaxial predictions (FE modeling) agree to a great extent with measurements. Discrepancy between mean tensile test curve and indentation-based model is in the same range as the variability of measurements data. An overestimation of uniaxial stiffness might be related to the different kinematic configurations of the two experiments. The linear elastic model is valid only for small strains.

### 6.5 Conclusion

This study underlines the importance of surface interaction when indenting very soft materials. Important effects influencing nanoindentation measurements and their interpretation (by decreasing order of importance) have been addressed, namely: (i) the relative humidity in the chamber, (ii) the surface roughness of the specimen, and (iii) materials and geometrical nonlinearities (need of a FE analysis).

To our knowledge, this work is the first to analyze and rationalize pull-in forces during indentation at the microscale showing that these are due to capillary effect. Larger capillary forces are related to the use of
larger indenter tip radius, such as in the present spherical tip, as compared to Berkovich indenter. The influence of surface topography could be understood through corresponding FE models explicitly representing an undulated surface. The findings of the present work might be applied to microindentation-based characterization of synthetic materials or biological tissues with very low stiffness, non-linear stress-strain behavior, and non-flat surface.
VISCOELASTIC CHARACTERIZATION
BY MEANS OF INDENTATION

All experimental data related to the present Chapter are reported in Appendix B.

7.1 Introduction

Over the last decade, several studies were performed aiming at a mechanical characterization of soft polymers and biological tissues by means of instrumented indentation testing devices; in addition to Atomic Force Microscopes (AFM) (Lee, 2007; Lekka and Laidler, 2009; Lekka et al., 1999; Zhou et al., 2012) and micro/macro-indenter apparatus (Akhter et al., 2004; Barnes et al., 2007; Kendall et al., 2007; Ling et al., 2007) nanoindenter machines have been shown to provide relevant insight for characterizing very soft materials (Constantinides et al., 2008; Ebenstein and Pruitt, 2004; Wahl et al., 2006; Yuan and Verma, 2006). The latter applications are related to challenges such as the inaccurate contact point detection (Ebenstein et al., 2004; Ebenstein and Pruitt, 2004; Kaufman and Klapperich, 2009; Wahl et al., 2006) and sensor-actuator ranges (al-
7. Viscoelastic characterization by means of Indentation

lowing for very low force sensing and large displacements) (Farine et al., 2013).

Beside difficulties related to experimental problems, major influences on data interpretation associated with soft material characteristics are the heterogeneity of the sample, its surface roughness as well as its time-dependent behavior when subjected to deformation; these concerns are of particular importance when testing biological tissues (Menzel, 2005). The analysis of experimental data by reliable models is the main prerequisite to the accurate characterization of soft time-dependent materials.

Time-dependent behavior can either be described by phenomenological viscoelastic approach with the relaxation function expressed in term of Prony series (Bembey et al., 2006; Oyen, 2005; Oyen and Cook, 2003), or by means of poroelasticity (Galli and Oyen, 2009; Hu et al., 2010) where the time-dependence is due to the flow of a fluid inside a poroelastic structure; the present work uses viscoelastic models to predict the behavior of a very soft time-dependent material based on spherical micro-indentation. Using Boltzmann operators, Lee and Radok (1960) provided a solution for the viscoelastic contact originating from the Hertz formulation assumed for linear elastic deformation; the model predicts the material response to an applied step load through the creep compliance function. Since the application of a step load function during the loading process of micro-indentation is unrealistic, the hereditary integral equations need to be solved for a ramp load function (Oyen, 2005).

A soft acrylic elastomer membrane (VHB4910) widely used for applications as Dielectric Elastomer Actuators (DEA) is taken as a model system for the present investigations. This material has been shown in previous investigation (Ha et al., 2007; Schmidt et al., 2011, 2012; Wissler and Mazza, 2005, 2007) to behave as a hyperelastic-viscoelastic material. It was selected in order to investigate the capability of linear and non-linear viscoelastic models to predict the time-dependent behavior in micro-indentation experiments at various maximum loads and loading rates.

Both a Quasi-Linear Viscoelastic (QLV) model (Nekouzadeh et al., 2007; Sarver et al., 2003; Schmidt et al., 2011; Yoo et al., 2009) and the Hertzian contact analytical solution Johnson modified for viscoelastic bodies (Oyen, 2005) were used in order to extract mechanical properties from
7.2 Experimental methods

7.2.1 Micro-indentation creep experiments

Experimental data were acquired using a modified force-driven Ultra Nanoindentation Tester (UNHT, CSM Instruments SA) (displacement range = 50 [µm], normal force range = 1-50000 [µN]) allowing a total immersion of samples in water; the corresponding experimental procedure has been previously described (Farine et al., 2013). Flat VHB4910 specimens (Diameter = 18 [mm], Height = 3 [mm]) were tested at different maximum loads (Pmax = 12.5, 25, 50, 100, 200, 400 [µN]) and loading rates (k = 32.5, 65, 130, 260 [µN/min]) with a spherical indenter tip (ruby sphere with a radius of 100 [µm]). A creep phase of 60 [s] was included at the end of the loading ramp to each measurement in order to investigate the time-dependent behavior of the material (Fig. 7.1).

7.2.2 Hertzian contact for viscoelastic bodies

Considering spherical indentation of soft materials, the small strain elastic deformation can be described by the modified Hertzian contact as Johnson

$$\frac{4\sqrt{R}}{3} h^{3/2} = \frac{(1 - \nu^2)}{E} P$$

(7.1)

where the force-displacement (P-h) relationship is non-linear. Recalling that geometric and material nonlinearities are not taken into account in this solution, the model is only valid for small deformations (shallow indentation). For an incompressible solid \(\nu = 0.5\) is used; replacing the elastic constant \((3/2E)\) by a corresponding linear viscoelastic integral operator (Lee and Radok, 1960), one can rewrite the contact equation as

$$\frac{16\sqrt{R}}{9} h^{3/2} = \int_0^t J_{AS}(t - u) \frac{dP}{du} du$$

(7.2)

where u represents the variable of integration and \(J_{AS}(t)\) is the creep compliance function, representing the time function of strain increase for
7. Viscoelastic characterization by means of Indentation

![Figure 7.1](image)

Figure 7.1  Force/time histories for different loading rates ($k_1 = 32.5$, $k_2 = 65$, $k_3 = 130$, $k_4 = 260$ [µN/min]) and maximum loads ($P_1 = 12.5$, $P_2 = 25$, $P_3 = 50$, $P_4 = 100$, $P_5 = 200$, $P_6 = 400$ [µN]); the creep time ($t_c$) was set to 60 [s].

A constant applied stress; in the present work, the particular form of creep compliance

$$J_{AS} = C_0 - \sum C_i \exp(-t/\tau_i) \tag{7.3}$$

for which $i = 1, 2$ was chosen. For the special case of micro-indentation ramp loading, the load is split into a ramp phase ($0 \leq t \leq t_R$) and an hold phase ($t \geq t_R$) giving rise to the relationship between the displacement and the creep function parameters (Oyen, 2005)

$$0 \leq t \leq t_R, \ P(t) = kt$$

$$h(t) = \left( \frac{9k}{16\sqrt{R}} \left\{ C_0t - \sum C_i\tau_i [1 - \exp(-t/\tau_i)] \right\} \right)^{2/3} \tag{7.4}$$
Experimental methods

Figure 7.2 (a) The axisymmetric FE model with its structured mesh representing the whole specimen on which kinematic boundary conditions are indicated, and (b) the representative indented volume considered for strain averaging at maximum load.

\[ t \geq t_R, \quad P(t) = P_{max} = kt_R = \text{const.} \]

\[ h(t) = \left( \frac{9k}{16\sqrt{R}} \left[ C_0 + \sum C_i \tau_i \exp(-t/\tau_i) \left[ \exp(t_R/\tau_i) - 1 \right] \right] \right)^{2/3} \]

(7.5)

7.2.3 Quasi-linear viscoelastic model

As the specimen indented undergoes large deformations, both material and geometrical nonlinearities might influence the experiment. The commercially available FE Software ABAQUS 6.10 (ABAQUS Inc., Providence, RI) was used together with an inverse analysis procedure to extract mechanical properties from measurements data. An axisymmetric model consisting of a perfectly rigid spherical indenter (radius = 100 [\mu m]) which is in frictionless contact with a specimen (width (W) = 3 [mm], height (H) = 3 [mm]) was created in order to perform the numerical calculations; the model included 3227 nodes assembled in 3135 quadratic elements (CAX8RH) (Fig. 7.2). A QLV material model was used to sim-
ulate the time-dependent response of VHB4910 undergoing large strains. The incompressible Yeoh formulation, which is a simplified form of the so-called reduced polynomial strain energy function, is used as a constitutive model in order to represent the nonlinear mechanical properties of VHB4910 (Wissler and Mazza, 2005):

\[ W = C_{10}(I_1 - 3) + C_{20}(I_1 - 3)^2 + C_{30}(I_1 - 3)^3 \] (7.6)

The time-dependent coefficients (Prony series relaxation function) are applied to the coefficients \( C_{10} \), \( C_{20} \) and \( C_{30} \) that define the energy function

\[ C_{ij}(t) = C_{ij}^0 \left[ 1 - \sum_{k=1}^{N} g_k (1 - \exp(-t/t_k)) \right] \] (7.7)

where the relation between the instantaneous elastic parameter \( C_{ij}^0 \) and the long-term elastic parameter \( C_{ij}^{\infty} \) is

\[ C_{ij}^0 = \frac{C_{ij}^{\infty}}{1 - \sum_{k=1}^{N} g_k} \] (7.8)

The linearized relaxation modulus related to the chosen material formulation can be calculated as

\[ E_{FE}(t) = 6C_{10}^0 \left[ 1 - \sum_{k=1}^{N} g_k (1 - \exp(-t/t_k)) \right] \] (7.9)

### 7.2.4 Tensile Relaxation Experiments

Uniaxial tensile tests were conducted on 4x1.5x0.1 [mm] VHB4910 samples stretched with a strain rate of 0.5%/s, similar to the maximum rate of deformation in micro-indentation experiments; after reaching a maximal strain of 18.5%, the deformation was hold constant for 35 [s], leading to relaxation of the material (Fig. 7.3). In the case of linear viscoelastic materials, the relaxation function can be determined from the creep compliance function \( J_{AS}(t) \) through the correspondence principle (Laplace domain)

\[ E_{AS}(t) = L^{-1}\left\{ \frac{1}{s^2} L\{J_{AS}(t)\} \right\} \] (7.10)
For a ramp-hold tensile experiment, the stress history is given for the ramp \((0 \leq t \leq t_1)\) and hold \((t_1 \leq t)\) phases

\[
0 \leq t \leq t_1, \quad \sigma(t) = \frac{\epsilon_1}{t_1} \int_0^t E_{AS}(t - \tau) d\tau \\
t_1 \leq t, \quad \sigma(t) = \frac{\epsilon_1}{t_1} \int_0^{t_1} E_{AS}(t - \tau) d\tau
\]

(7.11)

### 7.3 Results and inverse analysis

One set of indentation curve was used to determine the material model parameters, the rest of the data were used as a benchmark test for the verification of model predictive capability.

#### 7.3.1 Model parameters determination

Model parameters were determined from the case of a maximal load \(P_{max} = 50 \, [\mu N]\) applied at a deformation rate of \(k = 130 \, [\mu N/\text{min}]\). Following a previous approach (Oyen, 2005), the creep (hold) phase (Eq. 7.5) of the Hertzian contact formulation for viscoelastic bodies (ramp loading) was fitted to VHB4910 creep measurement data \((h - t)\); amplitude coefficients
7. Viscoelastic characterization by means of Indentation

<table>
<thead>
<tr>
<th>Hertzian Contact</th>
<th>$C_0$, $C_1$, $C_2$ [MPa$^{-1}$]</th>
<th>$\tau_1$, $\tau_2$ [s]</th>
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</thead>
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<tr>
<td></td>
<td>14.6, 3.5, 6.3</td>
<td>4.8, 51.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>QLV model (Yeoh, longterm)</th>
<th>$C_{10}$, $C_{20}$, $C_{30}$ [MPa]</th>
<th>$g_1$, $g_2$</th>
<th>$t_1$, $t_2$ [s]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.2E-2, -8.7E-8, 1.2E-7</td>
<td>0.6, 0.2</td>
<td>1.4, 28.8</td>
</tr>
</tbody>
</table>

Table 7.1 Material parameters for $P_{max} = 50$ [$\mu$N] and $k = 130$ [$\mu$N/min].

$(C_0, C_1, C_2)$ as well as time constants $(\tau_1, \tau_2)$ that were computed from the fit are listed in Table 7.1. The complete ramp-hold curves were then predicted for different maximum loads applied ($P_{max}$) and several loading rates ($k$) (Fig. 7.5, Fig. 7.8) using Eqs. 7.4 and 7.5.

FE inverse analysis was carried out for the full ramp-hold phases, leading to the QLV parameters of Eqs. 7.6 and 7.7 ($C_{10}, C_{20}, C_{30}, g_1, g_2, t_1, t_2$) listed in Table 7.1; FE simulations were subsequently performed for all different maximum loads and loading rates leading to FE predictions (Fig. 7.6, Fig. 7.9).

### 7.3.2 Predictions for Different Maximum Loads

Experimental load-displacement ($P-h$, ramp-hold) curves acquired for different maximum loads ($P_{max}$) at constant loading rate ($k = 130$ [$\mu$N/min]) are shown in Fig. 7.4; measurements data exhibits very good repeatability. Both models gave evenly rise to very good measurement predictions (Fig. 7.5, Fig. 7.6) for a wide range of maximum load applied ($P_{max} = 12.5, 25, 50, 100, \text{ and } 200$ [$\mu$N]). Predictions made through the linear viscoelastic (AS) model underestimated deformations for deeper indentation ($P_{max} = 400$ [$\mu$N]). Average strains at maximum load were determined from FE simulations according to the procedure by Pelletier et al. (2009); they averaged strain values over a sample volume proportional to the contact radius. Average strains for different values of indentation depth ($h$) are reported in Fig. 7.5. For strains exceeding 10%, the linear model deviates from the experimental data.
Results and inverse analysis

Figure 7.4  Force-displacement representative measurement curves among sets of 9 experiments for each different $P_{max}$ applied to VHB4910.

Figure 7.5  Data, creep phase fitting and analytical predictions for micro-indentation measurements at several $P_{max}$.

7.3.3 PREDICTIONS FOR DIFFERENT LOADING RATES

Fig. 7.7 shows the load-displacement ($P-h$, ramp-hold) curves acquired at different loading rates ($k$) for a constant maximum load applied ($P_{max} = $
7. Viscoelastic characterization by means of Indentation

Figure 7.6 Data, inverse analysis and FE predictions of micro-indentation measurements for different maximal loading.

Figure 7.7 VHB4910 Measurement data for different loading rates (k).

50 [µN]); measurement’s repeatability is shown to be very good. The modified Hertzian Contact for viscoelastic bodies and the QLV model provide very good prediction for a wide range of loading rate (k) configurations
Results and inverse analysis

Figure 7.8  Analytical solution predictions for different loading rate (k) exhibit good agreement with measurement data.

(Fig. 7.8, Fig. 7.9).

7.3.4 Relaxation functions

Relaxation module of analytical ($E_{AS}$) and FE ($E_{FE}$) based models (Eqs. 7.9 and 7.10) are compared in Fig. 7.10; curves exhibit an overall agreement, though instantaneous relaxation moduli ($E_{AS}$, $E_{FE}$ for $t = 0$) differ up to 25%.

7.3.5 Uniaxial tensile testing

Uniaxial data are shown in Fig. 7.11; corresponding tensile simulation using the previously established QLV material formulation (Table 7.1) as well as the stress history determined from the analytical expression given in Eq. 7.11 are displayed. Note that the analytical prediction provides good stress values in the initial phase, but diverges significantly from measurements for strains larger than 10%.
7.4 DISCUSSION

Linear viscoelastic models can be used to describe time-dependent materials behavior when limited strains are observed during the micro-indentation process (Hutchings, 2009). Stresses and deformations in an indentation test can be kept low either by decreasing the magnitude of the maximum load applied ($P_{max}$) to the sample or by increasing the contact area over which $P_{max}$ acts; accordingly, spherical tips are preferred to Berkovich indenters when aiming at a linear viscoelastic characterization. In the present work, the size of the spherical indenter ($R = 100 \text{ [\mu m]}$) lead to large local deformation during the micro-indentation process (local average strains up to 12%), when indenting up to 50 [\mu m] deep into the soft material (VHB4910) at larger forces.

Experimental data were acquired by means of surface scanning through a micro-indentation matrix (50 [\mu m] spacing) over a wide region of the specimen in order to evaluate data reproducibility; repeatability has been shown to be very good over the whole range of tests (Fig. 7.4, Fig. 7.7).
Figure 7.10  Relaxation module for VHB4910: Analytical Solution (AS) and Finite Element (FE). The corresponding relaxation module from Schmidt et al. (2012) is reported as dotted line.

Figure 7.11  Uniaxial tensile data (Nominal stress) compared with FE modeling and Laplace Transform analytical solution (0.5% strain/s).
7. Viscoelastic characterization by means of Indentation

Each measurement was followed by a retraction time of 60 [s], allowing the material to relax between two indentation processes. Challenges related to changes of the loading rate during the measurement procedure have been observed; the difficulty for the control unit of the nanoindenter to apply a perfect ramp load during the loading phase is visible in 7.9 (ramp) for low loading rate ($k = 32.5 \, [\mu N/min]$). Both models provide overall good predictions for a wide range of loads and loading rates; the linear viscoelastic model overestimated the response to indentation for strains larger than 10% (i.e. $P_{\text{max}} = 400 [\mu N]$).

Predictions for variable loading rates ($P_{\text{max}} = \text{constant}$) were satisfactory for both models with some deviations for low deformation rate ($32.5 \, [\mu N/min]$) (Fig. 7.8, Fig. 7.9). The linear viscoelastic and the QLV models provide overall good predictions of soft time-dependent material behaviors (VHB4910) over a large range of deformations. The corresponding relaxation functions obtained with both methods are plotted in Fig. 7.10; their instantaneous moduli exhibit up to 25% difference, but after 2 [s] the values coincide. Note that the values of relaxation times and instantaneous E modulus ($E(0)$) from the analytical solution were taken as initial values for the optimization, leading to the parameters of the QLV model.

Uniaxial data were used to compare the capabilities of the models to predict the behavior of VHB4910 undergoing larger strains up to 18% (Fig. 7.11). Predictions made through the QLV model agree to a great extent with measurements, unlike the linear viscoelastic model that diverges for strain larger than 10%; this observation is in line with the results of micro-indentation testing. As expected, the linear viscoelastic model is reliable for small strains; its limit of validity is shown for the present case to be at an average strain of about 10%.

The relaxation module obtained in this work is in line although not coincident with the results of multi-axial experiments over a wide range of deformation (Schmidt et al., 2012) (up to 800% strain), as shown in Fig. 7.10.
7.5 CONCLUSION

This study demonstrates the possibility to characterize the mechanical behavior of soft time-dependent materials using spherical micro-indentation. The model system selected is representative of the response of soft biological tissues such as porcine liver and skin (Constantinides et al., 2008).

The analytical viscoelastic approach has been shown to well describe the response to micro-indentation experiments for a wide range of deformation up to average strains of 10%; its ease of use and fast computation time make of it a straightforward analysis procedure of indentation data for materials exhibiting linear viscoelastic properties. FE modeling, through a more time-consuming inverse analysis method, could be shown to accurately describe the response to time-dependent deformations with strains larger than 10%; uniaxial data leading to large deformations have demonstrated the predictive capabilities of both analytical and QLV models.
In this context, modified nanoindenter machines allowing for low force sensing and large displacement have been shown to have a promising potential for characterizing such very soft materials at the microscale.

Another prerequisite to the accurate characterization of soft biological tissues by means of instrumented indentation is the use of appropriate models for extracting meaningful physical parameters from experimental observations; biological tissues, which are seen to a great extent as
anisotropic materials, demand for sophisticated constitutive models accounting for nonlinear stress-strain relation and time-dependent material response. Such time-dependent behavior can either be described by empirical viscoelastic approach with the time-dependence being a function of time-dependent elastic parameters (Chen, 2000; Nava et al., 2008), or by means of poroelasticity where the time-dependence is related to flow of a fluid inside a poroelastic structure and characterized by the Darcy permeability $\kappa$ (Biot, 1941).

The present chapter aims at stepping towards the characterization of soft biological tissues by investigating the behavior of synthetic homogeneous hydrogels undergoing micro-indentation testing; few analysis have been conducted in the past through conical and spherical indentation on compliant gels such as gelatin (Galli and Oyen, 2009), polyacrylamide (Galli et al., 2009), alginate (Hu et al., 2010), polydimethylsiloxane (Hu et al., 2011b) and agarose (Oyen, 2013) at both macro and microscopic lengthscale.

In the present work, agarose gels exhibiting porous structures (Normand et al., 2000) and mechanical properties similar to biological tissues in terms of stiffness and time-dependence were prepared with varying agarose concentrations (0.5, 1, 2 and 4% w/w) and indented at the microscale with the help of a modified nanoindenter machine at different maximal loads and several loading rates. Both Quasi-Linear Viscoelastic (QLV) and Linear PoroElastic (LPE) models were fitted to experimental data to obtain time-dependent material formulations which accuracies were subsequently tested on benchmark measurements at different maximal loads and for various loading rates.

Finally, an experimental force-controlled (creep) indentation procedure involving the use of spherical tips of different sizes ($R = 100, 200, 500$ and $1500 \, [\mu m]$) and allowing the differentiation between visco and poro-like behaviors is introduced.
8.2 EXPERIMENTAL METHODS

8.2.1 AGAROSE GELS PREPARATION

Agarose gel solutions were prepared by direct dissolution of varying amount of agarose powder (Life Technologies, Carlsbad, California, USA) in water-filled recipients in order to obtain gels with different agarose concentration (0.5, 1, 2 and 4% w/w). After microwaving the preparations up to a temperature of 99°C, the hot mixtures were poured into petri dishes and left for cooling down to room temperature to form water-filled percolating networks; a gel thickness of 3 [mm] was purposely achieved in order to avoid any influence of the substrate on subsequent micro-indentation measurements. Please see Chapter 3 for additional information about agarose gel composition.

8.2.2 MICRO-INDENTATION CREEP EXPERIMENTS

In the present work, spherical (ruby sphere with a radius of 100 [µm]) micro-indentation data were acquired with the help of a modified force-driven Ultra Nanoindentation Tester (UNHT, CSM Instruments SA, Peseux, Switzerland) (displacement range = 50 [µm], force range = 1-50000 [µN]) allowing full immersion of samples in water; the procedure has been previously described (Farine et al., 2013). Flat gels (Diameter = 18 [mm], Height = 3 [mm]) with varying agarose concentration (1, 2 and 4% w/w) were tested for different maximum loads ($P_{max} = 12.5, 25, 50, 100$ [µN]) and loading rates ($S = 32.5, 65, 130, 260$ [µN/min]); 0.5 % w/w agarose gels were tested for a special set of parameters ($P_{max} = 50$ [µN] and $S_3 = 130$ [µN/min]) only. Note that a creep phase of 60 [s] was coupled to each measurement in order to investigate the time-dependent behavior of the hydrogels (Fig. 8.1). Following the retraction of the indenter, an interval before the next step was enforced in order to ensure that time-dependent effects that may have raised during the indentation process are largely reduced. Note that experimental data were acquired by means of surface scanning through nanoindentation matrices (100 [µN] spacing) over a region of the specimen in order to evaluate data reproducibility. In order to observe and further classify the deformations of soft time-dependent ma-
terials undergoing force-controlled indentation procedures for either poro or visco-like behaviors, a simple experimental protocol assuming constant strains applied by spherical indenters of different sizes \((R = 100, 200, 500\) and 1500 [\(\mu N\)]) was proposed. Considering the example of doubling the size of the indenter \((R_2 = 2R_1)\), the representative strain given by Tabor (Hutchings, 2009) was assumed to stay constant and can be written as

\[
\epsilon = 0.2 \frac{a_1}{R_1} = 0.2 \frac{a_2}{R_2}
\]

(8.1)

where \(a_1, a_2, R_1,\) and \(R_2\) are the respective contact and sphere radius. Recalling the relationship between the contact radius and the indentation depth given by Hertz (Johnson, 1985) as

\[
a = \sqrt{hR}
\]

(8.2)

and replacing \(a_1, a_2\) and \(R_2\) in Eq. 8.1, we obtain with \(h_2 = 2h_1\). The force difference needed to reach the same amount of strain can be calculated
Experimental methods

<table>
<thead>
<tr>
<th>Microscopic</th>
<th>Spherical tips ( (R) ) [( \mu \text{m} )]</th>
<th>( P ) [( \mu \text{N} )]</th>
<th>( k ) [( \mu \text{N/min} )]</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>50</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>200</td>
<td>260</td>
<td></td>
</tr>
<tr>
<td>500</td>
<td>1250</td>
<td>1625</td>
<td></td>
</tr>
<tr>
<td>Macroscopic</td>
<td>Spherical tips ( (R) ) [( \mu \text{m} )]</td>
<td>( P ) [( \mu \text{N} )]</td>
<td>( k ) [( \mu \text{N/min} )]</td>
</tr>
<tr>
<td>200</td>
<td>88</td>
<td>116</td>
<td></td>
</tr>
<tr>
<td>1500</td>
<td>5000</td>
<td>6500</td>
<td></td>
</tr>
</tbody>
</table>

**Table 8.1** Constant loading time - \( t = 46.15 \) [s].

From Hertzian contact as

\[
P_2 = \frac{4\sqrt{(2R_1)}}{3} \frac{E}{(1-v^2)}(2h_1)^{3/2} = 4P_1
\]

(8.3)

Note that if an identical loading time is set for the different indentation setup (different indenter tips, different maximum loads reached), viscoelastic materials deformed with comparable strain level would creep according to the same time function; indeed, the relaxation/creep of viscoelastic materials is independent of the size of the indenter tip. As a result, normalized curves relative to the creep start would overlap. On the other hand, for materials displaying poro-elastic behavior, the relaxation/creep time scales with the radius of contact squared (Hu et al., 2010); as such, it is expected that the corresponding normalization of creep curves obtained with different tip geometry after an identical loading time would diverge.

In order to test this protocol, two sets of measurements were acquired with the UNHT, namely at microscopic and macroscopic ranges. Due to the complex structure of agarose gels, it is of particular interest to analyze both lengthscale in order to identify the time-dependence related to a relaxation/creep of the network (visco-elasticity) or to attribute it to fluid motion inside the structure. As a first step, microscopic measurements on 4% agarose gels where obtained with spherical tips of 100, 200 and 500 [\( \mu \text{m} \)]-radius; a comparison with tests using a tip of 1.5 [mm]-radius was conducted on the same 4% agarose gels. Details about experiments are summarized in Table 8.1.
8.2.3 FE MODELING

The commercially available FE Software ABAQUS 6.10 (ABAQUS Inc., Providence, RI) was used to extract mechanical properties from measurements data. Two axisymmetric models consisting of a perfectly rigid spherical indenter (radius = 100 [$\mu$m]) which penetrates a flat specimen (width (W) = 3 [mm], height (H) = 3 [mm]) consisting of 3227 nodes were created in order to perform the numerical calculations; the 3227 nodes constituting the gel were assembled in 3135 CAX4RH and CAX4RP elements for the QLV and LPE models, respectively. The sample was clamped to the bottom and constrained horizontally in its center accounting for axisymmetry; an additional boundary condition was applied to the top node layer ($p = 0$), allowing for flow of water across the upper surface of the gel.

8.2.4 LINEAR POROELASTIC MODEL

Agarose gels are made of a network of polymer chains containing water-filled cavities (Normand et al., 2000; Zhou et al., 2006); it appears thus justified to assume such materials to behave soil-like and to use the linear poroelastic (LPE) model to predict their behaviors. Indeed, the LPE material model assumes an elastic porous medium which is fully saturated by water. Basic properties such as the isotropy of the material, the linearity of the stress-strain relations, the application of small strains, the incompressibility of the liquid contained in the pores, and the flow of liquid through the porous skeleton described by Darcy’s law are many assumptions to be considered. The merge of the static equilibrium conditions assuming no translational nor rotational acceleration occurring given by

$$\sigma_{ij,j} = 0$$  (8.4)

with the kinematic relations representing the strains as functions of the displacement field

$$\epsilon_{ij} = \frac{1}{2} \left[ \frac{\partial u_i}{\partial x_j} + \frac{\partial u_j}{\partial x_i} \right]$$  (8.5)
and together with the constitutive equations of the isotropic, linear elastic material extended by an additional term accounting for pore pressure,

\[
\epsilon_{ij} = \frac{1 + \nu}{E} \sigma_{ij} - \nu \sigma_{kk} \frac{\sigma_{ij}}{E} + \delta_{ij} \frac{p}{3H} \tag{8.6}
\]

\[
\sigma_{ij} = 2\mu \epsilon_{ij} + \lambda \delta_{ij} \epsilon_{kk} - \delta_{ij} \alpha p \tag{8.7}
\]
give rise to a set of three differential equations

\[
\frac{E}{2(1 + v)} \nabla^2 u_j + \frac{E}{2(1 + v)(1 - 2v)} u_{k,kj} - \alpha p_{,j} = 0 \tag{8.8}
\]

containing four unknowns namely the displacement vectors \(u_1-u_2-u_3\) and the pore pressure \(p\), that FE models should simultaneously solve. Darcy’s law, which describes the flow of a liquid in a porous medium is then introduced to fully describe the system

\[
q_i = -\frac{\kappa}{\mu_f} p_{,i} \tag{8.9}
\]

where \(q_i\) denotes the volume of liquid flowing per second and per unit area in a certain direction, \(\kappa\) is called permeability and accounts for the resistance of the material to the liquid to flow, \(\mu_f\) characterizes the interaction properties of the liquid and is named dynamic viscosity. The hydraulic conductivity \((\kappa)\) can further be introduced in order to account for the porosity of a material; the permeability can be expressed as a function of the hydraulic conductivity according to

\[
\kappa = \frac{\mu_f \kappa}{\gamma_f} \tag{8.10}
\]

where \(\gamma_f\) denotes the specific weight of the wetting liquid. \(\gamma_f\) is defined in terms of the density of the liquid \(\rho_f\) and the magnitude of the gravitational acceleration \(g\). The porosity of the material is entirely described by the hydraulic conductivity \(\kappa\). The liquid properties are characterized by \(\gamma_f\) which in the case of water equals \(9807 [N/m^3]\) \((\rho_f = 1000 [kg/m^3], g = 9.807 [m/s^2])\). In general, the hydraulic conductivity \(\kappa\) is a function of the so-called void ratio which represents the ratio of void volume to solid volume. Considering, the gels to be fully saturated, \(e\) corresponds to the ratio of liquid (water) volume to solid volume

\[
e = \frac{V_{\text{water}}}{V_{\text{solid}}} \tag{8.11}
\]
8. Investigations of Agarose gels time-dependence

![Diagram](image)

**Figure 8.2** Illustration of the void ratio $e$ based on Helwany (2007).

Understanding that all change in volume is only due to the flow of the incompressible liquid out of the material (i.e. $\alpha = 1$), the void ratio is a function of the deformation. However, if the amount of liquid that flows out of the material is assumed to be very small, the void ratio can be considered constant; in this case, the hydraulic conductivity $k$ is constant as well. A fourth differential equation can be derived from Darcy’s law (Biot, 1941)

$$\frac{k}{\gamma_f} \nabla^2 p = \alpha u_{k,kt} + \frac{1}{Q} p_{,t} \quad (8.12)$$

The two constants characterizing an isotropic, linear elastic material ($E$ and $v$) have thus been complemented by three more constants ($\kappa$, $\alpha$ and $Q$) accounting for the permeability, the pore pressure and the flow of liquid within the material, respectively. If the flow of liquid is not restricted (i.e. $p = 0$), $\alpha$ measures the ratio of the liquid volume that is squeezed out of the material to the volume change of the material. In the present work, all changes in volume of the material have been assumed to be due to change in volume of the liquid (i.e. $\alpha = 1$); since incompressibility of the liquid is assumed, this means that the volume change of the material is caused by the flow of liquid out of the material only. $1/Q$ is a measure of the additional volume of liquid that can be forced into the material while the volume of the material is kept constant; if the material is fully saturated (i.e. contains no air bubbles) and the liquid considered incompressible, $1/Q = 0$. 

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8.2.5 Quasi-linear Viscoelastic Model

A Quasi-Linear Viscoelastic (QLV) material model incorporating nonlinear function of strain in a linear viscoelastic constitutive law was used to mimic the time-dependence response of agarose gels. The Yeoh formulation, which is a simplified form of the so-called reduced polynomial strain energy function, is used as a constitutive model in order to represent the nonlinear mechanical properties of VHB4910; it is described by six material parameters $C_{10}, C_{20}, C_{30}, D_1, D_2$ and $D_3$ as previously defined in (Eq. 2.76)

$$W_{Yeoh} = C_{10}(I_1 - 3) + C_{20}(I_1 - 3)^2 + C_{30}(I_1 - 3)^3 + \frac{1}{D_1}(J - 1)^2 + \frac{1}{D_2}(J - 1)^4 + \frac{1}{D_3}(J - 1)^6$$  \hspace{1cm} (8.13)

The time-dependent coefficients (Prony series relaxation function) are directly applied to the constants that define the energy function

$$C_{ij}(t) = C_{ij}^0 \left[1 - \sum_{k=1}^{N} g_k \left(1 - \exp \left(-\frac{t}{\tau_k}\right) \right) \right]$$  \hspace{1cm} (8.14)

where the relation between the instantaneous elastic modulus $C_{ij}^0$ and the long-term elastic modulus $C_{ij}^\infty$ is

$$C_{ij}^\infty = C_{ij}^0 \left(1 - \sum_{k=1}^{N} g_k \right)$$  \hspace{1cm} (8.15)

The relaxation function related to the chosen material formulation can further be calculated as

$$E_{FE}(t) = 6C_{10}^0 \left[1 - \sum_{k=1}^{N} g_k \left(1 - \exp \left(-\frac{t}{\tau_k}\right) \right) \right]$$  \hspace{1cm} (8.16)

8.3 Experimental Observations

1%, 2%, and 4% w/w agarose gels have been successfully tested (Fig. 8.3) by means of micro-indentation. A special focus is made on (i) the 2% agarose gels measurements data in order to analyze the influence of maximum load and loading rate, and (ii) on the 4% agarose gels to study effect related to indenter sizes. Although measurement’s repeatability has been
shown to be very good for all indentation data acquired, experimental displacements applied to 0.5% gels led to forces lower than the nanoin- dentor’s force resolution; trustworthy experiments were thus not possible and experimental data related to such gels are further disregarded (see Appendix B). Experimental data are shown in Figs. 8.4–8.7.

8.3.1 Models Parameters Determination

Three sets of parameters (for 1, 2 and 4% agarose w/w) were determined for both models from measurement data acquired for the special case of a maximal load of \( P_{\text{max}} = 50 \) [\( \mu N \)] applied at a deformation rate \( k = 130 \) [\( \mu N/\text{min} \)] (Fig. 8.8). FE inverse analysis was carried out for the full ramp-hold phases, leading to the LPE \((E, v, k)\) and QLV parameters \((C_{10}, C_{20}, C_{30}, g_1, g_2, t_1, t_2)\) listed in Table 8.2; FE simulations were sub-
Figure 8.4 Representative force-displacement measurement curves for different $P_{\text{max}}$ applied to various agarose gels (1, 2, 4% w/w) with the help of a modified nanoindenter machine.

Figure 8.5 Representative displacement acquired through microindentation ($R = 100$ [$\mu$m]) experiments performed on Agarose 1% for a maximum load of 50 [$\mu$N].
8. Investigations of Agarose gels time-dependence

Figure 8.6 Experimental displacement measured conducting indentation tests on Agarose 2% for different maximum loads \( P_{\text{max}} = 12.5, 25, 50, 100 \ [\mu \text{N}] \) and for several loading rates \( S = 12.5, 25, 50, 100 \ [\mu \text{N/min}] \).

Figure 8.7 Size effects investigated through indentation experiments conducted with increasing indenter tip sizes \( R \ [\mu \text{m}] \), different maximum loads \( P_{\text{max}} \ [\mu \text{N}] \) and several loading rates \( S \ [\mu \text{N/min}] \).
Figure 8.8  Gels (1, 2 and 4% w/w agarose) creep measurements curves for constants $P_{max}$ ($P_3 = 50$ [$\mu$N]) and $S$ ($S_3 = 130$ [$\mu$N/min]) served as holding phase for QLV and LPE inverse analysis.

<table>
<thead>
<tr>
<th>LPE model</th>
<th>$E$ [MPa]</th>
<th>$v$ [-]</th>
<th>$\dot{k}$ [\mu m/s]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1%</td>
<td>0.029</td>
<td>0.078</td>
<td>1.47E-4</td>
</tr>
<tr>
<td>2%</td>
<td>0.089</td>
<td>0.118</td>
<td>2.25E-5</td>
</tr>
<tr>
<td>4%</td>
<td>1.174</td>
<td>0.195</td>
<td>5.25E-8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>QLV model (Yeoh)</th>
<th>$C_{w0}$, $C_{w0}$, $C_{30}$ [MPa]</th>
<th>$g_1$, $g_2$ [-]</th>
<th>$t_1$, $t_2$ [s]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1%</td>
<td>7.0E-3, 4.1E-5, 3.2E-7</td>
<td>3.9E-6, 1.5E-1</td>
<td>0.35, 51.2</td>
</tr>
<tr>
<td></td>
<td>243.7, 42.6, 142.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2%</td>
<td>1.8E-2, 2.8E-4, 4.0E-7</td>
<td>3.6E-1, 8.5E-2</td>
<td>0.66, 23.8</td>
</tr>
<tr>
<td></td>
<td>41.7, 53.3, 64.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4%</td>
<td>2.3E-1, 2.4E-3, 3.1E-8</td>
<td>1.7E-3, 2.2E-1</td>
<td>0.27, 13.7</td>
</tr>
<tr>
<td></td>
<td>2.2, 3.5, 3.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 8.2**  Material model parameters determined from measurements at $P_{max}$ = 50 [$\mu$N] and $k = 130$ [$\mu$N/min].

Sequentially performed to evaluate predictions at different maximum loads and loading rates.
8. Investigations of Agarose gels time-dependence

8.3.2 Maximum load predictions

Experimental load-displacement ($P-h$, ramp-hold) curves acquired for different agarose concentration under constant maximum load ($P_3 = 50 \, [\mu N]$) and at fixed loading rate ($S_3 = 130 \, [\mu N/min]$) are shown on Fig. 8.4; measurements data for gels with 1, 2 and 4% w/w agarose concentration exhibits very good repeatability. Both models gave evenly rise to reasonable measurement predictions (Fig. 8.9) for a wide range of maximum load applied ($P_{max} = 12.5, 25, 50, 100 \, [\mu N]$) on 2% w/w agarose gels. The influence of the maximum load on the creep response of the soft hydrogels was investigated through creep experiment applying different maximum loads ($P_{max} = 50$ and $100 \, [\mu N]$); FE models showed their capabilities for predicting such discrepancies (Fig. 8.10).

8.3.3 Loading rate predictions

An attempt to analyze the effect of time-dependence of agarose gels on indentation data was made by varying the loading rates ($S_1 = 32.5$ to $S_4 = 260 \, [\mu N/min]$) for an identical maximum applied load of $50 \, [\mu N]$ and adding a holding phase (i.e. creep experiments) of $60 \, [s]$ (Fig. 8.11); the loading rate is not significantly affecting the observed response in the ramp loading phase. Using the parameter sets (LPE and QLV models) determined for the 2% w/w agarose gel, predictions of the material response for a constant maximal load of $50 \, [\mu N]$ at different loading rates were obtained (Fig. 8.12) and compared with acquired measurements. The creep displacements obtained for different loading rates were further compared to analyze the direct influence of the loading rate on the material creep (Fig. 8.13).

8.3.4 Size effects

The creep displacements obtained for different spherical indenter tip sizes were normalized as a function of the displacement achieved at the end of the loading phase ($h_c = 0$) and further compared for microscopic ($R = 100, 200, \text{and} \, 500 \, [\mu m]$) and macroscopic ($200 \text{ and} \, 1500 \, [\mu m]$) measurement sets (Figs. 8.14, 8.15).
Figure 8.9  (a) Inverse analysis ($P_{\text{max}} = 50 \, [\mu\text{N}], S = 130 \, [\mu\text{N/min}])$ and QLV predictions for micro-indentation experiment on 2% w/w agarose gels at several $P_{\text{max}}$, and (b) Inverse analysis and LPE FE predictions for micro-indentation experiment data on 2% w/w agarose gel for different maximal loading.

### 8.4 Discussion

Indentation measurement data were obtained for all % w/w of agarose gels in petri dishes allowing for full immersion of gels in water; specimens were
8. Investigations of Agarose gels time-dependence

![Figure 8.10](image1.png)

**Figure 8.10** 2% w/w agarose gel creep displacement for different maximum loads ($P_{max} = 50$ and $100 \ [\mu N]$).

![Figure 8.11](image2.png)

**Figure 8.11** 2% agarose gels behavior exhibits no strong difference among different loading rates.

thus maintain hydrated during the whole indentation process (i.e. to avoid sample damaging) and the influence of capillarity forces avoided. The
Figure 8.12  Model predictions of indentation experiments of 2% agarose gels at different loading rate; note the experimental difficulty to apply a constant ramp load over the whole loading procedure (ramp vs. applied load), particularly observable for small loading rate (k=32.5 $\mu$N/min)).

limits of the nanoindenter machine were reached for the very compliant 0.5% w/w gel, so that no trustworthy material parameters were acquired for this gel.
8. Investigations of Agarose gels time-dependence

![Figure 8.13] Creep measurements for two loading rates (32.5 and 260 [$\mu$N/min]). Model predictions are shown.

![Figure 8.14] Normalization of indentation curves acquired with different tip sizes overlap perfectly at the microscale: no size effect observed at low length-scale.
Figure 8.15 Divergences observed when normalizing creep displacements for microscopic and macroscopic indentation experiments: the effect of indenter size is clearly demonstrated here.

8.4.1 MODEL PARAMETERS

Overall, measurements variability become smaller with increasing percentage of agarose (Appendix B); it means that the variation among measurements is higher for more compliant behavior, which may be explained by the force resolution of the machine affecting the contact point detection to some extent.

Analyzing the constitutive parameters of the LPE model obtained through FE inverse analysis for the different agarose concentrations, the creep rate is lower for increasing percentage of agarose (Fig. 8.8); this can be easily explained by the decreasing permeability (i.e. $k \downarrow$) of gels with higher agarose concentration, since the network of agarose chains exhibits a higher density and the flow of water is therefore hindered; measurements are in line with these predictions.

Instantaneous young’s moduli (Fig. 8.16 (a)) obtained from the QLV model (Eq. 8.16) for 1, 2 and 4% w/w agarose gels are in line with values found in the literature for both micro ($R = 1500 \, [\mu m]$) and nano ($R = 400 \, [\mu m]$) indentation experiments (Oyen, 2013). Their elastic fraction
8. Investigations of Agarose gels time-dependence

![Graph](image)

**Figure 8.16** (a) The instantaneous modulus \(E_0\) for 1, 2 and 4% w/w agarose gels are in line with values found in the literature, (b) their elastic fraction \(E_\infty / E_0\) representation differing significantly.

\(E_\infty / E_0\) differ significantly (Fig. 8.16 (b)). These divergences are thought to be due to the different compositions and their associated protocols used to synthetize the gels.
8.4.2 Influence of maximum load on creep

Both the LPE and QLV models provided good predictions for 2% w/w agarose gels indentation for different maximum loads applied (12.5, 25 and 100 [µN]) (Fig. 8.9). A sensible increase of creep displacement was experimentally observed for higher maximal loads $P_{max}$ (50 vs. 100 [µN]) (Fig. 8.10); experimental observations were only qualitatively reproduced in FE simulations using LPE and QLV material formulation.

8.4.3 Influence of loading rate on creep

The influence of the loading rate ($S_1$, $S_2$, $S_3$, $S_4$) on the loading curve for a maximal load of 50 [µN] was not significant (Fig. 8.11); the representative curve with the largest loading rate ($S_4 = 260$ [µN/min]) tends to show a slightly stiffer behavior than the measurements at lower loading rates. At a loading rate of 260 [µN/min], time-dependent effects due to the flow of water within the material result in a stiffer behavior during the loading phase and a larger creep displacement in the subsequent creep phase (Fig. 8.12). Measurement data conducted with a loading rate of 32.5 [µN/min] shows a discontinuity at an indentation depth of about 1 [µm]. The nanoindenter machine was run in a displacement-controlled mode in order to reach the sample’s surface and subsequently the mode was switched to a load-controlled indentation; in the case of a loading rate of 32.5 [µN/min], this conversion took place too late, which yielded to the observed discontinuity in the measurements. Both models overestimate the indentation depths at all different loading rates considered. These discrepancies seem associated with a stiffer behavior of the measurements as compared to modeling at the end of the loading phase.

The 2% w/w agarose gel exhibits an increase in the rate of creep displacement ($h_c, t$) at the beginning of the creep phase for higher loading rates (Fig. 8.13); however, indenting the 2% w/w agarose gel with different loading rates leads to similar creep displacements after a sufficiently long time.

Models predictions differ considerably from measurement data, with an overestimated difference of the rate of creep displacement at the beginning of the creep phase. This yields larger deviations of the creep
displacements between the predicted curves with ongoing time.

8.4.4 Size effects

Normalized creep experiments data obtained through spherical indentation experiments ($R = 100, 200, \text{ and } 500 \ [\mu \text{m}]$) on 4% agarose gels exhibit size-independent visco-like behavior (i.e. curves overlap), similar to corresponding observations previously made for the acrylic elastomer VHB4910 (Fig. 8.14). It is hypothesized that, at the end of the loading period, the water has already diffused to a great extent so that the time-dependence observed is mainly due to the viscoelastic relaxation of the percolated network.

Clear divergences are observed between indentation experiments at different lengthscale (Fig. 8.15); the normalized creep displacements are indeed significantly larger for larger tip radius ($R = 1500 \ [\mu \text{m}]$). In line with poro-like behavior, the time needed for diffusivity ($t_D$) of water scales with the diffusivity constant ($D$) and the square of the radius of contact ($a_c$) (Galli et al., 2009; Hu et al., 2011a, 2010):

$$t_D = \frac{a_c^2}{D} \quad (8.17)$$

The radius of contact is directly dependent of the radius of the indenter tip, thus the time needed for the water to diffuse increases with increasing indenter tip size. At the end of the loading ramp, water still need to diffuse away of the contact zone, inducing larger creep displacement as compared to low lengthscale indentation testing dominated by viscoelastic effects of the solid matrix (Fig. 8.17); the interpretation of these data was formulated based on the input of Dr. Oyen of Cambridge University.

8.5 Conclusion

Smooth measurements data were acquired for three different agarose gel concentrations at different maximal loads, loading rates and for different tip sizes. Corresponding linear poroelastic (LPE) and quasi-linear viscoelastic (QLV) models were used to extract mechanical parameters from indentation data. The surface of agarose gels exhibited roughness (AFM,
sliding mode) in the order of few tenth of [nm], confirming the initial flat surface assumption (see Chapter 6). Predictive capabilities of the models were compared in applications to different loading conditions.

Finally, the size effect related to poroelastic behavior was analyzed thanks to a protocol involving force-controlled indentation experiments using different size tips. For the time scale of loading applied in the present experiments, agarose gels appear to behave visco-like at lower lengthscale and poro-like at larger lengthscale.

Figure 8.17 Representation of the different phases of creep response related to diffusivity of the water, for two different indenter tip sizes ($R = 200$ and $1500 \, [\mu m]$) based on the input of Dr. Oyen.
CHAPTER NINE

CONCLUSION

It is of particular interest to characterize mechanically the behavior of soft materials and biological tissues undergoing different kind of external stimuli. AFM has been extensively used for many years as a technique for interrogating biological tissues at a very low lengthscale, namely at the cell-level using nanometer tips working in a [nm] displacement range. However, aiming at characterizing tissues that are aggregate of cells, it appears more appropriate to use larger tips [µm] allowing for a more global interrogation of the tissue. Such a consideration may be taken into account by nanoindenter machines which were originally developed for hard materials characterization using larger tips ranging from few tenth of nanometers to hundreds of microns. The technique was originally developed to sense [mN] forces indenting materials at the nanometer scale; as such, the technique has been defined as nano-indentation (for [nm] penetration into materials). However, attempting to use nanoindenter machines for the characterization of soft materials and biological tissues, the forces and displacements respectively sensed [µN] and applied [µm] are both in a different range. As such, the suitability of the term nanoindentation might be questionable; thus, the term microindentation is often used in the present work for describing indentation at the microscale using a nanoindenter machine.
Due to their original design allowing for characterization of hard materials at the nanoscale, most nanoindenter machines exhibit working range limitations when intending to characterize very soft materials. These limitations related to both force sensitivity and displacement range of the apparatus lead to problems in contact point detection, reverberating on the accuracy of mechanical properties extracted from measurement data. In order to comply with the force range of their instruments, several researchers increased the contact area by increasing the size of the tips (e.g. $D = 1000, 2000 \, \mu m$). As such, they were able to sense higher forces for lower displacement, staying in a small deformation regime. However, by increasing the indenter tip size, the lengthscale interrogated is larger and the technique rejoin the lengthscale of other macroscopic mechanical testing techniques.

The UNHT nanoindenter provided by CSM Instruments allows to maintain a testing $\mu m$-lengthscale by sensing forces fitting in the working range of the machine. The setup used here enables indenting soft materials with elastic moduli in the order of few tenth of [kPa] with a spherical tip of 100 $\mu m$ in radius. For a relevant material characterization, larger strains have often to be applied on soft materials; as a result, non-linear deformations take place during the indentation process. FE based inverse analysis can be used for post-processing. As biological tissues might exhibit an untreatable roughnesses in the order of few tenth of $\mu m$, it is of major importance to account for it in the data analysis process.

The UNHT machine is able to record the initial phase prior to contact, allowing to observe and quantify surface interaction effects as well as their impact on measurement data; indeed, surface forces acting at the interface were not analyzed previously with nanoindenter machines because their magnitude were well below the overall forces sensed for hard material nanoindentation. As a result, the influence of these forces on the contact detection is commonly disregarded.

Special care was taken in this work in order to avoid damaging of the specimens undergoing microindentation experiments, namely by hydrating them sufficiently (i.e. liquid cell) and minimizing the stress concentration applied to them by indenter tips.
Main conclusions

The following exhaustive list aims at recapitulating the main conclusions arising from the present work:

The capabilities of a force-controlled nanoindenter machine to characterize very compliant materials exhibiting time-dependent behavior have been investigated. The drift stability offered by the present UNHT setup allowed acquiring a large number of measurement data for different maximum loads applied and at different loading rates.

The importance of pull-in forces when indenting very compliant materials under different environmental conditions has been highlighted; pull-in was shown to be due to capillary forces. Subsequently, indenting fully immersed in water has not only been shown to be a requirement to avoid dehydration of samples, but also a solution to avoid pull-in forces influences.

The need for characterizing the surface topography of biological tissues and accounting for it in the post-processing phase has been investigated through FE analysis relating its influence on acquired measurement data.

An FE based tool allowing for fully automated extraction of mechanical properties from microindentation experiments was developed in order to ease the analysis of non-linear behavior arising for large indentation depth.

Large strains (up to average strain > 10%) were achieved with a spherical indenter tip of $R = 100 \, [\mu\text{m}]$ indenting 45 $[\mu\text{m}]$-deep, highlighting non-linear elastic effects; subsequently, FE non-linear inverse analysis have been shown to provide a valuable alternative to analytical solution for such applications.

Extensive data were acquired and analyzed to characterize the time dependent mechanical behavior of soft elastomers and hydrogels; more than 1500 indentation experiments were indeed conducted on various
9. Conclusion

Experiment results were documented (see Appendix B) to allow researchers in this field to apply their model formulation for analysis of the present data.

TOWARDS MICRO-INDENTATION OF BIOLOGICAL TISSUES

Prenatal heart valve interventions aim at the early correction of congenital cardiac malformations; as such, it provides a promising treatment in maternal-fetal care (Weber et al., 2012). As part of a large collaboration with colleagues from different Universities in Europe aiming at investigating the fetal implantation of prenatally engineered living autologous cell-based heart valves, preliminary indentation experiments were conducted on a very compliant ($E=8 \text{ [kPa]}$) long term explant Tissue Engineering (TE) heart leaflet with a spherical tip of radius 1500 $\mu m$ (Appendix B). Few indents only were acquired due to sample movement difficulties related to sample hydration maintenance in air. Such a problem was overcome later by building a liquid cell allowing for full sample immersion in liquid.

Many organs have heavily branched structures arising by an at first sight similar process of branching morphogenesis (Iber and Menshykau, 2013); however, the regulatory components identified as responsible for the branching differ greatly among organs (e.g. lung, kidney, liver), and it is an open question whether a common principle (physical, geometrical factors) is driving the ramification process. Moreover, although the mechanical properties (e.g. ability to contract) of mesenchymal tissue have been shown to differ whether it induces or not epithelial layer branching (Nogawa and Nakanishi, 1987), an intrinsic comparison of pure mechanical properties has never been achieved (Iber and Menshykau, 2013). Attempts have thus been made in characterizing mechanical differences among mice lung mesenchymal tissues at different gestational stages (11.5, 12.5, and 13.5 days) by means of indentation experiments ($R = 100 \text{ [} \mu m \text{]}$). Unfortunately, the force resolution of the indenter machine was insufficient to capture trustworthy indentation data. On the other hand, meaningful data were acquired for indentation ($R = 1500 \text{ [} \mu m \text{]}$) of the whole em-
bryos at the previously mentioned gestational stages (E = 7.5, 12.5, 13.5 [kPa]), as well as for indentation measurements on adult mouse liver (E = 6 [kPa]) (Appendix B).

OUTLOOK

Based on the gained experience using the UNHT machine, few recommendations in order to further improve the machine are proposed.

Recalling that the maximum vertical distance between the reference tip and the indenter tip is of 50 [µm] and that the surfaces of soft materials are rarely flat, it has been very challenging to simultaneously position the tips of the UNHT as such that the reference lied on the sample holder of the liquid cell and that the indenter tip was in contact with the sample surface; this distance should be significantly increased.

In the present work, all indentation measurements were driven in a force-controlled mode (i.e. input forces, measured displacements). However, in order to empower the user to have a direct control on indentation strains and strain rates, it would be appreciable to have to possibility to drive the indentation head in a displacement-controlled mode.

The newly developed CSM Bioindenter makes use of a single indenter shaft with clear advantages for testing compliant materials, even though its capabilities to deal with thermal drift have still to be investigated. Moreover, the new machine allows for a maximal displacement of 100 [µm] which enables deeper indentation to characterize non-linear response of biological tissues.

Uncertainties remain concerning the accurate contact point detection. To tackle this problem, one could imagine the use of multiple force sensors with different working ranges. Alternatively, the coupling of the UNHT with a side microscope could allow the observation of the contact and determine the start of indentation.

The roughness of biological tissues is linked with their functionalities and as such cannot be eliminated (i.e. polished away). It has to be considered in data post-processing. One could imagine incorporating topographic information of sample surfaces into the FE automated tool previously introduced in the present work in order to run non-linear FE analysis including the real surface geometry. A possible procedure would
9. Conclusion

be:

Determine surface roughness through coupled AFM.

Incorporate surface geometry as 3D mesh in FE code.

Perform microindentation experiments.

Inverse analysis procedure including exact roughness of specimen.

The newly improved instrument enables thus characterizing the mechanical behavior of highly deformable materials with in-homogeneities at a length scale down to few tens of $\mu$m.

Based on the experience acquired in the present work, the newly developed CSM Bioindenter would additionally allow the mechanical characterization of a variety of compliant materials such as low density mineralized tissues (e.g. cartilage, dentin), loose connective tissues (e.g. blood vessels, skin), and technical biomedical or natural (e.g. nacre, spider silk fiber) materials. The Bioindenter is indeed expected to deliver essential information for a number of applications, such as the development of implants and devices interacting with soft human tissues (e.g. contact lenses, resorbable sutures, mesh implants and stents), fundamental investigations of cell biology (e.g. biological tissues), or tissue repair (e.g. mechanical characterization of ruptured fetal membrane).

In addition to life sciences or medical applications, the Bioindenter should enable the characterization of thin elastomer membranes, soft fibers and other components made of highly deformable synthetic materials.
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APPENDIX

A MECHANICAL ANALYSIS OF INDENTATION

A.1 INDEX NOTATION

In the present work, algebra has mainly been presented in symbolic notation, employing single (vector) or double (tensor) underlined symbols. However, in computational mechanics, vector and tensor quantities need to be referred to a basis; in order to get additional insights about these quantities and to ease mathematical operations among vectors and tensors, it is usually helpful to refer to their components.

The Cartesian basis, which consists of a fixed set of three basis vectors $\mathbf{e}_1$, $\mathbf{e}_2$ and $\mathbf{e}_3$, is introduced to present vector and tensor components relative to a right-handed and orthonormal system (Holzapfel, 2006), characterized by its mutually orthogonal vectors of unit length

$$
\mathbf{e}_1 \cdot \mathbf{e}_2 = \mathbf{e}_1 \cdot \mathbf{e}_3 = \mathbf{e}_2 \cdot \mathbf{e}_3 = 1 \quad (A.1)
$$

$$
\mathbf{e}_1 \cdot \mathbf{e}_1 = \mathbf{e}_2 \cdot \mathbf{e}_2 = \mathbf{e}_3 \cdot \mathbf{e}_3 = 0
$$

Any vector (i.e. $\mathbf{u}$) can thus be represented by a linear combination of the basis vectors $\mathbf{e}_1$, $\mathbf{e}_2$ and $\mathbf{e}_3$, such as

$$
\mathbf{u} = u_1 \mathbf{e}_1 + u_2 \mathbf{e}_2 + u_3 \mathbf{e}_3 \quad (A.2)
$$

where $u_1$, $u_2$ and $u_3$ are the Cartesian components of the vector $\mathbf{u}$ along the directions $\mathbf{e}_1$ (1,0,0), $\mathbf{e}_2$ (0,1,0) and $\mathbf{e}_3$ (0,0,1). Using index notation, the above relation can be written as

$$
\mathbf{u} = \sum_{i=1}^{3} u_i \mathbf{e}_i \quad (A.3)
$$
Appendix

or, in a more abbreviate form, as

\[ u = u_i \varepsilon_i \] (A.4)

where the Einstein summation (for \( i = 1, 2 \) and 3) convention is adopted. The summation convention stipulates that whenever an index (i.e. \( i \)) is repeated (only once) in the same term, then a summation over the range of this index is implied. The tensor product (dyad) of the vectors \( u \) and \( v \) is denoted by

\[ u \otimes v \] (A.5)

Occurring commas are used to separate component from differentiation indices; the index on the right of a comma designates a differentiation index, whereas the index on the left is a component index, i.e.

\[ q_{i,t} = \frac{\partial q_i}{\partial t} \] (A.6)

Higher order derivatives can be similarly represented

\[ u_{k,kt} = \frac{\partial^2 u_1}{\partial x_1 \partial t} + \frac{\partial^2 u_2}{\partial x_2 \partial t} + \frac{\partial^2 u_3}{\partial x_3 \partial t} \] (A.7)

A.ii SECOND-ORDER TENSOR

Second-order tensors (\( \mathbb{A} \)) may be thought as linear operator that act on vectors (\( u \)) to generate new vectors (\( v \)), i.e.

\[ v = \mathbb{A} u \] (A.8)

with Eq. A.8 defining a linear transformation that assigns a vector \( v \) to each vector \( u \). Any second-order tensor may be expressed as a linear combination of dyads (dyadic) formed by the (Cartesian) basis \( \varepsilon_i \), i.e.

\[ \mathbb{A} = A_{ij} \varepsilon_i \otimes \varepsilon_j \] (A.9)

where \( \mathbb{A} \) is denoted as a Cartesian tensor of order two that is resolved along orthonormal basis vectors. The nine Cartesian components of \( \mathbb{A} \) with respect to \( \varepsilon_i \) are respectively represented by \( A_{ij} \), i.e.

\[ A_{ij} = \begin{bmatrix} A_{11} & A_{12} & A_{13} \\ A_{21} & A_{22} & A_{23} \\ A_{31} & A_{32} & A_{33} \end{bmatrix} \] (A.10)
Einstein’s summation convention can be used for second-order tensor as well, i.e.

\[ A_{ii} = A_{11} + A_{22} + A_{33} \]  

(A.11)

**B Experimental data**

Figure B.1  Force-displacement (P-h) history resulting from indentation experiments on Ecoflex0030 in air (40% humidity) and fully immersed in water (sets of 9 curves) exhibit the good repeatability of measurements.
Figure B.2  Plotting the force vs. time ($P$-$t$) of Fig. B.1, one can observe the minimum contact load ($P_{min} = 2 \, [\mu N]$) serving as a switch between displacement-controlled and force-controlled mode (application of loads).

Figure B.3  Displacement vs. time curves for the two different configurations exhibit the large difference in displacement of the indenter before the initiation of the loading phase. As a result, experimental data acquired in the air (40% Humidity) are much more difficult to interpret.
B. Experimental data

Figure B.4  Experiments in the air (40% Humidity) and in water were conducted on the same compliant silicone (Ecoflex0030) with a radius 15x bigger, exhibiting maximum pull-in forces in the order of 230 [$\mu$N], in line with Eq. 2.4.

Figure B.5  Clear and repeatable pull-in effects among different experimental conditions (40%, 80%, 100% humidity) on fused silica.
Figure B.6  Paraffin wax exhibited no pull-in effects in air (40% Humidity) as compared to fused silica. Note that Paraffin wax plastified during indentation.

Figure B.7  Measurements on flat and rough Ecoflex0030 samples exhibited large and repeatable discrepancies.
B. Experimental data

**Figure B.8** Tensile tests on Ecoflex0030 exhibit good repeatability.

**Figure B.9** Sets of data (9 on 3 different samples) acquired through spherical ($R = 100 \, [\mu m]$) indentation on VHB4910 exhibit very good repeatability over a wide range of loads (12.5, 25, 50, 100, 200 and 400 [$\mu N$]).
Figure B.10 Various maximum loads have been applied to VHB4910 over a constant loading rates ($k = 130 \, [\mu N/min]$); very stable input forces were transmitted to the material.

Figure B.11 Measured displacements for individual maximum loads applied reveal good consistency over a wide range of deformations (i.e. curves all overlap during the loading phase of indentation).
B. Experimental data

Figure B.12  Force-displacement curves for different loading rates applied to VHB4910 exhibit good repeatability, though difficulties were observed in reaching constant maximum force among measurements.

Figure B.13  Difficulties to reach constant maximum loads can be attributed to problems related to accurate contact point detection, especially for low loading rates ($k_1 = 32.5$ [μN/min]).
Figure B.14 Measured displacements resulting from spherical indentation (R = 100 [μm]) of VHB4910 exhibit good repeatability.

Figure B.15 Uniaxial tensile measurements exhibit acceptable variability.
B. Experimental data

Figure B.16  Agarose 0.5% w/w experimental data are considered as untrustworthy measurements as the indenter tip was thought to be already in contact with the material prior the loading phase began (positive forces at \( h = 0 \ [\mu m] \)). A kick that seemed repeatable was however observed at \( h = 20 \ [\mu m] \); it might be that the brittle material fracture when reaching a specific deformation.

Figure B.17  Agarose 0.5% w/w measured displacement history exhibit a long time period prior switching (\( t = 40 \ [s] \)) to force-controlled mode.
Agarose 1% exhibits a shorter period prior applying loads (i.e. more consistent contact point detection), resulting in much more repeatable measurements.

A complete indentation analysis was conducted on the very challenging agarose 2%; as a first step, repeatable "creep"-measurements were acquired for different maximum loads (12.5, 25, 50 and 100 [μN]).
B. Experimental data

Figure B.20 Discrepancies among measurements on 4% agarose were very little.

Figure B.21 Creep displacement at the end of the loading phase ($P_{\text{max}} = 50 \mu N$) for 3 agarose gels (1, 2 and 4%) exhibited distinct behaviors.
Figure B.22  Agarose 2% creep displacements at the end of the loading phase are displayed for different maximum loads (12.5, 25, 50 and 100 [µN]), exhibiting a clear distinction between measurements acquired for $P_{\text{max}} = 100$ [µm] with the rest of the measurements.
Figure B.23  The loading rate was further varied for 2% agarose gels in order to observe its influence on indentation measurements. Force-displacement measurement curves do not allow to distinguish between the different measurements acquired at different loading rates.

Figure B.24  Displacement vs. time plots allow distinguish between measurements acquired at different loading rates. Note the larger discrepancies for lower loading rates.
Appendix

**Figure B.25** The different loading rates used to indent the 2% agarose gels lead to very little differences in terms of creep displacement at the end of the loading phase; indeed, only the fastest loading rate ($S_4 = 260 \, [\mu N/\text{min}]$) exhibits differences with other measurements ($S_1=S_2=S_3$).

**Figure B.26** The size effect is not observable on VHB4910 for small size radii ($R_1 = 100 \, [\mu m], P_1 = 22 \, [\mu N], k_1 = 29 \, [\mu N/\text{min}]), (R_2 = 200 \, [\mu m], P_2 = 89 \, [\mu N], k_2 = 116 \, [\mu N/\text{min}]), (R_3 = 500 \, [\mu m], P_3 = 556 \, [\mu N], k_3 = 722 \, [\mu N/\text{min}])$. 

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B. Experimental data

Figure B.27  No size effect is observed for VHB4910 when comparing small ($R = 200 \ [\mu m]$) to larger indenter size ($R = 1500 \ [\mu m]$), proving the material to behave viscoelastically.

Figure B.28  Initial spherical indentation measurements were conducted on Tissue Engineering heart leaflet (human). Simple Young’s modulus extracted from Hertzian contact agreed well with biological tissues corresponding values.
Figure B.29  Performing measurements on mice embryos (11.5 days) was very challenging (sample hydration, compliance of living samples), and as such the repeatability of measurements is worth than for isotropic silicones and gels. A simple Hertzian fit was used to easily extract mechanical properties from indentation data (E = 7.5 [kPa]).

Figure B.30  Although the repeatability of the measurements is not perfectly ensured, the measured displacement are rather stable.
B. Experimental data

Figure B.31  Measurements on older embryos (12.5 days) exhibit less stable measured signals and a stiffer behavior \((E = 12.5 \text{ [kPa]})\).

Figure B.32  Consistency is observed among measured displacements obtained for the 12.5 days embryos.
Figure B.33  Measurements instability when performing indentation experiments on embryos being 13.5 days old is dramatic; it might be explained by the fact that embryos have now formed muscles and tend to actively respond to the indenter deformation.

Figure B.34  Less stable measured displacements are visible when indenting embryos 13.5 days old.
Figure B.35  Preliminary spherical indentation tests were conducted on a mice liver ($E = 6 \ [kPa]$). The measurements acquired exhibit a kick at $14 \ [\mu m]$, similar to what was previously observed on 0.5% agarose gels.

Figure B.36  Measured displacements exhibit large discrepancies.
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