MAGNETIC STEERING AT THE NANOSCALE

ABHANDLUNG
zur Erlangung des Titels

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A mi madre y a mi padre
Abstract

The ability to manipulate individual objects in microscopical scenarios is a long sought part in the tool chest of engineers. Manipulation presupposes steering, which is obvious at the human scale, but cannot be taken as a given at scales that differ in several orders of magnitude from ours. As a rule of thumb, the smaller the scale of an object, the more challenging it is to steer it.

In response to the challenge of steering microscopic objects immersed in fluids, researchers have developed different concepts. These include microfluidic devices [1], microrobots governed by magnetic forces [2], and devices in which polarized objects move directed by electrical fields [3], to name a few. Of them, the ones based on magnetic fields have the appeal that they do not interfere with the internal activity of biological tissues, a property that makes them attractive for prospective biomedical applications. Until now, the efforts to attain magnetic steering have been successful at scales down to tens of microns [4].

The goal of this thesis was to investigate the challenges of (or limitations to) magnetic steering at the nanoscale. We did so by considering the magnetic forces acting on a magnetic nanoparticle that can move in the proximity of a flat surface. We started the study with theoretical considerations, essentially comparing the magnitude of magnetic steering forces with the magnitude of those forces that can undermine the guidance efforts (e.g. friction forces). These calculations encouraged an experimental realization, which we designed around a well-controlled model-system: microtubules propelled by surface-bound kinesins [5, 6]. In this system, we replaced the substrate by a magnetically patterned film, and the microtubules were decorated with magnetic nanoparticles, which mediate the steering. We describe the design and construction of the experiment, with particular attention to the fabrication and characterization of the magnetic films, their adaptation for use in protein assays, and the methodology to characterize the motion of the microtubules through their trajectories and speed.

The discrepancy between numerous experiments and our expectations raised more fundamental questions, such as the role played by Brownian motion in nanoscale systems. We present theoretical arguments to characterize the dynamics of magnetic steering acting on a particle which is also subject to Brownian motion. Because the ability of Brownian motion to
diffuse a system over the saddle point of a potential barrier depends primarily on the depth of the potential well, we studied the changes in the energy landscape that can be obtained with different selections of the magnetic components (geometry and material).

The theoretical arguments allow us not only to understand the experimental results, but also to outline the limitations to magnetic steering at the nanoscale. Furthermore, the analytical tools developed in this study enable us to outline design guidelines for future experiments involving magnetic steering at sub-micron scale. The design guidelines are presented as a recipe that can guide the selection of magnetic materials and geometric dimensions for both the magnetic film and the magnetic particles.


Zusammenfassung

Die Fähigkeit einzelne mikroskopische Objekte in kleinsten Anwendungen zu manipulieren ist eine große Herausforderung der Ingenieurswissenschaften. Manipulation benötigt eine Steuerung, welche auf einer üblichen makroskopischen Skala offensichtlich erscheint, dies aber ein Problem darstellt, wenn die Längenskala mehrere Größenordnungen kleiner ist. In der Regel ist die Steuerung eines Objektes umso schwieriger zu realisieren, desto kleiner dessen Abmessung ist.


List of abbreviations

ADP  adenosine 5’-diphosphate
AFM  atomic force microscopy
ATP  adenosine triphosphate
BRB80 80 mM PIPES, 2 mM MgCl, 1 mM EGTA, pH 6.85 with KOH
DMSO dimethylsulfoxide
DNA  deoxyribonucleic acid
DTT  dithiotreitol
EGTA ethylene glycol-bis(2-aminoethylether)-N,N,N′,N′-tetraacetic acid
GDP  guanosine 5’-diphosphate
GTP  guanosine 5’-triphosphate
MFM  magnetic force microscopy
NA  numerical aperture
nt  nucleotide
PBS  phosphate buffered saline
pI  isoelectric point
PIVES piperazine-N,N′-bis(2-ethanesulfonic acid)
PMT  photo multiplier tube
SUL  soft magnetic underlayer
TEM  transmission electron microscopy
VSM  vibrating sample magnetometry
XRR  x-rays reflectometry
Abstract

Zusammenfassung

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Chapter 1

Scope and structure of this thesis

1.1 Motivation

The control of motion is accomplished by forces that propel and orient the motion. In this work we are primarily interested in studying the forces that can orient the motion, that is, we are interested in steering mechanisms and not in propelling mechanisms.

Moreover, we focus on steering mechanisms that can be applied to guide the motion of nanoscopic objects immersed in a liquid. In other words, we want to procure a directional confinement, that allows motion in one direction, and averts it in other directions.

1.1.1 Magnetic forces applied to guidance

Among the possible forces that can be utilized in a guidance system, electrical and magnetic forces have the appeal not to necessitate a mechanical contact to the manipulated object. Electrical fields have been shown to work at the micrometer-scale [1], but flows of electrolytes are also generated [2]. In the case of magnetostatic fields, no measurable effect on biological tissues or fluids has been reported for moderate fields. This observation makes magnetic forces attractive for steering problems in which applications in the biomedical field may be sought.

Recent advances in the development of magnetic materials are worth mentioning here. For instance, the development of magnetic storage technologies led to the availability of magnetic films, in which its magnetic domains structure can be arranged in virtually arbitrary configurations\(^1\). In turn, each magnetic domain is the source of a magnetostatic field, that can

\(^1\)Digital information is typically stored in binary format. Each bit of information (0 or 1) is stored as the magnetization direction of a magnetic domain (e.g. up or down). A magnetic film such as those used for magnetic storage can be regarded as a matrix of magnetic domains, in which a bit (0 or 1) can be written by selecting the orientation of a magnetic domain (e.g. up or down). Currently, magnetic domains larger than 10 nm are possible [3].
exert forces on nanoparticles located nearby. Because the configuration of domains in one of these special magnetic films can be organized at will (at least theoretically), the magnetostatic fields can be designed to elicit directional confinement in a very flexible way. For us, it means that a practical way of generating magnetostatic fields for directional confinement at the nanoscale actually exists.

The range of distances between the manipulated object and the magnetic film is limited by the loss factor \( \sim \exp(-\pi z/w) \), where \( w \) is the characteristic domain width, which describes the decay of the stray field with the distance \( z \) to the film. For this reason, we restrict our attention to problems of magnetic steering close to the plane of the magnetic film.

1.1.2 Active transport at the nanoscale driven by biological motors

In order to test the steering ability of the magnetic field generated by a magnetic film, we preferable consider a system in which the motion is already setup, but the guidance is still missing. Moreover, it would be advantageous to focus on a system that has already been characterized. In the rest of this section we consider two candidate systems: microfluidic devices and biomolecular motors.

In microfluidic devices the fluid is pushed by pressure gradients along channels that have typical dimensions of a few tens of micrometers. Further miniaturization to nanoscale devices will necessitate higher pressure gradients which are impractical, for example because of the long transients they require\(^2\) [4]. Among other alternatives (e.g. peristaltic pumps [5]), the use of active transport via biomolecular motors has already been proposed (e.g. [6]) as a viable technology for cases in which transport through pressure-gradients is not an option.

Biomolecular motors (e.g. dyneins, myosins, kinesins) are used inside cells for a variety of active transport tasks [7]. Kinesins, for example, convert the free energy obtained from the hydrolysis of ATP into mechanical work, which is used, among other tasks, for the transport of organelles along microtubules, which form their natural intracellular tracks [8]. Shortly after the discovery of these proteins, researchers started to investigate the possibility of using them for active transport in synthetic environments (see e.g. [9]). A common realization of this idea uses kinesin motors adsorbed onto a surface which in turn propel the microtubules: the relative motion is the same, but here in this inverted assay, the microtubules move and not the kinesins. This well characterized system [10, 11, 12] can be used as a platform to study the effects of forces at the nanoscale, such as mechanical [13], electrical [1], or magnetic, as

\(^2\)A popular device to generate the pressure gradient is the syringe pump. The gradient is generated by compressing the fluid in the syringe (no sound waves) against a the resistance to the flow (dominated by the channel). For micrometer-scale devices the settling time can be minutes, or longer. See [4] and references therein for a more precise analysis.
it will be explained in the rest of this work.

1.2 Scope of this thesis

The motivation of combining active transport and magnetic guidance, both at the nanoscale, led us to work on a system where the motion of microtubules propelled by kinesin biomolecular motors is guided by magnetic forces. To that end, the substrate is replaced by a magnetically patterned surface, and the microtubules are decorated with magnetic particles. The goal is to show how magnetic guidance could be added to the toolbox of engineers designing nanodevices in which active motion is a requirement. Our preliminary studies suggested the viability of magnetic guidance at the nanoscale and, in consonance with the literature \[14\], that the possibility of applications should be explored. Hence the project was outlined to obtain first, a proof of concept (that magnetic pathways for biomolecular motors are actually viable), and then move to an optimization and possible development of applications.

Prompted by the discrepancies between our estimates and the results from experiments, we turned our attention to more fundamental questions, such as the role played by Brownian motion in nanoscale systems. Indeed, the assessment of the challenges to downscaling of magnetic steering became a central theme of this thesis:

1.2.1 Objectives of this thesis

- To investigate the challenges of (or limitations to) magnetic steering at the nanoscale. On the one hand we need a comparison between steering forces and forces that can undermine steering, such as friction forces. On the other hand we need to be able to assess the (im)balance between diffusion effects and steering mechanisms. We do this by studying the changes in the energy landscape as a function of the parameters (geometry and material of the magnetic components).

- To give design guidelines for future attempts of magnetic steering at sub-micron scale:
  - Understand the role of the geometrical parameters.
  - Enumerate the steps for the design of sub-micron magnetic steering.

1.3 Structure

In order to reach an audience broader than only those researchers interested in a single aspect (e.g. lab on chip, magnetic films, magnetic particles, or biomolecular motors), we decided
to organize the material around the theme magnetic steering at the nanoscale in chapter 2. Specific details were summarized into four subsequent chapters, each one dealing with a different domain of knowledge, with the intention to allow the non-specialist to skip that material easily.

Chapter 3 Microtubules propelled by surface attached kinesin biomolecular motors describes the main components of a motility assay, together with design issues and specific experimental techniques. Details about the work with cargo (stability, functionalization) are also addressed. This chapter contains protocols used to build a motility assay and to measure it in a confocal microscope.

Chapter 4 Forces acting on a microtubule in a motility assay presents the calculations (together with the necessary hypothesis) to assess the forces that are involved in magnetic steering. In this chapter we argue (i) that magnetic guidance is more effective if the particle is a single-domain ferromagnet with uniform magnetization, and (ii) if the magnetic film generates the highest possible gradient of magnetic density-flux.

Nowadays there is a wealth of sources of magnetic nanoparticles, but they usually fall into one of three cases: (i) fabricate them in house, (ii) obtain them from other researchers through collaborations, or (iii) purchase them from commercial sources. Because both fabricating and characterizing the particles was beyond the possibilities of this project, we balanced the advantages and disadvantages of the other two sources in favour of the last one, that offers the possibility of working with similar material over a long period of time. Thus the selection criteria should not only follow guidelines common to all types of particles (i.e. stability and functionalization, as described in chapter 3), but also presumes that an educated guess of the magnetic state is possible. The fundamental concepts of magnetism in single-domain ferromagnets that enable such an educated guess is the core of the first part of chapter 5 Criteria to choose magnetic particles and magnetic films.

A magnetically patterned substrate can be obtained by microfabrication [15]. However, microfabricating a several nanometers thick sample with nanometer scale features is challenging. In addition, the substrate must not impose topographic (rather than magnetic) guidance. An interesting alternative comes from the magnetic storage industry, which over the last decades has developed magnetic films on which the structure of magnetic domains can be arranged via a writing process. Direct use of hard-drive substrates is not possible because of two reasons. (i) These ubiquitous magnetic films (e.g. in the hard disk drive unit of almost every computer) make use of lubricants, whose chemical properties limit its use in protein assays. (ii) Besides, the mechanical rigidity is given by a substrate that turns out to be very fragile (cutting gen-

\[3\] We will argument in chapter 4 that a thick magnetic substrate leads to considerably stronger steering forces than a thin one.
erates magnetic debris that deposits on the surface, rendering it unusable). Fortunately, we can take advantage of the underlying know-how: A stripes-domain pattern can be arranged on magnetic films that can be manufactured by standard magnetron sputtering. Therefore, in the second part of chapter 5 Criteria to choose magnetic particles and magnetic films we deal with the concepts that enable us to understand the physics of exchange-coupled films of uniform anisotropy, the fabrication steps, and two complementary characterization techniques (MFM and VSM) to assess their magnetic properties.

A theoretical analysis of the interplay between the forces acting on a microtubule in a motility assay is presented in chapter 6 Theoretical analysis of magnetic steering at the nanoscale. The analysis is developed for a general problem of magnetic steering at the nanoscale, without assumptions that involve a motility assay, or any other specific system. In order to understand the role of Brownian motion, particularly when it limits downscaling, we studied the energy landscape for different choices of parameters (geometry, magnetic materials).

An appendix lists complementary experimental observations that are not essential for the development of the material, but might prove useful in future experiments.

Bibliography


Chapter 2

Magnetic steering at the nanoscale

2.1 Introduction

Magnetic forces are being employed for steering of matter at very different length scales. Ergeneman et al. [1], for instance, demonstrated the effectiveness of magnetic forces to steer millimeter size objects. At the scale of hundreds of microns, Yesin et al. [2] presented a model capable of untethered control of a microrobot using magnetostatic fields.

Probably the smallest scale example of magnetic steering is the one presented by Inglis et al. [3], based on a micrometer-scale patterned surface and magnetic nanoparticles. This device passes a blood cell suspension over magnetic stripes, and uses magnetic nanoparticles to tag leukocytes in the suspension. The authors show that the tags (about 5000 magnetic nanoparticles per leukocyte) allow the separation of the leukocytes from the red blood cells (which are not tagged). The system uses a bias external magnetic field supplied by a millimeter-scale magnetic circuit. The physics behind this example was thoroughly explained by Furlani [4]. The practical use of their construction depends on the use of quite a large number of nanoparticles, in such a way that the superposition of the individual forces is capable of macroscopic work. This example, and others [5, 6, 7, 8], illustrate the potential of magnetic manipulation albeit at a relatively coarse scale.

Dobson [9] reviewed the use of magnetic nanoparticles and magnetic field gradients to control cellular behaviour. The underlying idea is to exert magnetic forces and torques on particles that are in turn attached to cell membrane receptors or cellular components. Then the magnetic actuation can control specific cellular processes or examine the mechanical properties of cellular structures. These experiments make use of either micrometer sized particles or agglomerates of a large number of interlinked nanometer scale particles.

In all the examples reviewed above the magnetic objects are micrometer or even millimeter large. However, smaller magnetic particles would automatically enable actuation with finer
control.

To the authors’ knowledge there is no demonstration of a device capable of magnetic steering of nanometer-scale objects with nanometer-scale confinement. In order to assess the possibility of magnetic steering at the nanoscale, we continue the discussion in terms of the guiding mechanism (the generation of magnetic fields) and in terms of the manipulated objects (the magnetic particles).

As described by Furlani in extensive detail [10], what determines the force, apart from the particle magnetic moment, is the magnetic field gradient. Therefore we need to look for sources of magnetic fields that elicit the steepest possible gradients, and magnetic nanoparticles that carry the strongest possible magnetic moment.

The materials with spontaneous magnetization that carry the highest magnetic moment are ferromagnetic [11]. Therefore we consider ferromagnetic nanoparticles. In a brief excursion, we want to mention that the concept of using ferromagnetic particles as a handle for magnetic fields to manipulate non-magnetic structures is not new. For instance, high-gradient magnetic-separation procedures makes extensive use of magnetic nanoparticles (see e.g. [12]). As a slurry of particles flows e.g. around a magnetized wire, the suspended particles are attracted to it. Diffusion processes balance the tendency of the particles to agglomerate around the wire due to the magnetic forces. Thus a radial particle concentration gradient is formed [13, 14]. We remark that the goal in these examples is to drive a concentration gradient without confinement of any kind, and not magnetic steering of individual objects.

Regarding the generation of magnetic field gradients, they are typically provided by coils or magnets. An illustration of the problems encountered when reducing the dimensions of these magnetic field sources can be gained from comparing the field generated by them. Consider, for instance, a cylindrical permanent magnet of radius $r$ and height $h$, and a flat coil with the same external geometry and $n$ turns, with a total wire cross section $n \times w \times h/n$. While the moment of the former is $m_m = M_s \pi r^2 h$ ($M_s$ is a measure of the remanence), that of the latter is $m_c = J w h \pi r^2$, where $J$ is the current density. Both magnetic moments\footnote{If the magnetic moments are equal, the generated magnetostatic fields are also equal.} are equal when $J = M_s/w$. So as the wire width $w$ decreases consistent with smaller coil dimensions the current $J$ needs to increase as $1/w$. This is a problem, because there are always upper bounds on the current, such as those imposed by restrictions to heat dissipation. Therefore, if miniaturization is a goal it is more efficient to produce a given magnetic field, or it may first become possible to produce it, with small permanent magnets rather than with small coils.

Before we proceed with the calculation of the magnetic forces (see section 2.2.2 on page 10 and chapter 4), we need to study the requirements that magnetic steering has to satisfy. This is of course problem-dependent. For illustration purposes we select a well characterized...
test-model: motility assays of microtubules propelled by surface bound kinesins.

2.2 Magnetic guidance in motility assays: harnessing biological motors as a well controlled model system

2.2.1 Outline of the concept

The two main components of a motility assay are microtubules and the motor protein kinesin. Microtubules are proteinous filaments and a constituent of the cytoskeleton. They can form a tubular structure $\sim 25 \text{ nm}$ in diameter and several micrometers long. Kinesin is a biomolecular motor capable of transforming the chemical energy of ATP hydrolysis into mechanical work. Kinesins naturally adhere to microtubules and execute $\sim 8 \text{ nm}$ steps in a direction defined by the asymmetric nature of the microtubules [15]. During this highly processive motion kinesins follow from a linear path on the microtubules and do not switch direction.

This naturally occurring intracellular process can be reproduced in vitro, provided certain conditions are satisfied, e.g. that the surfaces have been properly passivated, the solution contains ATP and the necessary ions, and the oxygen concentration is kept low. In a standard motility assay, kinesins are adsorbed on a previously passivated surface, and stay at fixed positions. Subsequently a solution of microtubules is added. Some kinesins may be able to bind to a microtubule that floats by at a sufficiently close distance, and by executing steps in a single direction, as dictated by the asymmetry of the tubulin proteins constituting the microtubule, propel the microtubule. The motion can be easily observed in real time by adding fluorescent labels to the microtubules: under the microscope they resemble worm-like objects moving along erratic trajectories. The lack of a steady direction of motion stems from the fact that the leading end of microtubules, in essence the flexible polymer strand which forms a cantilever between the free end and the first attached kinesin, is subject to Brownian motion. The ensuing swiveling of the cantilever segment enables the microtubules to connect to motors other than those straight in front of their trajectories$^2$.

In contrast with that erratic motion, the separation between microtubules and the substrate is remarkably well defined. Kerssemakers et al [17] measured that separation to be $17 \pm 2 \text{ nm}$. In other words, the motion of microtubules is circumscribed to a quasi bidimensional space.

This bidimensionality of the motion is an advantageous feature for our test of magnetic control of motion-direction. We therefore introduce two modifications in the motility assay: First, the substrate, usually a cover slide, is replaced by a magnetic substrate as discussed$^2$.

$^2$It is worth mentioning that the persistence length of microtubules, $\sim 5 \text{ mm}$, is much bigger than the one of actin filaments, $\sim 18 \mu\text{m}$ [16].
below. Second, magnetic beads are attached to the leading end of the microtubule. The whole set-up and the most important geometrical parameters are sketched in Fig. 2.1.

A priori, we expect the magnetic force between the particles and the field gradient from the substrate to have a rectifying effect on the erratic motion of the microtubules, as particles are attracted to the substrate’s magnetic domain boundaries.

Figure 2.1: Sketch of a kinesin & microtubules motility assay. Microtubules attach to and are propelled by kinesins. The leading tip (length $p$) is subject to Brownian and friction forces, and also to the magnetic forces exerted on the bead (radius $r$) toward the lines dividing the magnetic domains. The magnetic bead attaches at a distance $d$ of the tip, within the biotinylated portion of the microtubule (of length $b$). Magnetic guidance is possible when the distances satisfy $d < p$. The spacing $\Delta$ can be less than $42 \text{ nm}$ if the bead does not attach to the top of the microtubule. The surfaces must be passivated (e.g. with casein) to avoid denaturation of the kinesin motors (not shown in the cartoons).

It is worth mentioning that neither kinesin nor microtubules (or any other essential component of the motility assay) is measurably affected by moderate magnetostatic fields.

In its simplest embodiment, the magnetic pattern is a stripe-domain pattern. Here we explored whether the domain boundaries can serve as rails for the magnetic guidance.

### 2.2.2 Forces acting on a microtubule in a motility assay

We turn the attention to the forces involved in the motion of a microtubule, in particular to those that can undermine guidance.

The molecular motor kinesin can generate forces up to $7 \text{ pN}$ with $8 \text{ nm}$ steps [19]. The force is always oriented tangential to the microtubule, and therefore is neither specifically

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3Van den Heuvel et al. [18] pointed out that the trajectory can only be effectively altered by the forces acting on the head of the microtubule.
contributing nor undermining guidance. This is because kinesin never changes its track as it walks along a microtubule.

When a microtubule moves, it experiences a friction force. The component of the friction force tangential to the microtubule opposes the force of the kinesin motors, and as such does not undermine guidance. It is the component of the friction force perpendicular to the microtubule that may undermine the guidance of the magnetic forces. We estimate it as the friction force on a very elongated ellipsoid (the geometry of the swiveling tip of a microtubule, \( p \sim 100 \text{nm} \) cf. Fig. 2.1) being translated in a direction perpendicular to its longest axis. Using expressions of section 4.1.2 (page 69), we obtain, for an ellipsoid of principal axis \( a = 100 \text{nm} \), \( b = c = 12.5 \text{nm} \) and a velocity \( v = 0.1 \mu \text{m/s} \), a friction force \( 10^{-2} \text{pN} \).

Elastic forces caused by bending microtubules will further affect guidance. To estimate them, a microtubule can be thought as a cantilevered beam clamped on one end, with the guiding forces bending the other end. Assuming small deformations, the arguments developed in section 4.1.3 (page 73) allow us to consider the beam equivalent to a spring of constant \( 3EI/L^3 \approx 10^{-7} \text{N/m} \) (for a 10 \( \mu \text{m} \) long microtubule). \( 10^{-2} \text{pN} \) would be required to transversely deflect its free end a distance of \( 100 \text{nm} \).

Brownian forces will tend to push the tip of the microtubule and the particle in random directions. On average the net Brownian force is zero, \( \langle F^B \rangle = 0 \), i.e. they should not favor any particular direction of motion. In 1908, Paul Langevin [20] presented a method to characterize the displacement of a free particle subject to Brownian motion by estimating the expectation, \( \langle Q \rangle \), and the dispersion, \( \langle Q^2 \rangle \), of the coordinate, \( Q \). The method has been extended to study, among other cases, the situation of a particle in a harmonic potential, i.e. quadratic in the coordinate (for a review see [21]). For times that are long compared with the relaxation times, \( \tau \), the expectation and the dispersion of the coordinate are (see Eq. 215 in [21]):

\[
\langle Q \rangle_{t \gg \tau} = 0, \quad \langle Q^2 \rangle_{t \gg \tau} = \frac{k_B T}{k}, \tag{2.1}
\]

where \( k \) is the curvature of the potential created by the magnetic forces. Here \( \langle Q^2 \rangle_{t \gg \tau} \) gives an estimate of the amplitude of the excursions of the particle around its equilibrium point, and can thus be interpreted as a comparison between the thermal agitation and the curvature of the potential created by the magnetic forces.

The force of the magnetostatic fields on a magnetic nanosphere is

\[
F = \nabla M(\nabla B), \tag{2.2}
\]

where \( B \) is the magnetic density flux evaluated at the center of the sphere, \( M \) is the magne-

\[4\text{The flexural rigidity and the persistence length are related to each other: } L_p = EI/k_B T.\]
Figure 2.2: Fields and gradients over a magnetic film, and the forces acting on an iron particle \((r = 25\text{ nm}, M_s = 1700\text{ kA/m})\). Here the spacing of the magnetic stripes is the domain width \(w = 100\text{ nm}\) (left panel) and \(w = 2\text{ \(\mu\)m}\) (right panel), the film thickness \(h = 30\text{ nm}\), the spacing \(\Delta = 40\text{ nm}\), \(\gamma\) is the thickness of a protecting layer \((5\text{ nm})\), and \(\delta\) is the thickness of the domain wall \((\delta \simeq 5\text{ nm})\). \(M_f = 600\text{ kA/m}\). 2001 domains of alternating orientation were used for the calculation. **A**: Geometry. **B**: Force components. **C**: magnitude of the magnetic flux density gradient in the direction of the field, and magnitude of the field. **D**: Individual components of the flux density field.

We calculated the curves in Fig. 2.2 following standard procedures (see [10] and section 4.2.1 on page 74). For design purposes, though, it is more practical to take advantage of the periodicity of the magnetic pattern and obtain mathematical expressions in which the relevant...
parameters are easier to identify. This is best accomplished by the Fourier components of the field, given outside of the sample as (see [22] and the section 4.2.1 on page 80):

\[ A_{H_x}(k_x, k_y) = i e^{-k_z(1 - e^{-kh})k_x} A_M(k_x, k_y), \]
\[ A_{H_y}(k_x, k_y) = i e^{-k_z(1 - e^{-kh})k_y} A_M(k_x, k_y), \]
\[ A_{H_z}(k_x, k_y) = e^{-k_z(1 - e^{-kh})} 2k_x A_M(k_x, k_y), \]

(2.3)

Here \( k = \sqrt{k_x^2 + k_y^2} \), and \( A_M \) are the Fourier coefficients that describe the magnetization of the film in terms of the two wave numbers \( k_x \) and \( k_y \) for the axes \( x \) and \( y \), respectively. The factor \( e^{-kz} \) (the so-called distance loss) in expressions 4.33 describes an exponential decay (the wider the domains, the smaller \( k \), the gentler the decay, as can be appreciated by comparing the left and right panels of Fig. 2.2). The so-called thickness loss \( (1 - e^{-kh}) \) indicates the dependence of the magnetostatic fields on the film thickness.

The spacing \( \Delta = 40 \text{ nm} \) used to estimate the magnetic forces displayed in Fig. 2.2 is the approximately the sum of the gliding plane height (\( \sim 17 \text{ nm} \)) and the estimated diameter of a microtubule, \( \sim 25 \text{ nm} \). Here we are neglecting the length of the biochemical link between the particle and the microtubule.

It is apparent from the values of the magnetic forces in Fig. 2.2 and our estimates of the friction and bending forces that magnetic forces are capable of influencing the trajectories of microtubules that carry a magnetic bead at their leading end.

Notice —in Fig. 2.2-A— that the horizontal component of the force changes sign upon crossing a domain boundary. Furthermore, the slope of the curve \( f_x \) vs. displacement at the domain boundary is roughly \(-1 \text{ pN/nm} \) (for \( w = 100 \text{ nm} \)), which according to formula 2.1 gives a root mean square displacement away of the domain boundary of \( \sim 2 \text{ nm} \). In the case \( w = 2\mu\text{m} \), \( k \approx -0.4 \text{ pN/nm} \) gives a root mean square displacement of \( \sim 3 \text{ nm} \). Therefore the domain boundary defines, in effect, a rail for the guidance.

It is instructive to compare the figures for the flux density gradients reported in Fig. 2.2 with those found in the literature for micrometer scale devices. In investigations upon design issues related to microrobots (equivalent in size to a 477 \( \mu \text{m} \) magnetic sphere) Yesin et al. [2] concluded that a field-gradient of \( 0.7 \text{ T/m} \) would be needed. Field-gradients in the range 30 – 80 T/m were needed in a magnetophoretic device to sort cells that endocytically incorporated \( \sim 50 \text{ nm} \) particles [23]. A remarkable gradient \( |\nabla \mathbf{B}| \sim 600 \text{ T/m} \) can be estimated from the data reported by Inglis et al. [3]: the authors estimate a \( \sim 5 \text{ pN} \) force on a group of \( \sim 5000 \) beads attached to a cell, each particle sporting a magnetic moment \( 1.8 \cdot 10^5 \mu_B \) (Fig. 1 of [3]). It should be noticed that the aforementioned applications entailed distances between magnets and particles of at least several micrometers.
2.2.3 Magnetic film design and fabrication

We deposit a magnetically patterned substrate by magnetron sputtering on glass slides (full details in sections 5.4 and 5.5). The film used is a stack of: glass$|_{Cr_{5\text{nm}}}^{Pt_{5\text{nm}}}(Co_{0.3\text{nm}}^{Pt_{0.7\text{nm}}})_{30}^{Pt_{5\text{nm}}}$.

The chromium layer is an adhesion layer that prevents delamination, and was found specially important when the film gets in contact with an aqueous solution. The platinum underlayer is a seed layer which usually has a $(1\ 1\ 1)$ texture, which in turn promotes the $(0\ 0\ 0\ 1)$ orientation of the hcp-Co layers. The protecting layer is an oxidation barrier. After in-plane demagnetization (following the protocol outlined in section 5.5.3 on page 107), a pattern of nearly parallel stripes is obtained, as shown in Fig. 2.3. The film was characterized with MFM and VSM measurements (see section 5.6 on page 109) that confirmed its perpendicular nature and $M_f = 600 \text{kA/m}$.

Figure 2.3: Characterization of a magnetic film with Magnetic Force Microscopy (MFM). The picture shows a $5 \times 5 \mu\text{m}^2$ frequency modulated MFM measurement of an in-plane demagnetized perpendicular $(Co_{0.3\text{nm}}^{Pt_{0.7\text{nm}}})_{20}$ film sputtered on SiO$_2$. In this type of measurements the frequency shift is proportional to the derivative of the force on the MFM cantilever tip along the $z$ axis, as it is scanned over the image area. The curve corresponding to the line between the two blue marks is shown as an example. Parallel stripe domains are clearly visible. The direction parallel to the stripes ($\sim 17^\circ$ counterclockwise from the vertical) naturally defines the direction along which microtubule guidance is expected to occur.
2.2.4 Magnetic tags that mediate steering

Commercially available magnetic particles were employed in the experiments to sensitize the microtubules to the applied magnetic field gradients [24]. They are known to have a bcc-Fe core, a thin protective layer is the oxidation barrier, and to be coated with streptavidin molecules. Their average size is 50 nm. Based on previous studies on iron particles (see [25, 26] and section 5.2.6 on page 97), it is reasonable to assume that at zero field they adopt a curled magnetization state (see section 5.2.3), and that for weak fields their magnetization becomes almost uniform in the direction of the field.

To bind the particles to the microtubule, we used a biotin-streptavidin linkage, which can withstand the magnetic forces (as explained in section 3.2.1 on page 39). The link is established by the streptavidin coated particles as they adhere to the biotinylated tip of the microtubules.

2.2.5 Conducting motor protein motility assays

Our motility assays follow previously published protocols [27] with minor modifications. The protocol is outlined here; full details can be found in section 3.6 on page 53. The diffusion chamber is made of two microscope cover-slides separated by double-side adhesive tape (∼100 µm thick). One of the cover slides has been previously coated with the magnetic film, as indicated above; the other is not coated and thus transparent, in order to allow light microscopy measurements. On the cover slide sporting the magnetic film, care is taken to mark the direction parallel to the domains. This direction is arranged perpendicular to the injection-flow direction in the diffusion chamber.

1. After rhodamine-labelled tubulin polymerized into microtubules, biotin-labelled tubulin was added for further polymerization at the microtubule ends. The microtubules were stabilized with paclitaxel. The length distribution of the biotinylated ends was measured before the experiments.

2. After the diffusion chamber is coated with casein, and the kinesin molecular motors adsorb to the surfaces, the microtubules are injected with a solution poor in ATP (slow microtubules).

3. The magnetic particles are injected subsequently. The diffusion chamber is already upside down, so the agglomerated particles will tend to precipitate onto the non-magnetic surface. The film, however, may catch some of them. The ATP concentration is kept low for the microtubules to move slower, in order to avoid the formation of microtubule ag-
glomerations (held together by the streptavidin-coated particles). Particles bind both to microtubules attached to the diffusion chamber wall and to microtubules in suspension.

4. After the particles were allowed to bind to the microtubules, a solution of fluorescent labelled biotin is introduced, after which the diffusion chamber is flushed with a solution of high ATP concentration. These labelled biotin molecules bind to the free streptavidin in the particles, preventing the formation of microtubule agglomerations, and providing a means of visualizing the particles.

5. The diffusion chamber is sealed (to avoid a flow being established by the action of surface tension forces at the ends) and taken to a confocal microscope for observation. A field of view $212 \times 212 \, \mu m^2$ is used. Frames are recorded every 5 s, for a total of 400 frames in each of the six experiments (out of two different batches of polymerized microtubules).

2.3 Measurements of particle trajectories and velocities when carried by microtubules

The trajectories of the magnetic particles carried around by microtubules were recorded using fluorescent confocal microscopy (particles and microtubules were labelled with different fluorescent tags). The data was divided in two groups: the group of the particles carried at the leading tip of microtubules, and the group of particles transported by the microtubules at their trailing end (tail)\(^5\). Recall that the magnetic forces can influence the trajectory of the microtubule only when the magnetic cargo is situated at the leading tip, that is swiveling due to thermal motion. For this reason we seek a statistical comparison between the trajectories with cargo either attached to the microtubule tip or at the tail.

Particle tracking has been done manually, by choosing the brightest pixel among those that represent a fluorescent labelled cargo. The uncertainty is of the order of a pixel. Note that detecting the fluorescence from particles situated at the far end of the assay cell (in terms of optical path; this is the side of the magnetic film) is affected by low signal to noise ratios. No suitable artifact-free post-processing algorithm was found that could warrant sub-pixel spatial resolution given the prevailing uncertainty.

Fig. 2.4 shows the digitized trajectories of the particles transported by microtubules at either end.

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\(^5\)The formation of microtubules chains, connecting the tail of a microtubule with the head of another via a nanoparticle has been observed, but ignored.
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Figure 2.4: Digitized trajectories of microtubules carrying magnetic nanoparticles. A trajectory across domains implies motion across the potential barriers. Trajectories were artificially translated to start at the origin, in order to facilitate their inspection.

From the trajectories alone we cannot discern if the speed depends on the direction of motion, which is another way in which the magnetic guidance effect could be manifested. To find out if the microtubules move faster along the stripes than across them, we measured the steps between frames\(^6\), counted the occurrence of steps of a given size in \(x\) and \(y\), in what amounts to the 2D histogram of Fig. 2.5. Longer steps correspond to faster speeds, given constant frame intervals.

Figure 2.5: Histograms of steps between consecutive frames for particles carried at the head/tail of microtubules. For each step \((\Delta x, \Delta y)\), the number of its occurrences was recorded. Each bin in the histogram contains a single pair of values \((\Delta x, \Delta y)\).

\(^6\)The steps between two frames, 5 s apart in time, are composed of many individual kinesin steps.
2.4 Discussion

A well established assay was utilized here to probe whether motor protein driven active transport of nanoparticles could be guided magnetically.

Although the measured microtubules velocities (0.15 ± 0.05 µm/s with cargo transport at the head, 0.16 ± 0.05 µm/s at the tail), are within the expected range\(^7\), the anisotropy of the magnetic pattern did not induce an obvious anisotropy in the motion.

However, we expected anisotropy in the motion of the microtubules. Furthermore we expected that anisotropy to reflect the anisotropy of the underlying magnetically patterned substrate.

Our consideration of the forces led us to expect the trajectories to show a tendency for alignment along the stripe domains if the particles are carried at the tip of a microtubule, and to be indifferent to the magnetic pattern if the magnetic cargo were carried at its tail. From Fig. 2.4 it would appear that this is not the case: the trajectories measured for particles carried at the head are comparable to those particles carried on the tail.

Although the histogram of Fig. 2.5 for the head trajectories might suggest a slight asymmetry in the direction of the magnetic stripes (vertical axis) compared to the Tails result, the effect is minor, at best. A much larger sampling would be necessary to make a significant statement, rendering the utility of such a guiding mechanism marginal.

The experimental results appear to contradict our initial expectations. Next in this section we examine some experimental uncertainties, and a rigorous analysis of the initial predictions.

2.4.1 Uncertainties in the experiment alone cannot explain the results

We briefly review some aspects of the experiment that might have led to the mismatch between the experimental results and the predictions.

- To know if a particle is attached close enough to the microtubule advancing tip to mediate magnetic guidance effectively, we need to estimate the distance \(d\) (see scheme right panel of Fig. 2.1) from the tip of the microtubule to the binding position of the particle, and the average length of the swiveling tip \(\langle p \rangle\) of the microtubules (\(\langle p \rangle\) is an average of the tip lengths in the experiment being considered).

Immediately before the experiments, the length distribution of biotinylated ends was measured by first adding fluorescent-labelled streptavidin to the microtubules and second

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\(^7\)Speed figures in table 3.2 in section 3.3 are larger because the ATP concentration is here 30 times lower than there.
measuring the segments from confocal microscopy images. Fig. 2.6 shows the length distribution of microtubule ends available for particle attachment, in the form of a histogram.

We use the tip-length histogram to estimate the probability that a particle attaches close to the tip of the microtubule. The probabilities that a particle attaches in the first 50 nm, 100 nm, 150 nm are 8%, 16%, and 24% respectively (averaged over the 352 tips of the first experiment, and 8% 17%, and 25% respectively if averaged over the 960 tips of the second experiment).

Van den Heuvel and colleges [18] deduced $\langle p \rangle = 100 \pm 20 \text{ nm}$ after measuring the erratic trajectories of very short microtubules\(^8\). Fallesen et al. [28] estimate the surface density $\sigma$ of kinesin by applying forces to the trailing end as the microtubule moves, finding $0.05 \leq \sigma \leq 20 \mu\text{m}^{-2}$ (it implies $\langle d \rangle \sim 220 \text{ nm}$). Other researchers estimate the kinesin density via landing rate experiments, as for example Hess et al [29]: $\sigma \sim 250 \mu\text{m}^{-2}$, from which an average distance $\langle d \rangle \sim 60 \text{ nm}$ between fully functional kinesin motors can be estimated. The literature contains many other examples with similar figures, the reported casein and kinesin concentrations being almost identical in all cases. Because we followed standard protocols, we consider that the figures above also characterize our experiment, and that we can reasonably estimate $50 \leq \langle p \rangle \leq 300 \text{ nm}$.

We have thus calculated the probability that a particle attaches to the first 50 nm of the microtubule, and can infer that in the worst case the proportion of trajectories influenced by the magnetic forces will be $1 : 12.5$ (roughly 8% of the trajectories). In the case where $\langle p \rangle$ is larger, the ratio can be as high as $1 : 6$ (if $\langle p \rangle \sim 100 \text{ nm}$) or even $1 : 4$

\(^8\)Here very short means that the microtubule is short enough to be supported at times by only one kinesin but not too short to be supported by none. Under these conditions the microtubule will diffusively pivot around single kinesin attachments and the trajectory will therefore exhibit sudden changes of direction.
From these figures we conclude that the magnetic beads attach to the leading, swiveling end of a significant portion of the microtubules that transport particles. Therefore they should be able to produce a guidance effect.

- We cannot know how many particles attach to the end of a microtubule, or if some particles agglomerated before attaching. Because the particles behave like a soft magnetic material, if more particles bind, the resulting magnetic forces will on average be stronger than the estimated force for one particle.

- There is a distribution of particle sizes. Particles that are larger than the average 25 nm are also soft magnetic, and the magnetic forces are at least as strong as the ones we computed. In the case of particles smaller than the average, we point out that their fluorescence signal decreases with the square of the radius, and therefore also the probability of measuring them decreases. However, because of the increasing importance of surface effects (with decreasing size), a smaller particle can be expected to be magnetically harder (see [25, 26] and the considerations in section 5.2.6). We will argue in section 2.5.2 that hard magnetic particles improve the effect of the magnetic forces. Therefore the distribution of particle sizes ought not decrease the effect of the magnetic forces.

This analysis reveals that the uncertainties in the experiment alone cannot explain the negative results.

### 2.4.2 Reviewing the preliminary theoretical analysis

**Relaxation times enable comparison between forces**

A rigorous comparison between the magnetic and the friction forces can be obtained by considering the dynamic equations with full account of all the forces applied on the particle: the magnetic forces, the friction forces, and the Brownian motion. In a first analysis we neglect the presence of the microtubule and concentrate on the problem of a single particle. We will reintroduce the bending forces at a later point.

The expected position and momentum of the particle at any given time can be computed from their values at \( t = 0 \) by solving the dynamic equations in a narrow-sense [30], that is, by first taking averages (the stochastic term vanishes) and then solving the system of ordinary differential equations (see section 6.1 on page 121) In this case the evolution of the system is an exponential decay to equilibrium, and the decay rate is given by the eigenvalues of a matrix formed with the coefficients of the differential equation.
If the particle moves not too close to the substrate (the spacing is larger than a radius), the dynamic equations for the translation and the rotation can be decoupled (see section 6.1), and the characteristic times $\tau$ reflect the relation of forces and of torques:

- **Translation:** $\tau_t^+ \sim m/\zeta < 10^{-6}\, \text{s}$. Here $m$ is the mass of the particle and $\zeta = 6\pi \nu r$ is the translational friction coefficient, $\nu$ is the dynamic viscosity (for water $\nu = 8.9 \times 10^{-4}\, \text{Pa} \cdot \text{s}$), and $r$ is the radius of the particle. This characteristic times expresses that the inertia term can be neglected in comparison to the friction, as it is well known in submicron systems. Most authors would have neglected inertia from the very start (for a rigorous treatment supporting this decision, see e.g. chapter 10 of [31]).

- **Translation:** $\tau_t^- \sim \zeta/k < 10^{-5}\, \text{s}$. Here $k$ is the curvature of the potential energy of the magnetic forces (or equivalently the slope of the curve $f_x$ vs. displacement). From Fig. 2.2 $k \sim 1\, \text{pN/\text{nm}}$ if $w = 100\, \text{nm}$ and $k \sim 0.4\, \text{pN/\text{nm}}$ in case $w = 2\, \text{\mu m}$. The relation expresses that the magnetic forces are larger in magnitude than the friction.

- **Rotation:** $\tau_r^+ \sim I/\gamma < 10^{-6}\, \text{s}$. Here $I = 2mr^2/5$ is the inertia moment of the bead, and $\gamma = 8\pi \nu r^3$ is the rotational friction coefficient. This small relaxation time expresses that the angular inertia is also negligible in comparison with the rotational friction.

- **Rotation:** $\tau_r^- = \gamma/g < 10^{-6}\, \text{s}$. Here $g$ is the proportionality factor between the torque and the departure from the equilibrium orientation of the particle. A free particle will rotate in spite of the friction forces and will orient itself to align its magnetization parallel to the applied field.

In the case the particle glides closer to the surface ($\Delta < r$), the characteristic times mix, but no significant changes in magnitude can be expected.

This analysis confirms the estimates made in the introduction, but does not account for the diffusive effects of the Brownian forces, that is, the ability of the thermal fluctuations to push the particle over a potential barrier.

**Brownian forces may allow the system to overcome the potential barrier**

In the periodic energy landscape of our magnetic stripe patterns, Brownian forces may cause the particle to diffuse away from an equilibrium position over an energy saddle point to the next equilibrium position. The analysis of section 2.2.2 did not consider this circumstance. In order to evaluate this possibility, we measure the depth of the potential well created by the magnetic forces in terms of the product $k_B T$, a measure of the thermal agitation that drives
The integral in expression 2.4 is calculated over a path parallel to the substrate that starts at the equilibrium position and ends at the point \( x = X \), where the horizontal force changes sign (and pushes the particle to the next equilibrium position). The situation is depicted in Fig. 2.7, which we calculated using the procedure of section 6.2.1 (on page 126).

Figure 2.7: A: The energy-barrier height depends not only on the intrinsic properties of the magnetic material, but also on the geometric parameters: the spacing of the magnetic stripes \( w + \delta \) (\( w \) is the domain width), the film thickness \( h \), the spacing \( \Delta \), the thickness of the protecting layer \( \gamma \), and the thickness of the domain wall \( \delta \). B: \( E_{\text{mag}}/k_B T \) is the depth of the potential well created by the magnetic forces, measured in units of \( k_B T \), at room temperature. The calculation was made with parameters \( M_f = 600 \text{kA/m} \) (Co/Pt), \( M_s = 1700 \text{kA/m} \) (iron), \( \delta = \gamma = 5 \text{nm} \), \( h = 30 \text{nm} \). The experiments presented above correspond to \( w \approx 100 \text{nm} \) and \( \Delta \approx 40 \text{nm} \). Curves of constant potential-depth are displayed for three different particle radii. The highlighted grey spot indicates the region of parameters probed by our experiment.

If the energy barrier is not high enough, Brownian motion does allow the particle to pass the energy barrier and thus to transition from one equilibrium position to the other. It is apparent form the Fig. 2.7 that the magnetic forces cannot stabilize the particle position given the level of thermal agitation driven diffusion at room temperature. In Fig. 2.7, our experiments correspond to \( w \approx 100 \text{nm} \) and \( \Delta \approx 40 \text{nm} \) (the highlighted grey spot), at which point the depth of the potential well created by the magnetic forces is about \( k_B T \). This fact (potential well too shallow in terms of \( k_B T \)) explains why the magnetic guidance of our experiment has
been ineffective.

The particles observed in our experiments are not free to move but are bound to the tips of the microtubules that transport them. In turn, the tip of each microtubule is also subject to Brownian forces, which explain the non straight trajectories of these semirigid filaments in motility assays. The Brownian forces acting on the tip of the microtubule get transmitted to the magnetic particle through the biotin-streptavidin link. Therefore these forces can be lumped together as an extra random force acting on the particle. We conclude that the magnetic forces that are not able to prevent diffusion of a free particle cannot be expected to stabilize the motion of the whole system (the microtubule and the particle bound to it) along a direction.

2.5 Outlook: design guidelines for magnetic steering at submicron scale

The analysis of the previous sections does not only reconcile the experimental results with the preliminary estimates, but also provides an analytical tool to identify opportunities for improvement.

In the following, we apply the tools developed in our theoretical analysis to outline guidelines for future designs of magnetic-steering systems at the sub-micron scale.

2.5.1 Use thicker films to generate stronger magnetostatic fields

The film thickness influences the magnetic steering forces through the steepness of the field gradients it produces. As anticipated above and explained in full detail in section 4.2.1 (page 80), the thickness loss factor \(1 - e^{-kh}\) leads to an exponential decay of the field and its gradient with decreasing thickness. Such exponential decay can be lower bound in the \(z\)-range of interest by using a film of sufficiently large thickness.

In order to assess the maximum performance gains attainable with thicker films, we calculated the ideal case of an infinitely thick film (left panel in Fig. 2.8).

In these estimates it is assumed that the magnetic properties and magnetic-domains structure remains unaltered. However, the field-amplitude increase gained with thicker films implies, at constant domains structure, a heightened magnetostatic energy, which in turn will drive the system to a configuration with smaller magnetic domains. In fact, in exchange-coupled films of uniform anisotropy the thickness and the domain width are not independent from each other (see [32] and section 5.3 on page 99). Most usually the search for a thicker film will
lead to a selection of a different magnetic material (with different saturation magnetization and anisotropy), or to a different fabrication strategy, as mentioned next.

The contemporary magnetic recording technology makes use of granular films. The grain boundary is engineered to remove the exchange coupling between neighboring grains in the film, and the grain size is adjusted for single domain magnetization. Assuming that a writing process is possible, in which the direction of magnetization of each individual grain can be selected at will, arbitrary domain configurations can be arranged. In practice, the writing process makes use of a soft magnetic underlayer (SUL) to help guiding the fields: the writing field must surpass the coercive field only in the target region and not on the surroundings. Incidentally, the use of a SUL results in increased stray fields, as if the film thickness were effectively doubled [33].

2.5.2 Use short links and hard magnetic materials

In our experiment the magnetization of the particle tends to align with the applied field. This happens because of two reasons: First and foremost, the particles are soft magnetic, i.e. even a small applied field induces the magnetization to rotate into alignment with it. This is a consequence of the particle material (iron) and their size ($r \sim 25 \text{ nm}$), as discussed in section 5.2.6 below (on page 97). Second, even if the anisotropy were sufficiently large to lock the magnetization direction to a given crystallographic axis (e.g. $\langle 100 \rangle$ directions in Fe) the particle itself might be able to rotate, given the additional degree of freedom conveyed by the link to the microtubule. Had the particle been bound to the microtubule via a short link, it would have not been able to rotate.

The magnetic forces can attain a more effective directional confinement if the link is short (in the sense that impedes particle rotation) and the particle is made of a hard magnetic material (a coercive field larger than the stray field at the particle position, say, 100 mT). This is because an orientation that is in equilibrium at one domain wall is not in equilibrium at the neighboring domain wall, but at the following: diffusion needs to overcome a higher and wider potential well. The situation is depicted on the right panel of Fig. 2.8.

2.5.3 Caveats to using thicker films and hard-magnetic particles

The use of thicker films and of tightly bound hard magnetic particles can make the magnetic guidance more robust against thermal agitation. However, it must be kept in mind that thicker films lead to stronger fields, which in turn may exceed the particle switching field. Therefore
Figure 2.8: Diffusion of a soft- (A, B) and of a hard- magnetic particle C, D). B,D: Depth of the magnetic potential well measured in units of $k_B T$. Comparison with Fig. 2.7 reveals that substantial improvements can be obtained by using either an infinite thickness magnetic film (as in A), or a hard-magnetic particle (as in C). In D the depth of the potential is independent of $\alpha$, but the equilibrium position depends on $\alpha$.

the film thickness cannot be considered independently of the particles switching field\(^9\).

On the other hand, if the rotation of the particle cannot be avoided (for example, when the problem at hand requires that the link between the particle and the object be long), the benefit of using hard-magnetic particles is marginal (it ensures a uniform magnetization even if embedded in weak magnetic fields).

In the next section we give a step-by-step guide to make effective use of the aforementioned engineering parameters.

### 2.5.4 Design guidelines for sub-micron magnetic steering

We illustrate the steps of the guideline with the example of guidance of cargo-transporting microtubules. The trajectories of microtubules can contain curves, but the radius of curvature is typically larger than 1 $\mu$m \(^{34}\). We assume that a lateral confinement in the range 200 – 500 nm can ensure unidirectional guidance.

\(^9\)As explained in detail in section 5.2.5 (page 96), the application of an external magnetic field $H$ modifies the free energy landscape. If the field is increased, it will eventually reach a critical strength, the so-called switching field, at which the magnetization suddenly rotates toward the direction of $H$. 

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1. **Specify the geometry of the desired directional confinement:**

   - the horizontal confinement, i.e. the range of acceptable path widths, \( w \), and
   - the vertical confinement, i.e. the range of acceptable spacings, \( \Delta \), between the particle and the magnetic substrate.

   The specification of these two parameters, \( w \) and \( \Delta \) should be used to define a rectangular window in Figs. 2.7 and 2.8. In our example, the choice \( 200 \leq w \leq 500 \text{ nm} \) and \( 5 \leq \Delta \leq 45 \text{ nm} \) leads to the situation depicted in Fig. 2.9 (calculated using the procedure of section 6.2.1 on page 126). Fig. 2.9 has been calculated for a very specific case of iron particles and \( \text{Co/Pt} \) magnets. However, the equations are linear in the magnetization, so to convert them to other cases only a factor must be applied. Below we will show how to do it; the rectangle in the picture is what we need for the time being.

   - It intersects curves of constant depth of the potential well. If the possible potential wells are considered deep enough\(^{10}\), the next steps are a guide to maximize the effectiveness of the magnetic steering.

   \[ E_{\text{mag}} = k_B T \]

   ![Figure 2.9: Design step 1 (section 2.5.4), after specification of the desired directional confinement. The rectangle specifies the region accessible with \( 200 \leq w \leq 500 \text{ nm} \) and \( 5 \leq \Delta \leq 45 \text{ nm} \). Three different situations are considered: A: hard-magnetic particle capable of rotating, or a soft magnetic particle. B: same as A, but an infinitely thick film is used. C: a tightly bound, hard-magnetic particle is used. In all cases the particle was assumed to be made of iron (\( M_s = 1.7 \text{ MA/m} \), and the film is a 30 nm thick \( \text{Co/Pt} \) film (\( M_f = 600 \text{ kA/m} \)).](image)

2. **Identify the set of available and suitable particles.** In practice, the actual selection depends on the availability of the particles in the desired size distribution, surface

\(^{10}\)Whether \( 10k_B T \) or \( 10^3k_B T \) are required is application dependent.
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functionalization, and stability.
In our example, the size of the path, $200 \leq w \leq 500 \text{nm}$ suggests an upper limit: magnetic forces are more effective if the particle is much smaller than the domain size ($r \leq 20 \text{nm}$); otherwise they sense attractive forces from neighboring domains as well.

3. **Determine if the particle can rotate.**

Note from Figs. 2.9-A-C that fixing the magnetization direction has a profound impact on the degree of attainable lateral confinement.

A necessary condition for a fixed magnetization orientation is that the particle does not rotate\(^{11}\).

If the particle can rotate or not may be estimated by evaluating the rigidity of the link between the particle and the manipulated structure. Usually a perfectly tight link is impossible, unless the particle is tethered by several links simultaneously.

4. **Select the particle**

- **Particle can rotate**: in this case the magnetization will rotate together with the particle. The nanoparticle with the highest possible magnetic moment should be selected. The magnetic moment of a single-domain nanoparticle can be estimated as the product of its saturation magnetization and its volume. Note that the size of the particle should exceed the superparamagnetic limit and, if possible, not exceed the critical single domain size (see Fig. 2.10).

- **Particle cannot rotate**: in this case one can take advantage of fixing the magnetization direction. This can be accomplished by selecting a particle with a switching field ($H_s \approx 2K_u/\mu_0 M_s$, see Fig. 2.10) greater than the maximum stray field. Apart from that, choose the particle with highest possible magnetic moment.

Fig. 2.9 has been computed for iron particles ($M_s^{\text{Fe}} = 1710 \text{kA/m}$). If, for illustration purposes, a non rotating CoFe\(_2\)O\(_4\) particle with $r = 22 \text{nm}$ and $M_s^{\text{CoFeO}} = 450 \text{kA/m}$ has been selected, a factor

$$\left(\frac{M_s^{\text{CoFeO}} r_{\text{CoFeO}}^3}{M_s^{\text{Fe}} r_{\text{Fe}}^3}\right) = \left(\frac{450 \times 22^3}{1710 \times 25^3}\right) \sim 1/6$$

should be applied to the black curves in Fig. 2.9.

\(^{11}\)Note that particle rotation is not the same as magnetization rotation. Whether the magnetization of the particle can rotate or not has a large impact on the attainable degree of lateral confinement, as depicted in Fig. 2.9. Magnetization rotation proceeds either by a physical rotation of the particle (the lattice rotates) or by rotation of the magnetic moments without rotating the lattice.
Figure 2.10: Range of sizes for ferromagnetic single-domain state (after Krishnan [35] and O’Handley [36]), bulk saturation-magnetization (after Coey [33]), magnetic crystalline anisotropy (after Coey [33]), and switching field (calculated as $H_s = 2K_u/\mu_0 M_s$, see section 5.2.5 on page 96). The critical radii for the super-paramagnetic limit (left limit of the bars), and for the single-domain/multidomain limit (right limit of the bars) have been estimated using simple theoretical models (Eq. 5.2 in section 5.2.2 and 5.3 in section 5.2.3); they are meant as a reference to be used in absence of experimental data.

It should be noted that the values stated in Fig. 2.10 are only representative. Actual experimental values can be very different. For example, Torres et al. [37] report particles of CoFe$_2$O$_4$ with a switching field of 84 kA/m. If possible, fully characterized particles should be used.

5. Select a magnetic film. Depending on the complexity of the guiding structures that are required by the application a film of exchange decoupled grains explicitly written with magnetic storage technology may be required. If not, i.e. in exchange-coupled magnetic films of uniform anisotropy, the thickness is not independent of the domains structure.

Either way, select the thickest possible film consistent with the desired structure.

One possible strategy to increase the thickness of a film is to fabricate it over a SUL (soft magnetic underlayer).

Note that in the specific case of a non-rotating magnetization a reconsideration of the previous design step may be necessary to ensure that the switching field is not surpassed by the stray field of the film.
2.5.5 The limits to downscaling: an outline

The above considerations allow us to outline the limits of downscaling. Assuming improvements in film fabrication (such as the use of in-plane magnetized domains written with conventional magnetic recording techniques, which remained beyond the scope of this work) that make feasible a very thick substrate with a saturation magnetization similar to that of iron, with $\sim 100 \text{ nm}$ domain width, hard-magnetic (coercive field $H_c > 1 \text{ T}$) particles tightly bound and unable to rotate, then it might be possible for the magnetic forces to generate potential wells deeper than $10^3 k_B T$ for spacings up to $50 \text{ nm}$ (Fig. 2.11). These considerations strongly suggest that magnetic steering at submicron scale is possible. Note, however, that the outlined sketch considers just the particle, and not the attached structure. The attached structure contributes to the stability of the particle by hindering rotations, but will likely sensitize the particle to Brownian forces.

![Diagram](image)

Figure 2.11: Ideal situation with hard-magnetic particles unable to rotate and an infinite-thickness substrate, with $M_f = M_s = 1700 \text{ kA/m}$. The depth of the potential is independent of $\alpha$, but the equilibrium position depends on $\alpha$.

2.6 Concluding remarks

We have attempted magnetic steering at the submicron scale by building a system using well characterized magnetic and biological components. The predicted magnetic steering
effects could not be observed (Figs. 2.4 and 2.5). The reason is that magnetic forces and
torques can overcome friction forces but they create potential wells that are too shallow
(compared with $k_B T$) to provide the directional confinement that is required for steering
(Fig. 2.7). In other words, our experiments and the theoretical considerations show that there
are fundamental limitations to downscaling of magnetic steering to the nanoscale. Furthermore
the limitation is set by the thermal agitation: magnetic forces downscale together with object’s
dimensions. The fundamental limitation is reached when the height of the confining energy-
barrier associated with the magnetic forces is of order $k_B T$.

It is instructive to revisit here our own review of previous experiments at the beginning of
this chapter. The purpose is not only to clarify why some experiments work, but also to state
realistic expectations for future experiments.

In the case of the successful experiment by Inglis et al., the authors do not calculate
the height of the energy-barrier. But it can be calculated from their diagrams of force as a
function of position to be $\sim 0.25 k_B T$ per particle. As 5000 particles attach to each cell, the
total energy-barrier is actually $\sim 1200 k_B T$ high. Thus diffusion forces cannot be expected to
overcome the energy-barrier within the measurement times.

In the case of medicine applications on living animals, the distance between the field-
gradient source and the manipulated magnetic object (at least several micrometers apart from
each other) make a downscaling to submicron scale impossible.

Our analysis allows us to understand the role of the geometrical parameters, and to state
design guidelines that would lead to successful magnetic steering at the sub-micron scale
($> 100 \text{ nm}$, section 2.5.4, Figs. 2.8 and 2.10), provided the distance between the magnetic
object and the source of magnetostatic fields can be made as small as a few nanometers.

Further downscaling ($< 100 \text{ nm}$) appears to be limited by the Brownian motion. For exam-
ple, the use of magnetic forces to guide macromolecular assemblies, in which the manipulated
objects and the confinement distances are in the order of a few nanometers, appears to be
impossible.

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Motility assay (microtubules propelled by surface bound kinesin motors)

In this chapter we describe the practical aspects of motility assays, including the design criteria (integration of magnetic components, measurement at the microscope) and the protocols.

3.1 A few words about microtubules and kinesin

The cytoskeleton is a scaffold made of proteins that can be found in the cytoplasm of most eukaryote cells. It consists of three types of filaments: actin filaments, microtubules, and intermediate filaments. A large set of accessory proteins connect the filaments to each other (cross-linking) or to other cell components (e.g. membrane-bound organelles). The organization and mechanical properties of the cytoskeleton determine the overall structure of a cell and its internal organization, to a large extent. For example, biomolecular motors, such as myosin and kinesin use the filaments (actin filaments, microtubules) as tracks for transportation of organelles from one part of the cell to another.

In this section, we focus our attention on microtubules and kinesin, with particular emphasis on the structural details and functional properties that are relevant for the construction of a motility assay.

3.1.1 Microtubules are tubular assemblies of $\alpha\beta$-tubulin proteins

A microtubule is essentially a hollow cylinder composed of $\alpha$- and $\beta$-tubulin, arranged in a helical structure [1]. During the polymerization, $\alpha\beta$-dimers\(^1\) associate in linear protofilaments

\(^{1}\)The word dimer describes the quaternary structure of a protein, which is formed by two macromolecules.
that later interact laterally to form a tube (Fig. 3.1). Usually microtubules are formed by 13 equally spaced protofilaments [2].

![Figure 3.1: Polymerization of αβ-tubulin dimers in a tubular filament. A: The GTP originally bound to β-tubulin hydrolyzes during the polymerization. B: Growth phase. C: Shrinkage phase. Catastrophe is the passage from growth to shrinkage phase, and the reverse transition is named rescue. Adapted from [3].](image)

At each polymerization step, αβ-tubulin dimers carrying GTP join the (+) end of the microtubules and create a nucleotide triphosphate (T) cap (composed of in which GTP has not been hydrolyzed), which favors the hydrolysis of GTP into GDP at internal subunits (sketched in Fig. 3.1-A). The addition of new subunits follows the asymmetry of the subunits already assembled, and the two ends are usually called the plus and the minus ends. The (T) cap is stable against dissociation, and promotes the association of further GTP carrying tubulin-dimers (see Fig. 3.1-B). A (D) cap (composed of dimers in which GTP was hydrolyzed into GDP) is unstable and the subunits tend to dissociate (or depolymerize), as depicted in Fig. 3.1-C. If the hydrolysis lags the polymerization, the cap size increases with the rate of polymerization. If the opposite happens, that is, if the hydrolysis proceeds faster than the polymerization, the filament enters the shrinkage phase, during which GDP containing dimers dissociate.

Dynamic instability (switching between phases of growth and shrinkage) is essential for cell activity, but is undesired in motility assays, in which microtubules of constant length are needed. In vitro, a shrinkage phase may be avoided if the supply of GTP and Mg$^{2+}$ is sustained. In practice, the addition of paclitaxel can be used to stabilize the microtubules (prevents both
shrinkage and growth) [4].

In vivo, γ-tubulin combines with other microtubule-associated proteins to form a circular structure on which the polymerization can start [5]. In vitro, the polymerization of tubulin can start if the concentration of tubulin is high enough (see e.g. [6]). The nucleated microtubules will remain in the growth phase until the concentration of tubulin is too low, or the GTP and MgCl₂ supplies are depleted. At that point, the microtubules are stabilized by the addition of paclitaxel.

From the practical point of view it should be noted that microtubules are fragile structures. They will break if subjected to excessive mechanical stress (e.g. vortexing, pipetting) or by temperature variations.

**Geometrical and elastic properties of microtubules**

Table 3.1 summarizes the geometrical and elastic properties of microtubules. The data were extracted from the work of Howard [1].

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outer diameter</td>
<td>∼ 25 nm</td>
</tr>
<tr>
<td>Inner diameter</td>
<td>∼ 15 nm</td>
</tr>
<tr>
<td>Flexural rigidity</td>
<td>∼ 30 × 10⁻²⁴ Nm²</td>
</tr>
</tbody>
</table>

Table 3.1: Geometric and elastic properties of microtubules. Extracted from [1].

We point out that the flexural rigidity of microtubules has been observed to depend on how the microtubules were stabilized (paclitaxel, MAPs, other other chemical compounds) [7]. The value of flexural rigidity in table 2.1 was obtained for paclitaxel stabilized microtubules [8], which is the stabilization method used in our experiments.

### 3.1.2 Kinesin is a biomolecular motor that moves along microtubule filaments

Kinesin is a family of molecular motors [9, 10] that moves along microtubule filaments, and thus plays an important role in the transportation inside eukaryote cells. Most kinesins move from the minus end toward the plus end of microtubules because they can attach to the microtubule in only one direction (in vivo this is the direction from the centrosome to the periphery of the cell). The steps are 8 nm long, which is the size of the αβ-tubulin dimers. The motion is called processive because kinesin makes a large number of steps before detaching.
One of the most prominent members of the family is kinesin-1 [11]. It is typically obtained from bovine brain and its geometry has been thoroughly characterized by in-vitro experiments [12].

3.1.3 Microtubules and kinesin for in-vitro cargo transport: motility assays

Dennis et al. [13] coined the idea of a nanoshuttle: microtubules glide over a kinesin coated surface (see left panel of Fig. 3.2). Efforts followed to evolve the system into a shuttle system by providing guidance (e.g. [14, 15, 16], cargo pickup stations [17], or even complete loading and unloading stations [18].

Figure 3.2: Idea of directed transport at the nanoscale (nanoshuttle). **Left:** A microtubule glides over a kinesin coated surface. Cargo is linked to the microtubule and transported along. In absence of guidance the trajectory is erratic. **Right:** A magnetic film replaces the substrate, and a ferromagnetic particle is attached to the leading tip of the microtubule. The magnetic forces on the particle are attractive to the line dividing the magnetic domains: the latter serves as a magnetic rail to guide the transport.

As we argued in chapter 2, we want to take advantage of the nanoshuttles idea, not for transport, but as a test-model. To that end we need to replace the substrate by a magnetic film that is divided in magnetic domains. Besides, the microtubule is decorated with a magnetic particle at the leading end. In the following sections, we explain the experimental techniques to build such a system and measure the trajectories of the microtubules.

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Because the microtubule moves and not the kinesin, some authors refer to these experiments as *inverted* motility assays.
3.2 Design of a motility assay

Motility assays comprising microtubules and kinesin have been studied extensively (see the excellent book of Howard [1] for an overview). The experience gathered in these studies has led to nearly standard protocols that each researcher adapts to the specific needs of her/his experiment. Here we present the criteria used to integrate the magnetic components in our experiments.

3.2.1 Binding the particles to the microtubules

There exist different methods to bind particles to microtubules. One popular method is to polymerize biotin-labelled tubulin, which can be obtained by published methods [19] or bought [20], and then use the exposed biotin as a binding point to either an antibody (e.g. Brunner et al. [17] used anti-biotin coated gold-nanoparticles) or to a protein of the avidin family (e.g. Ramachandran et al. [21] used streptavidin to bind commercially available antibodies).

The popularity of the biotin-avidin bond stems from the fact that it is among the strongest non-covalent protein-ligand linkages ($K_a \sim 10^{13} \text{M}^{-1}$) [22]. There is also a practical reason: the bond forms with high specificity and affinity at pH $\sim 7$ in buffers that are normally used in protein assays (e.g. PBS, BRB80), at room temperature. The members of the avidin family of proteins have a similar structure, and they typically differ in the carbohydrates that are added during the glycosylation. The glycosylation step not only modifies the isoelectric point, the added carbohydrates also lead to high levels of non-specific binding [23]. In order to avert undesired non-specific binding, most researchers prefer to use streptavidin, which is not glycosylated: Compared to avidin ($pI \sim 10$), streptavidin has a lower tendency to engage in non-specific binding and a nearly neutral isoelectric point.

Effect of forces on the streptavidin-biotin bond

The association of streptavidin and biotin creates a minimum in the free energy. As explained by Kramers [24], thermal agitation alone may drive the dissociation, if the experiment is long enough: The rate of escape is governed by the energy barrier, $U$, and is proportional to $\exp(-U/k_B T)$.

Many mechanisms are involved in the formation of a streptavidin-biotin bond, both of enthalpic and of entropic nature\(^3\), that create a succession of energy barriers instead of a single minimum.

---

\(^3\)An enthalpic reduction of the free energy can be due, for example, to an electrostatic interaction. An entropic one comes usually from an increase in the number of freedom degrees.
When a persistent force $f_b$ is applied to stretch the bond, and the force does not diminish when the bond is stretched, the energy landscape is tilted by $-f_b x$ ($x$ is the coordinate in the direction of stretching). If the force is weak, the escape rate will continue to be dominated by the steepest exponential of the outermost barrier, except that the latter has been weakened by an amount $f_b x$. For stronger stretching forces, the system may reach a situation in which the escape involves passing over a more shallow landscape, that includes some of the internal barriers [25].

In experiments, forces are applied in times longer than the relaxation times for thermal impulses ($\sim 10^{-12}$ s). Hence, the unbinding rate is not a simple exponentially decaying function of time governed by a constant escape rate. Evans and Ritchie [26] started the analysis of this situation by considering the case of a monotonically increasing force (as it is usually done in force-spectroscopy experiments) in a system, whose potential has a single minimum. The statistical distribution of unbinding events will be a product of a rupture rate that increases in time and the probability of bond survival, that decreases in time. The distribution will reach its maximum at a given time (or force, after a change of variables), at which most unbinding event will take place. Evans and Ritchie [26] showed theoretically that, in excess of a critical loading rate, the rupture force increases logarithmically with the loading rate. Next, the case of an energy landscape with many barriers is considered. When the time-increasing pulling force is applied, the ensuing cascade of barriers will lead to a sequence of linear regimes (rupture force vs. $\log[\text{loading-rate}]$) with ascending slopes [26].

The rupture force of Streptavidin-biotin bonds has been measured for different loading rates by Merkel et al. [27] (see Fig. 3.3).
In our experiments, the microtubules move forward at speeds in the range $0.08 - 0.8 \mu m/s$. The velocity of lateral motion, due to Brownian forces acting on the swiveling tip, is very likely in the same range. In terms of loading rate of magnetic forces, the velocity figures translate into $0.024 - 0.24 \text{pN/s}$, for which the rupture force can be expected to be larger than the magnetic forces (Fig. 3.3). These working conditions are adequate for this type of proof-of-concept experiments with cargo transported by microtubules, but a stronger bond is needed if robustness or reliability are an issue.

### 3.2.2 Positioning of a motility assay at the microscope

Fig. 3.4 depicts the essential elements of a motility assay in a diffusion chamber. The chamber consists of two parallel surfaces. One is the magnetic film (it is not transparent) and the other is a transparent cover (a glass microscope slide) that allows optical measurements inside the chamber. The distance between both surfaces is about $100 \mu m$, and the thickness of the microscope slide is roughly $130 \mu m$. These distances are adequate for work with standard microscopes, whose focusing range usually lies within $300 \mu m$. A relative tilt angle between both surfaces is unavoidable, but is in practice very small: it amounts to a few microns (less than $10 \mu m$ over a distance of $2 - 5 \text{mm}$).

As the magnetic substrate is not transparent, we are forced to invert the diffusion chamber (or use an upright microscope instead of an inverted one). This is not a problem from the point of view of the forces involved in the motility assay, because gravity plays no significant role (see section 4.1.1). However, the extra $\sim 100 \mu m$ optical path across the solution affects the quality of the measurements, requiring longer dwelling times per pixel and higher resolutions in order to capture the motion of the fluorescent labelled objects (see also next section and section 3.5).

### 3.2.3 Measuring the assay at the microscope: level of detail, speed, and field-of-view

Kinesin, microtubules, and the magnetic nanoparticles are very tiny objects whose dimensions are much smaller than the wavelength of visible light. In order to make them visible, we resort to the use of fluorophores and confocal microscopy, as explained below in section 3.5.

It is technically possible to measure the position of objects with better resolution than half wavelength (see e.g. [28]), but this comes at the cost of a very small field of view and a lengthy measurement.

We are interested in measuring a large number of events in an area $\sim 1 \text{cm}^2$, that changes significantly in $\sim 10 \text{s}$. Therefore, we would like to increase the field of view as much as
possible, such that the microtubules can be clearly distinguished.

On the other hand, we would like to be able to follow the trajectories very closely. However, a scanning confocal microscope needs a minimum dwelling time per pixel, such that enough photons reach the photo-multiplier, and a reasonable signal-to-noise ratio is attained.

Summarizing, the settings are arranged following the steps:

1. Select the largest field of view such that both microtubules and particles can be clearly distinguished in all frames.
2. Select a pixel size similar to the wavelength (half a micron).
3. Select the maximum scanning speed that both microtubules and particles can be clearly distinguished in all frames.
4. Take the longest possible sequence of frames such that the microtubules do not visibly depolymerize.
In practice, the signal-to-noise ratio diminishes as the experiment proceeds, and sometimes the focusing is less than perfect. As the images are then handled manually to extract the coordinates of the objects, small variations can be readily compensated. The few frames in which the tracking was not possible were discarded.

### 3.2.4 Engineering the polymerization: fluorescent labelled microtubules with biotinylated ends

In our experiments we need to engineer the microtubules such that: (a) the magnetic particles attaches to the leading tip of microtubules, and (b) the microtubules also carry fluorescent labels that enable the measurement of their trajectories.

To that end we take advantage of commercially available functionalized-tubulin:

- **Rhodamine-labelled tubulin**
  Cytoskeleton ([29]) supplies rhodamine-labelled bovine-brain tubulin. Rhodamine has been covalently bound to surface lysines. Labelling stoichiometry is $1 - 2$ dyes per $\alpha\beta$-tubulin dimer.
  The absorption peak of Rhodamine is at $535\,\text{nm}$ light, the emission peak is at $585\,\text{nm}$.

- **Biotin-labelled tubulin**
  Cytoskeleton ([20]) supplies biotin-labelled bovine-brain tubulin. Biotin has been covalently linked to random surface lysines of the protein using a long chain. The long chain has been selected because the large spacing between biotin and the protein enable both to develop their own functionality without impairing or interfering with the activity of the other one.

In both cases, the stoichiometry is $1 - 2$ labels per $\alpha\beta$-tubulin dimer, which does not affect the interaction between kinesins and microtubules.

The idea, inspired in previous work [30, 31, 32], is to design a hybrid polymerization in two steps. The first one is with rhodamine-labelled tubulin only, in a concentration such that the nucleation and elongation are possible. The second stage is entered when biotin-labelled tubulin is added, in a concentration that is not enough to nucleate new microtubules, but that can be high enough to elongate existing ones.

For this hybrid polymerization to be possible, a knowledge of the critical concentrations is needed. We do not need to find the exact numbers, just a working set of parameters. According to Brown et al. [6], the critical concentration for the nucleation of new microtubules is $\sim 10\,\mu\text{M}$, whereas the critical concentration for elongation of existing microtubules is $\sim 1\,\mu\text{M}$. The critical concentrations reported by Doot [30] are lower (he worked with a
2 µM tubulin concentration at the elongation stage), but our experience seems to be different (in agreement with values reported by Brown et al.), probably due to subtle differences in the concentrations of the incubation media.

In order to outline a recipe for the hybrid polymerization, we need to know the evolution of the polymerization in time. The supplier of Tubulin provides the polymerization curve shown in Fig. 3.5. It is apparent from Fig. 3.5 that the polymerization nucleates in the first \( \sim 10' \), the polymerization reaches its maximum rate \( \sim 10' \) later, and the saturation is established after \( \sim 30' \) from the start.

Figure 3.5: Microtubule assembly characteristics as determined by absorbance at 340 nm. A value of 0.8 OD unit indicates that > 90% of the tubulin has polymerized. Tubulin concentration is 5 mg/ml (We have used 3.2 mg/ml). Adapted from http://www.cytoskeleton.com/products/tubulins/abouttub.html.

The concentration values and incubation times given in section 3.6.1 below were calculated to follow the design outlined here.

### 3.2.5 Additional practical issues in a motility assay

The protocol to build a motility assay lists the exact amounts and concentration of all reagents except for the kinesin, ATP, and in the case of cargo transport, the amount of particles. Here we discuss the criteria used to fix the amounts of those components of the motility assay.

It is clear that different dilutions of kinesin lead to different surface densities. However, as already stated in section 2.4.1 above, the actual density of functional kinesin motors is very difficult to measure. In practice, and for each new batch of kinesin, several dilutions are tried in motility assays without cargo transport. Then, the highest dilution in which a reasonable amount of microtubules were observed, the trajectories are smooth, very low occurrence of buckling is observed, and sudden rotations of small microtubules do seldom take place, is selected as the dilution to use in all experiments.

When the trajectories of two microtubules cross, and one of them is carrying a streptavidin-coated nanoparticle, the particle will very probably attach to the second microtubule. This is an undesired effect in transportation systems. In order to diminish the probability of occurrence
of these collisions, we decided to use slow microtubules. But the velocity cannot be chosen to be arbitrary slow. The reason is that we need to measure trajectories in the first 40′, before the depolymerization of the microtubules becomes apparent.

The velocity of microtubules depends on the density of kinesin, and on the ATP and Mg\(^{2+}\) concentrations [33, 34, 35]. Variations in the kinesin concentration may lead to changes in the gliding speed of microtubules [36], but may also introduce undesired effects, as we have already mentioned before in this section. For these reasons, we control the velocity of the microtubules with the ATP concentration. The standard protocol dictates 1 µM ATP. We found through experience that 10 nM ATP is adequate at the point cargo is added (the particles diffuse in the solution and bind to the microtubules, which are nearly still). We also found that 40 nM ATP is appropriate at the last step, when the free pockets of the streptavidin in the assay had been passivated by flushing a biotin derivate.

### 3.3 Magnetic films should not undermine the functionality of the molecular shuttles

Biomolecular motors and their associated filaments have evolved to fulfill their function inside a cell. We need to recreate their functionality when the microtubules are decorated with magnetic nanoparticles and the kinesin motors bind to a magnetic substrate. Compatibility between biomolecular motors, protein filaments, and the synthetic world cannot be taken for granted [37]: an experimental proof is required.

In order to prove that the magnetic particles and the films do not undermine the functionality of the microtubules, we conducted motility-assay experiments on sputtered surfaces with different top-layers. The velocity measurements are summarized in table 3.2.

<table>
<thead>
<tr>
<th></th>
<th>glass surface</th>
<th>metal coated surface</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ti</td>
<td>0.7 ± 0.1 µm/s</td>
<td>0.3 ± 0.1 µm/s</td>
</tr>
<tr>
<td>Cr</td>
<td>0.7 ± 0.1 µm/s</td>
<td>0.3 ± 0.1 µm/s</td>
</tr>
<tr>
<td>Pt</td>
<td>0.7 ± 0.1 µm/s</td>
<td>0.7 ± 0.1 µm/s</td>
</tr>
<tr>
<td>Co(_3)O(_4)</td>
<td>0.8 ± 0.1 µm/s</td>
<td>0.8 ± 0.1 µm/s</td>
</tr>
</tbody>
</table>

Table 3.2: Microtubule-velocity measurement in inverted motility assays conducted on four magnetic films, each one having a different capping layer. In all cases, the diffusion chamber had a glass surface, on which the velocity of microtubules was also measured.

In all the experiments the population of microtubules kept increasing over the first ~ 10′ (microtubules in the solution land on the surface) and then decreased slowly, very much like it did on the glass surface.
It is apparent from table 3.2 that the speeds of the microtubules are affected by the constituents of the magnetic film. In this work we use films whose capping layer (oxidation barrier) is made of Pt.

### 3.4 Particles as cargo

#### 3.4.1 Stability

Coagulation of particles into larger aggregates (agglomeration) is often an undesired process because it usually alters the optical, electric, catalytic, and magnetic properties of nanoparticles.

In order to evaluate magnetic steering at the nanoscale, we need to work with single, non-aggregated nanoparticles, or using the jargon of particle manufacturers: stable nanoparticles.

**Electrostatic origin of the stability of nanoparticles**

When a particle is immersed in an ionic solution, the surface charge of the particle affects the distribution of ions around it, resulting in an increased concentration of counter-ions in the regions close to the surface.

![Electrostatic origin of stability](image)

**Figure 3.6:** Electrostatic origin of the stability of nanoparticles in an ionic solution. Inside the Stern zone some ions are bound to the particle. The \( \zeta \)-potential is the quantity usually measured in electrophoresis experiments and used to characterize the stability.

The solution layer immediately surrounding the particle can be divided in two parts (see Fig. 3.6): an inner region, called Stern layer, where the ions are strongly bound (and move together with the particle), and an outer region of less firmly attached ions (which do not move along with the particle). This double layer is known as the double electrical layer, and its presence is essential for the stability of the particles against coagulation: the colloids stabilize as a result of the repulsion force generated as their double layers overlap. When the
electrostatic repulsion force is stronger than the van der Waals (attractive) force, the particles are stable.

Colloid suspensions are often characterized by their $\zeta$-potential value (as depicted in Fig. 3.6, $\zeta$ is the apparent potential of a particle when it moves in an electric field).

It should be noticed that stability can also be attained using surfactants, which sterically avert the coagulation of particles. This strategy usually has the shortcoming that the requirements imposed on the solvent cannot be met without destroying the proteins involved in the experiment.

**Obtaining single (non-agglomerated) nanoparticles**

In practice, there are always particles that agglomerate. Next, we explain a popular technique used to obtain a slurry of single, non-agglomerated, particles.

Very small particles in a suspension stored inside a tube on a shelf are subject to gravity forces and Brownian motion. Gravity forces push them to sediment at the bottom of the tube, whereas Brownian forces favor their diffusion, in all directions. The upward flux of particles [38] is $-Dc_c/\partial z$, where $c = c(z)$ is the colloids concentration, $D = k_B T/\mu$ is the diffusion coefficient, and $\mu$ is the mobility of an individual particle. The flux due to the gravity force, $F$, is $Fc_c/\mu$. If both fluxes balance each other, $c(z) = c_0 \exp[-Fz/D\mu] = c_0 \exp[-Fz/k_B T]$, which is a Boltzmann distribution.

The role played by particle size is evident when we write the gravity force as $F = 4\pi r^3 \rho z/3$, where $r$ is the particle radius and $\rho$ is the density. Because the radius of the particle enters the formula amplified to the third power, larger particles will exhibit a much stronger tendency to sediment than small ones. In other words: particles that agglomerate do sediment faster than particles that do not.

The recipe to obtain single, non-aggregated nanoparticles is very simple: allow the particles to sediment as long as possible, then take a slurry of particles from the upper part of the tube.

**Practical issues**

In practice it is noticed that stable particles of up to $\sim 100 \text{ nm}$ in size can stay up to several months in suspension. Larger particles usually precipitate too quickly and the method cannot really be applied.

It must be kept in mind that long storage times may affect the particle composition, or its surface functionalization. For example, if the oxidation state of an iron particle is not the most stable one (e.g. it is $\alpha$-Fe instead of hematite), there might be more changes taking place than just a sedimentation.
If all the particles sedimented, it might be possible to restore the suspension by vortexing, or by sonication (in an ice bath to avoid overheating). However, only a small portion of the particles will remain in suspension after a few hours. Besides, the functionalization of the surface may have been damaged by the sonication.

As a rule of thumb, when either the sedimentation of the particles is evident, or the particles have started to change color, a new tube should be ordered.

### 3.4.2 Particle-mediated agglomeration of microtubules

Hess et al. [39] reported the formation of microtubule spools when streptavidin is added to a motility assay of biotin-labelled microtubules. The authors estimate the bending energy stored in a spool to be $\sim 10^4 k_B T$. Thermal agitation alone cannot disassemble the spool in the time frame of an experiment, but the spool may eventually disassemble the streptavidin-biotin links in a zipper-like mechanism if the tip of the microtubule finds a kinesin motor outside of the spool.

Yet another situation that takes place is the cross-linking of different microtubules. Cross linking may lead to configurations in which different microtubules prevent each other from moving.

Both situations can be observed when streptavidin coated particles are added to a motility assay in which the microtubules have biotinylated ends, and none of those situations is desired.

To avert the formation of spools and cross-linking, the following steps are followed: (a) the streptavidin coated particles are added to slow microtubules (the slower the microtubules move, the less probable a cross-link or a spool are). After the particles are allowed enough time to diffuse and bind to microtubules, a biotin solution is added to passivate the free streptavidin. Finally, the velocity of the microtubules is adjusted to a higher value. As explained in section 3.2.5, the velocity of the microtubules can be most efficiently adjusted by changing the ATP concentration.

### 3.5 Imaging of motility assays using scanning confocal microscopy

An optical microscope is a device that magnifies the image of a small sample using visible light and an arrangement of lenses. Typically, the light of the source is focused by a condenser onto the sample. An objective, with a very short focal distance and located very close to the sample, collects the light from the sample and focuses it inside the microscope tube. The objective is characterized by the range of angles from which it can take light (numerical aperture, NA) and
by its magnification. Usual range for the magnification is $4 \times$ to $100 \times$ and for the numerical aperture is $0.1 \sim 1.25$, or even as high as $1.6$ if oil is used at the interface. The image is then focused on the eye of the operator (or into a recording device, such as a camera) by the ocular.

The contrast in the image is formed according to differences in reflection or absorption of light in the sample.

Fluorescence microscopy makes use of fluorophores to form the image. In this case the specimen is illuminated with light in a narrow range of wavelengths, which is absorbed by the fluorophore. The fluorophore then emits light at a larger wavelength.

It is this emitted light that forms the image, and the contrast in the image reflects the different concentrations of fluorophores across the specimen. One limitation of fluorescent microscopy is that the objective collects emitted light from all the illuminated points in the sample, and not just the focus point.

The confocal microscope uses the objective to attain point illumination and a pinhole in an optically conjugated plane to eliminate the light that comes from points far from the focal plane. This way, it improves the lateral resolution and the contrast of a fluorescent microscope. Often, a pair of mobile mirrors add the ability to scan a sample, and a raster image is generated. Modern microscopes provide light splitters and filters that can be used to distinguish components of light in different wavelengths.

### 3.5.1 Measurement modes used in the experiments

A modern confocal microscope offers a wealth of different configurations that largely exceeds the scope of this text. We concentrate the exposition on the two modes that we used: interference and fluorescent modes.

**Interference mode**

The intensity of the light that reflects on the glass surface is about the same order of magnitude as the intensity of the light that reflects on the microtubule. Because both are located at slightly different distances along the optical path, their reflections interfere, as shown in Fig. 3.7-left.

A similar effect helps the observation of particles, as demonstrated by Jacobsen et al. [40]. This measurement mode is particularly useful to test the binding of particles to microtubules. In early stages of the development, when fluorophores had not yet been added to the particle, the interference mode allows us to distinguish bound particles that move along with a microtubule from artifacts.
Figure 3.7: A Rhodamine labelled microtubule transports two particles over a kinesin/casein coated glass surface. Field of view is 18.94 × 18.94 µm² (each picture). **Left:** Confocal microscopy: interference mode (λ = 546 nm). The light reflected at the glass interferes with the light reflected at the microtubule (contrast enhanced). The position of the beads is easy to spot. The interference pattern in the background is an artifact in the lenses and bears no relation to the sample. **Right:** Confocal microscopy: fluorescent mode. The intensity of the pixels reflect the intensity of rhodamine fluorescence.

The drawback of this measurement mode is that it works only when the two interfering components of light are about the same order of magnitude. Unfortunately this is not true in the case of motility assays on top of very flat metallic surfaces, that reflect a large portion of the visible light.

The interference mode gives an important piece of information about the position of microtubules in motility assays: as the gray shade of every microtubule keeps uniform along its length, it can be assumed that motion of microtubules is constrained to a layer not thicker than half a wavelength. This cannot be said of particles that lie atop of the casein coated surface, as depicted in Fig. 3.8: the gray shade of particles ranges from black to white depending on their position along the optical path.

**Fluorescent mode**

Confocal microscopes such as the Olympus FV-1000 [41] allow the use of bandpass filters, whose cutoff wavelengths can be selected. Typically, the wavelength of the source is left out of the band-pass selection, and the wavelength of the emitting fluorophore is allowed to pass. For example, in order to measure the position of the microtubules from the rhodamine labels, a 543 nm laser provides the excitation (peak absorption of rhodamine at 535 nm) and a band
pass-filter in the range $560 - 660 \, \text{nm}$ is selected (peak emission of rhodamine at 585 nm).

### 3.5.2 Settings used to measure with the Olympus FV-1000 scanning confocal microscope

All measurements reported in this Thesis were obtained with the Olympus FV-1000 scanning confocal microscope, using a $60 \times$ oil immersion objective ($\text{NA} = 1.35$, Olympus HPlanSApO).

The possibility of measuring the same signal at three different channels with three different filter settings enable us the following measuring strategy:

**Source 1** : $543 \, \text{nm}$ laser light

**Source 2** : $633 \, \text{nm}$ laser light

**Channel 1** : Band pass $460 - 560 \, \text{nm}$

This channel captures direct reflection of the source 1 laser. This reflection reaches its maximum amplitude when the system is focused on a reflecting surface (the magnetic film). During an experiment, the focusing is adjusted to keep the reflection at a maximum, thus ensuring that the signals measured correspond to objects located immediately above the magnetic film.

**Channel 2** : Band pass $560 - 660 \, \text{nm}$

This channel captures the fluorescence signal emitted by the rhodamine labels on the
Channel 3: The light signal whose wavelength is larger than 660 nm is measured here. This is particularly advantageous when a fluorophore marker, such as Cy5 (absorption maximum is 649 nm, close to source 2, and emission peak is 670 nm) is used as a readout of the position of the particles.

These settings are schematically represented in Fig. 3.9, where the channels are named PMT (Photo-Multiplier Tube).

Figure 3.9: Scanning confocal microscope settings (Olympus FV1000). The 543 nm laser source allows tracking of the surface (measured on channel 1) and position of rhodamine-labelled tubulin (channel 2). The 633 nm laser source allows tracking of the Cy5-labelled particles. The SDM560 splitter deviates wavelengths below 560 nm to PMT2. Similarly, the SDM640 splitter deviates photons with wavelengths above 640 nm to PMT3.

We give an example of pictures measured with the aforementioned settings in Fig. 3.10.

Figure 3.10: Example of pictures measured with the settings outlined in Fig. 3.9. **Left:** The gradient of gray shades indicates that the magnetic film is slightly tilted. **Center:** The microtubules appear slightly blurred, but they can be identified. **Right:** The level of contrast is adequate for manual tracking of the trajectories of the particles. The pictures are analyzed as a temporal sequence in order to sort out noise events.
3.6 Protocols of the motility assay

3.6.1 Protocol for microtubule hybrid polymerization

As already explained in section 3.2.4 above, a hybrid polymerization is carried out to obtain microtubules with biotinylated ends and a fluorescent labelled body.

The goal is to allow a standard incubation time of 30’ for the rhodamine-labelled tubulin to polymerize, and then replenish the reagents, allow additional incubation time under conditions in which only the biotin-labelled tubulin can elongate the existing microtubules. We typically produce four different polymerization, that differ from each other in the incubation time: a first one with a small amount of biotin (3’ additional incubation), a second one with some more biotin (5’ additional incubation), a third one with even more biotin (7’ additional incubation), and a last one with maximum amount biotin at the ends (10’ additional incubation).

Ingredients

In vitro polymerization of microtubules requires the presence of GTP and of the ion Mg$^{2+}$ [42, 43]. In order to stabilize the microtubules, paclitaxel is used [44].

- One red\textsuperscript{4}, one blue\textsuperscript{5}, flasks tubulin (usually kept in $-80^\circ$C refrigerator). Keep in ice.
- MgCl \textsuperscript{100 mM} (usually kept in a $-20^\circ$C freezer), keep in ice.
- GTP 25 mM (usually kept in a $-20^\circ$C freezer), keep in ice.
- DMSO (usually kept in a $-20^\circ$C freezer), keep in ice.
- Paclitaxel 10 mM (usually kept in a $-20^\circ$C freezer), keep at room temperature.
- BRB80, keep at room temperature.
- A small piece of aluminium foil to wrap aliquots.

Ingredients should never be vortexed to accelerate the thawing, because it generates bubbles. If bubbles form, spinning gets rid of them. A light spinning right before use ensures that the thawed solution is uniform.

\textsuperscript{4}Cytoskeleton Rhodamine tubulin (bovine brain) [29], 20 $\mu$g.
\textsuperscript{5}Cytoskeleton Biotin tubulin (bovine brain) [20], 20 $\mu$g.
MBI-1: buffer for the rhodamine-labelled tubulin incubation

Prepare 25 µl microtubule buffer by pipetting into an aliquot:
A) 21.8 µl BRB80 buffer, cool down 5’ in ice,
B) 1.0 µl MgCl (4 mM),
C) 1.0 µl GTP (1 mM), and
D) 1.2 µl DMSO (∼ 5%).

Label it MBI-1. Keep in ice.

MBI-2: buffer for the elongation of microtubules with biotin-labelled tubulin

Prepare 84.7 µl microtubule buffer by pipetting into an aliquot (figures in brackets indicate concentrations that will be attained when this incubation buffer is added in step C below):
A) 73.1 µl BRB80 buffer, cool down 5’ in ice,
B) 3.63 µl MgCl (4 mM),
C) 3.63 µl GTP (4 mM), and
D) 4.36 µl DMSO (∼ 5%).

Keep ∼ 5 µl aside for later use (see below) and add the rest to the (blue) tube with biotinylated tubulin. Vortex to mix, and spin to eliminate bubbles. Divide in two tubes containing ∼ 40 µl each. Label them B1 and B2. Keep in ice. All three tubes (MBI-2, B1, and B2) should be pre-warmed at the right time (see below).

BRB80T

BRB80T is a solvent for microtubules after polymerization. It contains paclitaxel to stabilize the microtubules.

Prepare four aliquots containing:
A) 79 µl BRB80, and
B) 1 µl paclitaxel.

Label them α, β, γ, and δ; and prepare pieces of aluminium foil to cover them. Keep at room temperature. All four tubes will be warmed together with the incubating solution. The goal is to avoid depolymerization of not-yet-stabilized microtubules that would take place if they come in contact with a cold stabilizing solution.
Incubation stages

A) Pipette 6.25 µl of MBI-1 in the (red) tube with rhodamine labeled tubulin. Just leave the drop near the top of the vial (do not risk touching the tubulin with the tip of the pipette) and light spin to get the fluid go to the bottom. Vortex a few seconds. If bubbles are produced, light spin, and keep in ice. The tubulin (monomer) concentration is now ∼ 58.2 µM. Incubate it for 30 minutes at 37°C.

B) About 5′ before the previous step is over, pre-warm at 37°C. the tubes MBI-2, B1, and B2.

C) When the timer goes off, add the remains of MBI-2 (about 5 µl) to the incubated solution (Rhodamine labelled tubulin), and stir the solution with the tip of the pipette. The idea is to reduce the density of tubulin around the microtubules before the biotin-labelled tubulin is added. Then, add ∼ 5.5 µl (half the tube) on the B1-tube, and the other half into the B2-tube. Each tube should contain a total volume of ∼ 43.4 µl. Now the concentration of biotin-labelled tubulin is 4 µM. A light stirring with the pipette is needed to mix both suspensions, till the red colour is uniform (but no pistoning: the microtubules are too fragile for that). Incubate further at 37°C.

D) After 3′ (total incubation 33′) take 20 µl of B1 into the aliquot labelled α, containing 480 µl of warmed BRB80T. Keep at room temperature in aluminium foil to prevent bleaching of the rhodamine. The tubulin (dimer) concentration is now ∼ 0.6 µM.

E) After 2′ (total incubation 35′) take 20 µl of B2 into the aliquot labelled β, containing 480 µl of warmed BRB80T. Keep at room temperature in aluminium foil to prevent bleaching of the rhodamine. The tubulin (dimer) concentration is now ∼ 0.6 µM.

F) After 2′ (total incubation 37′) take 20 µl of B1 into the aliquot labelled γ, containing 480 µl of warmed BRB80T. Keep at room temperature in aluminium foil to prevent bleaching of the rhodamine. The tubulin (dimer) concentration is now ∼ 0.6 µM.

G) After 3′ (total incubation 40′) take 20 µl of B2 into the aliquot labelled δ, containing 480 µl of warmed BRB80T. Keep at room temperature in aluminium foil to prevent bleaching of the rhodamine. The tubulin (dimer) concentration is now ∼ 0.6 µM.

Characterization

In order to measure the geometric composition of the hybrid microtubules, they can be observed at the microscope. The use of fluorescent labelled streptavidin (e.g. Alexa-Fluor-488 labelled streptavidin [45]).
The exact microscope settings should be adjusted each time, but the following settings were found adequate for measurements with the Olympus FV-1000 scanning confocal microscope [41]: Lasers 488 nm at 5% power, and laser 543 nm at 5% power. After the filter DM488/543/633, the splitter SDM560 is used to separate the first channel\(^6\) (filtered in the wavelength range 460–528 nm, \(h\nu \simeq 680–800\) V), and the splitter SDM640 was used to separate the second channel\(^7\). (filtered in the wavelength range 560–660 nm, \(h\nu \simeq 750–900\) V).

For these measurements, the sample is prepared as follows: Introduce a 25× dilution of fresh microtubules in CT solution (see below) in a diffusion chamber, await 3′, add a 25× dilution of 1 mg/ml Streptavidin-Alexa-Fluor-488, wait 3′ and wash with CT.

Figure 3.11: 50×20 \(\mu\text{m}^2\) fluorescent confocal microscopy of microtubules (taken from the \(\alpha\) tube, prepared as indicated in the text). Green: Alexa-Fluor-488. Red: Rhodamine. The yellow portion is the superposition of both types of fluorescence because the tips also include traces of Rhodamine labelled tubulin. Images were acquired with an Olympus confocal microscope [41], and then blended as green and red channels.

3.6.2 Protocol for an inverted motility assay

Assay solutions without cargo transport

Ingredients

A) BRB80, keep at room temperature.

B) Casein, \(\sim 20\ \text{mg/ml}\) (abbr.0 =CS, usually stored in \(-20^\circ\text{C}\) freezer), keep in ice.

C) MgATP, 100 mM (abbr.0 =MA, usually stored in \(-20^\circ\text{C}\) freezer). Keep in ice.

D) D-glucose, 2 M (abbr.0 =DG, usually stored in \(-20^\circ\text{C}\) freezer). Keep in ice.

E) Glucose oxidase, 2 mg/ml (abbr.0 =GO, usually stored in \(-20^\circ\text{C}\) freezer). Keep in ice.

F) Catalase 0.8 mg/ml (abbr.0 =Cat, usually stored in fridge). Keep in ice.

\(^6\)Alexa-Fluor-488 has a maximum absorption at 495 nm and maximum emission at 519 nm.

\(^7\)Recall that rhodamine has maximum absorption at 535 nm, and maximum emission at 585 nm.
G) DTT 10 mM (abbr.0 = DTT, in −20°C freezer). Keep in ice.

H) Paclitaxel\(^8\), 1 mM in DMSO (abbr.0 = TX, usually stored in −20°C freezer). Keep in ice.

I) Kinesin (usually stored in −80 refrigerator). Label K. Keep in ice.

**Paclitaxel solution** In an aliquot:

A) 485 µl BRB80,

B) 10 µl casein, and

C) 5 µl paclitaxel.

Label it CT and *keep at room temperature*. This solution can be prepared a few minutes in advance.

**Casein solution** Pipette into an aliquot:

A) 95 µl BRB80, *keep in ice* for 5’.

B) 5 µl casein

Label it CS1 and *keep in ice*. This solution can be prepared a few minutes in advance, and essential to prevent denaturation of the kinesin motors [36].

**ATP solution** In an aliquot combine MA with casein to produce CA:

A) 97 µl BRB80, *keep in ice* for 5’,

B) 2 µl casein, and

C) 1 µl MA.

Label it CA and *keep in ice*. Its only use is to dilute the kinesin.

---

\(^8\)Taxol
**Kinesin solution**  Each assay uses 20 µl of this solution. It does not contain anti-fades, so it can be prepared a few minutes in advance. However, in practice the five minutes before its usage (see below) are more than enough to prepare it right before usage. If two or more experiments are made one after the other, then it is just better to prepare some more solution and keep it in ice.

A) 47 µl CA,  
B) 3 µl kinesin.  
Label it K5d and keep in ice.

**Motility solution**  
A) 85 µl CT,  
B) 1 µl DG, and  
C) 1 µl GO, and  
D) 1 µl DTT, and  
E) 1 µl Cat, and  
F) 1 µl MA, and  
G) 10 µl MT100H.  
Label it MT1000 and keep at room temperature.

**Assay solutions for cargo transport**  
**Ingredients**  
A) BRB80, keep at room temperature.  
B) Casein, ~ 20 mg/ml (abbr. =CS, usually stored in −20°C freezer), keep in ice.  
C) MgATP, 100 mM (abbr. =MA, usually stored in −20°C freezer). Keep in ice.  
E) Glucose oxidase, 2 mg/ml (abbr. =GO, usually stored in −20°C freezer). Keep in ice.  
F) Catalase 0.8 mg/ml (abbr. =Cat, usually stored in fridge). Keep in ice.
G) DTT 10 mM (abbr. DTT, in −20°C freezer). Keep in ice.

H) Paclitaxel, 1 mM in DMSO (abbr. TX, usually stored in −20°C freezer). Keep in ice.

I) Kinesin (usually stored in −80 refrigerator). Label K. Keep in ice.


Paclitaxel solution (CT) and casein solution (CS1) are prepared as indicated in the previous section.

Diluted ATP solutions The purpose of a low ATP concentration is to build an assay in which the microtubules move at very low speed.

Here, two aliquots are prepared (typically a few minutes before the experiment). In the first aliquot prepare diluted MgATP (MAd):

A) 97 µl BRB80, keep in ice for 5’, and

B) 3 µl MgATP.

Label it MAd and keep in ice. MAd is an ingredient of CAd, the motility solution, and the washing solution. In a second aliquot combine MAd with casein to produce CAd:

A) 97 µl BRB80, keep in ice for 5’,

B) 2 µl casein, and

C) 1 µl MAd.

Label it CAd and keep in ice. Its only use is to dilute the kinesin.

Kinesin solution Each assay uses 20 µl of this solution. It does not contain anti-fades, so it can be prepared in advance. However, in practice the five minutes before its usage (see below) are more than enough to prepare it at that very moment. It contains diluted ATP (from CAd) because the intention is that the microtubules move slowly.

A) 47 µl CAd,

B) 3 µl kinesin.

Label it KSd and keep in ice. If two experiments are made one after the other, then it is just better to prepare some more solution and keep it at ∼ 4°C.
Pre-motility solution  Many of the solutions used here contain an anti-fade, or oxygen scavenger, that removes (excess) oxygen from the solution. The action of the anti-fade declines after $\sim 1.5$ hours. We choose to prepare the anti-fade component in one step. In one aliquot pipette the quantities of one column, depending on the number of derived products intended:

<table>
<thead>
<tr>
<th></th>
<th>1 pr.</th>
<th>2 pr.</th>
<th>3 pr.</th>
<th>4 pr.</th>
<th>5 pr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT</td>
<td>85 µl</td>
<td>170 µl</td>
<td>255 µl</td>
<td>340 µl</td>
<td>425 µl</td>
</tr>
<tr>
<td>DG</td>
<td>1 µl</td>
<td>2 µl</td>
<td>3 µl</td>
<td>4 µl</td>
<td>5 µl</td>
</tr>
<tr>
<td>GO</td>
<td>1 µl</td>
<td>2 µl</td>
<td>3 µl</td>
<td>4 µl</td>
<td>5 µl</td>
</tr>
<tr>
<td>Cat</td>
<td>1 µl</td>
<td>2 µl</td>
<td>3 µl</td>
<td>4 µl</td>
<td>5 µl</td>
</tr>
<tr>
<td>DTT</td>
<td>1 µl</td>
<td>2 µl</td>
<td>3 µl</td>
<td>4 µl</td>
<td>5 µl</td>
</tr>
</tbody>
</table>

Normally the fourth column is used. Label it PT and keep in ice. Possible derived products are: motility solution (both rich and poor in ATP), wash solutions (both rich and poor in ATP).

In the particular case of the wash solution rich in ATP, that is used at the very end, it is worth to prepare it from scratch just before using it, which extends the life of the experiment.

Motility solution  In an aliquot pipette:

A) 89 µl PT (first column of PT table),

B) 1 µl MAd, and

C) 2 – 10 µl MT-100-B.

Label it MT1000 and keep at room temperature. A range of volumes is given for the microtubules, the exact amount must be selected depending on the amount of microtubules that is desired during the experiment. This is trial and error, but is an efficient way to cope with the visibilities of the polymerization.

Wash solution poor in ATP  25 µl of this solution are needed per wash operation, and in practice it has been seen that more than one wash might be very necessary, specially when rinsing unreacted streptavidin. This solution is also the solvent to dilute the particles.

In an aliquot pipette:

A) 356 µl PT (fourth column of PT table),

B) 40 µl CT, and

C) 4 µl MAd.

Label it WSd and keep at room temperature.
Particle solution  
Particle solution has been previously prepared from Miltenyi-Biotec particles [47]. 100 µl slurry of particles was left stored in an aliquot, in vertical position over a stack of three NdFeB magnets. After two days the particles precipitated at the bottom. We then took out 90 µl of supernatant, and introduced 30 µl of BRB80. The particles can be very easily resuspended by a light pistoning with a pipette. The final volume of 40 µl is a 2.5 concentration of the initial suspension.

The particles are kept at 4°C and were observed to remain in stable suspension, during months after the preparation, suggesting that the agglomeration was kept at a minimum.

In an tube pipette:

A) 99 µl WSD,
B) 1 µl of a 1 : 100 particle dilution, taken from the upper part of the aliquot.

Label the aliquot PSd and keep at room temperature. The aliquot should be kept vertical, so the agglomerated particles, if any, go to the bottom.

Fluorescent-labelled biotin solution  The fluorescent-labelled biotin solution is injected some time after the particle suspension. The objective is to passivate the streptavidin molecules that were not used for cargo binding, and at the same time provide a readout for the position of the particles. This way, particles do not favor the cross-linking of microtubules, and we can identify the position of the microtubule where the particle is bound.

In an tube pipette:

A) 99 µl WSD,
B) 1 µl of 100 µM Biotin–DNA–Cy5 [46].

Label it BS and keep at room temperature.

Wash solution rich in ATP  It takes 20 µl of this solution to speed up the movement of slow microtubules. In an aliquot pipette:

A) 95 µl CT,
B) 1 µl DG,
C) 1 µl GO,
D) 1 µl Cat,
E) 1 µl DTT, and
F) 1 µl MA.

Label it WS and keep at room temperature. Although the experiments are never as long as 1.5 hours (the estimated time that the anti-fades are effective), preparing this solution right before usage helps to ensure that the whole sequence of measurements were made under the same conditions.

**Flow cells**

Use a microscope slide (Menzel Glaeser #1 24 × 50 mm²), and a round glass coated with the magnetic film (as explained above) separated by two pieces of double sticky tape (thickness ∼ 100 µm).

If the channel width is ∼ 5 − 7 mm, then the volume of the channel is ∼ 10 − 15 µl, and the surface ∼ 2 − 4 × 10⁸ µm².

Right after construction, the flow cells are kept inside Petri dishes. A small piece of parafilm between the dish and the big glass is used to ease the handling. The environment of the flow cell is kept humid by including a small piece of moist tissue-paper inside the dish at all times (the idea is to prevent the solutions inside the cell from drying out).

**Running the assay**

As a first step, ingredients are collected and the flow cells are built. Solutions are pipetted into the flow cell (with the help of a tissue paper on the other side, which absorbs the outgoing fluid, such that capillary forces push the flow), avoiding bubbles¹⁰, in the order indicated below.

A) 20 µl CS1, and wait 5′,

B) 20 µl KSD, and wait 5′.

C) 20 µl MT1000, and eventually, watch at microscope (this check is not essential). If the check is not made, then wait 5′ before the next step to allow microtubules in the solution enough time to land on the surface.

D) 20 µl particle solution and wait 5′.

E) 20 µl biotin–DNA–Cy5 solution and wait 2′.

F) Wash with 20 µl WS and take it to the microscope.

The flow cell is sealed with transparent foil to prevent evaporation.

¹⁰Better bubble-free results can be attained if the pipette and the tissue paper do not work simultaneously: first a few micro-liters are given at one side, and then part of the fluid is absorbed through the diffusion chamber from the other end.
Bibliography


[45] Invitrogen. Streptavidin, Alexa Fluor 488 conjugate, 1 mg. cat. no. S-11223. [http://products.invitrogen.com/ivgn/product/S11223](http://products.invitrogen.com/ivgn/product/S11223), 2009. Invitrogen’s streptavidin, Alexa Fluor 488 conjugate is a bright, photostable probe with excitation and emission characteristics similar to those of fluorescein. Streptavidin is a nonglycosylated biotin-binding protein that is isolated from bacteria and has a near-neutral isoelectric point. It is widely used to detect biotinylated probes, because it reportedly exhibits less nonspecific binding than does the glycosylated, biotin-binding protein, avidin. See also [http://www.invitrogen.com](http://www.invitrogen.com).


Chapter 4

Forces acting on a microtubule in a motility assay

The dynamics of a magnetic object in a fluid depends not only on the magnetic forces exerted by other magnetic objects in its proximity, but also on other volume forces, such as gravity, and the forces exerted by the fluid and by other objects that may come in contact.

In this section we briefly review all the forces that affect the dynamics of a particle, and we discuss their relevance in the case of a fine particle. Magnetic forces and torques are considered in a separate section.

4.1 Non-magnetic forces

4.1.1 Gravitational force is irrelevant

In the case of tiny transition-metal particles \( r < 100 \text{ nm} \), the gravitational force

\[
F_g = -\mathcal{V}(\rho_p - \rho_f)g
\]

\( (\rho_p \text{ is the density of the particle, } \rho_f \text{ is the density of the fluid, } g \simeq 9.8 \text{ m/s}^2, \text{ and } \mathcal{V} \text{ is the volume of the particle}) \) is at most of order \( 10^{-3} \text{ pN} \). It is very small compared to any other force that will be discussed here, and therefore it can be safely ignored.

4.1.2 Translational and rotational friction

When a particle moves in a fluid, there is momentum transfer from the particle to the fluid: the fluid also moves. Hence we need to consider not only the motion of the particle, but we also need a description of the motion state of the fluid around the particle, \( \mathbf{v}(r) \), at all points \( r \) of the
fluid. Every steady state flow depends on three parameters: the kinematic viscosity $\nu = \eta/\varrho$, the velocity of the object $u$, and a characteristic length $l$. The simplest non-dimensional parameter that can be built with them is the Reynolds number, $Re = ul/\nu = gul/\eta$. The steady state solution of the motion equations will then be a function of the Reynolds number $v = u(x/l, Re)$. In other words, similar flows will be calculated when the Reynolds number are equal. Table 4.1 gives examples of different Reynolds numbers.

<table>
<thead>
<tr>
<th>Example</th>
<th>$Re$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td>$\sim 10^5$</td>
</tr>
<tr>
<td>Typical pitch (baseball)</td>
<td>$\sim 2 \times 10^5$</td>
</tr>
<tr>
<td>Ciliate</td>
<td>$\sim 10^4$</td>
</tr>
<tr>
<td>Person swimming</td>
<td>$\sim 4 \times 10^6$</td>
</tr>
<tr>
<td>Smallest fish</td>
<td>$\sim 1$</td>
</tr>
<tr>
<td>Fastest fish</td>
<td>$\sim 10^6$</td>
</tr>
<tr>
<td>Blood flow in brain</td>
<td>$\sim 10^2$</td>
</tr>
<tr>
<td>Blue whale</td>
<td>$\sim 3 \times 10^8$</td>
</tr>
<tr>
<td>Blood flow in aorta</td>
<td>$\sim 10^3$</td>
</tr>
<tr>
<td>A large ship</td>
<td>$\sim 5 \times 10^9$</td>
</tr>
</tbody>
</table>

Table 4.1: Reynolds number in common examples. The onset of turbulent flow is estimated to be $\sim 10^4$ for pipe flow and $\sim 10^6$ for boundary layers. Data taken from Wikipedia.

In the case of a small bead ($r < 100 \mu$m) moving slowly ($u < 1 \mu$m/s), $Re < 10^{-6}$.

The Navier-Stokes equation simplifies for low Reynolds numbers to the linear equality $\eta \Delta v - \nabla p = 0$, which together with the continuity equation for incompressible flow, $\nabla \cdot v = 0$, and the border conditions, completely determines the flow [1]. This is the so called Stokes approximation. In consequence, the drag forces and torques are proportional to the speed (here proportional means that the drag force and the velocity vectors are related to each other by a second order tensor).

Brenner [2] showed that for an arbitrary body, whose movement in a fluid can be described with the Stokes approximation, a hydrodynamic centre (or centre of hydrodynamic stress) can be defined. This is the point where the hydrodynamic force resulting from the relative translational motion of particle and fluid is applied. The torque exerted by the hydrodynamic force around the hydrodynamic centre is zero, by definition. It can also be shown that an object rotating around its hydrodynamic centre in an otherwise quiescent flow does not experience any translational force. We only mention the physical intuition behind the derivations and proofs, but deliberately omit them. The technical details can be found in the work already cited.

The location of the hydrodynamic centre depends only on the geometry of the particle\(^1\). In particular, the position of the hydrodynamic center does not depend on the fluid properties or the relative velocity between particle and fluid. A particularly interesting case is given when the hydrodynamic center and the mass center coincide: in such a case, stability (when a force

\(^1\) $\eta$ is the dynamic viscosity.

\(^2\) This contention is not true in the case of a high Reynolds number system, in which the non-linear effects would cause the position of the hydrodynamic centre to change according to the flow conditions.
on the hydrodynamic center does not elicit a torque that reorients the object) is achieved independently of the object orientation\(^3\). This situation is called \textit{neutral stability}.

Neutral stability is attained by any system composed of an object of uniform density, and possessing three orthogonal symmetry planes (ellipsoid, right angle prism), or three non-coplanar axes of symmetry that are not mutually perpendicular in any pairing, except perhaps one, (tetrahedron, octahedron).

In the case of neutral stability, the force and the velocity are parallel and the relation between them can be expressed with scalars (the same holds for the torque and the angular velocity). In the body-fixed coordinate system, the forces and torques generated by friction are \([3]\):

\[
\begin{align*}
    f_x &= -\alpha_x \dot{x}, \\
    f_y &= -\alpha_y \dot{y}, \\
    f_z &= -\alpha_z \dot{z}, \\
    \tau_x &= -\beta_x \omega_x, \\
    \tau_y &= -\beta_y \omega_y, \\
    \tau_z &= -\beta_z \omega_z,
\end{align*}
\]

(4.2)

where the motion along and around the axes is measured relative to the fluid.

Expressions for the friction coefficients are given in a sub-section below. For a sphere of radius \(r \simeq 100 \text{ nm}\) at a velocity \(u \simeq 1 \mu\text{m/s}\), the friction force is \(\sim 10^{-3} \text{ pN}\).

If an object moves close to a surface, linear and angular velocity are coupled [4]. This effect becomes relevant when the separation is smaller than the radius, \(\Delta < r\). For our estimates we will assume larger separations, and we will keep in mind that corrections are needed when the particle gets closer to the substrate.

\textbf{Friction coefficients}

In the specific case of an ellipsoid of semi axes lengths \(a\), \(b\), and \(c\), there exist closed formulas for all drag coefficients \(\alpha_x\), \(\alpha_y\), \(\alpha_z\), \(\beta_x\), \(\beta_y\), and \(\beta_z\). They can be expressed in terms of four constants \(P\), \(Q\), \(R\), and \(S\) (defined below):

\[
\begin{align*}
    \alpha_x &= \frac{16\pi\eta}{S + a^2P}, \\
    \alpha_y &= \frac{16\pi\eta}{S + b^2Q}, \\
    \alpha_z &= \frac{16\pi\eta}{S + c^2R}, \\
    \beta_x &= \frac{16\pi\eta}{3} \frac{b^2 + c^2}{b^2Q + c^2R}, \\
    \beta_y &= \frac{16\pi\eta}{3} \frac{c^2 + a^2}{c^2R + a^2P}, \\
    \beta_x &= \frac{16\pi\eta}{3} \frac{a^2 + b^2}{a^2P + b^2Q}.
\end{align*}
\]

(4.3)

\(3\)If gravity is important, then the orientation independent stability is achieved when also the buoyancy center (the mass centre of the fluid displaced by the object) superimposes with the mass and hydrodynamic centres.

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where \( \eta \) is the dynamic viscosity constant. The constants \( P, Q, R, \) and \( S \) can be defined in terms of the particles geometry, with two integrals

\[
P = \int_0^\infty \frac{ds}{\sqrt{(a^2 + s)^3(b^2 + s)(c^2 + s)}}, \tag{4.5}
\]

and

\[
S = \int_0^\infty \frac{ds}{\sqrt{(a^2 + s)(b^2 + s)(c^2 + s)}}, \tag{4.6}
\]

together with the linear equations system

\[
P + Q + R = \frac{2}{abc},
\]

\[
a^2 P + b^2 Q + c^2 R = S. \tag{4.7}
\]

Additionally, whenever the determinant of the equations system vanishes \( (b = c) \) it is \( R = Q \).

The elliptic integrals can be expressed in terms of the elliptic integral of the first kind \( F(\varphi, k) \) and the elliptic integral of the second kind \( E(\varphi, k) \). Under the condition \( a^2 > b^2 > c^2 \geq 0 \), we define the auxiliary quantities

\[
\vartheta = \sin^{-1} \sqrt{(a^2 - c^2)/a^2},
\]

\[
\kappa = \sqrt{(a^2 - b^2)/(a^2 - c^2)}, \tag{4.8}
\]

so the four auxiliary constants become:

\[
P = \frac{2}{(a^2 - b^2)\sqrt{a^2 - c^2}} \left[ F(\vartheta, \kappa) - E(\vartheta, \kappa) \right],
\]

\[
S = \frac{2}{\sqrt{a^2 - c^2}} F(\vartheta, \kappa),
\]

\[
Q = \frac{S - 2c/ab - P(a^2 - c^2)}{b^2 - c^2},
\]

\[
R = \frac{S - 2b/ac - P(a^2 - b^2)}{c^2 - b^2}. \tag{4.9}
\]

\footnote{The functions \( F(\varphi, k) \) and \( E(\varphi, k) \) are:

\[
F(\varphi, k) = \int_0^\varphi \frac{d\alpha}{\sqrt{1 - k^2 \sin^2 \alpha}} = \int_0^{\sin \varphi} \frac{dx}{\sqrt{(1 - x^2)(1 - k^2 x^2)}},
\]

\[
E(\varphi, k) = \int_0^\varphi \sqrt{1 - k^2 \sin^2 \alpha} \, d\alpha = \int_0^{\sin \varphi} \frac{\sqrt{1 - k^2 x^2}}{\sqrt{1 - x^2}} \, dx.
\]

These definitions agree with the definition of the \texttt{C} function calls \texttt{gsl_sf_ellint_F() and gsl_sf_ellint_E()} in the GNU Scientific Library [5].}
if $a^2 > b^2 > c^2 ≥ 0$. However, if the ellipsoid is a revolution body around axis 'a' (i.e. $b = c$), then [3]

$$S = \begin{cases} \frac{2}{\sqrt{a^2-b^2}} \log \frac{a+\sqrt{a^2-b^2}}{b} & \text{if } a > b \\ \frac{2}{\sqrt{b^2-a^2}} \tan^{-1} \frac{\sqrt{b^2-a^2}}{a} & \text{if } a < b \end{cases}$$

$$P = \frac{1}{a^2-b^2} \left( S - \frac{2}{a} \right)$$

$$Q = \frac{1}{2} \frac{1}{a^2-b^2} \left( \frac{2a}{b^2} - S \right)$$

$$R = Q.$$ (4.10)

The expressions do simplify in the case of a sphere$^5$ of radius $a$:

$$\alpha_x = \alpha_y = \alpha_z = 6\pi \eta a,$$

$$\beta_x = \beta_y = \beta_z = 8\pi \eta a^3.$$ (4.11)

### 4.1.3 Bending forces on a microtubule

We consider a straight rod$^6$ of length $L$, initially at rest along the $x$ axis ($0 ≤ x ≤ L$). The mathematical description is easier when the arc length $s$ and the tangential angle $\theta(s)$ are used to describe the position of the rod. Initially $\theta(s) = 0$ for $0 ≤ s ≤ L$.

When a torque$^7$, $T(s)$, is applied to the rod, it will deform. In the linear approximation, the deformation can be described as [6]:

$$\frac{d\theta}{ds} = \frac{1}{EI} T(s),$$ (4.12)

where $EI$ is the flexural rigidity, $E$ is the Young modulus (an intensive property of the material) and $I$ is the second moment of inertia of the cross section (depends only on the geometry of the sample and not on the material). In the case of a microtubule, $EI \sim 30 \times 10^{-24}$ Nm$^2$ (see table 3.1 on page 37).

Assuming that the deformations are very small, $x \simeq s$, $dy/dx \simeq dy/ds = \sin \theta \simeq \theta$, and

---

$^5$This is the result obtained for the non slip boundary condition. If the slip boundary condition is assumed, then the friction coefficient would have been $4\pi \eta a$.

$^6$Here the word rod is meant for an object whose length is much larger than its lateral dimensions. We cannot neglect the lateral dimensions because of their essential role in bending problems.

$^7$A torque is a pseudo vector. Here we restrict the analysis to a bidimensional problem, which means that the only non-zero component of the torque is along the $z$ axis, which we treat as an scalar quantity.
\[ d^2 y / dx^2 \simeq d\theta / ds, \] expression 4.12 reduces to

\[ \frac{d^2 y}{dx^2} = \frac{T(x)}{EI}. \] (4.13)

We consider now the following problem: One end of the rod is held fixed at the origin, and a force, whose only non zero component, \( F \), points in \( y \) axis, is applied at the other end. The torque along the rod is then \( T(x) = (L - x)F \), and equation 4.13 can be integrated to yield a total deflection at the free end \( y(L) = FL^3/3EI \), proportional to the force. Because of the proportionality between the bending force and the displacement, the analogy to a spring of stiffness \( 3EI/L^3 \) is immediate. Note that the stiffness of the equivalent spring increases as the length of the rod decreases.

### 4.1.4 Forces of biomolecular motors

Working like tiny engines, biomolecular motors convert chemical energy (e.g. ATP hydrolysis) into mechanical work in the form of directed stepwise motion. Particular examples are kinesin, myosin, and dynein. Kinesin can generate forces up to \(~7\ \text{pN}\) with \(~8\ \text{nm}\) steps [7]. Myosin V makes \(~36\ \text{nm}\)-long steps over a wide range of forces, from \(~5\ \text{pN}\) forward to \(~1.5\ \text{pN}\) backward load [8]. Dynein makes \(~8\ \text{nm}\)-long steps and can revert its direction of motion for forces above its stall force of \(~7\ \text{pN}\) [9].

Kinesin motors are, among other functions, in charge of intracellular transport. This task is accomplished in a highly cooperative fashion [10], so the limit value \(~7\ \text{pN}\) has indicative value. Because kinesin motors walk along a single filament, the direction of the force is always tangential to the microtubule: as such it can neither help nor undermine guidance efforts. Nevertheless, the forces range mentioned above serve as a reference for the assessment of forces in nanoscale systems.

### 4.2 Magnetic forces acting on a particle

In this section we derive expressions for the calculation of the magnetic forces between a stripe domains pattern and a magnetic particle.

#### 4.2.1 Magnetostatic fields of a stripe domains pattern

A stripe domains pattern can be described with two alternative formulations: as a superposition of individual alternating magnets, or via Fourier coefficients. Before introducing both approaches, we introduce useful notation with the help of more basic formulas.
For a magnetostatic field (absence of currents) $\nabla \times H$ and $\nabla B = 0$, so the magnetic field can be expressed in terms of a potential $\varphi$:

$$H = -\nabla \varphi.$$  \hspace{1cm} (4.14)

As $\text{div} B = \mu_0 \text{div}(H + M) = 0$,

$$\nabla^2 \varphi = \nabla M.$$  \hspace{1cm} (4.15)

It can be proved that if the magnetization is confined to a volume $V$ and falls abruptly to zero outside of this volume, then

$$\varphi(x) = -\frac{1}{4\pi} \int_V \frac{\nabla' M(x')}{|x - x'|} dV' + \frac{1}{4\pi} \oint_S \frac{M(x') \cdot \hat{n}}{|x - x'|} dS',$$  \hspace{1cm} (4.16)

where $S$ is the surface surrounding the volume $V$ and $\hat{n}$ is the outward normal to $S$. If the magnet is in free space, then $B = \mu_0 H$ and:

$$B(x) = -\frac{\mu_0}{4\pi} \int_V \frac{\rho_m(x')(x - x')}{|x - x'|^3} dV' + \frac{\mu_0}{4\pi} \oint_S \frac{\sigma_m(x')(x - x')}{|x - x'|^3} dS',$$  \hspace{1cm} (4.17)

where $\rho_m = \nabla M$ is the volume magnetic-charge density and $\sigma_m = M \cdot \hat{n}$ is the surface magnetic-charge density.

**An array of alternating magnets**

![Diagram of an array of alternating magnets](image)

**Figure 4.1:** A uniformly magnetized slab and a slab with magnetic charges on its surfaces are equivalent to compute the field outside of the slab. The slab is infinitely long and spans the whole $y$ axis.

Expressions for the magnetostatic fields in direct space were derived by other authors (e.g.
by Furlani [11] or Bertram [12]).

For an infinite slab magnet of rectangular section as shown in Fig. 4.1, the volume density of magnetic charges \( \rho_m \) is zero everywhere; the surface density of magnetic charge \( \sigma_m \) is zero on the side surfaces, \( M \) at the upper face and \( -M \) at the bottom face.

The first term on the right hand side of equation 4.17 is zero, whereas the integral of the second term can be calculated by inspection, yielding the expressions:

\[
B_\perp^x(x, z) = \frac{\mu_0 M_s}{4\pi} \left\{ \ln \left[ \frac{(2x + w)^2 + (2z - h)^2}{(2x + w)^2 + (2z + h)^2} \right] - \ln \left[ \frac{(2x - w)^2 + (2z - h)^2}{(2x - w)^2 + (2z + h)^2} \right] \right\},
\]

\[
B_\perp^y(x, z) = 0,
\]

\[
B_\perp^z(x, z) = \frac{\mu_0 M_s}{4\pi} \left\{ \ln \left[ \frac{(2x + w)^2 + (2z - h)^2}{(2x + w)^2 + (2z + h)^2} \right] - \ln \left[ \frac{(2x - w)^2 + (2z - h)^2}{(2x - w)^2 + (2z + h)^2} \right] \right\},
\]

(4.18)

for the case of perpendicular magnetization (\( M \) parallel to the \( z \) axis), and

\[
B_\parallel^x(x, z) = \frac{\mu_0 M_s}{2\pi} \left\{ \tan^{-1} \left[ \frac{w - 2x}{2x + w} \right] + \tan^{-1} \left[ \frac{w - 2x}{w + 2x} \right] + \right\},
\]

\[
B_\parallel^y(x, z) = 0,
\]

\[
B_\parallel^z(x, z) = \frac{\mu_0 M_s}{4\pi} \left\{ \ln \left[ \frac{(2z + h)^2 + (2x - 2x)^2}{(2z + h)^2 + (2x + w)^2} \right] - \ln \left[ \frac{(2z - h)^2 + (2x - 2x)^2}{(2z - h)^2 + (2x + w)^2} \right] \right\}
\]

(4.19)

for the case of in-plane magnetization (\( M \) parallel to the \( x \) axis). Notice that the expressions given here differ from those given by Furlani [11] or Bertram [12] because of the geometry conventions taken (they consider magnets of width \( 2w \) and height \( 2h \)).

**Derivatives of the field for perpendicularly magnetized beam**

The partial derivatives of equations 4.18 are:

\[
\frac{\partial B_\perp^x}{\partial x} = \frac{4\mu_0 M_s y h}{\pi} \left\{ \frac{2x + w}{[(2x + w)^2 + (2y - h)^2] [2x + w)^2 + (2y + h)^2]} - \frac{2x - w}{[(2x - w)^2 + (2y - h)^2] [2x - w)^2 + (2y + h)^2]} \right\},
\]

(4.20)
\[
\frac{\partial B_z^\perp}{\partial y} = \frac{\mu_0 M_s h}{\pi} \left\{ \frac{4y^2 - h^2 - (2x + w)^2}{(2x + w)^2 + (2y - h)^2} \left[ \frac{(2x + w)^2 + (2y + h)^2}{4y^2 - h^2 - (2x - w)^2} \right] - \frac{(2x - w)^2 + (2y - h)^2}{(2x - w)^2 + (2y + h)^2} \right\}, \tag{4.21}
\]

\[
\frac{\partial B_y^\parallel}{\partial x} = \frac{\mu_0 M_s}{\pi} \left\{ \frac{2}{(h - 2y)^2 + (w - 2x)^2} + \frac{2y - h}{(h - 2y)^2 + (w + 2x)^2} \right\}, \tag{4.22}
\]

\[
\frac{\partial B_y^\parallel}{\partial y} = \frac{\mu_0 M_s}{\pi} \left\{ \frac{2x - w}{(h + 2y)^2 + (w - 2x)^2} - \frac{2x + w}{(h + 2y)^2 + (w + 2x)^2} \right\}. \tag{4.23}
\]

**Derivatives of the field for in-plane magnetized beam:**

The partial derivatives of equations 4.19 are:

\[
\frac{\partial B_z^\perp}{\partial x} = \frac{\mu_0 M_s}{\pi} \left\{ \frac{h + 2y}{(w - 2x)^2 + (h + 2y)^2} + \frac{2y - h}{(w - 2x)^2 + (h - 2y)^2} \right\}, \tag{4.24}
\]

\[
\frac{\partial B_z^\perp}{\partial y} = \frac{\mu_0 M_s}{\pi} \left\{ \frac{4x^2 - w^2 - (2y + h)^2}{(2y + h)^2 + (2x - w)^2} \left[ \frac{(2y + h)^2 + (2x + w)^2}{4x^2 - w^2 - (2y - h)^2} \right] - \frac{(2y - h)^2 + (2x - w)^2}{(2y - h)^2 + (2x + w)^2} \right\}, \tag{4.26}
\]

\[
\frac{\partial B_y^\parallel}{\partial x} = \frac{2\mu_0 M_s w}{\pi} \left\{ \frac{4y + 2h}{(2y + h)^2 + (2x - w)^2} + \frac{4y - 2h}{(2y - h)^2 + (2x + w)^2} \right\}. \tag{4.27}
\]

The magnetostatic field of an array of alternating magnets can be computed by direct superposition, essentially following the procedure:

1. Assign values to the thickness of the magnets \(h\), the width of the magnets \(w\), the thickness of the capping layer (oxidation barrier) \(\gamma\), the separation between magnets
(the domain wall thickness, in the case of a thin film of uniform anisotropy) \( \delta \), and
the saturation magnetization \( M_s \). Decide which is the direction of the magnetization
(perpendicular or in-plane).

2. Consider an array of \( 2N + 1 \) magnets, and identify each magnet with an index, \( n \), such
that \(-N \leq 2n \leq N\). Let the center position of the magnets with magnetization \(-M_s\)
be \((x_{2n}, y_{2n}) = 2n(w + \delta, -h/2 - \gamma)\) and the center position with magnetization \( M_s \) be
\((x_{2n+1}, y_{2n+1}) = (2n + 1)(w + \delta, -h/2 - \gamma)\).

3. Calculate the fields as a superposition, by adding the fields of all magnets. Use formula
4.18 for the case of perpendicular magnetization and formula 4.19 if the magnetization
is in-plane. In each term added, use the coordinate \( x = x' - x_n \), where \( x' \) is the
measurement coordinate (the point at which the total field needs to be calculated).

4. Calculate the derivatives of the fields, adding the individual contribution of the mag-
nets (as done in the previous step), except that formulae 4.20-4.23 are used for the
case of perpendicular magnetization, and formulae 4.24-4.27 in the case of in-plane
magnetization.

The calculation is illustrated in Figs. 4.2-4.3 for a choice of geometrical parameters and mag-
netic material from the case study of chapter 2.

---

**Figure 4.2:** The array of magnets shown in **A** generates the fields de-
picted in **B**. The magnets extend
infinitely in the \( y \) axis are mag-
netized in the \( z \) direction. Here
\( M_s = 600 \text{kA/m} \), the magnet width
\( w = 2 \mu\text{m} \), the magnet height
\( h = 30 \text{nm} \), and the spacing be-
tween magnets \( \delta = 5 \text{nm} \). 2001 do-
 mains of alternating orientation were
used for the calculation. Extending
the number of magnets to the sides
changes the values less than 1%.
The symmetry between Figs. 4.2 and 4.3 just mirrors the symmetry between expressions 4.18 and 4.19.

The expressions 4.18 and 4.19 are very practical for quick calculations with the help of a computer, but it is very difficult to understand the influence of the parameters, or even to answer some practical questions, such as the dependence on the coordinate $z$.

It is in the interest of applications to consider magnets as thick as possible (for reasons that will be easier to understand with the mathematical tools developed in the next section). The expressions given above allow the computation of the fields for any value of the thickness, but it will prove useful to also count on expressions for the case of infinite thickness. The expressions for infinite thickness are obtained in two steps. First we change coordinates $z \rightarrow z - h$ (top surface will be at the $z = 0$ plane) and then we take the limit as every dimension becomes negligible compared to $h$:  

\[
\begin{align*}
B_x^{\infty}(x, z) &= \frac{\mu_0 M_s}{4\pi} \ln \frac{(x + w/2)^2 + z^2}{(x - w/2)^2 + z^2}, \\
B_y^{\infty}(x, z) &= 0, \\
B_z^{\infty}(x, z) &= \frac{\mu_0 M_s}{2\pi} \left\{ \tan^{-1} \left( \frac{2z}{2x - w} \right) - \tan^{-1} \left( \frac{2z}{2x + w} \right) \right\},
\end{align*}
\]  

(4.28)
and

\[
B_{x}^{\infty}(x, z) = \frac{\mu_e M_s}{2\pi} \left\{ \tan^{-1} \left[ \frac{2z}{2x + w} \right] - \tan^{-1} \left[ \frac{2z}{2x - w} \right] \right\},
\]
\[
B_{y}^{\infty}(x, z) = 0,
\]
\[
B_{z}^{\infty}(x, z) = \frac{\mu_e M_s}{4\pi} \ln \left[ \frac{(x - w/2)^2 + z^2}{(x + w/2)^2 + z^2} \right].
\]

The superscript \(\infty\) reminds that \(h \to \infty\) is assumed in the expression.

**Description via Fourier coefficients**

The advantage of this approach is that the final expressions are easier to handle when discussing dependence on parameters. Although this approach allows an arbitrary geometry of the magnets (as depicted in Figs. 4.4), some further assumptions are necessary:

- The magnetization is parallel to the \(z\) axis everywhere inside the sample.
- The surfaces of the film are perfectly flat and parallel.

We follow the method presented by Hug et al. [13] and start calculating the Fourier coefficients of the magnetization:

\[
A_M(k_x, k_y) = \frac{1}{2\pi} \int_{-\infty}^{\infty} dx \int_{-\infty}^{\infty} dy M_z(x, y) e^{-ik_x x - ik_y y}. \tag{4.30}
\]

---

Hug et al. use the positive sign convention in the forward Fourier transform (as used in Numerical Recipes [14]), and we use here the negative sign convention, as in FFTW [15] or GSL [5] numerical methods libraries, and notably Matlab [16]. Besides, a preceding factor \(1/\sqrt{2\pi}\) per dimension is introduced, in order to render both the transform and its inverse as unitary operators, thus enabling an immediate geometrical interpretation without scaling factors. The choice of conventions plays no role in [13].
The magnetostatic potential \( \varphi \) can also be written in terms of its Fourier coefficients (see chapter four of [17]):

\[
\varphi(x, y, z) = \frac{1}{2\pi} \int_0^\infty dk_x \int_0^\infty dk_y A_\varphi(k_x, k_y) \cdot e^{ik_x x + ik_y y} \cdot e^{-z\sqrt{k_x^2 + k_y^2}}. \tag{4.31}
\]

The boundary condition at the surface, \( \partial \varphi / \partial z |_{z=+0} = -M \cdot \hat{n} / 2 \), leads to a relation between the Fourier coefficients of the magnetization \( M_z \) and those of the magnetostatic potential \( \varphi \). If the surfaces of the magnetic film are both perfect planes, then \( \partial \varphi / \partial z |_{z=+0} = -M_z / 2 \), yielding:

\[
\sqrt{k_x^2 + k_y^2} A_\varphi(k_x, k_y) = \frac{A_M(k_x, k_y)}{2}. \tag{4.32}
\]

The influence of waviness in the surfaces is treated in a separate section. The Fourier coefficients of the magnetic field can then be readily expressed in terms of \( A_M \) by just adding the contributions of the two faces (as dictated by the second term of equality 4.31):

\[
\begin{align*}
A_{H_x}(k_x, k_y) &= i \frac{e^{-kz}(1 - e^{-kh})k_x}{2k} A_M(k_x, k_y), \\
A_{H_y}(k_x, k_y) &= i \frac{e^{-kz}(1 - e^{-kh})k_y}{2k} A_M(k_x, k_y), \\
A_{H_z}(k_x, k_y) &= \frac{e^{-kz}(1 - e^{-kh})}{2} A_M(k_x, k_y),
\end{align*}
\tag{4.33}
\]

where \( k = \sqrt{k_x^2 + k_y^2} \). Outside of the magnetic film it is \( B = \mu_0 H \), where \( \mu_0 \) is the permeability of vacuum.

Expressions 4.33 predict not only an exponential decay of the fields as the distance to the surface grows (derivatives on \( z \) just add a \( k \) factor), but they also portray the dependence on the magnets thickness, \( h \): the fields grow exponentially fast with the thickness.

It is instructive to consider once again the array of side-by-side parallel magnets. The magnetization of the array is:

\[
M_z(x) = \frac{4M_s}{\pi} \left( \sin kx + \frac{\sin 3kx}{3} + \frac{\sin 5kx}{5} + \cdots \right), \tag{4.34}
\]

where \( k = 2\pi / w' = \pi / w \). It is apparent that the first term of equation 4.34 is three times more important than the second. Many properties of a magnets array can be stated by considering the first term and neglecting the effect of higher harmonics, whose effect decays with \( 1/n \).
The field in direct space can be computed as an inverse Fourier transform:

\[ H_x(x, y, z) = \frac{1}{2\pi} \int_{-\infty}^{\infty} dk_x \int_{-\infty}^{\infty} dk_y A_H e^{ik_x x + ik_y y}, \]

\[ H_y(x, y, z) = \frac{1}{2\pi} \int_{-\infty}^{\infty} dk_x \int_{-\infty}^{\infty} dk_y A_H e^{ik_x x + ik_y y}, \]  
\[ H_z(x, y, z) = \frac{1}{2\pi} \int_{-\infty}^{\infty} dk_x \int_{-\infty}^{\infty} dk_y A_H e^{ik_x x + ik_y y}, \]

(4.35)

but we will not use this approach to estimate the fields.

4.2.2 Magnetostatic forces and torques on a particle

Expressions for magnetostatic forces and torques

The force per volume unit exerted by a magnetic density flux field on a material point with magnetization \( M \) in a non magnetic medium is [18]:

\[ f = M \cdot \nabla B, \]  
\[ (4.36) \]

whereas for the torque density we have:

\[ t = M \times B. \]  
\[ (4.37) \]

The total force and the total torque can be calculated by integration over the volume of the particle.

It is worth mentioning that the force and the torque are exerted on the magnetic moments of the atoms, and not the atoms themselves. Of course the magnetic moment is geometrically tied to the atoms. For the force it does not make any difference, but for the torque the distinction is important because the magnetic moment of an atom in a lattice may be rotated without rotating the lattice.

Maximum force and torque are attained for uniformly magnetized particles

The total force on a particle is the volume integral of equation 4.36 over the volume of the particle (and similarly the total torque is a volume integral of Eq. 4.37). It is apparent that the force and the torque will be stronger if all material elements of the particle are magnetized in the same direction, otherwise they tend to cancel each other out.

For uniform magnetized particles, the force and the torque are proportional to the saturation magnetization |\( M \)|. In practical terms, the higher the saturation magnetization of uniformly magnetized particle, the stronger the magnetic forces and torques that can be applied on them.
The magnetic state of a particle is discussed in chapter 5, but we can mention here that sufficiently small ferromagnetic nanoparticles can sport a state of uniform magnetization. We also anticipate part of the vocabulary. In the event that the magnetization has a fixed direction (when embedded in weak magnetic fields), it is said to be hard magnetic. But if the magnetization rotates (and the particle itself does not) towards the applied magnetic field, then the particle is called soft magnetic.

Simplifications for uniformly magnetized spherical particles

The assumption that the particle is a sphere and uniformly magnetized leads to important simplifications. If the particle is free to rotate, it will exhibit a tendency to align its magnetic moment with the applied magnetic field, as shown below: Each dipole element $δm$ in the particle will be subject to a torque $δm × B$. The equilibrium orientation of a nanoparticle with center at $x_p$ is:

$$0 = \int_{V_p} δm × B = \mu_0 m × \int_{V_p} (H + M) dV = \mu_0 m × \int_{V_p} (H_a - \frac{1}{3}M + M) dV = \mu_0 m × \int_{V_p} H_a dV = \mu_0 V_p m × H_a(x_p).$$

Recall that the field $H$ inside the particle equals the external applied field minus the demagnetization field ($-M/3$ in the case of an sphere). In the last equality we have used that the magnetic field is a gradient of a potential and the results obtained by Hu [19] (the average of the magnetostatic magnetic field in a spherical region equals its value at the center of the sphere).

Equation 4.38 says that the equilibrium position is the one at which the permanent magnetization of the particle points in the same direction as the magnetostatic field at the center of the particle.

The total force on a particle of volume $V$ is:

$$F = \int_V f = M \int_V \cdot \nabla B,$$

(because of the assumption that the particles magnetization is uniform). The magnetostatic fields exhibit an exponential decay $e^{-kz}$ (see equation 4.33 on page 81). Whenever the charac-
teristic length $1/k$ is much larger than the particle radius ($1 \gg kr$) it is legitimate to assume that the force is the one on an effective dipole located at the particle center, and the dipole field is the one of the actual particle when located in an uniform field of magnitude equal to the field at the center of the particle [20]:

$$F = \nabla M \cdot \nabla B.$$  \hspace{1cm} (4.40)

This is the so-called effective dipole located at the particle center.

Equation 4.40 will be applied in the following scenarios:

A Free, hard-magnetic particle. This is the case of a particle that can rotate, so its magnetization can always be assumed to be aligned with the external field.

B Tightly bound, hard-magnetic particle. The particle will not be able to rotate, and its magnetization cannot be assumed to be aligned with the external magnetic field.

C Soft-magnetic particle. The magnetization of the particle will point in the direction of the external magnetic field, even if the particle cannot rotate.

From the analytical point of view, cases A and C are equivalent. From a quantitative point of view, the actual value of the force depends on the volume of the particle and on its saturation magnetization.

The gradient of the magnetic flux density

From expression 4.36 we learn that the gradient of the magnetic density flux plays an essential role in the calculation of the force.

The gradient of a vector is a tensor. From $\nabla \cdot \mathbf{B}$ it follows that the gradient tensor is traceless. In absence of currents $\nabla \times \mathbf{H} = 0$, which together with $\mathbf{B} = \mu_0 \mathbf{H}$, indicate that the gradient tensor is symmetric. The geometry of the problem allows further simplifications. Because the magnets extend along the $y$ axis, the magnetostatic field does not depend on $y$ and all derivatives with respect to that coordinate vanish. From these observations it follows that for any magnetization vector in the $x - z$ plane

$$M = M_s (\cos \phi, 0, \sin \phi)$$  \hspace{1cm} (4.41)

the modulus of $M \cdot \nabla \mathbf{B}$ is independent of $\phi$:

$$|M \cdot \nabla \mathbf{B}| = M_s |(B_{x,x} \cos \phi + B_{x,z} \sin \phi, 0, B_{x,z} \cos \phi - B_{x,x} \sin \phi)|$$

$$= M_s (B_{x,x}^2 + B_{x,z}^2).$$  \hspace{1cm} (4.42)
In the event the magnetization of the particle sports a $y$ component, we need to add a pre-factor $\cos \theta$ (here $\theta$ is the angle between $\mathbf{M}$ and the $x-z$ plane).

Fig. 4.5 illustrates the magnitude of the flux-density field and the magnitude of its gradient for a specific case, relevant for this work.

![Figure 4.5: Magnitude of the flux-density field and of its gradient (in the $x-z$ plane) as a function of the distance between the substrate and the measurement point. The maximum value has been taken for all $x$-coordinate positions, as calculated from expressions 4.18 and superposition of 2001 magnets, with parameters $w = 100 \, \text{nm}$, $h = 30 \, \text{nm}$, $\delta = \gamma = 5 \, \text{nm}$, and the magnetization of the film is $M_f = 600 \, \text{kA/m}$. The apparent exponential-like decay can be readily understood from expression 4.33 and the subsequent discussion.](image)

**Free and bounded magnetic-particle**

The particle to be steered may be free to rotate, or it may be bound to some useful load. In the latter case the load may just prevent the particle from rotating: the magnetic torque might not be strong enough to rotate the particle together with the load, even if the connecting link is not perfectly rigid. This realization prompts us to distinguish between free and bound particles.

In the event a particle embedded in a magnetic field is free to rotate, we assume that the magnetic torque will rotate the particle until its magnetization is parallel to the magnetic field.
Hard and soft magnetic material

A soft magnetic material is a material whose magnetization aligns with the applied magnetic field. In the case of a multidomain object, its net magnetization at zero applied field will be negligible. But as soon as a magnetic field is applied, a non zero magnetization can be measured.

A hard magnetic material tends to keep the orientation of its magnetization (almost) irrespective of the applied magnetic field.

Actually, whether a material is regarded as a hard or soft magnetic material depends on the situation. For example, if the applied field is very weak, every material could be ranked as hard, and at sufficiently strong fields every material would be classified as soft. In practice, the limit between both behaviours is given by the switching field, as described in section 5.2.5 (on page 96).

4.2.3 Example: magnetic forces on an iron nanoparticle

To calculate the force on a particle, we follow the procedure:

1. Calculate the gradients of the magnetostatic field generated by the stripe-domains configuration using the procedure listed on page 77.

2. Select a magnetization direction and apply formula 4.40 to calculate the force. If the particle is assumed to be soft magnetic, the direction of the magnetization should be chosen parallel to the magnetic flux-density field.

We use this procedure to calculate the magnetic force on a single-domain ferromagnetic iron particle ($M_s = 1.7 \times 10^6 \text{ A/m}$, as in bulk iron). Furthermore it is assumed that the particle is either free to rotate or it is soft magnetic (and thus it will align its magnetization with the applied magnetic field). The geometry of the problem, the magnetostatic fields, and the magnetic forces on the particle are illustrated Fig. 4.6.
Figure 4.6: Fields and gradients over a magnetic film, and the forces on an Iron particle \( r = 25 \text{ nm} \). Here the domain width \( w = 100 \text{ nm} \), the film thickness \( h = 30 \text{ nm} \), the gap \( \Delta = 10 \text{ nm} \), \( \gamma \) is the thickness of a capping layer \( (5 \text{ nm}) \), and \( \delta \) is the thickness of the domain wall \( (\delta \approx 5 \text{ nm}) \). 2001 domains of alternating orientation were used for the calculation. \( M_f = 600 \text{kA/m} \) and \( M_s = 1.7 \times 10^6 \text{ A/m} \). The particle is assumed free to rotate and align itself with the field. 

Bibliography


Chapter 5

Criteria to select magnetic particles and magnetic films

Magnetic particles and magnetically patterned films are essential components of a magnetic steering system. There exist a wealth of magnetic nanoparticles and magnetic films. In order to make a selection, we need to understand how they work. In this chapter we review magnetism concepts for the selection of magnetic particles, and for the fabrication and characterization of the magnetic films.

5.1 Ferromagnetic nanoparticles and ferromagnetic films

We are primarily interested in obtaining magnetic nanoparticles and magnetic films such that the forces between them are the maximum possible ones.

Because the forces and torques are stronger for particles with a higher magnetization, we draw our attention to ferromagnetic particles and ferromagnetic films, which are the ones that support the higher values of magnetization.

5.1.1 Magnetic state of a ferromagnetic object

The magnetic state of a rigid ferromagnetic body is determined by the exchange interactions between neighbouring atoms, by the magnetostatic interactions, and by the magnetic-anisotropy interactions. The dominant interactions are the exchange interactions, that keep neighbouring spins aligned and thus generate a spontaneous magnetization. The dipole-dipole magnetostatic interactions have a negligible short-range effect but dominate at large distances; their role is thus different in bulk material and in small particles, where they only can determine the direction of the spontaneous magnetization (depending on the specimens geometry). In
larger bodies they can have the effect that the magnetization gradually changes direction, in such a way that the net magnetization of the body is considerably less than the corresponding to uniform magnetization. The crystalline-anisotropy interactions are also generally smaller than the exchange interactions in the short range (although they can be derived from a local free-energy density). Crystalline anisotropy can determine the direction of the spontaneous magnetization. In this case the preferred directions are in general independent of specimens' shape and dimensions. Here we further assume that the particle is a rigid body, that is, magnetostriction effects will not be considered.

If the magnetostatic interactions force the magnetization to change its direction along a spatial extension, the crystalline anisotropy will favor abrupt changes: At each side of the transition region the magnetization tends to follow an easy axis of magnetization, as defined by the magneto-crystalline anisotropy. The transition regions tend to be sharp because of the anisotropy, whereas the exchange interaction broadens the transition. Each region where the magnetization is uniform (or very close to uniform) is called a magnetic domain, and the transition regions, which are typically thin, are called domain walls.

If an external magnetic field is applied in a fixed direction but the field magnitude is varied between a large positive and a large negative value, the magnetization will rotate, following the applied field, mostly continuously, but irreversible jumps in the orientation are also possible. The magnetization curve, that is, the plot of the magnetization in the direction of the field, versus the magnitude of the applied field will be a hysteresis loop, in which the irreversible jumps are vertical lines connecting the curves of reversible change. Typically, the magnetization curve crosses the axis of zero magnetization at a field value other than zero: this is the coercive field. Also, at the zero applied field the magnetization curve will cross the field axis at a non-zero value: this is the remanent magnetization. The shape of the magnetization curve depends on the direction at which the magnetic field has been applied (see also section 5.6.2 on page 112).

The magnetostatic interactions depend on the geometry of the object. If the object’s shape has a particular symmetry, e.g. if it is ellipsoidal, the minimization of the magnetostatic energy will tend to align the magnetization in the direction of a specific principal axis. This effect is usually called shape anisotropy, because the direction of the easy axis of magnetization is related to the shape. The orientation of the lattice might be related to the macroscopic geometry of the specimen in particular manufacturing processes (more commonly in the case of films than in the case of particles), but in general no relation between crystalline and shape anisotropy can be assumed.
5.1.2 Characteristic energies help characterize ferromagnetic particles and films

The actual properties of a magnetic film or of a magnetic nanoparticle depend not only on the intrinsic properties of the constituent material, but also on the defects, surface effects, shape, size, temperature, or applied magnetic field, to name but a few of the most important variables.

One popular way to gain insight into a particular problem of magnetism is to use characteristic energies and distances [1]. Here we list the three aforementioned interactions energies and two characteristic lengths that can be derived from them:

- **exchange stiffness**, $A$, with units of $J/m$.
- **crystalline anisotropy**, $K$, with units of $J/m^3$.
- **magnetostatic energy**, $\mu_0 M_s^2$, with units of $J/m^3$ ($\mu_0 = 4\pi \times 10^{-7}$ Tm/A is the permeability of vacuum, $M_s$ is the saturation magnetization).
- **exchange length**, $l_{ex} = \sqrt{A/M_s}$. Roughly speaking, for distances much shorter than $l_{ex}$ exchange dominates over magnetostatic interactions, and for distances much larger than $l_{ex}$ the opposite will be the case.
- **wall width**, $l_w = \sqrt{A/|K|}$ gives a measure of the abruptness of a change in magnetization direction between two magnetic domains. (See also section 5.3.1 on page 101).

The use of these quantities assumes the simplifications used in the models to coin them, and hence they can be most useful when only order-of-magnitude estimations are sought. Most of the time they are used to find out the dominant effect in a particular setting.

It should be pointed out that the characteristic energies depend on the variables usually not considered or not even mentioned, most notably the temperature. In the following sections, additional relations will be given to compute other characteristic quantities (e.g. critical radii).

5.2 Ferromagnetic nanoparticles

The magnetic force that can be exerted on a ferromagnetic particle is maximum when the particle is uniformly magnetized, because in that case all material elements of the particle add to the force in the same direction. For this reason we concentrate our study of ferromagnetic nanoparticles on the conditions under which they sport a uniform state of magnetization.
5.2.1 Magnetic state of a ferromagnetic nanoparticle

Because the exchange, magnetostatic, and crystalline anisotropy interactions have different distance ranges, the size of the ferromagnetic specimen can be important. This is the so-called size effect. For example, sufficiently small particles cannot accommodate a multidomain structure: they are single-domain particles. Further, in sufficiently small particles, it may happen that thermal agitations alone can flip all spins in a particle at once, a situation known as superparamagnetism.

In a nanoparticle the lattice cells located at the surface or very close to it, where the symmetry of the crystal structure breaks, may amount to a significant portion of the total. This is the so-called surface effect. For example, in a maghemite ($\gamma$-Fe$_2$O$_3$) nanoparticle with $r = 4$ nm, 50% of the atoms lie at the surface [2]. In general, the outer shell of a nanoparticle may have different chemical and magnetic structures than the inner core, even if the bulk material is the same.

The importance of size and surface effects increase when the size of the particle decreases. Usually the magnetization curve of a single particle cannot be measured. Instead, one measures the magnetization curve of a powder containing many particles. The interpretation of such measurements (see also section 5.6.2 on page 112) is complicated by several facts: the particle-particle interaction cannot be neglected (unless the particles are far away from each other), and the particles have a distribution of sizes and orientations. In other words, it is not possible to infer the magnetization curves of a single particle from the powder measurements, except in a first approximation.

5.2.2 Critical radius of superparamagnetism

For a small enough particle, thermal agitation alone may induce simultaneous flips involving all spins within short periods of time. The probability of a flip is (see e.g. page 307 of [3])

$$p(\text{flip}) = \nu_0 \exp \left( -\Delta W_K V / k_B T \right),$$

(5.1)

where $\nu_0$ is an attempt frequency factor equal to approximately $10^9 s^{-1}$, $\Delta W_K$ is the energy barrier to surmount per volume unit, $V$ is the volume of the particle, and $T$ is the absolute temperature. In spherical nanoparticles $\Delta W_K$ is typically the barrier imposed by the magneto crystalline anisotropy.

The probability of a turnover of all spins within a time $\tau$ is $1/2$ when the radius of the
particle satisfies:

\[ r_{sp}^3 = \frac{3kT \ln (2\nu_0\tau)}{4\pi \Delta W_K}. \quad (5.2) \]

Estimations of the superparamagnetic radius are given in Fig. 2.10 on page 28.

These estimations describe an ideal case in which defects and surface effects (which can increase or decrease the local anisotropy) play no role. As such they are useful to guide the intuition, either at the design stage of an experiment, or when the results are analyzed, but they cannot be taken to hold exactly.

### 5.2.3 Critical radius for single domain particle

For a sufficiently large particle, the magnetostatic energy contribution can be reduced if the particle does not have uniform magnetization. This is specially true when the magnetostatic term of the energy dominates over the anisotropy terms (be it crystalline or shape). In this case the magnetization will curl and tend to follow the surface of the particle, in an attempt to avoid poles at the surface\(^1\) that is, a solenoidal distribution of magnetization in which \(\nabla \mathbf{M} = 0\) inside the particle and \(\hat{n} \cdot \mathbf{M} = 0\) at the surface, where \(\hat{n}\) is the normal to the surface. The net magnetization of a particle demagnetized by curling will be somewhat lower than in the case of uniform magnetization.

If the particle is even bigger, further reductions of the magnetostatic energy can be attained if the particle divides in magnetic domains. The formation of a multidomain state implies an energy penalization in terms of the exchange interaction. However, the energy gains due to minimization of the magnetostatic interaction may compensate for that. For example, it can be argued (see chapter 8 of [3]) that the single domain state of a spherical particle of radius \(r\) will be stable if the energy to create a domain wall, namely, \(\sigma_{dw}r^2 = 4\pi r^2 \sqrt{AK}\) \((\sigma_{dw}\) is the surface density of energy of the domain wall, \(A \sim 10^{-11}\) J/m is the volume density of exchange energy (or exchange stiffness), and \(K\) is a constant that describes the crystalline anisotropy), exceeds the magnetostatic energy gained by passing from a single- to a multi-domain state, \(\mathcal{V}\mu_0M_s^2/(2/3)\), where \(\mu_0 = 4\pi \times 10^{-7}\) Tm/A is the permeability of vacuum. Here we assumed that the magnetostatic energy of two semi-spherical domains of opposite orientations is negligible in comparison to the single-domain case. The critical radius is then the one that makes both energies equal:

\[ r_{sd} \simeq 9\frac{\sqrt{AK}}{\mu_0 M_s^2}, \quad \text{for } K > \mu_0 M_s^2/6. \quad (5.3) \]

\(^1\)For a rigorous derivation see e.g. page 102 of [4].
This model makes the assumption that the anisotropy is strong enough to hold the direction of \( \mathbf{M} \) uniform regardless of poles at the surface \((K > \mu_0 M_s^2/6)\). When the anisotropy is not as strong, the magnetization tends to twist all over the particle, instead of only at the domain wall. This twisting has a corresponding exchange energy cost. The critical radius is calculated by equating the exchange energy cost to the magnetostatic energy of a uniformly magnetized sphere (assumed to be gained when \( \mathbf{M} \) is allowed to avoid poles at the surface by curling):

\[
r_{sd} \simeq \sqrt{\frac{9A}{\mu_0 M_s^2} \left[ \ln \left( \frac{2r_c}{a} \right) - 1 \right]}, \quad \text{for } K < \mu_0 M_s^2/6. \tag{5.4}
\]

where \( a \) is the radius of the singularity at the symmetry axis of the particle.

It should be noted that the comparison between only two magnetization states cannot lead to the conclusion that one of them minimizes the energy, because there may exist another configuration, not considered in the analysis, for which the total energy is even lower [5, 6]. However, formulas 5.3 and 5.4 are intended for order-of-magnitude estimates only.

### 5.2.4 Coercive field

### 5.2.5 Equilibrium and stability of a magnetic state

When no field is applied, the free energy landscape presents energetically equivalent minima along the easy axis of magnetization (two orientations in the simplest case of a single easy axis). Application of a magnetic field along an easy axis decreases the free energy in the same direction and increases it in the opposite direction, which becomes a meta-stable state. At a critical value of applied field, \( H \), the equilibrium becomes unstable: if the magnetization, \( \mathbf{M} \), pointed initially opposite to \( H \), it will rotate irreversibly towards the direction of \( H \).

![Figure 5.1: A magnetic field is applied at angle \( \varphi \) on a sample with uniaxial anisotropy, whose magnetization points at angle \( \theta \).](image)

We analyze the behaviour of the magnetization in the sample with the model of Stoner and Wohlfarth [7] (see Fig. 5.1). Initially the sample is magnetized to saturation, \( M_s \), with
Magnetic steering at the nanoscale  P. Stickar

M at an angle $\theta$ to the easy axis $x$. We assume a negligible demagnetization field, so the internal field is approximately equal to the applied field, $H_a = H_a(\cos \varphi, \sin \varphi)$. The energy of this single domain particle has two terms: the magneto crystalline energy density $K \sin \theta$, and the magnetostatic energy density $-\mu_0 M_s H_a \cos(\varphi - \theta)$. The values of the angle $\theta$ that represent energy minima can be obtained by minimization of the expression containing both terms. When $\theta = 0$ (positive orientation along the easy axis), a minimum of the energy occurs when $-2K/\mu_0 M_s < H_a \cos \theta = H_a$ and we have $M_x = M_s$. Similarly, for $\theta = \pi$ (the negative orientation of the easy axis of magnetization), a minimum takes place when $H_a < 2K/\mu_0 M_s$ and we have $M_x = -M_s$. Therefore the switching field is:

$$H_s = \frac{2K}{\mu_0 M_s}. \quad (5.5)$$

5.2.6 Example: Iron particles

We take the case of iron for a deeper analysis. For single-domain Fe particles, the critical radii computed with formulas 5.3 and 5.4 are 3 nm and 25 nm, respectively.

It is instructive to briefly review the values reported in the literature, both as a result of theoretical considerations and from measurements.

Butler and Barnejee [8] compute the differences in free energy for different magnetization configurations and conclude that the transition between the uniform magnetization state and the circular spin state takes place at a radius $r_c = 17.3$ nm, smaller than the superparamagnetic radius that they also report $r_{sp} = 26$ nm. They conclude that the lowest energy configuration is the one of the circular spin state, and that no uniform-magnetization state exists at zero field.

Sato et al. [9] make a similar estimates but for particles of tetragonal shape. For unit aspect ratio the find a critical size $l_c \approx 20$ nm for the transition between uniform-magnetization and the circular configuration of spins. The particle becomes a multidomain one when the size reaches $\sim 900$ nm.

Lu et al. [10] measured the magnetization curves of Iron particles of average sizes 15, 21, 28, 35, 43, and 50 nm, as determined by TEM. The coercive field values (Fig. 5.2) decays rapidly as the diameter of the particles increases. The samples used for VSM measurements were checked with TEM to have a large proportion of isolated particles. For this reason, Lu et al. infer that the decrease of the coercive field is an indication of the magnetic state transition between single-domain and multi-domain states, which they estimate to lie at $\sim 40$ nm.

Bødker et al. [11] measured the Mössbauer spectra of $\alpha$-Fe nanoparticles. They found that “(1) metallic iron particles with diameters down to about 2 nm have magnetic properties similar to bulk $\alpha$-Fe, (2) the magnetic anisotropy energy constant increases with decreasing
Figure 5.2: Coercive field of Iron nanoparticles, as a function of the size, at 5.5 K and at 300 K, after Lu et al. [10]. The particles have a \( \alpha-\text{Fe} \) core and a 2 nm thick magnetite shell. Adapted from [10].

Particle size, presumably because of the influence of surface effects, (3) chemisorption of oxygen results in formation of a surface layer, which is ferromagnetically coupled to the core of the particles, but with magnetic hyperfine fields similar to those found in thicker passivation layers, which have a disordered spin structure”. Hence we can assume a saturation magnetization \( M_s \simeq 1700 \text{kA/m} \). The conclusions of Bødker et al. also suggest that the tendency measured by Lu et al. for the coercive field (Fig. 5.2) may be due to surface effects\(^2\) and cannot be directly related to the magnetic state of the particle.

From the considerations above we may assume that iron nanoparticles with \( r \geq 20 \text{nm} \) are weak magnets when immersed in fields of \( \sim 50 \text{ mT} \) or stronger. The data reported in the literature suggest that for particles with \( r \geq 20 \text{nm} \) the role of the surface effects can be neglected. It is not clear if such particles sport uniform magnetization, but nothing seems to argument against assuming that they do when a field of a few milli-Tesla is applied.

TEM characterization of iron magnetic nanoparticles

The particles analyzed in this section are those used in the motility-assay experiments described in chapter 3, and they were acquired from Miltenyi Biotec GmbH [12].

The crystalline structure of iron nanoparticles can be revealed by TEM measurements, as shown in Figs. 5.3 (general view), (the crystal lattice can be easily recognized), and , obtained by Dr. Magdalena Parlinska-Wojtan. Figure 5.5 illustrates two particular cases in which the particle is seen as a square/cube (plane (100) or equivalent), or hexagon (plane (111))

The distances calculated from electron diffraction measurements agree \(^3\) with the known values corresponding to metallic Fe [13]. The structure corresponds to the hexoctahedral

\(^2\)The proportion of atoms at the surface of a particle increases as the size decreases.

\(^3\)Dr. Myriam Aguirre’s help is gratefully acknowledged.
5.3 Thin magnetic films

In a thin film, as for example a Co/Pt multilayer \((A \sim 10^{-11} \text{ J/m}, K \sim 4.9 \text{ MJ/m}^3, M_s \sim 800 \text{ kA/m} \text{ after Coey [14]})\), both characteristic distances \(l_w\) and \(l_{ex}\) are a few nanometers long. Therefore, magnetostatic interactions between portions of the sample separated further apart than \(l_w, l_{ex}\) will overpower the exchange interactions and the film will divide in magnetic domains.
In this section we present the elementary concepts needed to describe the fabrication and the characterization of a magnetic film. We emphasize the case of perpendicular films of Co/Pt, and the formation of a pattern of stripe domains.

Figure 5.5: View of crystals (scale bar of direct space is 50 nm long; scale bar of reciprocal space is 21/\(\text{nm}\)). Left: distance between points and center is \(\approx 5.1/\text{nm}\), corresponding to a separation between planes \((110)\) of \(\approx 2.00\,\text{Å}\). Right: distance between points and center is \(\approx 3.5\,\text{nm}\), corresponding to a separation between planes \((100)\) of \(\approx 2.85\,\text{Å}\). In both cases an outer shell of a different material is apparent (aside of the soft one). Measurements made by Dr. Magdalena Parlinska-Wojtan using a JEOL JEM-2200 FS field emission electron microscope. The wavelength is 0.0251 Å.
5.3.1 Domain walls

Inside a domain wall, the magnetization takes intermediate orientations that are not aligned with any easy axis of magnetization. Two of the simplest possible descriptions are represented in Fig. 5.6.

**Figure 5.6:** 180° rotation of the magnetization vector inside a domain wall for two simple models, in an infinite uniaxial material. **Panel a):** Bloch wall (infinite planar wall). **Panel b):** Néel wall. Adapted from [15].

### Infinite planar walls

The simplest case of a domain wall is a 180° wall in an infinite uniaxial medium, that separates two domains of opposite magnetization. If the wall contains the easy axis there is no charge formation (the component of the magnetization perpendicular to the wall will be the same at both sides of the wall). Furthermore, if the magnetization rotates inside the wall, there will be no magnetic charges inside the wall either. The magnetostatic energy of such a wall, denominated Bloch wall, attains the minimum possible value: zero.

A domain wall is sometimes characterized by a domain-wall energy density $\gamma_w$ (the exchange energy lost because of the spins misalignment between neighbouring lattice sites and the crystalline anisotropy energy penalization because of the departure from the easy axis) and a domain-wall thickness $\delta_w$ (the distance between sites at which the spin orientations are those of the respective domains). In the case of an infinite 180° wall $\gamma_w = 4\sqrt{AK}$ and $\delta_w = \sqrt{A/K}$. 

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Domain walls in thin films

A magnetic film is named thin when its thickness is comparable to the Bloch domain wall thickness. The concept of an infinite 180° domain wall becomes questionable. In this case an in-plane rotation of the spins has a lower energy than an out of the plane rotation (Fig. 5.6). This type of wall is usually named Néel wall.

When a magnetic filed is applied, the angle between the end positions reduces and the Néel wall may be energetically favorable compared to the Bloch mode.

The structure of a real domain wall cannot be classified in one of the two pure categories, but is usually a combination of both. Close to the surface the spins will tend to avoid the formation of surface magnetic charges, and far from the surfaces the spins will tend to follow Bloch modes.

The role of defects

If the ferromagnetic body contains non-magnetic regions (defects), domain walls will tend to comprise many of such regions, because therein no wall energy is incurred and so the overall energy is reduced. Indeed, to move a domain wall away from a defect demands the Zeeman energy to provide the wall energy created when depinning from the defect. This is one of the origins of irreversibility in magnetization loops and demagnetization procedures.

In-plane demagnetization of perpendicular films

When a field is applied perpendicular to the easy axis (in-plane field) the domain wall energy can be minimized if the intermediate spins point in the direction of the field. This has the additional effect that the chirality (the 180° rotation of the spins has two possibilities) of the walls is the same for the whole sample, provided the applied field is strong enough to overcome the stabilizing effect of the defects. The field is not turned off at once, but it is gradually reversed. During the process the domain walls change their orientation, and their position (they can move perpendicular to the field), but they keep their chiralities as dictated by the applied field. Domain walls of the opposite chirality may annihilate each other as they are pushed together, whereas domain walls of equal chirality will tend to stay parallel to each other. After successive reversals of the field, but with decreasing end intensities, a state is reached in which the domain walls are mostly parallel to each other.
5.3.2 Parallel stripes in high-anisotropy exchange-coupled perpendicular films

The lowest energy state of a film with perpendicular magnetization at zero applied field is an arrangement of parallel stripe domains which subsequently point in opposite directions (see e.g. page 11 of [16]).

A minimization of the magnetostatic energy would lead to very thin domains, or equivalently, to a large density of domain walls, which in turn entail an energy penalization. On the other hand, if the domains are very wide the energy penalization of the domain walls is minimized but the magnetostatic energy may be too high. There is an intermediate point at which the consumption of energy for the formation of the domain walls is compensated by the diminution in magnetostatic energy.

The domain width at zero applied field increases with decreasing film thickness (e.g. [16, 15]). In theory, arbitrary large domains can be obtained if the film sufficiently thin, and a parallel-stripe domain pattern minimizes the total magnetic energy in all cases. However, as explained by Hubert & Schäfer ([15], page 290), there exists a critical thickness $l_c = \mu_0 \sigma_{dw}/M_s^2$, below which the saturation field becomes negligible, as the domain width enlarge. From the experimental point of view, when the saturation field lies below the Zeeman field needed to overcome the pinning of the domain walls at the defects, the energy minimum becomes impossible to reach.

5.4 Design of a magnetic film

The magnetic films studied here consist of four layers:

- An adhesion layer that holds the film attached to the glass substrate, thus preventing delamination of the film, specially when it is put in contact with an aqueous solution.
- An underlayer, acts as a seed layer that through its texture promotes a preferred crystallographic orientation in the next layer.
- A magnetic layer.
- Capping layer, that protects the magnet against oxidation and wear.

5.4.1 The adhesion layer

It has been observed that the magnetic films deposited atop glass substrates delaminates when they get exposed to an ionic solution such as those typical of protein-assays. The use of an
adhesion layer is essential to avoid this problem.

Figure 5.7: $2 \times 2 \mu m^2$ MFM measurement and profile lines, before and after the exposure to a ionic solution. **Top:** A film before the contact with the ionic solution. **Middle:** A film, without Chromium adhesion layer, after the contact with BRB80. **Bottom:** A film, with a $5 \text{ nm}$ Chromium adhesion layer, after the contact with BRB80.

We tested the system by covering the film ($\text{Pt}_{5\text{ nm}}(\text{Co}_{0.4\text{ nm}}\text{Pt}_{0.7\text{ nm}})_{30}\text{Pt}_{5\text{ nm}}$) with a drop of BRB80 (a buffer developed for tubulin polymerization and kinesin-microtubules motility assays, see [17] and [18]), with and without adhesion layer.

As adhesion layer we used Chromium. Chromium is known to react with glass: it reduces the silicon and forms crystals of chromium silicide, that grow into the glass. When the metallic seed layer is deposited upon, the chromium silicide anchors it [19].

After one hour of BRB80 incubation, the substrates were rinsed with ethanol, and dried with an air jet. Finally, the samples were investigated with MFM to verify if their magnetic properties were preserved. The results (shown in Fig. 5.7) indicate that the use of a chromium adhesion layer permits the preservation of the magnetic properties of a film.

5.4.2 The seed layer

The goal of a seed layer is that its crystalline texture induces the desired orientation in the magnetic layer that is grown on top of it.

In the case of Co/Pt multilayers, it has been observed that regular layers of Co and Pt begin to form early in the sputtering process when deposited on Si [20]. To ensure that the hcp structure of the Co layers forms with its $c$ axis perpendicular to the film, regardless of the adhesion layer, we decided to use a $5 \text{ nm}$ Pt seed-layer, which also has the added benefit of being an oxidation barrier.
5.4.3 The magnetic layer

The three room-temperature magnetic transition metals Fe, Ni, and Co are natural candidates to be the main component of the magnetic layer. Nickel has been used in similar constructions, albeit several micrometers large [21]. Because of its relatively low saturation magnetization, we make it our last choice. Iron has a very high saturation magnetization but it has rather low intrinsic anisotropy. Cobalt sports a high intrinsic anisotropy and relatively high saturation magnetization. Besides, the R&D efforts on magnetic recording media established an extensive body of knowledge that guides us to prefer a cobalt based film with perpendicular anisotropy: Co/Pt multilayers. Previous studies by Johnson et al. [22] indicate that the anisotropy of Co/Pt strongly depends on the growing conditions. For this reason we should verify the perpendicular character of the films after sputtering.

Examples of different magnetic layers are given in section 5.5.2 on page 106 (effect of the sputtering temperature on the perpendicularity of the film), in section 5.5.3 on page 107 (influence of the number of repeats and the demagnetization parameters on the domains structure), and in section 5.5.3 on page 107 (on an attempt to improve the perpendicularity of the films even further by sputtering on a sample mounted on top of a magnet).

5.4.4 The capping layer

In this particular application there is no wear that may affect the film. The sole purpose of the capping layer is to avoid oxidation (which in turn may negatively affect the magnetic properties of Co). It has been observed that a 2 nm Platinum capping layer successfully protects a cobalt sample for a few days, but the sample appears to have oxidized after a few months. We have used a capping layer of 5 nm platinum and verified that it prevents oxidation at least over a year. The results shown above in Fig. 5.7 are a verification that a 5 nm Platinum capping layer actually protects the multilayer.

5.5 Fabrication of a magnetic film

5.5.1 Substrate preparation

The substrate is, in all experiments reported here, a round microscope slide [23], 18 mm in diameter, 0.13 – 0.16 mm in thickness.

Microscope slides are cleaned before use: After 15’ ultrasonication in acetone bath, the substrates are rinsed with \(d\text{H}_2\text{O}\) and immersed 4 hours in Piranha solution (70% sulfuric acid [24], 30% hydrogen peroxide [25]), rinsed in \(d\text{H}_2\text{O}\), then rinsed with ethanol. They are
subsequently immersed in 2% Hellmanex [26] solution in dH$_2$O (pH $\sim$ 12) for 4 hours. Finally, they are rinsed in dH$_2$O, then in ethanol, and then let dry (only exceptionally with the help of argon flow, as the latter usually contaminates the surface).

5.5.2 Sputtering

Selection of sputtering parameters

A first selection of the temperature and gas pressure during sputtering is based on the Thornton diagram [27] (see Fig. 5.8).

Figure 5.8: Microstructure zone diagram for metal films deposited by magnetron sputtering (adapted from [27]). $T$ is the substrate temperature and $T_m$ is the coating material melting point. Zone 1: atomic shadowing during transport, zone 2: surface diffusion, zone 3: bulk diffusion, zone T: transition zone between zones 1 and 2.

It is known that the relative diffusion rates (of the add-atoms at the surface, at the grain boundary, in bulk) determine whether the deposited film looses all memory of early growth steps (this is the case when bulk diffusion dominates) or not. As activation energies for surface, grain boundary, and bulk diffusion are typically in the ratio 1:2:4, the single most relevant parameter is the ratio $T/T_m$, where $T$ is the substrate temperature and $T_m$ is the
coating melting temperature. At intermediate temperatures surface chemistry and diffusion dominate over other mechanisms such as atom shadowing. At lower temperatures, when $T/T_m < 0.5$, surface and grain boundary diffusion are orders of magnitude more important than bulk diffusion rates. At even lower temperatures the coating microstructure depends as well on other parameters, such as the substrate surface morphology, and the working gas pressure.

Magnetron sputtering is carried out starting with a base pressure better than $5 \times 10^{-8}$ mbar, working with a pressure of $3.3 \mu\text{bar}$, Argon flow of $15$ sccm, and typical sputtering rates of $152$ s/nm (Cobalt target at $12$W), $70.4$ s/nm (Platinum target at $15$W), and $133$ s/nm (Chromium target at $12$W). The sputtering rates were determined by x-ray reflectometry (XRR).

In our case the working gas pressure is the lowest possible such that the sputtering rates are repetitive. Melting temperatures of Co, Cr, and Pt are much higher than room temperature. In order to assess if a temperature higher than room temperature may favor the anisotropy, we sputtered Co/Pt multilayers at various temperatures and checked their magnetic properties afterwards.

It is apparent from Fig. 5.9 that sputtering at $250^\circ\text{C}$ affects the perpendicularity of the film (see section 5.6.2 on page 112 for the interpretation criteria).

It is concluded that room temperature sputtering at the lowest possible pressure produces the films with the highest perpendicularity.

We point out that the sputtering conditions change along the time, mostly as a consequence of the target getting worn out. It follows that sputtered samples should always be characterized.

### 5.5.3 Generating the stripe-domains pattern

The objective is to get straight magnetic pathways ($\sim 2$ $\mu\text{m}$ wide) drawn on a thin magnetic film. In this section, a cost effective strategy to render such a pattern is considered. The idea is to demagnetize the sample in a particular geometrical configuration. Such a procedure would have an influence on the direction of the spins at domain walls, ultimately influencing the layout of the domain walls.

The steps are as follows: The sample is placed between the poles of a yoke, as depicted on the left panel of Fig. 5.10 (the applied magnetic field is parallel to the surface of the film). Then, an oscillating magnetic field is imposed, as shown schematically on the right panel of Fig. 5.10. The parameter $\Delta t$ is chosen such that the current stabilizes before the next demagnetization step takes place.

The maximum achievable magnetic field of $0.8$ Tesla was applied because at that field the magnetization very nearly saturates. The field amplitude of each demagnetization cycle is a
Figure 5.9: VSM magnetization curves of two samples of identical layer structure, Pt_{18nm}(Co_{0.2nm}Pt_{0.7nm})_{50}Pt_{5nm}, but different sputtering temperature. The type of measurement is indicated with the symbol $\perp$ (sample surface arranged perpendicular to the field) or $\parallel$ (parallel to the field). Sputtering temperature, $T$, and coercive field, $H_c$, are indicated in each picture. Insets show raw data (refer to section 5.6.2 on page 112 for details about the data processing).

Figure 5.10: Sample demagnetization process. **Left:** Position of the sample (red broken line) between the poles (P) of a yoke, over a non magnetic table. A Hall sensor (H) measures the applied magnetic field. $y-$axis is perpendicular to $x-$axis. **Right:** Demagnetization process: applied magnetic field as a function of time. $H_0$ should be larger than the field that brings the sample to saturation.
portion of the previous cycle: \( v_n = v_i \exp(-\alpha n) \), where \( v_i \) is the initial amplitude (0.8 T in this case). A total of 1000 demagnetizing cycles were applied with a decaying constant of \( \alpha = 0.186 \).

Throughout a demagnetization process the system of spins takes on different configurations. A demagnetization process that consists of more cycles will visit more states, and the final configuration will be correspondingly closer to the minimum energy state. At the same time, the applied magnetic field sets a preferential orientation for the spins located in the domain walls. In the case of samples with perpendicular magnetization the spins at the domain walls are the only ones that can preserve their orientation after the applied field is suppressed. If, as a result of the sputtering conditions, the natural magnetization of the sample is mixed (some grains perpendicular and some other grains in-plane), it is possible for the domain walls to assemble themselves in combination with the in-plane domains, such that the final geometry is only marginally influenced by the externally applied magnetic field.

As shown in Figure 5.11, the success of the demagnetization strategy to generate a stripes domain pattern depends not only on the number of demagnetization cycles but also on the number of repeats\(^4\).

For thicker multilayers (larger number of repeats), the dependence on the number of demagnetization cycles becomes harder to notice.

We are not only interested in a regular domain geometry, we are also interested in achieving different domain widths. It is apparent from Fig. 5.12 that the domain size can be tuned within the range \( 80 - 130 \, \text{nm} \). Actually, this range and the minimum repeats’ number that admits parallel domains depend on the exact sputtering conditions. The tendency could be measured with samples sputtered the same day (in random order), but cannot be easily repeated after the same targets had been used under different conditions, because the sputter rate can change noticeably in that case.

### 5.6 Characterization of magnetic films

#### 5.6.1 Domain structure of the film measured with magnetic force microscopy

Magnetic force microscopy (MFM) is a scanning probe microscopy technique that resembles AFM, and in which the measurement reflects the forces between the sample and a magnetized tip (see e.g. [29]).

The magnetized tip is one difference between AFM and MFM. In the case of AFM the

\(^4\)These data has been referred to in [28]
Figure 5.11: $5 \times 5 \mu m^2$ MFM measurements showing that the resulting configuration depends on the number of demagnetization cycles. In both cases the film is a $(Co_{0.3\text{nm}}Pt_{0.7\text{nm}})_N$ multilayer, for $N = 3, 5, 7$. The demagnetizing field was applied in the vertical direction (parallel to the resulting stripes). **Left:** 100 demagnetization cycles. **Right:** 1000 demagnetization cycles. The stripes on the right appear to be more regular than those on the left. A higher number of cycles does not significantly affects the domain geometry.

tip-sample interaction forces are larger ($\sim 1 \text{nN}$) and decay very rapidly with the distance (short range van der Waals). For this reason AFM measurements are carried out with the tip very close to the sample or in contact. In MFM the forces are smaller ($1 - 10 \text{pN}$) and their decay is much more gradual.

Typically the result of an MFM scan is presented as a raster picture, in which each pixel represents a measurement point, and the pixel value is a signal proportional to the gradient of magnetic force along the $z$ axis.
Examination of Fig. 5.11 reveals that the gray shades exhibit contrast in some regions and not in others. The reason for this is that, when the stray field is uniform (e.g. at the center of a large domain) the gradient of the field is very small, and the force on the cantilever (and its derivative) is negligible. At the border between domains the stray field presents abrupt variations, the gradient of the field is steep, and the force on the cantilever changes very rapidly with the height. This is the so-called domain-wall contrast. If the domains are small, the stray field is inhomogeneous all over the sample and the so-called domains-contrast is obtained.

In the event there are closure domains (section 5.6.2 on page 114), the intensity of the stray field is drastically reduced, and for all practical purposes the sample appears to have an in-plane magnetization. For this reason MFM measurement complement the information obtained from magnetization curves.
5.6.2 Magnetization curves measured with a vibrating sample magnetometer

A vibrating sample magnetometer (VSM) is an instrument to measure magnetization curves [30]. The sample is magnetized in an uniform field (neither forces nor eddy currents are generated) and makes an oscillatory movement inside a coil system, which is designed to generate a signal proportional to the magnetic moment of the sample.

To measure a magnetization curve, the field is allowed to change from a large negative value to a positive value and back.

As such, the system measures the magnetic moment, not the magnetization. The magnetization can be obtained by dividing by the volume of the (magnetic part of the) sample.

We used a VSM module of a Physical Properties Measurement System manufactured by Quantum Design [31].

Data processing

The hysteresis loops measured reflect not only the magnetic properties of the sample, but also those of the sample holder. For this reason, a non-magnetic sample holder is used, that does not dominate the magnetic response of the sample.

The first step of data processing is to subtract the effective-diamagnetic (or paramagnetic-like) response of the sample holder, which is assumed to be a straight line of negative slope (this is right only if no magnetic impurities are present). The slope of the line can be estimated as the slope of the magnetization curve where the sample is magnetically saturated. This is the procedure used for all measurements presented in this work. For example, in the insets of Fig. 5.9 the raw data appears tilted with a negative slope. The diamagnetic response can be subtracted after a linear fitting of the ends.

Occasionally, artifacts due to internal changes in the equipment could not be avoided. Those effects are less noticeable when the magnitude of the signal is large, but it is not always immediately clear if such deviations are the byproduct of sample-coil collision, an internal mis-calibration, a thermal deviation, dilatation affecting position of the sample, impurities, or even all of them combined. In all cases a repetition of the measurements was enough to distinguish between real features and artifacts.

Interpretation of VSM measurements

The magnetization curves in Fig. 5.9 exhibit different characteristics. For example, the curve of the sample fabricated at room temperature and measured with a field perpendicular to it exhibits shoulders, or sudden changes of the magnetization. The magnetization curve of
the same sample measured with an in-plane field exhibits a more gradual variation of the magnetization.

What the magnetization curves reveal is the relative orientation of the magnetization to the applied field. We recall the mechanism of magnetization reversal mentioned in section 5.2.5 (page 5.2.5): When no field is applied, the free energy landscape presents energetically equivalent minima along the easy axis of magnetization (two orientations in the simplest case of a single easy axis). Application of a magnetic field along an easy axis decreases the free energy in the same direction and increases it in the opposite direction, which becomes a meta-stable state. At a critical value of applied field, $H$, the equilibrium becomes unstable: if the magnetization, $M$, pointed initially opposite to $H$, it will rotate irreversibly towards the direction of $H$. If all the spins in the sample point along the same direction, then the magnetization curves will depict an abrupt shoulder whenever the field is reverted beyond a critical value (the anisotropy field). This ideal case rarely happens in in actual macroscopic samples, because lower barrier reversal processes may exist, notably reversal through domain wall nucleation and motion.

If instead, the field is applied in a direction other than an easy axis of magnetization, the spins will tend to rotate towards the direction of the field. Domain wall motion, if possible, will only occur when the field exceeds the coercivity. Otherwise a smooth magnetization rotation process will set in. When a magnetic field is applied to a spin at angle $\theta$ (to the easy axis, see left panel of Fig. 5.13) the energy can be written as:

$$G = -M_s H \cos \alpha + K_u \sin^2 \beta,$$

(5.6)

where $K_u$ is the crystalline anisotropy. Then $\beta$ takes the value that minimizes the energy. If $\theta = \pi/2$, then $\sin \beta = M_s H / 2K_u$. Therefore, for $\theta = \pi/2$ the measured value of the magnetization in the direction of the applied field is:

$$M = M_s \sin \beta = M_s^2 H / 2K_u,$$

(5.7)

which is the linear relationship between $M$ and $H$ observed. If $\theta < \pi/2$, then $\beta$ satisfies

$$\cos^2 \beta = [4M_s^2 \cos \theta K_u \cos \beta + 4K_u^2 \cos^2 \beta] \sin^2 \beta + M_s^2 H^2 \cos^2 \theta,$$

(5.8)

and a non linear relation takes place between $M$ and $H$. Figure 5.13 shows the curves for three representative cases.

In practice, most spins in a sample are aligned along a common easy axis of magnetization,
and a portion of the spins point in other directions. The magnetization curve will exhibit a combination of both effects: sudden jumps as the magnetization is reversed in the easy axis, and a gradual change for the rest, that will 'round' the shoulder. Therefore, the magnetization curves reveal the predominant orientation of the magnetization in a sample.

It should be pointed out that if the sample contains closure domains (Fig. 5.14) that fill a small portion of the total volume, these may go unnoticed in the magnetization curves.

Figure 5.14: The formation of closure domains leads to a minimization of the magnetostatic energy. If the volume portion occupied by the closure domains is small, the magnetization curves may not have the accuracy to reveal their presence. In this case, the use of a supplementary technique, such as MFM, should be used.
5.7 Example: Co/Pt multilayers

To explore the possibility of increasing the quality of the films, an experiment was made to compare two situations:

- The sample is mounted on a sample-holder with perpendicular magnetization. The sample is $\sim 1 \text{ cm}^2$ and the magnet diameter is 6 cm. With this option we want to check if the applied magnetic field during deposition promotes a different arrangement of the add atoms.

- The sample is mounted on a non-magnetic sample-holder.

The magnetization curves corresponding to some selected samples is shown in Fig. 5.15. The saturation magnetization that can be read out from the magnetization curves, $\sim 600 \text{ kA/m}$ agrees with the values found in the literature: Angelakeris et al. [32] report values half as large, Donnet et al. [33] report about the same values, whereas Coey [14] reports a figure $\sim 30\%$ higher.

It is apparent from the magnetization curves of Fig. 5.15 that:

- The magnetization reaches its saturation value forming a shoulder, and not asymptotically. We infer that the magnetization of the sample is perpendicular.

- The central part of the magnetization curves features a slope that decreases as the number of repeats increases. We infer that the slope is a consequence of the shape anisotropy of the sample.

- The presence of the magnet during sputtering does not improve the perpendicularity of the samples.
Figure 5.15: Magnetization curves of samples sputtered on a magnet (left) and without the magnet (right). The applied magnetic field is perpendicular to the flat surface of the sample. The inset shows raw data (after division by the volume). The number of repeats is indicated in each picture.
Bibliography


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Chapter 6

Theoretical analysis of magnetic steering at the nanoscale

6.1 Equations of motion for magnetic steering

We undertake a rigorous analysis of the problem of magnetic steering in order to understand the discrepancy between our expectations and the experimental results. Firstly, we write down the dynamic equations, in which all forces are correctly described. In order to keep the theoretical description at its simplest possible level, we adopt the following simplifications:

- The problem is reduced to a two dimensional problem, where the coordinates are \( Q \) (the position along the \( x \) axis) and the inclination of the particle \( \Omega \) (the angle between the \( z \) axis and the magnetization, which is assumed to lie on the \( x-z \) plane).

Here we are assuming that the motion along the \( z \) axis is impossible, for example, because of mechanical constraints.

There are actually no reasons to assume that the magnetization is in the \( x-z \) plane, except if the particle is made of a soft magnetic material. If the particles magnetization can be oriented out of the \( x-z \) plane, the corresponding term in the equations should be affected by a factor \( \cos \alpha \), where \( \alpha \) is the angle between the magnetization and the \( x-z \) plane. Because the equations are linear, we can leave out that factor now, and re-introduce it later.

- Usually the particle is not isolated, but transmits the magnetic forces to a body. In the case of the test-model selected in chapter 2 and described in chapter 3, the structure attached to the particle is a microtubule. In the interest of generality, we ignore the forces exerted by the microtubule (or of any other structure that might be attached to
the particle. The effect of these forces can still be considered at a later point, once the free-energy landscape (without them) is known.

We calculate the evolution of a single magnetic nanobead in the very close vicinity of an equilibrium point, that is, the mid-line between two magnetic domains. We assume that at time $t$ the state of the particle can be completely described with a distance $Q_t$, and an inclination angle $\Omega_t$. In this section, the term equilibrium point is defined as the point where the horizontal component of the magnetic force vanishes. Similarly, the equilibrium orientation is the orientation of the magnetization that is parallel to the externally applied magnetostatic field.

We will assume that the system may depart from its equilibrium point and orientation, but it is still very close to them. In other words, we are assuming that the horizontal location of the bead is very close to the border between two magnetic domains, and that the orientation of the bead is such that the direction of the magnetic flux density $B$ is very close to one of the easy axes of the magnetization of the bead (a spherical bead does not have geometrical anisotropy, but a single crystal particle will definitely present crystal anisotropy).

We further simplify the analysis by assuming that in the very close vicinity of the equilibrium point and equilibrium orientation, the magnetic force is proportional to the distance $Q_t$, whereas the magnetic torque is proportional to the inclination angle $\Omega_t$, of the magnetization to the direction of the flux density generated by the substrate. We denote the proportionality constant between force and displacement by $k$, and between torque and change of inclination by $g$.

The stochastic differential equations that describe the system are \cite{1, 2}:

$$
\begin{align*}
    dQ_t &= m^{-1}P_t dt, \\
    dP_t &= -kQ_t dt - \zeta (m^{-1}P_t + r\gamma I^{-1}L_t) dt + \sqrt{2\pi k_BT} \zeta dX_t, \\
    d\Omega_t &= I^{-1}L_t dt, \\
    dL_t &= -g\Omega_t dt - \gamma (I^{-1}L_t + r^{-1}\zeta' m^{-1}P_t) dt + \sqrt{2\pi k_BT} \gamma dY_t.
\end{align*} \tag{6.1}
$$

where $r$ is the radius of the bead, $m$ and $I = \frac{2mr^2}{5}$ are the mass and the moment of inertia of the bead, and $\zeta = 6\pi \nu r$ and $\gamma = 8\pi \nu r^3$ are the translational and rotational friction coefficients. Here $dX_t$ and $dY_t$ are Wiener stochastic processes defined in the same probability space as the coordinate $Q_t$, the inclination $\Omega_t$, the moment $P_t$, and the angular moment $L_t$.

In the event the bead moves immersed in a quiescent fluid and close to a plane with $\Delta < r$, a pure translation generates a torque and a pure rotation generates a translating force.

\footnote{If the particle moves, it transfers part of its moment to the fluid, which will then also move. Hence the fluid is not at rest, even if the volume occupied by the fluid is orders of magnitude larger than the volume of the particle. Here the term quiescent fluid is meant to describe this state of motion compatible with the steady motion of the particle, as is done in fluid mechanics textbooks.}
as exposed in the work of Goldman et al. [2]. Thus in our problem $\zeta'$ and $\gamma'$ are not zero. However, in the range $r > \Delta > r/10$, both $\zeta', \gamma' < 0.1$, so we may still gain useful insight into the rheology with the assumption $\Delta > r/10$ and $\zeta' = \gamma' \simeq 0$. This assumption leads to a considerable simplification because of the decoupling of rotation and translation. Additionally, a correction factor in the range $1 - 3$ should be applied to the friction coefficients, but as we will see below, such correction does not play an essential role.

We assign numerical values for the sake of definiteness: for an aqueous solution we may assume $\nu = 8.9 \times 10^{-4}$ Pa $\cdot$ s, and for a metallic iron particle we can take the bulk value $\varrho = 7800$ Kg/m$^3$ to calculate the mass $m = \varrho 4\pi r^3/3$.

Under the assumptions stated at the beginning of this section, the linear parameters $k$ and $g$ can be calculated directly from the mathematical expressions for the magnetic flux density over the substrate (see section 4.2.2 on page 83).

6.1.1 Relaxation times enable comparison between forces

Equations 6.1 are stochastic. The term that describes the Brownian motion has infinite variation in any time interval, and therefore the traditional tools of mathematical analysis cannot be applied to it.

We are interested in calculating the most probable value that we would obtain if we make a measurement. This is the expected value, and can be obtained by taking expectation over the probability space.

Because the dynamic equations are linear, by taking the expectation $\langle \cdot \rangle$ over the probability space we obtain equations in which the Brownian term vanishes (its average is zero), and that can be solved in a traditional way, provided the initial expected values are known [3]:

$$
\begin{align*}
\langle Q_t \rangle &= \exp \left[-t \begin{pmatrix} 0 & -m^{-1} \\ k & \zeta m^{-1} \end{pmatrix}\right] \cdot \langle Q_0 \rangle, \\
\langle P_t \rangle &= \exp \left[-t \begin{pmatrix} 0 & -m^{-1} \\ k & \zeta m^{-1} \end{pmatrix}\right] \cdot \langle P_0 \rangle, \\
\langle \Omega_t \rangle &= \exp \left[-t \begin{pmatrix} 0 & -I^{-1} \\ g & \gamma I^{-1} \end{pmatrix}\right] \cdot \langle \Omega_0 \rangle, \\
\langle L_t \rangle &= \exp \left[-t \begin{pmatrix} 0 & -I^{-1} \\ g & \gamma I^{-1} \end{pmatrix}\right] \cdot \langle L_0 \rangle.
\end{align*}
$$

(6.2)

This approach enables studies about the evolution of the system after a perturbation. If the system undergoes a perturbation that takes it from its equilibrium position, it will decay back exponentially fast. The characteristic times of decay are given by the inverse of the eigenvalues of the matrix in equation 6.2. We have for the translation and for the rotation:

$$
\begin{align*}
\lambda_{\pm}^t &= -\zeta/2m[1 \mp \sqrt{1 - 4km\zeta^{-2}}], \\
\lambda_{\pm}^r &= -\gamma/2I[1 \mp \sqrt{1 - 4gI\gamma^{-2}}].
\end{align*}
$$

(6.3)
The decay can be oscillatory provided the dimensionless parameter $4km\zeta^{-2}$ (or $4gI\gamma^{-2}$) is greater than one, but it turns out that this is never the case for the numerical values considered above. Actually, it is quite realistic to assume $4km\zeta^{-2} \ll 1$ and $4gI\gamma^{-2} \ll 1$, so the characteristic decay times are:

\[
\tau_\pm^t = -(2m\zeta^{-1})/[1 \mp (1 - 2km\zeta^{-2})],
\]

\[
\tau_\pm^r = -(2I\gamma^{-1})/[1 \mp (1 - 2gI\gamma^{-2})].
\] (6.4)

The eigenvector of the smallest translation eigenvalue, $\tau_+^t \simeq m/\zeta$, is essentially the case of a particle almost at the equilibrium position but with a non-vanishing moment. For particles a few nanometers big, this characteristic time is at most a few microseconds (i.e. the bead will forget its initial speed in that time interval), which is why most of the literature discards the inertia term in equation 6.1 from the very start: If the time intervals of interest are long compared to $\tau_+$, the Ornstein–Uhlenbeck description of Brownian motion (Eq. 6.1) can be adequately approximated by the Smoluchowski description (see chapter 10 of [1]). A similar situation is observed for the smallest rotation eigenvalue, $\tau_+^r \simeq I/\gamma$.

The other eigenvectors, associated to the largest eigenvalues ($\tau_-^t = \zeta/k$ and $\tau_-^r = \gamma/g$) describe the cases where a particle is initially away from the equilibrium position (either shifted or rotated) and animated with a small moment that relaxes back to the point of equilibrium. For the cases considered in this work, $\tau_-^t < 1\mu$s, and $\tau_-^r$ is even smaller.

The characteristic decay times allow a comparison between the forces, as also pointed out in section 2.4 above (partially repeated here, for completeness).

- **Translation:** $\tau_+^t \sim m/\zeta < 10^{-6}$ s. Here $m$ is the mass of the particle and $\zeta = 6\pi\nu r$ is the translational friction coefficient, $\nu$ is the dynamic viscosity (for water $\nu = 8.9 \times 10^{-4} \text{Pa} \cdot \text{s}$), and $r$ is the radius of the particle. This characteristic times expresses that the inertia term can be neglected in comparison to the friction, as it is well known in submicron systems.

- **Translation:** $\tau_-^t \sim \zeta/k < 10^{-6}$ s. Here $k$ is the curvature of the potential energy of the magnetic forces. The relation expresses that the magnetic forces are larger in magnitude than the friction.

- **Rotation:** $\tau_+^r \sim I/\gamma < 10^{-6}$ s. Here $I = 2mr^2/5$ is the inertia moment of the bead, and $\gamma = 8\pi\nu r^3$ is the rotational friction coefficient. This small relaxation time implies that the angular inertia is also negligible in comparison with the rotational friction.

- **Rotation:** $\tau_-^r = \gamma/g < 10^{-6}$ s. Here $g$ is the proportionality factor between the torque and the departure from the equilibrium orientation of the particle. A free particle will
rotate in spite of the friction forces and will align itself to align its magnetization parallel
to the applied field.

In case the particle glides closer to the surface ($\Delta < r$), the characteristic times mix, but no
significant changes in magnitude can be expected.

So far the calculation of the relaxation times confirms the predictions made before the
experiment. We will now take into account the effect of Brownian forces.

6.2 Effect of Brownian motion

We have already considered the effect of Brownian forces in section 2.2.2. However, the
potential created by the magnetic forces was assumed to have no rim. This is a reasonable
assumption when the potential well is very deep compared to $k_B T$.

If the potential well is not much deeper than $k_B T$, the nanoparticle might be able get over
the saddle point of the potential within the time span of an experiment. This characteristic
exit-time is known as the first-passage time, and it is a quantity that can be used to characterize
a system. The mean first-passage time can be estimated using analytical expressions [4]. There
is a difficulty with this approach: the potential $U = - \mathbf{M} \cdot \mathbf{B}$ depends on the orientation of
$\mathbf{M}$ relative to the flux-density field $\mathbf{B}$. Hence, the calculation of the first-passage time must
assume a particular orientation of the particle at each point.

For design purposes, it is more useful to know how the energy landscape is affected by the
choice of the design parameters (geometry of the magnetic film and of the particle, constitutive
material of the film and of the particle). In particular, we want to measure the depth of the
potential well generated by the magnetic forces in terms of the product $k_B T$.

6.2.1 Potential well created by magnetic forces might be too shallow
for effective guidance

We measure the depth of the potential well created by the magnetic forces, $E_{\text{mag}}$, as the
mechanical work to be performed to take a particle to the rim, assumed to be at position
$x = X$.

$$\frac{E_{\text{mag}}}{k_B T} = \int_0^X f_x dx \, \frac{k_B T}{k_B T},$$

(6.5)

The situation is depicted in Fig. 6.1, below.

It is apparent from Fig. 6.1 that the potential well of the magnetic forces can be too
shallow for guidance, specially when the domain size is smaller than 200 nm.
Figure 6.1: Left (A and B): the magnetization can rotate and is always parallel to the field. Right (C and D): fixed magnetization. A,C: Brownian forces may cause the particle to diffuse away, from an equilibrium position to another one. B,D: $E_{\text{mag}}/k_B T$ is the depth of the potential well created by the magnetic forces, measured in units of $k_B T$. The calculation was made with parameters $M_f = 600 \text{kA/m (CoPt)}$, $M_s = 1700 \text{kA/m (iron)}$, $\delta = \gamma = 5 \text{nm}$, $h = 30 \text{nm}$. Curves of constant potential depth are displayed for three different particle radii.

Particle size does not seem to play an important role in establishing this condition. This is actually a simplification in the presentation of the data, which we attained by choosing the separation $\Delta$ as a parameter and not the distance to the center, $z = r + \Delta$.

Procedure to calculate the depth of the potential well

To calculate the depth of the potential well, we follow the following procedure:

1. Calculate the forces on a particle (with the procedure of page 86) for positions of the particle along a horizontal line parallel to the magnetic film.

   Here the horizontal line is the positions that the particle might take if the magnetic forces were not there.

2. Find the position of the rim, that is, of the saddle point that the particle must reach in order to escape from the potential well. This is the position at which the forces change sign.
The depth of the potential well is simply estimated as the work that has to be done against the magnetic forces to take a particle from a potential minima to the potential saddle-point. In other words, we compute the integral in equation 6.5 from a potential minimum to the point $X$ at which the force stops opposing the escape.

Bibliography


Appendix A

Additional results

A.1 If particle concentration is too high, microtubules stop moving

We report here the results of an experiment, whose objective was to determine the optimal concentration of particles to be used. The experiment does not use hybrid microtubules: the microtubules were polymerized from a single initial incubation solution containing 20% rhodamine-labelled tubulin and 80% biotin-labelled tubulin.

The experiment follows the protocols described in section 3.6.2 and the particles were prepared as indicated on page 61 of that section. The particle suspension used in each sub-experiment is presented in table A.1.

<table>
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<th>3rd exper.</th>
<th>4th exper.</th>
<th>5th exper.</th>
<th>6th exper.</th>
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<td>100 µl</td>
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</tr>
<tr>
<td>10 µl</td>
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<td>1 µl</td>
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<td>1 µl</td>
<td>1 µl</td>
<td>1 µl</td>
<td></td>
</tr>
<tr>
<td>[c]_{rel} 8.3 \times 10^{-1}</td>
<td>8.3 \times 10^{-2}</td>
<td>2.5 \times 10^{-2}</td>
<td>2.5 \times 10^{-4}</td>
<td>2.5 \times 10^{-3}</td>
<td></td>
</tr>
</tbody>
</table>

Table A.1: Composition of the particle suspension used in the experiment to determine the optimal concentration. The concentrations mentioned are relative to the concentration received, which is not known. Experiments are labelled from 2nd onwards because the first experiment was made without particles.

The trajectories were measured with confocal microscopy (Fig. A.1).

It is apparent from Fig. A.1 that the magnetic forces toward the film may be too strong if the concentration of particles is too high.

More importantly, we point out that the measurements summarized in Fig. A.1 effectively demonstrate the effect of the magnetic forces. It is the gradient of the stray field which
Figure A.1: $212 \times 212 \, \mu m^2$ confocal microographies of microtubules polymerized with 20\%-rhodamine, 80\%-biotin labelled tubulin. The gray scale has been inverted. $c_o$ is the original, unknown concentration of particles. Upper row: no motion observed. Lower row: motion is observed.

We point out that this result contrasts with those published by Hutchins et al. [1], in which a microtubule decorated with magnetic nanoparticles over lengths $\sim 1 \, \mu m$ aligns itself with the stray field of a millimeter-sized magnet. In the experiment of Hutchins et al. the effect of the torque on a micrometer-sized magnetic structure is shown.

**Bibliography**

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