

A Magnetic Pincher for the dynamic measurement of the actin cortex thickness in live cells

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Abstract

The actin cortex is an essential element of the cytoskeleton allowing cells to control and modify their shape. It is involved in cell division and migration. However, probing precisely the physical properties of the actin cortex has proved to be challenging: it is a thin and dynamic material, and its location in the cell – directly under the plasma membrane – makes it difficult to study with standard light microscopy and cell mechanics techniques. In this chapter we present a novel protocol to probe dynamically the thickness of the cortex and its fluctuations using superparamagnetic microbeads in a uniform magnetic field. A bead ingested by the cell and another outside the cell attract each other due to dipolar forces. By tracking their position with nanometer precision, one can measure the thickness of the cortex pinched between two beads and monitor its evolution in time. We first present the set of elements necessary to realize this protocol: a magnetic field generator adapted to a specific imaging setup, and the aforementioned superparamagnetic microbeads. Then we detail the different steps of a protocol that can be used on diverse cell types, adherent or not.

Introduction

The actin cortex is a thin and dense meshwork of actin filaments located just underneath the plasma membrane of eukaryotic cells. It is a key element of the cytoskeleton in many processes that involve a control of the cell shape. Indeed, the cortex is an active material, due to the actin filament fast turnover and their association with myosin II microfilament. The gradients of tension that these motors generate are the source of cell-scale deformations that occur for instance during bleb-mediated amoeboid migration or furrowing during cell division (Chugh and Paluch, 2018). Because the cortex is very thin relatively to the cell and the tensile forces it generates are mainly parallel to the cell surface, the cortex is often viewed as a two-dimensional sheet of actin. However this point of view neglects the role of the 3D architecture of the cortex, which is key for many of its emerging properties. This architecture is very challenging to study experimentally: single-actin filaments are below the diffraction limit, their density in the cortex is high and their typical turnover rate is a few tens of seconds (Fritzsche et al., 2013). As a whole, the cortex is very dense in actin filaments and contains a host of other proteins that associate with actin to produce different types of structures. This explains why the most precise observations of the cortex have been obtained with electron microscopy on fixed samples, cells (spread cells: Svitkina et al., 1986; round cells: Chugh et al., 2017) or cellular blebs (Charras et al., 2006). More recently techniques compatible with live cells were developed: atomic force microscopy (Eghiaian et al., 2015) or sub-resolution microscopy (Clark et al., 2013; Chugh et al., 2017) helped to better understand the regulation mechanisms of cortical actin architecture, by combining observations with perturbations of actin binding proteins activity using drugs or siRNA.

We propose here a new approach, relying on magnetic microbeads to pinch the cortex of live cells. These beads, commonly used for cell separation, acquire a magnetization when placed in an external magnetic field. If a bead is inside a cell and another outside, they will form a pair pinching the cortex by attracting each other. When the pinching force is kept low and constant, the beads do not significantly indent the cortex. In this configuration, by tracking their relative position and subtracting the size of the beads, one can probe the thickness of the cortex at the beads location with a very high spatial (10 nm) and temporal (sub-second) resolution. In particular, this allows us to monitor the fluctuations of the thickness in time, which are significant, and correlated with the activity of Myosin II motors in the actin meshwork (Laplaud et al., 2021). Thanks to its versatility, this technique can be applied to many cell types in diverse contexts and setup. By basing this thickness measurement on a force application, our approach also open new ways to directly measure cortex mechanics.

[Fig. 1]

1. Method

1.1. Overview

The setup consists in an inverted microscope used in bright field illumination, with a system mounted on the microscope stage to generate a uniform magnetic field at the location of the imaged sample. This system can be an array of permanent magnets or a pair of electromagnetic coils. The protocol, whose main steps are summarized on Fig. 1, is based on 4.5 μm superparamagnetic microbeads, the M-450 Dynabeads. Cells are incubated with these beads until a significant uptake is reached. Then, cells are transferred in the experimental chamber, together with additional beads. The chamber, placed on the microscope, is exposed to a controlled uniform magnetic field. The beads magnetize and attract each other at short range, forming pairs or chains of beads. This self-organization generates simultaneously a large number of pinching events in the chamber (Fig. 2A, 2D), meaning a situation where a bead inside the cell forms a pair with another outside the cell, which pinches the cell cortex. Such beads can then be filmed under bright field illumination, and the images analyzed with a simple algorithm tracking the beads positions (Fig. 2B). This way we compute the cortical thickness and its evolution in time (Fig. 2C) with a high spatial ($\approx 30\text{nm}$) and temporal resolution (up to 100 Hz; more details in Laplaud et al., 2021).

[Fig. 2]

1.2. Specific setup

1.2.1. Magnetic field generation

This protocol requires the generation of a uniform magnetic field over the experimental chamber. When exposed to an external magnetic field, superparamagnetic beads will become magnetic dipoles, with a magnetic moment that is a function of the applied field. Because the gradient of the external field is negligible, there is no long-range magnetic force and the beads do not drift. Instead, the dipolar force generated by each bead causes them to attract each other. This leads to a self-organization of the beads present in the experimental chamber, forming pairs or chains aligned with the magnetic field (Fig. 5A, 4B). We insist here on the importance of the field uniformity: a too strong gradient of magnetic field would cause superparamagnetic beads to drift toward the higher field regions.

We propose below two options to generate a controlled and uniform magnetic field on a microscope. The first is the Halbach array (Halbach, 1985), which consists in a set of permanent magnets arranged in a specific geometry around the sample. The second relies on two copper coils placed on both sides of the sample, in a pseudo-Helmholtz configuration.

- Halbach Array

The term “Halbach array” refers to an arrangement of permanent multipole magnets. These arrays can have very diverse properties and application depending on their geometry. Here we use a set of dipolar magnets arranged in a circle (for more details see Tretiak et al., 2019). It generates a uniform magnetic field inside the circle, and a negligible field outside. The magnets have to be identical in size and magnetization, and arranged so that their direction vector rotates twice faster than their position vector on the circle (Fig.2A).

In such geometry, the field generated in the center of the Halbach array has the following magnitude:

$$B_0 = \frac{3}{8} N \mu_0 \frac{m}{\pi \cdot R^3}$$

[Eq. 1]

Where N is the number of magnets used in the array, μ_0 is the vacuum magnetic permeability, R is the radius of the circle joining the center of magnets, and m is the magnitude of the magnetic moment of the magnets.

Another useful relationship in Halbach array design is the following: for a given magnet of volume V that contains a uniform field of magnetization M, the resulting magnetic moment m is simply:

$$m = V \cdot M$$

[Eq. 2]

As a reference the neodymium N42, of which the cubic magnets we use are made, has a magnetization of $M_{N42} \approx 1.01 \times 10^6$ A/m. Their magnetization is simply deduced from this value and their geometry.

A slightly more complex application of this concept is the nested Halbach array (Fig. 3B). The idea is to use two concentric Halbach arrays that generate the same field B_0 and can be rotated independently. This is possible using larger magnets for the outer array, since as stated by Eq. 1, the generated field magnitude decreases rapidly when the radius of the array increases; this decrease is compensated by increasing the volume of the magnets so they possess a larger magnetic moment (Eq. 2). Therefore, this system can generate uniform magnetic field of any magnitude between ≈ 0 mT (when the two arrays' fields are in opposite direction) and $2 \cdot B_0$ (when they are in the same direction).

[Fig. 2]

We detail here the specifications of a nested array that we have optimized for this protocol and which is easy to mount on many microscopes, given that it has the outer dimension of a 6 well plates (Table 1, Fig. 3C). The body of the device, meaning the rectangular support and the two rings bearing the magnets, have been 3D-printed (Printer: Fortus 250mc, Stratasys, USA; Material: ABS X-TREME, iSQUARED, Switzerland). The neodymium cubic magnets are commercially available (Supermagnete, Germany). This instance of the nested Halbach array is designed to generate a maximum field of $2.B_0 = 8.6 \text{ mT}$.

[Table 1]

- Coils

As a consequence of Ampere's law, an electric current circulating through a coil of conductive wires generate a magnetic field that curls around the coils. A pair of such coils positioned symmetrically along a common axis, with a distance between the coils of the same order of magnitude than the radius of the coils, are called pseudo-Helmoltz coils. If the same current circulate through both coils in the same direction, a quasi-uniform magnetic field will be generated in the space between the coils. The magnetic field magnitude in the center of this system is proportional to the intensity of the electric current. Hence, such system can generate a field that can be tuned directly by controlling this current supplied to the coils.

[Fig. 4]

In our case, the two coaxial coils (custom made by SBEA TechnologiesF, France) are completed with a mu metal core (750 spires; length: 40 mm; inner diameter: 46 mm; outer diameter: 86 mm, see Fig. 4A) to increase the generated field. The coils are powered by a bipolar operational power supply amplifier 6A/36V (Kepco, USA) controlled by the computer through a data acquisition module (National Instruments, USA). The maximum field generated is 55 mT (which correspond to the maximum supplied current, 6 A) with a gradient less than $0.1 \text{ mT}\cdot\text{mm}^{-1}$ over the sample.

- Comparison of the two solutions

If both options can produce a similar uniform magnetic field in the sample zone, they offer different advantages and disadvantages, which are summarized in the Table 2 below. The most crucial difference is that the Halbach array is easier to make and to use on any microscope, while the coils require special manufacturing and a custom made microscope stage (Fig. 4B). On the other hand the magnetic field generated by the coils can be dynamically adjusted during an experiment (opening the doors to rheology experiments which are beyond the scope of the method described here), which is impossible with a Halbach array.

[Table 2]

1.2.2. Superparamagnetic Beads

The properties of the superparamagnetic beads are crucial to the feasibility and the precision of this technique. The size of the beads in particular is key: they need to be big enough to generate a sufficient dipolar force and for their position to be accurately determined with bright field microscopy. We have identified the M-450 Epoxy Dynabeads (Dyna, Thermo Fisher, USA) as the best choice since they ally many important features.

- Beads from the Dynabead line are very monodisperse in diameter. A given batch of M-450 Dynabeads typically has an average diameter close to $4.5\ \mu\text{m}$ with a standard deviation of less than 25 nm (Fig. 5D).
- M-450 possess one of the highest density of magnetic particles (Fonnum et al., 2005) and the largest size of all Dynabeads. Thus they can acquire high magnetic moment magnitudes, which result in a large range of pinching forces: from 1 pN to more than 1 nN. Moreover, like other Dynabeads, they have a very small residual magnetization, which ensures that they stop attracting when the field is brought back to zero.
- Optical properties: when observed under transmitted bright light illumination at high magnification, an intense light spot forms roughly $3.7\ \mu\text{m}$ below the bead center (Fig. 5C). Computing the center of mass of this light spot, using the light intensity (pixel value) as a weight allows the center of the beads to be localized with a resolution that overcomes the diffraction limit: the precision of their localization in 2D is 2 nm (Bauër et al., 2017) and 10 nm in 3D (Laplaud et al., 2021).
- They are relatively easy to uptake for cells. This depend on the cell type and can be tuned by coating the beads with diverse molecules, but every cell type considered for this experiment so far have proved able to uptake M-450 beads.

This technique have also been performed successfully using M-270, which are smaller (typical diameter: $2.7\ \mu\text{m}$) and magnetize less strongly. The method did not work when attempted with MyOne (typical diameter: $1\ \mu\text{m}$). The M-450 are nonetheless the best choice in terms of robustness and precision.

[Fig. 5]

When using superparamagnetic Dynabeads, it is possible to determine the value of the attractive force one bead apply on its neighbor as a function of the external magnetic field and the beads positions. This way, the magnitude of the pinching force applied to the cortex can be computed,

which is useful when interpreting the thickness measurements. However since it is not essential for the use of this technique, we will not present this aspect here; the details are available in Bauër et al., 2017 and in Laplaud et al., 2021.

1.2.3. Imaging system

To monitor the actin cortex thickness in time, a pair of beads pinching the cortex must be tracked in 3D. This is done by acquiring a time-lapse movie of the beads illuminated in bright field, with the focus on the light spot below the beads. To further improve the precision of the bead tracking, a Z-stack of 3 images is acquired at each time-point (see next section). A high magnification and a high dynamic range are required to produce images where the beads can be tracked precisely.

In total, in addition to the magnetic field generator, the imaging setup must include the following elements:

- High-magnification objective (63X or 100X, preferably NA = 1.4).
- Bright-field light source with a field diaphragm and a condenser diaphragm on the light path.
- Camera with a 16-bits dynamic range. We use an Orca Flash4 (Hamamatsu, Japan).
- Piezo element to control the position of the focal plane along the Z-axis. This can be a piezo controlled stage, or a PIFOC focus scanner. We use a PIFOC P-721.CDQ (Physik Instrumente, Germany).
- Image acquisition software which can control both the camera and the Z-axis, and save along with the images a log file containing the precise time of each image acquired. We use a custom-made Labview program, along with a data acquisition module (NI 6343, National Instruments, USA).
- An environment control chamber to maintain the temperature of the sample to 37°C throughout the experiment, and ideally the CO₂. We use The Box and The Cube (Life Imaging System, Switzerland).

1.2.4. Guidelines for bead imaging

Before imaging M-450 Dynabeads, the microscope and camera setting have to be adjusted following these principles:

1. The brightness of the light spot below the beads is maximum around 3.7 μm below the equatorial plane for M-450 Dynabeads. We call it the plane of maximum intensity.
2. First the focus must be made on a bead in the plane of maximum intensity. Set the exposure time to 5 ms.

3. Set the Köhler illumination conditions on the microscope, then slightly open the field diaphragm to make the light spot sharper. Verify by moving the focal plane up and down the light spot spread a bit as you get further from the maximum intensity plane (see Fig. 5C for a reference).
4. Adjust the light source power to use a significant fraction of the camera dynamic range. Typically, adjusting the maximum intensity to roughly 40'000 grey levels on a 16-bit camera is ideal. The image should never become saturated throughout the light spot.

When acquiring a time-lapse, the following points are keys to image the beads in a way that will allow a precise 3D tracking:

1. The tracking of the beads in the XY plane will be more precise when the focus is done on the plane where the light spot is the brightest (plane of maximum intensity).
2. The tracking of the beads along the Z-axis will be more precise when the focus is done on a plane located above or slightly below the plane of maximum intensity. This is because the shape of the light spot does not vary much when moving up and down from the plane of maximum intensity, but starts forming specific patterns when one move further below or – even better – above. These patterns facilitate the tracking along Z.
3. As mentioned before, the ideal solution to optimize tracking in both XY and Z is to acquire a small stack of 3 images for each time point. Typically 3 planes 0.5 μm apart in Z and centered on the plane of maximum intensity will allow a very precise 3D localization of the beads.
4. If the conditions of imaging do not allow such Z-stack to be acquired at each time-point, the best plane to localize the bead precisely in XY and Z on a single image is one located slightly above the plane of maximum intensity, where the light spot below the bead starts spreading a little bit: it is the best compromise between points (1) and (2).

1.3. Protocol

1.3.1. General comments

This protocol was optimized for two cell types: primary mouse dendritic cells (DC) and 3T3 fibroblasts (3T3). In the following protocol, some steps will differ according to which cell type they are applied to. It will then be clearly stated. We believe that together, these two cases are representative of a large number of cell types. Importantly, they encompass the cases of strongly adherent cells (3T3) and weakly-adherent ones (DC).

1.3.2. Materials

- Beads preparation
 - o M-450 Epoxy beads (Dynal, Thermo Fisher, USA)

- Culture Medium
- Magnet
- PBS
- Rotating wheel for aliquots
- Experimental chamber preparation (For DC)
 - Glass-bottom petri dish (35mm)
 - Bovine Serum Albumin (Merck, Germany)
- Experimental chamber preparation (for 3T3)
 - Holed petri dish (35mm) OR Petri dish (35mm) & access to a laser cutter.
 - 25 mm round glass coverslip (Thickness #1)
 - PLL(20)-g[3.5]-PEG(2) (SuSoS, Switzerland)
 - HEPES buffer (pH 7.4)
 - Fibronectin (from bovine serum, F1141, Merck, Germany)
 - NaHCO₃ buffer (pH 8.3)
 - Quartz photomask (JD Photodata, UK)
 - Deep UV-source (UVO Cleaner: $\lambda = 254 \text{ nm}$, $P = 7 \text{ mW/cm}^2$; Jelight, USA)
 - Non-toxic glue (silicon glue "SA 500", Zolux, France)
- Cell handling
 - TrypLE (Thermo Fischer, USA)
 - PBS
 - Sterile HEPES solution (H0887, Merck, Germany)
 - Culture medium
 - Imaging medium
 - Cell culture hood
 - Incubator with CO₂
- Imaging Setup: Refer to section 1.2.
- Other equipment
 - Gaussmeter (model GM08 with transverse probe, Hirst Magnetic Instruments Ltd., UK)

1.3.3. Bead size measurement

The distribution of diameters of the M-450 Dynabeads has a very low variance, with the exception of rare and obvious outliers, and varies only slightly from one batch to another. Nonetheless, for each vial of beads newly purchased, it is necessary to characterize precisely the average diameter, as it is

one of the main sources of uncertainties of the technique. This is done easily using long chains of beads that form under specific conditions.

Step 1 – Image acquisition

1. In a 35 mm glass-bottom petri dish, coat the glass surface with 1 % bovine serum albumin (Merck, Germany) by incubating for 5 minutes. Rinse twice with PBS.
2. Adjust the volume of PBS contained in the dish to approximately 2 mL, and add 400'000 beads of the population you want to characterize. Rock gently the dish to homogenize as the beads sediment.
3. Place the dish in the experimental setup equipped with the magnetic field generator. Apply a field of 5 to 10 mT.
4. The beads will self-organize in long chains. Acquire several images of such chains in the plane of the beads light spot. Several criteria have to be filled:
 - a. As per usual, the light spot maximum intensity should be close to the maximum of the pixel range, but never saturating.
 - b. The chains must clearly be separated from each other, do not consider chains that are stacking on each other.
 - c. Within a chain, the bead aspect must be identical, indicating that they are all in the same plane along the Z-axis. If it is not the case, two explanations are probable. Either the dish is not horizontal enough, in which case it need to be rectified. Or the beads are badly aligned, and in this case one can switch off the field, mix the solution to resuspend the beads and switch the field on again during the beads sedimentation, if possible with a high field value (30 mT).
5. Acquire images of chains to gather a total of several hundreds of beads.

Step 2 – Image Analysis

1. In the Fiji software, open the images of a chain of beads.
2. Use the method detailed below in section 1.4.1. to detect the position of the center of each bead in the chain.
3. In the results table, use the columns “XM” and “YM” – the position in X and Y of the center of mass of each light spot – compute the series of neighbor-to-neighbor distance in the chain. Ordering the table row by XM value beforehand might simplify the task.
4. Repeat steps 1 to 3 for each of the acquired images.

5. Compute the mean and the standard deviation of the distribution of neighbor-to-neighbor distances. These values are used to characterize respectively the central tendency and the variability of the bead population.

1.3.4. Bead preparation

Before using M-450 Dynabeads it is necessary to rinse them from the stock solution medium and coat them with a molecule to tune the ability of cells to ingest them. Our standard coating protocol uses complete medium (with 10% FBS) to increase moderately the uptake rate by cells. Other options are fibronectin coating, to increase bead ingestion, and mPEG coating to decrease it (see Section 2).

Step 1 – Rinsing

1. The commercially available M-450 Dynabeads are conserved in distilled water with a concentration of 4×10^8 beads/mL. Vortex the stock solution vial to resuspend the beads and pipet 30 μ L of the solution in an aliquot.
2. Add 1 mL of PBS to this aliquot and vortex the mix for 20 seconds.
3. Hold the aliquot vertically above a magnet (typically the W-10-N 1 cm neodymium cube from Supermagnete) for 20 seconds. The lower tip of the aliquot should be in contact with the magnet. The beads will sediment rapidly.
4. While holding the aliquot vertically above the magnet, gently remove the supernatant to disturb as little as possible the bead pellet.
5. Repeat steps 2 to 4 twice.

Step 2 – Coating

With complete medium

1. In the aliquot containing your rinsed pellet of beads, add 100 μ L of complete medium used for the culture of your cells of interest.
2. Vortex the mix for 30 seconds.
3. Before the beads sediment, place the aliquot on a rotating wheel for 3 hours.
4. Conserve the aliquot at 2 to 8°C, with the cap wrapped in Parafilm for improved conservation. If manipulated only in sterile conditions, it can last up to one month.

The resulting concentration expected in the aliquot is 120'000 beads/ μ L. Accounting for the loss of a fraction of the beads during the rinsing steps, the actual concentration should be around 100'000 beads/ μ L. To verify one can dilute 100 times the solution and count the concentration of beads with a typical cell-counting slide.

1.3.5. Experimental chamber preparation

While non- or weakly-adherent cells do not require a very specific experimental chamber, adherent cells such as 3T3 fibroblasts or macrophages do. Indeed, in the absence of surface treatment of their 2D substrate, those cell types would spread until they become flat and their cortex undergoes large reorganization as the cells produce stress fibers. To prevent the cell to spread excessively one need to control the shape that the cells will adopt while adhering. This can be done by micropatterning a molecule that favors adhesion on the chamber's bottom side, such as fibronectin (Azioune et al., 2009).

Case of weakly adherent cells

1. In a 35mm glass-bottom petri dish, coat the glass surface with 1% bovine serum albumin (Sigma-Aldrich) by incubating for 5 minutes. Rinse twice with PBS.
2. Before adding the cells, rinse twice with warm imaging medium.

Case of adherent cells

This step is a variation on the technique described in Azioune et al., 2009. Hence we will not detail here all the steps of the micropatterning protocol itself, but rather how we adapted it to fit the constraints of our technique. Indeed, the original protocol used magnets to mount the coverslip on the microscope stage, which is obviously not possible in this method. Thus we fabricate our own glass-bottom petri dish by assembling a holed dish and the micropatterned coverslip.

Step 1 – Petri dish preparation

We use 35mm petri dishes with a 20mm hole cut in the plastic bottom. Such dishes can either be bought or fabricated. To make them in the lab, we suggest to use a laser cutter which can be programmed to cut a circular hole in a batch of precisely positioned petri dishes. One can custom make a guide to help with the positioning in the laser cutter.

Step 2 – Micropatterning on glass coverslip

We perform the aforementioned micropatterning protocol (Carpi et al., 2011) on 25 mm round glass coverslips (thickness #1), using PLL-PEG to passivate the surface and custom made photomask to burn the micropattern with deep UV light ($\lambda = 254$ nm). The micropatterned shape are discs of identical diameters. For 3T3 fibroblasts we used 20 μm large discs. The diameter of the discs should be roughly equal to the typical diameter of the cell type used (when our 3T3 cells are in suspension, they adopt a spherical shape with a typical diameter of 18 μm). Importantly, the micropatterned

slides can be conserved several days but the incubation with fibronectin should be done just before the experiment (see below).

Step 3 – Assemble the chambers

To stick the micropatterned coverslip on the bottom of the holed petri-dish, use non-toxic silicon glue.

1. If they have been fabricated in the lab, the holed dish and its lid must be cleaned using 70% ethanol, and dried thoroughly.
2. Pour some glue in a syringe (roughly 1 mL of glue for 4 dishes).
3. Place the holed dish upside down. Use the syringe to apply a thin and continuous ribbon of glue on the edge of the hole.
4. Using a plastic tweezer, place the coverslip on the glue circle, the micropatterned side facing down (toward the inside of the dish). Very gently press on the edges of the coverslip to push it against the dish and spread the glue. The circle of glue should be continuous so that the dish does not leak.
5. Let the assemble chamber dry overnight at 4°C. At this stage the assembled chamber can be conserved for approximately a week.

Step 4 – Application with the protein of interest

Because of the shorter conservation time of fibronectin, this step is only performed one day before or the day of the experiment.

1. Prepare a 10 µg/mL fibronectin solution in NaHCO₃ (pH 8.3).
2. Prepare a disc of Parafilm of 18 mm in diameter.
3. Pipet 150 µL of the fibronectin solution on the micropatterned glass surface of a dish.
4. Using a tweezer, place the Parafilm disc as a cap over the fibronectin droplet, in order to spread it on the entire surface of the coverslip as a uniform layer. Incubate for 20 to 30 minutes.
5. Without removing the Parafilm disc, add rapidly 2 mL of PBS in the dish. The disc of Parafilm should get detached and float at the surface. Remove it and rinse twice more with warm imaging medium.

1.3.6. Cells handling

Goal: Have the cell intake M-450 Dynabeads, and transfer them to the experimental chamber. This part of the protocol is very dependent on the cell type used. We propose two options: one for the

primary mouse Dendritic Cells (DC), that can intake beads fast and are not strongly adherent, and another for the 3T3 fibroblasts, that intake the beads less efficiently and are strongly adherent.

Dendritic Cells

Single Step – Transfer in experimental chamber & Incubation with beads.

1. In the BSA coated petri dish, transfer 1 mL of cell in suspension in imaging medium at 2.5×10^5 cells/ml.
2. Mix 2.5 μ L of the coated M-450 Dynabeads solution (see 1.3.4) with 0.5 mL imaging medium. Vortex the mix, and add it in the chamber, in order to obtain a 1:1 bead-to-cell ratio. Let incubate at 37°C, 5% CO₂ for 1 hour.
3. After 1h, roughly 25% of cells are expected to have ingested at least one bead. Verify that it is the case using a simple phase contrast microscope.
4. Optional - Only if the environment chamber on the microscope does not control CO₂. Add 0.5 mL of imaging medium supplemented with HEPES to bring the concentration of HEPES in the medium to 20 mM.

3T3 Fibroblasts

Step 1 – Incubation with beads

1. 2 days before the experiment, pass approximately 2×10^5 cells in a 25 cm² culture flask.
2. Mix 6 μ L of the coated M-450 Dynabeads solution (see 1.3.4) in 1 mL of warm culture medium. Vortex the mix and add it to the flask. Gently rock the flask to homogenize the distribution of beads.
3. After 2 days, a significant fraction of cells are expected to have ingested at least one bead (10 to 50% according to bead preparation). Verify that it is the case using a simple phase contrast microscope.

Step 2 – Transfer in experimental chamber

1. On the day of the experiment, detach the 3T3 cells from their flask with TrypLE and resuspend them in warm imaging medium.
2. In a micropatterned dish with 20 μ m fibronectin discs (as prepared in 1.3.5) transfer 2 mL of cell suspension at 1.5×10^5 cells/ml. Let them sediment and adhere to the patterns for 20 min, at 37°C 5% CO₂.
3. Check that most micropatterned discs are occupied by a cell that has started to adhere on the substrate.

4. Flush the bottom of the chamber to remove the non-adherent cells. In order to do this, hold a pipet in both hand and inject warm medium on one side as you aspire on the other side of the chamber. Never let the chamber dry. The flushing will generate a flux of medium in the chamber and efficiently remove the excess of cells, leaving only the adherent ones.
5. Let the cells adhere completely for 2 hours at 37°C and 5% CO₂.

Step 3 – Outer beads addition

1. Prepare a mix of 0.5 mL of warm imaging medium and 2.5 µL of the coated beads solution (see 1.3.4.). Vortex the mix and add it to the dish.
2. Optional - Only if the microscope incubator does not control CO₂. Add 0.5 mL of imaging medium supplemented with HEPES solution to bring the concentration of HEPES in the medium to 20 mM.

1.3.7. Magnetic Pincher imaging

After placing the sample onto the microscope stage where the magnetic field generator is mounted, the first step is to acquire Z-stacks of beads. They will be used to compute a “Depthograph” (see section 1.4 and Fig. 8). It is preferable to perform this step in the experimental dish containing the cells, in the absence of magnetic field: the formation of chains of beads would hinder the acquisition of clean Z-stacks. Then, the magnetic field can be applied to trigger the beads self-organization in the whole chamber, and many pinching event should occur simultaneously. For each imaged cell, acquire a 10 min time-lapse with a stack of images at different Z-positions for each time point (Fig. 6A). Together with a log file indicating the precise time for the acquisition of each image, these movies are the raw data processed by the image analysis software to determine the cortex thickness as a function of time.

[Fig. 6]

Step 1 – Z-scan of beads (reference Depthograph)

1. Set the microscope light power and the field aperture as explained in 1.2.4. These setting should not change throughout the whole imaging session.
2. Position one or several beads in the field of view. These beads should be clearly separated from each other, perfectly still, and their image not affected by any object in the chamber or on the optical path. Position the focus in the equatorial plane of the beads (see 1.2.4).
3. Acquire a Z-stack of these beads: 401 steps every 20 nm, for a total course of 8 µm. The piezo element must be used to ensure the precision of the displacement along Z.
4. Repeat steps 2 and 3 until at least 8 Z-stacks of beads have been successfully acquired.

Step 2 – Magnetic Pincher on live cells

1. “Switch on” the magnetic field generator. This is done by either placing the Halbach array on its support, around the sample; or by sending current in the Helmholtz coils, according to the chosen option. The field magnitude should be comprised between 3 and 10 mT, typically 5 mT, and be kept constant across experiments to obtain comparable results. It is strongly recommended to use a Gaussmeter to measure in situ the applied field.
2. Position a cell whose cortex is pinched by a pair of beads in the field of view. In addition to the guidelines defined in section 1.2.4, the following criteria must be met:
 - a. These beads should be as much as possible aligned along the magnetic field lines and in the same plane.
 - b. The light spot below them should not be hindered by extra or intra-cellular objects.
 - c. The pair of beads can be part of a longer chain that extend into, or out of the cell, or even both. However, beads pinching the cortex must have a maximum of 2 nearest-neighbors (one of them being automatically the other bead of the pair) that are well aligned with the line of magnetic field. Otherwise the magnetic field in the region of the beads will not be properly defined.
3. Acquire a time-lapse of these beads (Fig. 6A, 5B):
 - a. 2 to 10 minutes, one z-triplet of image every 600 ms, for a total of 200 to 1000 time-points. Shorter durations allow for more movies of different cells to be acquired. Longer time-lapse movies allow for a more accurate measurement of each cell cortex behavior.
 - b. Each z-triplet is a short Z-stack of 3 images, with a step of 0.5 μm , ideally centered on the plane of maximum intensity. The piezo element must be used to ensure the precision of the displacement along Z.
 - c. Allow at least a delay of 50 ms between each step of the Z-stack, to ensure the precision of the positioning along Z.
4. Make sure that the software used for the acquisition also saves the precise time at which each image was taken. This can be attached to the images metadata or saved in a separate log file.
5. Repeat steps 2 and 3 to acquire a dataset representative of your cell population. A given chamber should not be imaged for longer than 2 hours. Typically 10 to 20 cells should be acquired in each chamber, depending on the set duration of the acquired time-lapse movies.

1.4. Image analysis

This section details the image analysis workflow used to extract the cortical thickness as a function of time from the acquired time-lapse. It requires two different softwares: ImageJ (in our case, Fiji: Schindelin et al., 2012) and Python. To analyze a group of time-lapse imaged from the same dish, the first task is to generate a Depthograph. It simply consists in a YZ-plane averaged profile of the bead, used to determine the relative positions of the beads along the Z-axis (Gosse and Croquette, 2002). Then a simple tracking algorithm is applied to the time-lapse movies of Magnetic Pincher, to compute the trajectories of the beads in 3D. As many other tracking algorithms, ours proceed in two classic steps: (i) Objects segmentation and (ii) Frame-to-frame matching. Step (i) is done semi-automatically with Fiji while step (ii) has been automatized in a Python function.

The Python code can be found on Github on the following repository: https://github.com/jvermeil-biophys/CortExplore_MIMB.git. The main libraries required to run the code are the following: os, time, numpy, pandas, scipy, scikit-image (Van Der Walt et al., 2014), matplotlib and pyautogui.

1.4.1. An important method: Detect beads position in the XY plane using Fiji

This detection method is routinely used across many of the image analysis workflows presented in this chapter. While simple, it allows a detection of the center of a bead in the XY plane with a very high precision. It consists in segmenting of the light spot below a bead, then computing its center of mass, using the pixel value as weight (see Fig. 7).

1. In the Fiji software, open an image or a movie containing beads (Fig. 7A).
2. In “Set Measurement” select “Area”, “Standard deviation”, “Center of Mass”, “Stack Position”.
3. Use “Image/Adjust/Threshold” to segment manually the light spots under the beads (Fig. 7B, 6C). Enable the “Stack histogram” option. If the file analyzed is a movie, the same threshold value is used to segment the beads in all the frames of the movie. The regions segmented should cover each light spot, be roughly circular and have a diameter around 1 to 2 μm . Do not click on any of the buttons and let the “Threshold” dialog box opened with the thresholded regions visible on the image.
4. Use the “Image Particle” tool to measure the properties of each region (Fig. 7D), with the following options enabled: “Display results”, “Exclude on edges”, “Include holes”, “Clear results”. Adjust the area and circularity criterion so that the only objects analyzed are the segmented light spots. Select “Show: Outlines” to visually check that all the light spots were analyzed.
5. Save the Results table (Fig. 7E) in .txt format.

[Fig. 7]

1.4.2. Make a Depthograph from the bead Z-stacks

In order to make a reference Depthograph, process bead Z-stacks acquired for a given experiment to compute a typical YZ profile of the beads.

Step 1 – Detection of the beads center, in Fiji.

1. In the Fiji software, open a Z-stack of one or several beads (acquired as specified in 1.3.7. – Step 1). For each bead visible, crop a small rectangular region of interest around the bead and save the resulting Z-stack in .tif format.
2. Open one of these single-bead Z-stack.
3. Use the method detailed in section 1.4.1. to detect the position of the center of the bead on most frames of the Z-stack. Not managing to detect the light spot on the first and the last frames is normal. The light spot should be successfully segmented typically from frame 100 to frame 300 over a total of 401 in the stack, without any misdetection within this range. Check visually with the “Outlines” that it is the case.
4. Save the Results table in .txt format in the same folder as the corresponding .tif single-bead Z-stack. By convention, the name of this .txt file should be the same as the .tif file, appended with “_Results.txt”.

Step 2 – Computation of the Depthograph, in Python.

This task is performed using the Main_DepthoMaker.py script from the CortExplore_MIMB package.

In order to run it follow these steps:

1. Gather all the .tif bead Z-stack files for a given experiment in a folder. Each .tif file must be accompanied with the corresponding “_Results.txt” file generated in the previous step. The naming convention must be strictly respected.
2. Open the Main_DepthoMaker.py script in any development environment (e.g. Spyder). Run the line importing the depthoMaker() function from SimpleBeadTracker.py. If it returns an error, check that the working environment is defined as the “CortExplore_MIMB” folder (where the .py files are).
3. Indicate the paths of the relevant directories by filling the dictionary “dictPaths” according to the instructions in comment.
4. Indicate the value of the relevant parameters by filling the dictionary “dictConstants” according to the instructions in comment.
5. Run the line calling the depthoMaker() function. The resulting Depthograph, along with a “_Metadata.csv” file will be saved in the specified directory.

[Fig. 8]

The precise instructions to run this code are detailed in the Github repository documentation.

As a reference, here are the outlines of the functioning of the `depthoMaker()` function.

- i. Open a .tif Z-Stack of a bead. Using the previously computed Results file, on each frame, translate the image so that the center of mass of the bead is exactly in the center of the image. This is done using `skimage.transform.warp()` function with a bi-linear interpolation.
- ii. On each frame, take the vertical intensity profile of the bead. This can be done by averaging the 5 central vertical lines of the frame. This intensity profile is a 1D array of pixel value.
- iii. Concatenate all these arrays along a second dimension to obtain a 2D array of intensity profiles. The first dimension is Y the second is Z. This constitutes a Depthograph for a given bead.
- iv. Repeat the steps 1 to 3 for each stack of beads corresponding to an experiment. Average all the Depthographs, by using the plane of maximum intensity to match them. The resulting 2D array is the average YZ profile of beads imaged to produce the Depthograph of a given experiment.

1.4.3. Segment the beads with ImageJ

As a first step to track the beads positions in the Magnetic Pincher time-lapse, segment the light spots of the beads of interest in all the frames in ImageJ / Fiji and determine the position of their center.

1. In the Fiji software, open a Magnetic Pincher time-lapse. If necessary convert it to the .tif format.
2. If the images are organized as Z-T hyperstacks, flatten it to obtain a 1-dimensionnal stack where images are ordered in the following way: (t1, z1), (t1, z2), (t1, z3), (t2, z1), (t2, z2), (t2, z3), ..., (tN, z1), (tN, z2), (tN, z3); where t1 ... tN are all the successive time-points and z1, z2, z3 are the lower, middle and upper Z-planes, in that order. To do so, use the function "Image/Hyperstack/Hyperstack to Stack".
3. Use the method detailed in section 1.4.1. to detect the position of the center of each bead of interest in the time-lapse. Check visually with the "Outlines" plot that the light spots corresponding to the beads of interest were analyzed in nearly all the frames. Save the Results table in .txt format. By convention, the name of this .txt file should be the same as the .tif file, appended with "_Results.txt".
4. Repeat steps 1 to 3 for each of the acquired Magnetic Pincher time-lapse.

1.4.4. Track the beads in 3D with a custom-made python algorithm

The second step of the tracking is performed using the `Main_3DTracker.py` script from the `CortExplore_MIMB` package.

1. Gather all the .tif Magnetic Pincher time-lapses files for a given experiment in a folder. Each .tif file must be accompanied with 2 other files: (i) the corresponding “_Results.txt” file generated in the previous step and (ii) a file containing the precise date in millisecond of each image as a single text column. By convention, the name of this .txt file should be the same as the .tif file, appended with “_Timepoints.txt”.
2. Open the `Main_3DTracker.py` script in any development environment (e.g. Spyder). Run the line importing the `mainTracker()` function from `SimpleBeadTracker.py`. If it returns an error, check that the working environment is defined as the “CortExplore_MIMB” folder (where the .py files are).
3. Indicate the paths of the relevant directories by filling the dictionary “dictPaths” according to the instructions in comment.
4. Indicate the value of the relevant parameters by filling the dictionary “dictConstants” according to the instructions in comment.
5. If necessary set optional parameter values by filling the dictionary “dictConstants” according to the instructions in comment.
6. Run the line calling the `depthoMaker()` function.
7. For each Magnetic Pincher time-lapses file, the program will start by displaying the first frame, where detected beads labeled with orange crosses. If both of the beads pinching the cortex are properly detected on this first image, click on “Yes” then click on the positions of the two beads (the clicks on the image do not have to be very precise). If one of the two beads is not detected, click on “Next Frame” and continue.
8. As the program matches the positions of the beads frame by frame to build their trajectories, it might happen that the algorithm lose track of one of the beads of interest, either because it was not detected on a given frame, or because its frame-to-frame motion was significant. Then, the program displays this frame. Once again, if both of the beads pinching the cortex are properly detected on this first image, click on “Yes” then click on the positions of the two beads (the clicks on the image do not have to be very precise). If one of the two beads is not detected, click on “No”.
9. Once the program have built the trajectories, it will ask the user to assess for each of the beads of interest whether it is in or out of the cell (useful to save the trajectories), and whether it has 1 or 2 neighbors (useful to compute the pinching force).

10. [Automatic step] After these steps building the trajectories of the beads in the XY plane, the program compute automatically the motion of the beads along the Z-axis by comparing for each frame the vertical profile of the beads of interest to the Depthograph generated for this experiment. This step can be time-consuming.
11. [Automatic step] From the XY and Z positions of the beads, the program simply compute the 3D center-to-center distance between the beads.
12. [Automatic step] The program computes the force applied by the beads on the cortex for each time-point, using the method detailed in (Laplaud et al., 2021).
13. [Automatic step] Finally, the program saves a “_timeseries.csv” file in the specified folder.

The resulting “_timeseries.csv” file is a table containing the evolution with time (column “T”) of different quantities: the beads center-to-center distances along each axes (“dx”, “dy”, “dz”), in the XY-plane (“D2”) and in 3 dimensions (“D3”). It also contains the magnetic field (“B”, constant) and the force (“F”, varying due to the fluctuations in distance). To compute the time-resolved thickness of the cortex, subtract the beads average diameter (see 1.3.3) to the distance in 3 dimensions (“D3”). Average thickness of the cortex and fluctuation amplitude can be readily obtained from this time-series.

2. Limitations and pitfalls

Tracking along the Z-axis

One of the main difficulties with the deployment of the Magnetic Pincher comes from the tracking of beads along the Z-axis: the fact that it requires stacks of three images at different Z adds complexity to the setup. This may be solved in future developments of the technique, either using deep learning to better analyze the features of the images, or improving the setup with quantitative phase imaging to obtain an additional source of information to track the beads along the Z-axis (see Bon et al., 2015). Both of these upgrades could enable the tracking of microbeads based on a single Z-plane.

Throughput

Since this technique involves a magnification that do not usually allow several pinched cells to be observed in the same field of view, it does not have a very high throughput: within a day of experiment, a few tens of cells can be probed. This can be mitigated by acquiring shorter time-series.

Micropatterned chamber manufacturing

For adherent cells that requires a micropatterned substrate, the experimental chamber manufacturing can prove challenging. To adopt a “quality-control” approach when optimizing this step, it is useful to make the micropatterns fluorescent, by adding 3 µg/mL of labeled fibrinogen in

the fibronectin solution (Fibrinogen from Human Plasma, Alexa Fluor Conjugate, Thermo Fischer, USA).

Beads imaging

If the bead appearance in trans-illumination seems very different than on Fig. 5C, one should adjust the field diaphragm and light source intensity. Setting the Köhler conditions is a good starting point. The “dotty” effect due to the pinholes when using a spinning disc microscope in bright field mode can be problematic (this might be the case when performing simultaneous fluorescence imaging using such a microscope).

Bead uptake by the cells

The uptake of M-450 Dynabeads by cells is a crucial step that affects a lot the overall success and throughput of the technique. It is recommended to optimize carefully this step when using the technique for the first time or when applying it to a new cell type. We suggest two alternative coating options (see section 1.3.4) to increase or decrease the affinity of cells for beads. Increasing it is useful to ensure enough beads are ingested. Decreasing it is useful for outer beads if the cells are very prone to ingest them during time-lapse acquisitions.

- To increase bead uptake: coat the beads with fibronectin.
- To decrease bead uptake: coat the beads with mPEG.

With fibronectin

The protocol is the same than with complete medium (section 1.3.4), but replacing the medium in step 1 by 100 μ L of fibronectin diluted in NaHCO₃ (pH 8.6) at 25 μ g/mL (see Materials section).

With mPEG

Beads with a less-adhesive surface can be made by using the Dynabeads included in the CELlection™ Biotin Binder Kit (11533, Thermo Fisher, USA). These are M-450 Dynabeads functionalized with streptavidin via a DNA linker, which allows to bind biotinylated proteins to the surface.

Follow the rinsing protocol mentioned above (section 1.3.4) but replace the complete medium in Step 2 with a solution of mPEG(5K)-Biotin (JKA3097, Merck, Germany) in HEPES (10 mM, pH 7.4) at a concentration of 1 mg/mL.

Assess if a bead is inside a cell

To ensure a bead is inside a cell at high magnification, focus on the equatorial plane of the bead (Fig. 1E). At low magnification, gently moving the experimental chamber to induce sloshing can help to distinguish the floating beads from the engulfed ones.

Difficulty to find an adequate pinching event

In such case, one can adjust the cell-to-beads ratio suggested in section 1.3.6. If many cells have a bead inside but none in their close environment, one can add additional beads during the imaging, or induce some gentle motion of the experimental chamber to make free beads move.

Motion of the cells during imaging

Depending on cell types, the cell activity can be very likely to cause a motion of the beads in X, Y and Z. In this case one should follow the beads with the stage and with the focus to ensure the imaging conditions of the beads stay as good as possible throughout the acquisition: as the thickness is computed from the beads relative distance, their absolute position in the field of view is not important.

Difficulty when segmenting beads

In the method described in section 1.4.1 the thresholding should ideally be stringent and allow to segment only the beads light spots. However it is often not possible, for instance because the beads moved up or down during the acquisition. Then a solution is to increase significantly the threshold value and define a rectangular ROI around the beads, before running the “Analyze Particle” tool.

Difficulty when making a Depthograph

If Depthographs look noisy or discontinuous, it might be because of one of the Z-stacks used to compute it. Select only the good beads for those Z-stacks: as fixed to the substrate as possible. Do not hesitate to rule them out a posteriori if a given Z-stack gives a very noisy Depthograph.

Difficulty when tracking the beads

If one of the bead of interest is not detected on too many frames of a time-lapse, the tracking step in python will take a very long time. Do not hesitate to re-do the segmentation step described in section 1.4.1 to try to improve the beads detection. Or adjust the imaging parameters for next experiment.

3. Discussion

The Magnetic Pincher can probe the actin cortex thickness in live cells and monitor its fluctuations with high precision and time resolution. One of the key feature of this technique is its flexibility: the version using the Halbach array to generate the field can easily be installed on any microscope, and the requirements regarding the imaging setup are easy to fulfil (simple bright field illumination). It is also surprisingly easy to apply to many different cell types: while one might think that having non-immune cells to ingest $4.5\text{ }\mu\text{m}$ large beads is a hard task, some adjustments to the beads coating (see Section 2) can make it very robust. As of today this technique have been successfully applied to

mouse primary dendritic cells and 3T3 fibroblasts, but also to Dictyostelium amoeba, to HoxB8 immortalized macrophages, RPE1 human retinal pigment epithelial cells and to MDCK epithelial cells. It is also simple to use the Magnetic Pincher in combination with other classic tools in cell biology: chemical drugs, epifluorescence imaging or optogenetic systems.

An important point to be made regards the definition of the cortical thickness we measure with the Magnetic Pincher. As recent observations have shown, the actin cortex is not a homogeneous layer: the density of actin (Clausen et al., 2017) and other components (e.g. myosin II, see Truong Quang et al., 2021) varies along the cortex depth. It is also connected to the rest of the actin cytoskeleton. Here we define the thickness of the actin cortex based on its mechanical properties: it is defined as the layer that withstand the small stress applied by the beads pinching it. This means the measured value is not exactly the thickness as it could be defined based on actin density. Future developments should allow to compare the various measures. The Magnetic Pincher based definition is convenient when considering the mechanical function of the actin cortex, because a fundamental attribute of the cortex is its density in branched and crosslinked actin filaments, which can withstand the applied stress, as opposed to the more loosely connected filaments present in the cytoplasm.

A natural extension of this technique consists in using the beads to indent the cortex more significantly by applying a controlled force. This way, by tracking the beads to compute the cortex deformation in response, we can compute mechanical characteristics of the cortex, such as an elastic modulus (Laplaud et al., 2021). The cortex being a very complex material (viscoelastic, non-linear, active and non-uniform) interpreting this type of measurement is challenging and their in-depth characterization is still under development. Yet the Magnetic Pincher, which can apply a large range of forces directly on the cortical layer, seems like a promising tool for these rheology experiments.

References

- Azioune, A., Storch, M., Bornens, M., Théry, M., Piel, M., 2009. Simple and rapid process for single cell micro-patterning. *Lab. Chip* 9, 1640. <https://doi.org/10.1039/b821581m>
- Bauër, P., Tavacoli, J., Pujol, T., Planade, J., Heuvingh, J., du Roure, O., 2017. A new method to measure mechanics and dynamic assembly of branched actin networks. *Sci. Rep.* 7, 15688. <https://doi.org/10.1038/s41598-017-15638-5>
- Bon, P., Bourg, N., Lécart, S., Monneret, S., Fort, E., Wenger, J., Lévêque-Fort, S., 2015. Three-dimensional nanometre localization of nanoparticles to enhance super-resolution microscopy. *Nat. Commun.* 6, 7764. <https://doi.org/10.1038/ncomms8764>
- Carpi, N., Carpi, N., Piel, M., Azioune, A., Fink, J., 2011. Micropatterning on glass with deep UV. *Protoc. Exch.* <https://doi.org/10.1038/protex.2011.238>
- Charras, G.T., Hu, C.-K., Coughlin, M., Mitchison, T.J., 2006. Reassembly of contractile actin cortex in cell blebs. *J. Cell Biol.* 175, 477–490. <https://doi.org/10.1083/jcb.200602085>
- Chugh, P., Clark, A.G., Smith, M.B., Cassani, D.A.D., Dierkes, K., Ragab, A., Roux, P.P., Charras, G., Salbreux, G., Paluch, E.K., 2017. Actin cortex architecture regulates cell surface tension. *Nat. Cell Biol.* 19, 689–697. <https://doi.org/10.1038/ncb3525>
- Chugh, P., Paluch, E.K., 2018. The actin cortex at a glance. *J. Cell Sci.* 131, jcs186254. <https://doi.org/10.1242/jcs.186254>
- Clark, A.G., Dierkes, K., Paluch, E.K., 2013. Monitoring Actin Cortex Thickness in Live Cells. *Biophys. J.* 105, 570–580. <https://doi.org/10.1016/j.bpj.2013.05.057>
- Clausen, M.P., Colin-York, H., Schneider, F., Eggeling, C., Fritzsche, M., 2017. Dissecting the actin cortex density and membrane-cortex distance in living cells by super-resolution microscopy. *J. Phys. Appl. Phys.* 50, 064002. <https://doi.org/10.1088/1361-6463/aa52a1>
- Eghiaian, F., Rigato, A., Scheuring, S., 2015. Structural, Mechanical, and Dynamical Variability of the Actin Cortex in Living Cells. *Biophys. J.* 108, 1330–1340. <https://doi.org/10.1016/j.bpj.2015.01.016>
- Fonnum, G., Johansson, C., Molteberg, A., Mørup, S., Aksnes, E., 2005. Characterisation of Dynabeads® by magnetization measurements and Mössbauer spectroscopy. *J. Magn. Magn. Mater.* 293, 41–47. <https://doi.org/10.1016/j.jmmm.2005.01.041>
- Fritzsche, M., Lewalle, A., Duke, T., Kruse, K., Charras, G., 2013. Analysis of turnover dynamics of the submembranous actin cortex. *Mol. Biol. Cell* 24, 757–767. <https://doi.org/10.1091/mbc.e12-06-0485>
- Gosse, C., Croquette, V., 2002. Magnetic Tweezers: Micromanipulation and Force Measurement at the Molecular Level. *Biophys. J.* 82, 3314–3329. [https://doi.org/10.1016/S0006-3495\(02\)75672-5](https://doi.org/10.1016/S0006-3495(02)75672-5)
- Halbach, K., 1985. Application of permanent magnets in accelerators and electron storage rings (invited). *J. Appl. Phys.* 57, 3605–3608. <https://doi.org/10.1063/1.335021>
- Laplaud, V., Levernier, N., Pineau, J., Roman, M.S., Barbier, L., Sáez, P.J., Lennon-Duménil, A.-M., Vargas, P., Kruse, K., Roure, O. du, Piel, M., Heuvingh, J., 2021. Pinching the cortex of live cells reveals thickness instabilities caused by myosin II motors. *Sci. Adv.* 7, eabe3640. <https://doi.org/10.1126/sciadv.abe3640>
- Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.-Y., White, D.J., Hartenstein, V., Eliceiri, K., Tomancak, P., Cardona, A., 2012. Fiji: an open-source platform for biological-image analysis. *Nat. Methods* 9, 676–682. <https://doi.org/10.1038/nmeth.2019>
- Svitkina, T.M., Neyfakh, A.A., Bershadsky, A.D., 1986. Actin cytoskeleton of spread fibroblasts appears to assemble at the cell edges. *J. Cell Sci.* 82, 235–248. <https://doi.org/10.1242/jcs.82.1.235>
- Tretiak, O., Blümmler, P., Bougas, L., 2019. Variable single-axis magnetic-field generator using permanent magnets. *AIP Adv.* 9, 115312. <https://doi.org/10.1063/1.5130896>

Truong Quang, B.A., Peters, R., Cassani, D.A.D., Chugh, P., Clark, A.G., Agnew, M., Charras, G., Paluch, E.K., 2021. Extent of myosin penetration within the actin cortex regulates cell surface mechanics. *Nat. Commun.* 12, 6511. <https://doi.org/10.1038/s41467-021-26611-2>

Van Der Walt, S., Schönberger, J.L., Nunez-Iglesias, J., Boulogne, F., Warner, J.D., Yager, N., Gouillart, E., Yu, T., 2014. scikit-image: image processing in Python. *PeerJ* 2, e453. <https://doi.org/10.7717/peerj.453>

Figure 1 – Overview of the Magnetic Pincher steps

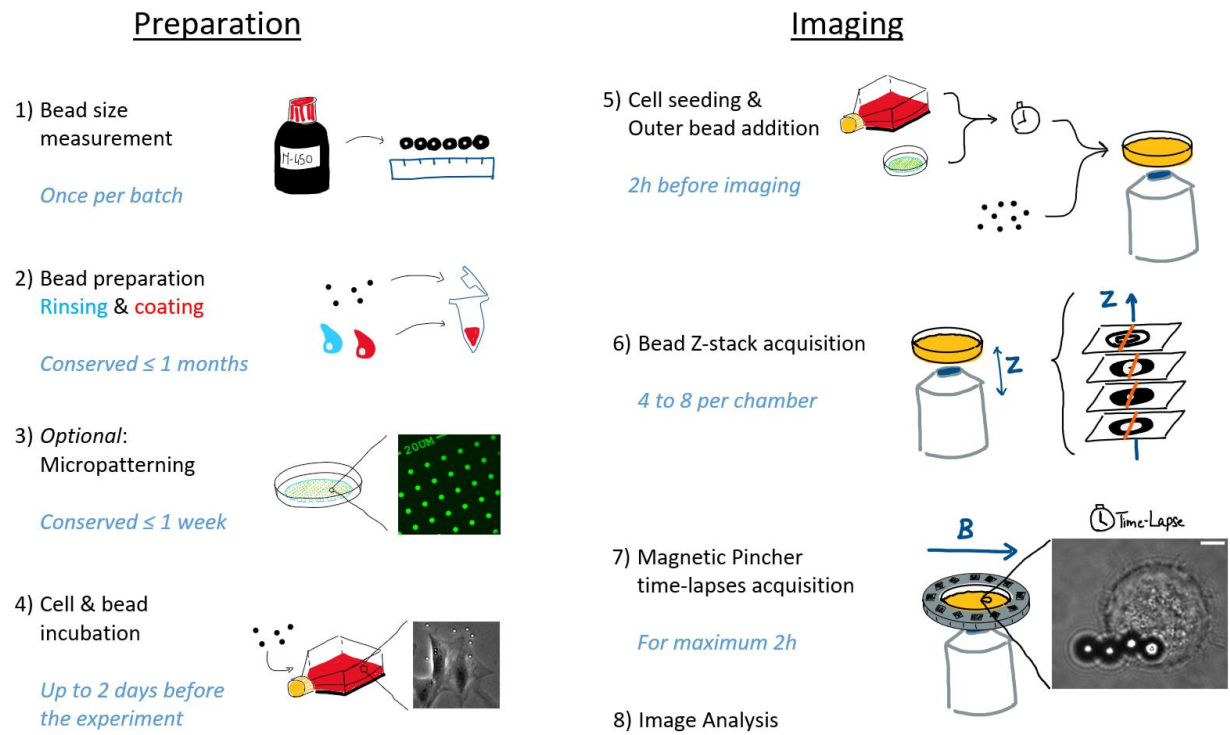
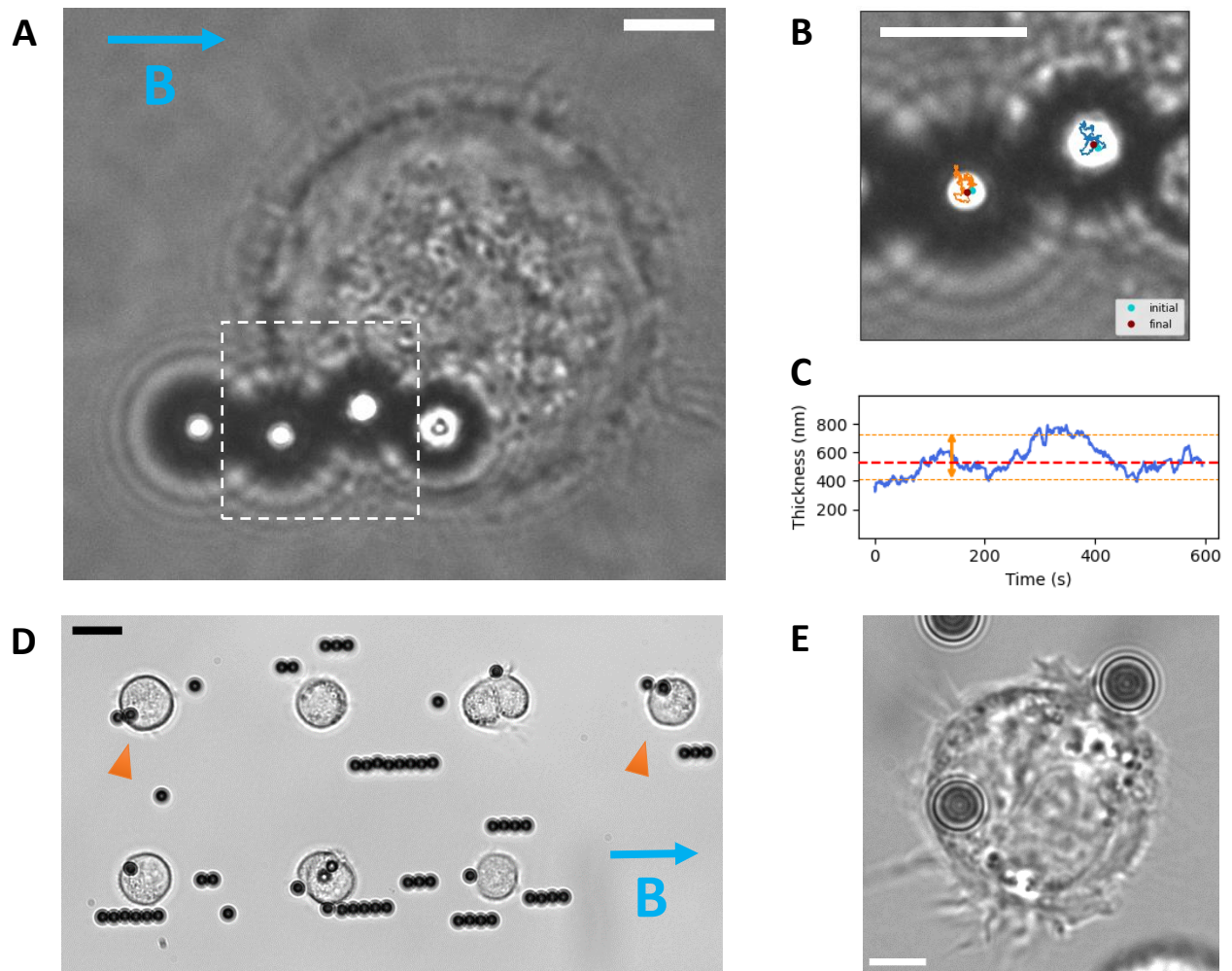
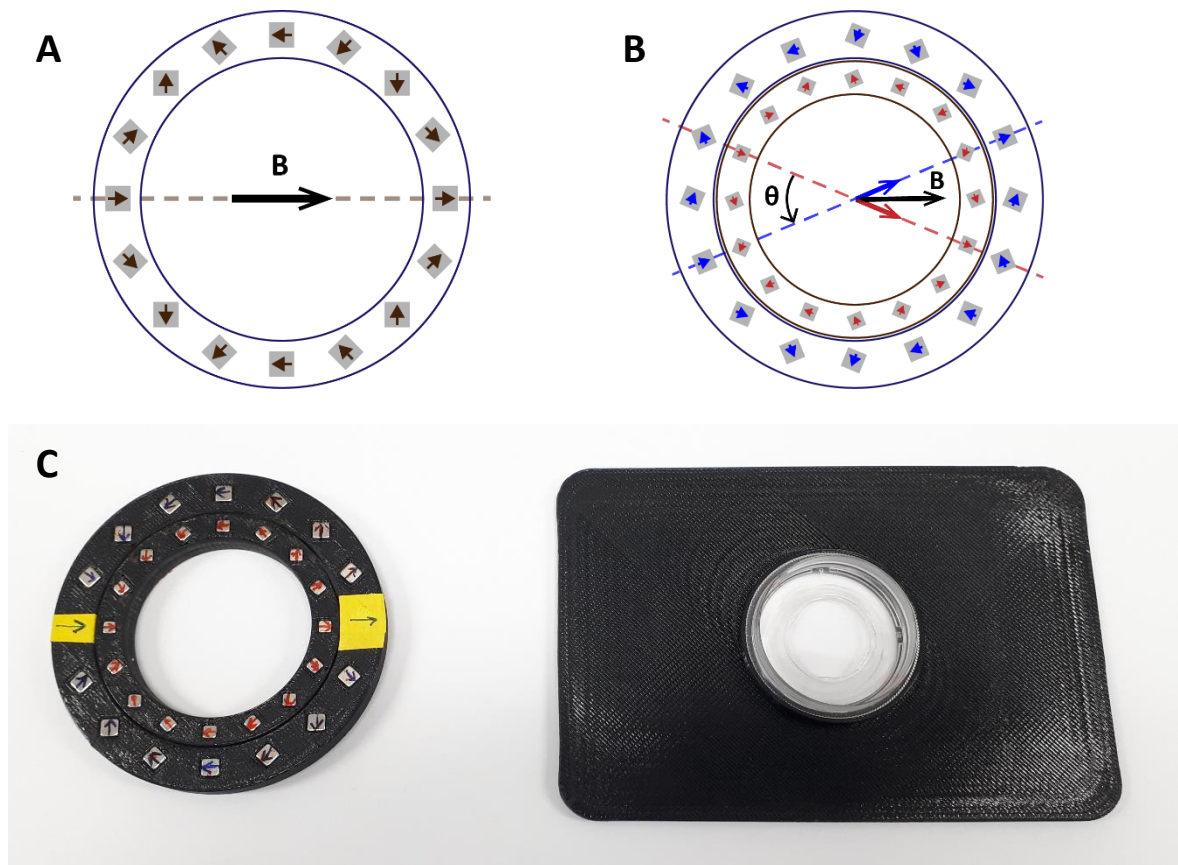


Figure 2 – Principle of the Magnetic Pincher technique



A – 3T3 fibroblast with its cortex pinched by superparamagnetic M-450 Dynabeads. The image was acquired with brightfield microscopy, objective 100X, NA = 1.4, scale bar = 5 μm . The cell is adhering on a 20 μm micropatterned fibronectin disc and cannot spread more because the surrounding substrate is coated with PLL-g-PEG. The cell has ingested two beads and two outer beads have been attracted toward them, the ensemble forming a chain aligned with the magnetic field (blue arrow). The two beads in the white frame are the ones pinching the cortex. Here the focus is made on the light spot below the beads. NB: The bright spot is saturated in this image for better visualization, but should not be saturated during the course of the experiment. B – A zoom on the region marked by the white frame, scale bar = 5 μm . The trajectories of the beads within a 10 minute long observation are shown in orange and blue. C – Cortical thickness as a function of time (blue line) for this 3T3 fibroblast. The median (red dashed line) and the inter-decile difference (D9 - D1, orange lines and arrow) are the metrics used to characterize respectively the typical cortical thickness and the fluctuation amplitude. Here the median is 525 nm and the fluctuations amplitude 320 nm. D – A larger field of view of a Magnetic Pincher experiment, with 3T3 cells adhering on 20 μm fibronectin discs. The orange arrows point to two pinching event suitable for image acquisition. Magnification 40X, scale bar 20 μm . E – A 3T3 cell with one M-450 inside, with the focus made on the bead equatorial plane. The cell is adhering on a 20 μm fibronectin disc; scale bar 5 μm .

Figure 3 – Halbach Array



A – Schematic of a simple circular Halbach array. On the circle, the magnetic moment vectors of magnets are represented as arrows; in the center, the resulting magnetic field created inside the Halbach. Note the way magnets are arranged: as one rotate along the circle, the magnets' moment direction rotate twice as fast. B – Schematic of nested circular Halbach arrays. The two arrays are designed to generate fields of equal magnitudes B_0 (blue and red arrows in the center). The direction of this field is figured for the outer and inner arrays by the dashed lines (red and blue respectively). Rotating one with respect to another by an angle θ allow to tune the magnitude of the total field (black arrow): $B = 2B_0 \cdot \cos(\theta/2)$. C – 3D-printed Halbach corresponding to Table 1 specifications. Right: the nested Halbach arrays. Left: The associated rectangular support which has the same outer dimensions as a 6-well plate. A central cylindrical extrusion allow to fit perfectly an experimental chamber inside (here a micropatterned chamber, see section 1.3.5) and the Halbach arrays outside.

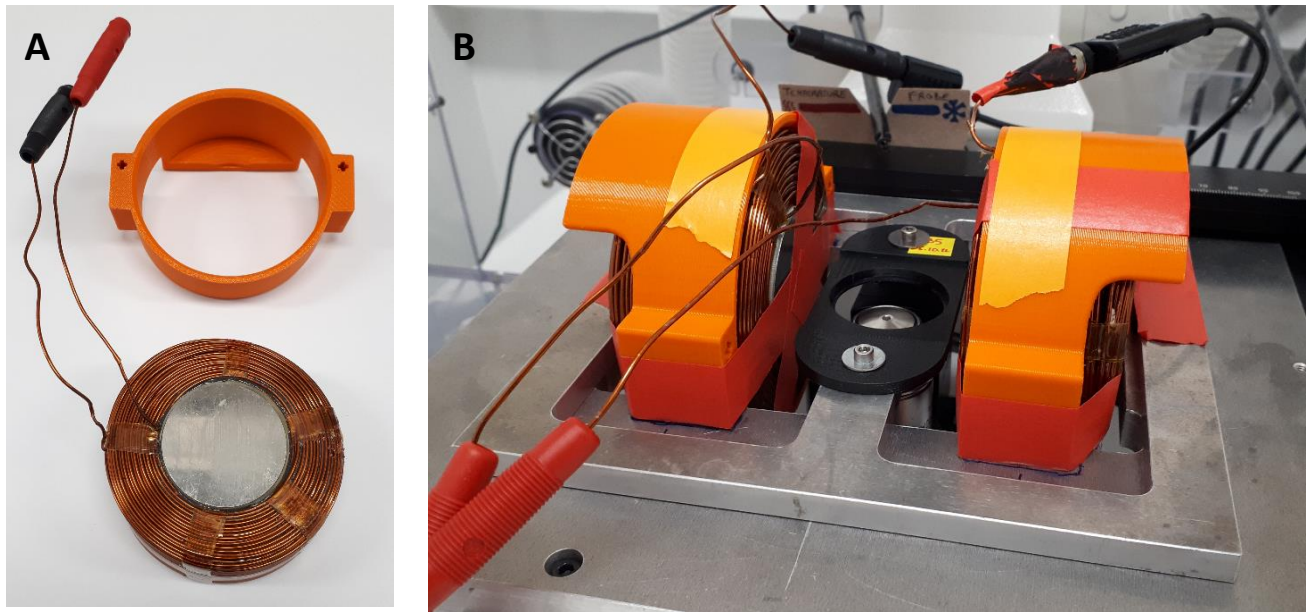
Table 1 – Example of nested Halbach array design – Technical specifications

Support	
Length x Width x Thickness	127 x 85 x 3.5 mm
Inner array	
Inner / Central / Outer radius	21 / 24.75 / 28 mm
Height	5 mm
Magnet side length	3 mm
Number, type of magnets	16, N42 Neodymium
Outer Array	
Inner / Central / Outer radius	28 / 33 / 38 mm
Height	5 mm
Magnet side length	4 mm
Number / type of magnets	16 / N42 Neodymium
Magnetic Properties	
Maximum magnetic field magnitude (arrays in the same direction)	Ideally: 8.6 mT Experimentally: 8.3 mT
Minimum magnetic field magnitude (arrays in opposite directions)	Ideally: 0 mT Experimentally: 0.2 mT
Gradient over the central 1 cm region	< 0.11 mT.mm ⁻¹

Table 2 - Comparison of the two magnetic field generation solution

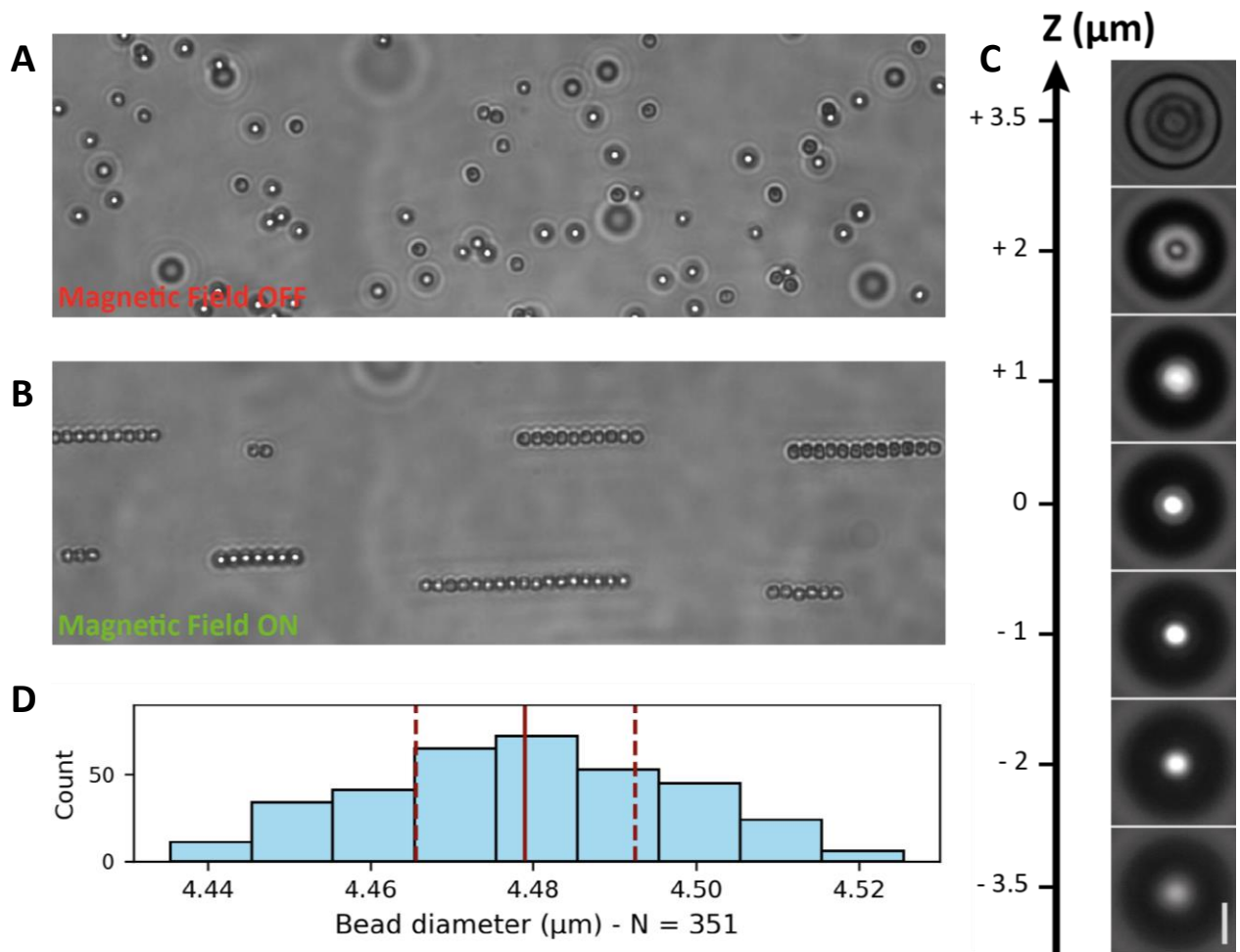
	Halbach array	Coils
Fabrication	<ul style="list-style-type: none"> - The body can be 3D-printed - Magnets are available at low cost 	<ul style="list-style-type: none"> - Need of a custom manufacturing
Size	Can be designed with the size of a 6-wells plate or a 10 cm petri dish.	Each coil is 40 x 86 mm (length x outer diameter)
Mounting on a microscope	Simple, given the flexible design options.	Require a $\approx 140 \times 95$ mm rectangular hole in the microscope stage.
Generated field	<p>With a simple array: 1 fixed field, from 1 to 90 mT.</p> <p>With a nested array: tunable field, from 0 to 30 mT. The adjustments cannot be done live during an experiment.</p>	Field adjustable in live during the experiment by tuning the intensity of the current supplied to the coils. The field can go from 0 to 60 mT, but high magnitudes cannot be maintained too long, due to the Joule effect heating the coils.

Figure 4 – Coils and imaging setup



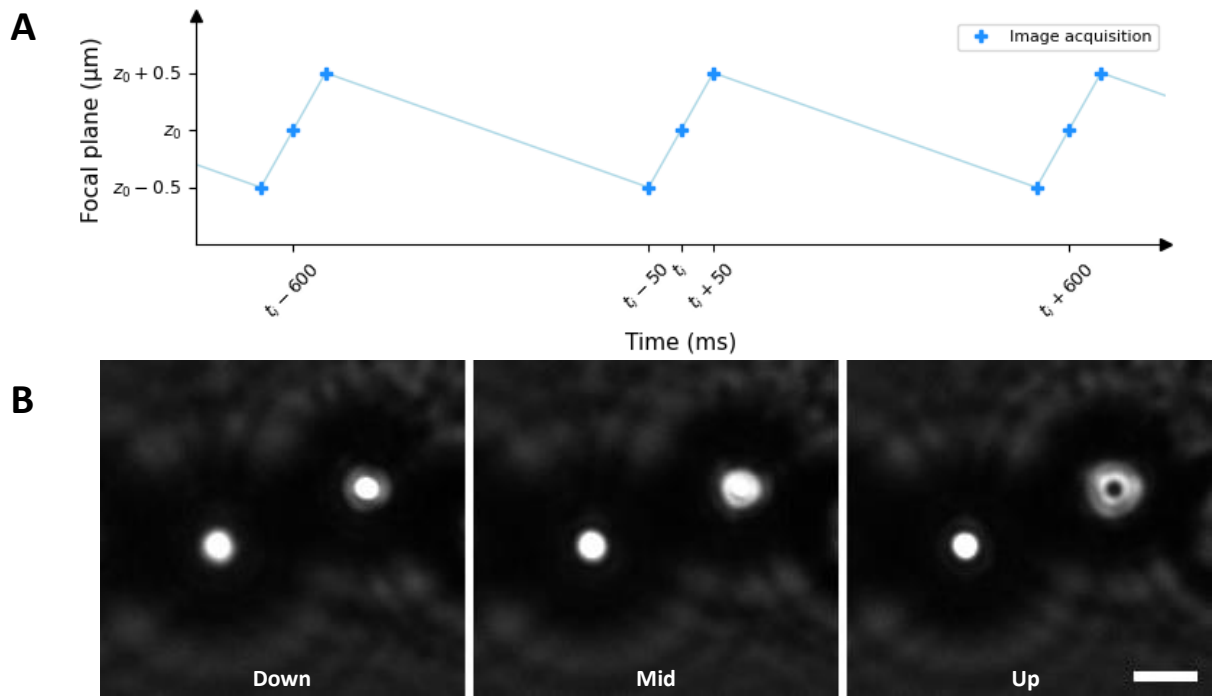
A – A coil and its case. The coil (bottom) itself is comprised of the mu-metal core, the spires, and the electric connectors. The role of the case (top) is to support the coil when it is mounted on the microscope stage. B – A pair of coils mounted on an inverted microscope stage. The coils in their cases are coaxial and equidistant from the objective. The stage comprises a rectangular hole to allow the coils to be mounted. Its mobile part is attached to an XY micromanipulator and support a 3D-printed dish-holder.

Figure 5 – M-450 Dynabeads



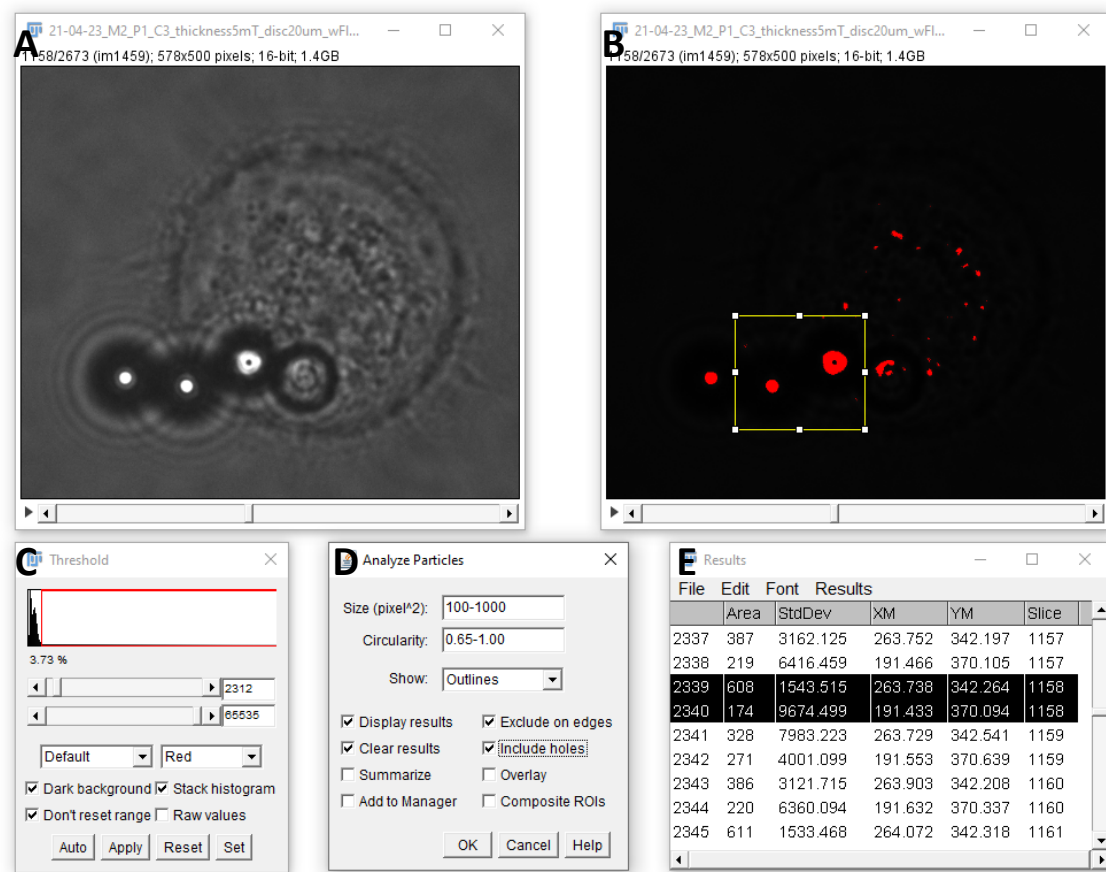
A, B – M-450 Dynabeads before (A) and after (B) exposition to an external magnetic field. Without field, the beads undergo Brownian motion. When exposed to an external field, the beads magnetize, and attract each other to form pairs or chain aligned with the field direction (horizontal here). C – Aspect of a bead along the vertical direction (scale bar: 2 μm). The zero of the Z-axis is here the plane where the light spot observed below the bead is the brightest. In planes higher or lower, the intensity decrease progressively, and the shape of the light spot changes. Here the pixel value range is the same on all the images but has been adjusted to increase the contrast. D – Histogram of the bead size distribution within one batch of M-450 Dynabeads, with a bin size of 10 nm. The red line is the median, the dashed lines are the quartiles. Over N = 351 beads, the average diameter is 4476 nm with a standard deviation of 19 nm.

Figure 6 – Time-lapse Acquisition



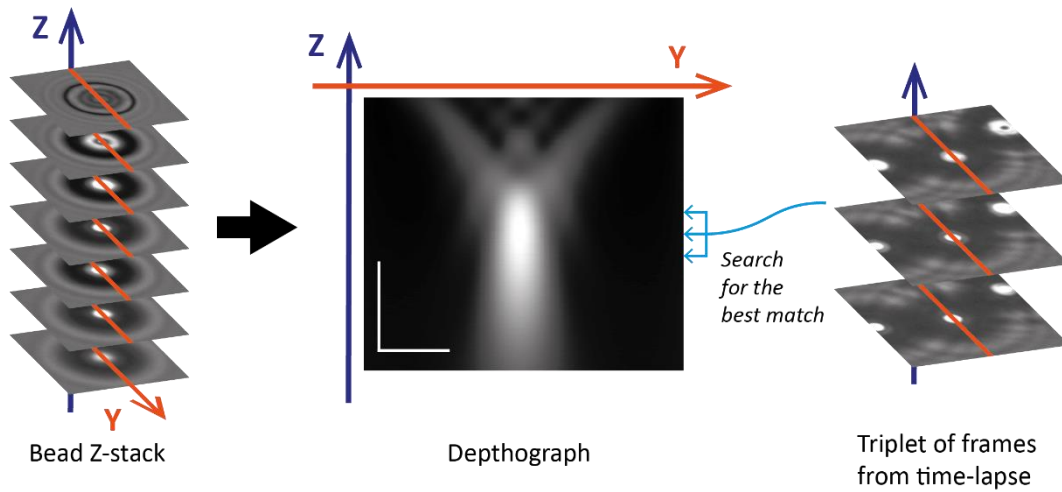
A – Acquisition scheme. For each time-point t_i , a stack of three frames is acquired. Those three frames are acquired every 50 ms and are distant of $0.5\ \mu\text{m}$ along the Z-axis. B – Example of such triplet of frames, taken from the same film as Fig. 1 (Scale bar: $2\ \mu\text{m}$). Note that here the two beads do not have the same Z coordinate: the left bead light spot is at its brightest in the “Up” frame, while for the right bead it is in the “Down” frame. The left bead is therefore slightly above the right bead. NB: The bright spot is saturated in this image for better visualization, but should not be saturated during the course of the experiment.

Figure 7 – Localize bead centers with Fiji



A – Raw frame of a Magnetic Pincher time-lapse. Only the contrast have been modified. The image has no scale (results in pixel values). B – The same frame, where the threshold have been applied and a rectangular region of interest have been drawn around the beads pinching the cortex. C – The “Threshold” dialog box. It has been manually adjusted to segment properly the beads light spot in all frames of the time-lapse. D – The “Analyze Particles” dialog box. The parameters shown here are the set typically applied. E – The “Results” windows that opens after running “Analyze Particles”. The two selected lines correspond to the two light spots segmented inside the ROI in (B). The columns XM and YM contain the coordinates of the centers of mass of the light spots (center weighted by the pixel values). Note that here, running “Analyze Particles” with the option “Show: Outlines” would also open an “Outlines” window (not shown here) useful to assess the quality of the detection.

Figure 8 – Depthograph



Computation and application of the Depthograph. On the left, a Z-stack used to generate the Depthograph. In the center, an example of such Depthograph, with the depth (Z) on the vertical axis and the profiles (orange lines) on the horizontal axis (vertical scale bar: 2 μm ; horizontal scale bar: 1 μm). On the right, a typical application of the Depthograph: to locate the bead along the Z-axis, one can take its profiles (orange lines) on the frames of a Z-triplet, and compare them with every row of the Depthograph to find the best match. This approach, using the fixed distance between the 3 frames (0.5 μm) as an additional information, ensure the uniqueness of the best match and improve the precision. Therefore each bead can be located within a common reference Depthograph and the distance ΔZ between the beads pinching the cortex can be computed.