

TensioX

Application tutorial

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I. Installation

TensioX can be installed and downloaded from <http://www.msc.univ-paris-diderot.fr/spip.php?rubrique274&lang=en>. The application is coded in MATLAB and the source code can be obtained at the same location.

No MATLAB license is required to run the application but MATLAB Runtime is required and is installed simultaneously with the application.

II. Introduction and principle

This application is designed to measure the surface tension and the Young modulus of magnetic multicellular spheroids by flattening them with an external magnet as described in [1].

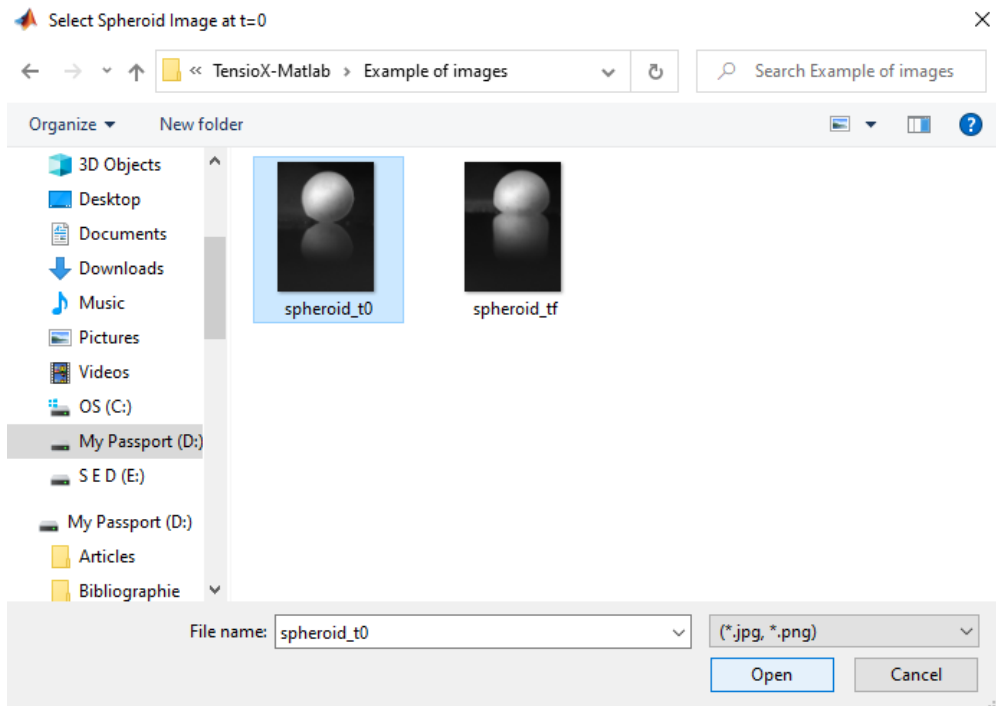
Briefly, iron oxide superparamagnetic nanoparticles (γ -Fe₂O₃) obtained via Massart's procedure are incorporated into the cells then spheroids are formed by magnetic molding (overnight formation). Finally, spheroids are flattened with an external magnet and the side profile of each spheroid is monitored with a camera. The equilibrium shape of the spheroid is determined by the competition between surface tension and magnetic forces. Surface tension is measured by fitting the spheroid side profile at equilibrium while Young modulus is determined thanks to the radius of the contact zone using Hertz theory.

To obtain the measurements, four elements are needed:

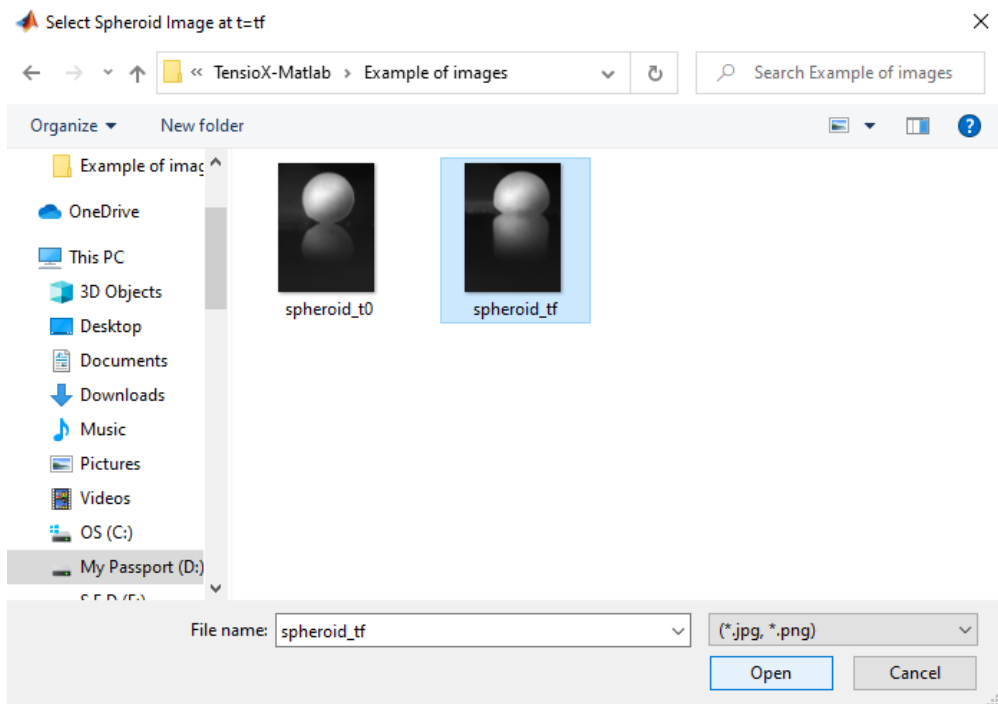
- A picture of the spheroid at t_0 (the initial spheroid needs to be spherical to ensure the accuracy of the measurements).
- A picture of the spheroid at t_f when the equilibrium shape of the spheroid under flattening is reached.
- The scale factor of the imaging system in m/pixels.
- The magnetic force per unit of volume applied on the spheroid $f_v = M_v \times grad(B)$, with M_v the magnetic moment per unit of volume (that can be measured using VSM, Vibrating Sample Magnetometry, measurements for example) and $grad(B)$ the magnetic field gradient of the external magnet at the position of the spheroid.

III. Step-by-step tutorial

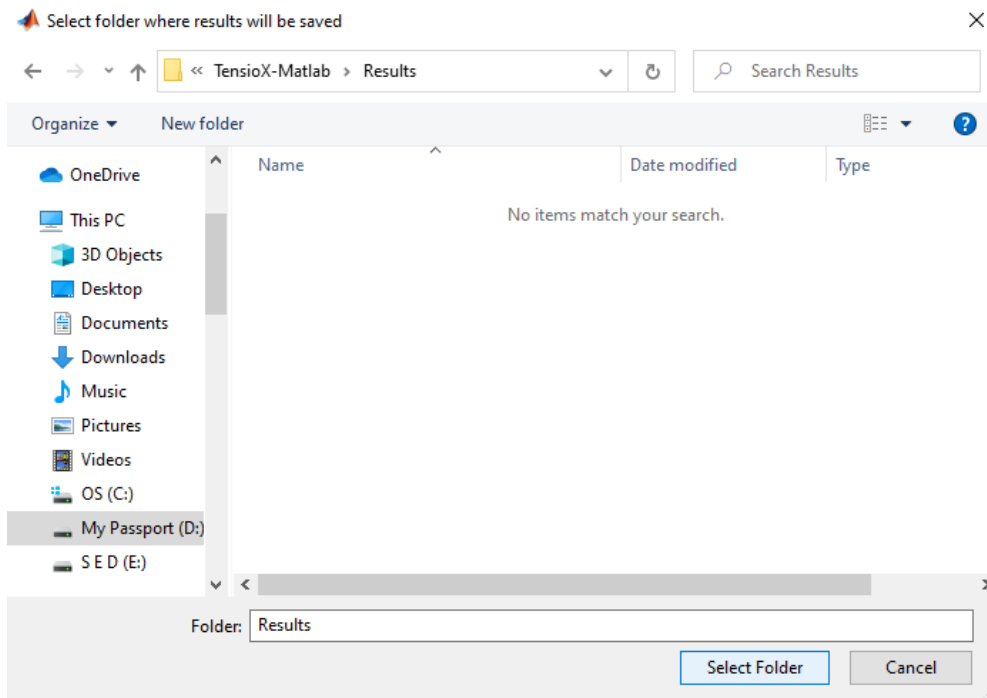
- Open TensioX.
- Select the spheroid image at $t = t_0$.



- Then select image of spheroid at $t = t_f$ when the equilibrium shape is reached.



- Select folder where results will be saved.

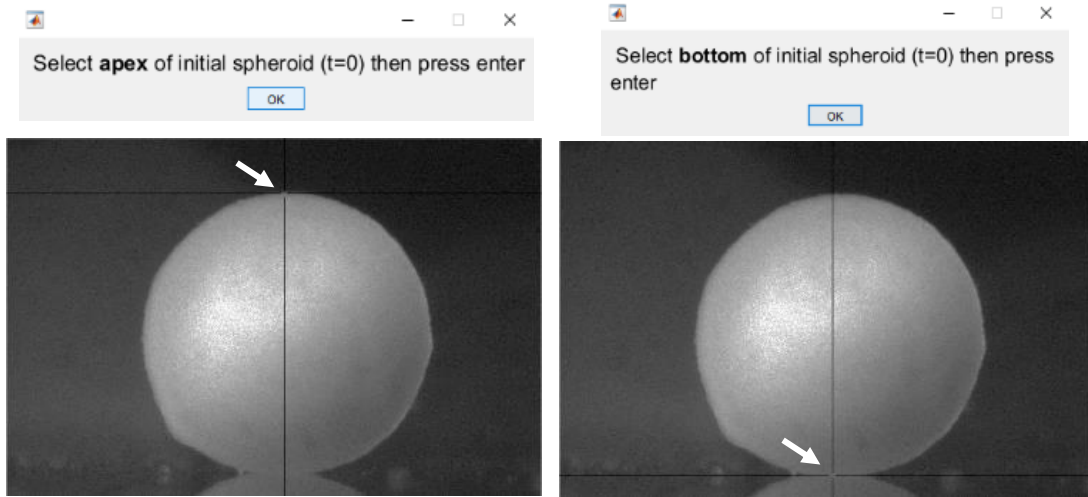


- Spheroid image at $t = t_0$ opens and several windows open asking to select successively the left side, the right side, the apex and the bottom of the spheroid.

For each point, press OK then click on the left, right, apex or bottom of the initial spheroid (as shown on the below figure) using the crosshair cursor, then press ENTER.

From this, the initial volume of the spheroid is estimated

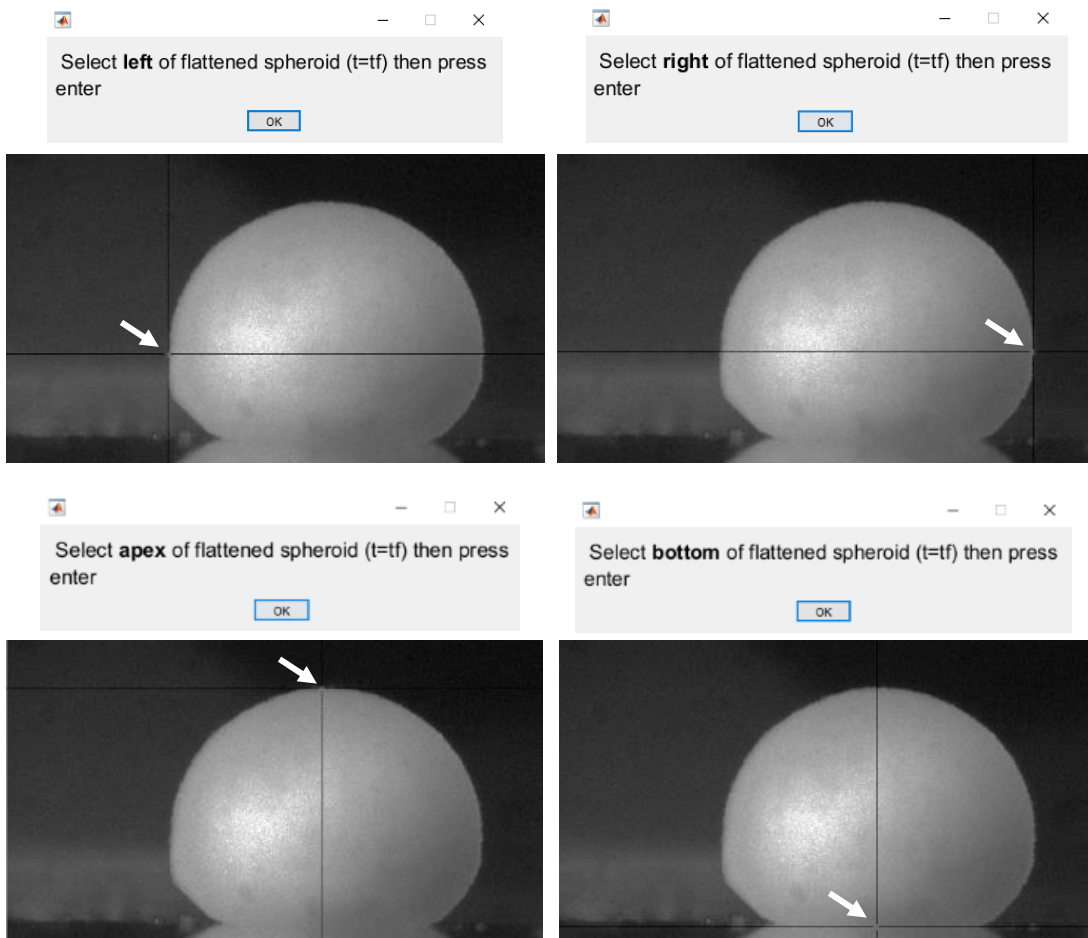




- Spheroid image at $t = t_f$ opens and several windows open asking to select successively the left side, the right side, the apex and the bottom of the spheroid.

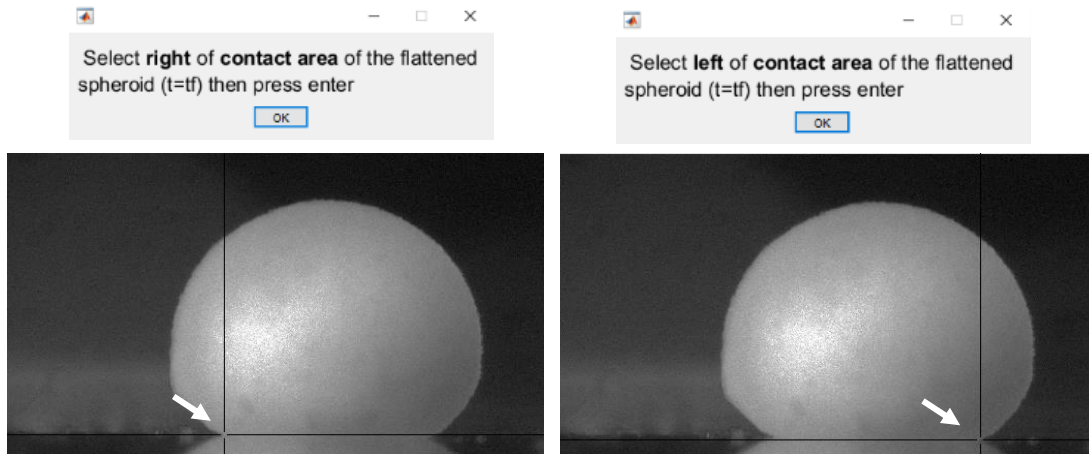
For each point, press OK then click on the left, right, apex or bottom of the flattened spheroid (as shown on the below figure) using the crosshair cursor, then press ENTER.

From this, the theoretical profile is optimized by minimizing the width the height and the volume of the spheroid, to obtain the surface tension of the spheroid.



- Finally, two windows open asking to select the right and left limit of the contact area of the flattened spheroid.

As previously, for each point, press OK then click on the left or right of the contact area of the flattened spheroid (as shown on the below figure) using the crosshair cursor, then press ENTER.



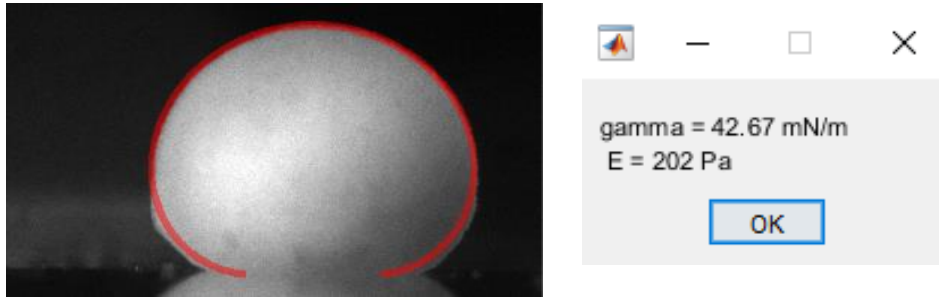
- Enter the image scale in m/pixel, the measured value of the magnetic force per unit of volume in N/m^3 and an estimated value of gamma in mN/m . If the estimated value of gamma is unknown, leave the default value. Then press OK.

The image shows an "Input" dialog box with a title bar containing a small icon, the word "Input", and standard window controls (minus, maximize, close). It contains three text input fields:

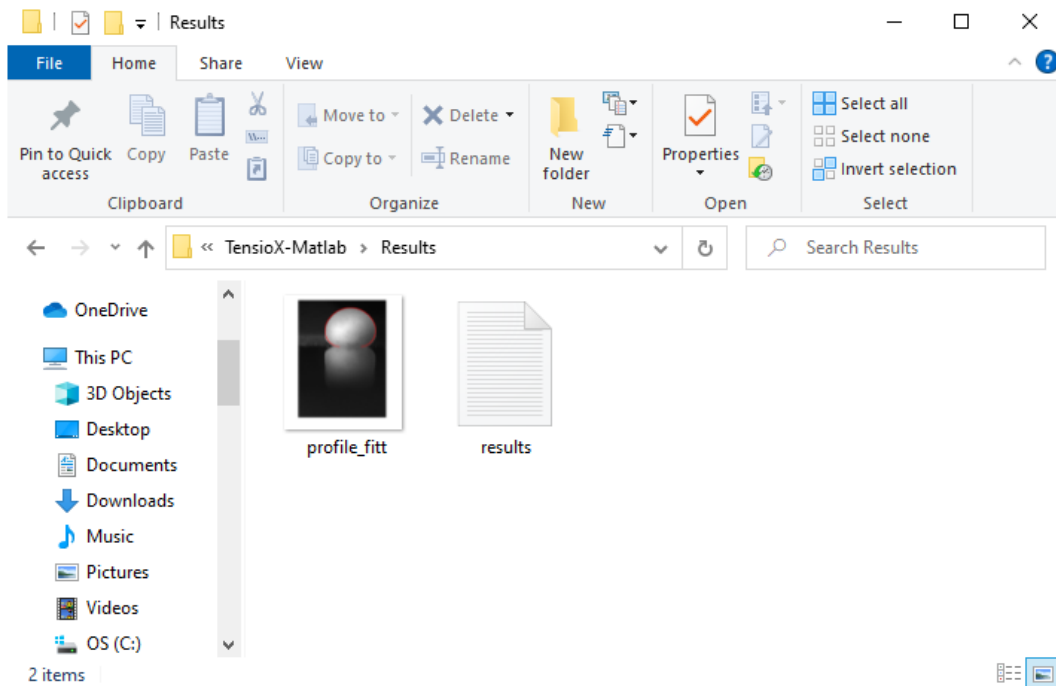
- The first field is labeled "Enter the image scale in m/pixel" and contains the text "2.21e-06".
- The second field is labeled "Enter the measured value of f_v ($\text{grad}(B) \cdot M_v$) in N/m^3 " and contains the text "50000".
- The third field is labeled "Enter an estimated value of gamma in mN/m " and contains the text "10".

 At the bottom right of the dialog box are "OK" and "Cancel" buttons.

- Two windows open containing the computed surface tension and Young modulus values and an overlay of the flattened spheroid image and the fitted profile for the corresponding surface tension. A good agreement between the experimental and the computed profile (in red) is required to guarantee the reliability of the surface tension results.



Both files are saved in the previously selected folder. The computed surface tension and the Young modulus are saved in a results.txt file.



IV. Detailed description of the MATLAB code

Surface tension and Young modulus are determined as described in [2]. The following paragraphs describe what the MATLAB code contains.

1) Surface tension measurement

Theoretical profiles are obtained by resolving numerically (ode45 function) the classical Laplace equation of capillarity describing the mechanical equilibrium conditions for two homogeneous fluids separated by an interface and in non-wetting conditions. To adjust the numerical profile to the experimental profile, the quadratic error e on the height h , the width w and the volume V of the flattened spheroid is minimized with respect to the curvature at apex of the flattened spheroid (b) and $c = \frac{f_b}{\gamma}$ the capillary constant of the system.

$$e = \left(1 - \frac{h_{th}}{h_{exp}}\right)^2 + \left(1 - \frac{w_{th}}{w_{exp}}\right)^2 + \left(1 - \frac{V_{th}}{V_{exp}}\right)^2$$

h_{exp} , w_{exp} are computed from the extracted values on the flattened spheroid image while V_{exp} is computed from the extracted values on the initial spheroid image (volume of a sphere).

The initial parameters for the minimization are taken such as $b_0 = \frac{1}{R_0}$ with $2R_0$ the average between the width and the height of the initial spheroid and $c_0 = \frac{f_v}{\gamma_0}$ with f_v and γ_0 the magnetic force per unit of volume and the estimated surface tension respectively, entered manually. The quadratic error e is minimized with the function `fminsearch`, to obtain the capillary constant of the system from which the surface tension γ is deduced. The final numerical profile and the image of the flattened spheroid are superimposed to check for the reliability of the result.

2) Young modulus measurement

The Young modulus E is computed using Hertz theory for an elastic sphere with an initial radius R_0 which gives $E = \frac{(1-\sigma^2) \pi M_v grad(B) R_0^4}{L}$ where σ stands for the Poisson ratio ($\sigma = 1/2$) and L for the radius of the contact zone computed from the extracted values of the contact area of the flattened spheroid image.

V. Cautions and remarks

- The initial shape of the spheroid at t_0 has to be spherical to give reliable measurements.
- The substrate on which the spheroid is has to be flat and the camera has to be correctly aligned to provide accurate measurements.
- This application can be adapted to other systems than multicellular aggregates such as any type of viscoelastic fluid or material in non-wetting conditions with respect to the substrate.

VI. Authors, copyright, distribution policy

This application was written by Irène Nagle.

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References:

[1] Irène Nagle, Florence Delort, Sylvie Hénon, Claire Wilhelm, Sabrina Battonnet-Pichon, Myriam Reffay, Fluid-like properties of tissue models: intermediate filament disorganization pointed out, under submission

[2] Francois Mazuel, Myriam Reffay, Vicard Du, Jean-Claude Bacri, Jean-Paul Rieu, Claire Wilhelm, Magnetic flattening of stem-cell spheroids indicates a size-dependent elastocapillary transition, PRL, 114(9), 2015