TractionsForAll

v1.0

September 1st, 2013

TractionsForAll v1.0 is freely distributed program that calculates tractions exerted by an adherent cell on the soft hydrogel substrate underneath. It is created to allow the students and researchers in the field of mechanobiology to study contractile responses of variety of cell types. Although helpful for understanding the process, no background in applied mechanics and computer programming is needed for using the software. The program is written and compiled in Matlab (Mathworks).

More details about the background theory and implemented experimental techniques can be found in following references[1-4]:

1. Marinković A, Mih JD, Park JA, Liu F, Tschumperlin DJ. *Improved throughput traction microscopy reveals pivotal role for matrix stiffness in fibroblast contractility and TGF-beta responsiveness.* American journal of physiology Lung cellular and molecular physiology 2012; 303:L169-80.

2. Mih JD, Sharif AS, Liu F, Marinković A, Symer MM, Tschumperlin DJ. *A multiwell platform for studying stiffness-dependent cell biology.* PloS one 2011; 6:e19929.

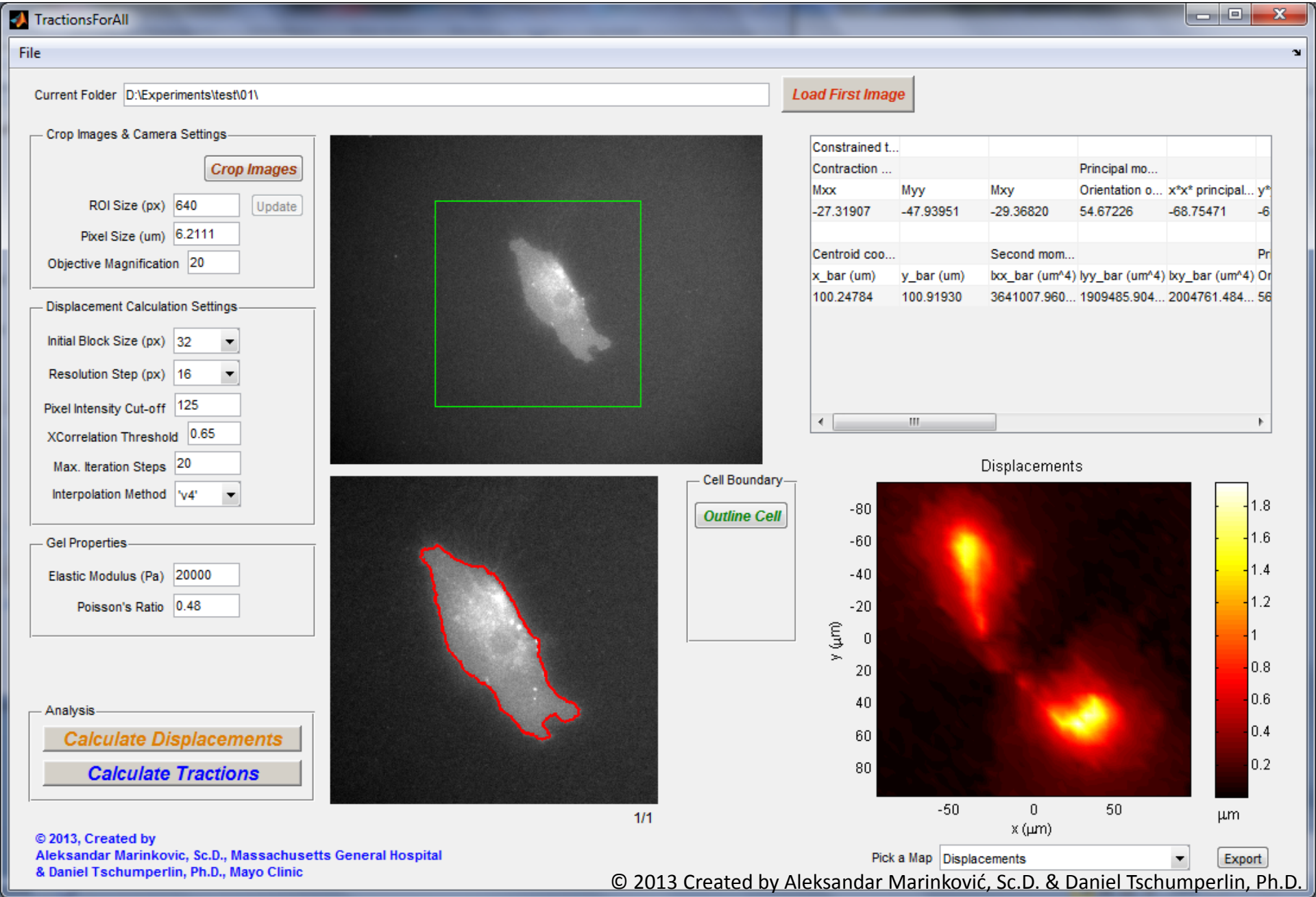
3. Butler JP, Tolic-Norrelykke IM, Fabry B, Fredberg JJ. *Traction fields, moments, and strain energy that cells exert on their surroundings.* American journal of physiology 2002; 282:C595-605.

4. Tolic-Norrelykke IM, Butler JP, Chen J, Wang N. *Spatial and temporal traction response in human airway smooth muscle cells.* American journal of physiology 2002; 283:C1254-66.

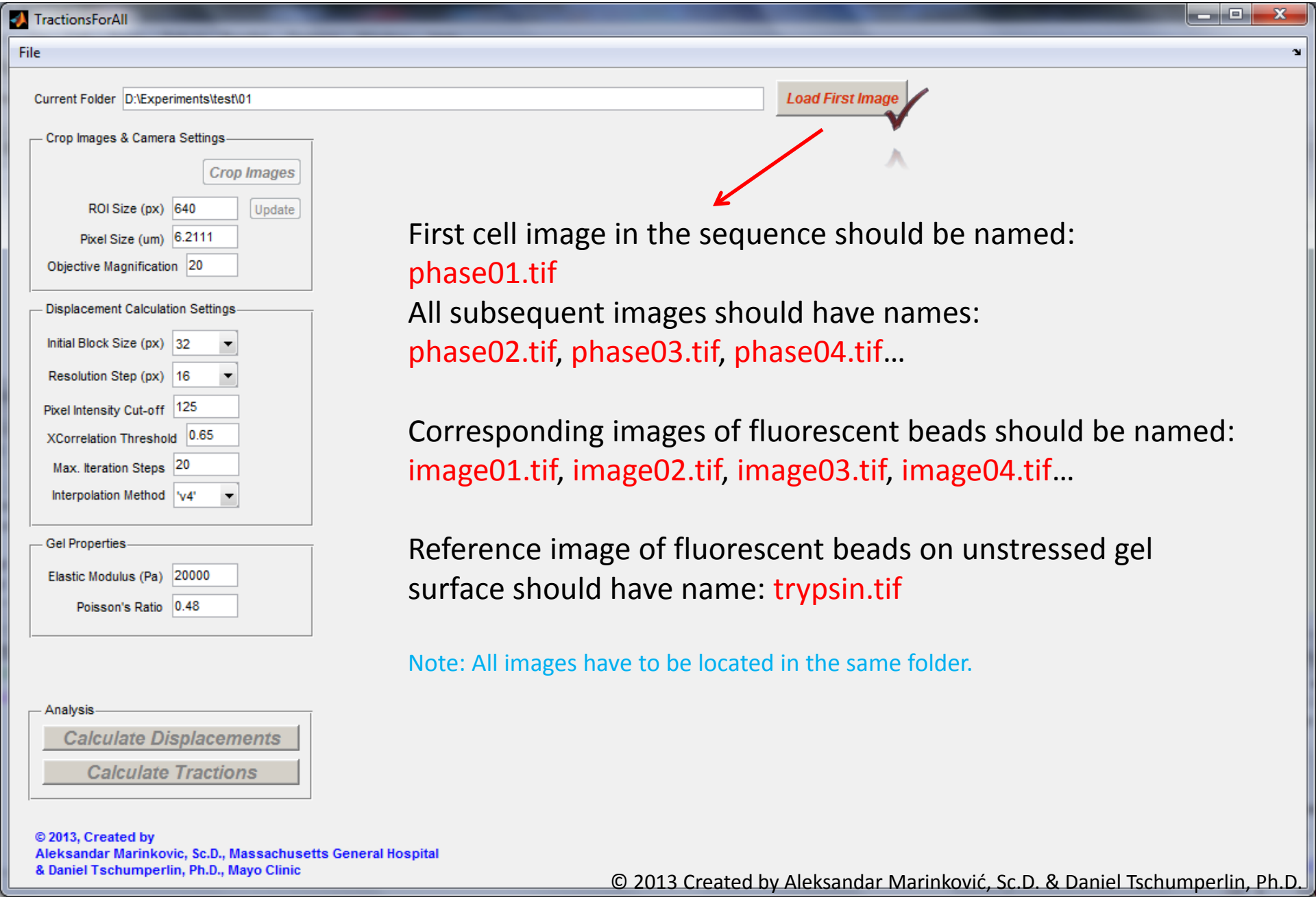
If you find this program useful please cite these papers in your publication.

Traction calculation in few simple steps

Outlook of the main window



1. Select a sequence of images for analysis ✓



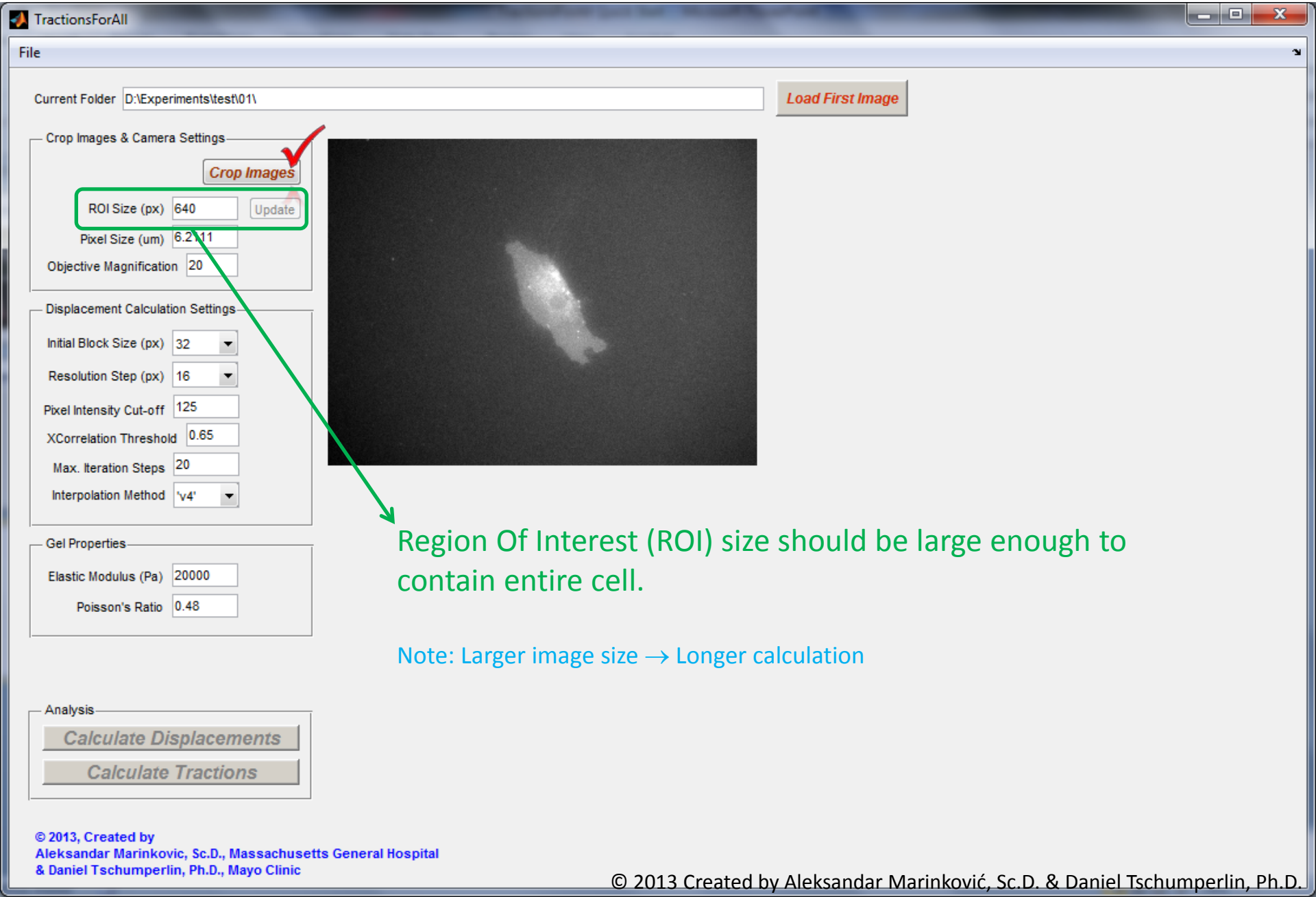
First cell image in the sequence should be named:
phase01.tif
All subsequent images should have names:
phase02.tif, phase03.tif, phase04.tif…

Corresponding images of fluorescent beads should be named:
image01.tif, image02.tif, image03.tif, image04.tif…

Reference image of fluorescent beads on unstressed gel surface should have name: trypsin.tif

Note: All images have to be located in the same folder.

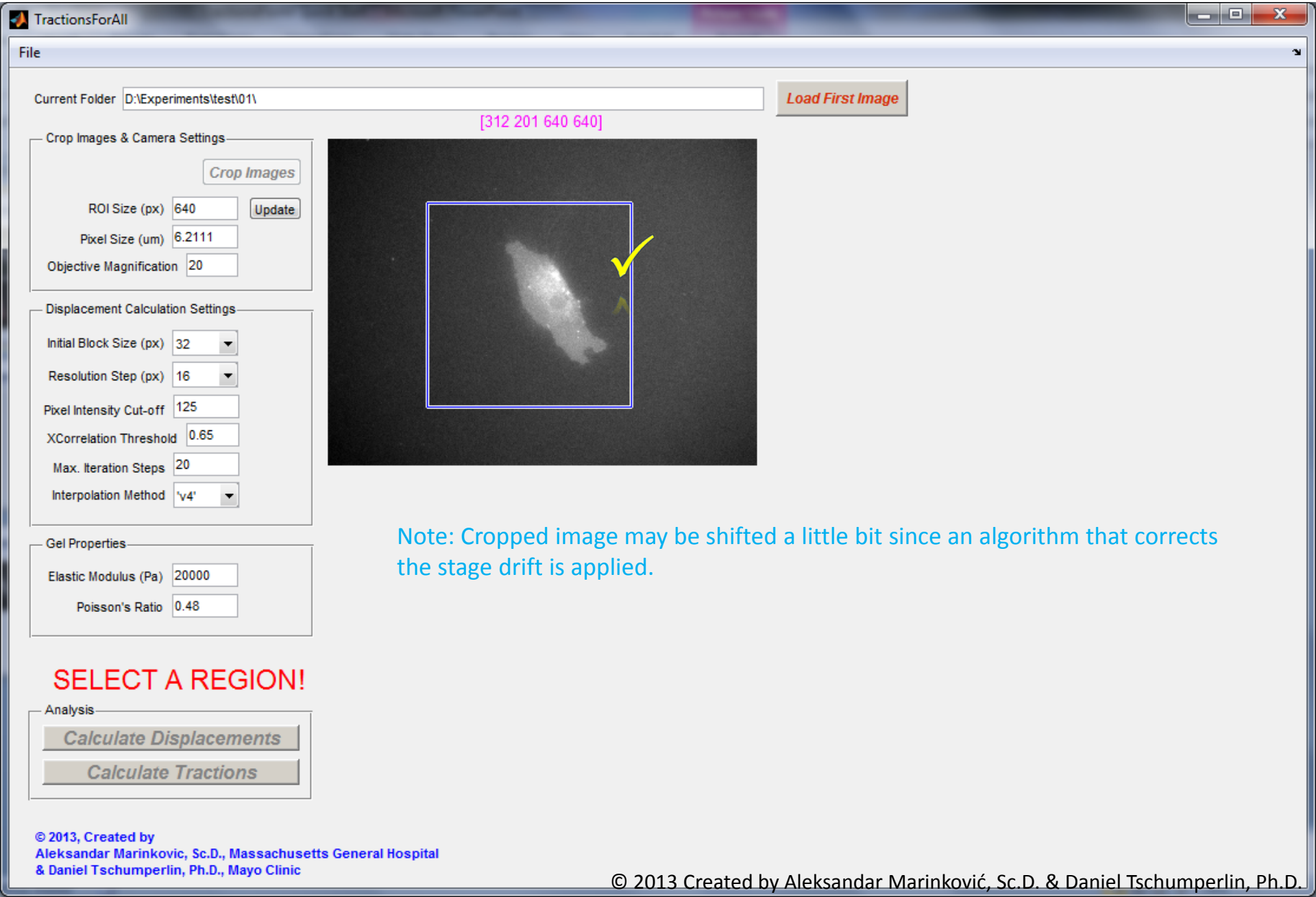
2. Crop the images to a square shaped format ✓



Region Of Interest (ROI) size should be large enough to contain entire cell.

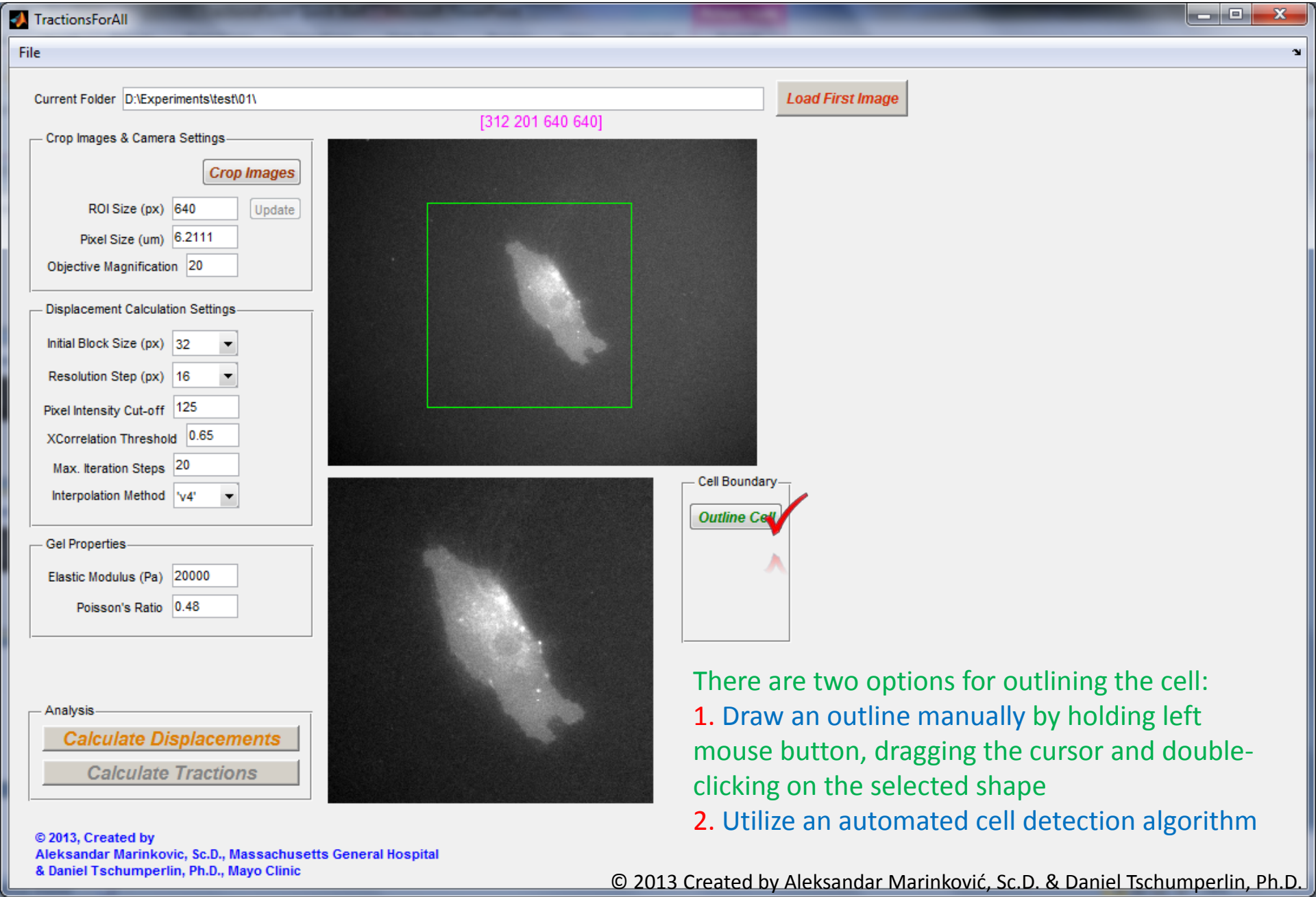
Note: Larger image size → Longer calculation

3. Drag the square and double click to select the region of interest ✓



Note: Cropped image may be shifted a little bit since an algorithm that corrects the stage drift is applied.

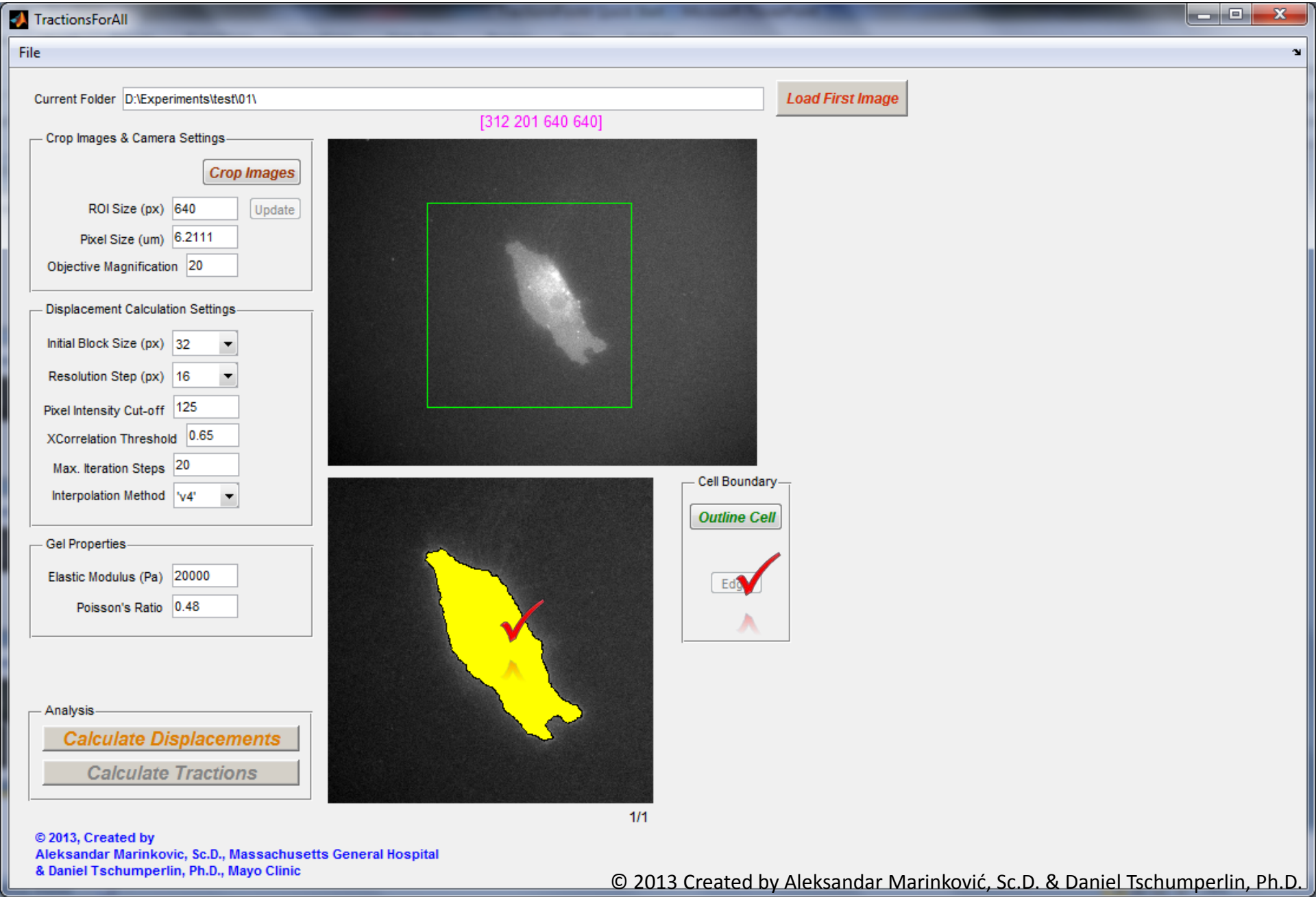
4. Outline a cell on the cropped image ✓



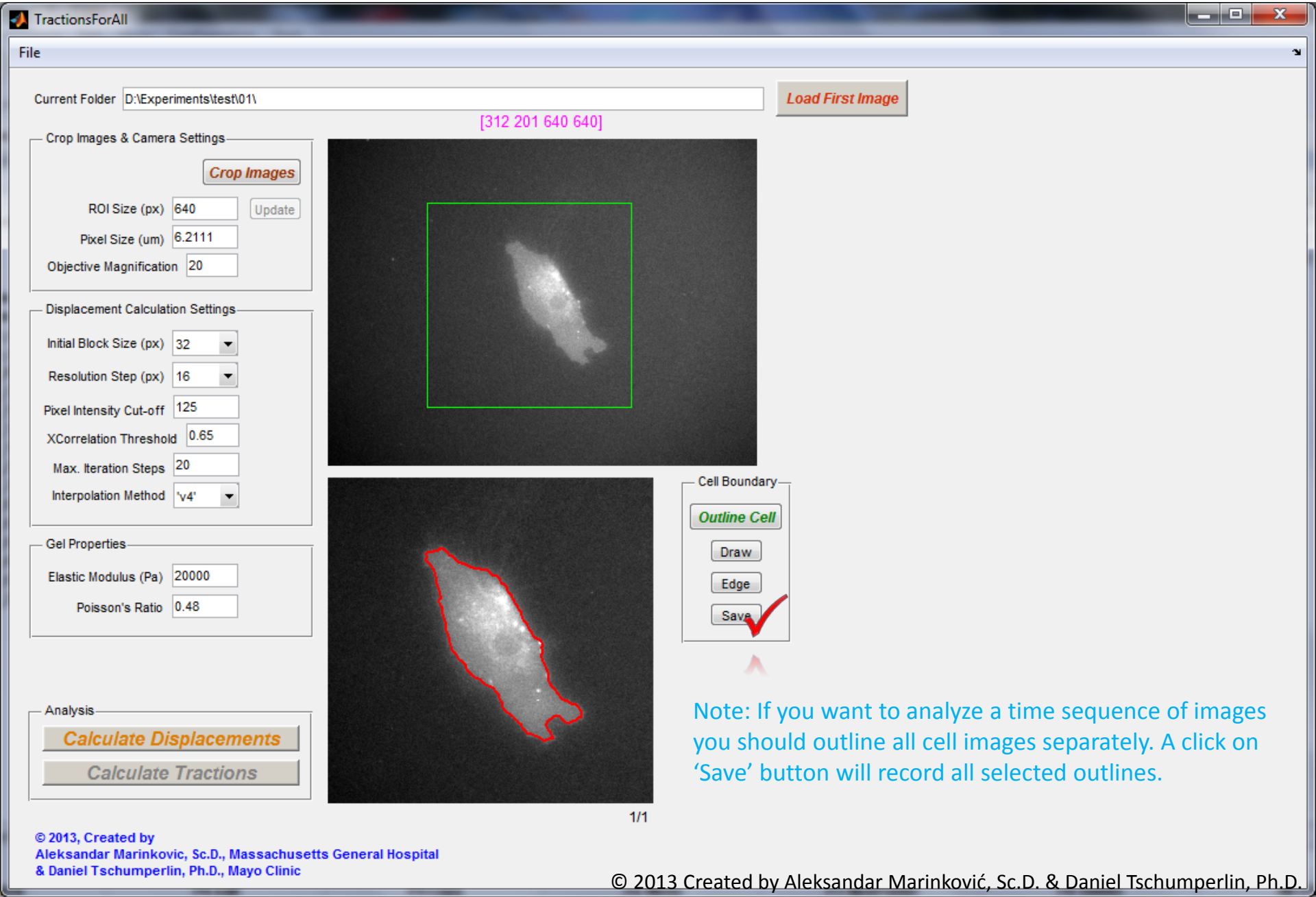
There are two options for outlining the cell:

1. Draw an outline manually by holding left mouse button, dragging the cursor and double-clicking on the selected shape
2. Utilize an automated cell detection algorithm

5. If cell detection filter was used click on a yellow painted cell ✓

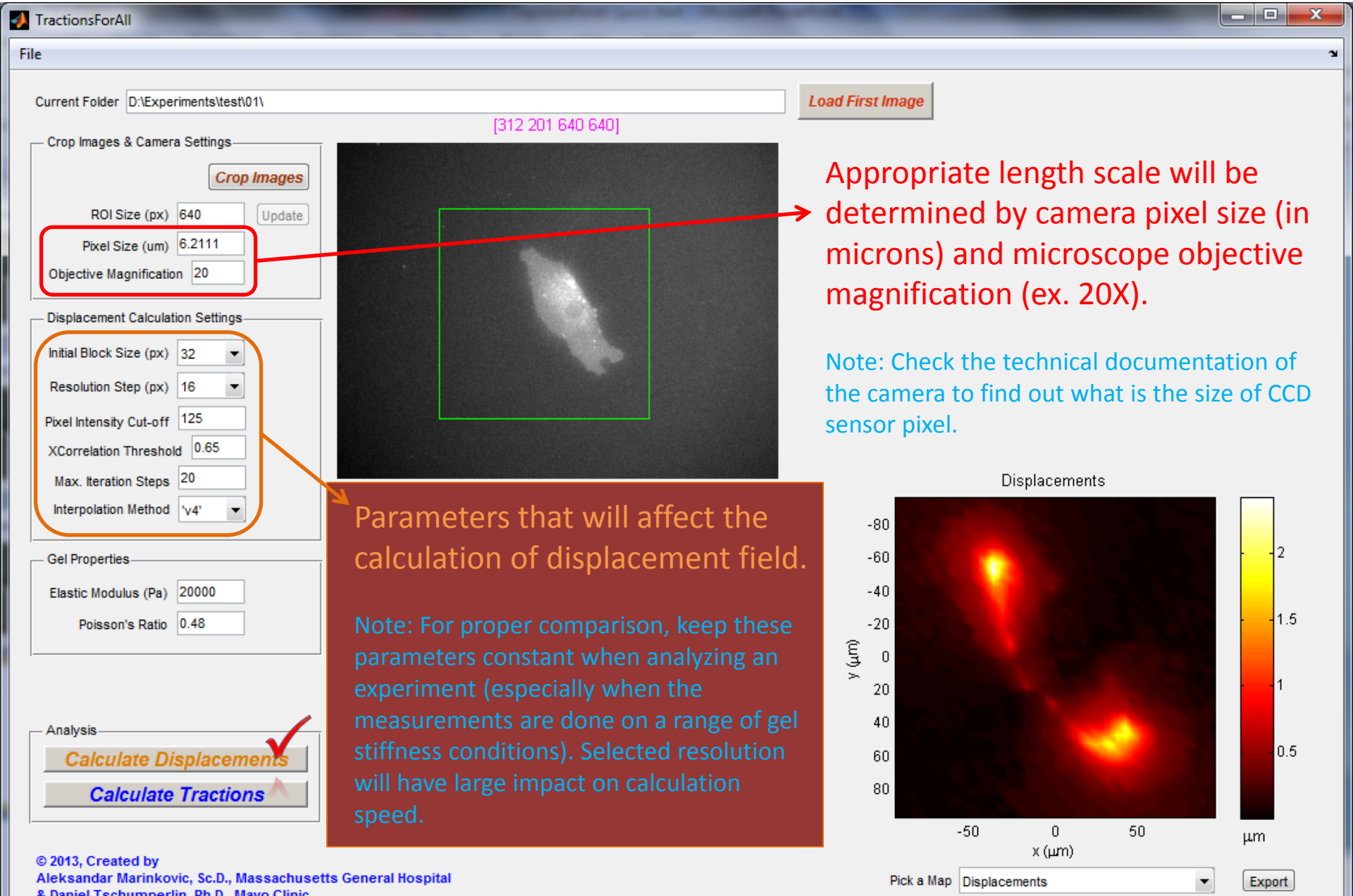


6. Save cell outline by clicking on the ‘Save’ button ✓

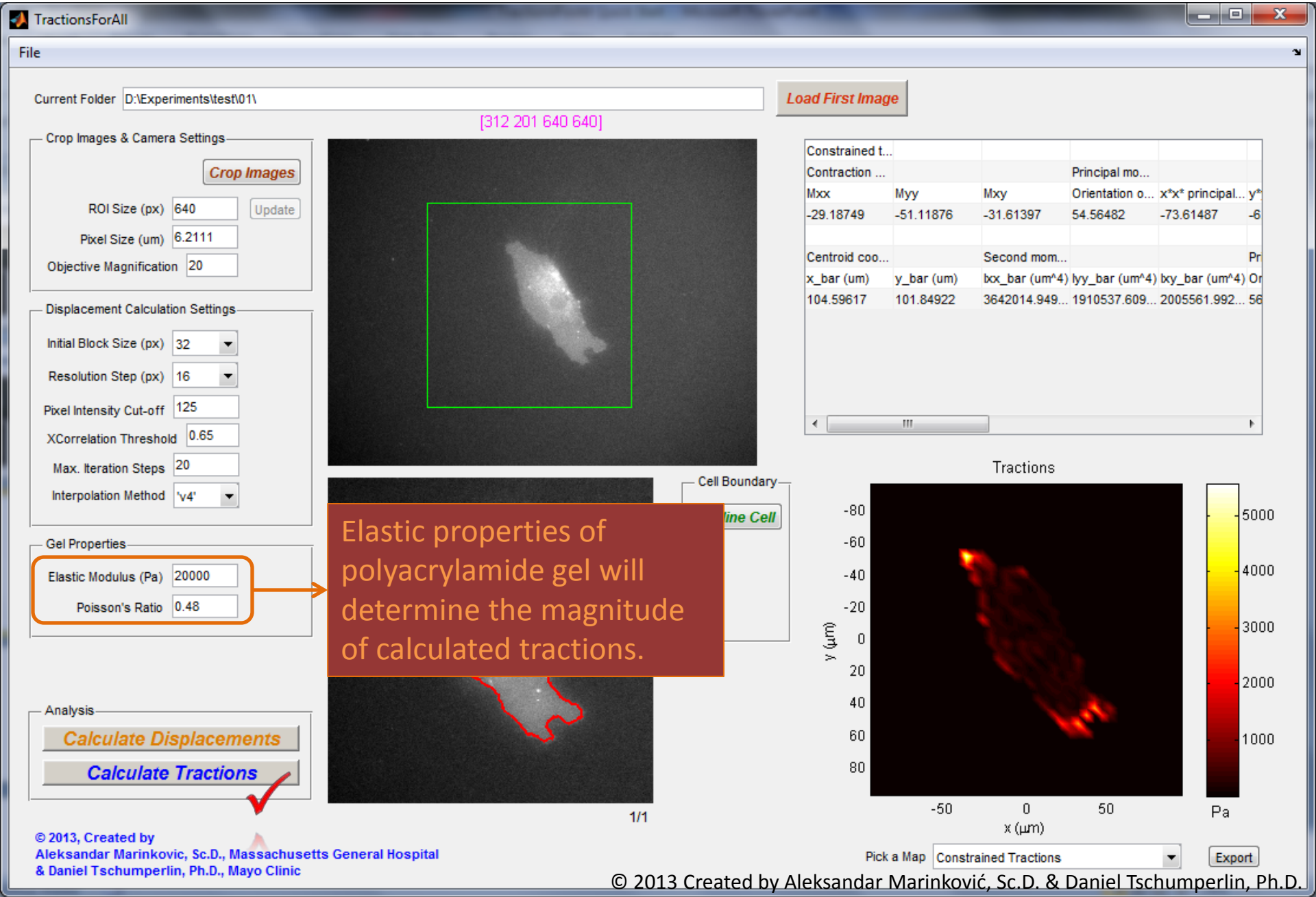


Note: If you want to analyze a time sequence of images you should outline all cell images separately. A click on ‘Save’ button will record all selected outlines.

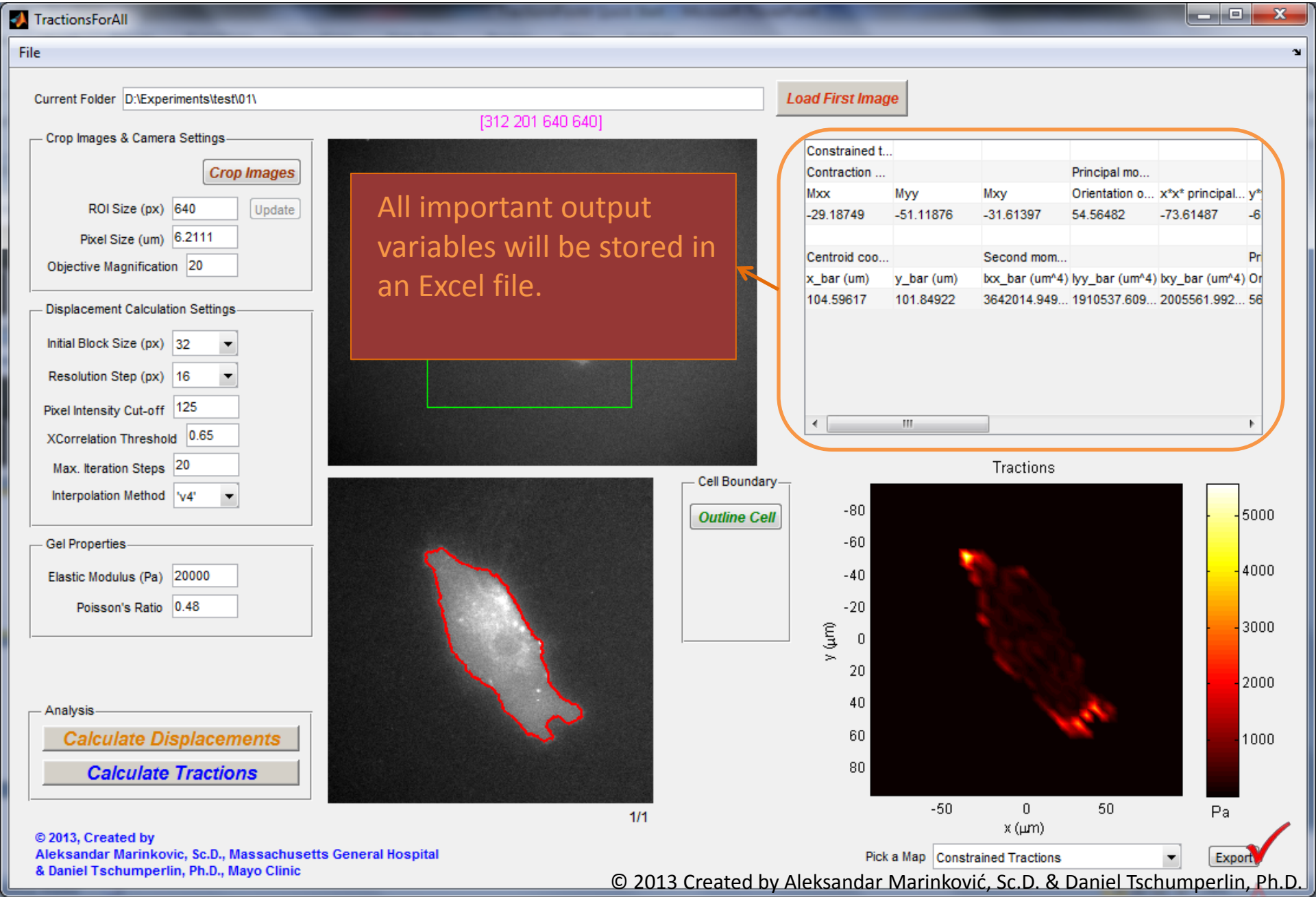
7. Calculate displacement field for each image in the sequence ✓



8. Calculate corresponding tractions ✓



9. Edit and Export the plots ✓



Polyacrylamide Gel Preparation and Surface Conjugation of Fluorescent Microspheres

- Treat glass-bottom 24-well plates (In Vitro Scientific) with 0.4% aqueous solution of 3-methacryloxypropyltrimethoxysilane (Acros Organics) **at pH 3.5** for 1 h. Rinse three times in distilled water and air dry. Treated plates can stay on the shelf for months.
- Seven prepolymerization solutions of variable ratios of acrylamide:bisacrylamide (Bio-Rad) can be prepared in advance and stored at 4 °C [% acrylamide:% bisacrylamide (Young's modulus, kPa); 3:0.05 (0.3), 3:0.11 (1), 7.5:0.05 (6), 7.5:0.12 (13), 7.5:0.19 (17), 7.5:0.34 (20), 12:0.242 (75)] (see **Table 1** for detailed recipe; these solutions can be kept for weeks at 4 °C). Solutions could be sterilized by filtering through 0.22 μm filters.
- Prepare 1% ammonium persulfate (APS) aqueous solution (by mass, APS density is 1.98 g/ml) and 100 mM sodium bisulfite (SB) solution (SB MW ≈ 104 g/mol). These solutions can be mixed together in volume ratio 9 x APS:1 x SB and kept at 4 °C for several days – test it!). Solutions can be sterilized by filtering.
- Add ~20 μl of polymerization mixture (**ratio of prepolymerization solution:APS:SB = 90%:9%:1%**) in the well and sandwich it with SurfaSil-treated (Thermo Scientific), hydrophobic glass coverslips (15 mm in diameter for 24-well plate) **for 5 min**. The thickness of resulting gel will be ~100 μm.

- After polymerization, the gel surface can be derivatized with 1 mg/ml 3-Hydroxytyramine hydrochloride (Sigma) in 50 mM HEPES solution, **at pH 8.5-8.7 for 5 min** (**Important**: remove solution and wash the gels with water, or PBS, because oxidizing 3-Hydroxytyramine hydrochloride will become dark and it will be quickly absorbed in the gel).
- Fluorescent, 200 nm in diameter, sulfate-modified latex microspheres of chosen color (FluoSpheres, Invitrogen) will be conjugated to the gel surface when sonicated aqueous suspension of beads (diluted at 1:200) is delivered on top of the gels for 60 min. The gels should be rinsed three times in distilled water to remove all remaining nonattached beads.
- Gels should be UV sterilized for 1 h (microwaving the plate full of water for 30 sec, or until boiling point is reached, is an attractive alternative; **Be careful! Violent boiling can ruin your gels.**)
- Functionalize gels by incubation with 10 μg/ml of sterile collagen I (PureCol) in PBS for 2 h.

Note: THIS PROTOCOL DOESN'T REQUIRE ANY VACUUM DEGASSING OR POLYMERIZATION IN NITROGEN CHAMBER. POLYMERIZATION OCCURS IN 1-2 MINUTES, ON THE BENCH! PLAY WITH YOUR GEL SYSTEM! FIND OPTMAL POLYMERIZATION CONDITIONS FOR YOUR APPLICATION.

Table 1.

For 1 ml of gel solution:

Measured E (Pa)	300	1000	6000	13000	17000	20000	75000
% Acrylamide	3	3	7.5	7.5	7.5	7.5	12
% Bisacrylamide	0.048	0.107	0.053	0.117	0.192	0.236	0.242
POLYMERIZATION MIXTURE (all in **µl) – PREPARED IN ADVANCE**							
40% Acrylamide	75	75	187.5	187.5	187.5	187.5	300
2% Bisacrylamide	24.5	53.5	27	58	88	118	120.5
Water	799	770	684	653	623	593	478
TEMED	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Total	900	900	900	900	900	900	900
Ammonium persulfate (APS) and sodium bisulfite (SB) solutions can be prepared in advance, but should be mixed together with **prepolymerization mixture just before gel casting**							
1% APS (vol)	90	90	90	90	90	90	90
100 mM SB	10	10	10	10	10	10	10

For 50 ml of gel solution:

Measured E (Pa)	300	1000	6000	13000	17000	20000	75000
% Acrylamide	3	3	7.5	7.5	7.5	7.5	12
% Bisacrylamide	0.048	0.107	0.053	0.117	0.192	0.236	0.242
POLYMERIZATION MIXTURE (all in **µl) – PREPARED IN ADVANCE**							
40% Acrylamide	3750	3750	9375	9375	9375	9375	15000
2% Bisacrylamide	1225	2675	1350	2900	4400	5900	6025
Water	39950	38500	34200	32650	31150	29650	23900
TEMED	75	75	75	75	75	75	75
Total	45000	45000	45000	45000	45000	45000	45000
Ammonium persulfate (APS) and sodium bisulfite (SB) solutions can be prepared in advance, but should be mixed together with **prepolymerization mixture just before gel casting**							
1% APS (vol)	4500	4500	4500	4500	4500	4500	4500
100 mM SB	500	500	500	500	500	500	500