

SOFAST ImageJ Plugin Manual

Install

It is very easy to install the SOFAST plugin. You can simply use ImageJ's *Install plugin* routine. Start ImageJ and click *Plugins* → *Install...*. Choose the file *sofast.jar*, and save it to the suggested folder. You will now see a new entry called *SOFAST* in the *Plugins* menu.

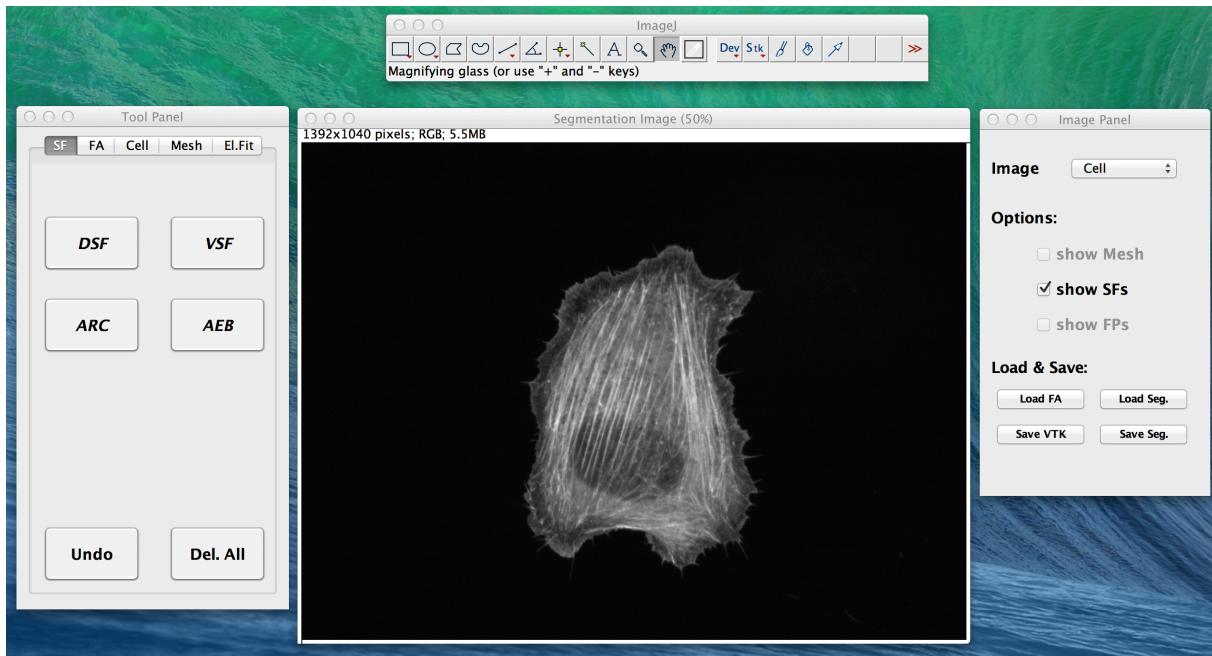
Alternatively, you can just copy the *sofast.jar* file to ImageJ's *plugin* folder in the ImageJ installation path, and restart ImageJ.

Uninstall

Find ImageJ's installation folder and navigate to *plugins*. Remove the file *sofast.jar*.

Segmentation with SOFAST

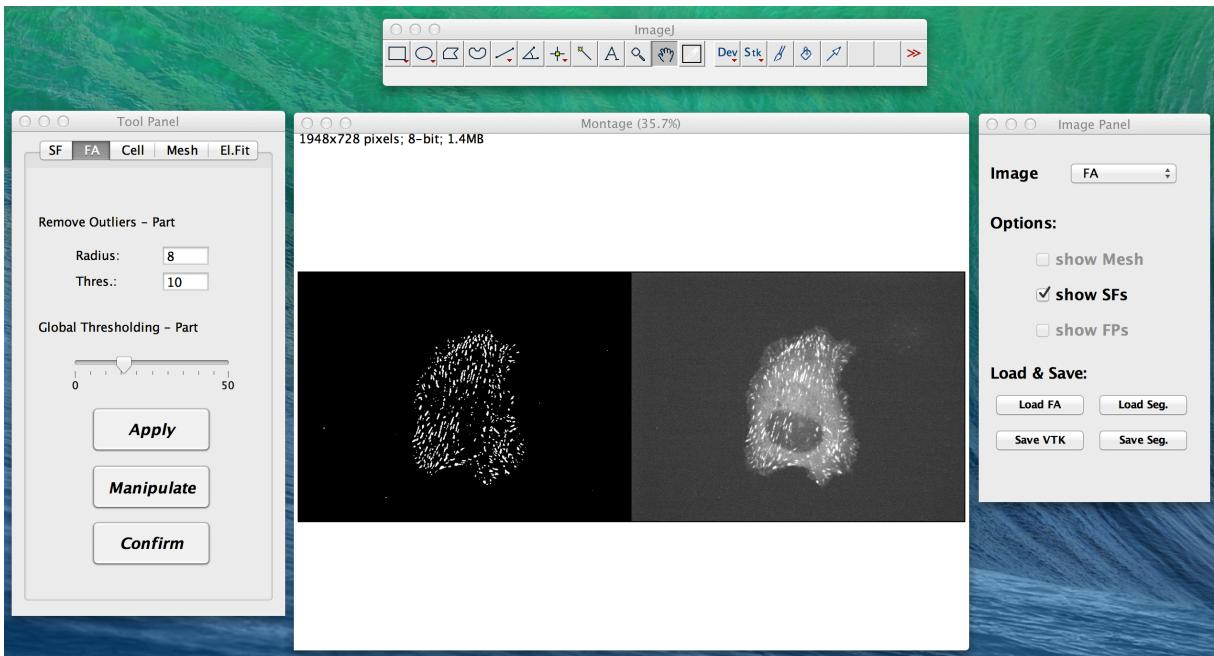
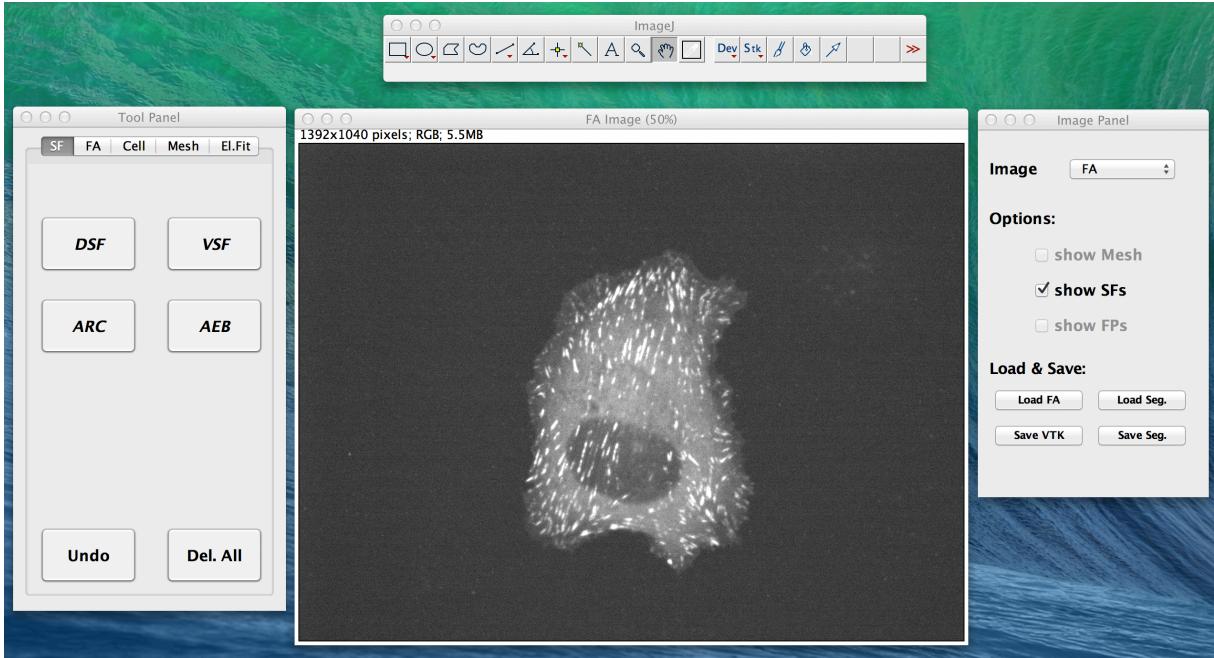
Start ImageJ and open the actin fluorescence image. Click *Plugins* → *SOFAST* and wait for the plugin to start. You should see a resulting screen like this:



We also want to segment focal adhesions (FAs) here. So click on *Load FA* in the *Image Panel*, and choose the corresponding fluorescence image (here, we use paxillin as the FA marker). The resulting display is shown on the next page. Note that the *Image* dropdown menu in the *Image Panel* has turned from *Cell* to *FA*. You can switch between different images using this menu.

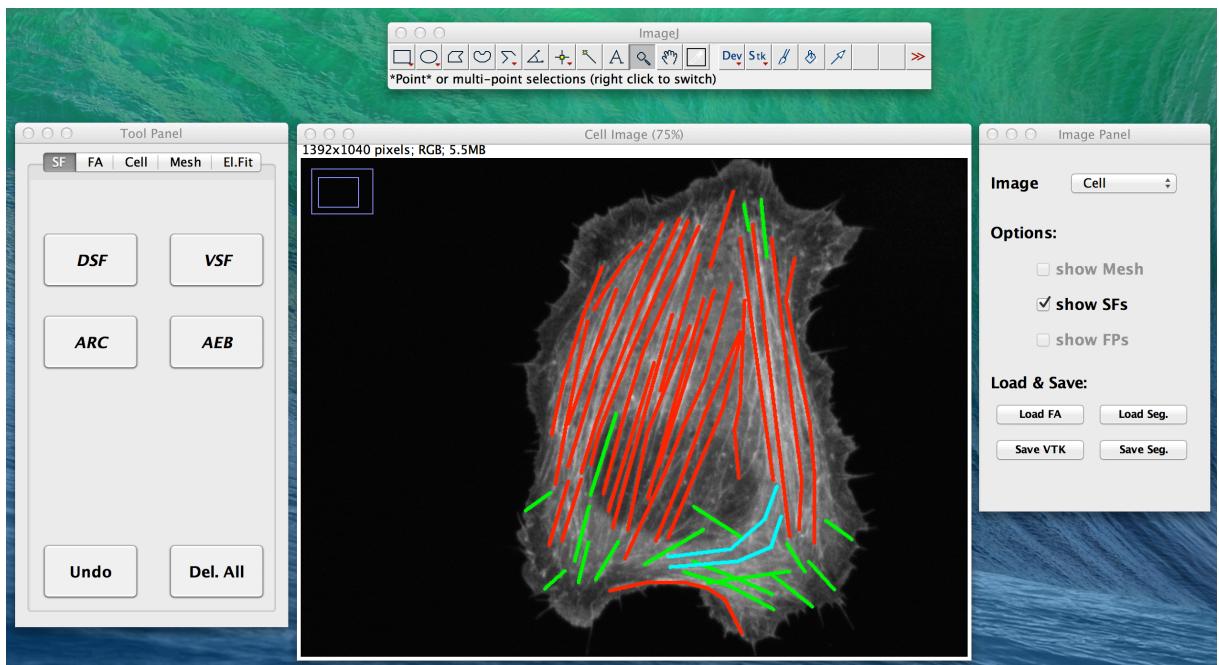
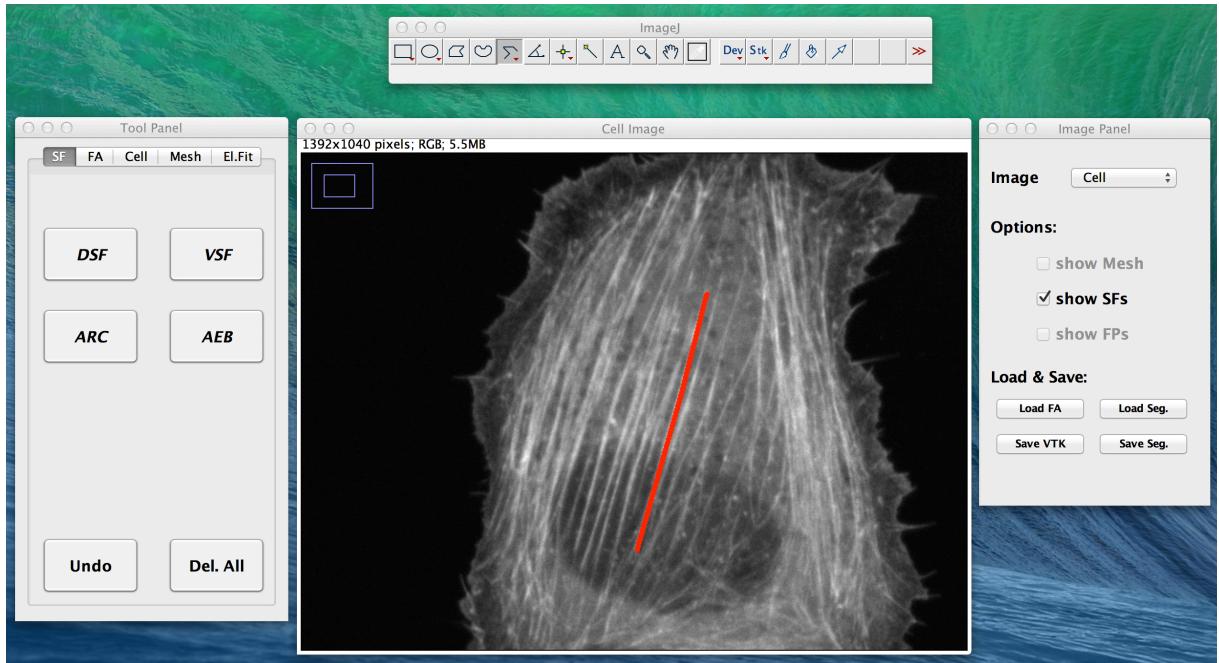
We now start by segmenting FAs (bright white spots). Select *FA* in the *Tool Panel*. Adjust the parameters for outlier removal and global thresholding and click *Apply*. Everytime you do so, the segmented image will be calculated from the original fluorescence image. You can see original and segmentation side by side to decide whether you are satisfied with the segmentation. In the end, you can hit *Manipulate* and use ImageJ's build in tools

to improve the segmentation. Click *Confirm* when the segmentation is done. We recommend saving the segmented image using ImageJ's build-in *save* functions. If you have a segmented FA image at hand, you can simply load this into the plugin (instead of the raw data) and skip the FA segmentation in the tool.

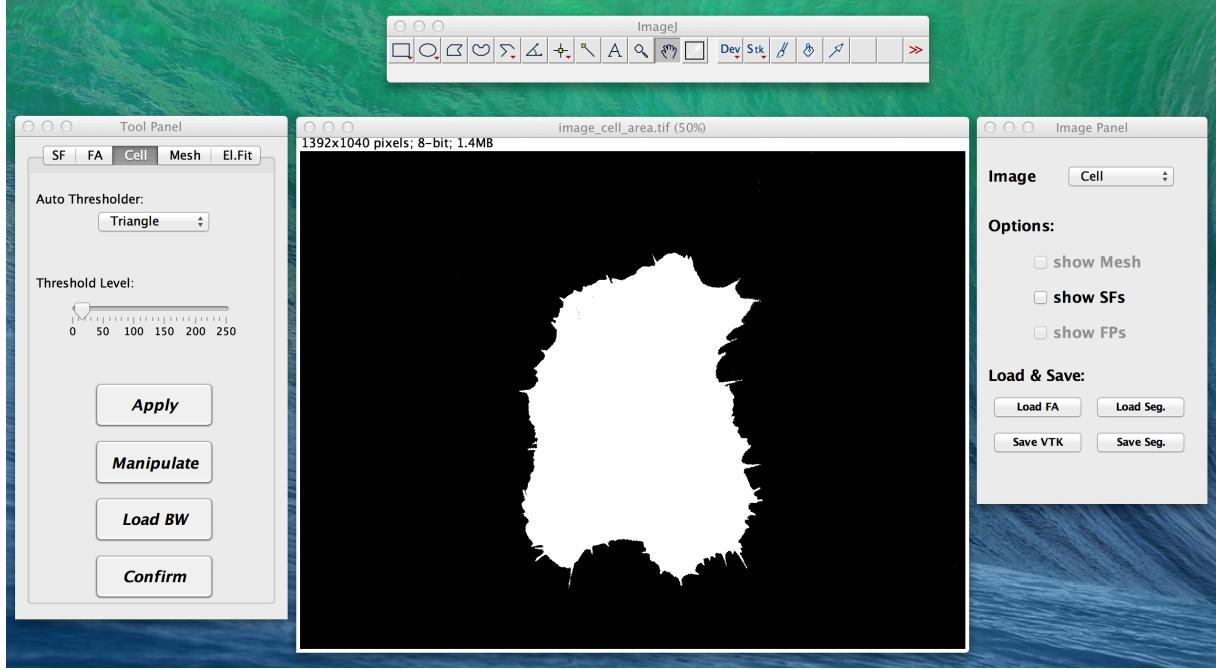


Next, we want to segment stress fibers (SFs). Switch back to the actin cell image (in the *Image Panel* or by hitting the *space* key on your keyboard when the image window is active) and to the *SF* tab in the *Tool Panel*. The four buttons in the upper part represent four different types of SFs: dorsal stress fibers (DSF), ventral stress fibers (VSF), transverse arcs (ARC) and actin edge bundles (AEB). Click on one of the four (e. g. *DSF*) and use the line tool (which is automatically selected) to draw a line where one stress fiber runs. Note that this line can consist of many piecewise straight parts. While you segment, you can again hit the *space* key on your keyboard to change views between the

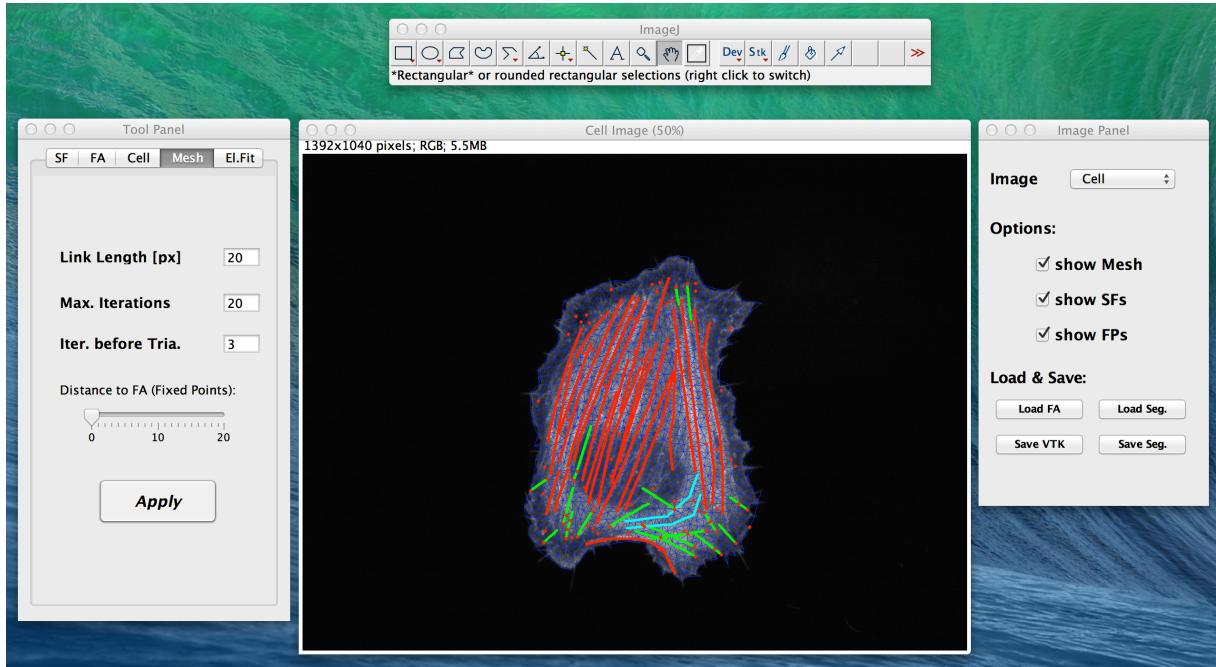
actin and the FA image. When you have finished, click on the button for the type of the SF that you have segmented. Be careful with this choice, as it might introduce fixed points in the network we are generating later. A VSF and an AEB fix both of their end points, a DSF fixes only the starting point and an ARC does not introduce any fixed points. What these fixed points are will become clear later. Instead of clicking on the buttons, you can use shortcuts to finish your segmentations: *d* for DSF, *v* for VSF, *a* for ARC, and *e* for AEB. You can also zoom in the image to be more precise and it is always possible to save and reload your segmentation with the corresponding buttons in the *Image Panel*.



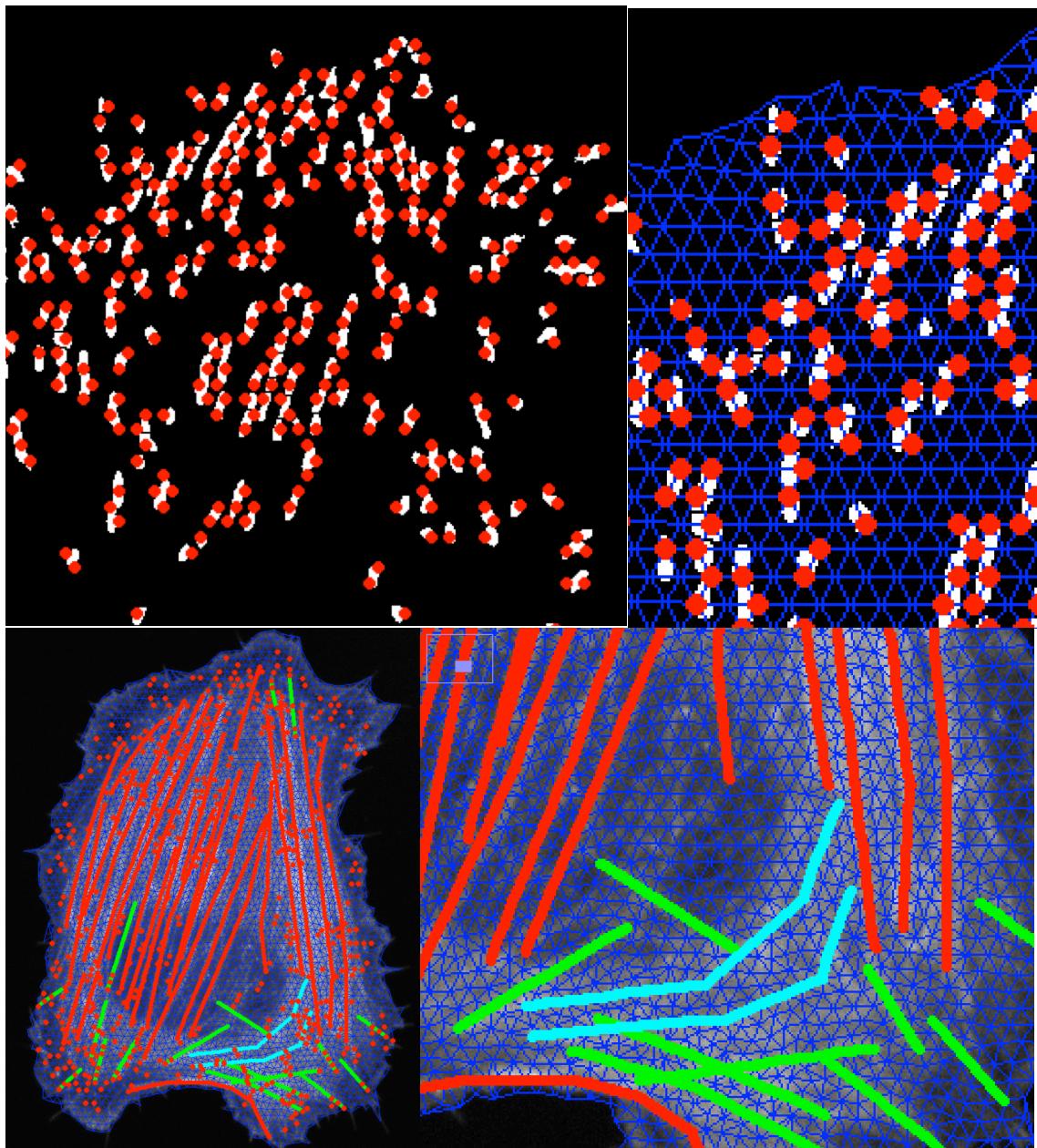
Segment cell area from actin data. Click on the *Cell* tab in the *Tool Panel* and turn off SF visualization (Uncheck *show SFs* in the *Image Panel*). Set a thresholding method and level and hit apply. Again, after clicking *Manipulate* you can use ImageJ's build-in functions to improve cell area segmentation. You can save the image via ImageJ's *save* function and reload a segmented cell area binary image via the button *Load BW* in the *Cell* tab of the *Tool Panel*. When you have arrived at a binary image representing cell area, hit *Confirm*.



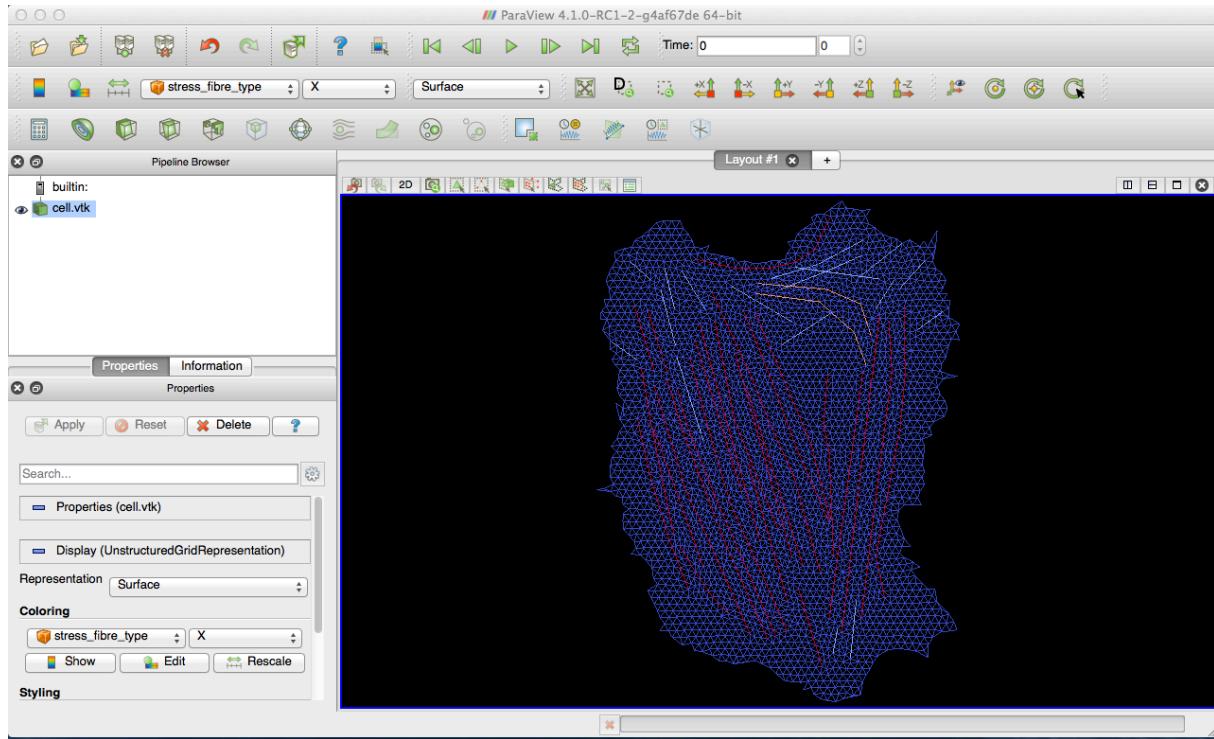
Mesh generation. In the next step, we want to provide the segmented information in a triangular network that can be used in model computation assays like Model-based traction force microscopy. Switch to the *Mesh* tab in the *Tool Panel*. Adjust the parameters that control mesh size and homogeneity and hit *Apply*. The result should look like the following:



The SFs are displayed in different colors and network links run along all of them. The network spans the whole cell area and is displayed in blue. Small red dots mark the sites where network vertices lie above focal adhesions. As the distribution of fixed points might not be sufficient to describe FA locations, you can define a distance to FAs, up to which a network vertex is fixed. We show some detailed images of the resulting network with SFs and fixed points.



You can now save the segmentation in a standard VTK file format (www.vtk.org) by clicking *Save VTK* in the *Image Panel*. The output file can be displayed with standard viewers (e. g. ParaView, www.paraview.org, see next page) and is also supported as an active cable model description for Model-based traction force microscopy.



Fit ellipses to FAs. In a final step we want to highlight, that it is possible to fit ellipses to all segmented focal adhesions in the SOFAST plugin. Just switch to the *El.Fit* tab in the *Tool Panel* and hit *Fit Ellipses*. Follow the dialog to save the output file and open it with a standard reader. Each row represents one focal adhesion, and the columns bear the following information (from left to right): X position, Y position, angle to the x-axis in rad, length of semi-major axis, length of semi-minor axis, and distance to cell edge in pixel.

shapes.txt						
791.4425287356322	243.00574712643677	0.8526442411078239	14.674445771776341	7.548621741136478	25	
799.078947368421	243.60526315789474	1.3470363392163156	5.549949353974384	4.358877857623014	23	
723.1551724137931	249.1206896551724	1.5723844633281359	10.785087360895632	6.84722569786106	10	
802.43939393934	248.83333333333334	2.9835890742344926	7.049163542847284	5.960551875533448	23	
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753.1715328467153	265.3540145985402	1.39432219251203	19.379236091010306	9.0010677825233	30	
767.5344827586207	263.78735632183907	1.143199694124109	16.458629372983278	6.730319875468509	37	
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805.6496062992126	265.79133858267716	1.242070501187042	15.093815651975733	10.713091103653401	27	
710.0 269.6914893617021	1.0480743297453636	15.805915429025696	7.5721344798112	17		
783.5579710144928	276.8768115942029	1.6007842012523708	23.098735350021943	7.606782558045351	51	
679.5227272727273	272.25 2.0351801918080685	8.174246244233586	6.853542001853383	6		
692.7083333333334	272.0 1.5707963267948893	8.336886035938123	7.330734507325033	12		
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625.5 275.875 1.7030522529549819	8.613140462382127	5.9130095476605025	7			
755.2115384615385	280.2692307692308	1.3846234601836724	10.524548531235089	6.290859520456665	45	
715.4270833333334	285.6666666666667	1.405530154328588	19.455378895881758	6.2826325279354505	33	
702.3928571428571	282.2142857142857	0.8217211172679464	6.990012711820757	5.100234966997402	25	
773.628 290.212 1.7788492472605514	20.338331223524435	7.82536882415446	62			
624.0 285.7 1.498315628599149	10.209301645338515	6.235683835027608	13			
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825.425 285.7 0.947314382880906	7.2947675572299815	6.981659304405011	21			
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832.125 288.125 0.7854088409041973	7.20609224425855	5.654058267709201	17			
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