SOFAST ImageJ Plugin Manual

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Install

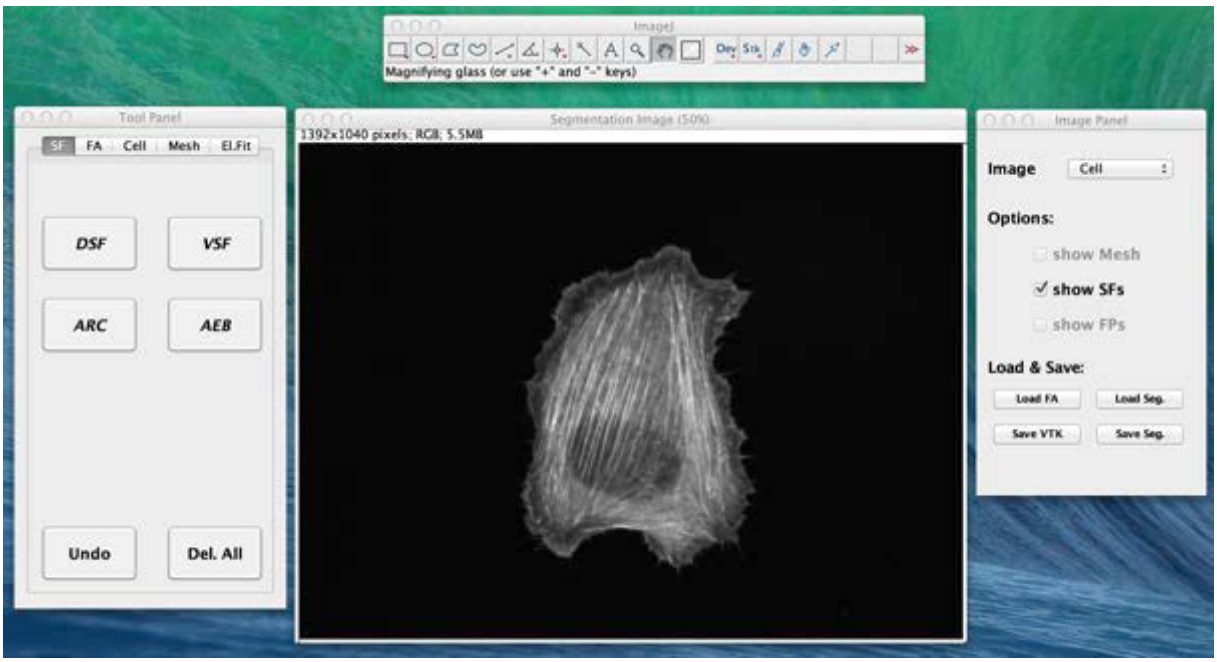
The SOFAST plugin can be installed using ImageJ's *Plugins* menu. Start ImageJ and go to *Plugins* → *Install*. Choose the file *sofast_.jar,* and save it to the suggested folder. You will now see a new entry called *SOFAST* in the *Plugins* menu. Alternatively, you can copy the *sofast_.jar* file to ImageJ's *plugin* folder in the ImageJ installation path, and restart ImageJ.

Uninstall

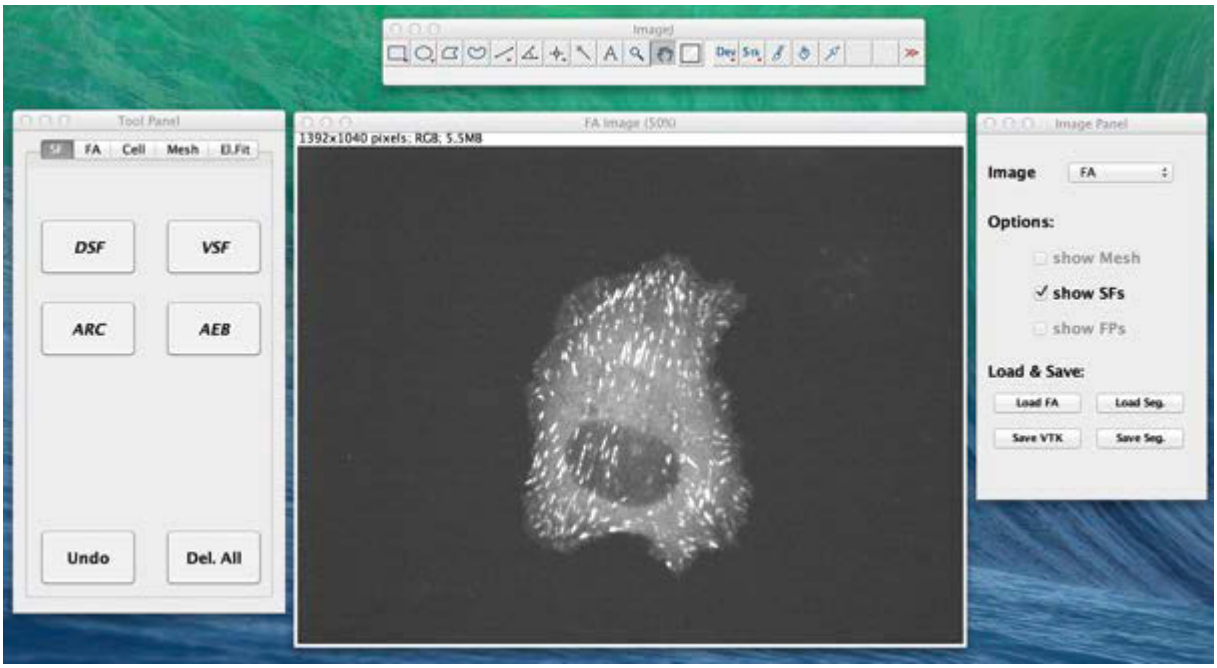
Find ImageJ's installation folder and navigate to *plugins.* Remove the file *sofast_.jar*.

Segmentation with SOFAST

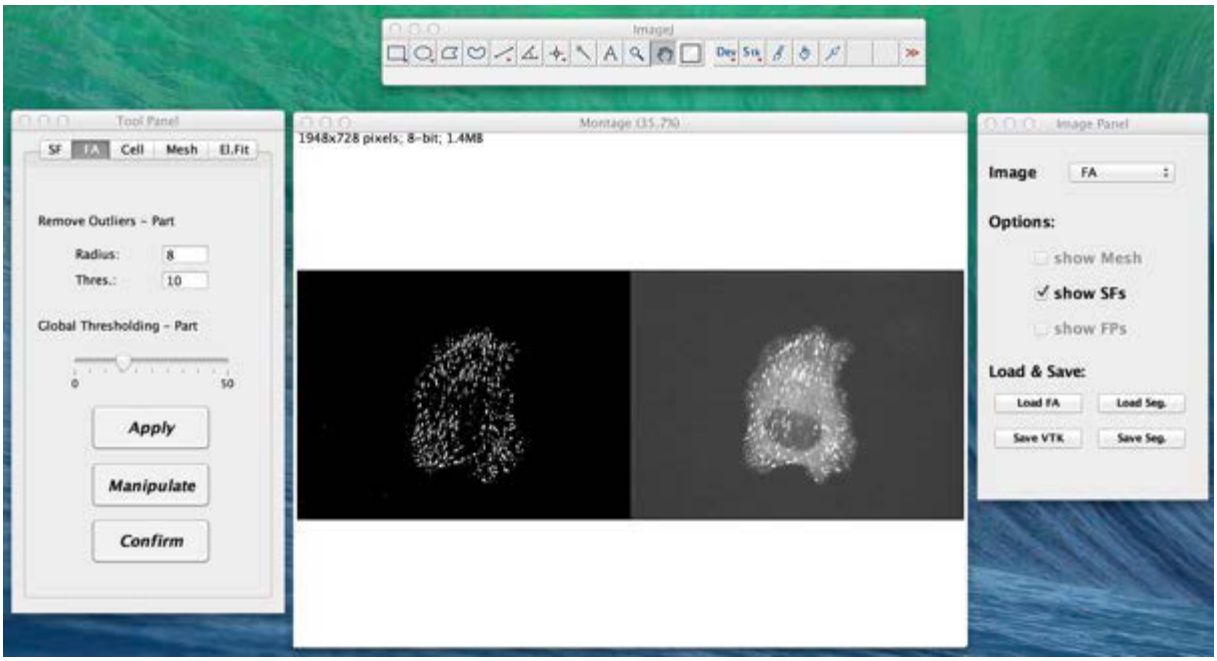
FA segmentation. Focal adhesions (FA) and stress fibers (SF) will be segmented from the paxillin and actin fluorescence images, respectively (instead of paxillin, one could also use other FA-markers, e.g. vinculin or zyxin). Moreover the actin image will be used to give cell shape. Therefore we first start ImageJ and open the actin fluorescence image. Then click *Plugins* → *SOFAST* and the plugin will start. You should see a screen like this:



Note the *Tool Panel* at the left and the *Image Panel* at the right. Now click on *Load FA* in the *Image Panel*, and choose the corresponding fluorescence image. Note that the *Image* dropdown menu in the *Image Panel* now turns from *Cell* to *FA*. You can switch between different images using this menu. You should now see the following screen:

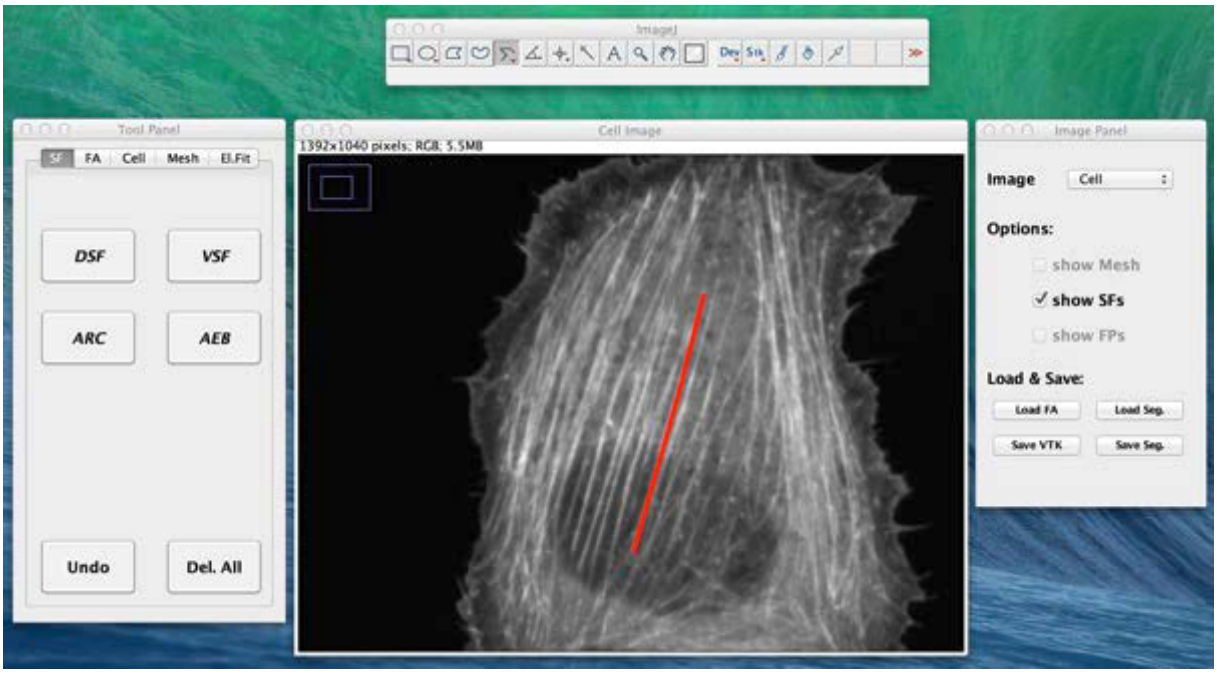


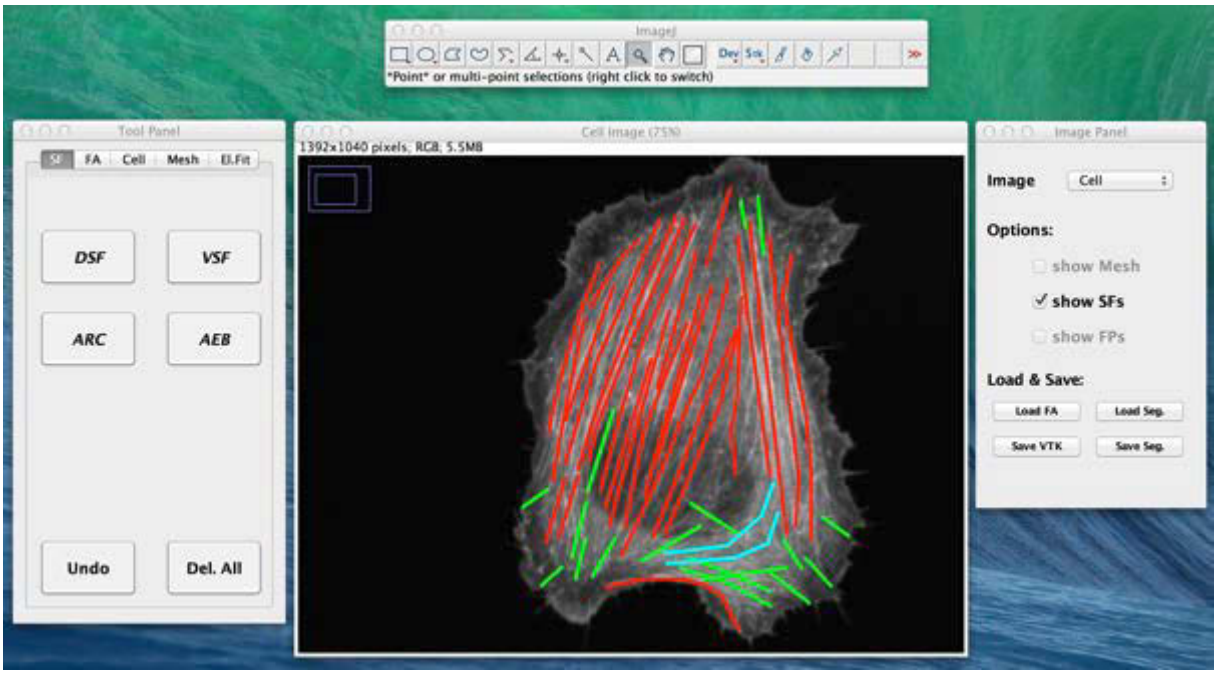
We now segment the FAs. Select *FA* in the *Tool Panel*. Adjust the parameters for outlier removal and global thresholding and click *Apply*. Everytime you do so, the segmented image will be calculated from the original fluorescence image. You can see original and segmentation side by side to decide 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.



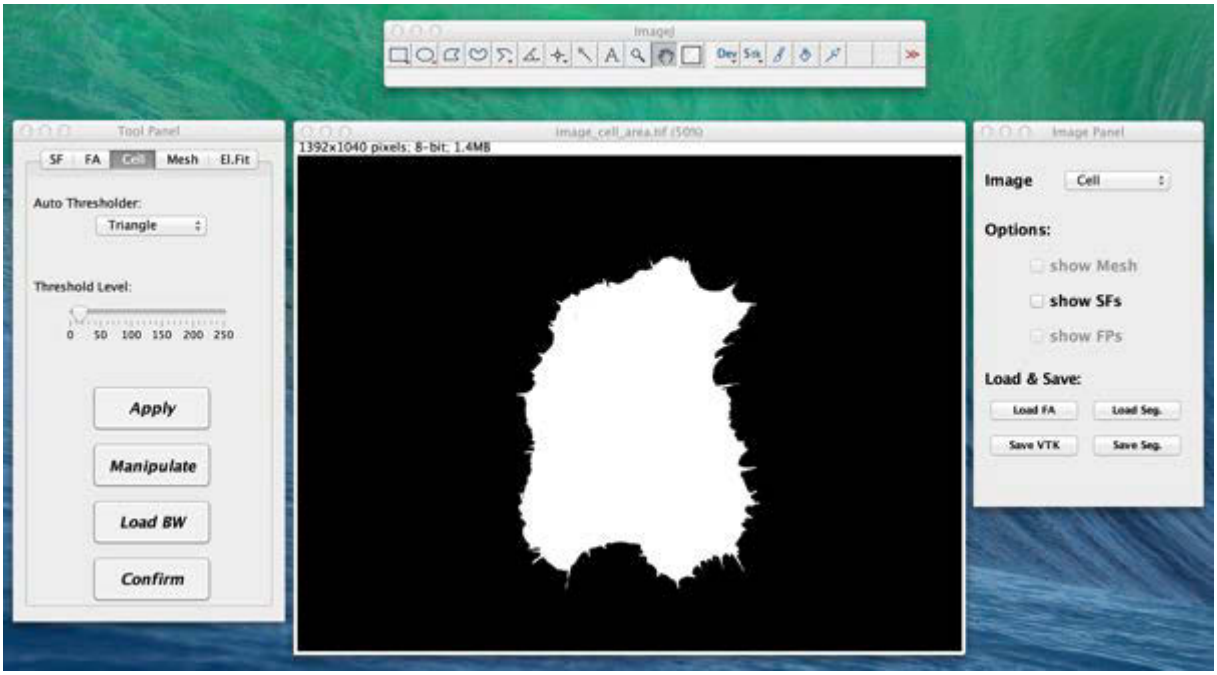
SF segmentation. In order to segment the SFs, we switch back to the actin cell image (in the *Image Panel* or by hitting the *space* key on your keyboard when the image window is active) and to the *SF* tab in the *Tool Panel*. The four buttons in the upper part represent

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 such 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 into the image to be more precise. It is always possible to save and reload your segmentation with the corresponding buttons in the *Image Panel*.

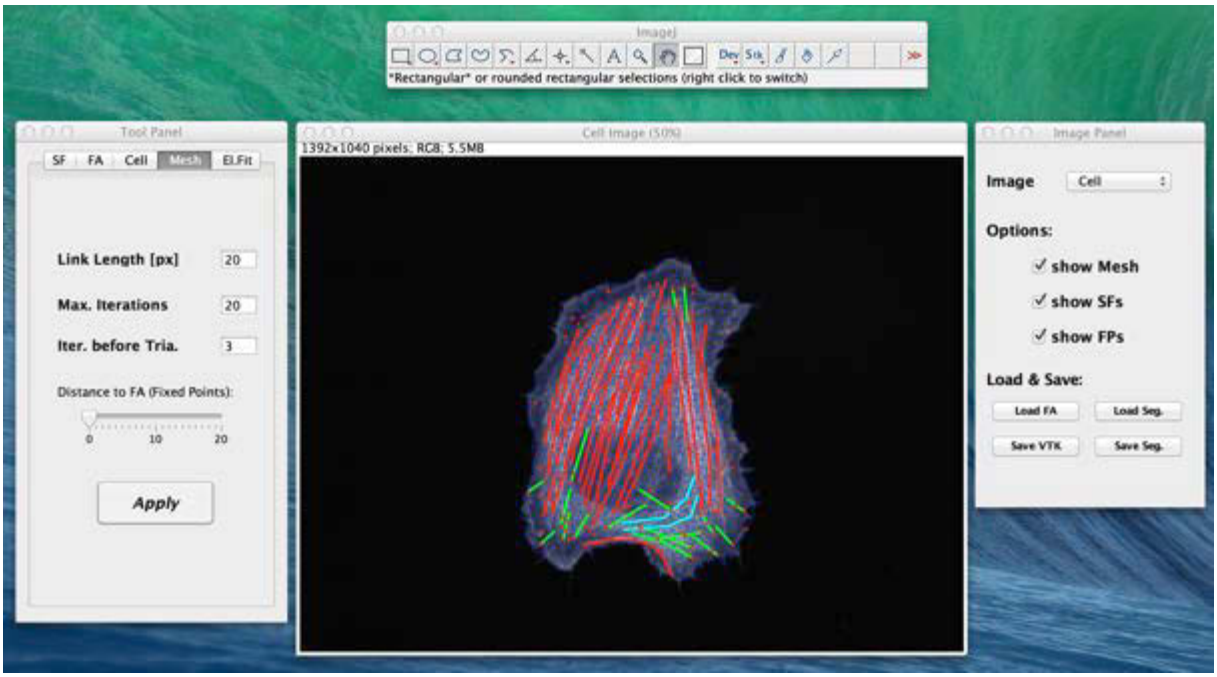




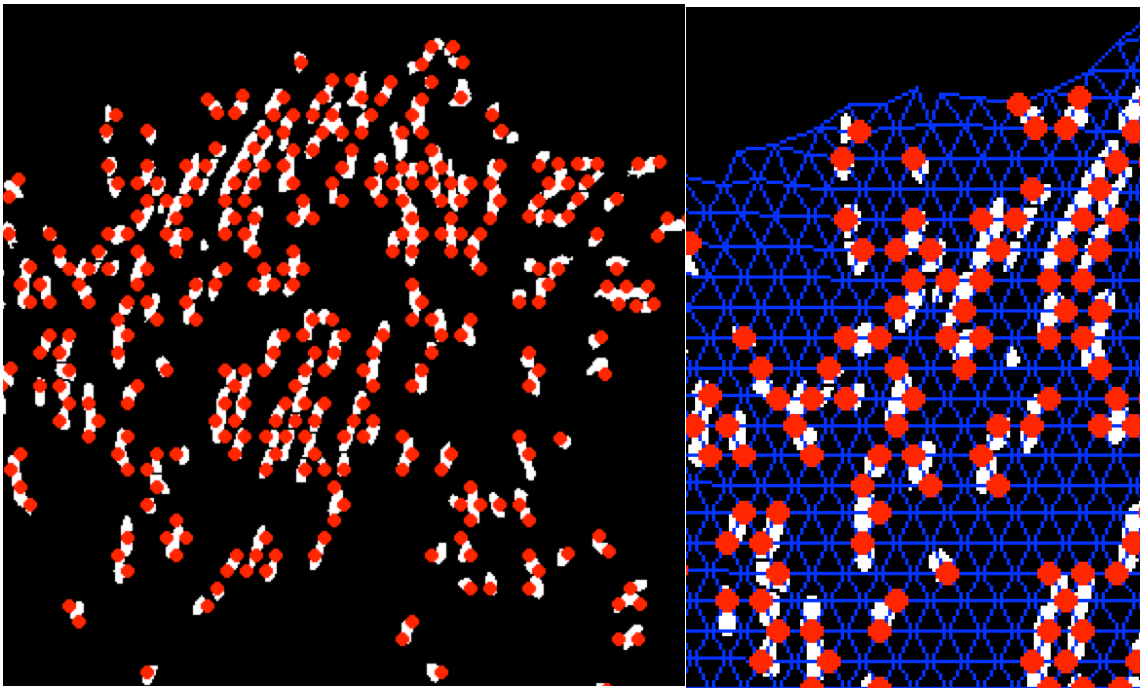
Cell segmentation. In order to segment the cell area from the actin image, click on the *Cell* tab in the *Tool Panel* and turn off SF visualization (uncheck *show SFs* in the *Image Panel*). Set a thresholding method and level and hit apply. Again, after clicking *Manipulate* you can use ImageJ's build-in 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*.

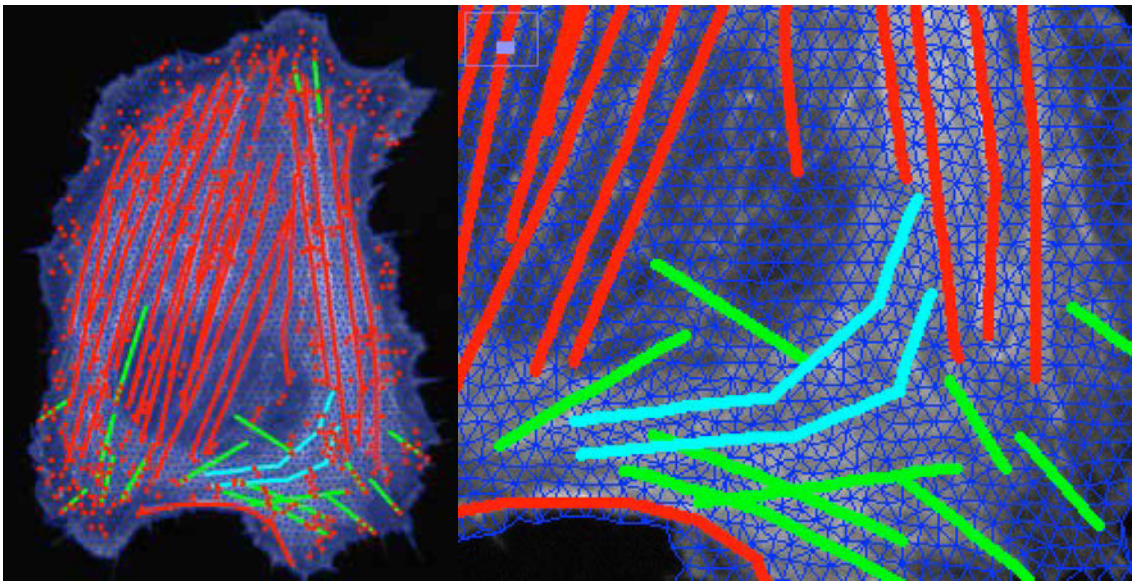


Mesh generation. In the next step, we want to transfer the segmented information into a triangular network that can be used for computational purposes, in particular for model-based traction force microscopy. Switch to the *Mesh* tab in the *Tool Panel*. Adjust the parameters that control mesh size and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

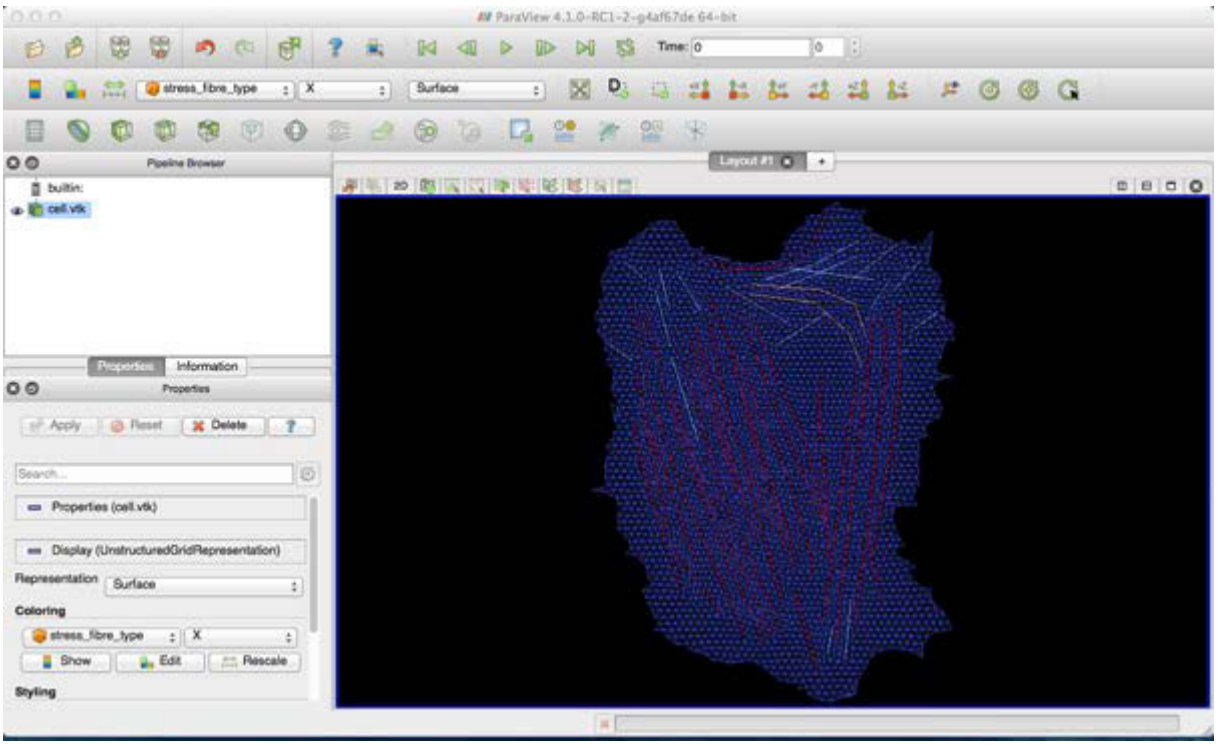


The SFs are displayed in different colors and network links run along all of them. The network spans the whole cell area and is displayed in blue. Small red dots mark the sites where network vertices lie above focal adhesions. As the distribution of fixed points might not be sufficient to describe FA 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 the 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. with ParaView, www.paraview.org, see below) and is also supported as an active cable model description for model-based traction force microscopy.



Fit ellipses to FAs. It is also possible to fit ellipses to all segmented focal adhesions in the SOFAST plugin. 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 contain 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.