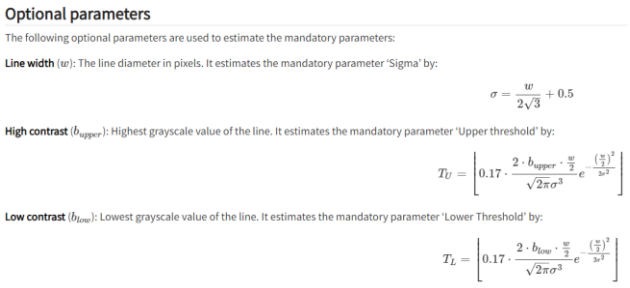
Ridge Detection Usage Tutorial

1. Load the image into ImageJ and if it is a video use **Image->Stacks->Z-project->Avg. Intensity**
2. Save the original image with an indicative name, which we'll use to pair with the results
3. Optional. While trying to use the RidgeDetection you may receive a warning for the type of file it can process. If so: **Image->Type->8-bit**
4. Before opening the RidgeDetection plugin we need to find the **Optional Parameters**, which are used to calculate the Mandatory Parameters.



Optional parameters

The following optional parameters are used to estimate the mandatory parameters:

Line width (w): The line diameter in pixels. It estimates the mandatory parameter 'Sigma' by:

$$\sigma = \frac{w}{2\sqrt{3}} + 0.5$$

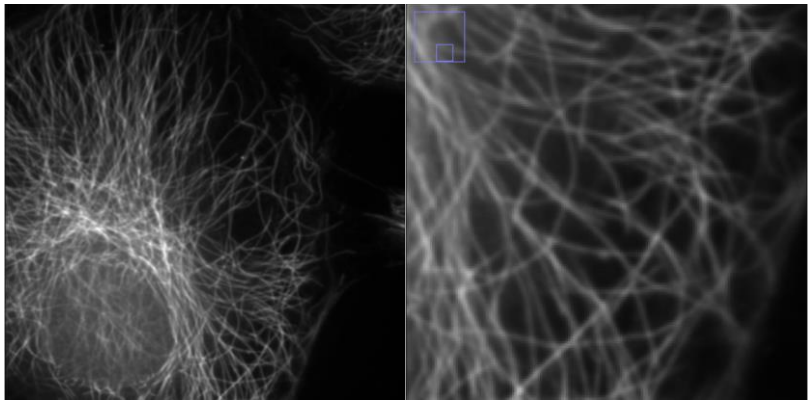
High contrast (b_{upper}): Highest grayscale value of the line. It estimates the mandatory parameter 'Upper threshold' by:

$$T_U = \left\lfloor 0.17 \cdot \frac{2 \cdot b_{upper} \cdot \frac{w}{2}}{\sqrt{2\pi\sigma^3}} e^{-\frac{\left(\frac{w}{2}\right)^2}{2\sigma^2}} \right\rfloor$$

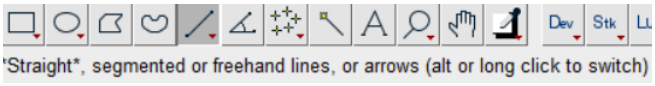
Low contrast (b_{low}): Lowest grayscale value of the line. It estimates the mandatory parameter 'Lower Threshold' by:

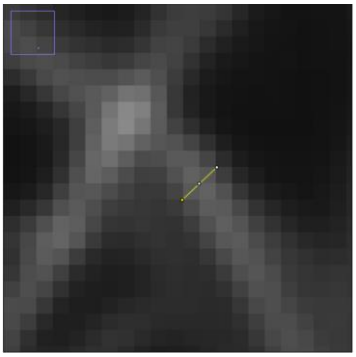
$$T_L = \left\lfloor 0.17 \cdot \frac{2 \cdot b_{low} \cdot \frac{w}{2}}{\sqrt{2\pi\sigma^3}} e^{-\frac{\left(\frac{w}{2}\right)^2}{2\sigma^2}} \right\rfloor$$

   a. To find all of these parameters I suggest to zoom in a section of the image with high SNR (Signal-to-Noise ratio), normally being the **corners of the images**.



   b. **Line width**: Zoom in until you can clearly see the filament in **PIXEL detail**. Then use the **"Straigth"** line function and draw it for the **width of the filament**.

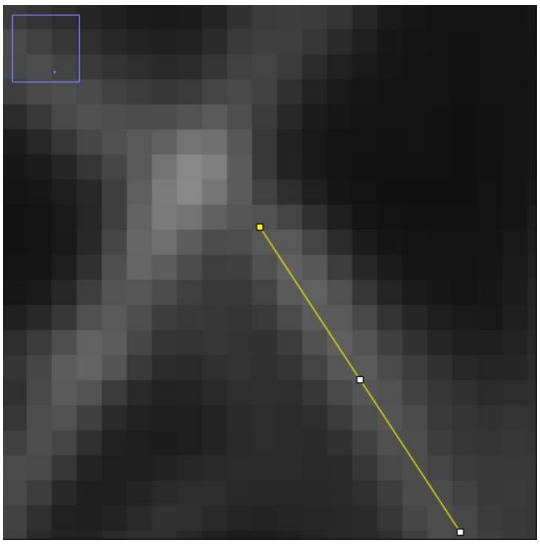




i. Press **Ctrl+M** or **Analyze->Measure** and you will receive the Line Width as the **Length**.

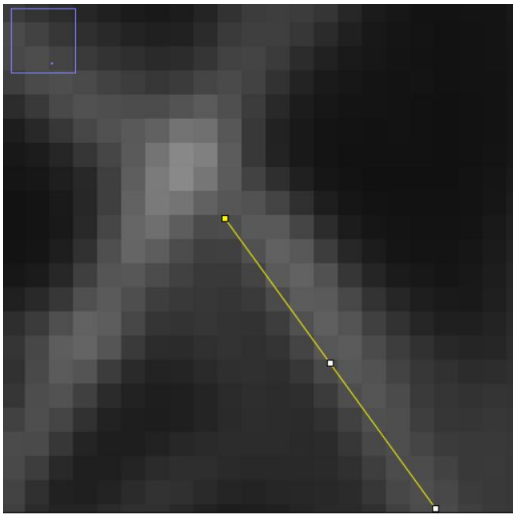
	Area	Mean	Min	Max	Angle	Length
1	4	79.730	64.417	91.956	43.831	2.888

c. **Low Contrast**: Once again draw a line but through the **middle of the filament**. Press **Ctrl+M** and use the **Mean** as your Low Contrast.



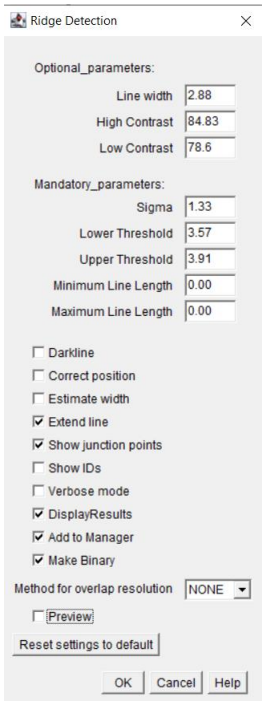
	Area	Mean	Min	Max	Angle	Length
1	16	84.831	73.882	95.294	-56.811	14.538

d. High contrast: Now draw a line at the **side of the filament**. It should be through what is still considered filament (**not on the background**). Press **Ctrl+M**

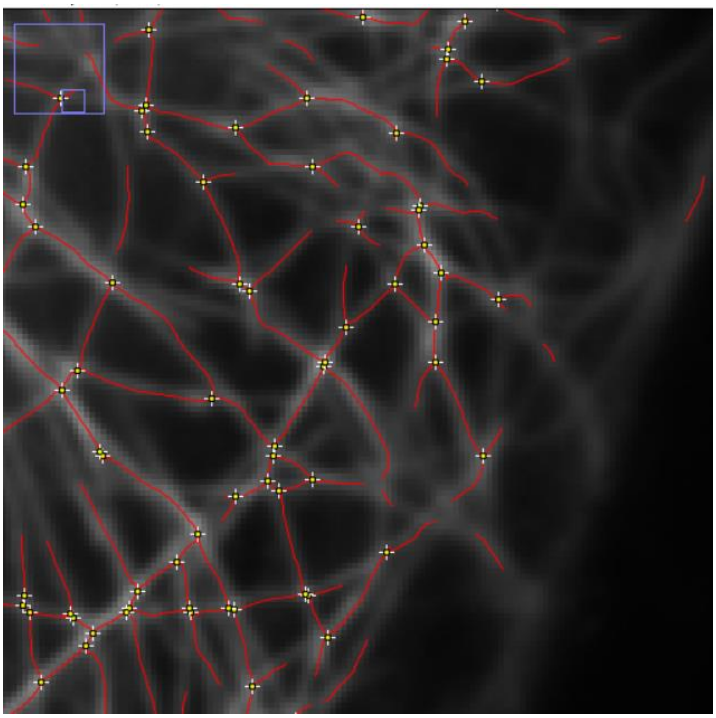


	Area	Mean	Min	Max	Angle	Length
1	16	78.605	74.722	82.015	-53.996	14.885

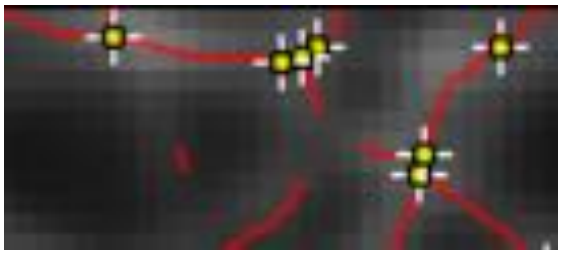
5. Zoom out into the **initial corner section studied** (Before zooming in to Pixel Resolution). Open **Plugins->Ridge Detection** and enter the parameters found before, you will see that the mandatory parameters are calculated in real time. **Make sure to have the same boxes checked as seen in the image**:



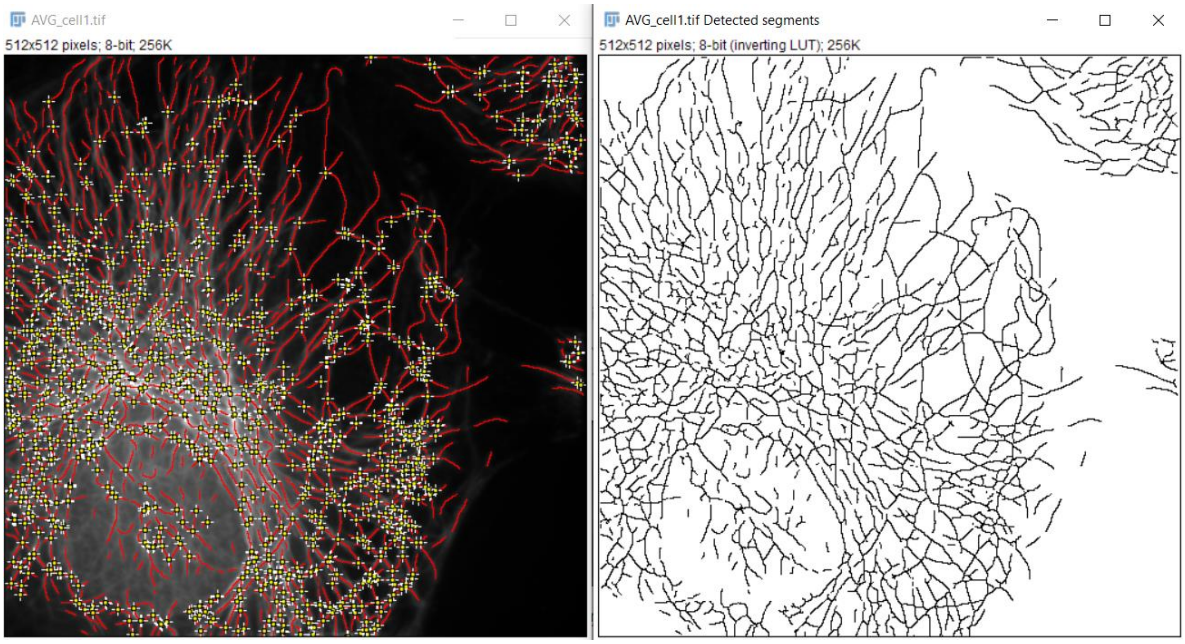
6. Select **Preview**, you will see in the image the proposed filaments, with the junction points (connections between branches)



7. We can see in the picture that filaments are correctly detected but there are still some with lower intensity that are not being detected. **You could change the parameters, I recommend to adjust first the contrast and then the line width**.
    a. You should adjust prioritizing that it is able to reconstruct more filaments but without the creation of many junction points close together. **When you start seeing many junction points close together it is doing overfitting**. For example:



8. From experimenting, while doing the initial estimation process, the parameters shouldn't change that much, **normally lower the values**. After being happy with the result of the zoomed in area look at the whole picture and once again adjust parameters
    a. **The ridge detection doesn't let you interact with the image while open so you would need to close it (Take note of your latest parameters)**
9. Before proceeding, **check the box Estimate Width** (We want to analyze this value, the resulting binarized image will not look as great so if you want it make sure to run it without Estimate Width). Select **OK** in the ridge detection and you will receive a binarized image as well as some tables.



10. Save the **Results table** according to the name used for saving the file CellX_results
11. Save the **Junctions table** according to the name used for saving the file CellX_junctions

Additional Information:

Ridge Detection Wiki: https://imagej.net/plugins/ridge-detection#optional-parameters

Ridge Detection Video Tutorial: https://www.youtube.com/watch?v=55MPB3YblGQ

For referencing the use of ridge detection they request to reference the formal method and implementation:

Method: Steger, C. (1998). An unbiased detector of curvilinear structures. *IEEE Transactions on Pattern Analysis and Machine Intelligence*, *20*(2), 113–125. doi:10.1109/34.659930

Implementation: Wagner, T., Hiner, M., & Xraynaud. (2017). thorstenwagner/ij-ridgedetection: Ridge Detection 1.4.0 (Version v1.4.0). Zenodo. doi:10.5281/ZENODO.845874