

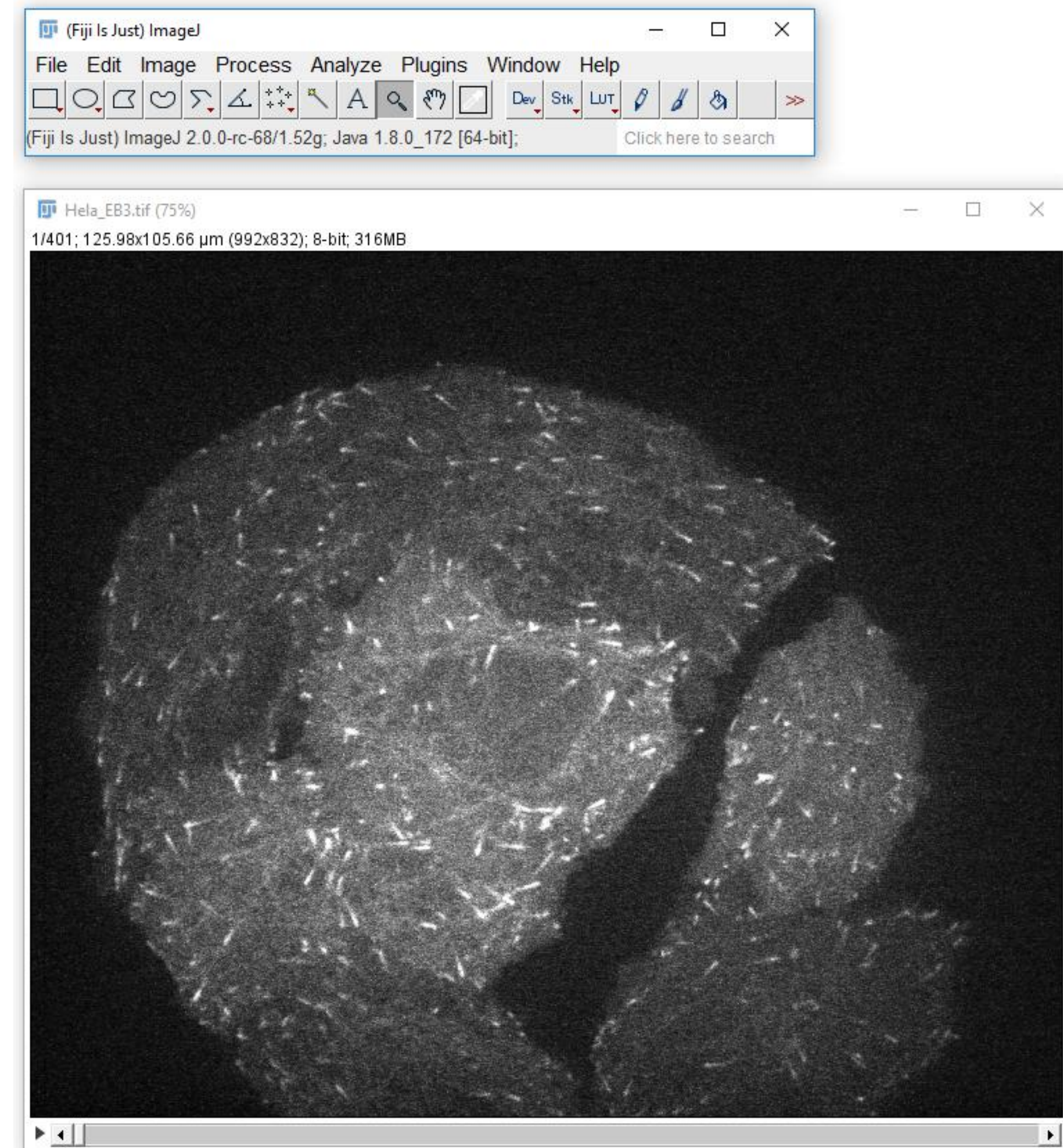
Exercise: Reslicing/Kymograph

Image Processing & Analysis for Life Scientists

Olivier Burri, Romain Guiet & Arne Seitz

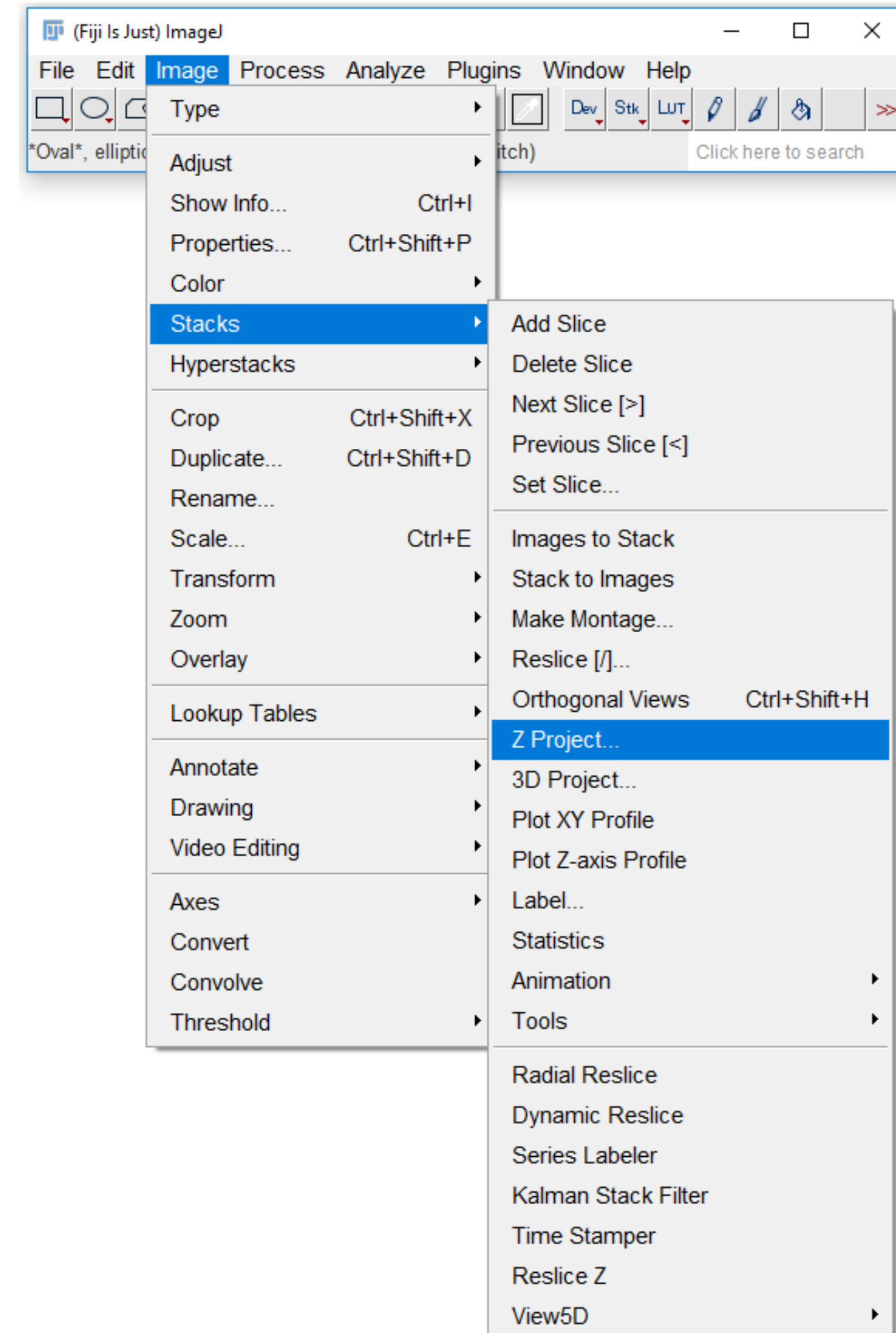
Reslicing

1. Open *Hela_EB3.tif*
EB3 is a protein that binds to the growing ends of microtubules.
2. Try to find a suitable projection method in order to find the trajectories of the EB3 tips.



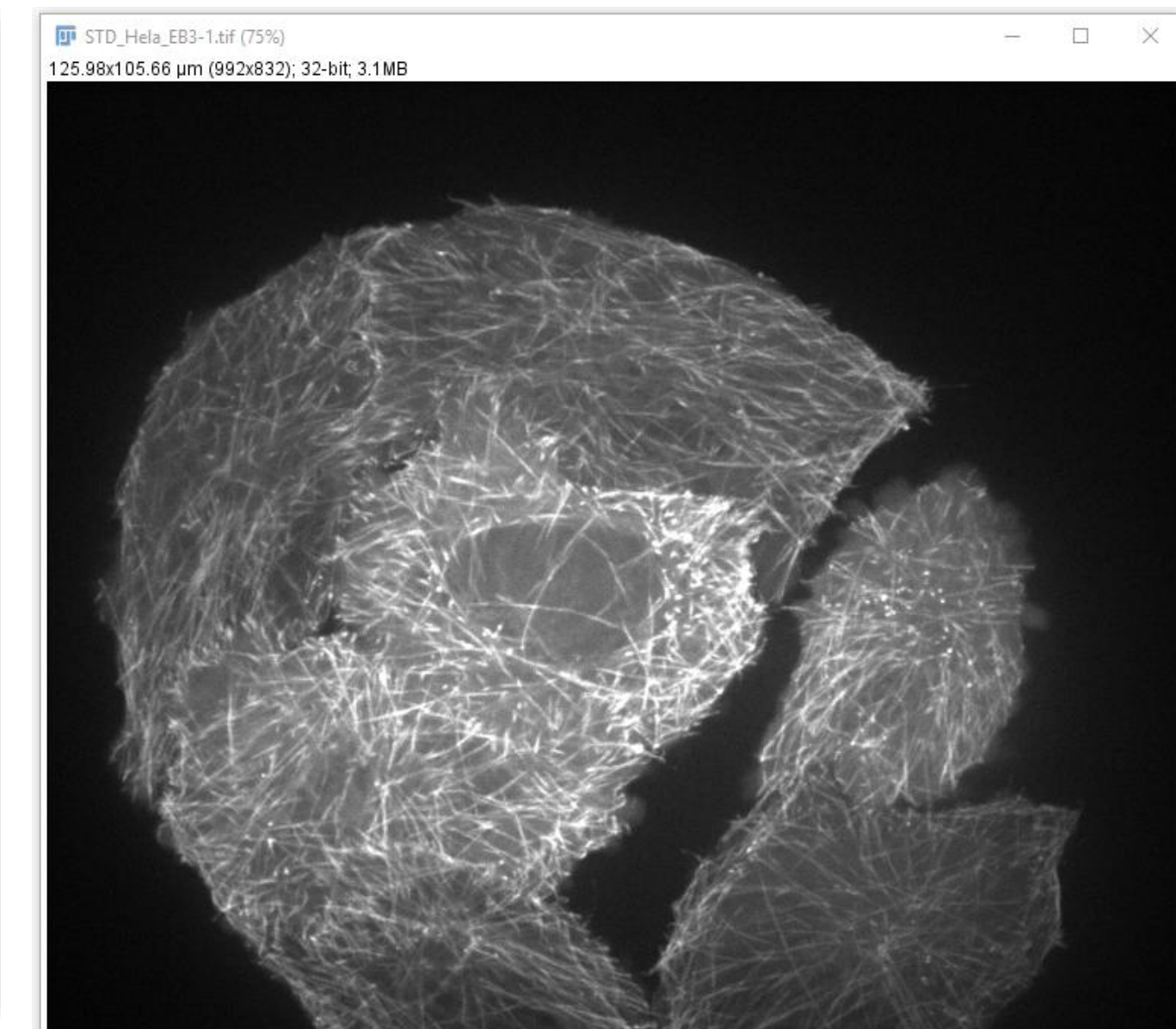
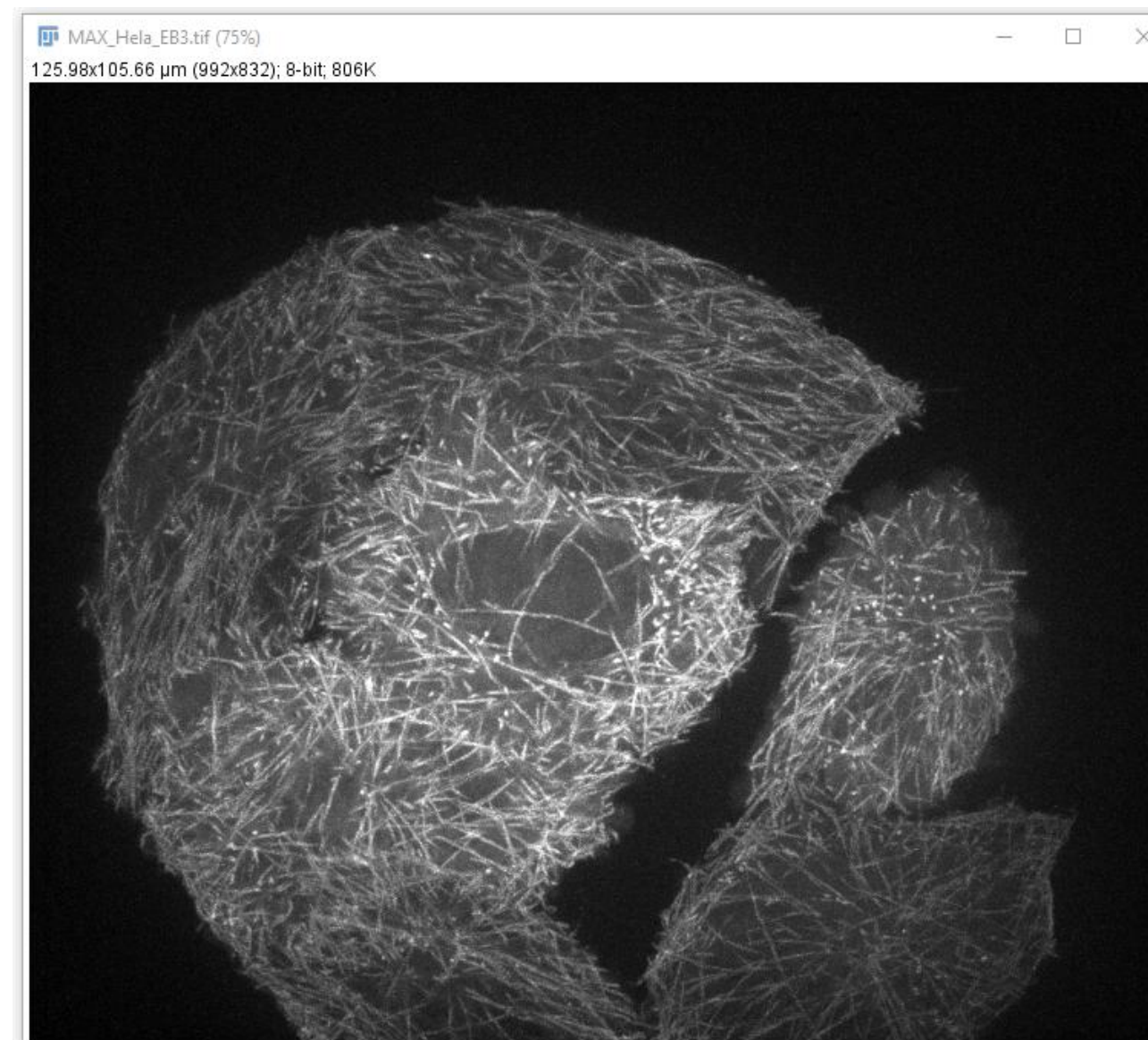
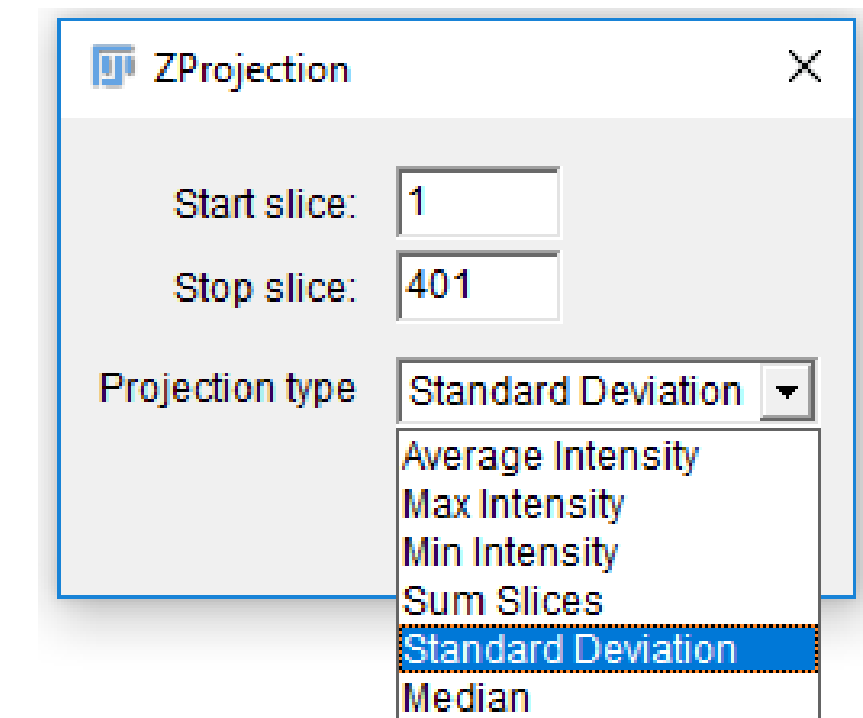
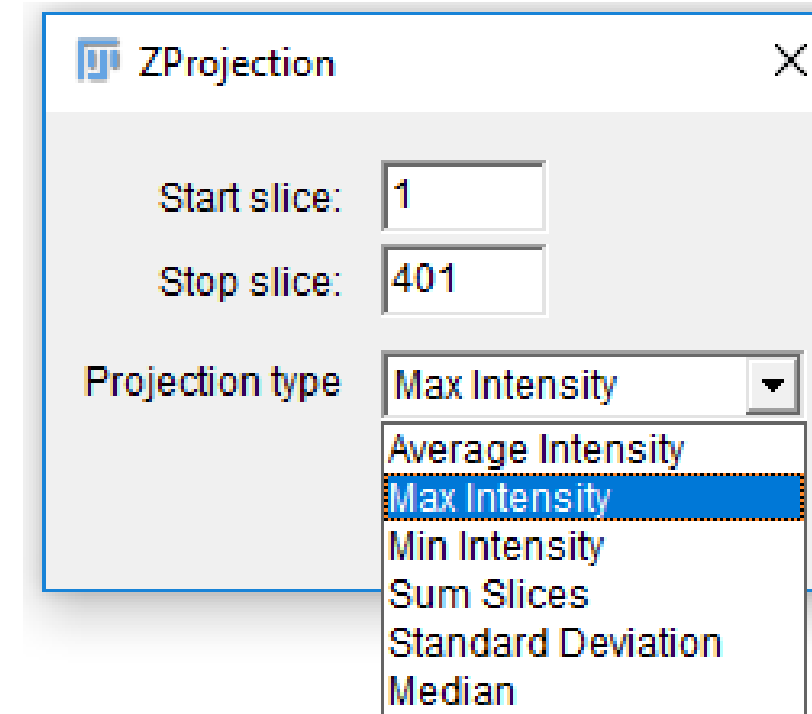
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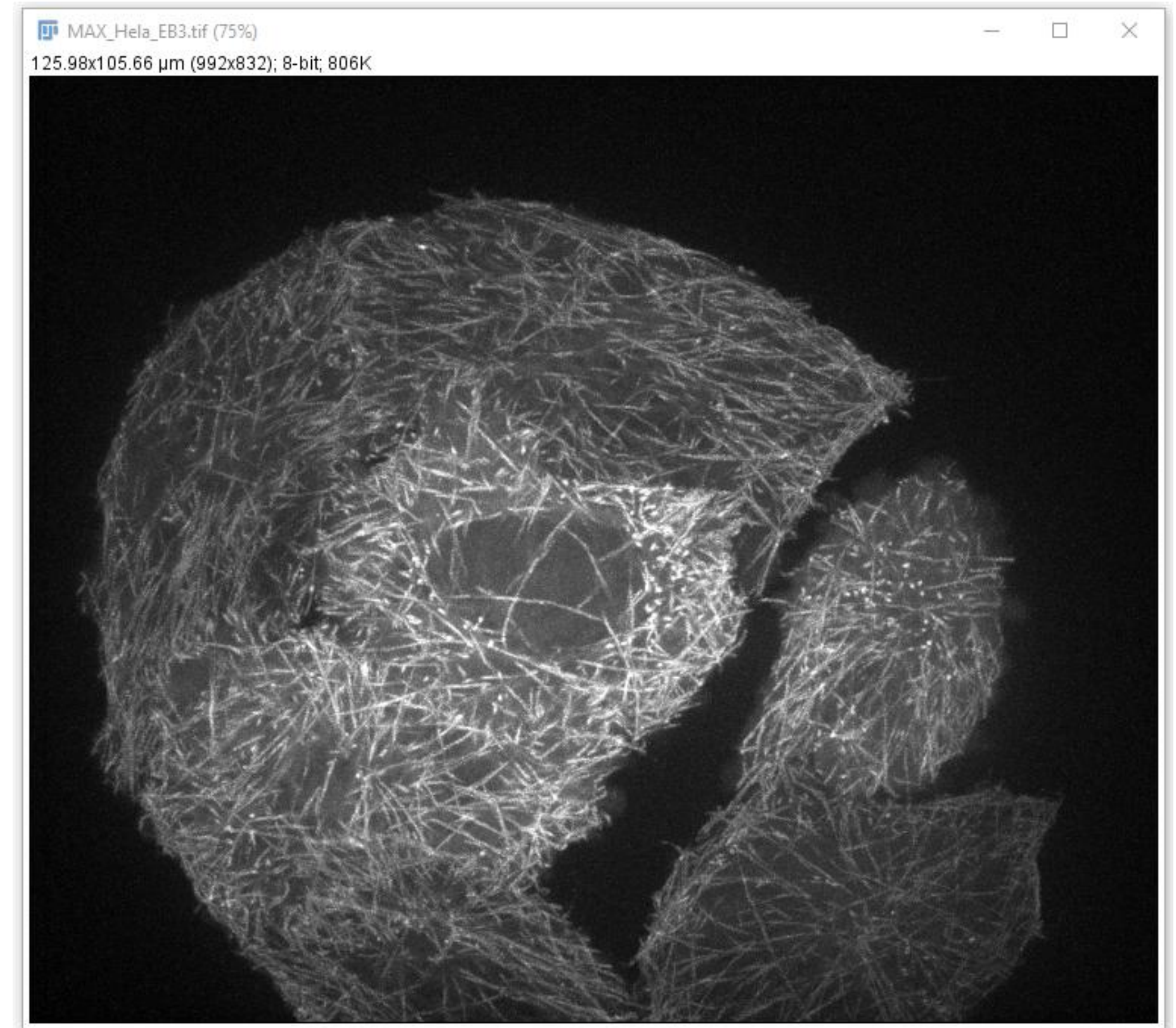
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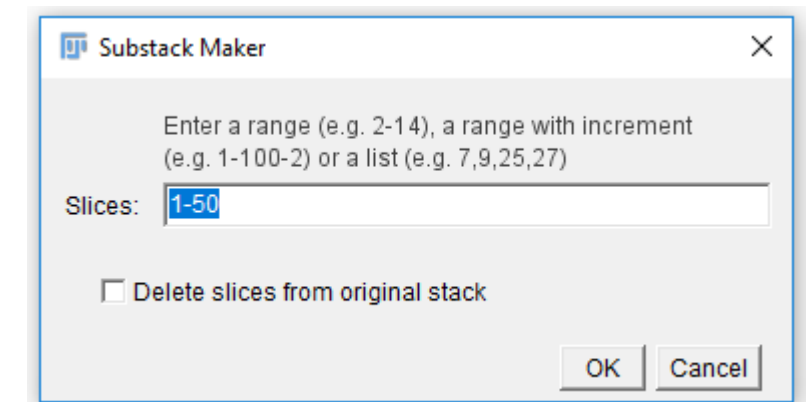
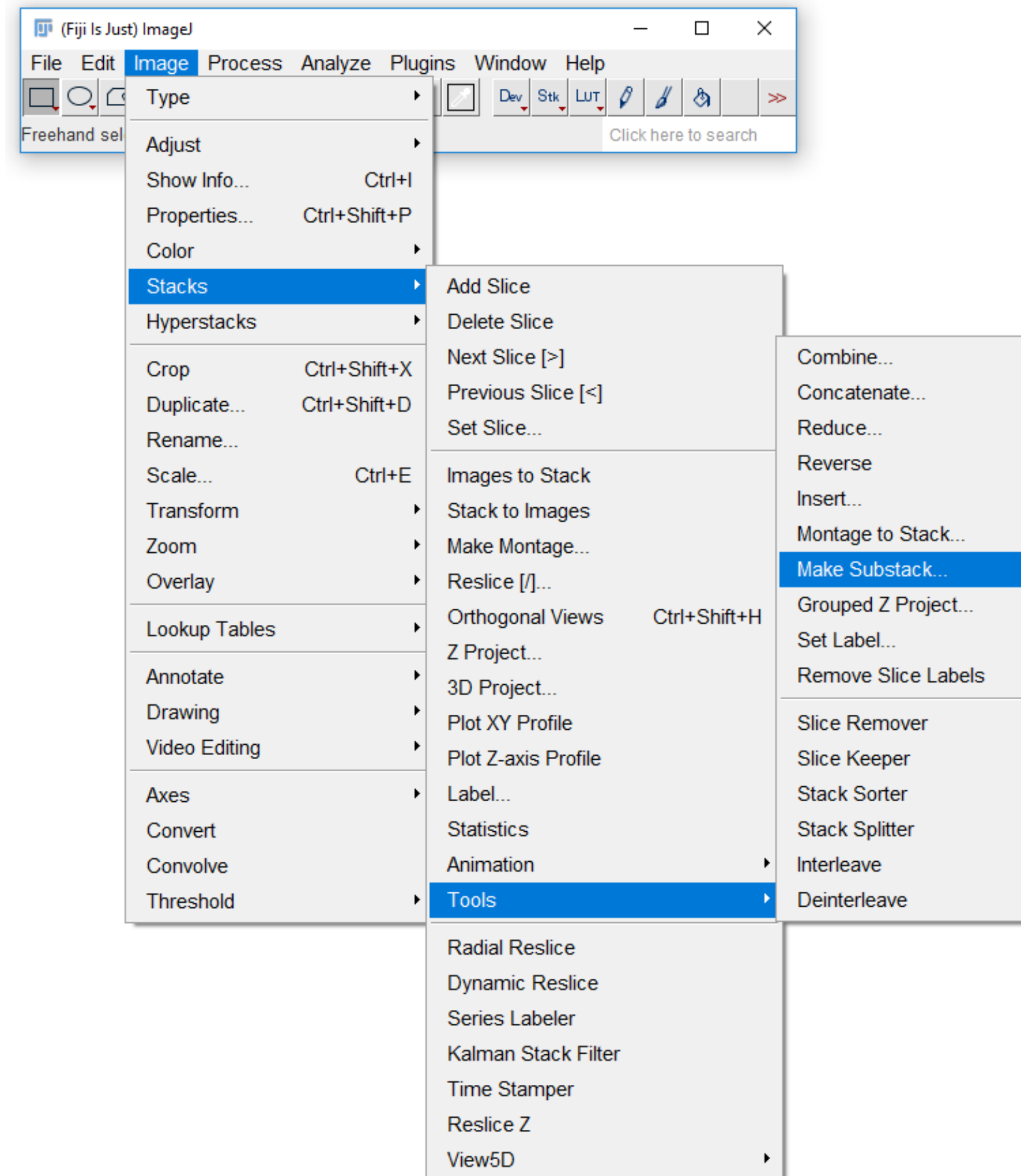
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4. What can be done to reduce the number of trajectories?



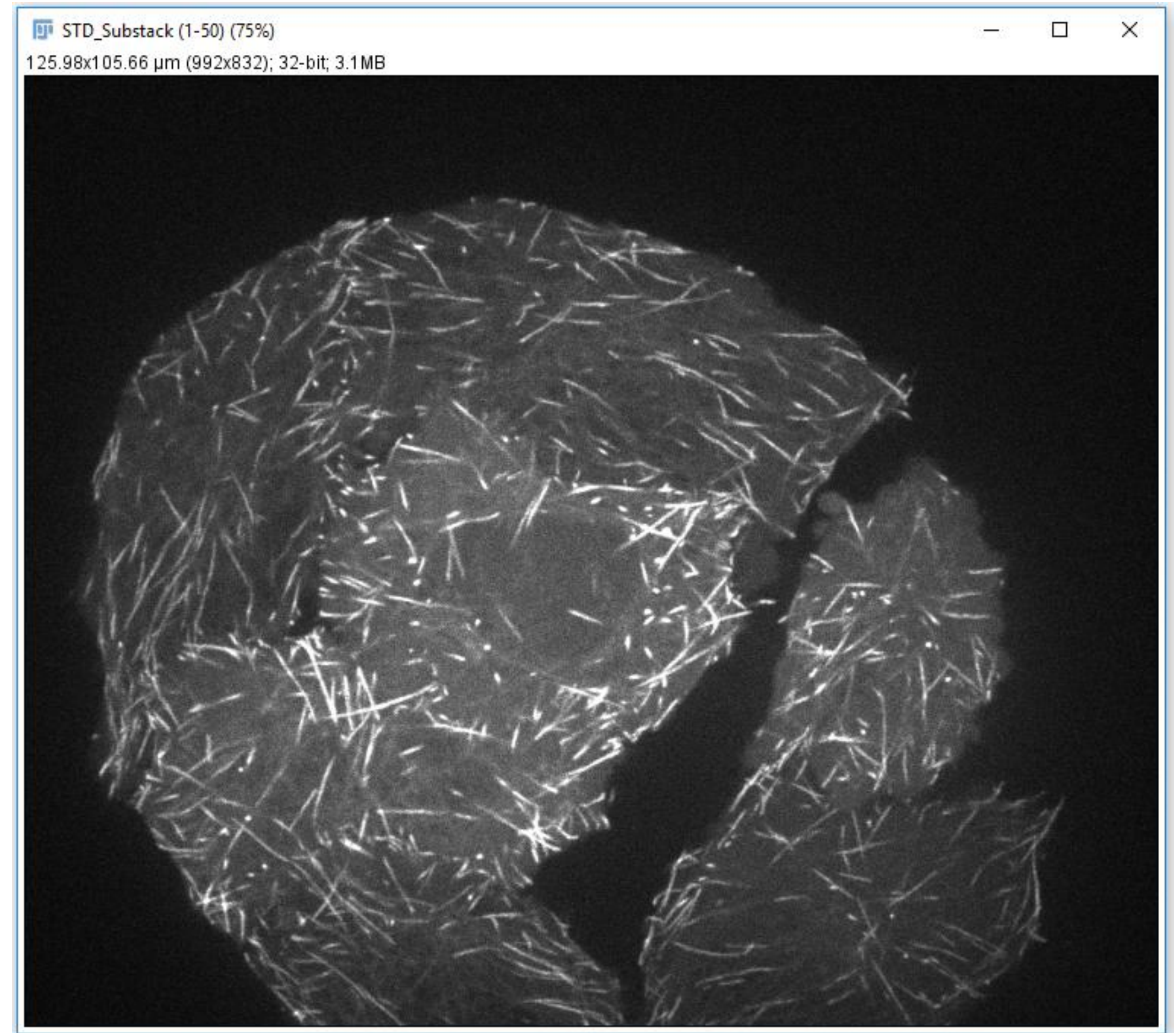
Reslicing

4. What can be done to reduce the number of trajectories?
5. Make a substack of the first 50 slices.
Image → Stacks → Tools → Make Substack...
6. Perform a Z-projection



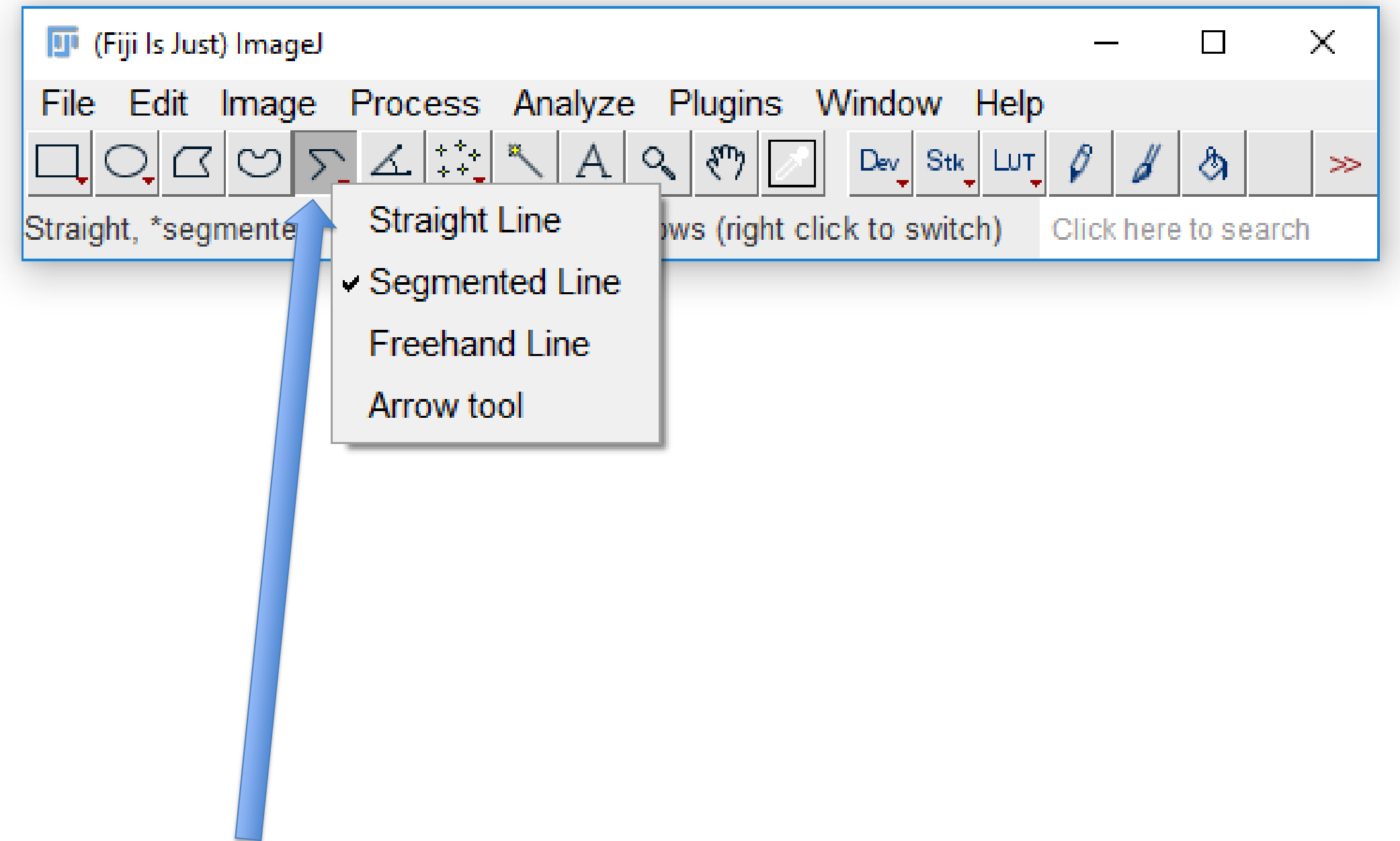
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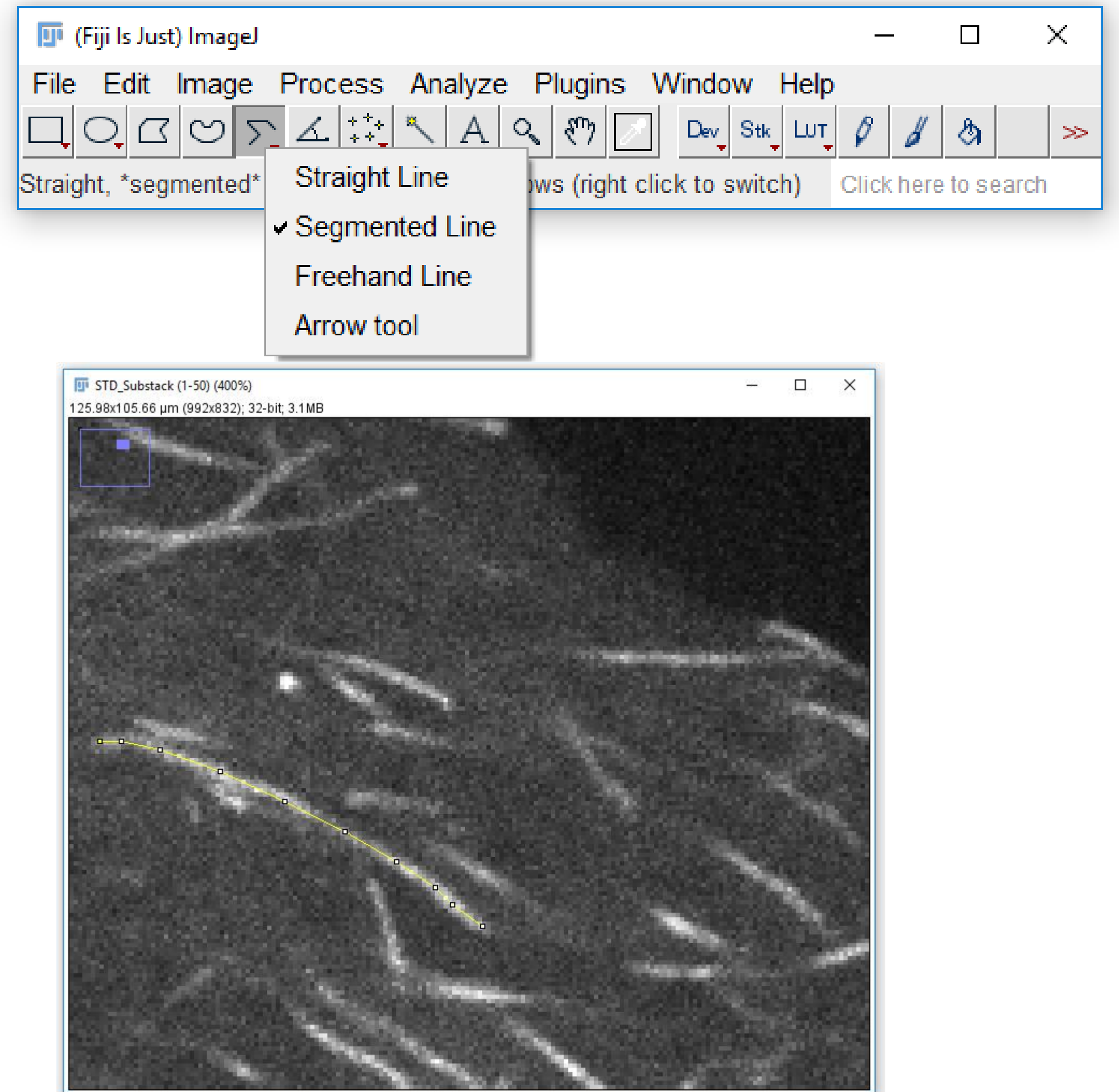
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7. Zoom in into the projected image and draw a line with the segmented line tool.



A mouse right-click opens the selection window.

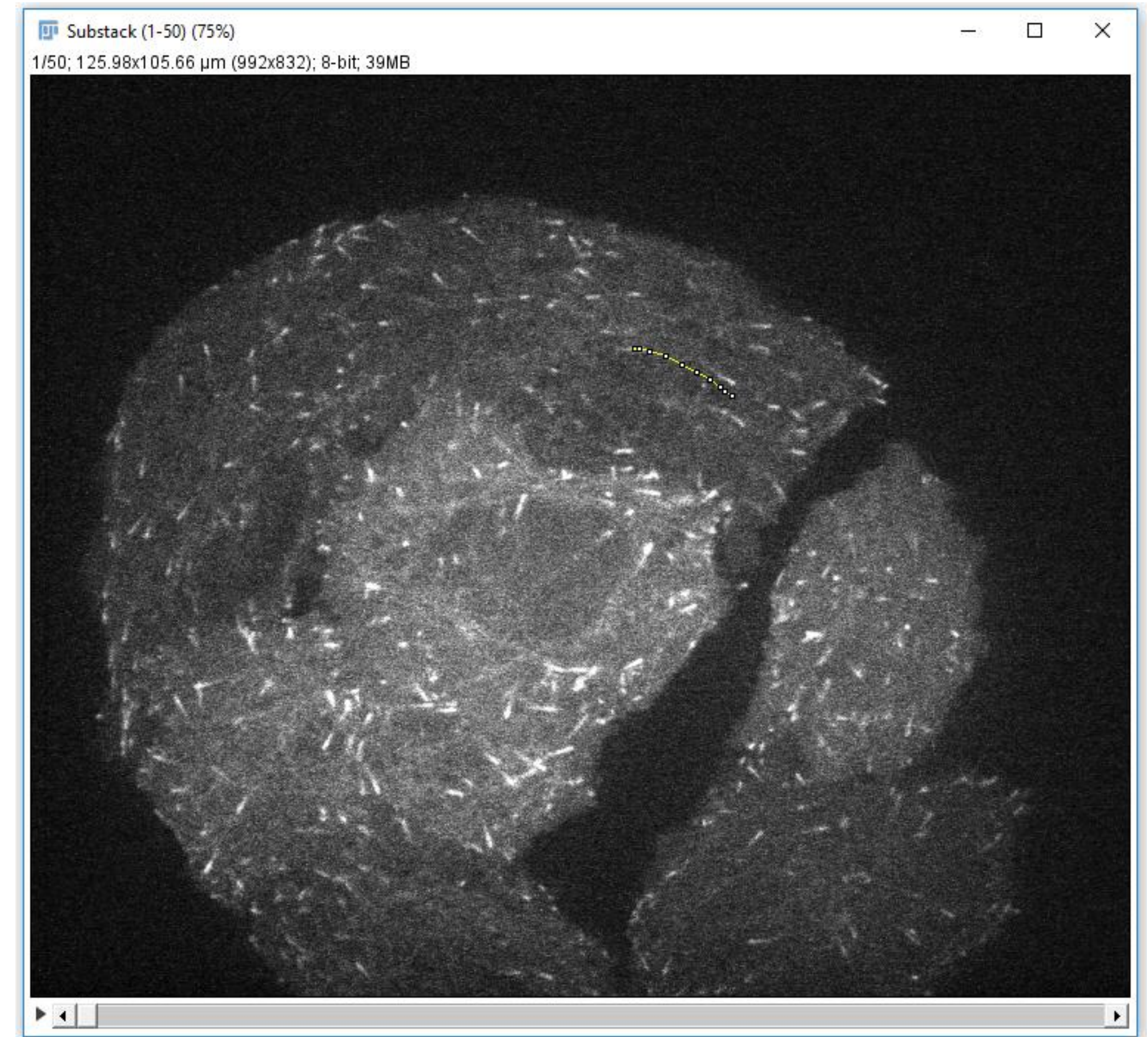
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8. Transfer the selection to the stack and perform the Reslice operation.



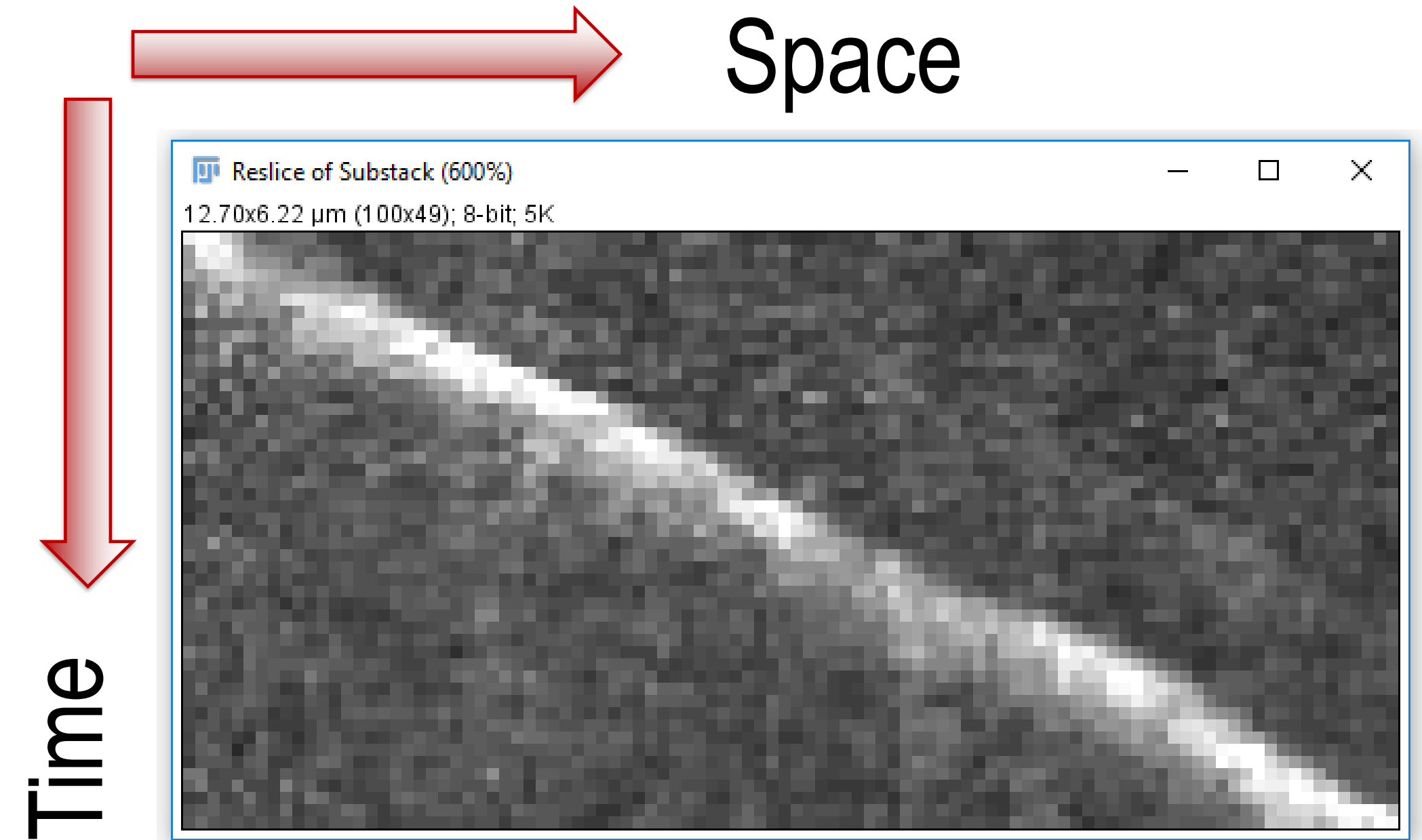
Reslicing

8. Transfer the selection to the stack and perform the Reslice operation.
9. Use: Edit→Selection→Restore Selection to transfer the selection to the stack.
10. Use: Image→Stacks→Reslice [/]... to create a kymograph



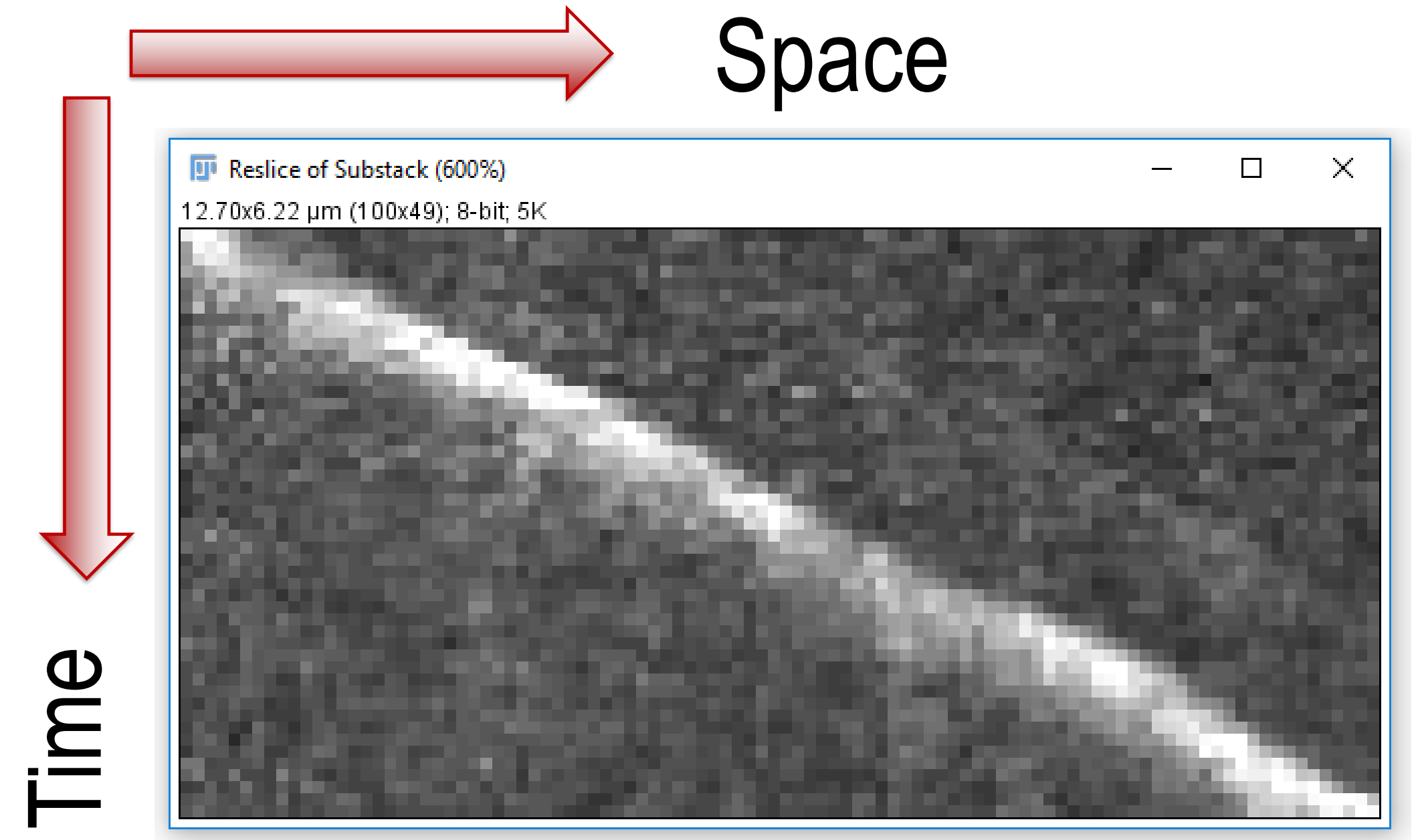
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11. Calculate the speed of the growing microtubule tip.



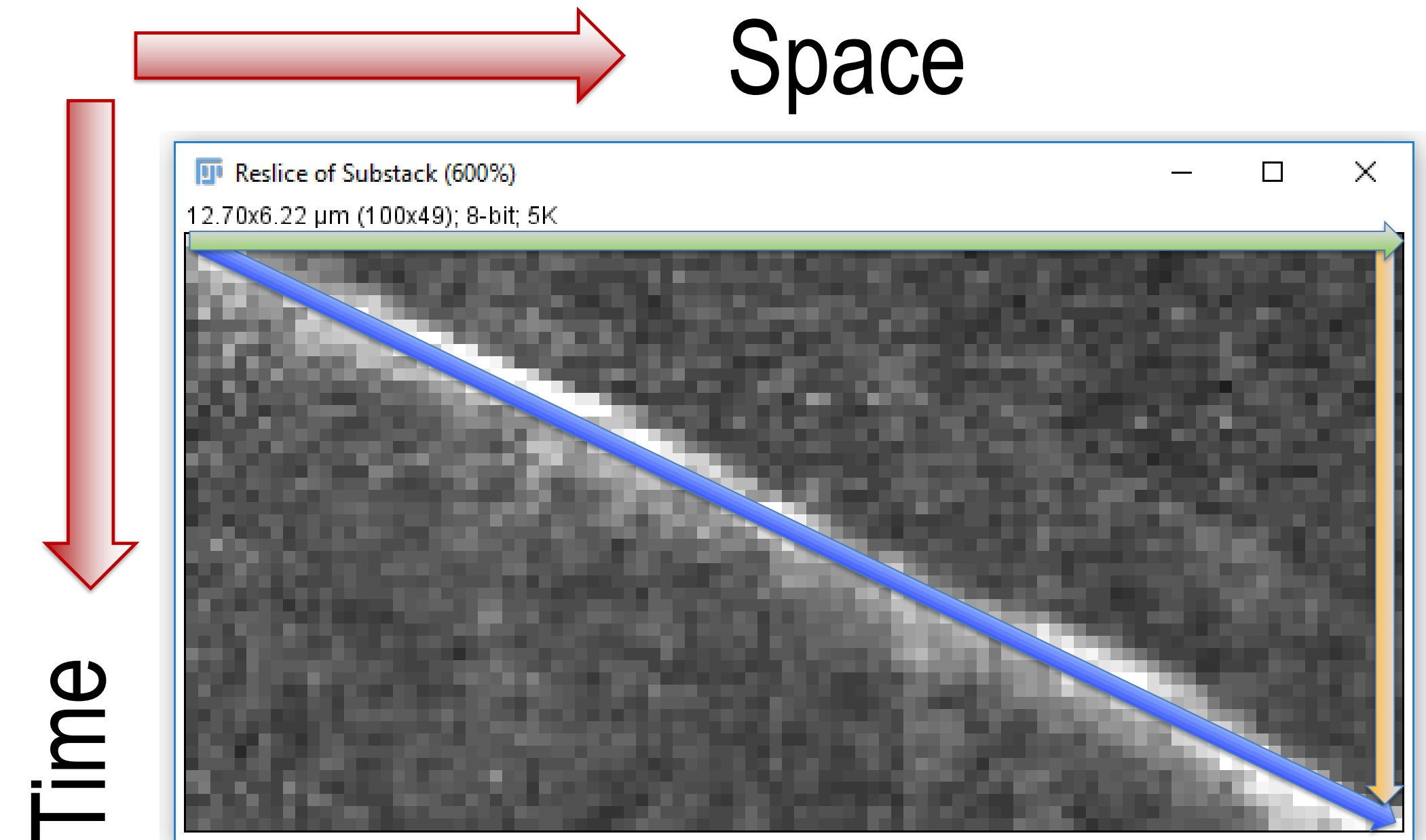
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For the units see: Image → Properties of the time-stack.

Space axis: 1 pixel $\approx 0.127 \mu m$

Time axis: 1 pixel $\approx 0.3 s$

$$v = \frac{100 \times 0.127 \mu m}{49 \times 0.3 s} = 0.86 \mu m s^{-1}$$



→ trajectory of moving structure

→ distance travelled

→ travel time

distance traveled = width of the image.

travel time = height of the image.

Reslicing

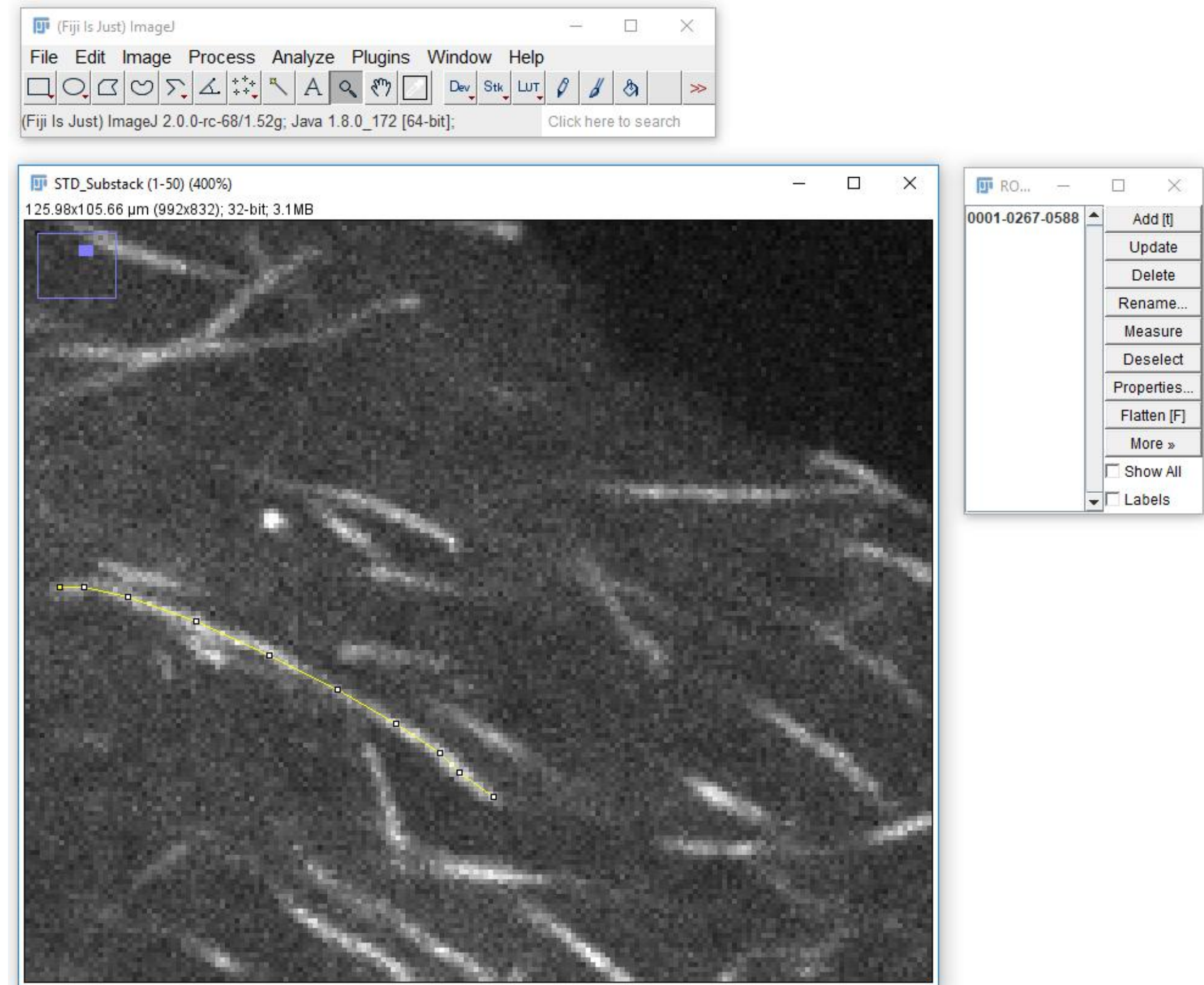
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Space axis: 1 pixel $\approx 0.127 \mu m$

Time axis: 1 pixel ≈ 0.3

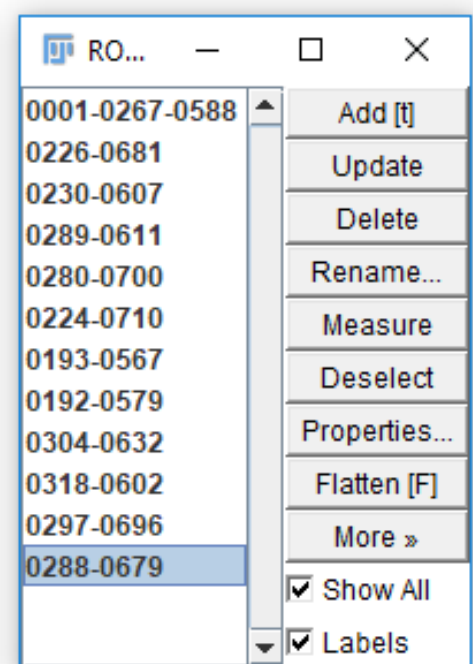
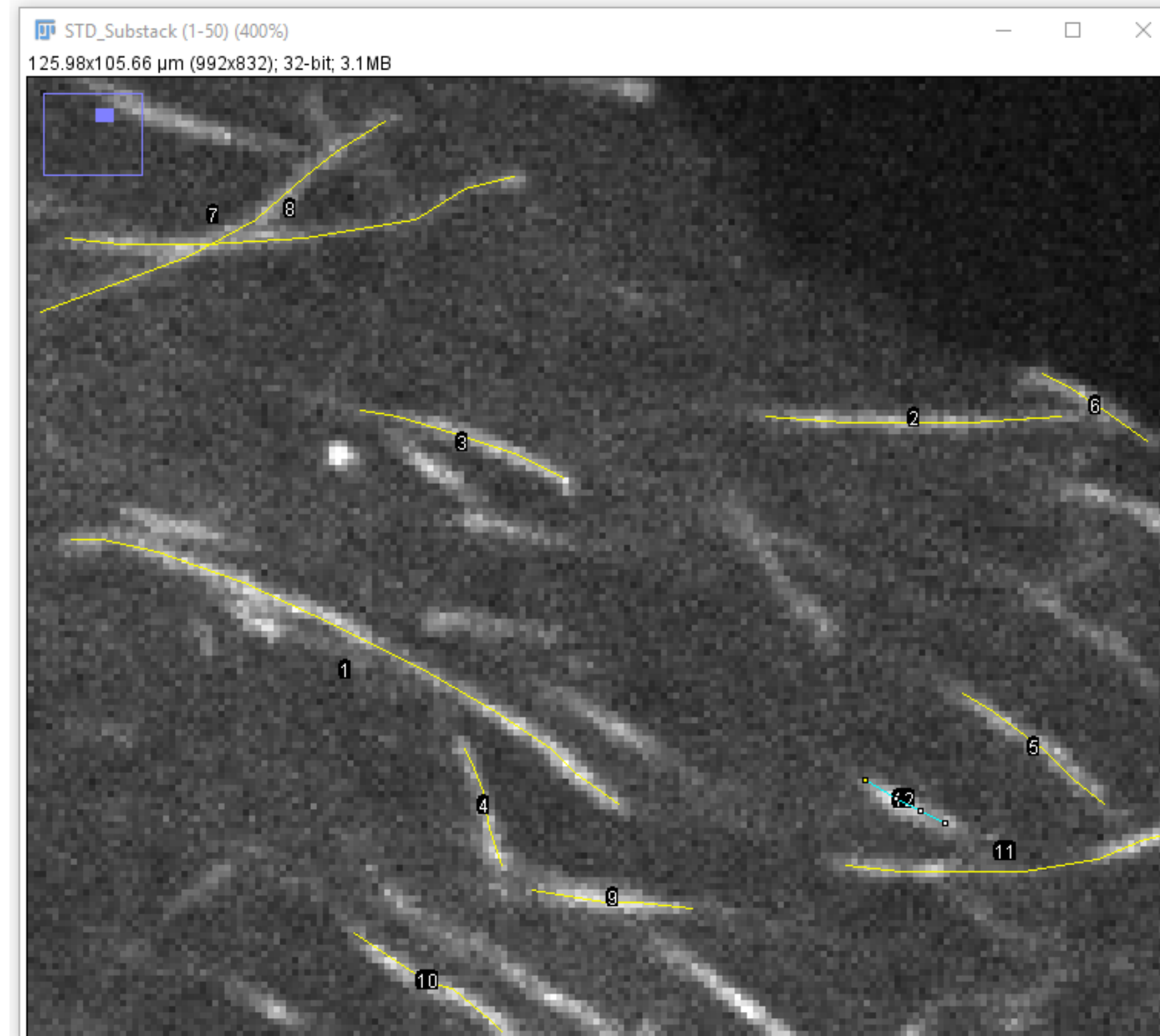
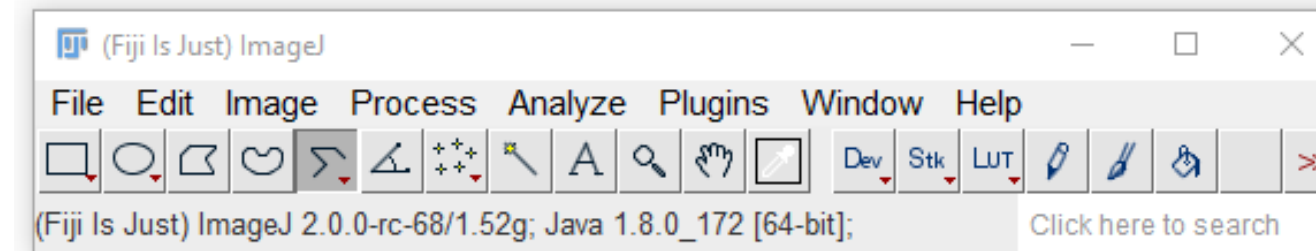
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12. Alternatively you can use the ROI Manager to store the selections.



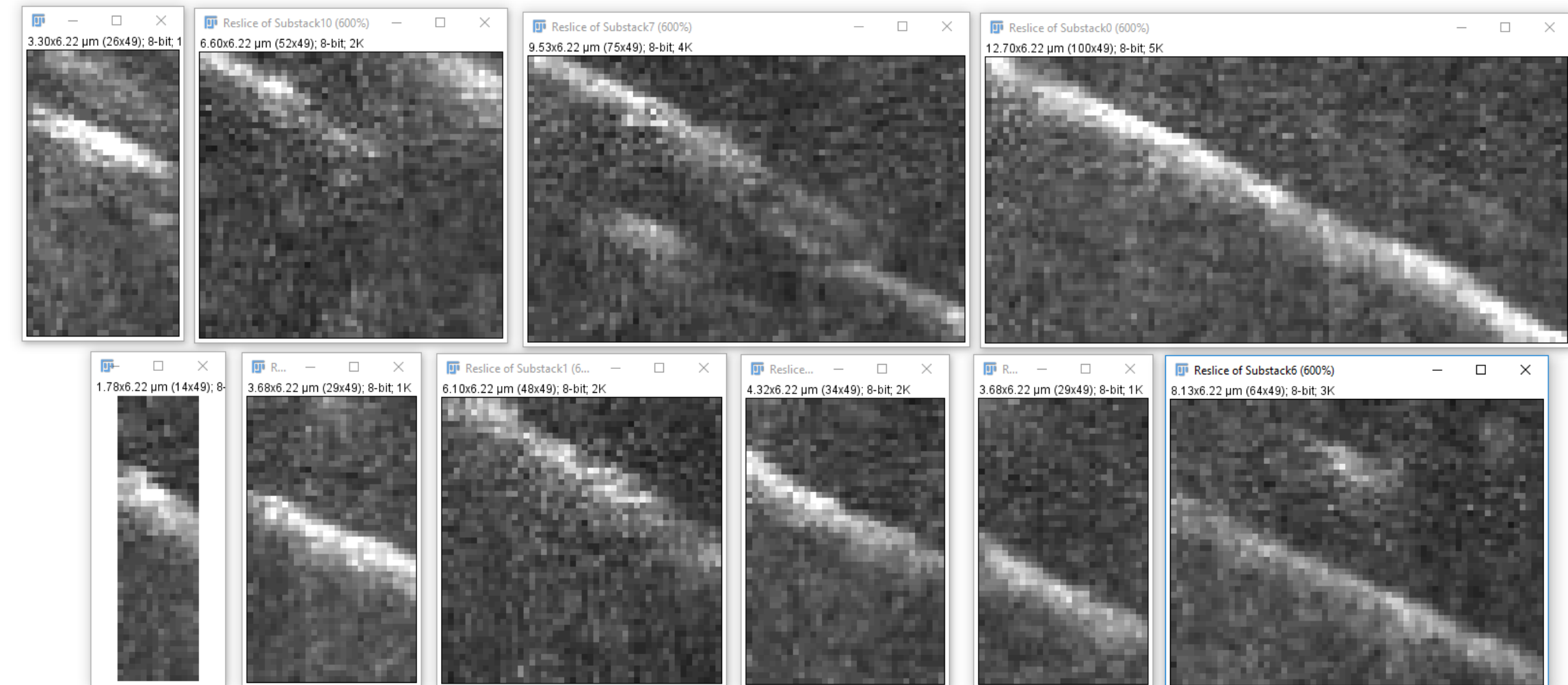
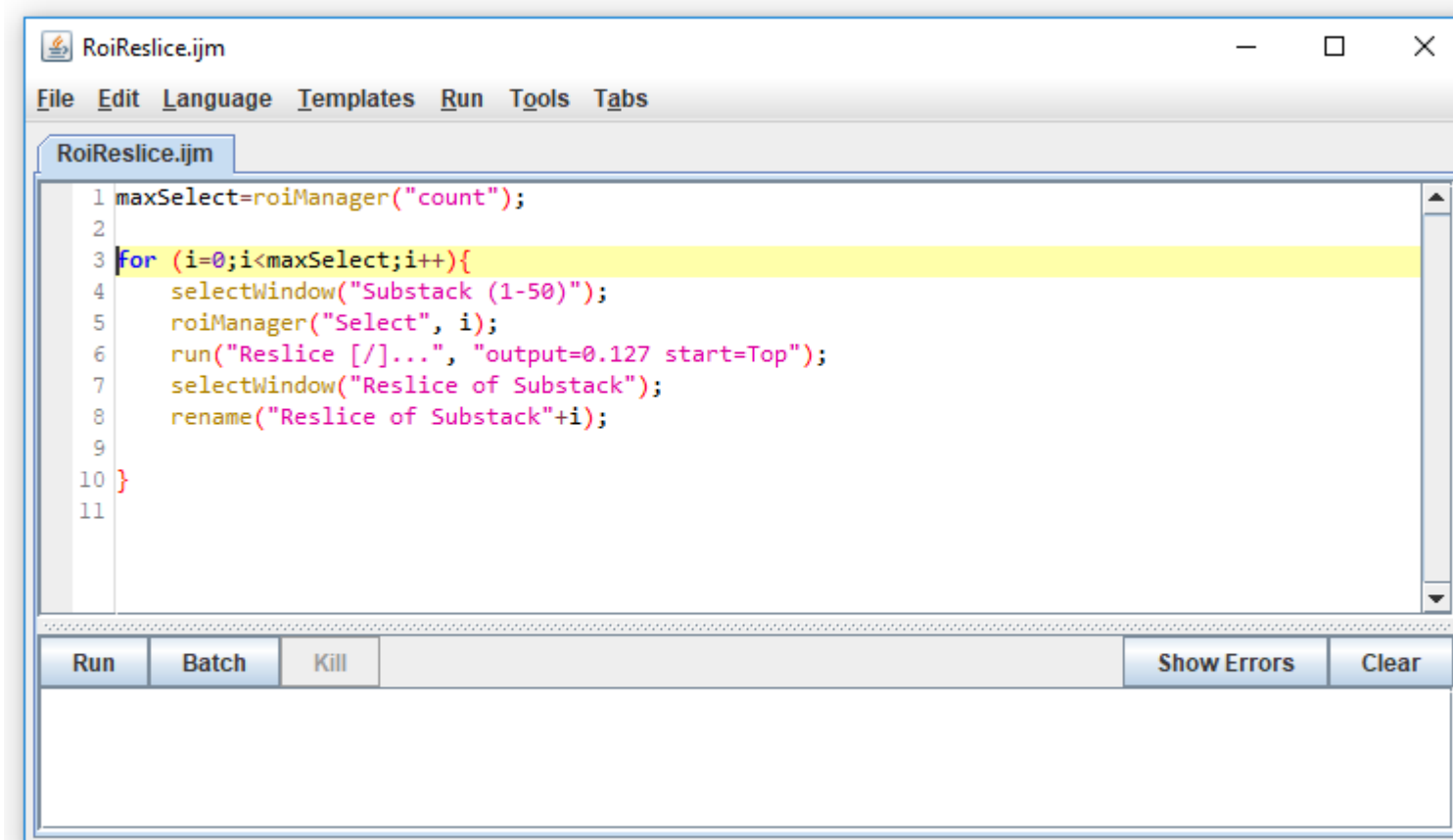
Reslicing

12. Alternatively you can use the ROI Manager to store the selections.
13. This is useful in case you want to analyse more trajectoires.
14. The kymographs can then be calculated via a small macro.



Reslicing

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13. This is useful in case you want to analyse more trajectoires.
14. The kymographs can then be calculated via a macro.



Note: The slope of the trajectory is corresponding to the speed of the moving structure. It is very similar for the different trajectories.