

Adipose Tissue Segmentation in Fiji



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BEFORE YOU BEGIN

Ensure that you have the most up-to-date versions of **Fiji** (<http://imagej.net/Fiji>) and the plug-in **MorphoLibJ** (<http://imagej.net/MorphoLibJ>)

Under “Help” in Fiji,
choose “Updates”

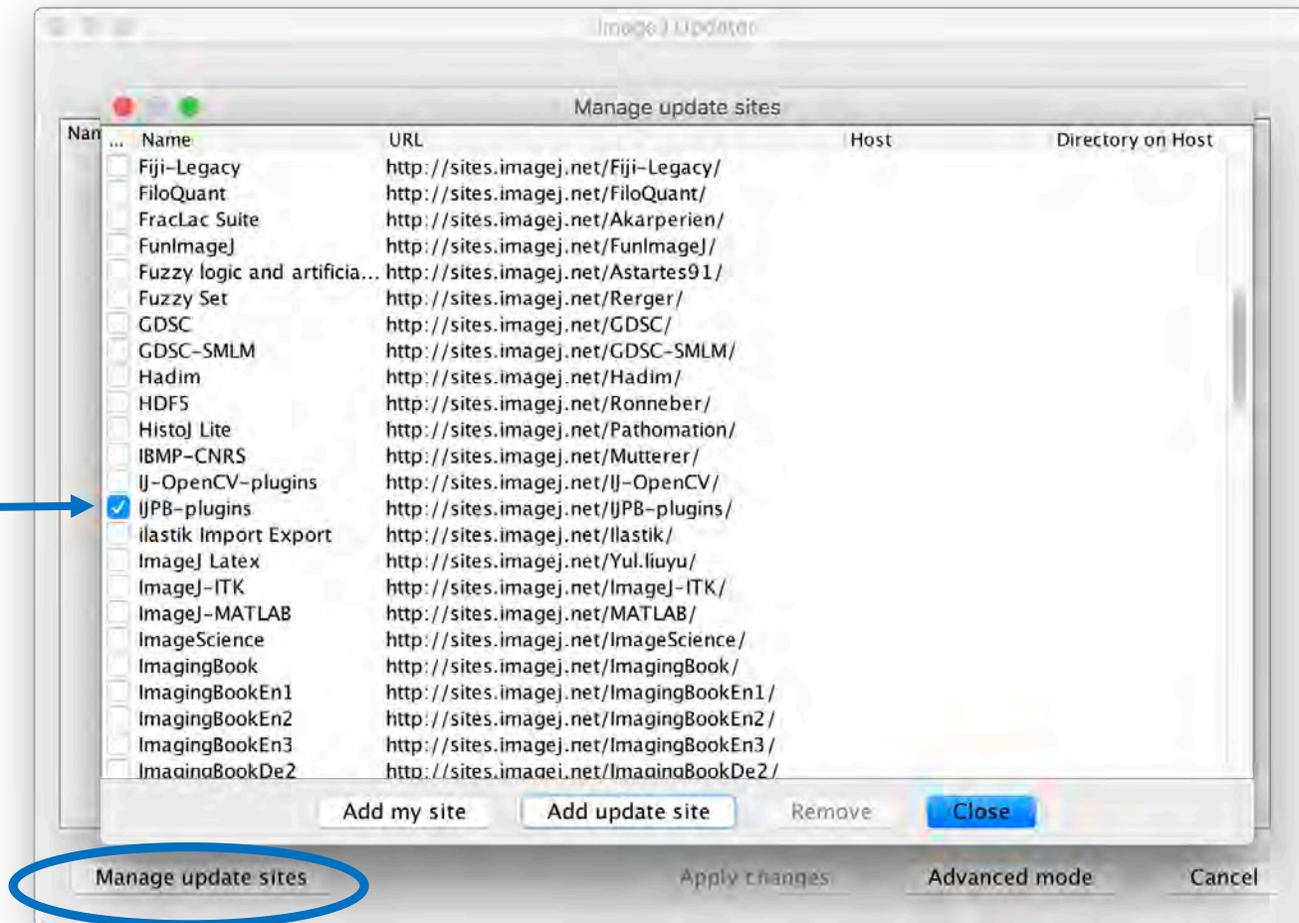
Click the “Manage
update sites” button
on the bottom left of
the ImageJ Updater
window

Check the IJPB-
plugins option

Click “Close”

Allow Fiji to update

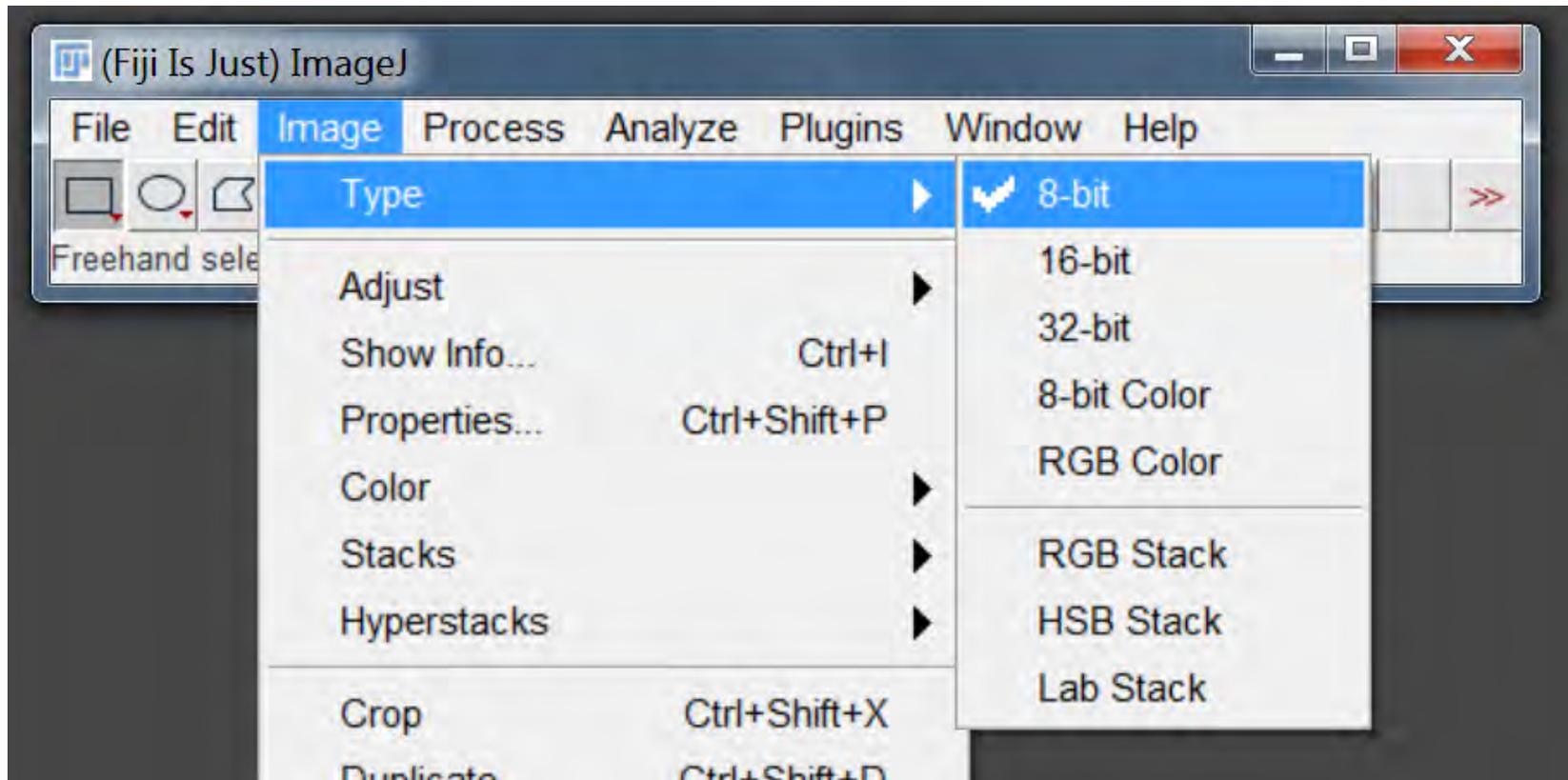
Restart Fiji



Step 1: Open Fiji

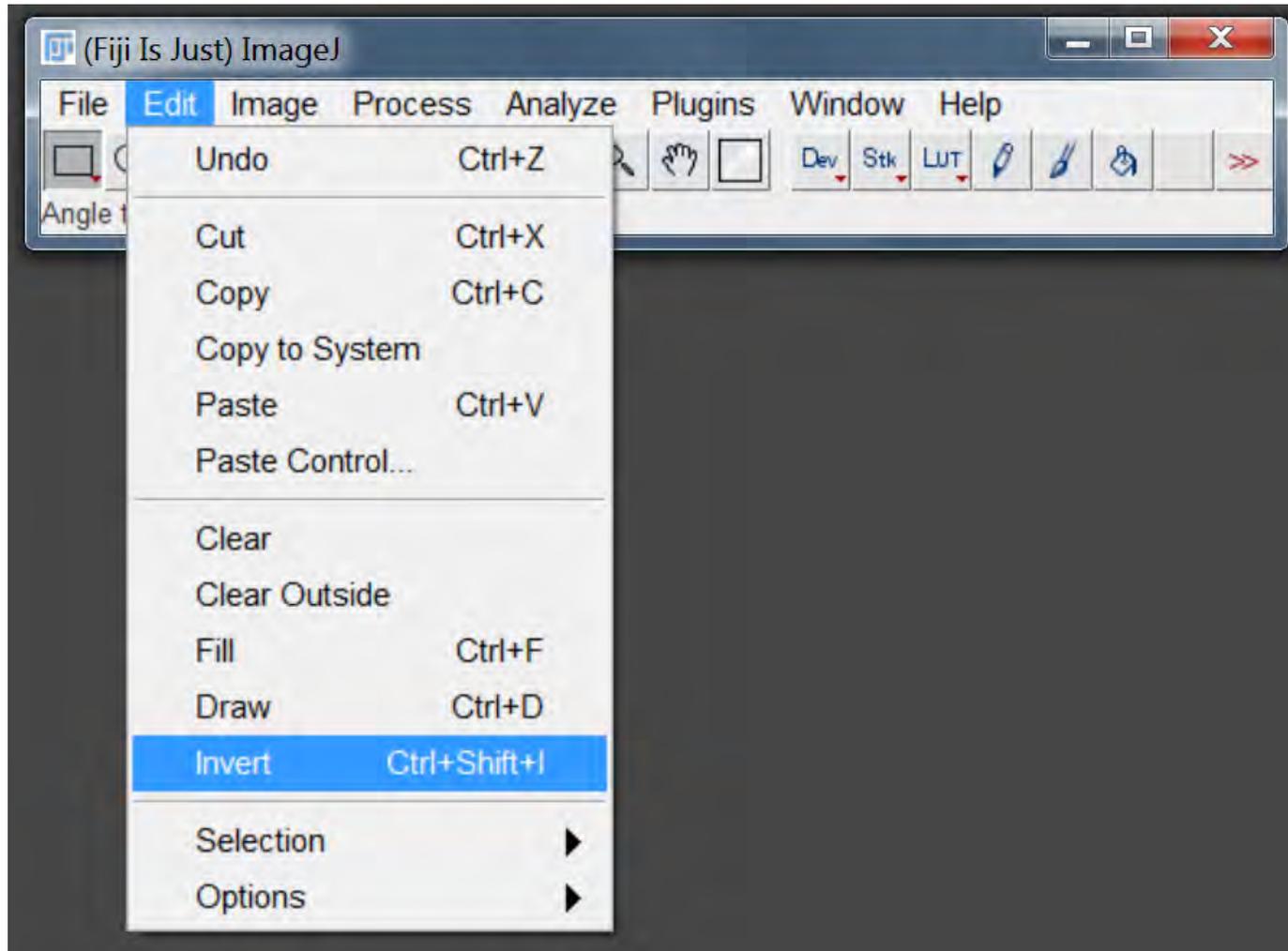
Step 2: Drag the file of interest into Fiji to open it

Step 3: Convert the image to 8-bit grey if it is not already

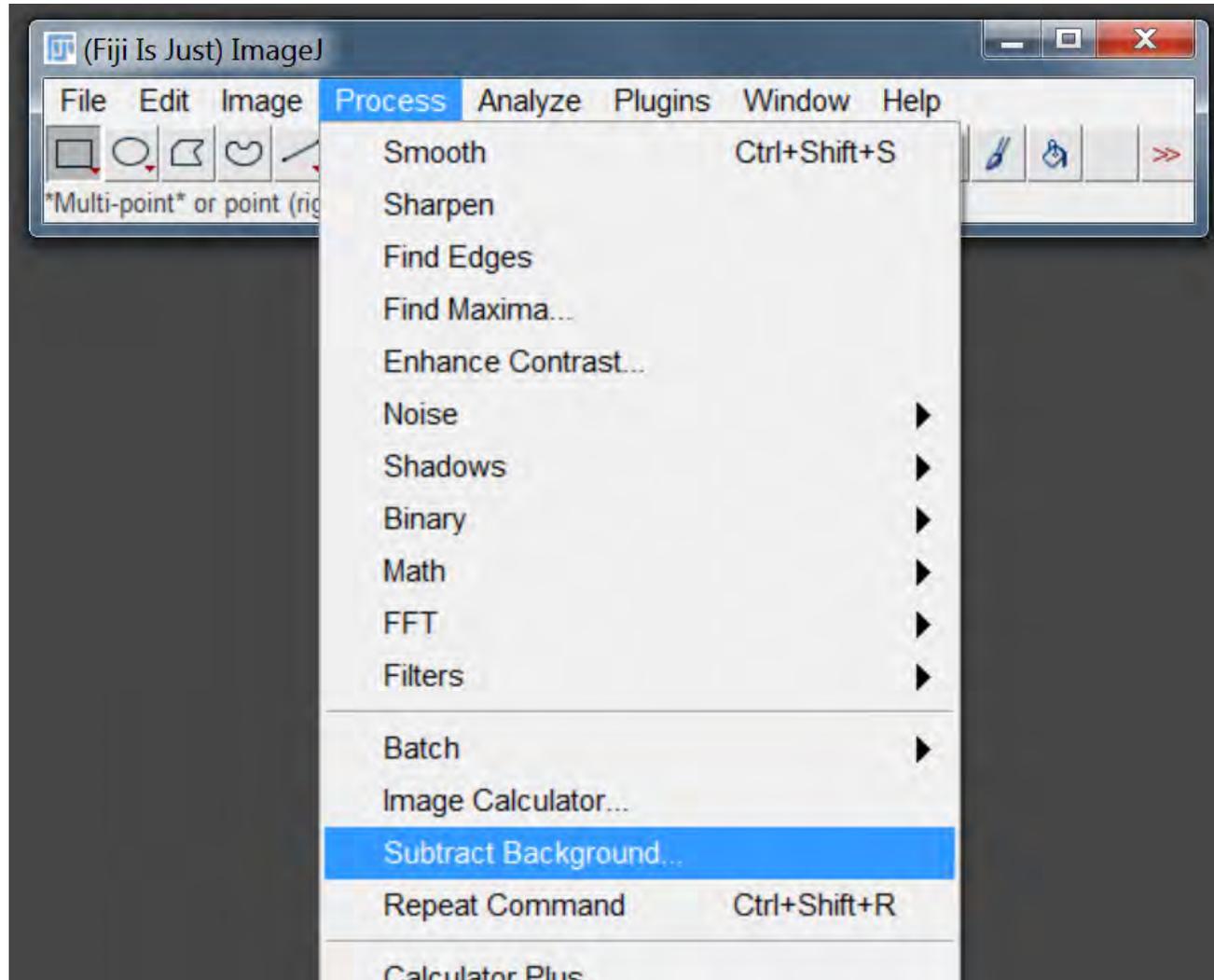


Step 4: Invert the image

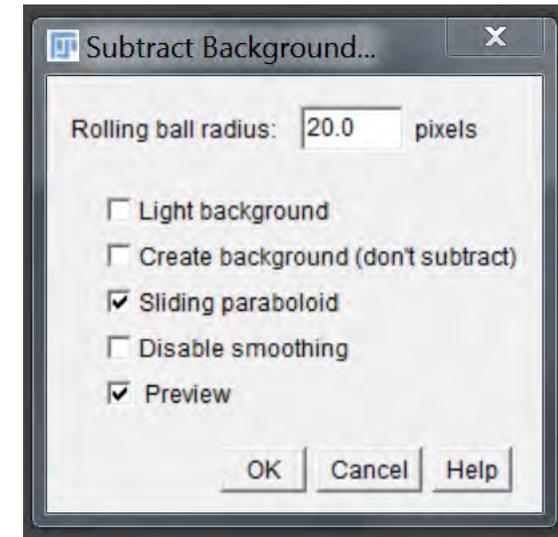
Do this step if you are analyzing a bright field image.
Skip this step if you are analyzing a fluorescent image.



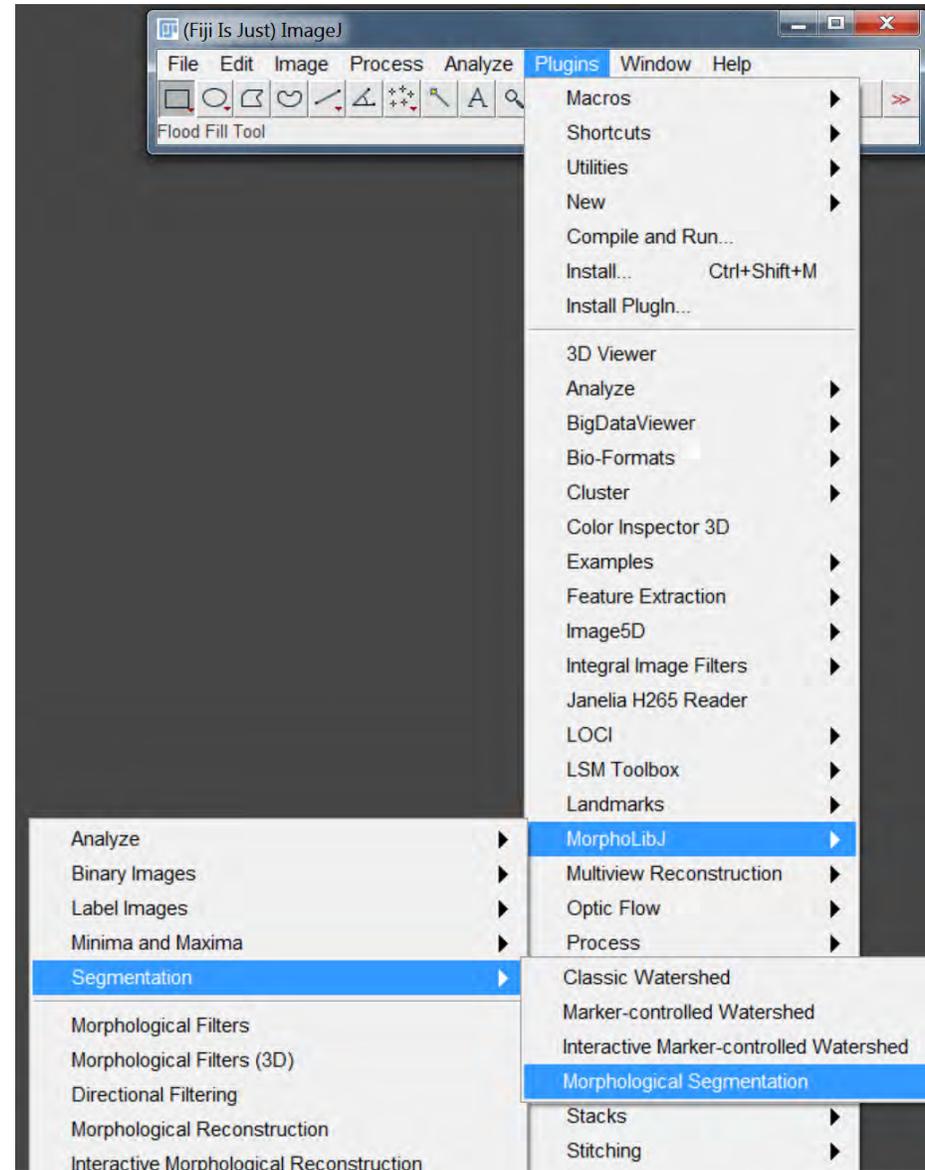
Step 5: Subtract background



Subtract Background Settings



Step 6: Morphological Segmentation



Step 6: Morphological Segmentation - Settings

The image displays the Morphological Segmentation software interface. On the left, a settings panel is shown with a blue border. A red arrow points to the 'Run' button, which is circled in red. The settings are as follows:

- Input Image:** Border Image, Object Image
- Gradient type:** Morphological
- Gradient radius:** 3
- Show gradient
- Watershed Segmentation:** Tolerance 4, Advanced options, Calculate dams, Connectivity 4
- Results:** Display Overlaid basins, Show result overlay, Create Image
- Post-processing:** Merge labels, Shuffle colors

The main window on the right shows the processed image with a dark background and white, intricate, branching structures. The window title is 'Morphological Segmentation' and it displays '1677x1271 pixels, 8-bit, 2MB'.

Step 6: Morphological Segmentation - Outcome

Input Image

Border Image 
 Object Image 

Gradient type **Morphological** ▼
Gradient radius
 Show gradient

Watershed Segmentation

Tolerance
 Advanced options
 Calculate dams
Connectivity
Run

Results

Display **Overlaid basins** ▼
 Show result overlay
Create Image

Post-processing

Merge labels
Shuffle colors

Morphological Segmentation
1677x1271 pixels, 8-bit 2MB

Input Image

Border Image 
 Object Image 

Gradient type **Morphological** ▼
Gradient radius
 Show gradient

Watershed Segmentation

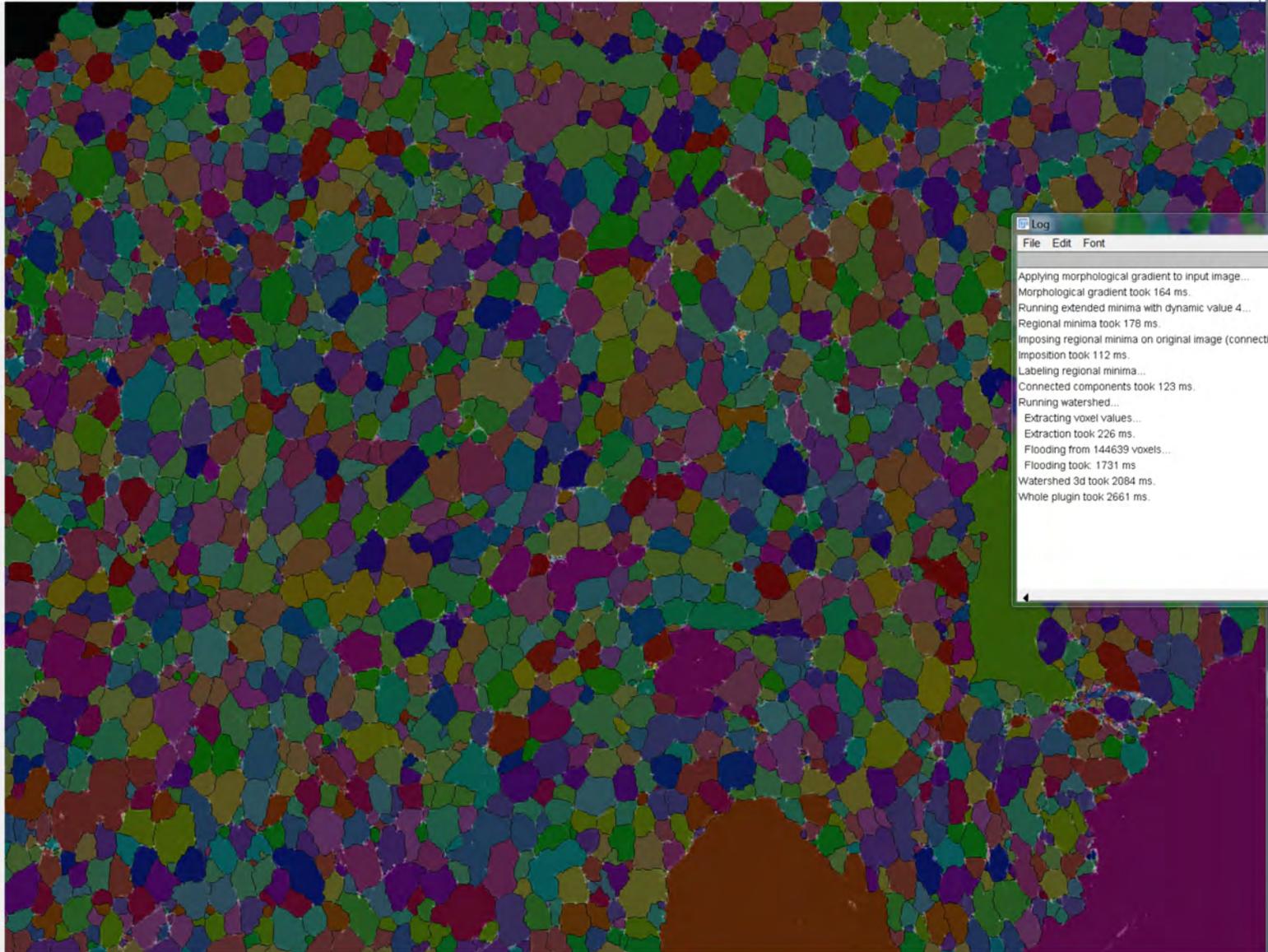
Tolerance
 Advanced options
 Calculate dams
Connectivity
Run

Results

Display **Overlaid basins** ▼
 Show result overlay
Create Image

Post-processing

Merge labels
Shuffle colors

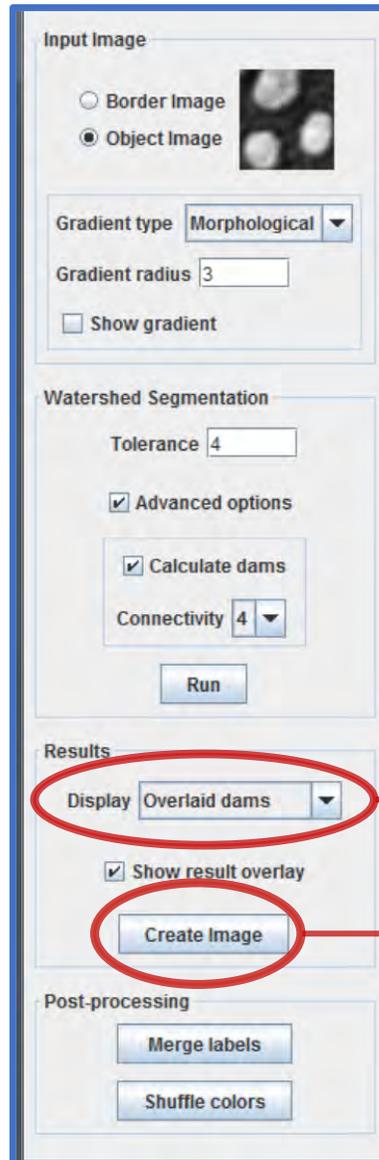


Log
File Edit Font

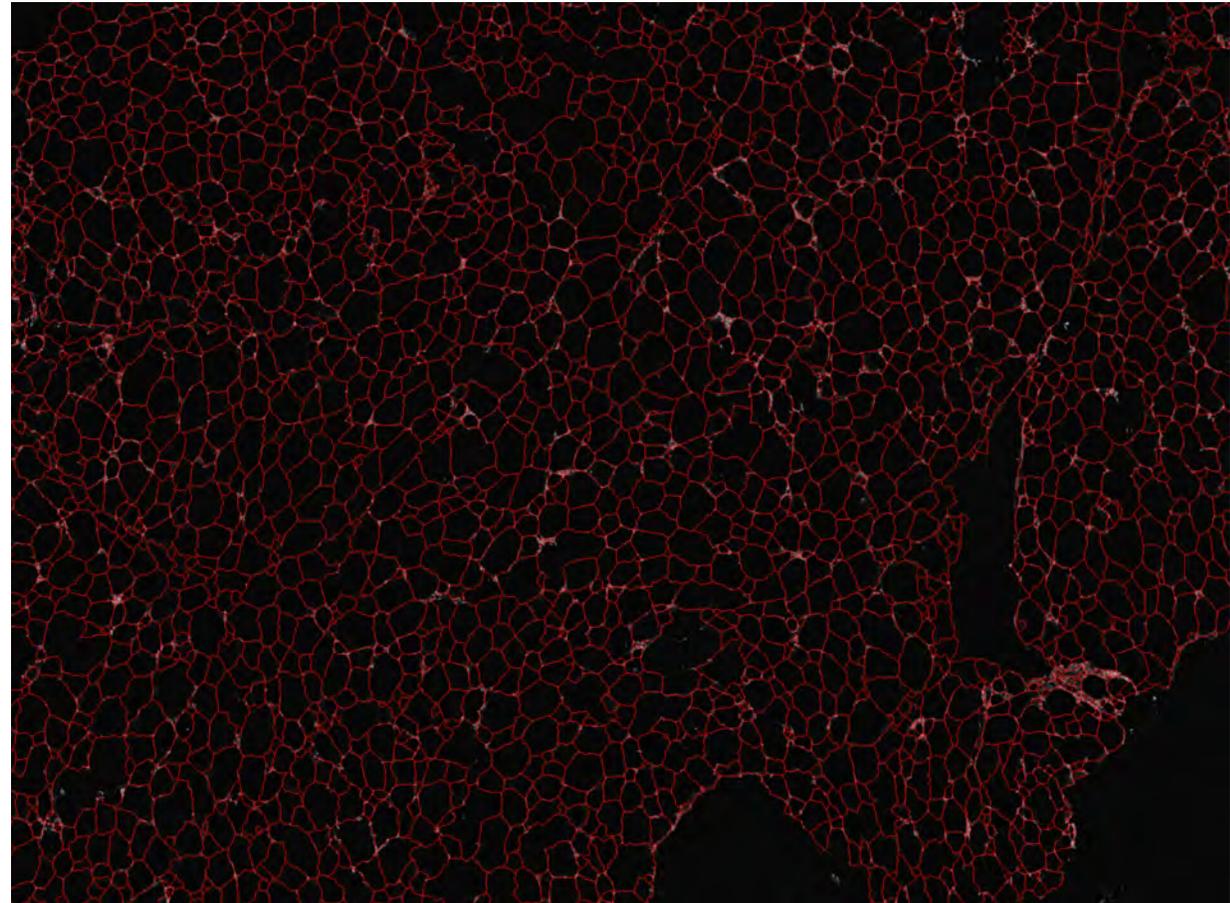
```
Applying morphological gradient to input image...  
Morphological gradient took 164 ms.  
Running extended minima with dynamic value 4...  
Regional minima took 178 ms.  
Imposing regional minima on original image (connect...  
Imposition took 112 ms.  
Labeling regional minima...  
Connected components took 123 ms.  
Running watershed...  
Extracting voxel values...  
Extraction took 226 ms.  
Flooding from 144639 voxels...  
Flooding took: 1731 ms  
Watershed 3d took 2084 ms.  
Whole plugin took 2661 ms.
```

Step 7: Display “Overlaid dams” option

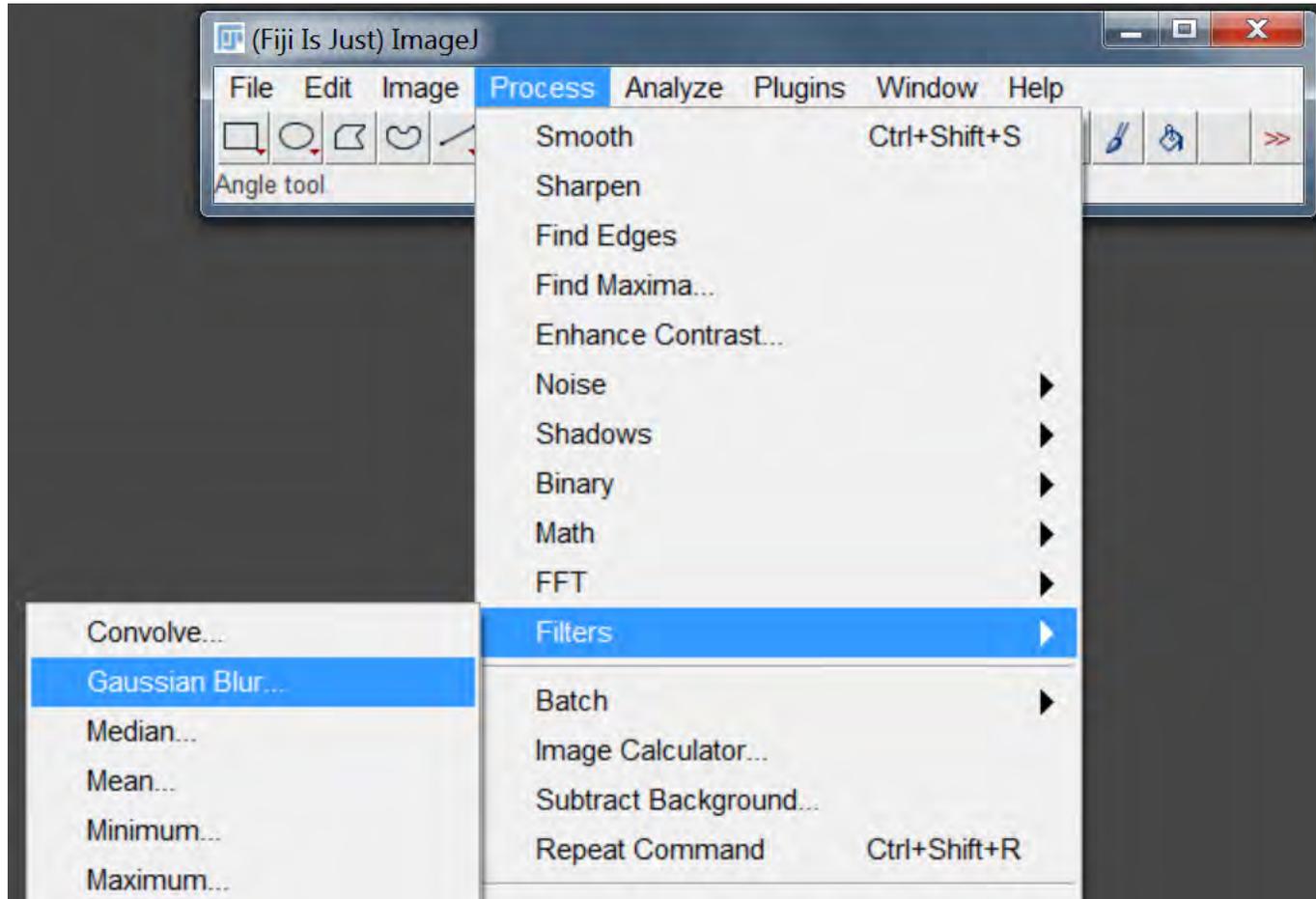
Step 8: Click “Create Image”



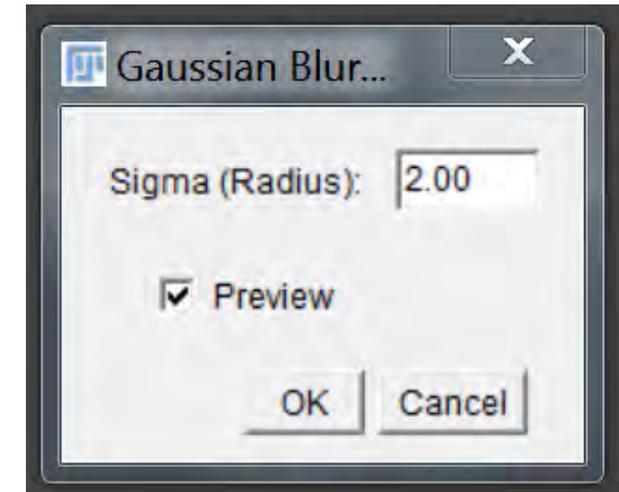
Overlaid dams output



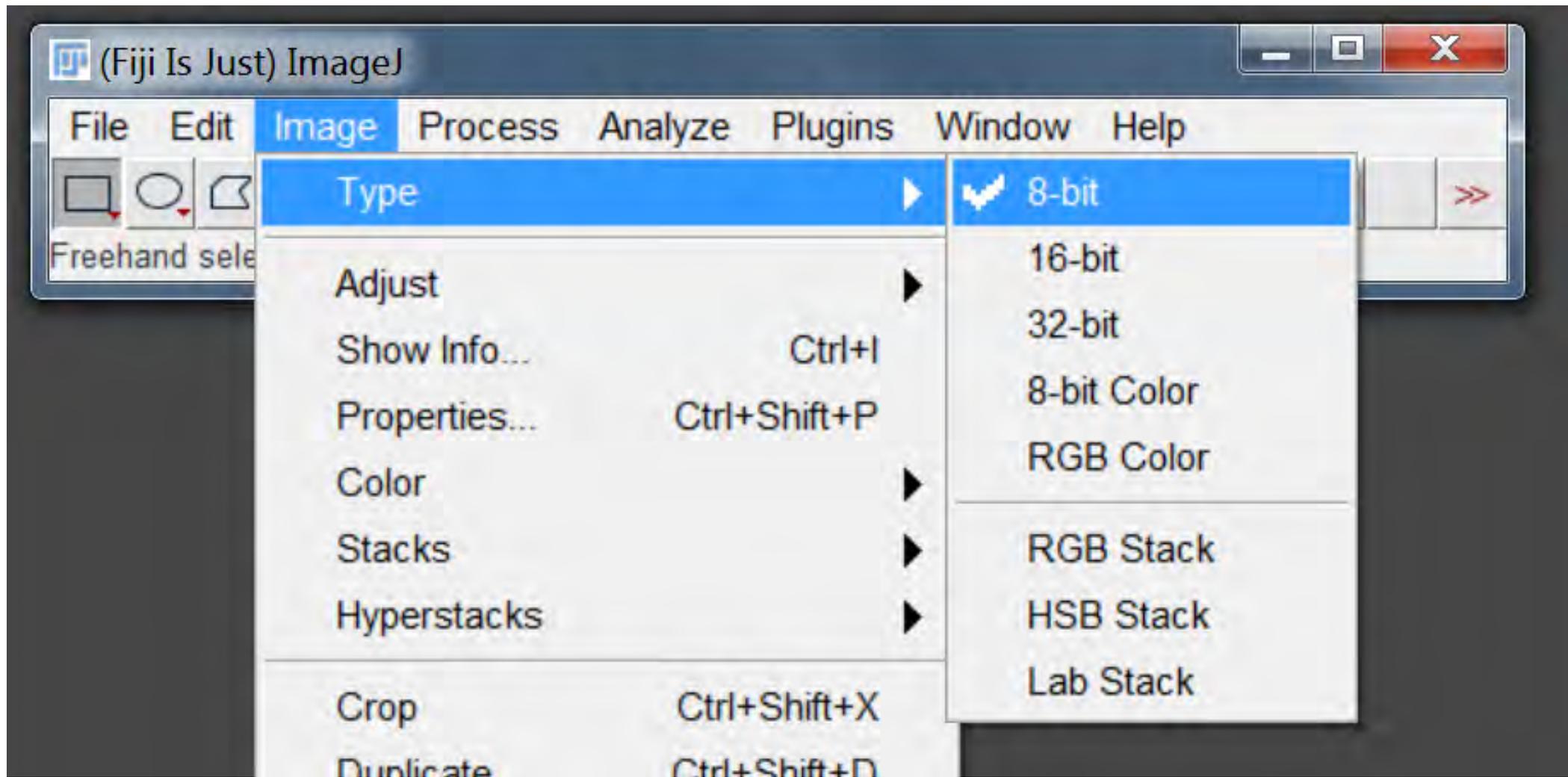
Step 9: Perform Gaussian Blur



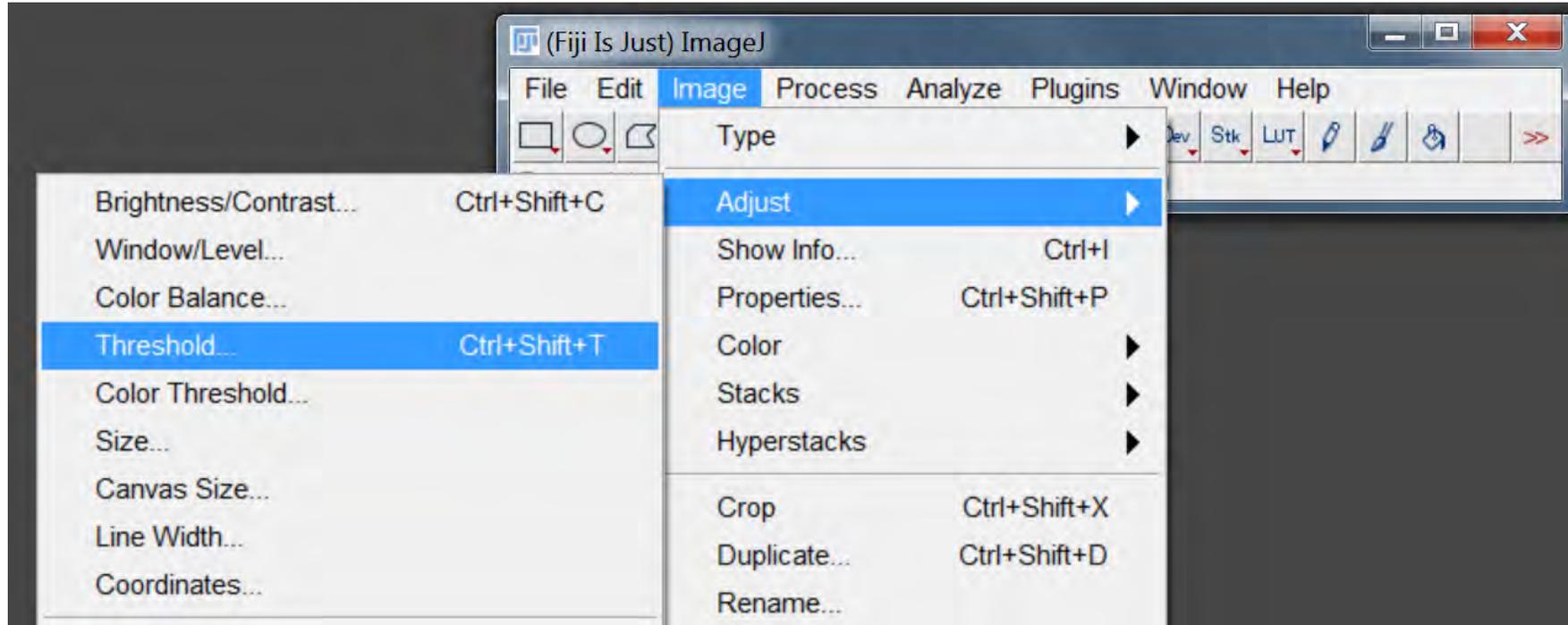
Gaussian Blur Settings



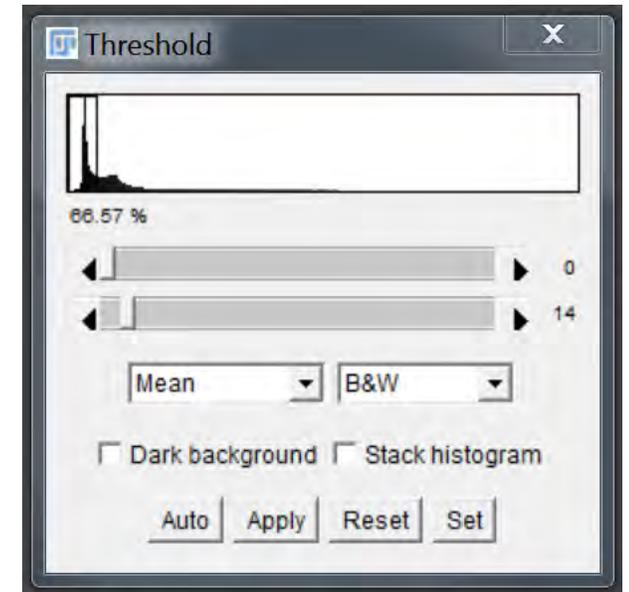
Step 10: Convert the image to 8-bit



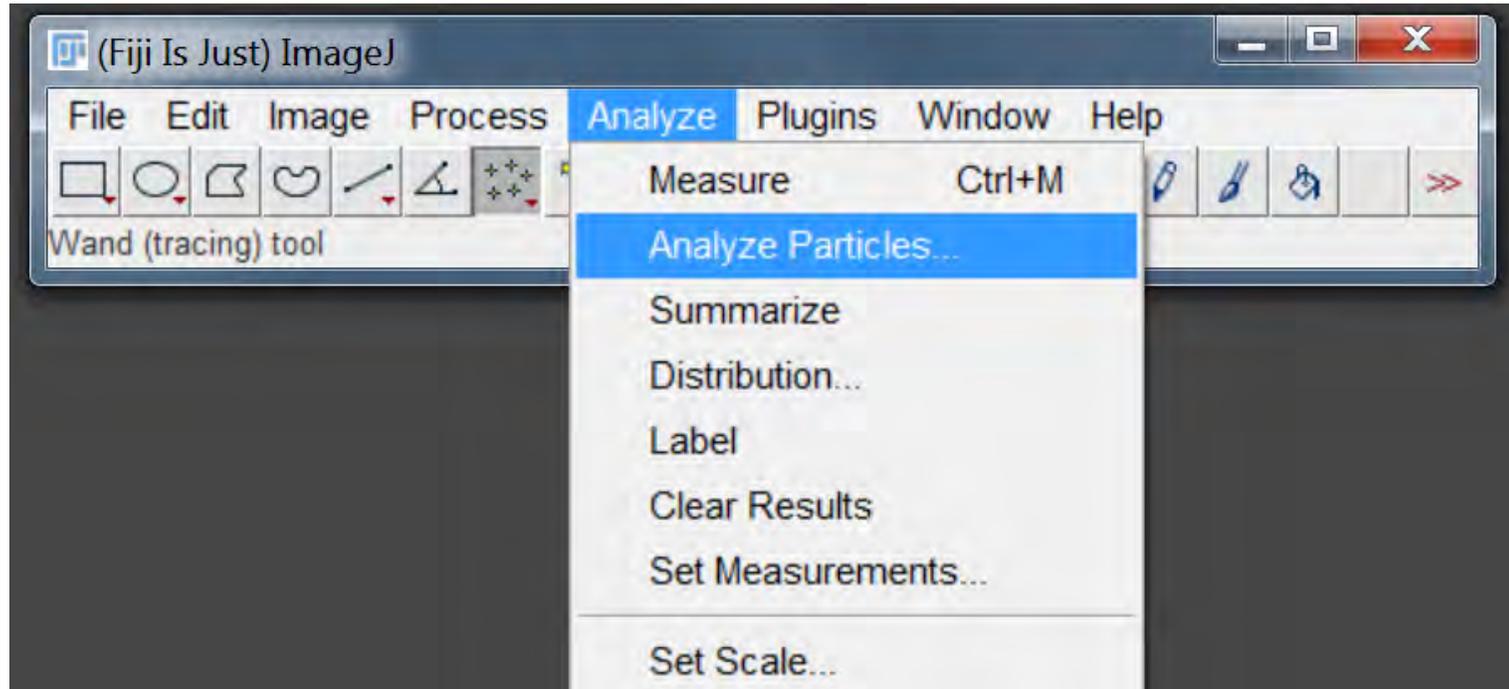
Step 11: Threshold the image



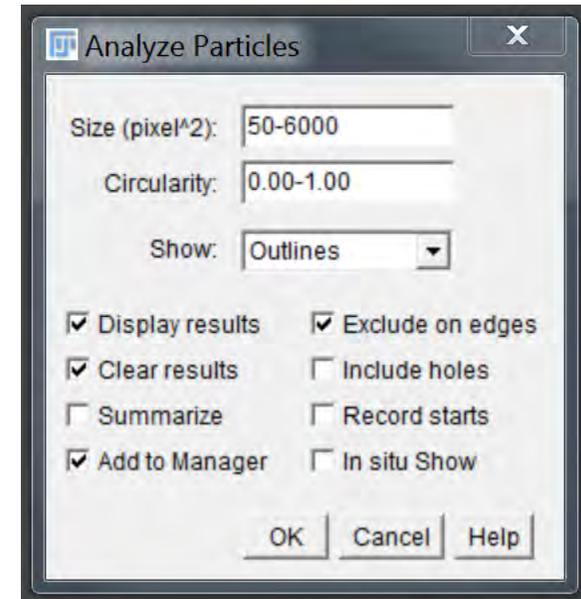
Threshold Settings



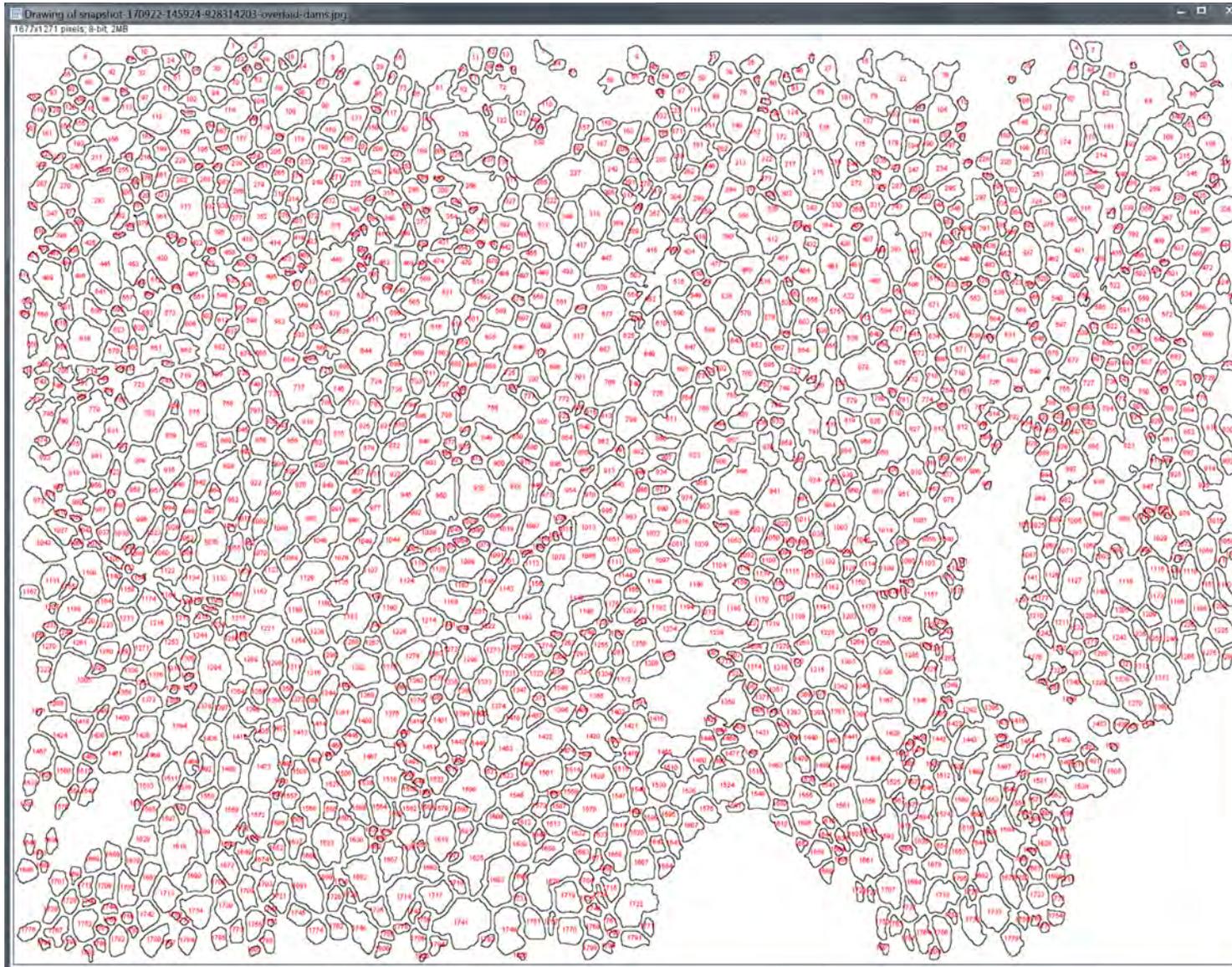
Step 12: Analyze Particles



Analyze Particles Settings



Step 12: Analyze Particles - Output



Save this image, if you choose to.

Note that large open spaces, as well as cells which were joined together due to segmentation issues, are not counted.

Step 13: Export Results Data

Export your data in .CSV format

