Adipose Tissue Segmentation in Fiji

Joseph Roland, Ph.D.

DHSR Digital Histology Shared Resource

Vanderbilt University Medical Center

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"

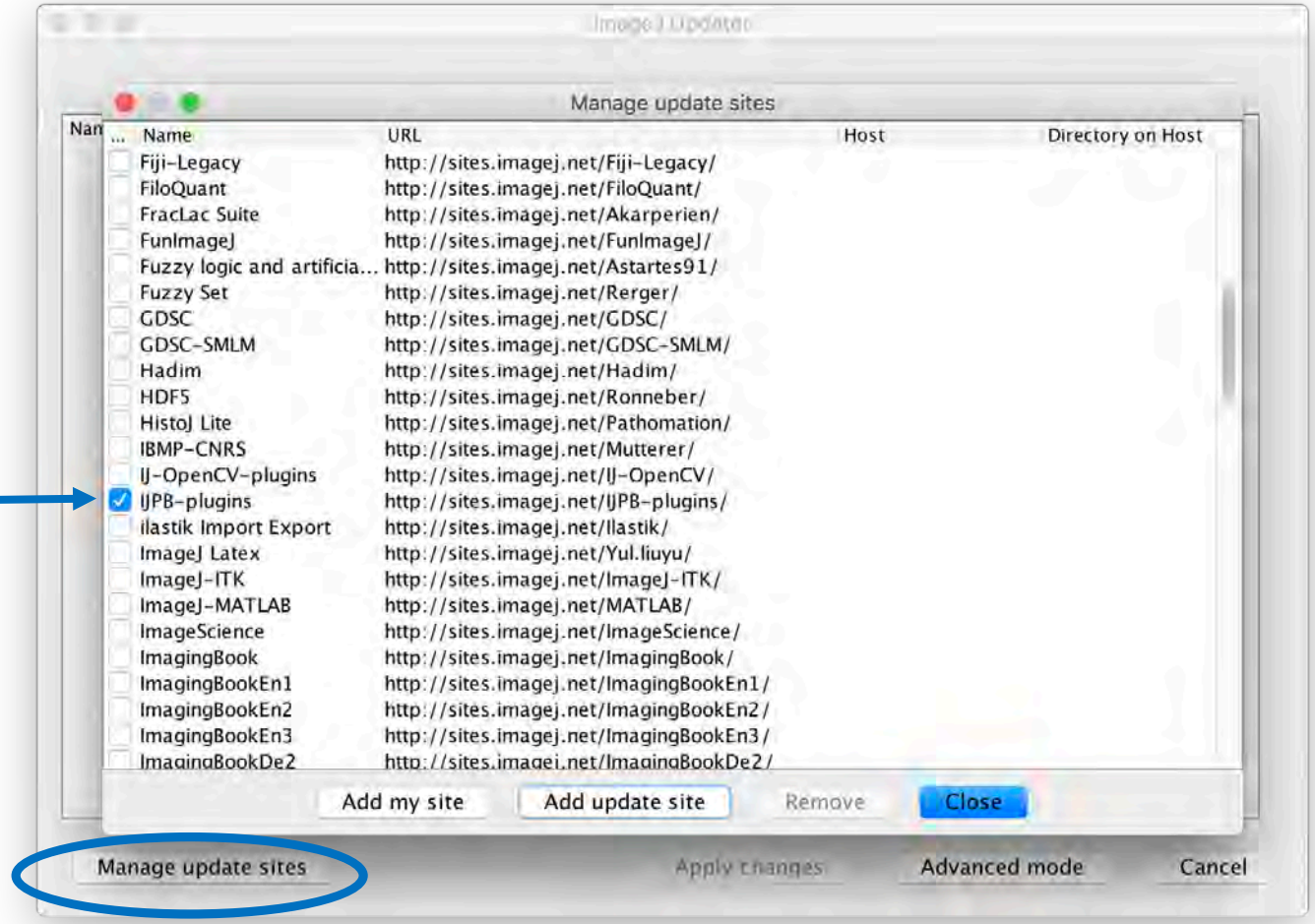
Click the "Manage update sites" button on the bottom left of the ImageJ Updater window

Check the IJPB-plugins option

Click "Close"

Allow Fiji to update

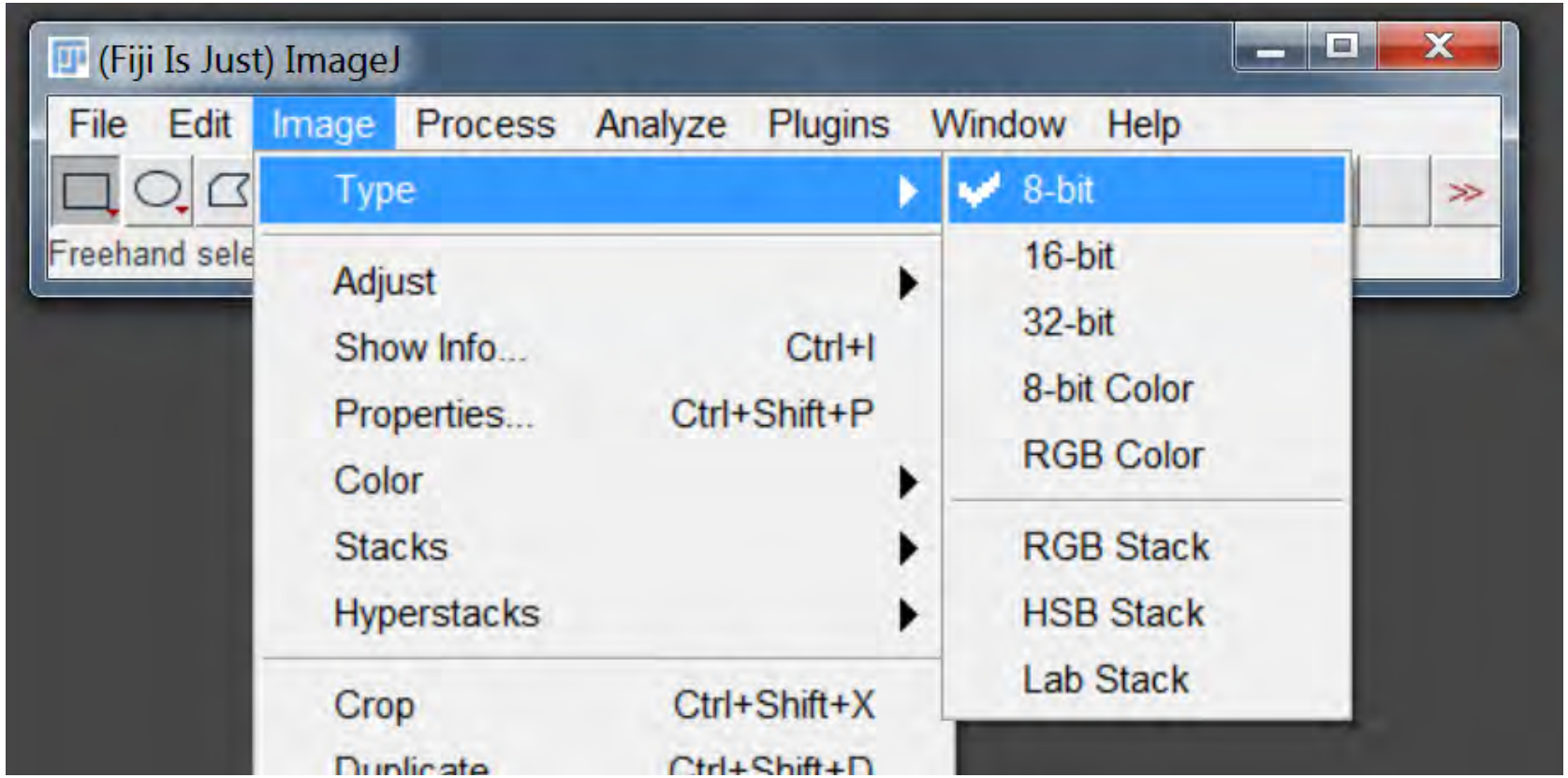
Restart Fiji



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

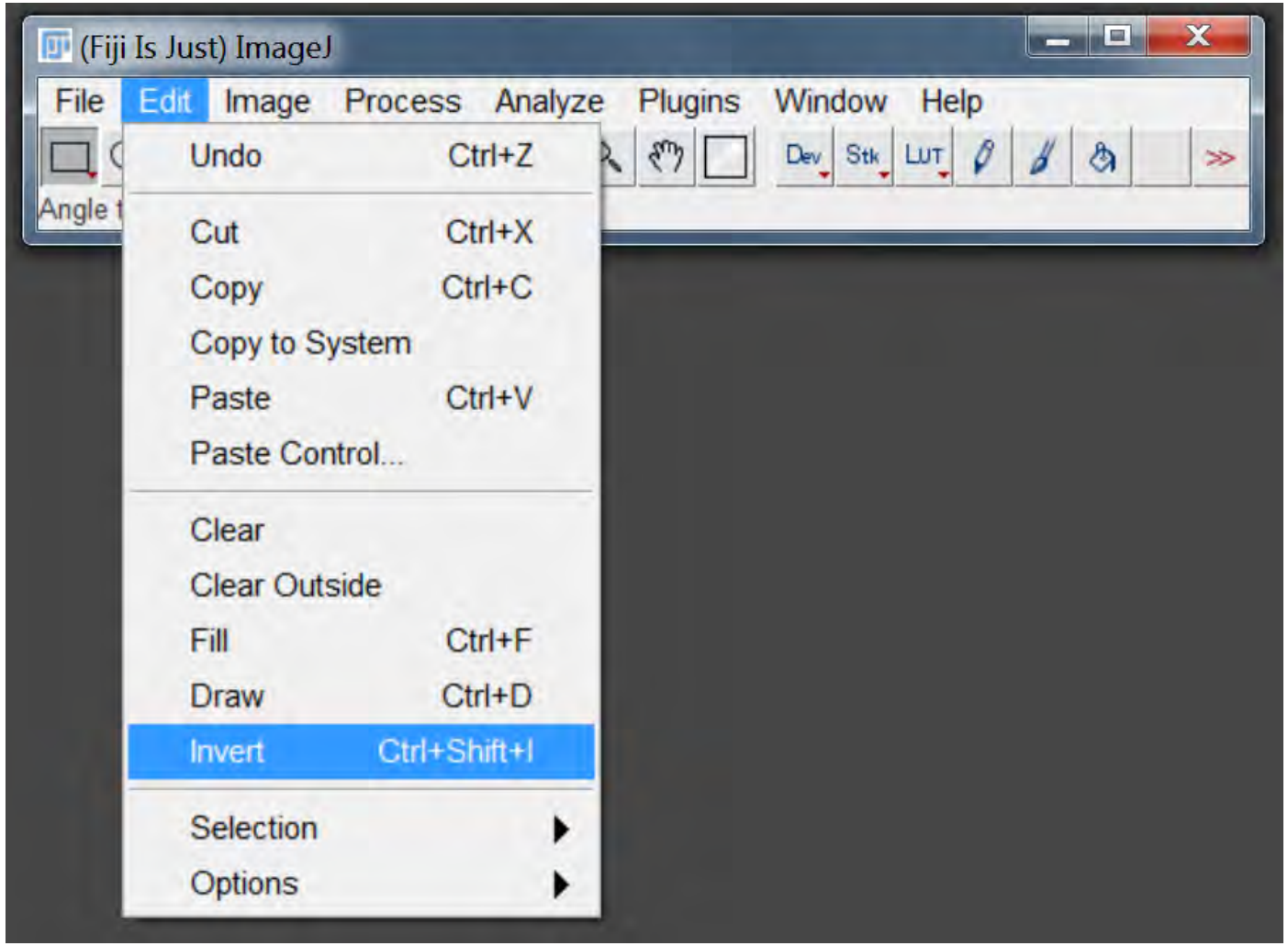


Joseph Roland, Ph.D.
DHSR

Step 4: Invert the image

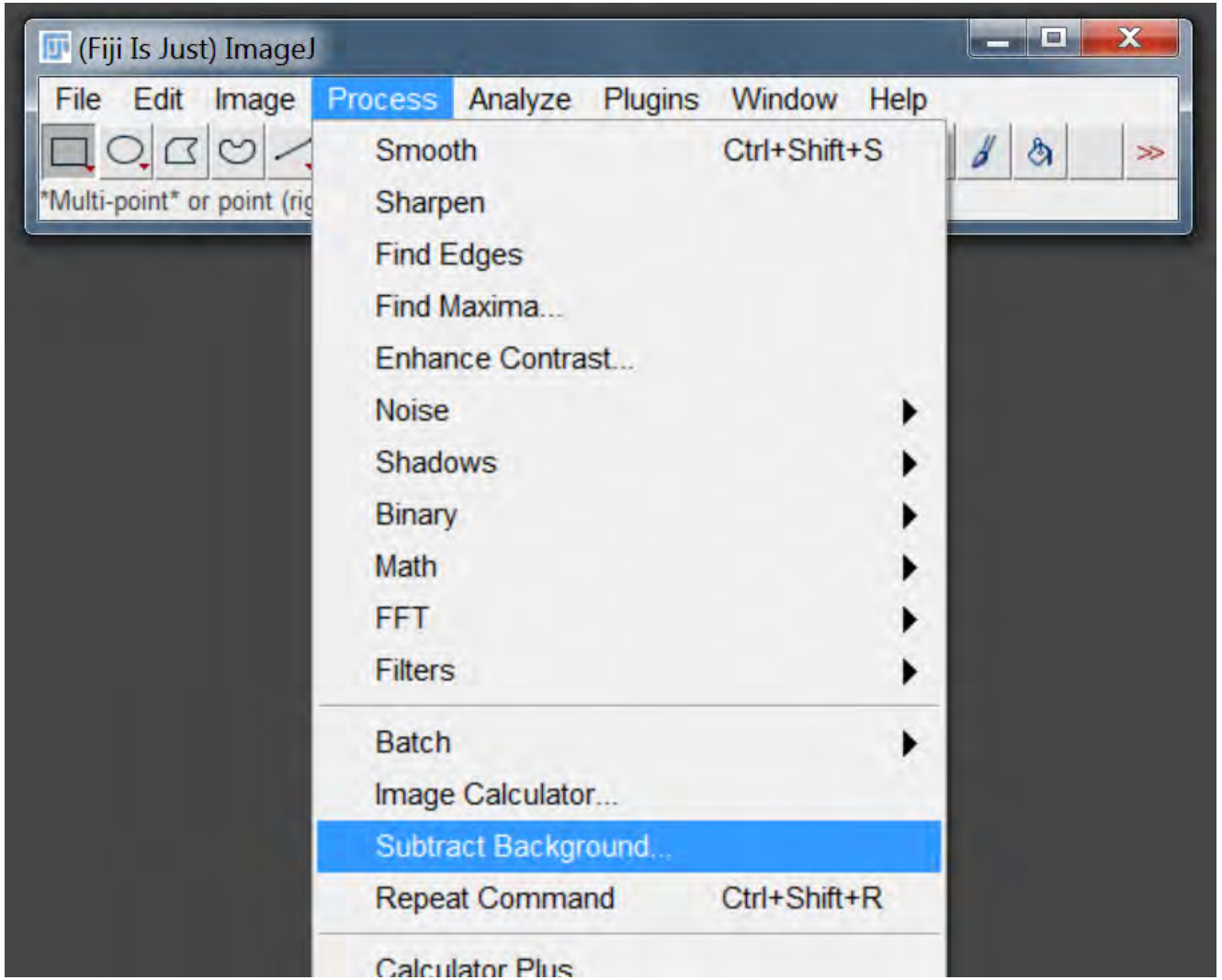
Do this step if you are analyzing a bright field image.

Skip this step if you are analyzing a fluorescent image.

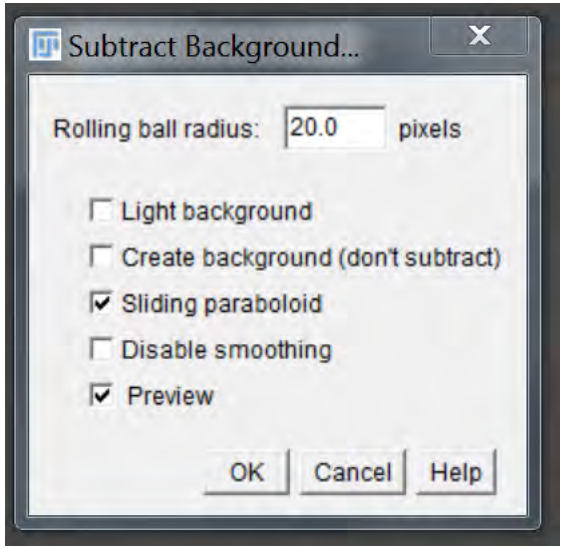


Joseph Roland, Ph.D.
DHSR

Step 5: Subtract background

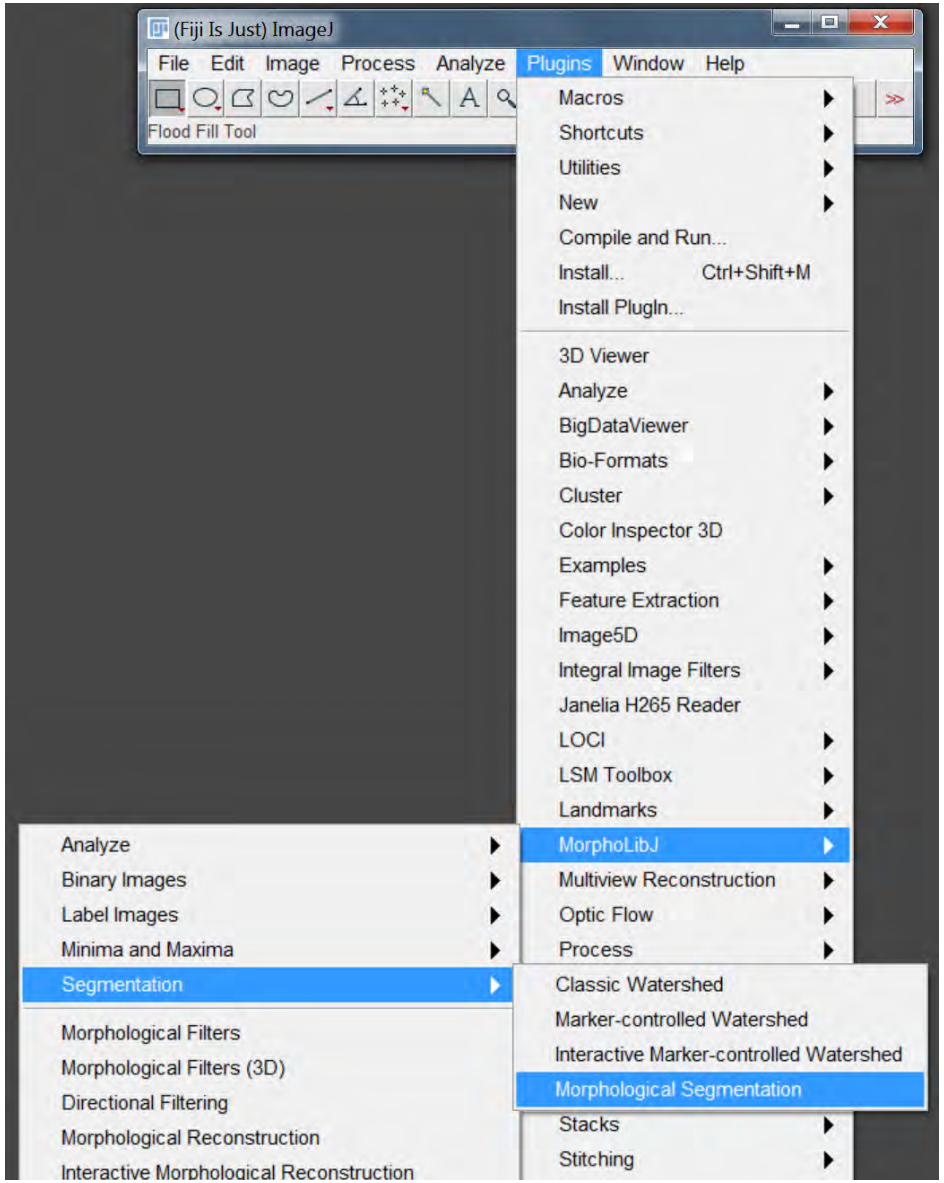


Subtract Background Settings



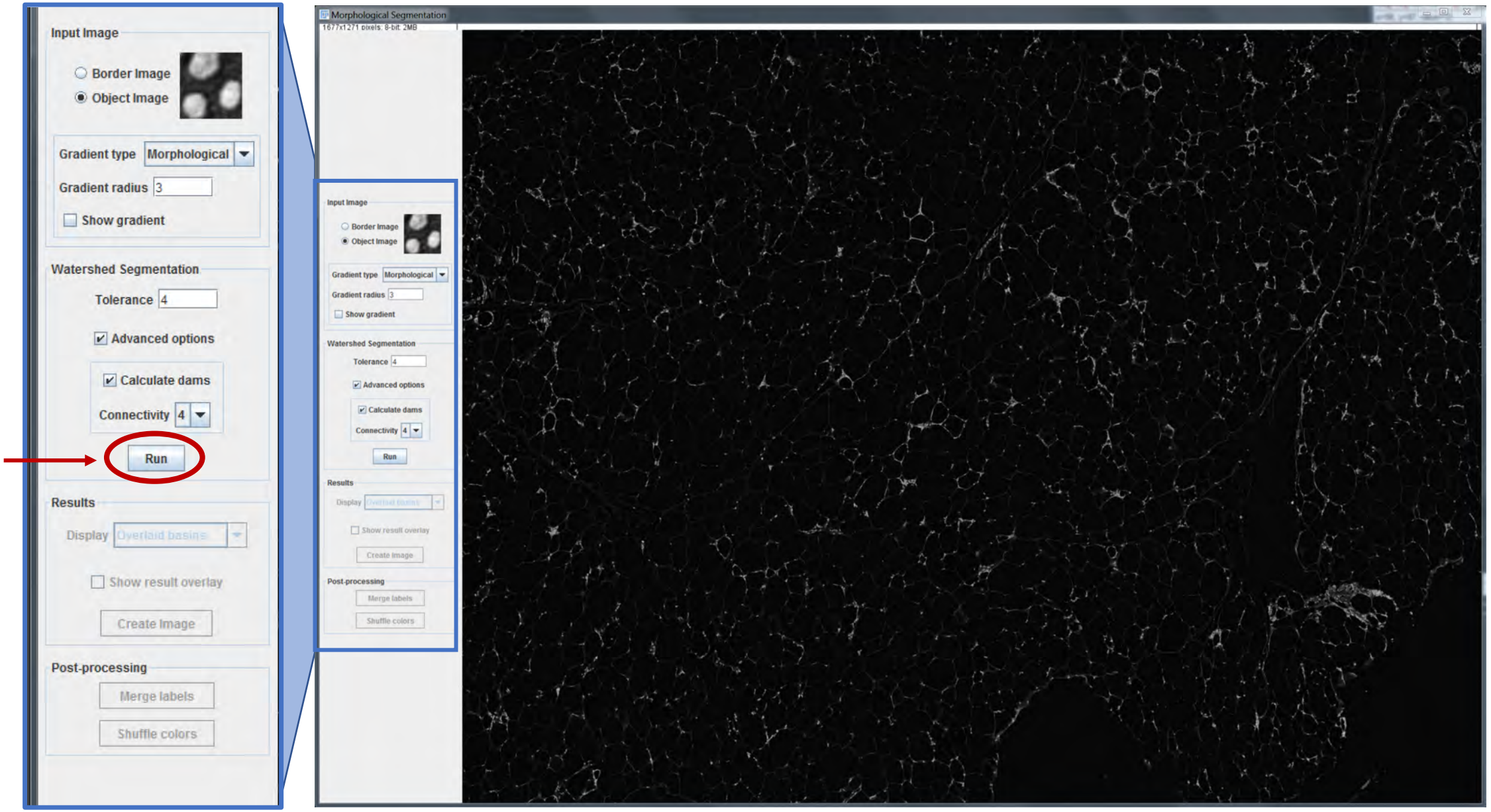
Joseph Roland, Ph.D.
DHSR

Step 6: Morphological Segmentation



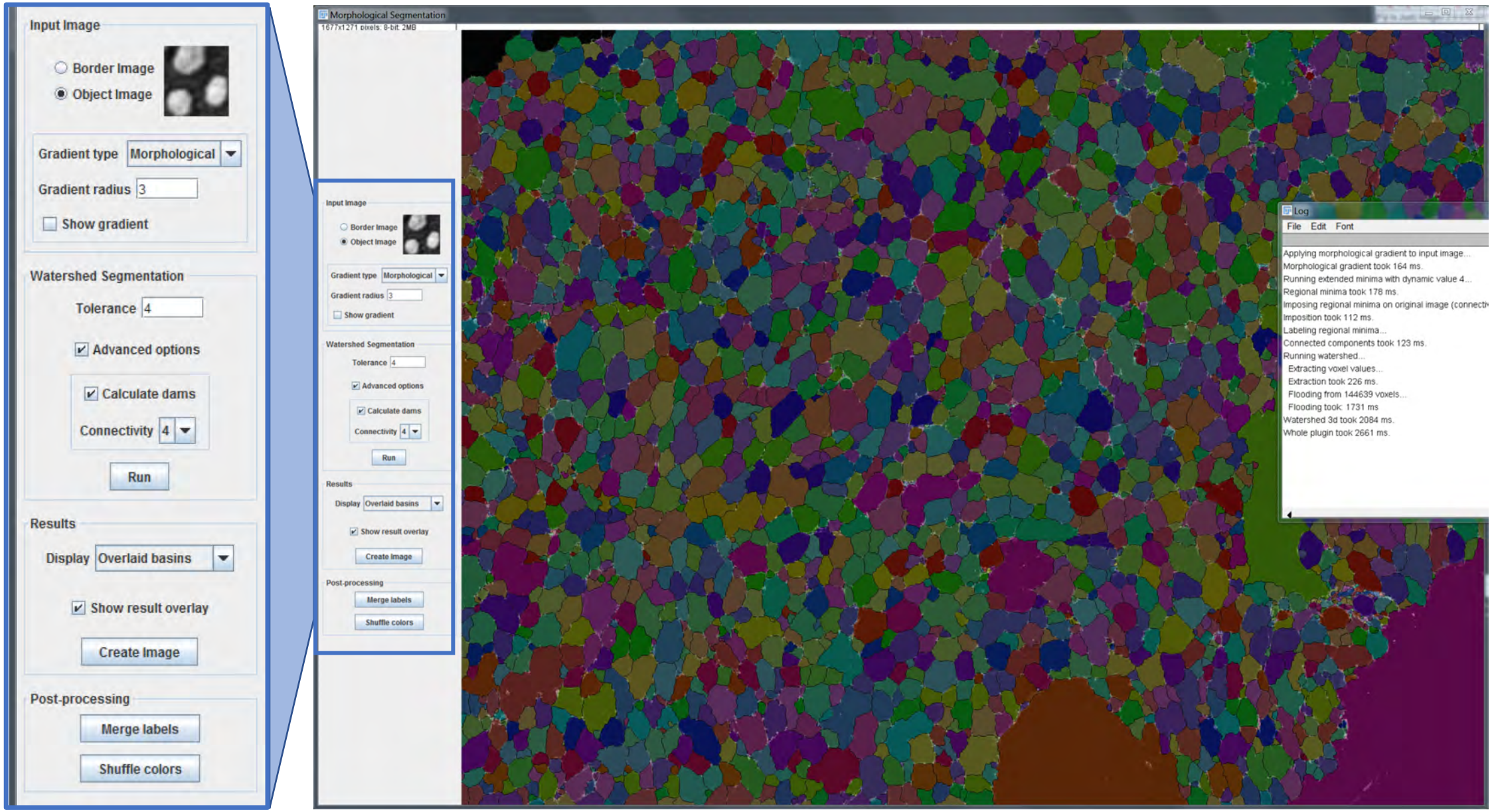
Joseph Roland, Ph.D.
DHSR

Step 6: Morphological Segmentation - Settings



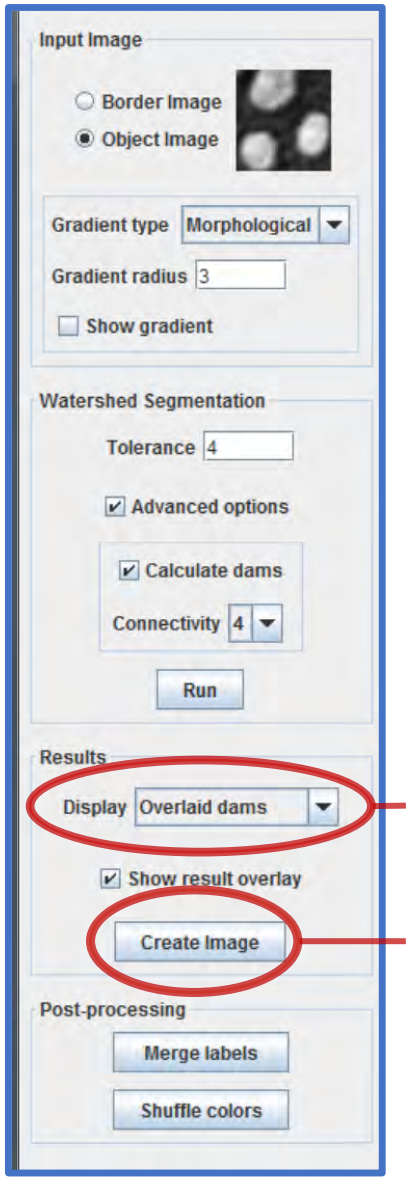
Joseph Roland, Ph.D.
DHSR

Step 6: Morphological Segmentation - Outcome



Joseph Roland, Ph.D.
DHSR

Step 7: Display “Overlaid dams” option

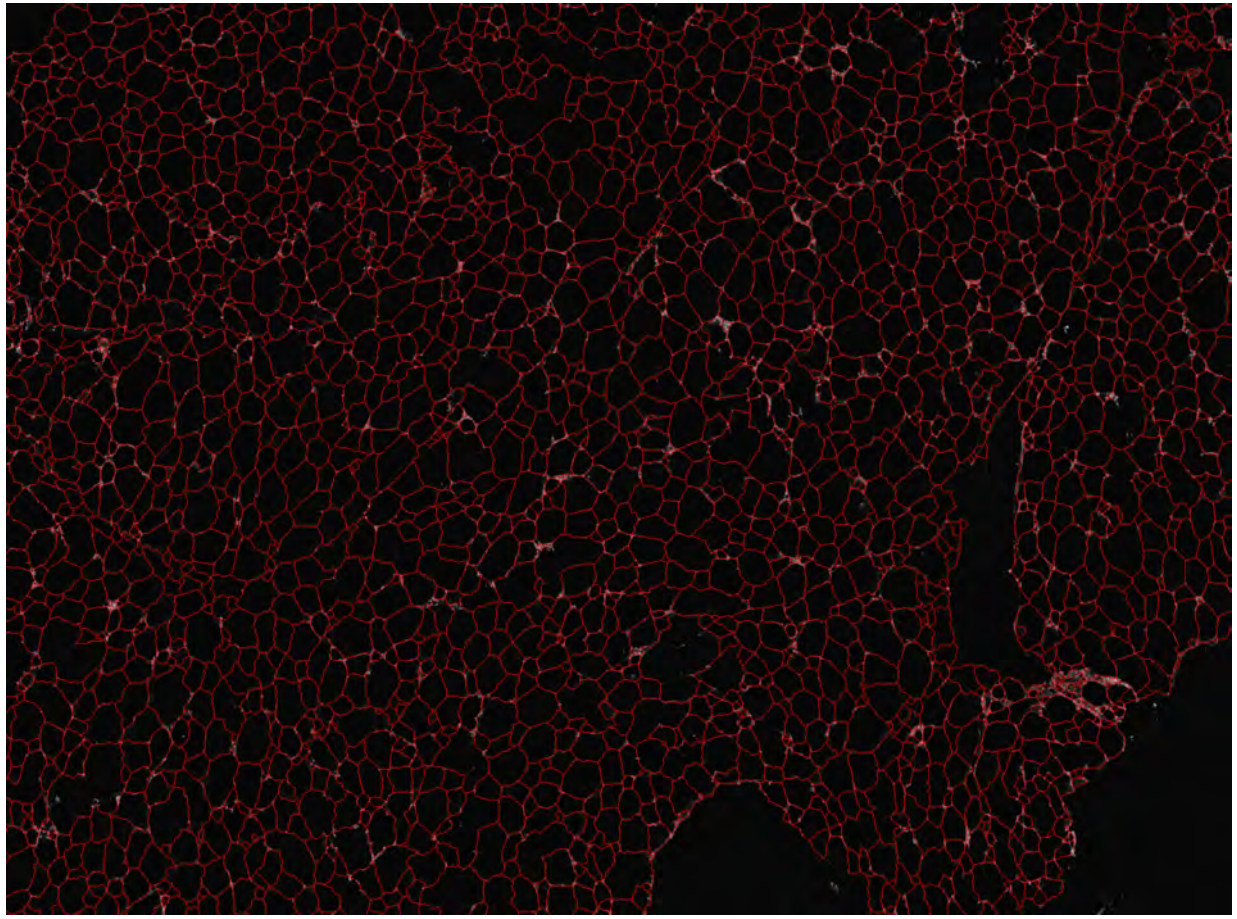


Step 7

Step 8

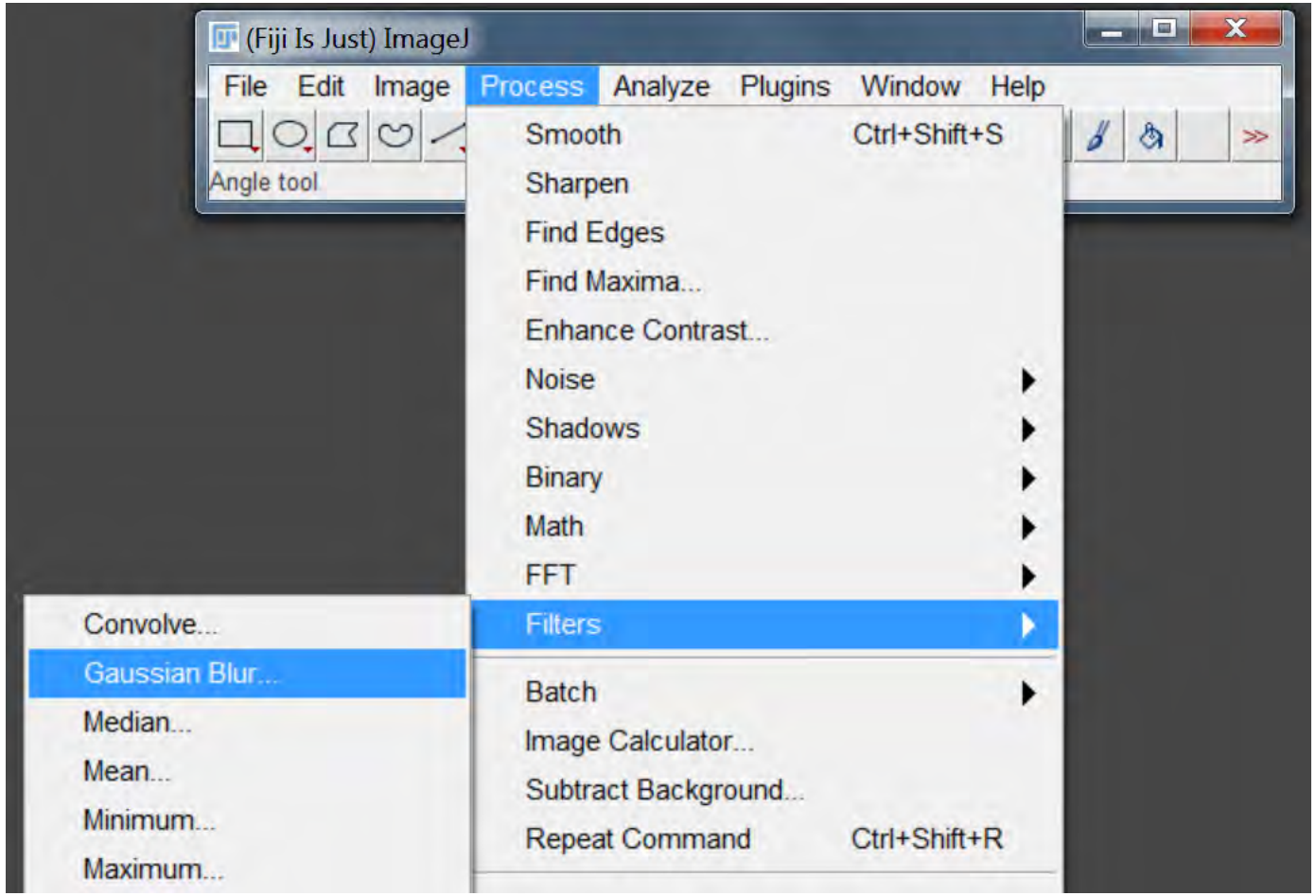
Step 8: Click “Create Image”

Overlaid dams output

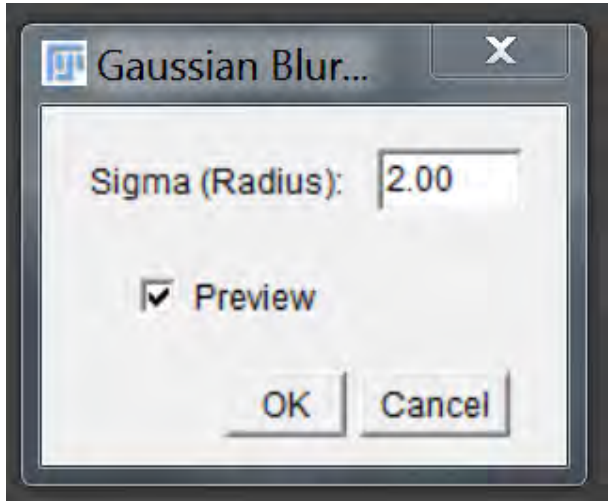


Joseph Roland, Ph.D.
DHSR

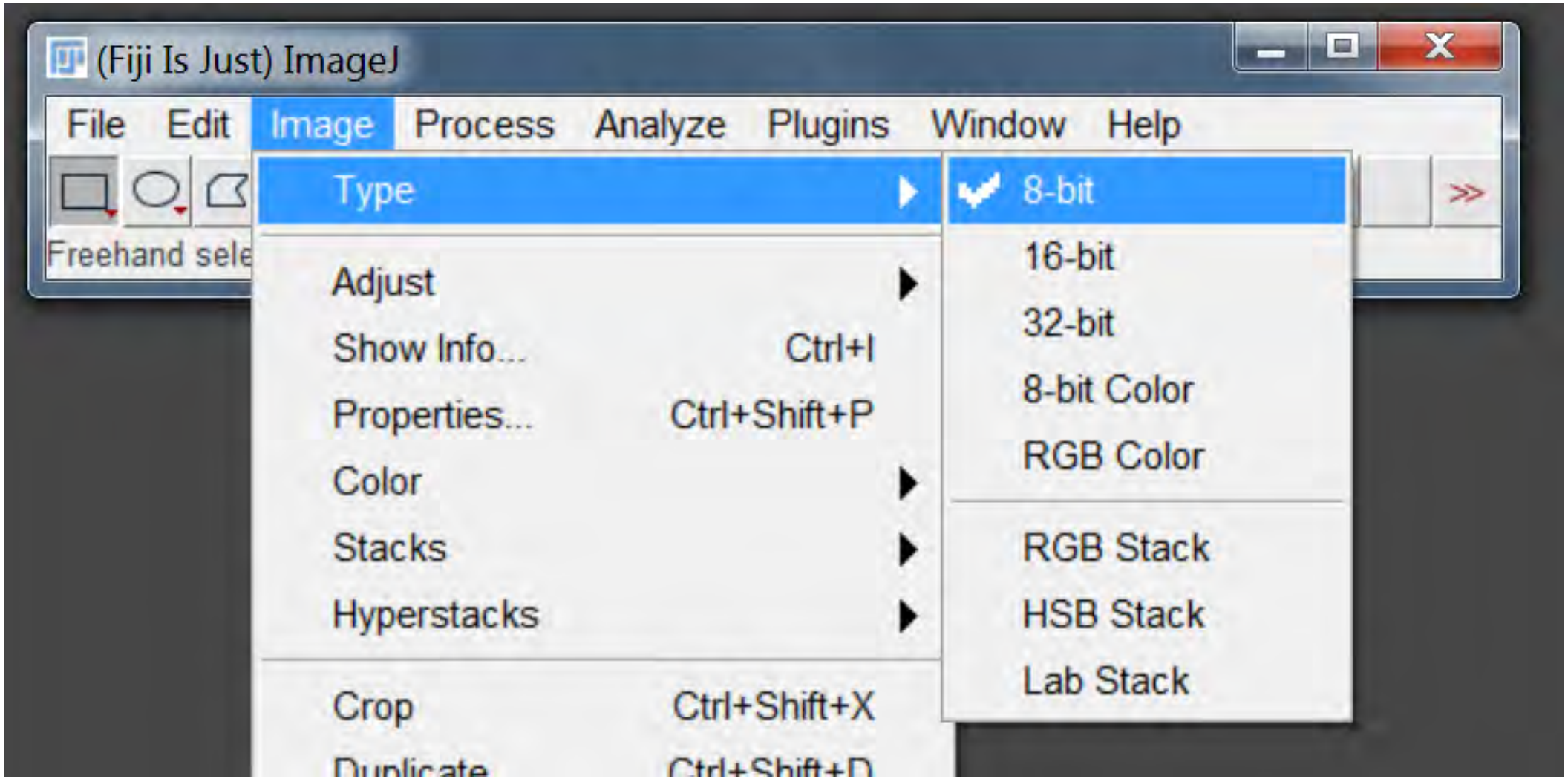
Step 9: Perform Gaussian Blur



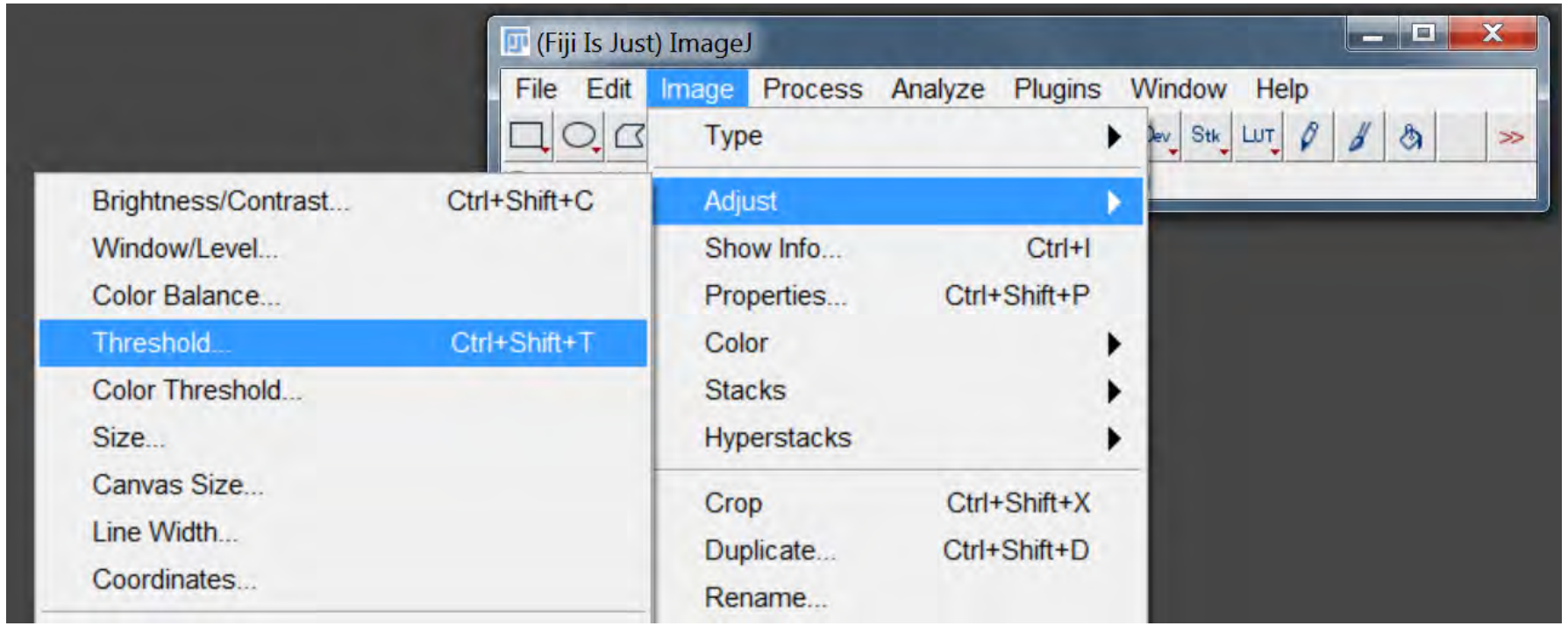
Gaussian Blur Settings



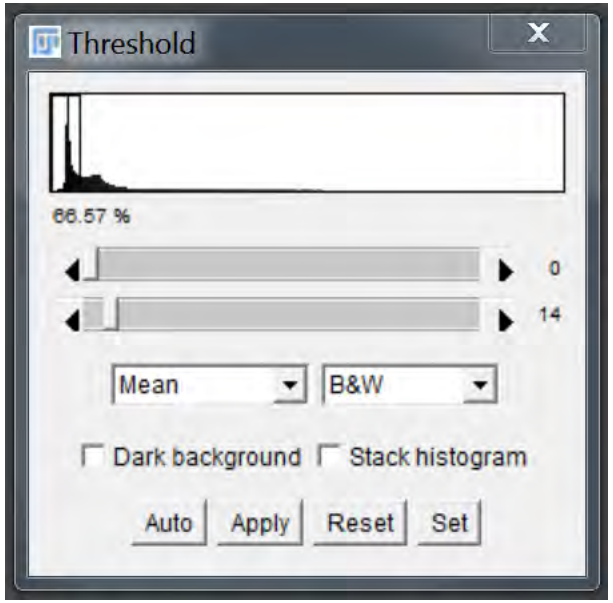
Step 10: Convert the image to 8-bit



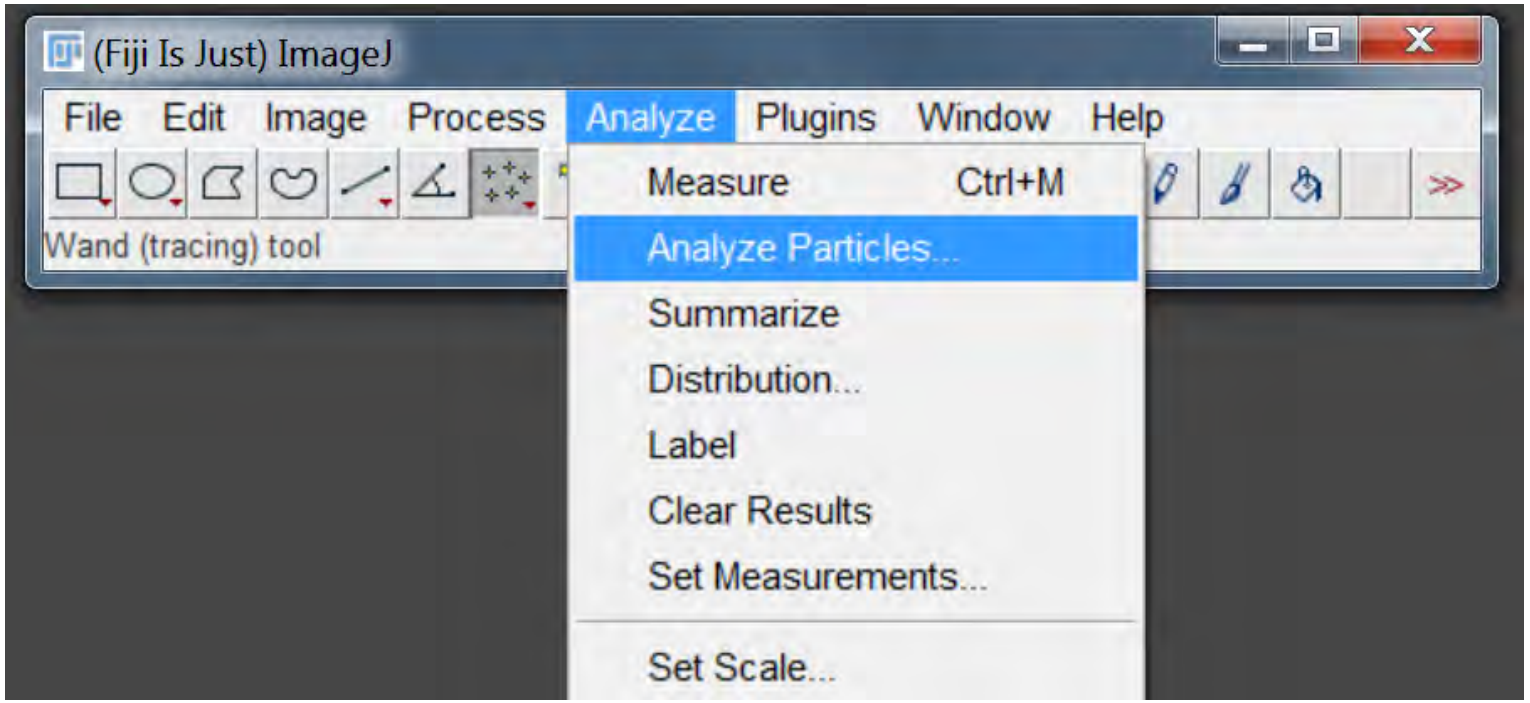
Step 11: Threshold the image



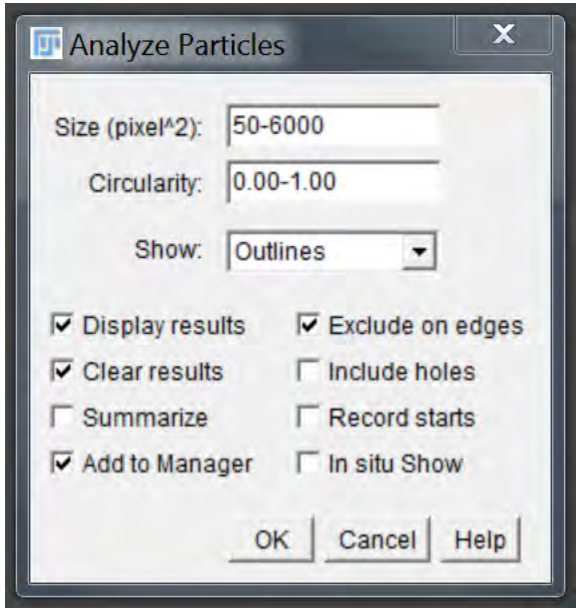
Threshold Settings



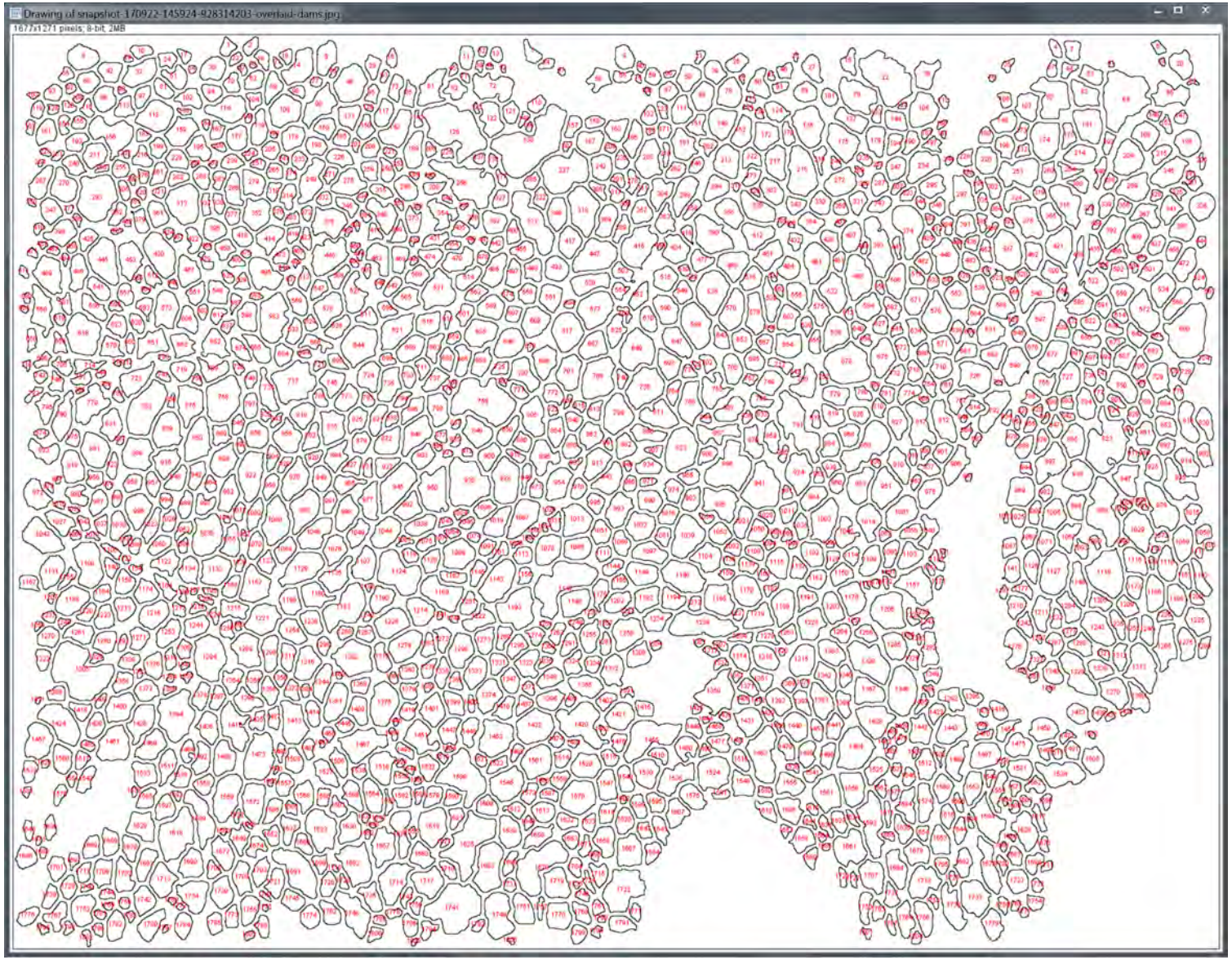
Step 12: Analyze Particles



Analyze Particles Settings



Step 12: Analyze Particles - Output

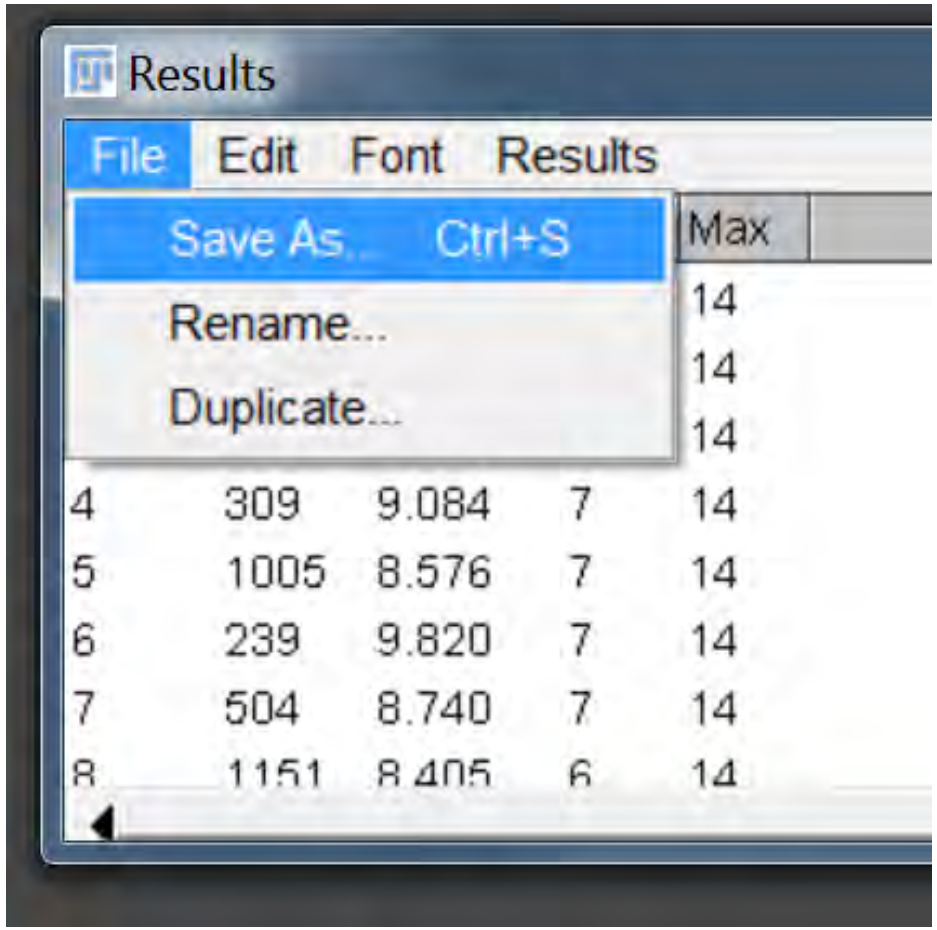


Save this image, if you choose to.

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



Joseph Roland, Ph.D.
DHSR