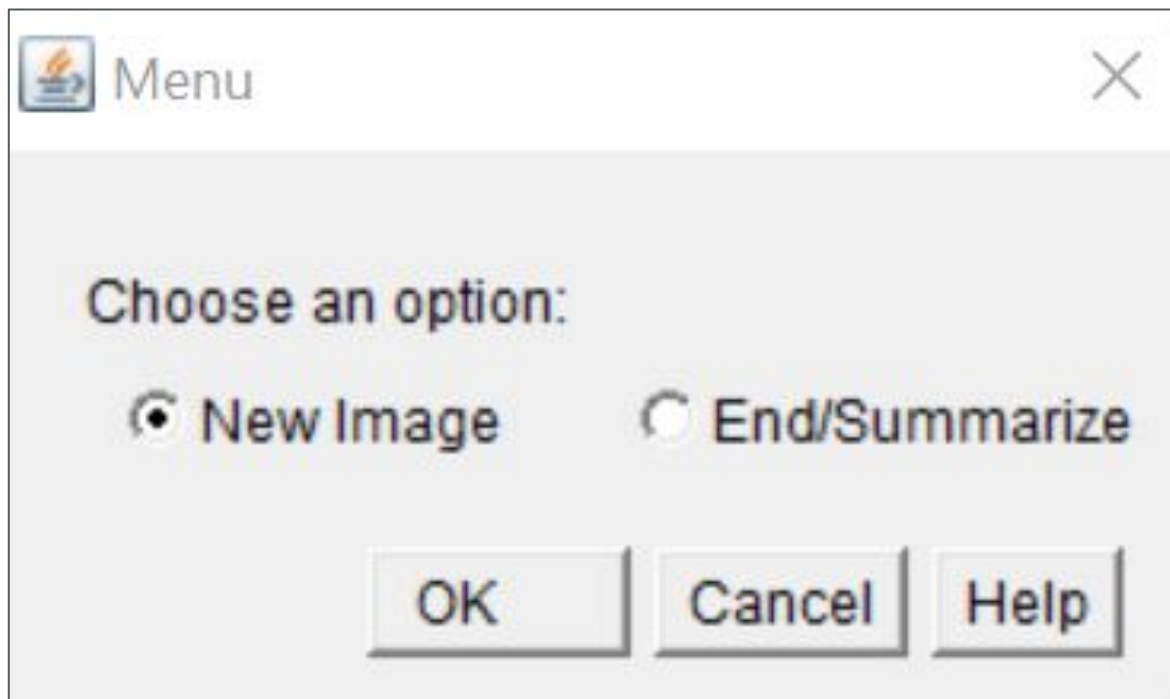


ImageJ Brightfield Cell Segmentation Workflow

Benjamin Ngu (bnngu@usc.edu)

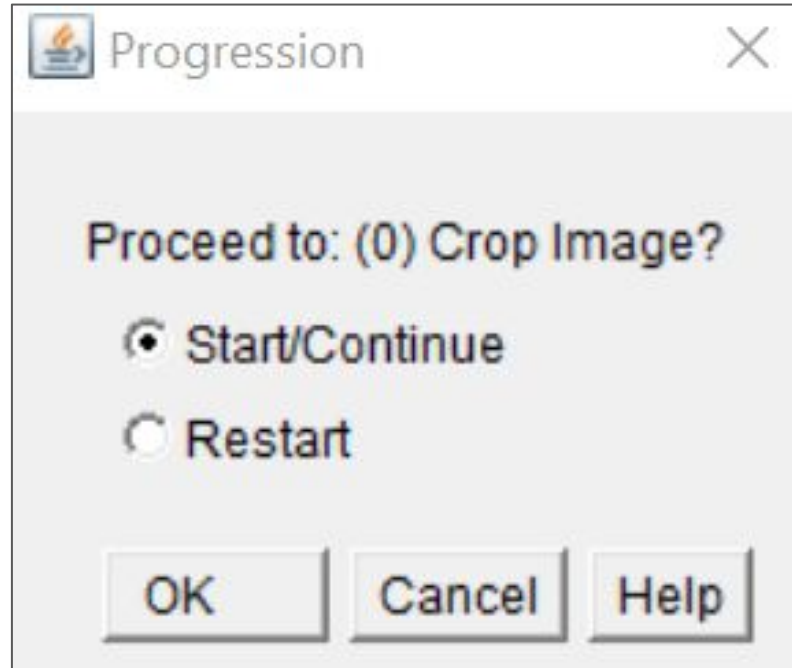
Start-up



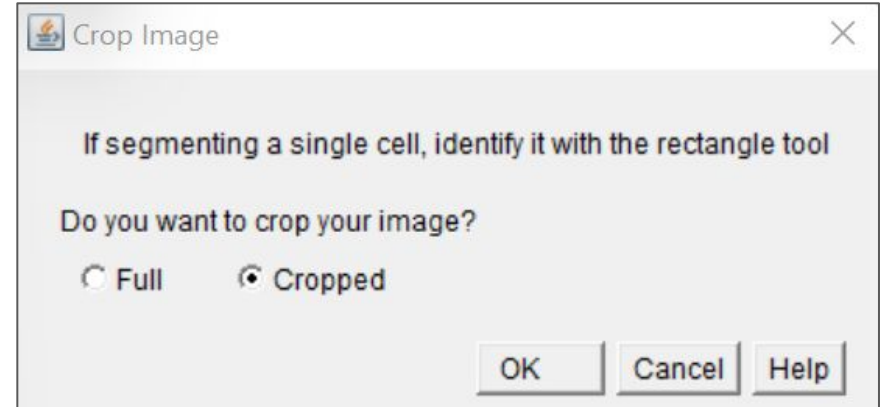
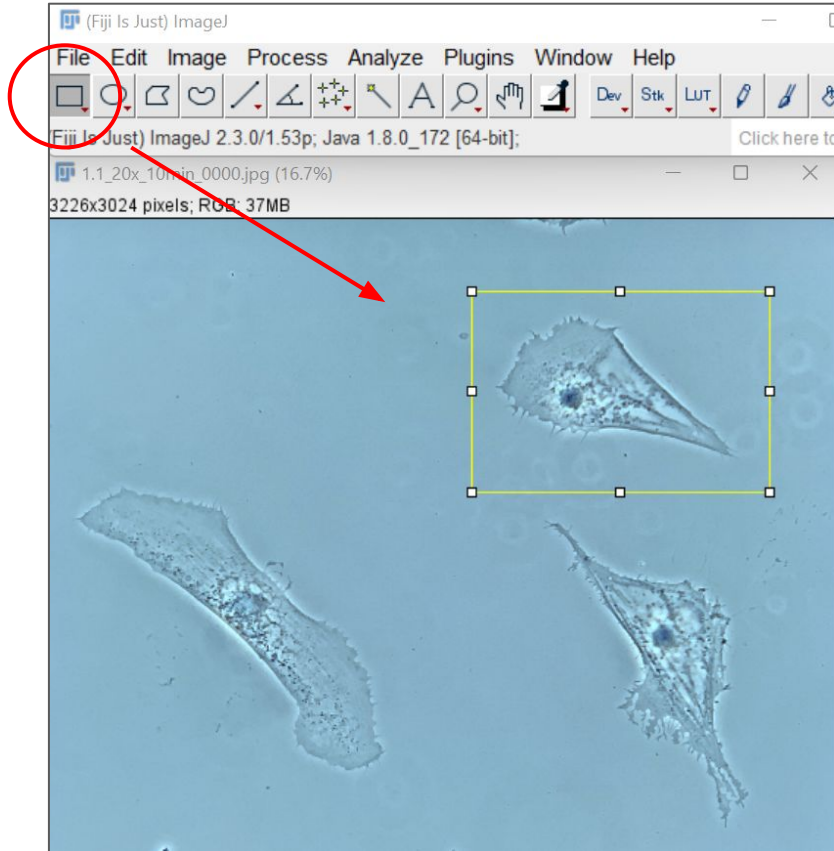
Make sure an image is open before proceeding



Checkpoint #0: Crop Image

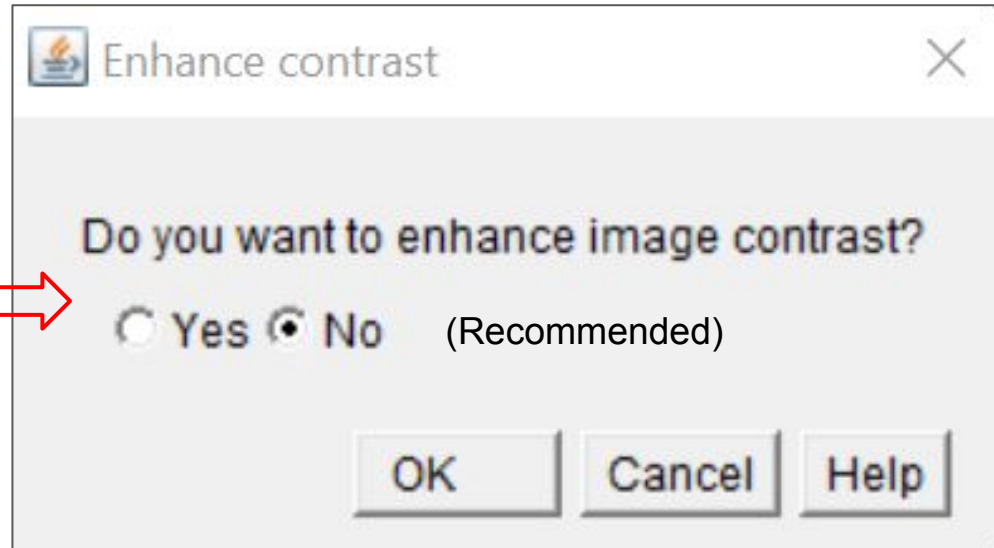
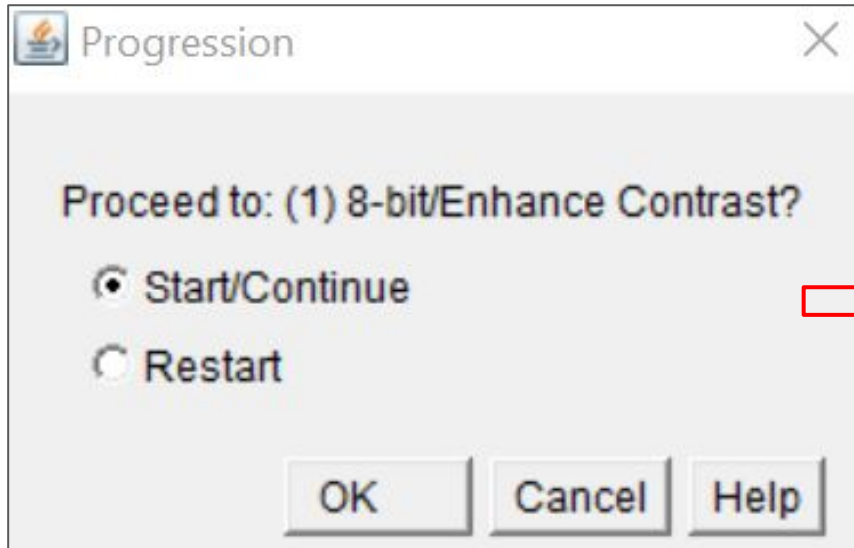


Checkpoint #0: Crop Image

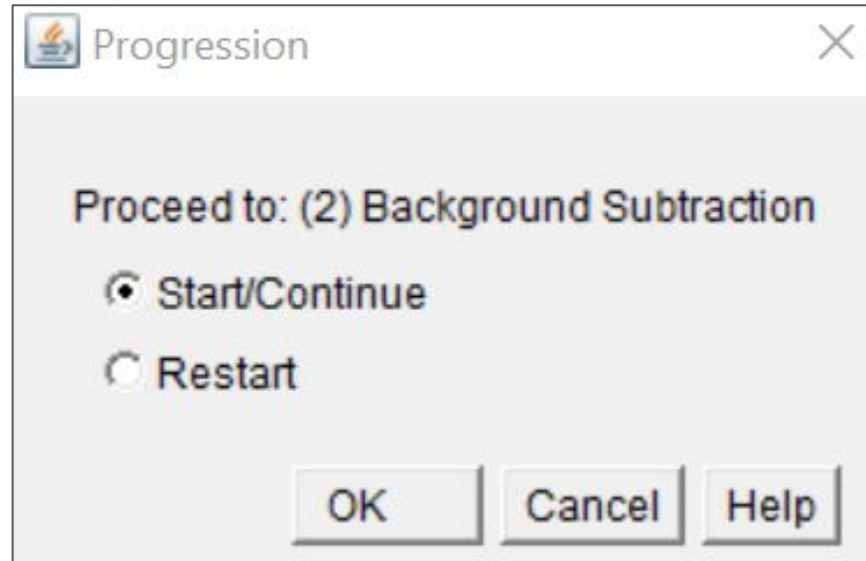


Box cell before hitting "OK"

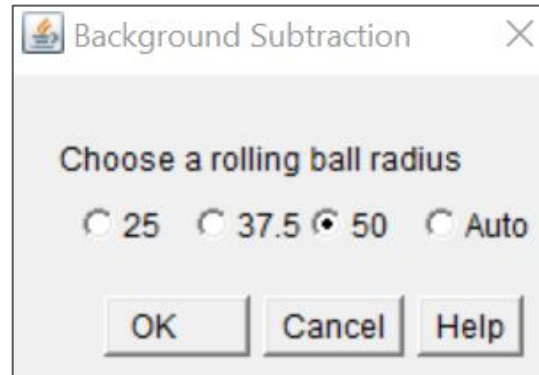
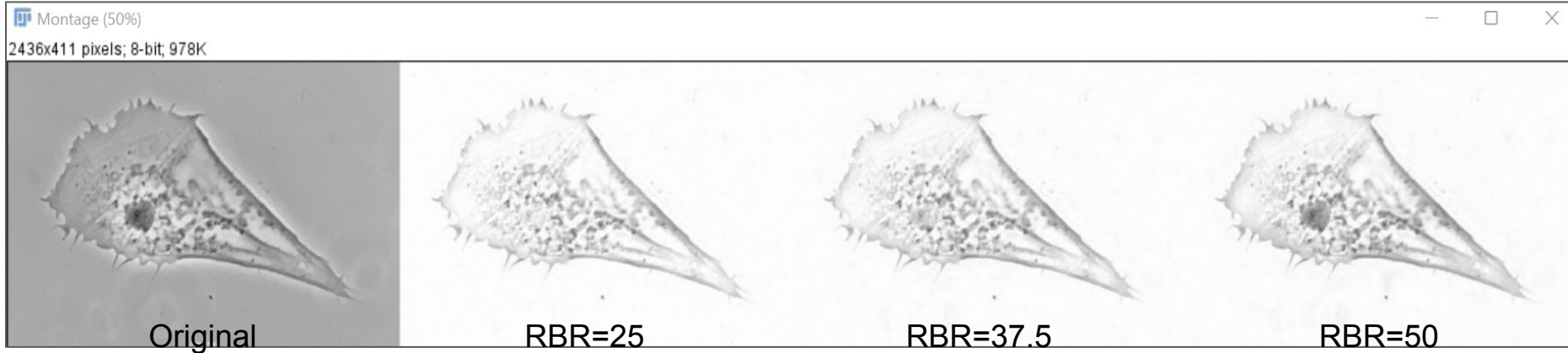
Checkpoint #1: Convert to 8-bit Image/Enhance Contrast



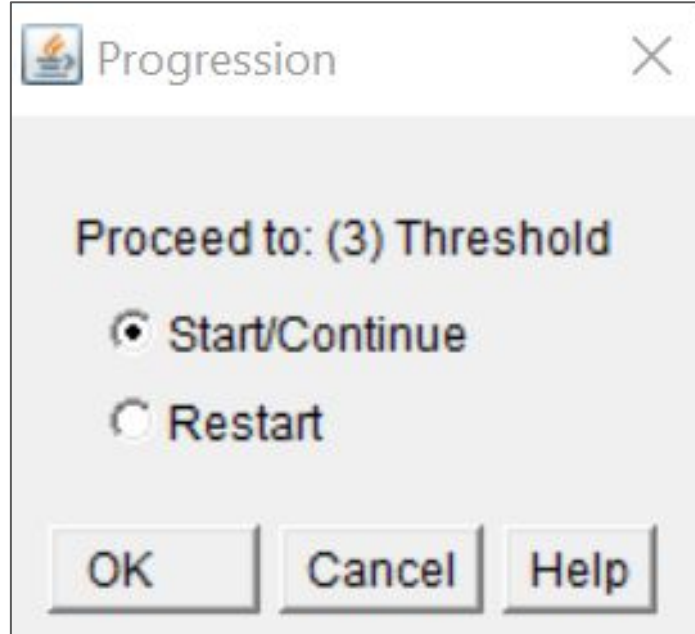
Checkpoint #2: Background Subtraction



Checkpoint #2: Background Subtraction



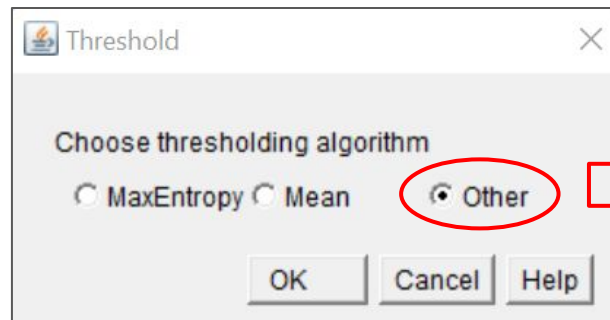
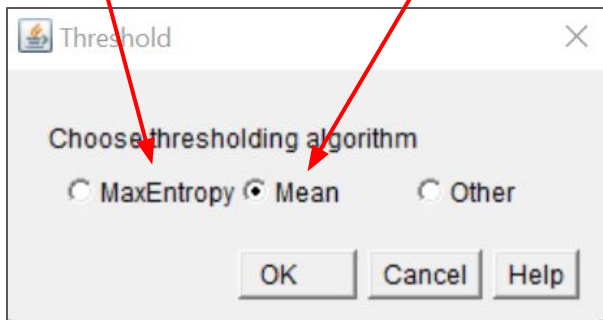
Checkpoint #3: Threshold



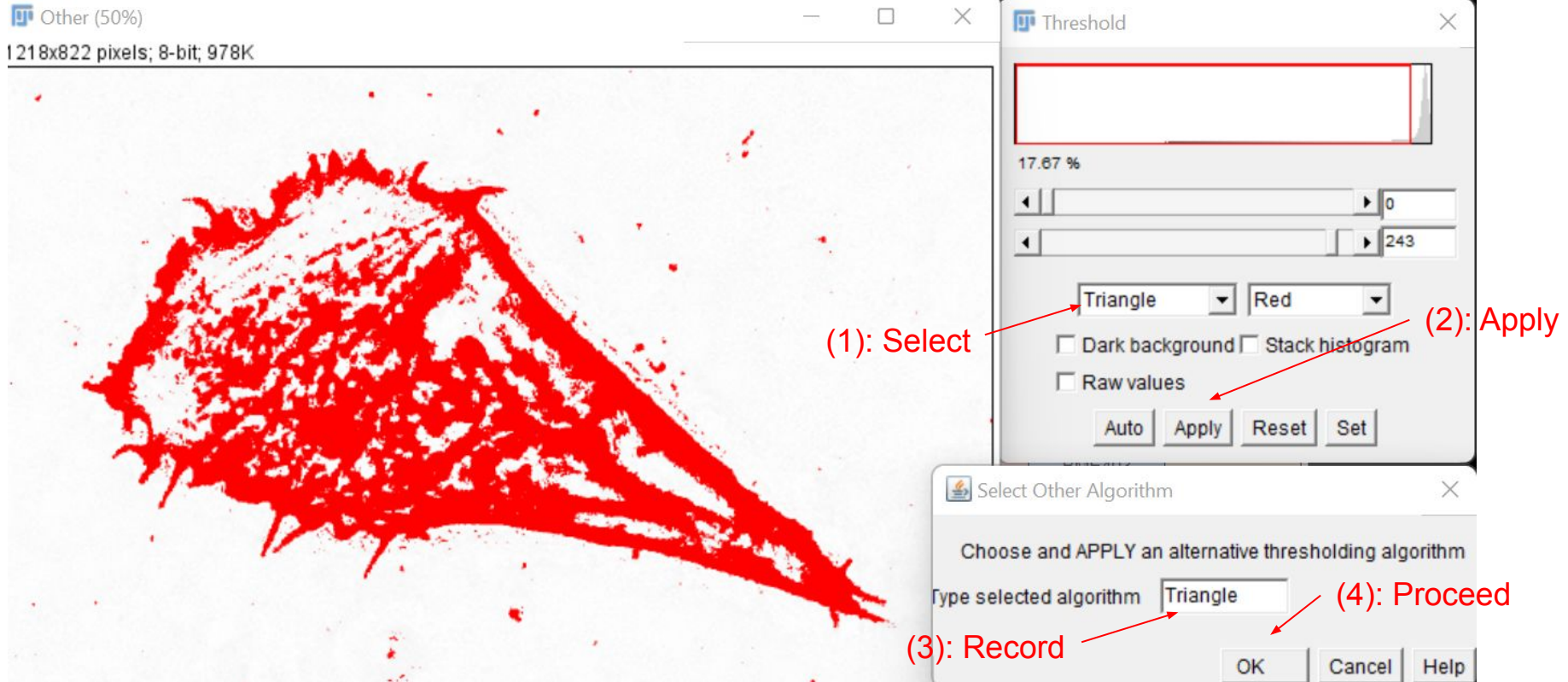
Checkpoint #3: Threshold



“Other” (Use to evaluate first two alg.)



Checkpoint #3: Threshold (“Other”)



Other (50%)
1218x822 pixels; 8-bit; 978K

Threshold

17.67 %

0 243

Triangle Red

☐ Dark background ☐ Stack histogram

☐ Raw values

Auto Apply Reset Set

Select Other Algorithm

Choose and APPLY an alternative thresholding algorithm

Type selected algorithm Triangle

OK Cancel Help

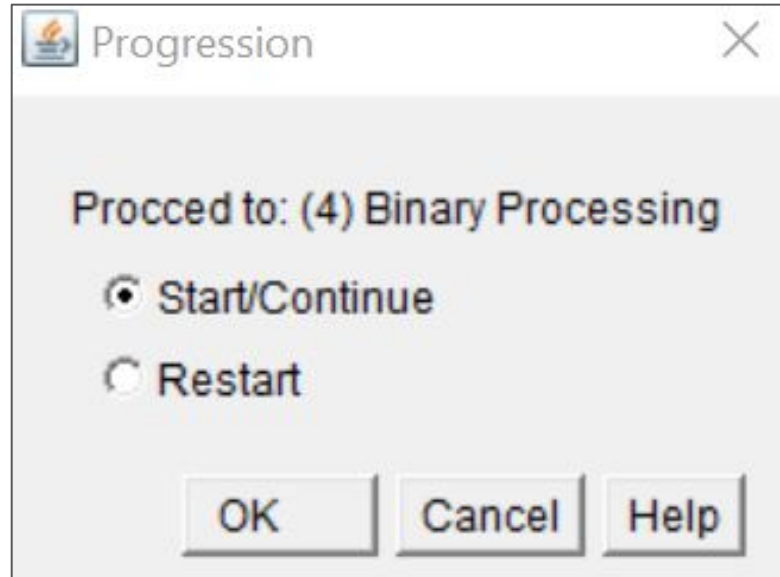
(1): Select

(2): Apply

(3): Record

(4): Proceed

Checkpoint #4: Binary Processing

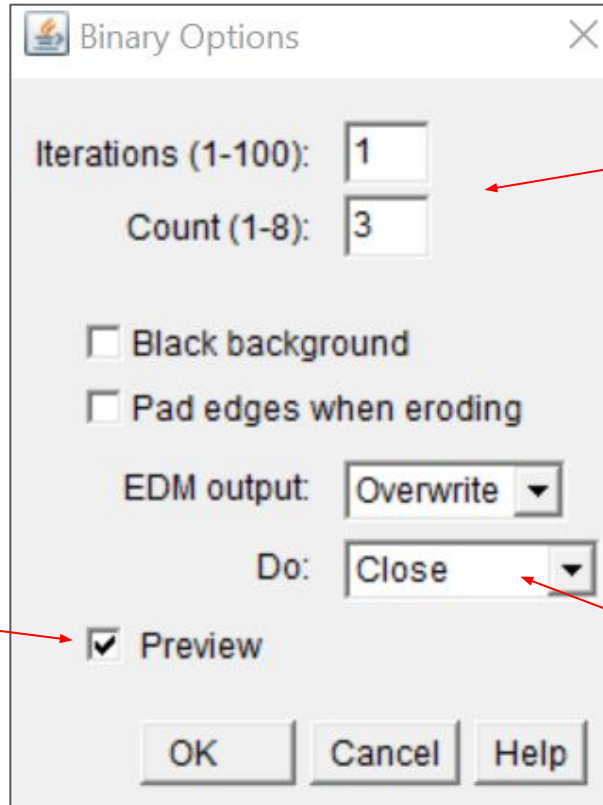


Checkpoint #4: Binary Processing



Program will “fill in” the initial binary image produced by the thresholding algorithm.
“Check to start” if it is not satisfactory. Else, you will proceed to checkpoint #5

Checkpoint #4: Binary Processing



The image shows a 'Binary Options' dialog box with the following controls:

- Iterations (1-100):** A text input field containing the value '1'.
- Count (1-8):** A text input field containing the value '3'.
- ☐ **Black background**
- ☐ **Pad edges when eroding**
- EDM output:** A dropdown menu currently showing 'Overwrite'.
- Do:** A dropdown menu currently showing 'Close'.
- ☒ **Preview**
- Buttons at the bottom: **OK**, **Cancel**, and **Help**.

Iterations Specifies the number of times erosion, dilation, opening, and closing are performed. Iterations can be aborted by pressing **Esc**.

Count Specifies the number of adjacent background pixels necessary before a pixel is removed from the edge of an object during erosion and the number of adjacent foreground pixels necessary before a pixel is added to the edge of an object during dilation.

Recommendation:

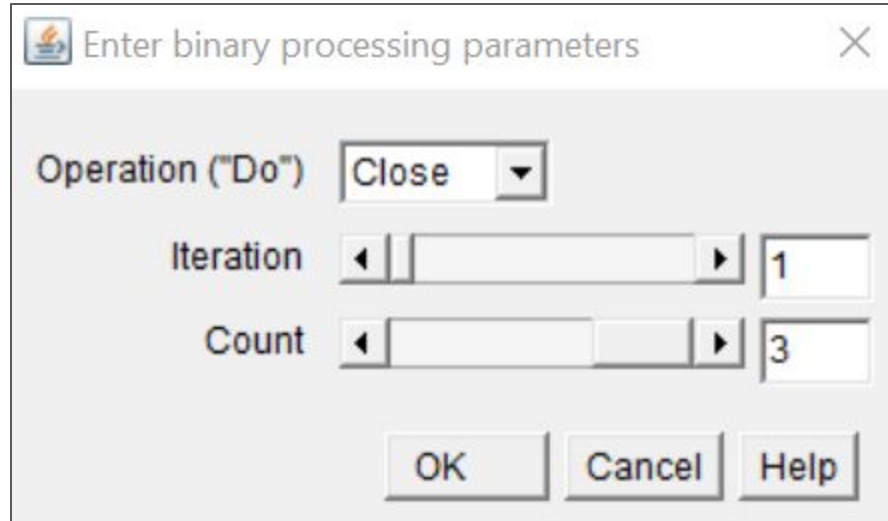
- Iteration: 1-10
- Count: 3

Select to preview
the effects of
your binary
operation

Select binary processing operation

Recommendation: "Close" and "Open"

Checkpoint #4: Binary Processing

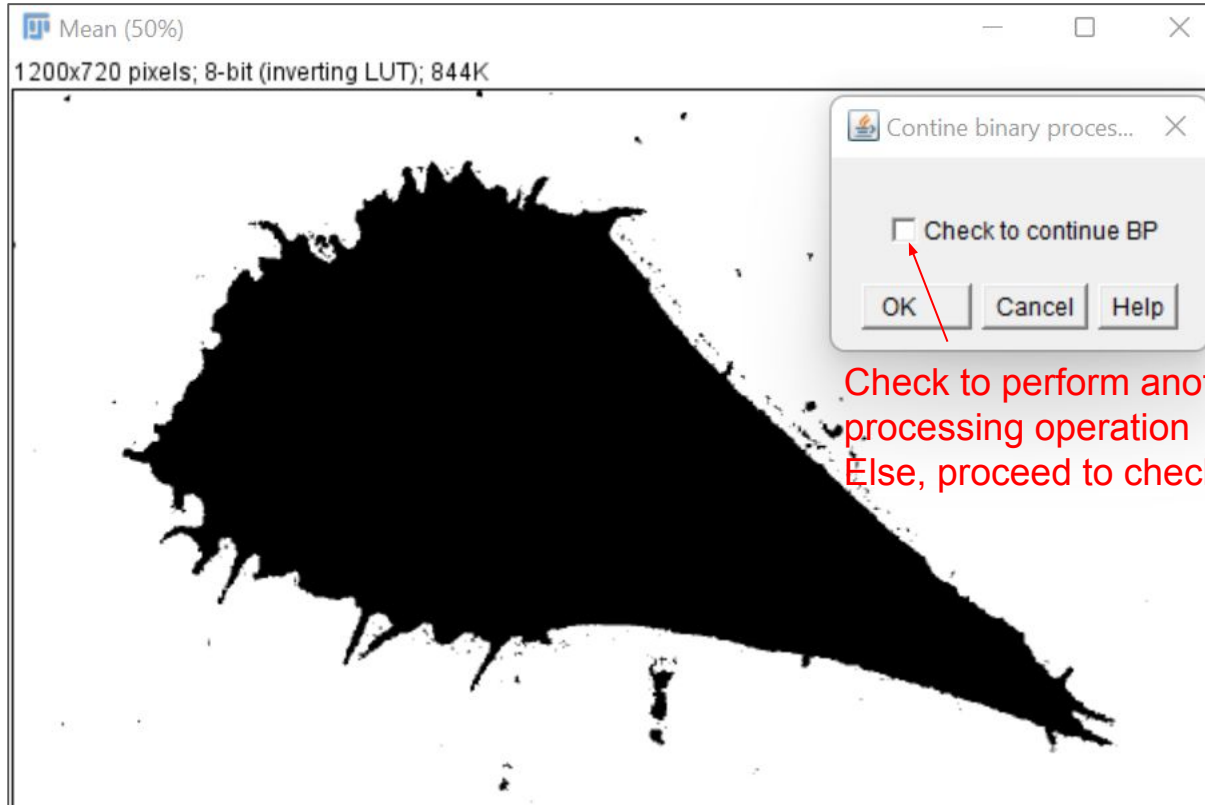


A screenshot of a Windows-style dialog box titled "Enter binary processing parameters". The dialog box has a standard title bar with a close button (X) in the top right corner. Inside the dialog, there are three main input fields: "Operation ('Do')", "Iteration", and "Count". The "Operation ('Do')" field is a dropdown menu currently showing "Close". The "Iteration" field is a numeric input box with a value of "1". The "Count" field is a numeric input box with a value of "3". At the bottom of the dialog, there are three buttons: "OK", "Cancel", and "Help".

Field	Value
Operation ("Do")	Close
Iteration	1
Count	3

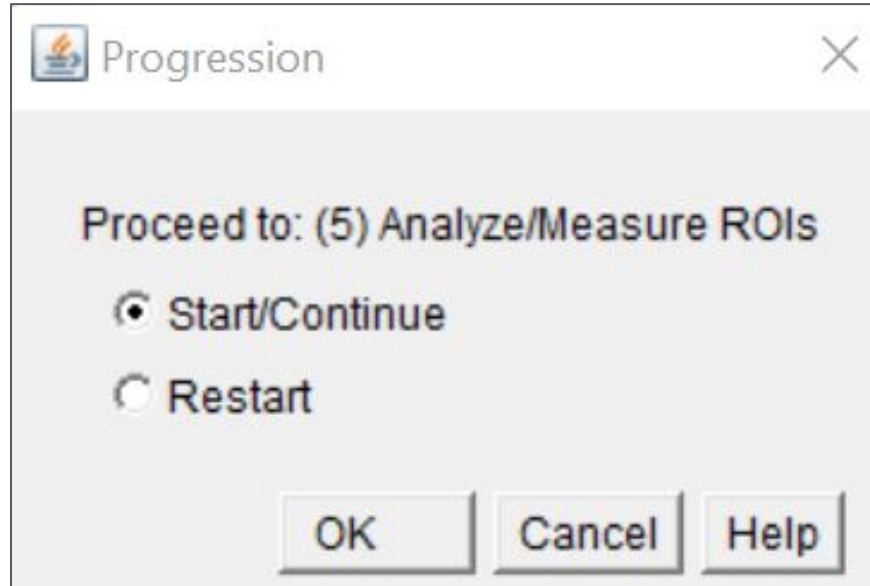
Record binary processing operation and parameters

Checkpoint #4: Binary Processing

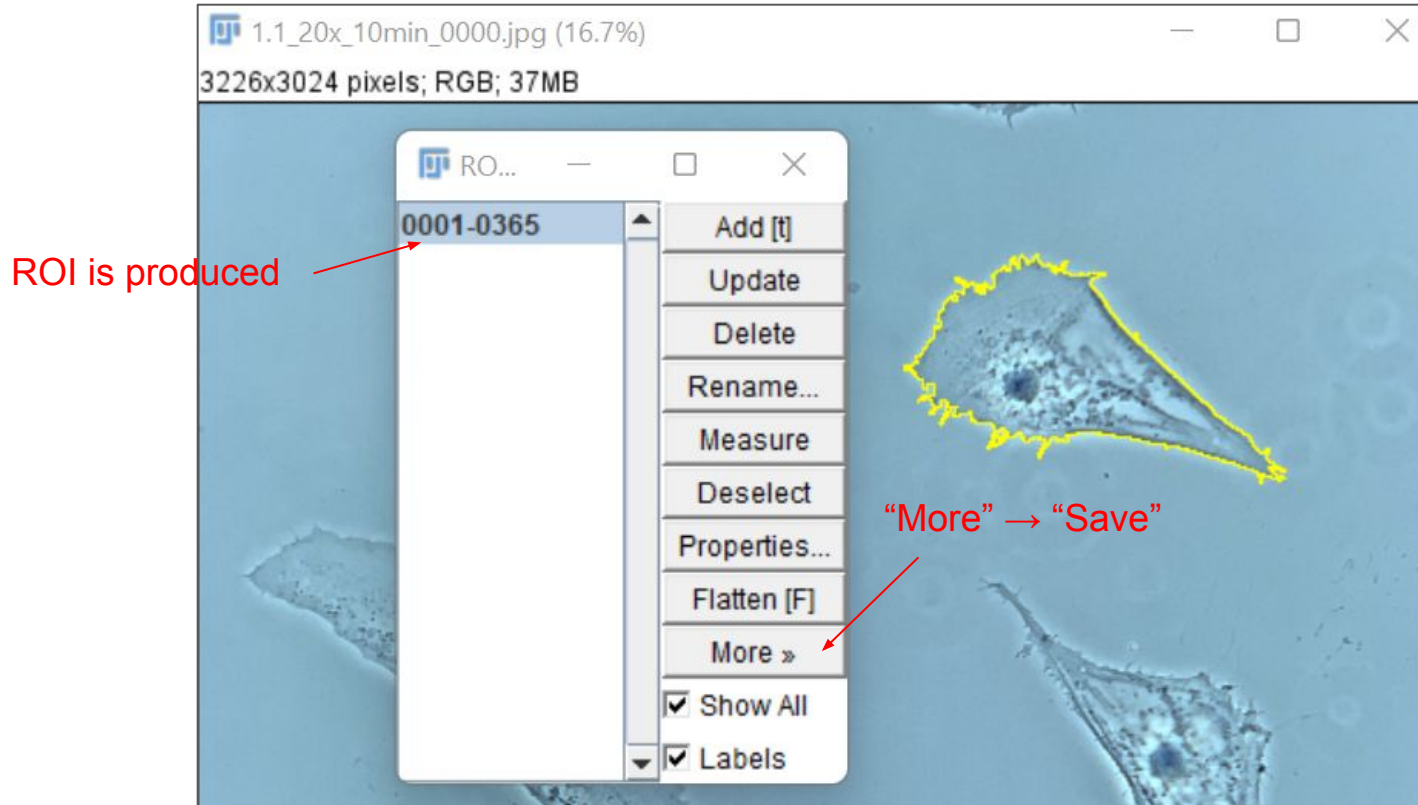


Check to perform another binary
processing operation
Else, proceed to checkpoint #5

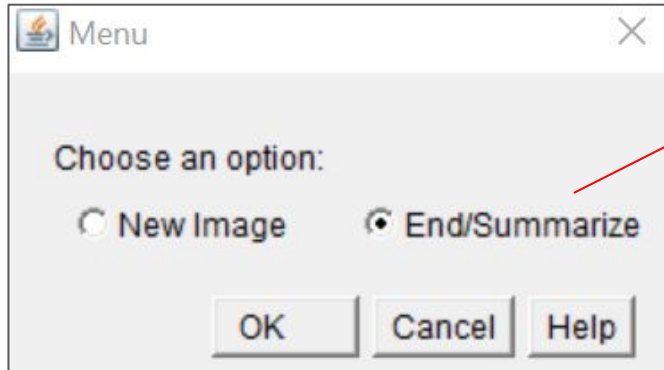
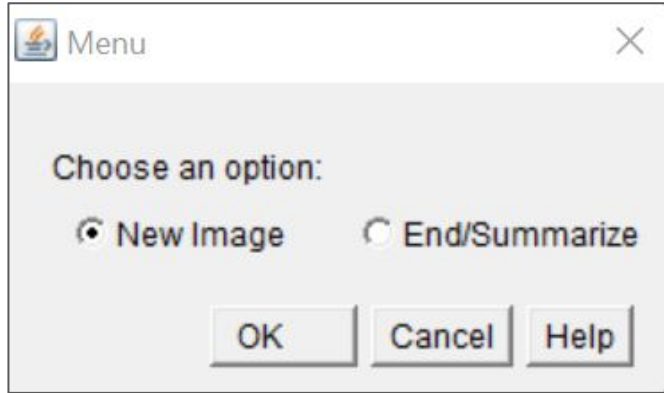
Checkpoint #5: Analyze Particles



Checkpoint #5: Analyze Particles



Process another image or STOP



A screenshot of a 'Summary of Parameters' window. It has a title bar with a small icon and a close button. Below the title bar is a menu bar with 'File', 'Edit', and 'Font'. Below the menu bar is a table with 7 columns: 'Images', 'Enhance Contrast?', 'RBR', 'Threshold', 'Binary Operation', 'Iterations', and 'Count'. The table contains one row of data.

Images	Enhance Contrast?	RBR	Threshold	Binary Operation	Iterations	Count
1.1_20x_10min_0000.jpg	No	50	Mean	Nothing	1	3