Example Use of ImageJ and Diameter J for SEM measurements

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Example Use of ImageJ and DiameterJ for SEM Measurements

Downloads for ImageJ (aka Fiji) and DiameterJ:

https://imagej.net/ij/download.html, https://imagej.net/plugins/diameter

Microsoft Excel Spreadsheet Template for analysis (click on 'view raw'):
https://github.com/OSEL-DBCMS/Respirator-Filtration-Efficiency-Calculator-Support-Files/blob/main/Superpixel_Fiber%20Diameter_Measurements%20Template.xlsx

Material Characteristics used in the Respiratory Filtration Efficiency Calculator

The following slides will instruct the user in determining

- Mean Material Thickness (slides 5-7)
- Packing Density (equation is on slide 8, and incorporates Mean Material Thickness, found on slides 5-7)
- Mean Fiber Diameter (slides 11-30)

Overview of Instructions

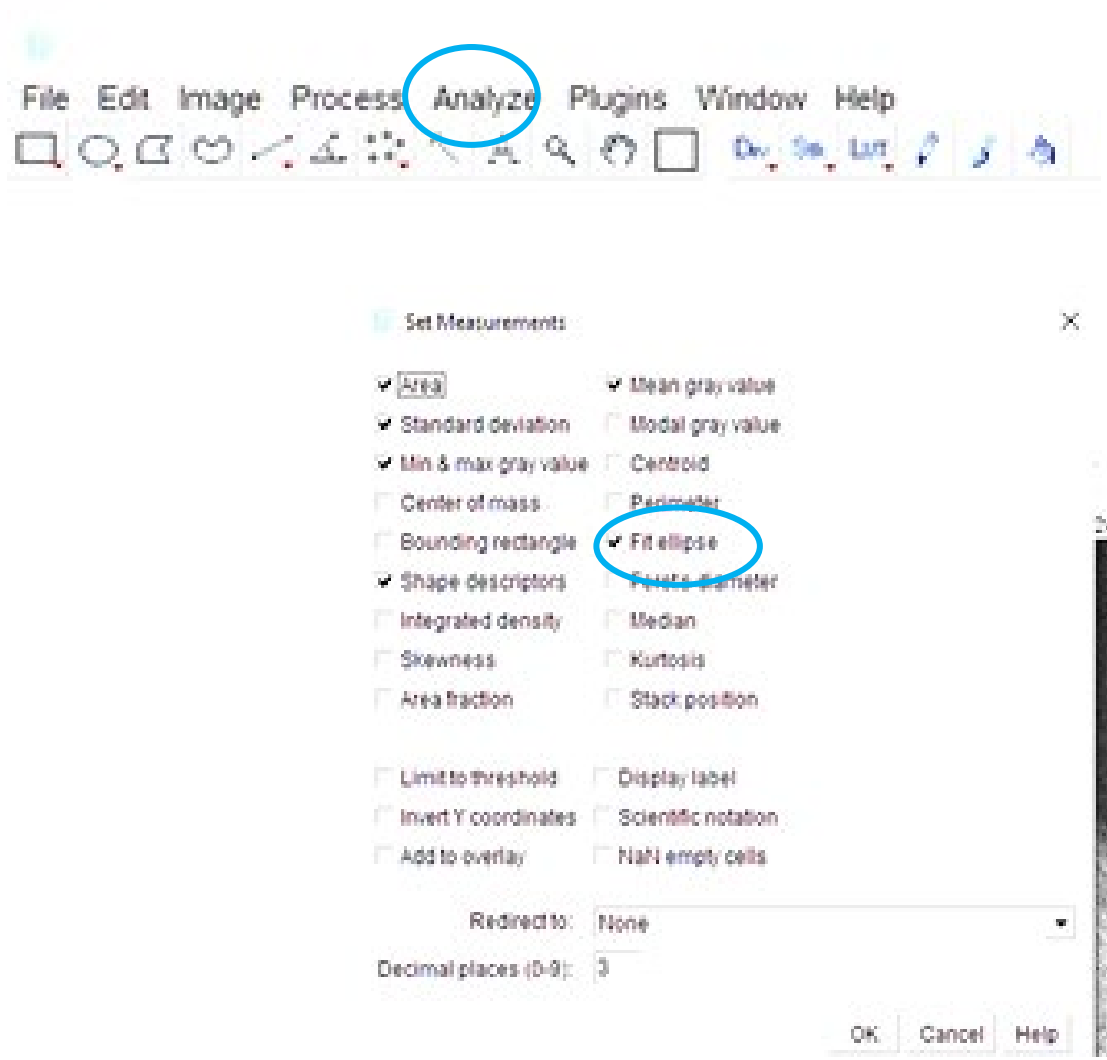
- * **Before this point**, you should have:
  - SEM image files of your respirator material ready to analyze
  - Fiji/ImageJ and DiameterJ downloaded and set up on your workstation
  - Excel template named “"**SuperPixel_FiberDiameter_Measurements**” for fiber analysis (link on slide 1) downloaded on your workstation
- **Step 1**: Use ImageJ and DiameterJ to measure Material Thickness and calculate Packing Density
- **Step 2**: Use Image J and DiameterJ to examine SEM image Meta information and create segmented images in preparation for fiber diameter analysis
- **Step 3**: Use Excel to filter DiameterJ output of segmented images and to extract fiber diameter means and standard deviations
- (Repeat Steps 2 and 3 for each SEM image of the respirator/mask.)

Step 1: Use ImageJ and DiameterJ to measure Material Thickness and calculate Packing Density

- The following instructions in Step 1 use ImageJ and DiameterJ to measure mean material thicknesses on cross-sectional SEM images, and then uses that quantity to calculate Packing Density.

Step 1.1: Determine Mean Material Thickness

- Use cross-sectional SEM images to generate a side profile; determine the width (material thickness) and calculate packing density of fibers.
  - The equation for packing density is provided in slide 8
- **Five measurements are taken** from a cross-sectional image.
- **Cross-sectional thickness is measured in ImageJ.**
  - From the tool bar go to the '**Analyze**' tab (circled in blue)
    - Scroll down to '**Set Measurements**' and make a tick mark for '**Fit ellipse**' (circled in red)
    - Leave other tick-marks as-is
    - Hit '**OK**'

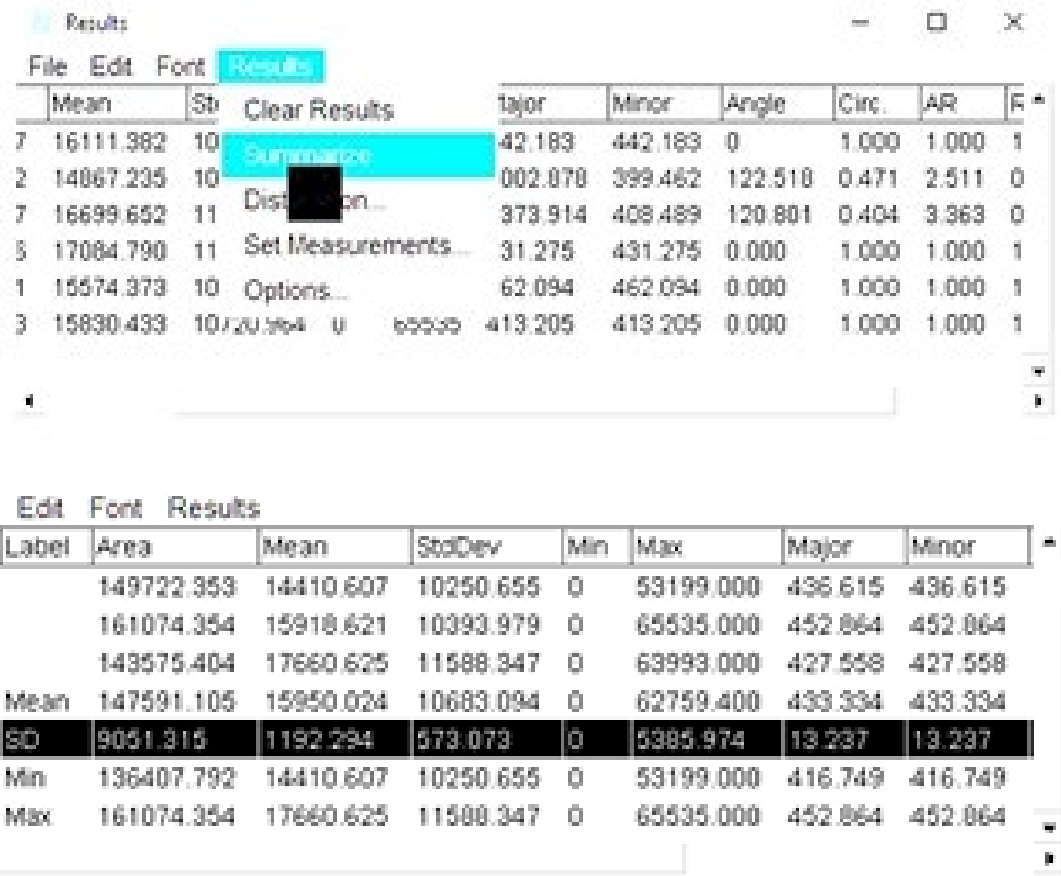


Step 1.1: Determine Mean Material Thickness, cont'd

- From the tool bar choose the ellipse icon (circled in **blue**).
- Use the ellipse function to draw a circle such that its diameter reaches both inner and outer surface edges of the material (example shown to the left in **yellow**)

Step 1.1: Determine Mean Material Thickness, cont'd

- In the "Results" panel look for '**Major**' value as the diameter value of the circle
  - 'Major' and 'Minor' should be the same value since it is a perfect circle
  - **CTRL + M** allows you to measure a new circle on the same image
    - Perform this task for each of 5 circles
  - Then go to the '**Summary**' from the '**Results**' panel (highlighted in light blue)
    - The table provides you with the following:
      - Mean, Standard Deviation (SD), etc.
      - Take the mean of your 5 circles
        - This value is your mean material thickness in microns
        - Keep records of the **mean** and standard deviation (**SD**) for future use with the Respirator Filtration Efficiency Calculator



Step 1.2: Calculate Packing Density

Packing density α is calculated using the equation described by Appert-Collin et al. It is determined by (1) measuring (m^2) and cutting a square swatch of filtration layer material, followed by (2) weighing the swatch (g). The grammage (i.e., the mass per area of the swatch) (g/m^2) is divided by the product of the measured thickness of the material (m) and the density of the polymer from which the fiber is made (g/m^3).

The equation is as follows:

$$\alpha = \frac{10^{-3}\ G_{\left(\text{in } g.m^{-2}\right)}}{Z\ \rho_{Fi}}$$

Where:

Packing density α [=] unitless, a value between 0 and 1.
Grammage G [=] g/m^2
Thickness of the material Z [=] m (Determined on Slides 6 and 7; convert microns to meters)
The density of the polymer from which the fiber is made ρ_{Fi} [=] g/m^3

Repeat this procedure for a total of 5 swatches to obtain a mean and standard deviation of packing density. Keep record of these two quantities for future use with the Respirator Filtration Efficiency Calculator

Citation: Jean-Christophe Appert-Collin, Dominique Thomas, 2 - Fibrous Media, Aerosol Filtration. 2017. 31-47. https://doi.org/10.1016/B978-1-78548-215-1.50002-0:

Mean Fiber Diameter

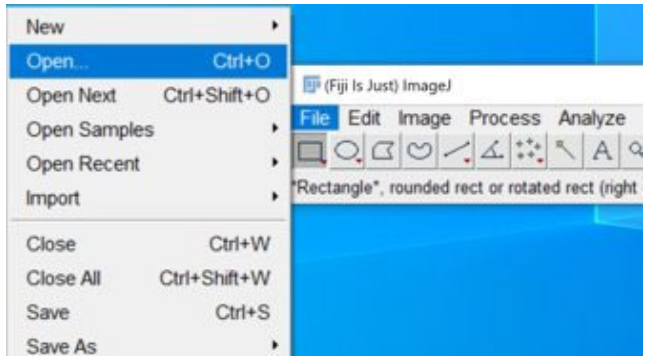
- Mean fiber diameter for your material fabric is found through following Steps 2 and 3. Slides 11-30 instruct the user in determination of this value. The units should be in microns (µm).

Step 2: Use ImageJ and DiameterJ to examine SEM image Meta information

- **Meta information:** information on the image data including but not limited to pixel size, bit depth, dimension and objective information. Metadata is essential to read image data correctly; for example, to have accurate measurements, the image needs to be adjusted according to the correct/associated pixel size. Saving and preserving metadata is key in quantitative image analysis. https://imagej.net/formats/metadata

Step 2.1: Use DiameterJ- Set & Gather Meta Information

1. Open SEM image

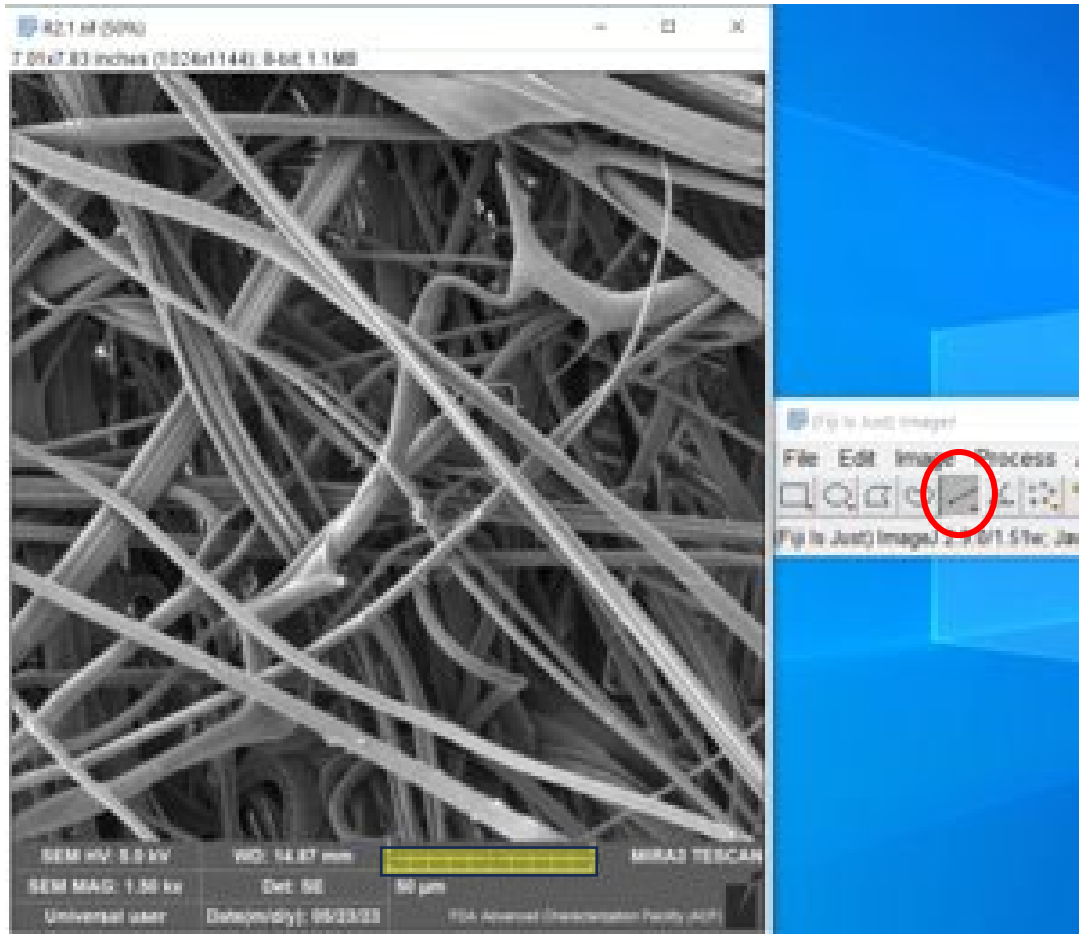


2. Check image and ensure that the file is '<u>8-bit</u>' and a '<u>.tif</u>' . If the image does not indicate this information, save the file as an '8-bit' '.tif' (see circled information in image below).

Step 2.2: Using DiameterJ- Set & Gather Meta Information

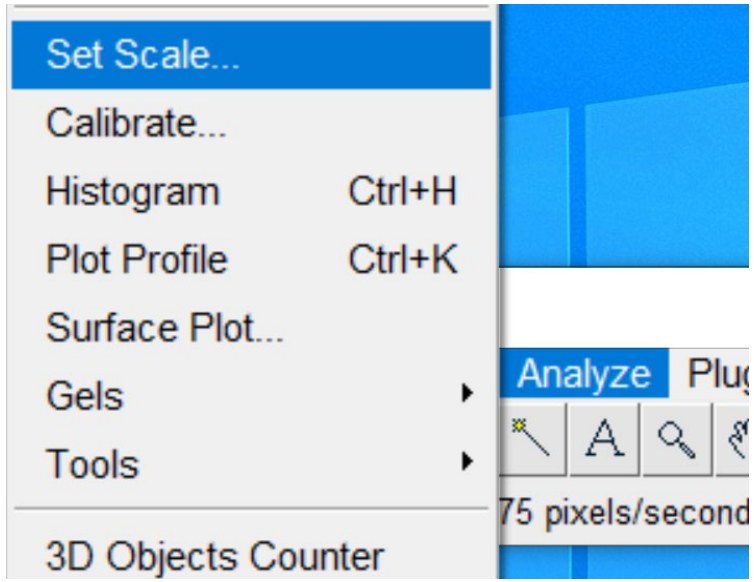
Instructions for determining pixel-micron calibration

1. Select the *Straight* tool (line segment) on the main bar. (circled in the image)
2. Go to the SEM image and find the scale bar at the bottom (highlighted in yellow in the image). While holding “Shift”, left click and hold down on the left side of the scale bar and extend the line to the opposite end of the scale bar.
3. While still holding ‘Shift’, let go of the left mouse button. This will draw a line across the scale bar. ‘Shift’ keeps it straight.



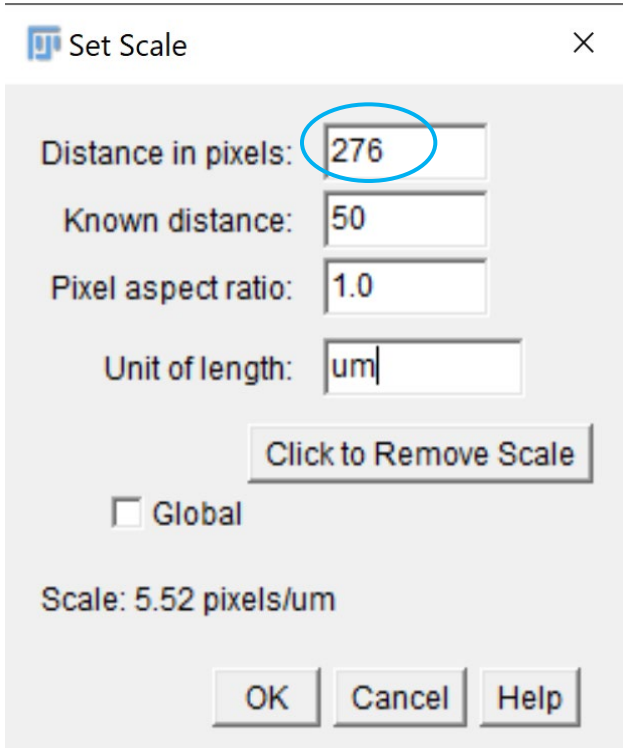
Step 2.3: Use DiameterJ – Set & Gather Meta Information

4. At the top of the ImageJ (Fiji) menu, go to **Analyze > Set Scale**



5. Record the "**Distance in pixels**" in a separate location. Your known distance is on the scale bar from the SEM image (example: 50 um for image shown in example).

- **This information will be used later, in step 3.2.**

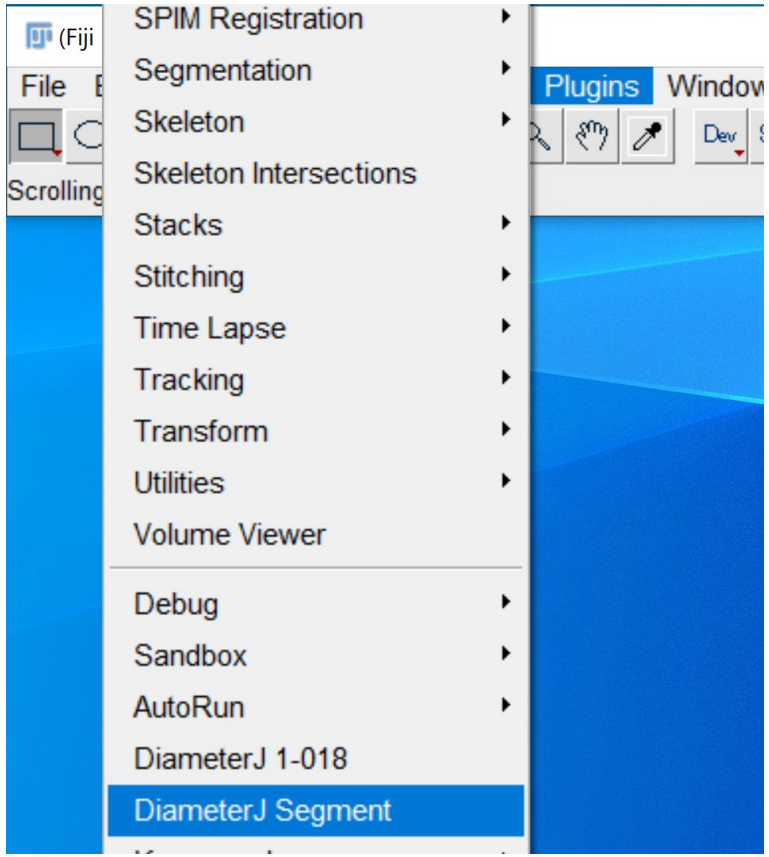


Step 3: Use DiameterJ and Excel to determine mean fiber diameters and standard deviations

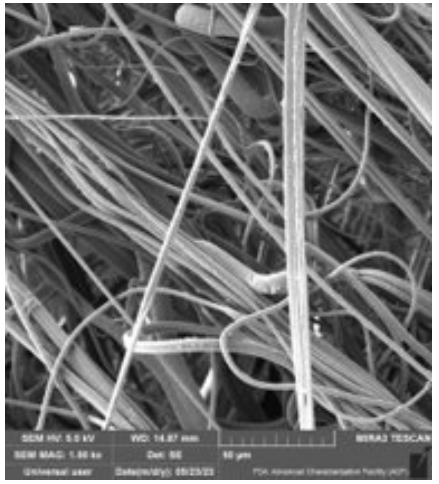
- The following instructions for Step 3 use Excel to analyze DiameterJ output for mean fiber diameters and standard deviations.

Step 3.1: Use DiameterJ – Create Segmentation

1. In Fiji, select Main Bar > Plugins > **DiameterJ Segment**

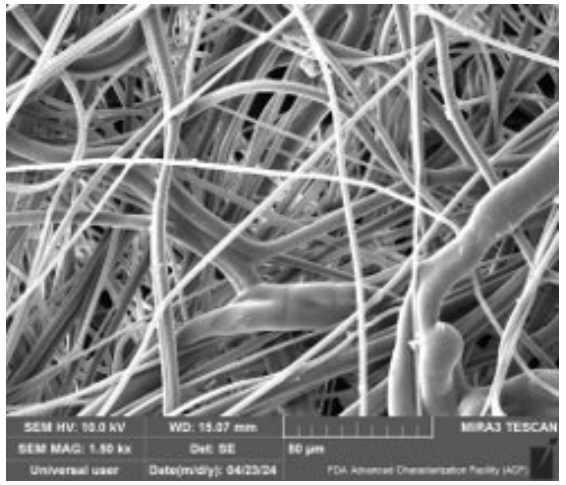


2. *Determine if your SEM image is portrait **vs** landscape



<Portrait

Landscape>



Step 3.1, cont'd: Use DiameterJ – Create Segmentation

3. **Set "basic image" information**
   If photo is "portrait" use: 1024x1144
   If photo is "landscape" use: 1024x888

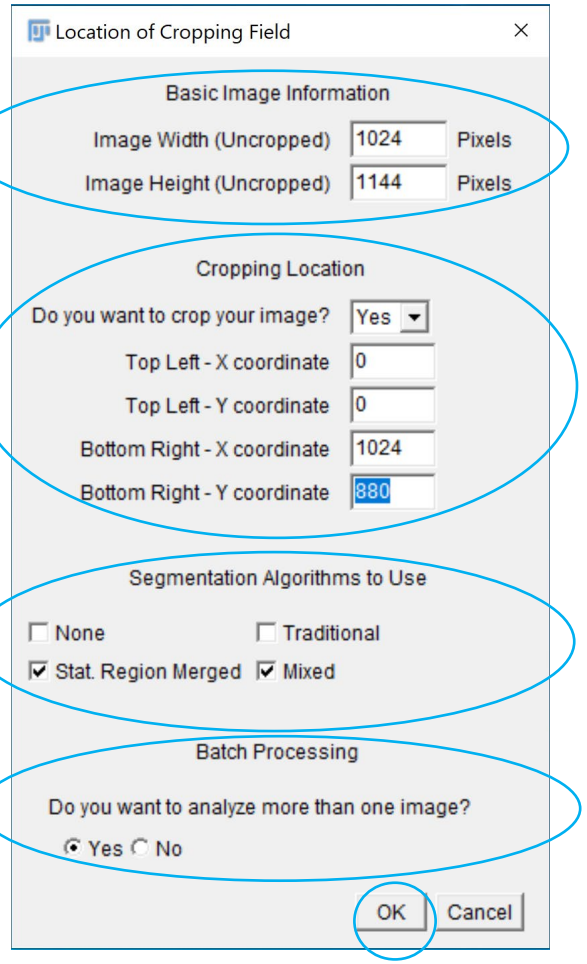
4. **Set "cropping location" information** – Yes
   "portrait" use: 1024x880
   "landscape" use: 1024x700
   (This step removes the bottom SEM data from the SEM image so that ImageJ can work only with the fabric images.)

5. In "**Segmentation Algorithms to Use**", Select "Stat. Region Merged" and "Mixed" (default).

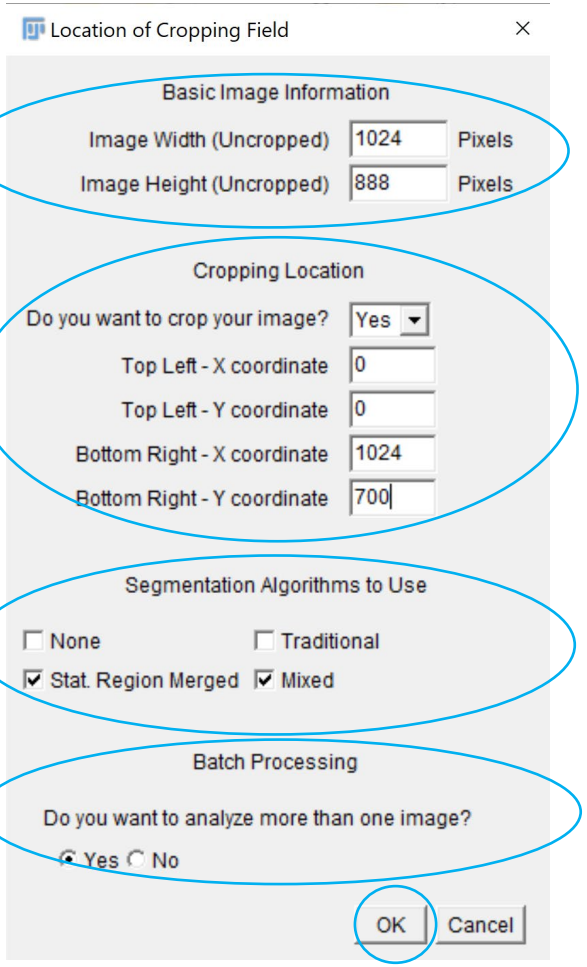
6. In "Batch Processing", select "Yes"

7. Hit 'OK'.

Portrait:

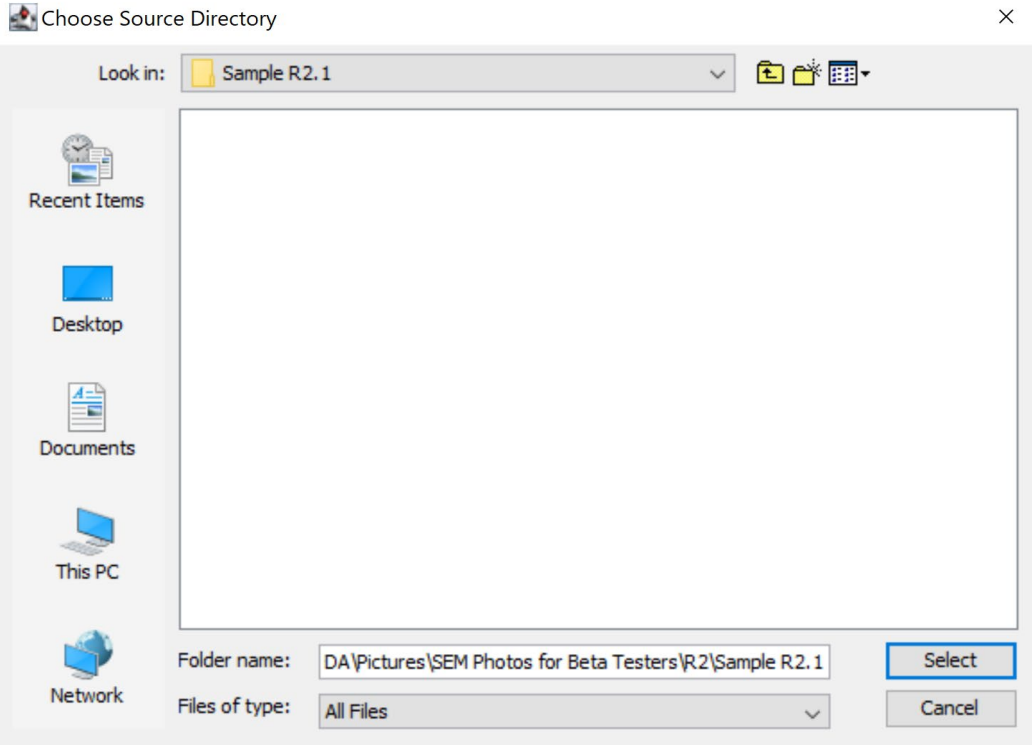


Landscape:

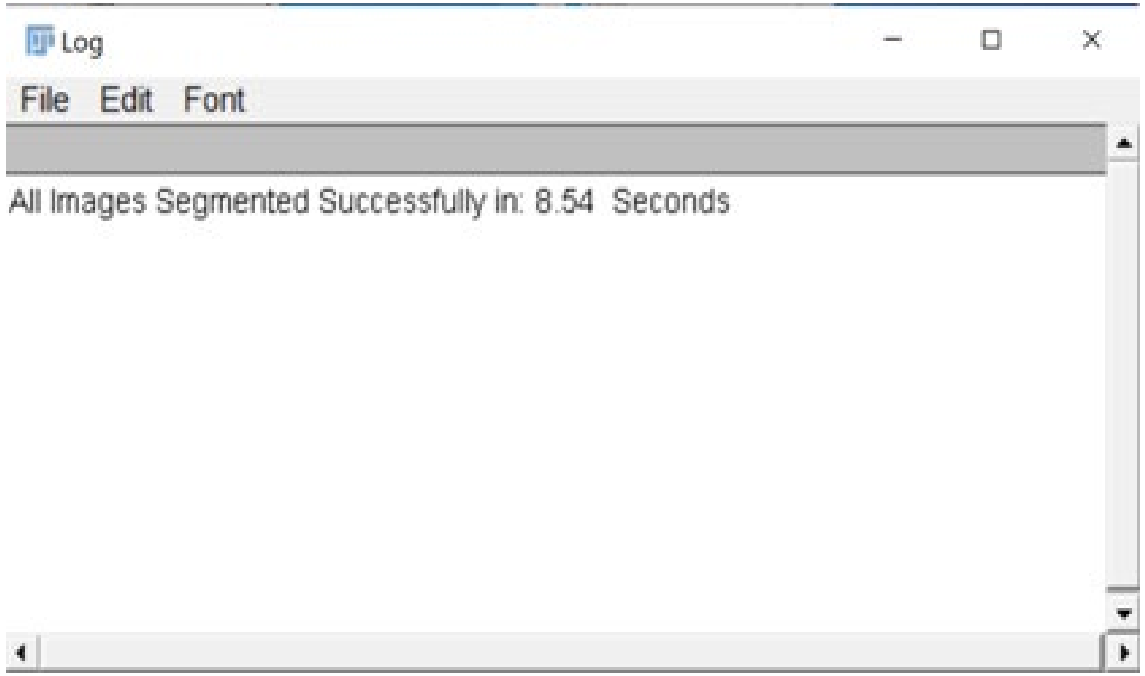


Step 3.1, cont'd: Use DiameterJ – Create Segmentation

8. Select folder containing your image, Hit 'Select'

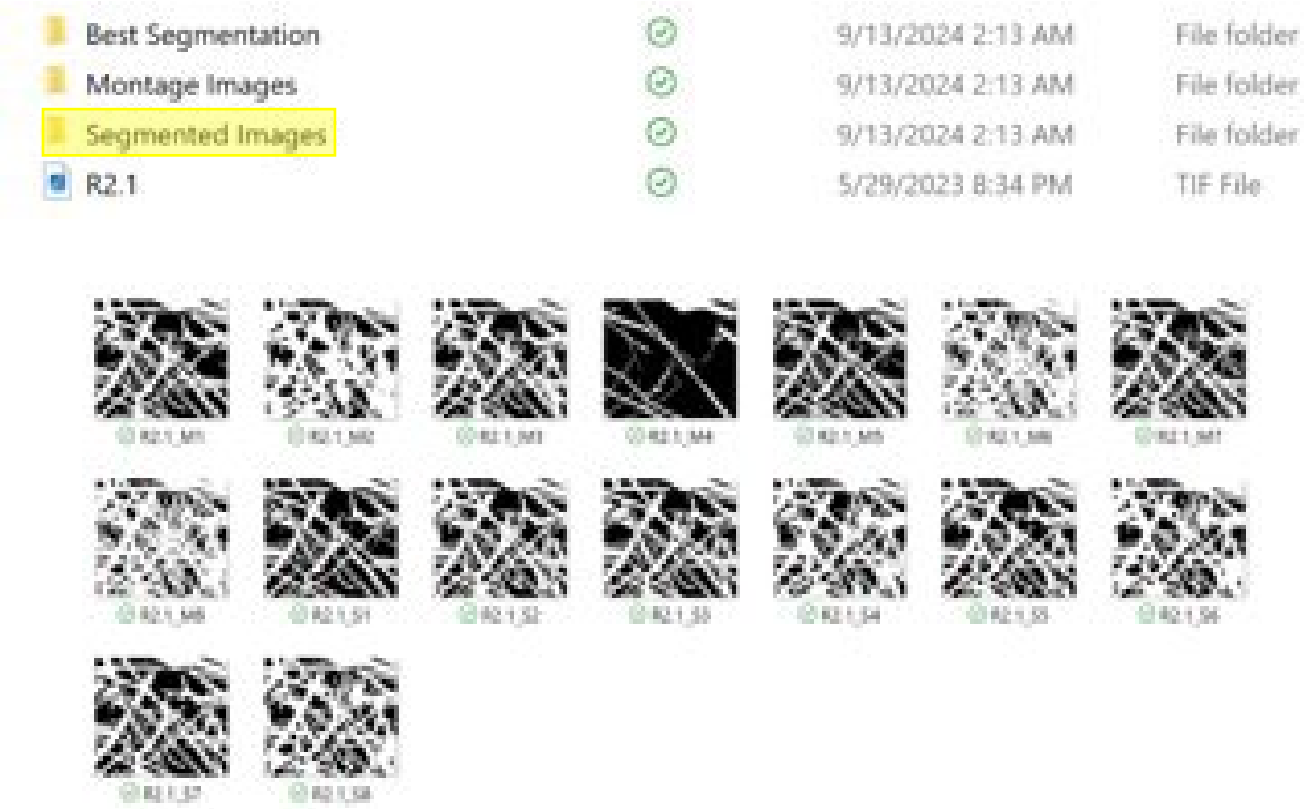


9. If DiameterJ was successful in analyzing images, the dialog box will indicate "All Images Segmented Successfully" and a time.



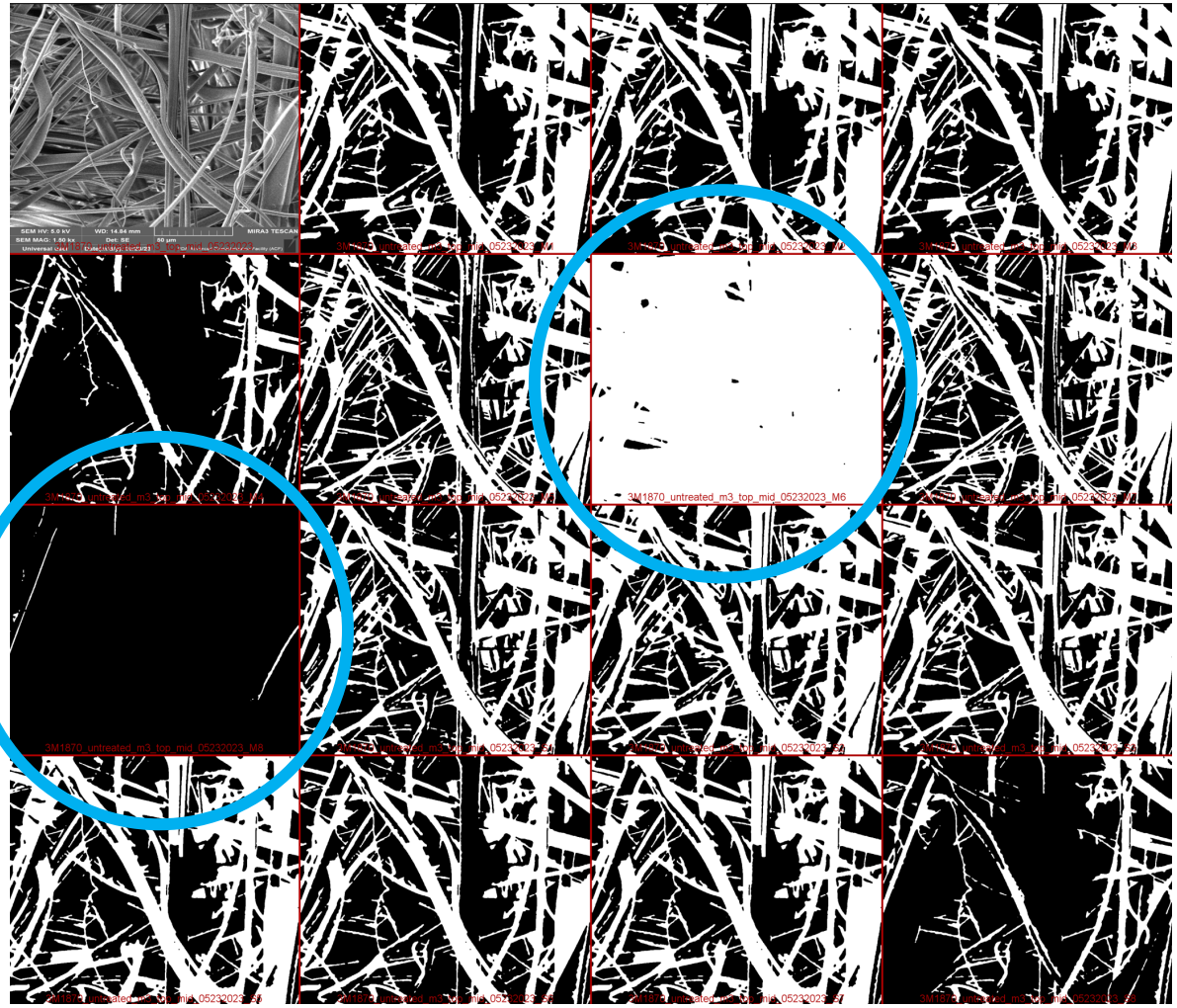
Step 3.2: Use DiameterJ – Fiber Diameter Analysis

- **Output** is found in the same folder as the SEM image that was segmented.
  - Open the folder "**Segmented images**".
  - Verify segmented images are present.
  - See Warning on the next page. If you are returning to this step after DiameterJ has failed, remove images that may be causing DiameterJ to fail.



Step 3.2, cont'd: Use DiameterJ – Fiber Diameter Analysis

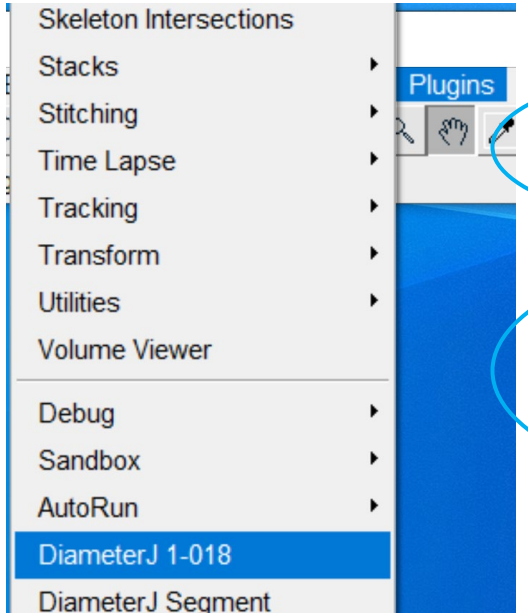
- WARNING: Poorly segmented images that are too white or too black will stop DiameterJ from working. If this happens, re-start the program and follow the steps up to here and delete (i.e., trash or move to trash) these files from the set.

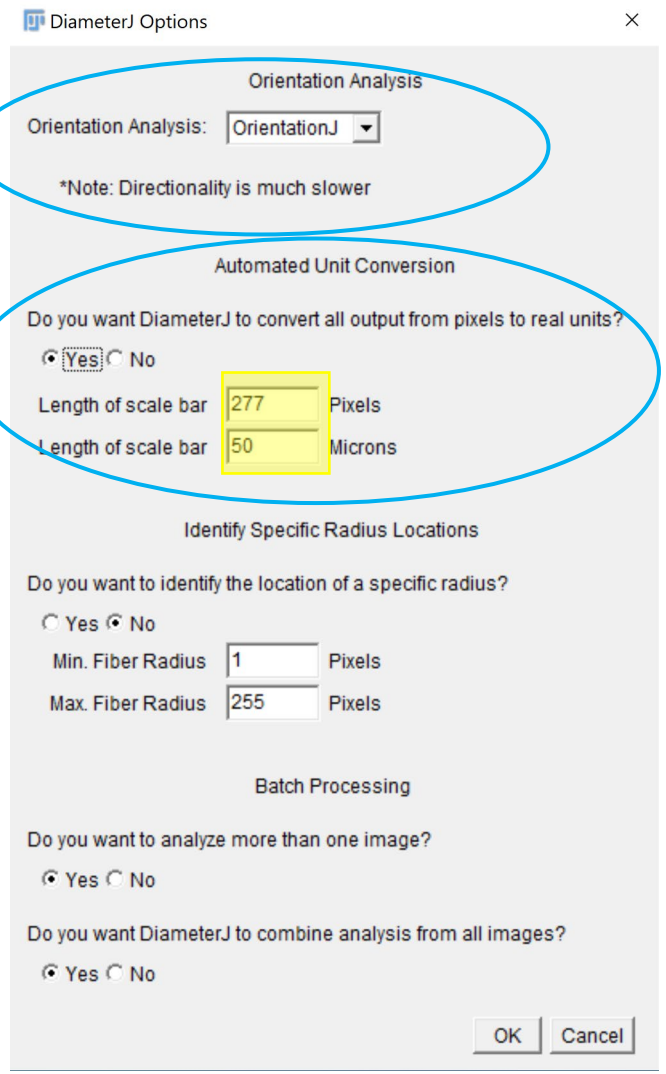


Step 3.2, cont'd: Use DiameterJ – Fiber Diameter Analysis

Go back to Fiji/ImageJ's main bar. **Click Plugins > DiameterJ 1-018**

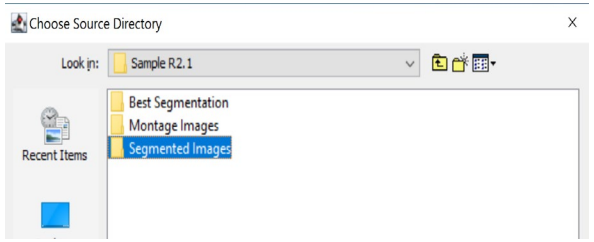
- In "Orientation Analysis", leave selection as "OrientationJ" (default).
- In "Automated Unit Conversion":
  - "Do you want Diameter J to convert all output from pixels to real units?" Select '**Yes**'.
  - "Length of scale bar" – here input the numbers previously recorded in **Step 2.3**; e.g., "Distance of pixels" in Step 2.3 should be recorded here in "length of scale bar, pixels" and "Known Distance" in Step 2.3 should be recorded here in "Length of scale bar, microns"
- **Leave all other settings as default**





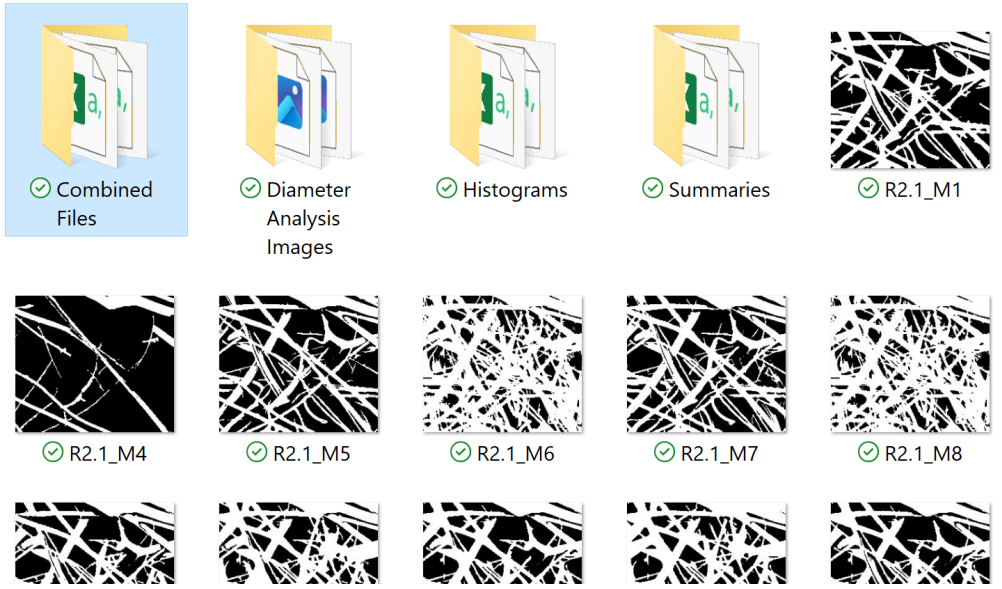
Go back to the file folders.

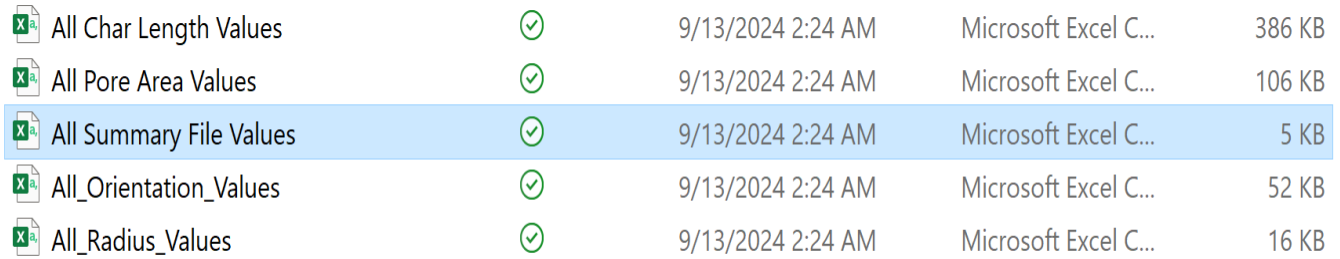
Choose "Segmented Images".



Step 3.3: Use Excel - access fiber measurements

- Four new file folders (CSV format) are generated in the '**Segmented Images**' Folder:
  - Combined Files
  - Diameter Analysis Images
  - Histograms
  - Summaries
- Open '**Combined Files**' folder
- Open '**All Summary File Values**' spreadsheet

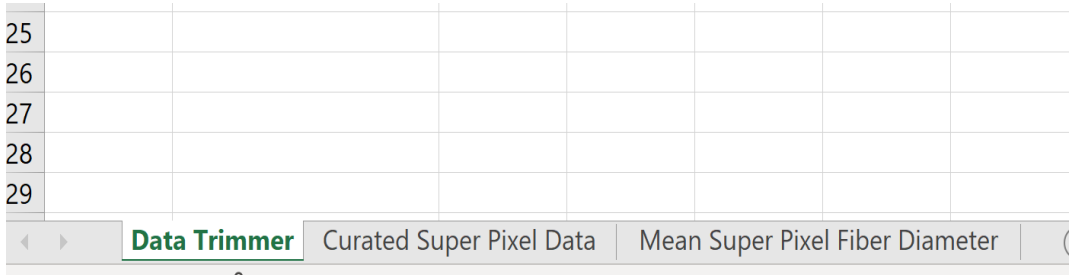




Step 3.3, cont'd: Use Excel - access fiber measurements

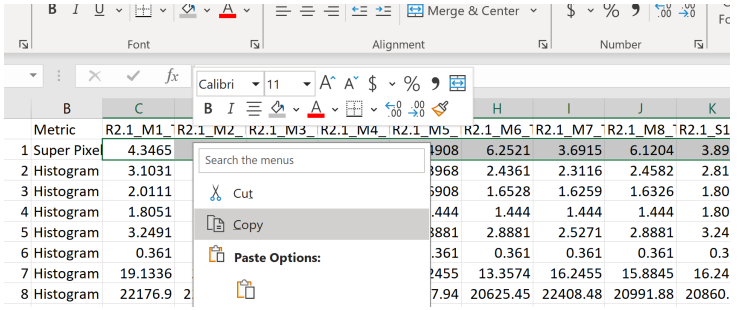
- The next actions outlined in Step 3.4, '**Use Excel - Sort Fiber Measurements**' are an example of how to analyze data using Microsoft Excel. A downloadable template named "**SuperPixel_FiberDiameter_Measurements**" is linked on the title page of this presentation.

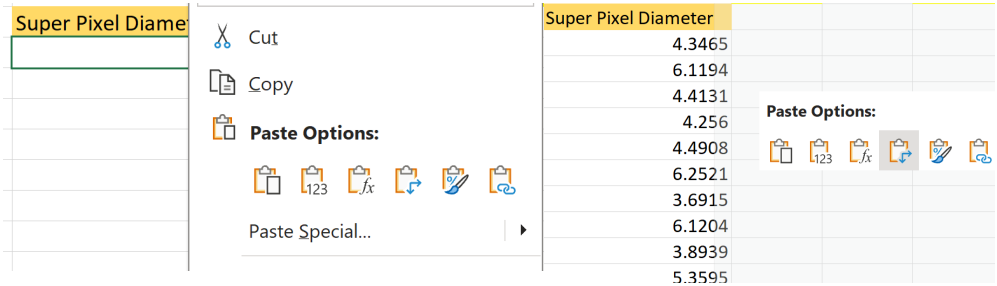
- **SuperPixel_FiberDiameter_Measurements** has 3 tabs at the bottom:
  - 'Data Trimmer' for filtering out data;
  - 'Curated Super Pixel Data' for storing the filtered data;
  - 'Mean Super Pixel Fiber Diameter' for storing the final mean and standard deviation that will be used for the Filtration Efficiency Calculator tool.



Step 3.4 Use Excel – Sort fiber measurements

- Go back to the spreadsheet '**All Summary File Values**'. Each row represents a metric. **Choose the first row**. Highlight first entry in the **"Super Pixel"** row **(C2).** Hold Ctrl + Shift and hit "->" right arrow or manually highlight the entire row using your mouse.
- **Copy** this selection (CTRL + C).
- Go to the "**SuperPixel_FiberDiameter_Measurements**" spreadsheet and select the **"Data Trimmer" tab**; select the cell under "**Super Pixel Diameter".** Right click -> Paste Options -> Paste Transpose (T) **(This step is important because Excel does not filter rows, only columns.)**

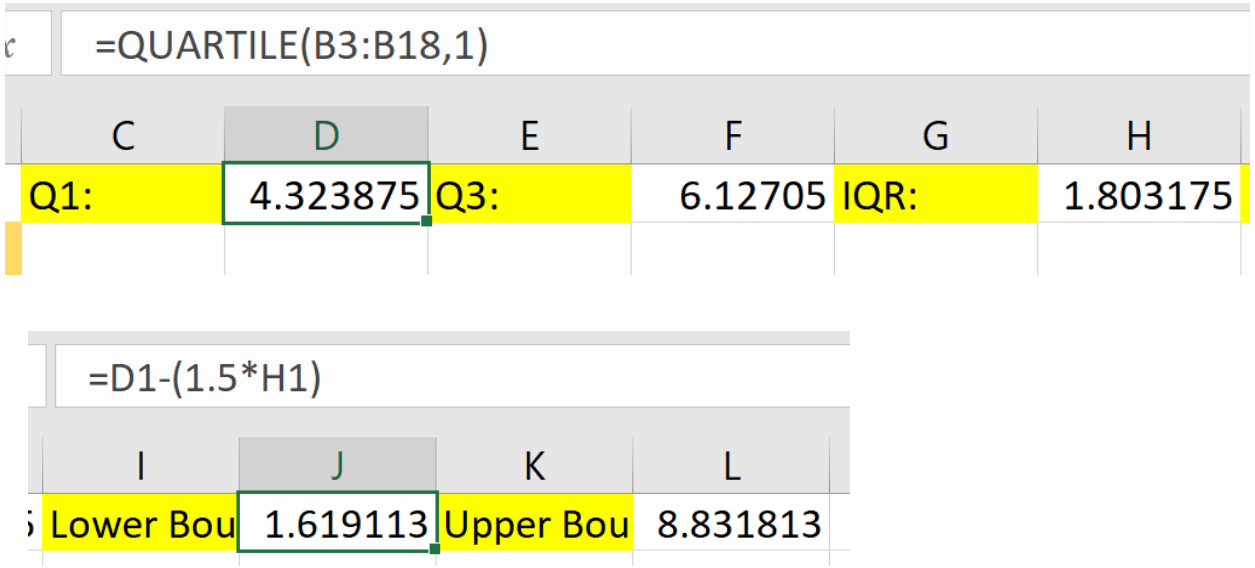




Step 3.4, cont'd : Use Excel – Sort fiber measurements

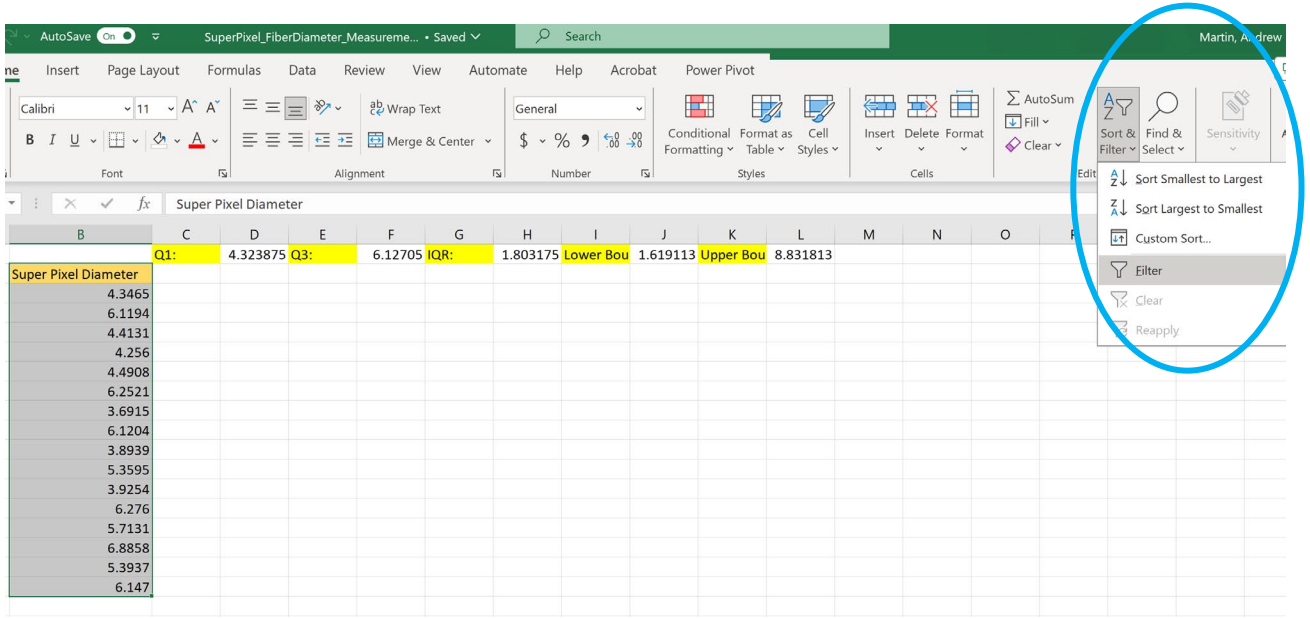
- Still in "**SuperPixel_FiberDiameter_Measurements**" spreadsheet and **"Data Trimmer"** tab**:**
- Select the cells next to those labelled "**Q1**" and "**Q3**" and type "=QUARTILE([DATARANGE],1) and "=QUARTILE(DATARANGE,3)" respectively. Replace [DATARANGE] with the range of cells containing your data under the Super Pixel Diameter column (in Column B).
- The range of data (Interquartile range, or IQR) and Upper/Lower Bounds are calculated by the spreadsheet using Q1 and Q3.
  - IQR = Q3 – Q1
  - Upper bound = Q3 + 1.5 * IQR
  - Lower bound = Q1 – 1.5 * IQR

Upper and Lower bound values will be used on slide 26.



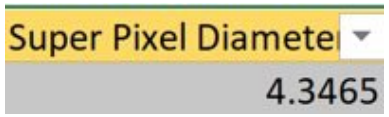
Step 3.4, cont'd: Use Excel – Sort fiber measurements

1. **Please note:** this method is specific to EXCEL.
2. Highlight the cells in the column named 'Super Pixel Diameter' – Open the "**Home**" Ribbon on the top of Excel and look for "**Sort and Filter**". Or "Ctrl + Shift + L".
3. Click '**Filter**', this generates a drop-down arrow on the column name.

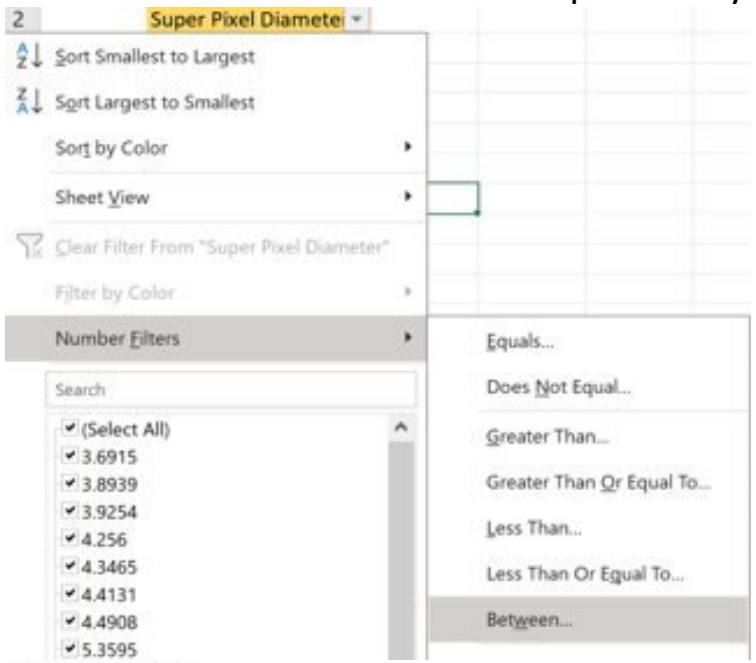


Step 3.4, cont'd: Use Excel – Sort fiber measurements

- Click on the drop-down arrow on the cell named 'Super Pixel Diameter'.



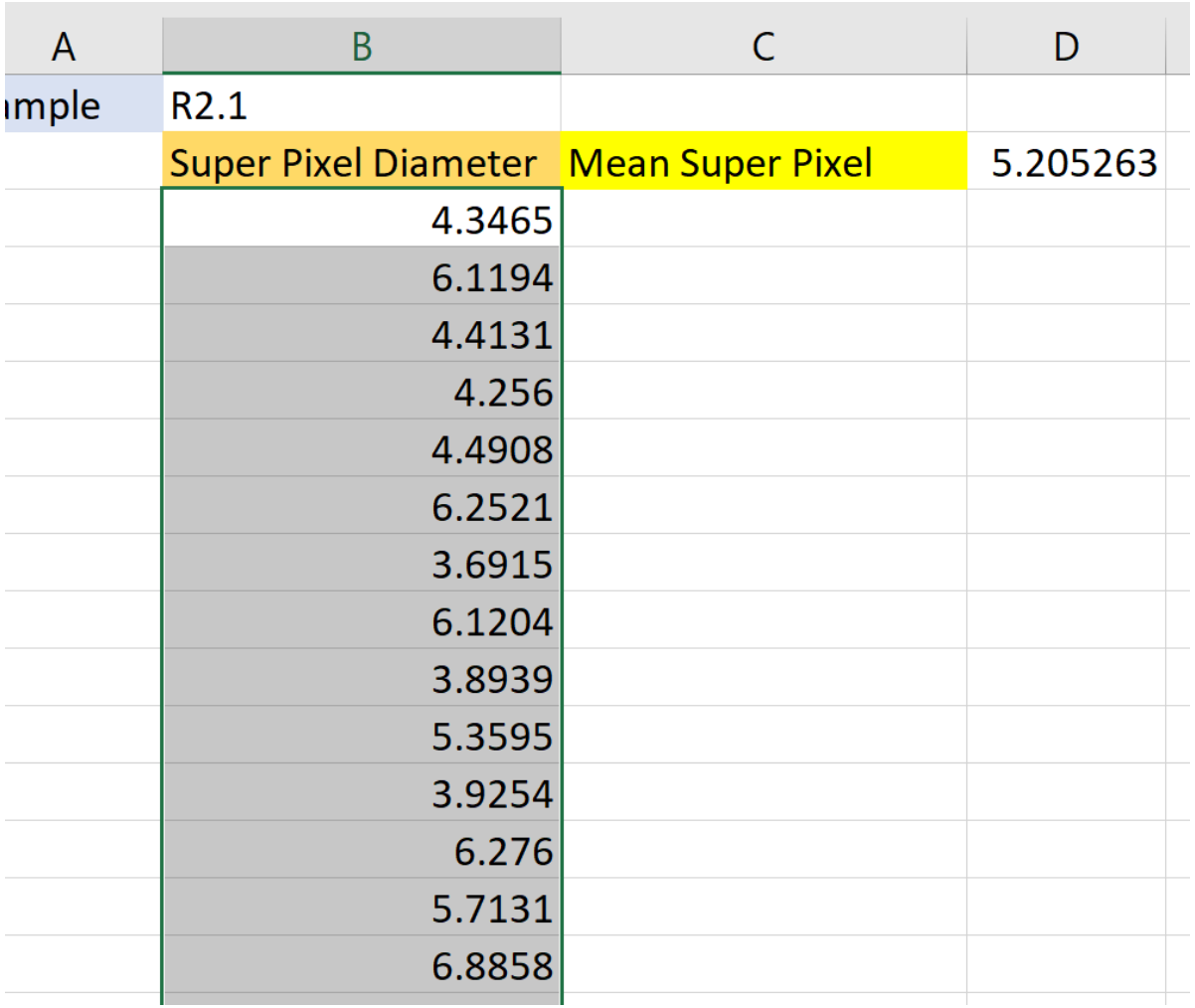
- Find "**Number Filters**" and select "**Between**." Enter the value for Upper and Lower Bound values calculated two slides previously in Step 3.4 . Hit "**OK**".



You have now sorted out appropriate segmentations to use for material fiber diameter analysis.

Step 3.5: Use Excel – Calculate Fiber Diameter Means

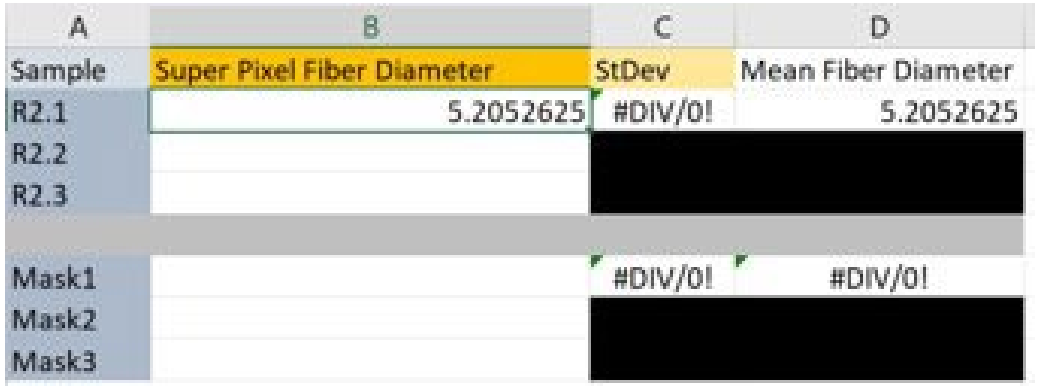
1. Highlight your new, filtered data, and copy (Ctrl + C).
2. Within the '**SuperPixel_FiberDiameter_Measurements**' Excel file, **open** the tab "**Curated Super Pixel Data**" Paste the new, filtered list in the column "**Super Pixel Diameter**".
3. Where it says "**Mean Super Pixel**" in yellow, type "=AVERAGE([DATARANGE])" into the empty adjacent cell, and replace [DATARANGE] with the cells containing data, to get the mean fiber diameter from the filtered data.
4. Copy the Mean Super Pixel value



A	B	C	D
mple	R2.1		
	Super Pixel Diameter	Mean Super Pixel	5.205263
	4.3465		
	6.1194		
	4.4131		
	4.256		
	4.4908		
	6.2521		
	3.6915		
	6.1204		
	3.8939		
	5.3595		
	3.9254		
	6.276		
	5.7131		
	6.8858		

Step 3.5: Use Excel – Calculate Fiber Diameter Means

- After copying the Mean Super Pixel value, click on the **“Mean Super Pixel Fiber Diameter”** tab on the same Excel file named "**SuperPixel_FiberDiameter_Measurements**”. This sheet contains 6 rows for “super pixel fiber diameter”, 2 rows for “**stdev**”, and 2 rows for “**mean fiber diameter**” values.
- Paste in the appropriate sample cell under the “**Super Pixel Fiber Diameter**” column.

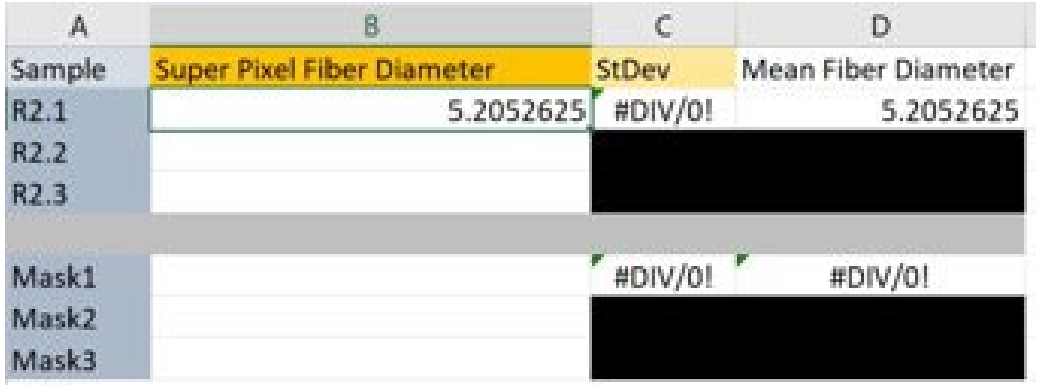


A	B	C	D
Sample	Super Pixel Fiber Diameter	StDev	Mean Fiber Diameter
R2.1	5.2052625	#DIV/0!	5.2052625
R2.2			
R2.3			
Mask1		#DIV/0!	#DIV/0!
Mask2			
Mask3			

Repeat Steps 2.1 through 3.5 (slides 11-28) in their entirety, for each SEM image from a respirator/mask product.

Step 3.6: Calculate Mean Fiber Diameters and Standard Deviations

- In the StDev column, select the empty cell C2 and type “=STDEV(B2:B4)”. Then in cell C6 type “=STDEV(B6:B8)”. In the adjacent cells under “**Mean Fiber Diameter**”, type in D2 “=AVERAGE(B2:B4)” and in D6 “=AVERAGE(B6:B8)” to find the means.
- **You now have the mean fiber diameters and the standard deviations of the means for input into the Respirator Filtration Efficiency Calculator.**



A	B	C	D
Sample	Super Pixel Fiber Diameter	StDev	Mean Fiber Diameter
R2.1	5.2052625	#DIV/0!	5.2052625
R2.2			
R2.3			
Mask1		#DIV/0!	#DIV/0!
Mask2			
Mask3			