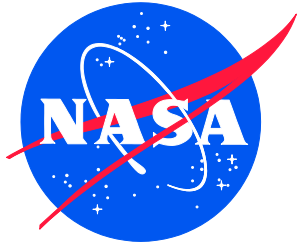


NASA/CR-2017-219365



# Tutorial for Collecting and Processing Images of Composite Structures to Determine the Fiber Volume Fraction

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January 2017

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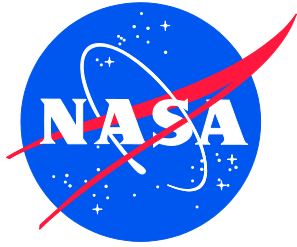
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**Technology/Programs Used:**

Zeiss LSM 5 Exciter Microscope

AxioVision Release 4.8.2

ImageJ



## 1.0 Introduction

Fiber-reinforced composite structures have become more common in aerospace components due to their light weight and structural efficiency. In general, the strength and stiffness of a composite structure are directly related to the fiber volume fraction, which is defined as the fraction of fiber volume to total volume of the composite. The most common method to measure the fiber volume fraction is acid digestion, which is a useful method when the total weight of the composite and the fiber weight can easily be obtained. However, acid digestion is a destructive test, so the material will no longer be available for additional characterization. Acid digestion can also be difficult to machine out specific components of a composite structure with complex geometries. These disadvantages of acid digestion led the author to develop a method to calculate the fiber volume fraction. The developed method uses optical microscopy to calculate the fiber area fraction based on images of the cross section of the composite. The fiber area fraction and fiber volume fraction are understood to be the same, based on the assumption that the shape and size of the fibers are consistent in the depth of the composite. This tutorial explains the developed method for optically determining fiber area fraction performed at NASA Langley Research Center.

The first step in the optical fiber-area-fraction determination is to section and polish a piece of the composite structure in the area of interest. After polishing, the optical microscopy method uses a confocal microscope, image collecting software, and image processing software. The confocal microscope is a Zeiss LSM 5 Exciter<sup>1</sup> microscope. The microscope has an automatic stage and attached camera to easily collect images. The Zeiss image-collection software, AxioVision Release 4.8, is connected to the microscope. It can create stitched mosaics and panorama images of the specimen underneath the microscope. After the images are collected from the microscope and AxioVision, they are processed using the Java-based image-processing and analyzing software, ImageJ.<sup>2</sup> This software is open source, user-friendly, and has hundreds of plug-ins available to users. After identifying the fibers in the image processing software, the fiber area fraction can be calculated.

This tutorial first explains how to collect and process relatively small images. Then, a specific example is used to describe how to collect and process images of complex structures. The complex structure used in this example is a fluted-core sandwich structure that is comprised of facesheets, webs, and noodles. The noodle specifically has complex geometry that would be difficult to separate from the rest of the structure for acid digestion. Therefore, the developed optical method is an ideal way of calculating the fiber volume fraction of this structural component.

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<sup>1</sup> is not an endorsement by the National Aeronautics and Space Administration

<sup>2</sup> <http://imagej.nih.gov/ij/>

## 2.0 Collecting Images from the Microscope

1. Images can be collected using the **Zeiss LSM 5 Exciter** microscope (Fig. 1) currently located in in **Room 253** of **Building 1205** at NASA Langley Research Center.



Figure 1. Covered microscope and computer.

2. Turn on the computer and the microscope.
  - a. The power switch is near the floor on a power strip on the left side of the microscope (Fig. 2).

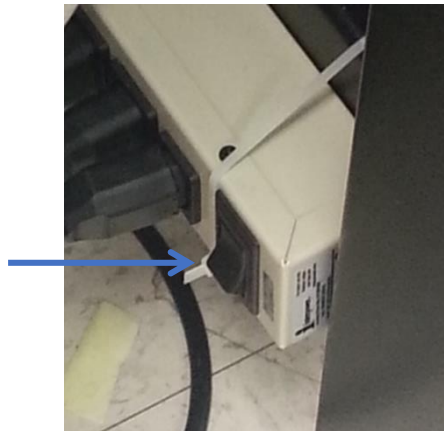


Figure 2. Microscope power switch.

- b. The computer monitor also needs to be powered on. If the computer asks for a password to log in under the username “administrator,” just press **Enter** on the keyboard. A password is not required to log in.

3. Set up the microscope.

- a. Take the blue cover off the microscope (Fig. 1). There are three buttons on the left side of the microscope labeled **LSM**, **TV**, and **VIS** (Fig. 3). These buttons represent the different viewing systems for the image. Press the **VIS** button in order to see the image through the lens of the microscope (Fig. 4).

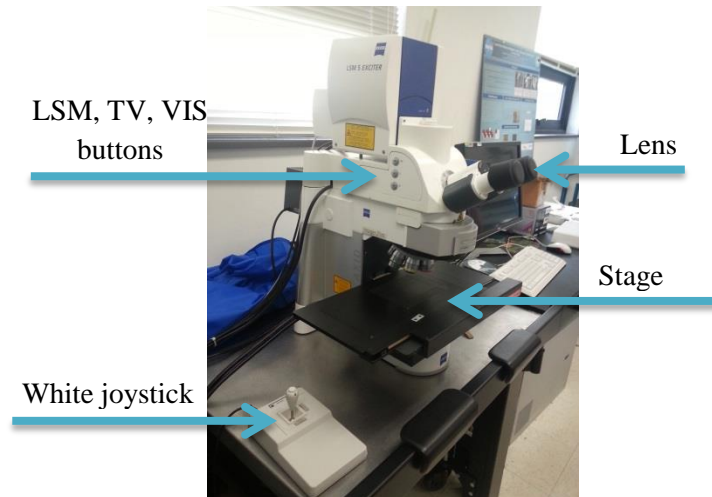


Figure 3. Zeiss LSM 5 Exciter microscope.



Figure 4. LSM, TV, and VIS buttons.

- b. On the digital screen for the microscope settings, click **Open** for the **RL-Shutter** (Fig. 5) in order to turn the light on. Then, manually move the specimen under the microscope, so that the area of interest is under the light source (Fig. 6). Click on **Microscope** in order to change to a different magnification. Starting at **5X**, work up to **100X** (Fig. 7). Use the course and fine adjustment knobs (Fig. 8) in order to focus on the specimen. These adjustment knobs are located on the right side of the microscope, below the stage. Use the white joystick (Fig. 3) that is connected to the microscope in order to look at a different area on the specimen. **Note: If the light does not appear to turn on, press on Reflector (next to Objectives at the top**

of the screen). Then, click on **BF**, which stands for Brightfield (Fig. 9). This should turn on the correct light.

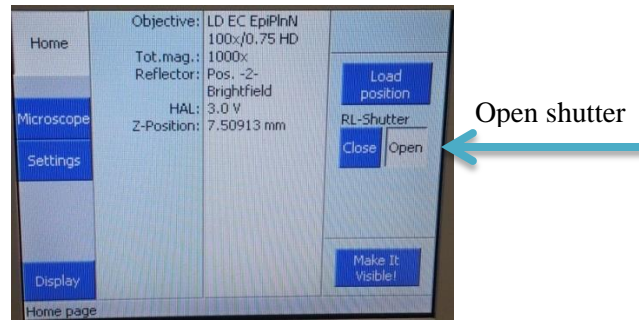


Figure 5. Microscope touch screen.

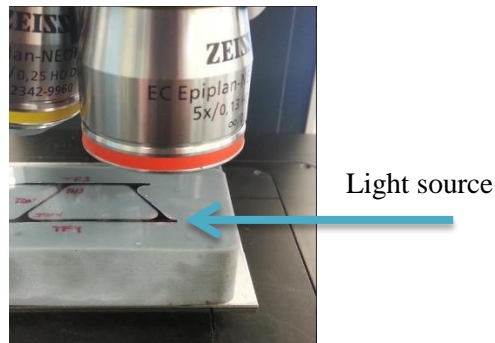


Figure 6. Light source placed over area of interest.

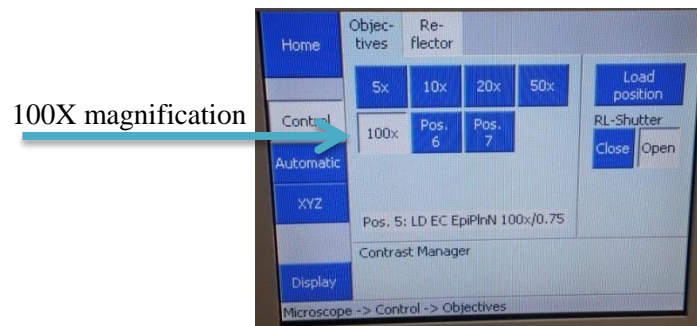


Figure 7. Microscope magnifications.

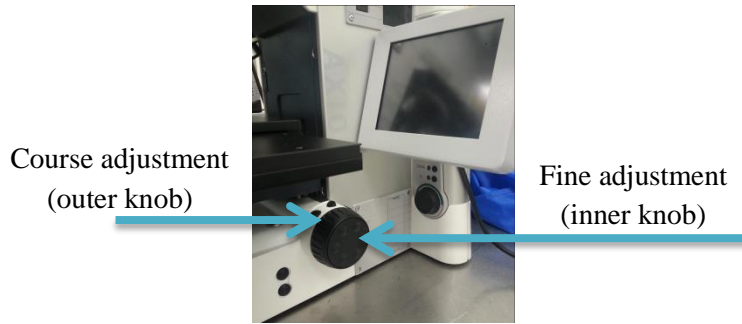


Figure 8. Course and fine adjustment knobs.

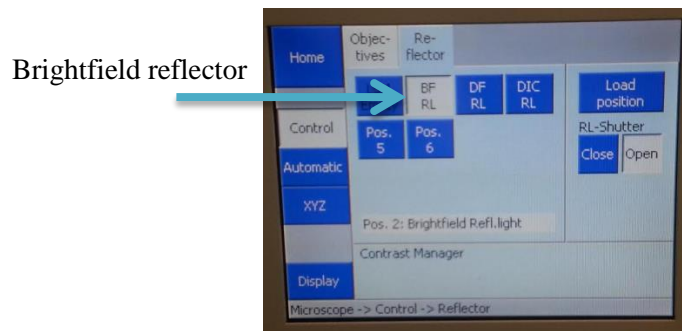


Figure 9. Reflector settings.

4. Open **AxioVision Rel. 4.8** on the computer desktop.

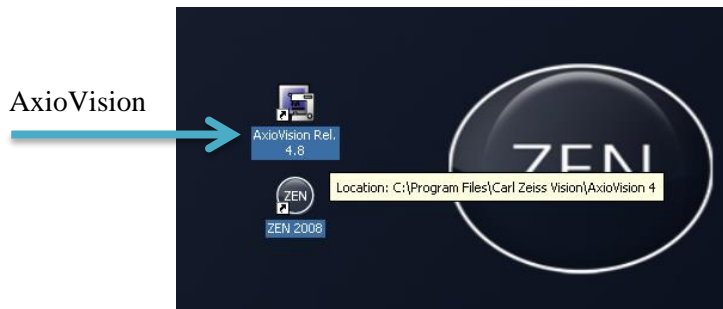


Figure 10. Computer desktop.

- a. Instead of **VIS**, press the **TV** button on the left side of the microscope (Fig. 4). This will allow the microscope image to be seen on the computer screen. Press the **Live** button (Fig. 11) on the left hand side of the AxioVision window in order to see the live image.

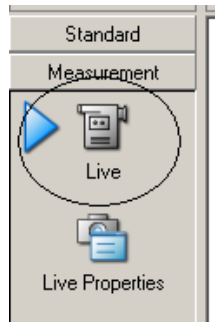


Figure 11. Live button in AxioVision window.

- b. In order to create the most optimal image, there are some options under the live feed button (Fig. 12). Turn on the **White Balance** and **Exposure** options to optimize the balance of color channels and adjust the exposure time, respectively. Select the **A. Min/Max** and **G. 0.45** (which is usually selected by default) in order to optimize the contrast and brightness as well as the color reproduction on the monitor, respectively.

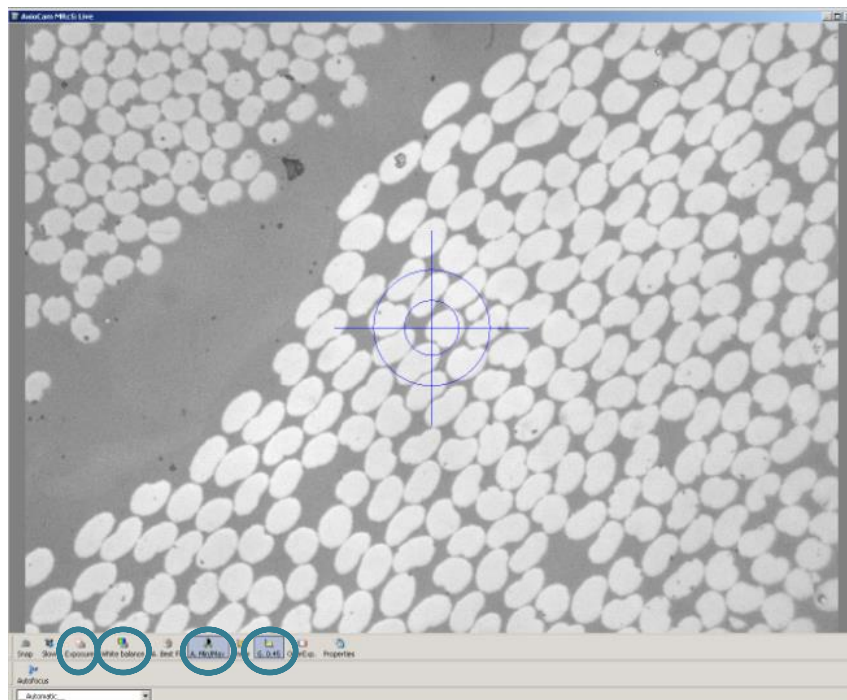


Figure 12. Live image in AxioVision window.

5. Eliminate gradients that occur in the live image (Fig. 13).
  - a. Click on the **Live Properties** tab on the left hand side, underneath **Live**.
  - b. Click on the **General** tab.
  - c. Using the white joystick that is connected to the microscope, move the slide out of the field of view (a.k.a. the specimen). For this specific type of specimen, move to a region with uniform coloration. If there are any dust particles or anything that

would affect the shading, move the course adjustments to make the live image out of focus.

- d. Click on **Shading Correction** and wait for the system to calibrate. **Note:** If a region with uniform coloration was properly selected, then this function will correct any optical inhomogeneities. The ideal image will produce the same colored pixels for all of the fiber cross-sections. This is optimally necessary for thresholding in **Step 17** of processing.

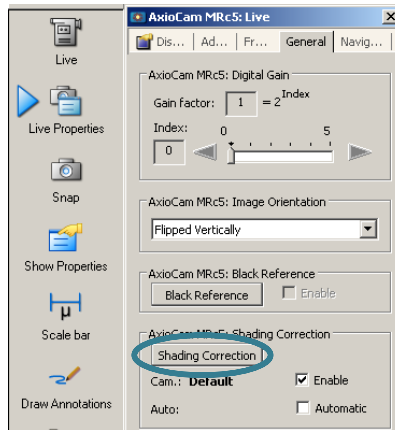


Figure 13. Shading correction function.

6. Create a stitched image of multiple microscope images.
  - a. Under the **Work Area**, click on **MosaiX**. Once it expands, click on **Acquisition** (Fig. 14). Parameters and MosaiX settings will appear below.
  - b. Change the **Autofocus** from **off** to **every tile** (Fig. 14).
  - c. **Note:** If the resulting image looks like the image below (Fig. 15), then the amount of rows chosen needs to be re-evaluated. If the camera starts to go beyond the specimen area of interest, it will result in these unfocused areas towards the bottom of the stitched image.

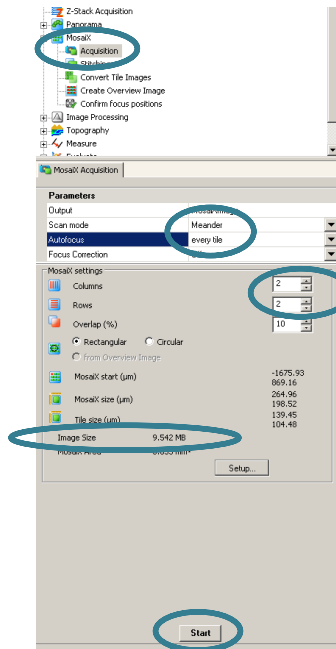


Figure 14. MosaiX acquisition settings.

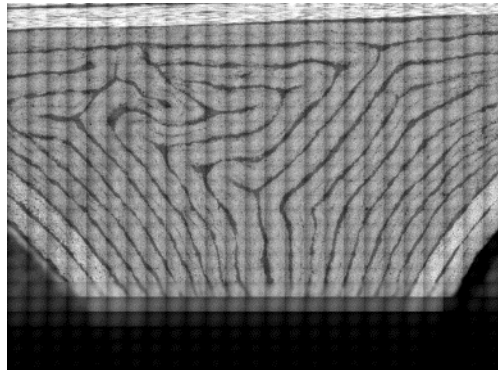



Figure 15. Results from autofocus error.

- d. Under the MosaiX settings, change the columns and rows as necessary. The image needs to be less than 2000 MB (or 2 GB) in order to export it later. The **image size** will display. Anything larger than a 29 X 29 image is not advised because of 2GB memory limit of the computer. For this example, a 2 X 2 image will be obtained (Fig. 14).
- e. At the bottom of the acquisition settings (Fig. 14), press **Start**, and the microscope camera will start capturing images and placing them together. This may take up to an hour for a mosaic image that is relatively larger. For example, a 20 X 20 image will take a longer amount of time than a 10 X 10 image.
- f. **Note:** If the captured images are all the same and the stage is not automatically moving, then the program will need to be restarted. **Cancel** the current project and exit out of **AxioVision**. Open **AxioVision** again and it should start working properly.



- g. To see the entire image, click on the **magnifying glass with an arrow**  going through it (Fig. 16).

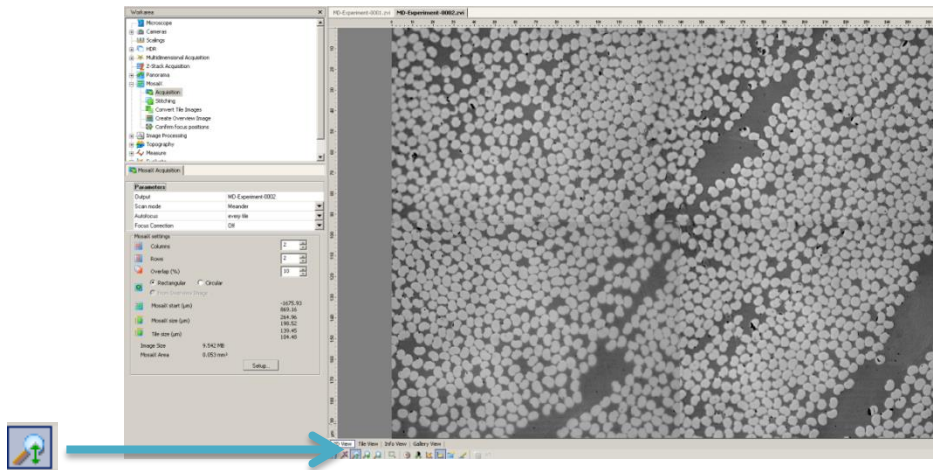


Figure 16. Full-scale image view.

- h. After the system has rendered multiple images, the images may not be correctly aligned. In order to align images, click on **Stitching**, which is right below **Acquisition** (Fig. 17). Do not change any parameters and press **Start** at the bottom of the page (Fig. 17). The images should now be noticeably aligned. This can be seen in the screenshot below (Fig. 17). As a result, the outer edges of the entire image may be skewed. The alignment process is a much faster process than the previous step of collecting all of the individual images.

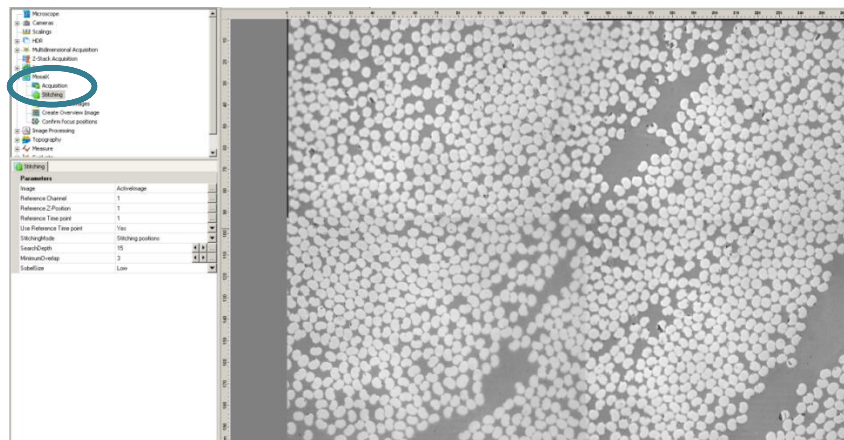


Figure 17. MosaiX stitching function and results.

## 7. Save the project. **File > Save As...**

- a. In order to save the project in **AxioVision**, save it as a **.zvi** file (the default file type) (Fig. 18).

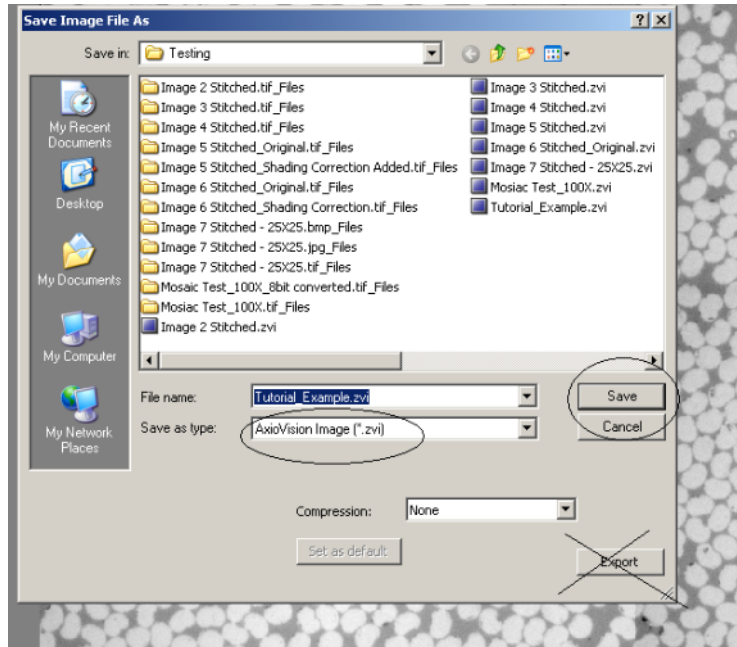


Figure 18. Save As... window.

8. Export the stitched image. **File > Export**
  - a. **Note:** In order to save the image as high resolution, **export** the image as a **.tif** file **instead of saving** as a **.tif** file.
  - b. Set the location for the exported file and set the **File Type** to **TIF Tagged Image File** (Fig 19).
  - c. To save time and memory space, only export the stitched image. In order to do this, all of the following choices need to be checked in the export menu (Fig. 19):
    1. **Generate merged image(s)**
    2. **Merged images only**
    3. **Gray scale**
    4. **Convert to 8 bit**
    5. **Apply display mappings**
  - d. Click **Start** at the bottom of the window (Fig. 19). Remember that it will not export an image that is above 2 GB. Under **Output Files**, the **Status** of the image will say OK if the file was exported correctly. It will say **Not enough memory space** if there is not enough memory on the computer or if the image is too large. After the process is complete, verify that the file is in the designated location and **Close** the export window.

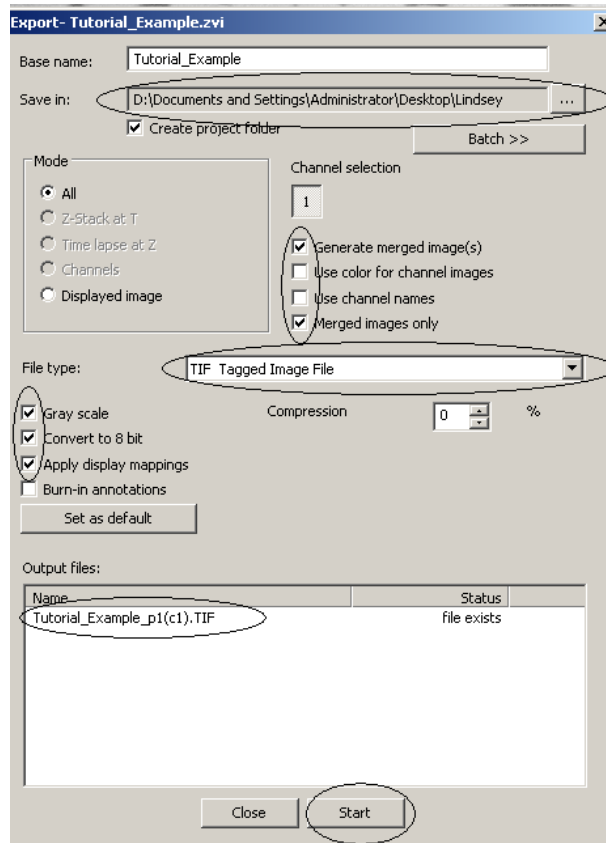


Figure 19. Export window.

- e. **Note:** If an error report message appears stating that AxioVision is going to close after trying to export an image, follow these steps. In the Work Area, click on **Cameras > AxioCam MRc5**. There will be three options: **Adjust**, **Frame**, and **General**. Click on **Frame** and change the **Camera Mode** to **B/W 1292 X 968 fast standard mode**.
9. Copy the files onto a removable disk/USB drive.
  - a. **Start > My Computer > Removable Disk**
  - b. Drag the image files onto the **Removable Disk** Folder.
  - c. Once the files have been copied, safely remove the disk by clicking the icon on the bottom-right of the desktop.

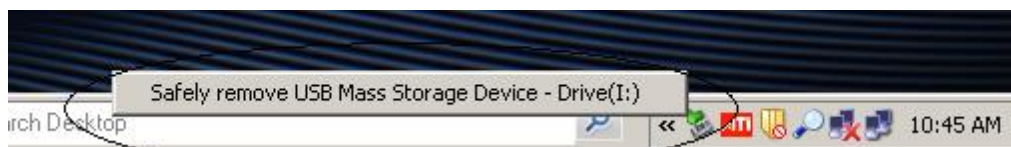


Figure 20. Safely removing USB drive on desktop.

10. Shut down the computer.
  - a. Exit AxioVision.
  - b. **Start > Shut Down**

- c. Turn off the monitor.
11. Turn off the microscope.
- a. On the digital touch screen, set the magnification to **5X** (Fig. 7), and **Close the RL-Shutter** (Fig. 7).
  - b. Click **Load position** (Fig. 7), then **Set Work position** (Fig. 21). A message should appear stating **Lower stage limit reached** (Fig. 22). This means that the stage is at the correct height for placement of subsequent specimens.

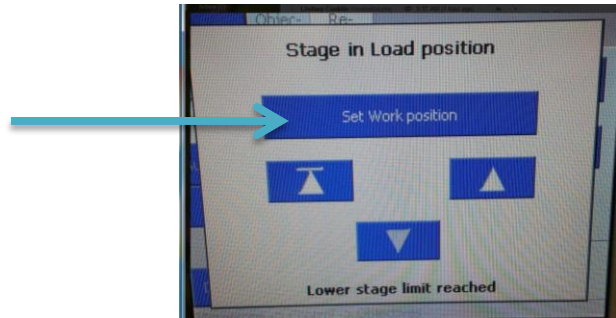


Figure 21. Set Work position button.

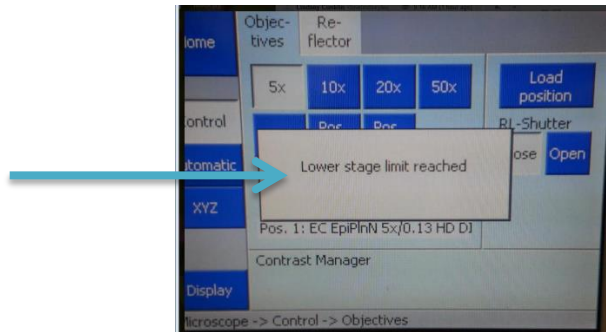


Figure 22. Stage height confirmation.

- c. Turn the power off on the power strip and cover the microscope with the blue cover.

### 3.0 Image Processing Using ImageJ

- 12. Download **ImageJ** using the following web address:  
<http://imagej.nih.gov/ij/download.html>
- 13. Install **ImageJ**
- 14. Open/Run **ImageJ**. An illustration of the desktop image (Fig. 23) and the toolbar workspace (Fig. 24) are provided below.



Figure 23. ImageJ desktop icon.

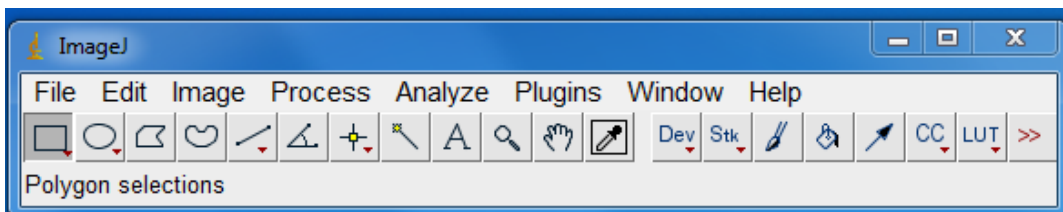


Figure 24. ImageJ workspace.

15. Open a file in ImageJ using **File > Open** (Fig. 25).

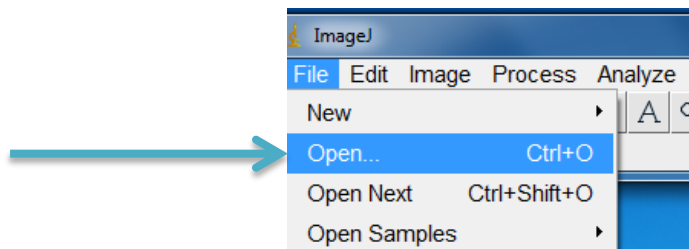


Figure 25. Opening a file in ImageJ.

a. Select the image to be used in processing (Fig. 26).

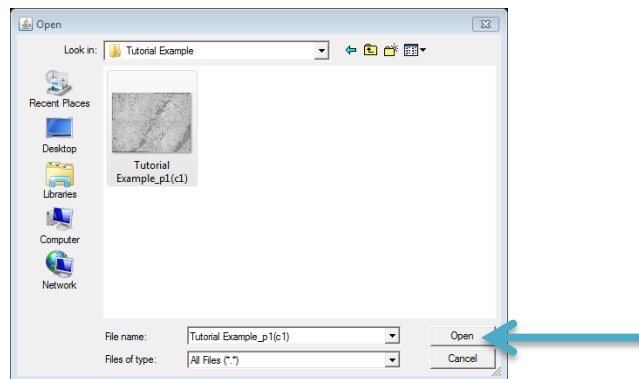


Figure 26. Selecting a file to open in ImageJ.

b. Ensure the photometric interpretation of the image is 8-bit and not RGB. This information can be found directly above the displayed image. If the image is RGB (Fig. 27), right click the image and select **Control Panel** (Fig. 28). Expand the **Image** option, expand **Type** and select **8-bit** (Fig. 29). Then, exit out of that window. The description should now say **8-bit** above the image (Fig. 30).

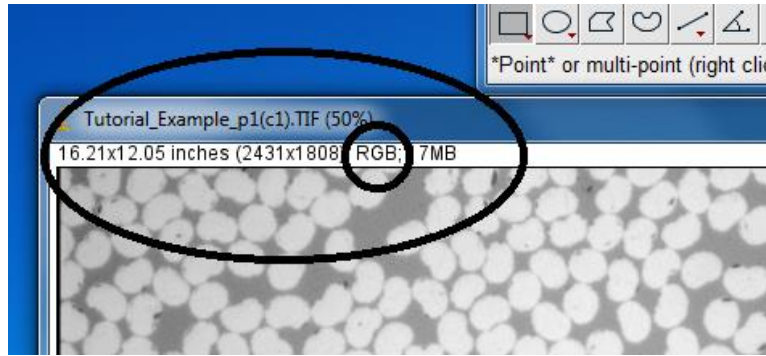


Figure 27. RGB photometric interpretation.

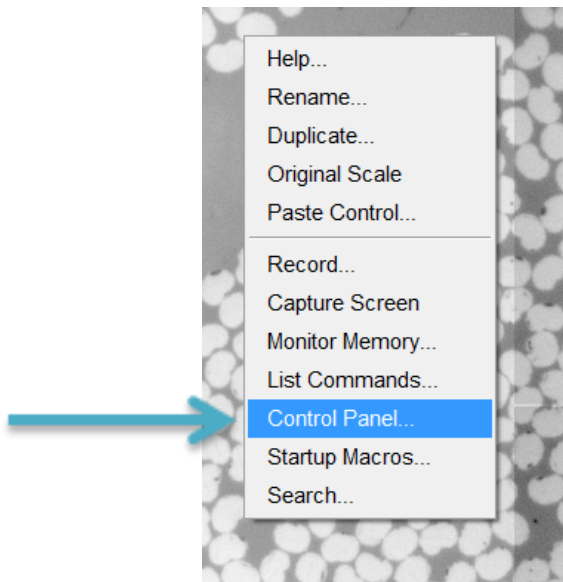


Figure 28. Control Panel.

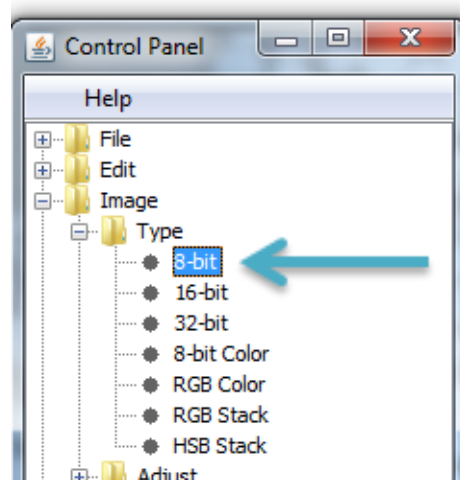


Figure 29. 8-bit image type selection.

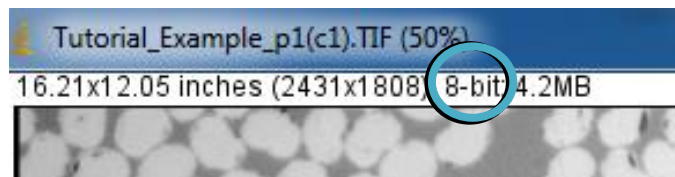


Figure 30. 8-bit photometric interpretation.

## 16. Process > Enhance Contrast

- a. Set the **Saturated Pixels** to **20%** for high contrast. This setting can be changed depending on how much contrast is needed. Also, check the box that says **Normalize** (Fig. 31). The image before enhancing contrast (Fig. 32) and the image after enhancing contrast (Fig. 33) are below.

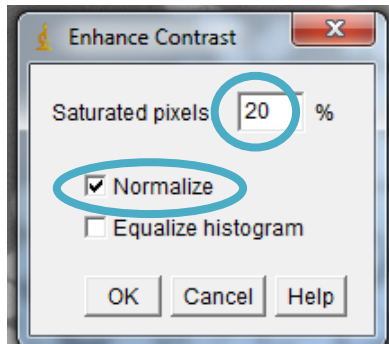


Figure 31. ImageJ enhance contrast window.

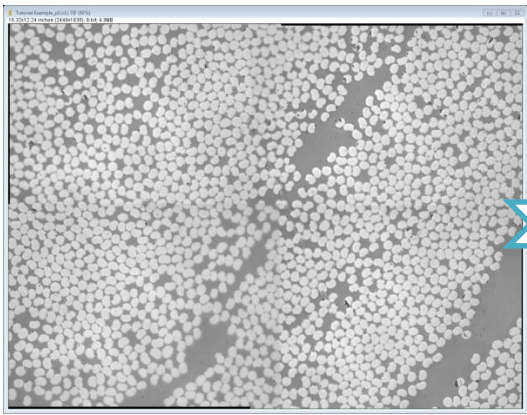


Figure 32. Example image (original).

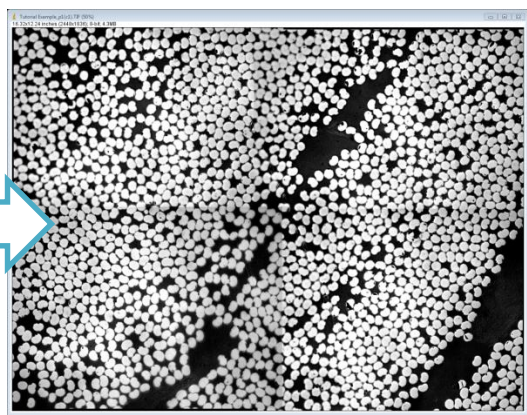


Figure 33. Example image (after enhancing contrast).

#### 17. Image > Adjust > Threshold (Ctrl+Shift+T)

- a. The threshold will usually select the red background; however, the opposite is needed. In order to threshold (select in red) the fiber cross sections, check the box that says **Dark background** (Fig. 34). This will invert the threshold selection (Fig. 35).
- b. **Note:** The threshold value bars are automatically set to default. It is recommended not to change these. If the bars need to be adjusted, then the region of uniform coloration (Step 5(c)) was incorrectly selected. This problem indicates that optical inhomogeneities are present in the image. The fiber cross sections should ideally be the same color, so the processor can automatically select all fiber cross section pixels.

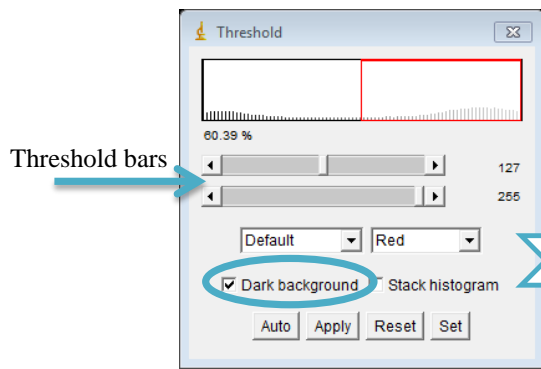


Figure 34. ImageJ threshold window.

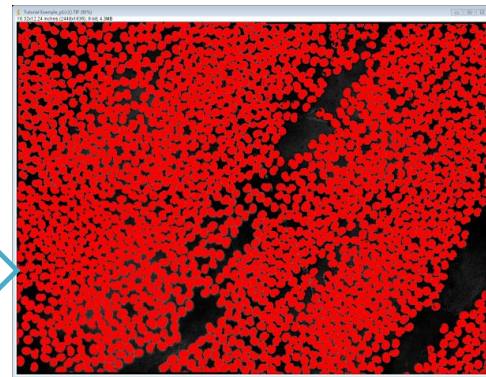


Figure 35. Example image (after auto-thresholding).

18. **Process > Smooth**

19. **Process > Binary > Make Binary**

- a. Keep all of the boxes checked and press **OK** (Fig. 36).

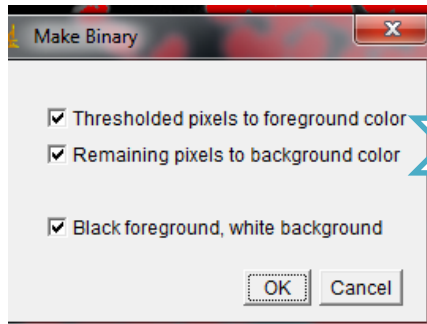


Figure 36. ImageJ Make Binary window.

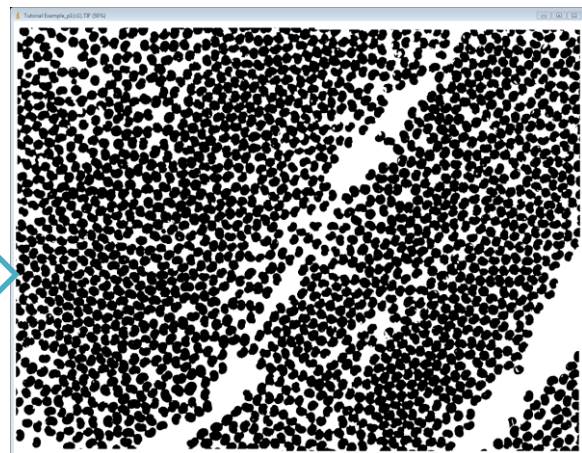


Figure 37. Example image (after making binary).

20. **Process > Binary > Open**

- a. Use this option if there are unnecessary and isolated pixels that are artifacts of the image collection process. For example, see the images below (Figs. 38 and 39):





Figure 38. Sample image *before* opening.



Figure 39. Sample image *after* opening.

- b. This will remove isolated pixels that appear in the binary image.

21. **Process > Binary > Close**

- a. Use this option if holes need to be filled in various regions. For example, see the images below (Figs. 40 and 41):



Figure 40. Sample image *before* closing.



Figure 41. Sample image *after* closing.

- b. This process will fill small holes that appear in the binary image.
- c. **Note:** Sometimes, this option will merge 4–5 cross-sections together because it will eliminate the small gaps in between them. This should not happen! Click **Edit > Undo** in order to undo this process.

22. **Process > Binary > Watershed**

- a. After completing **Step 21**, the fiber cross sections might be touching/overlapping in the binary image. The watershed option (Figs. 42 and 43) uses segmentation to separate the cross sections from one another. It works best when the objects are not significantly overlapped.

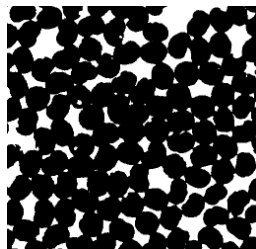


Figure 42. Sample image *before* watershedding.

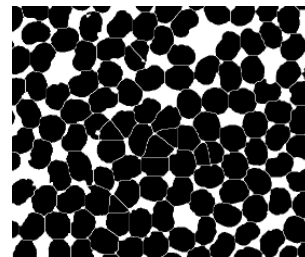



Figure 43. Sample image *after* watershedding.

- b. This step takes a while for the larger images.

23. Is there unwanted area that should not be in the fiber-volume-fraction calculation?

- a. Use the **polygon selection**  tool in order to isolate the desired area directly before analyzing the particles in **Step 24**. Outline each part that is to be included with a left click and end the shape with a right click. Then, it will calculate the area of the cross-section in the selected area.

**24. Analyze > Analyze Particles**

- a. Only check the box (Fig. 44) that is labeled:
  1. **Summarize**
- b. Then, click **OK** (Fig. 44).

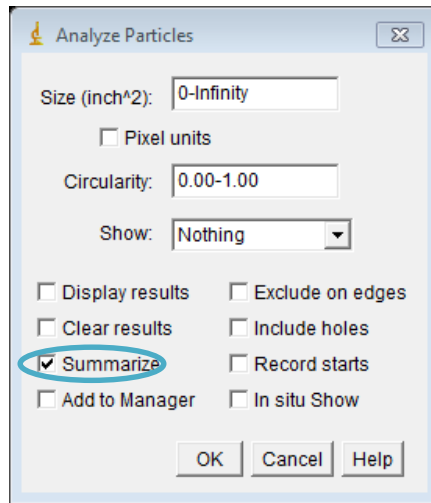


Figure 44. ImageJ analyze particles window.

- c. A window will open that is labeled as the **Summary** (Fig. 45) of the final image (Fig. 46). The most important categories for fiber-volume-fraction measurement are **Total Area** and **% Area** (Fig. 45). **Note: The Total Area represents the fiber cross-sectional area (not the total area of the selected area) and the % Area represents the percentage of fiber cross sections compared to the total area in the selected space. Dividing the Total Area by the % Area (once turned into a decimal) will give the area of the entire selected area.**

Slice	Count	Total Area	Average Size	%Area	Mean
Tutorial Example_p1(c1).TIF	1866	31303.121	16.776	59.850	0

Figure 45. Summary results from analyzing particles.

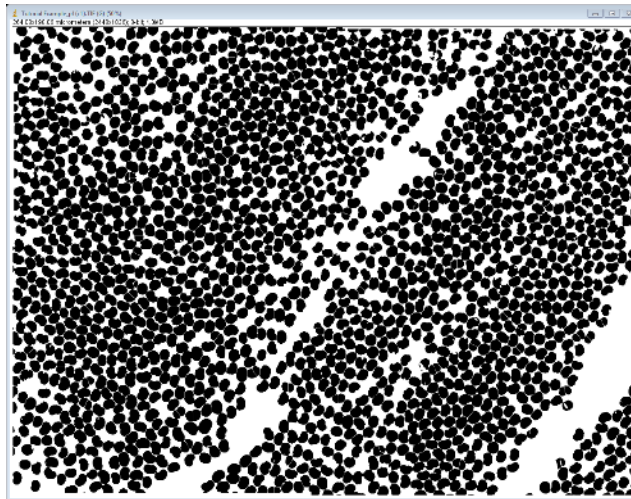


Figure 46. Example image (result before analyzing particles).

#### 4.0 Collecting Images of a Large Area of Interest

Here are tips on how to use the microscope to collect images of a large area of interest, such as a unidirectional carbon fiber noodle.

25. Obtain a **5X** image of the entire noodle to find measurements.
  - a. Change the magnification of the microscope to **5X** (Fig. 7).
  - b. Make a **3 X 3** or **4 X 4** image in order to create a mosaic of the entire noodle.
  - c. The noodle needs to be split into two halves because of the 2 GB memory restrictions on the computer. Use the **Alig. Rectangle** tool (Fig. 47) in order to create an outline for the top and bottom halves (Fig. 48). It will create red rectangles around the selection and post the area for that square in  $\mu\text{m}^2$  (Fig. 48).



Figure 47. Alig. Rectangle tool.

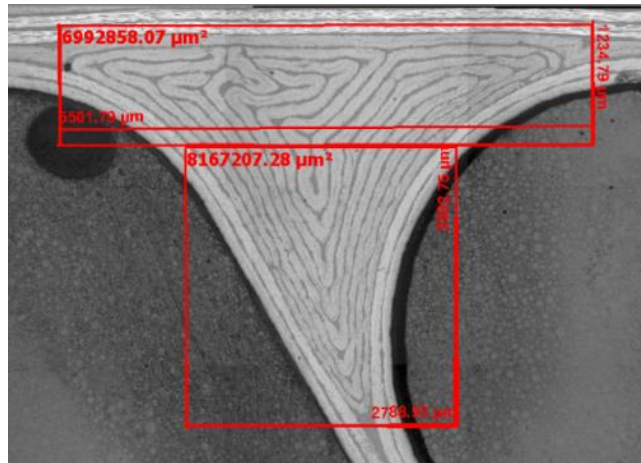


Figure 48. Noodle measurements (3 X 3 image, 5X magnification).

- d. Now that the rectangles have been created, use the **Distance** tool (Fig. 49) to find the length and width for each rectangle (Fig. 48).



Figure 49. Distance tool.

- e. Save or export the image in order to use this as a reference for deciding how many columns and rows the image needs to be in **Step 26(d–e)**. Exporting the image will additionally require checking the box that says **Burn in Annotations** in the export window (Fig. 19). This will permanently put the measurements on the image. Notice that the top rectangle (Fig. 48) has dimensions of 5501.79 μm (horizontal distance) X 1234.79 μm (vertical distance). The final image should look similar to figure 48.
26. Create a small 100X image that includes a scale bar.
- a. Change the magnification of the microscope on the microscope’s touch screen from **5X** to **100X** (Fig. 7).
  - b. Under **MosaiX > Acquisition**, create a 3 X 3 image of any part of the specimen that is outside of the noodle area. A white scale bar will be easier to view without the fiber cross sections in sight. (Fig. 50).

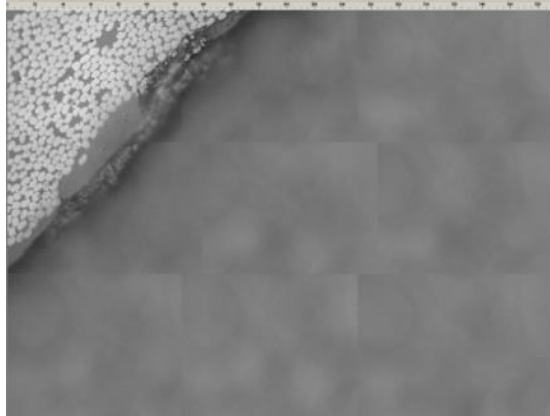


Figure 50. Collected image to insert scale bar.

- c. Click on the **scale bar** on the far left side of the AxioVision window (Fig. 51). Click on the top-left of the 3 X 3 image and hold down the click. Drag a line towards the top-right corner of the 3 X 3 image (Fig. 52). Drag the line far enough to make a **100  $\mu\text{m}$**  scale bar.

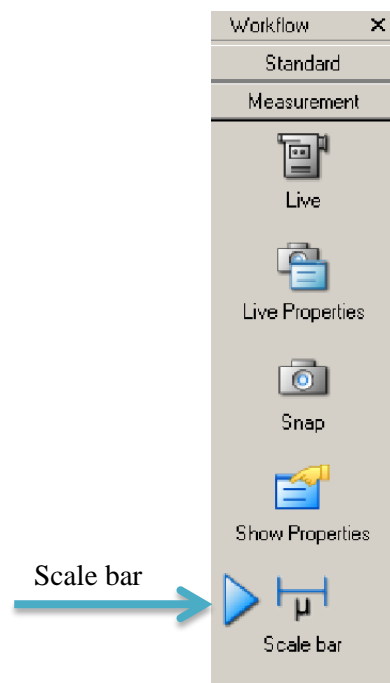


Figure 51. Scale bar location in AxioVision window.

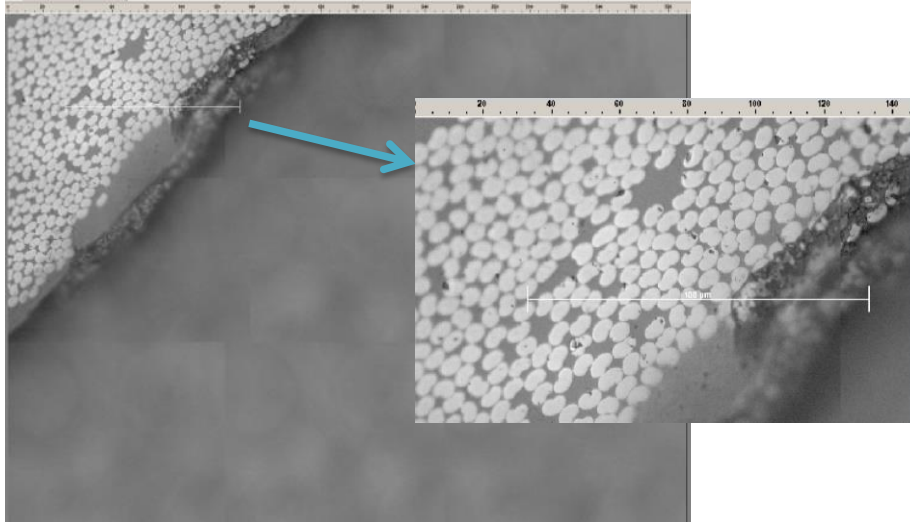


Figure 52. Scale bar placement on AxioVision window.

- d. **Export** the image with the same options found in **Step 8** (Fig. 19). Additionally, click on **Burn in Annotations** in order to keep the scale bar in the exported image. **Note:** Verify that the image was exported to the correct location. After the image file was opened, note that the scale bar has moved toward the bottom-right of the image (Fig. 53). This is why the placement of the scale bar (Fig. 52) in **Step 26(c)** is important.

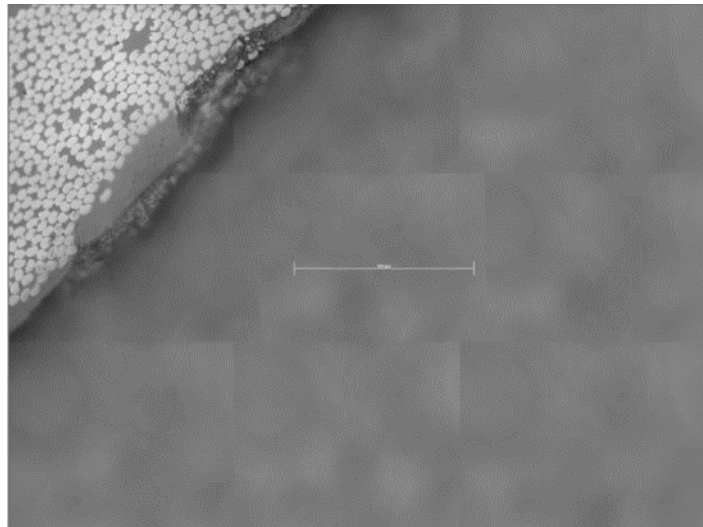


Figure 53. Scale bar's automatic movement after exporting.

- e. Copy this image onto a flash drive in order to be used in **Step 34**.
27. Setting up the mosaic image to make the first (or top) half of entire area of interest.
    - a. Under **MosaiX > Acquisition**, click on **Setup...** under **MosaiX Settings**. A window will appear that looks similar to the image below (Fig. 54).

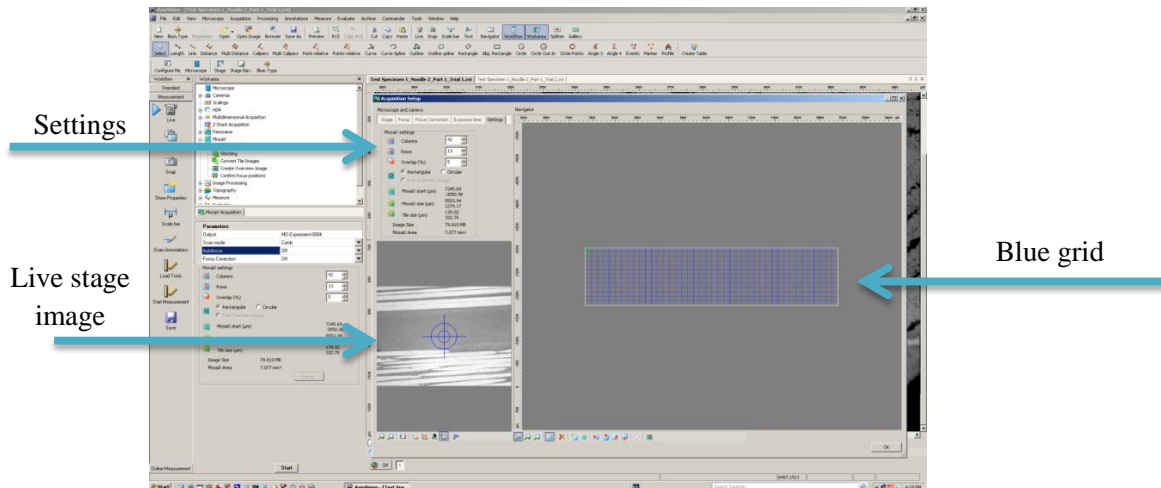


Figure 54. MosaiX Acquisition Setup window.

- b. Use the white joystick connected to the computer (Fig. 3) and coarse and fine adjustments (Fig. 3) to move the specimen to the starting point of the top half. The live stage image can be seen below the settings (Fig. 54). The starting point will be the top-left corner of the first image.
- c. Click on the **Settings** tab (Fig 55).

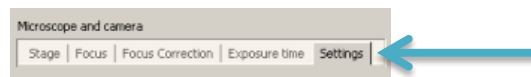


Figure 55. Settings option in MosaiX Acquisition Setup window.

- d. Start to increase the number of columns until the top number of the **MosaiX size ( $\mu\text{m}$ )** is slightly larger than the horizontal distance found in the top rectangle (Fig. 49). The example uses 42 columns because the corresponding MosaiX size of  $5553.94 \mu\text{m}$  (Fig. 56) is slightly larger than the distance of  $5501.79 \mu\text{m}$  (Fig. 49).
- e. Start to increase the number of rows until the bottom number of the **MosaiX size ( $\mu\text{m}$ )** is slightly larger than the vertical distance for the top half. The example uses 13 rows because the corresponding MosaiX size of  $1274.17 \mu\text{m}$  (Fig. 56) is slightly larger than the distance of  $1234.79 \mu\text{m}$  (Fig. 49).

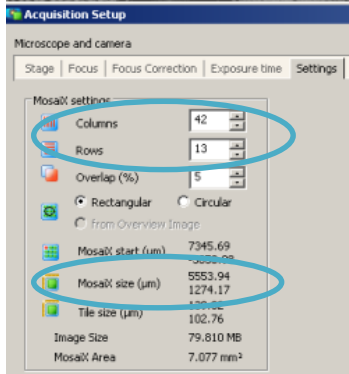



Figure 56. Selected columns and rows in MosaiX Acquisition Setup window.

- f. In the grid that is created on the right of the settings window (Fig. 57), there is a **blue grid** that contains the selected number of rows and columns with a **green plus sign** in the middle. The plus sign represents where the current stage position is located. The current stage position needs to be placed at the top-left corner of the grid. In order to do this, press the button that looks like a **square with a green plus sign on the top-left corner** . After hovering over the icon, a text box will appear stating “**Moves the MosaiX image to the current stage position.**” The icon is located below the grid space in the row of options. **Note:** Every time the stage position is moved to the desired starting point, this button needs to be selected again. The stage position can only be selected on the grid, but the current live image can be seen under the settings.

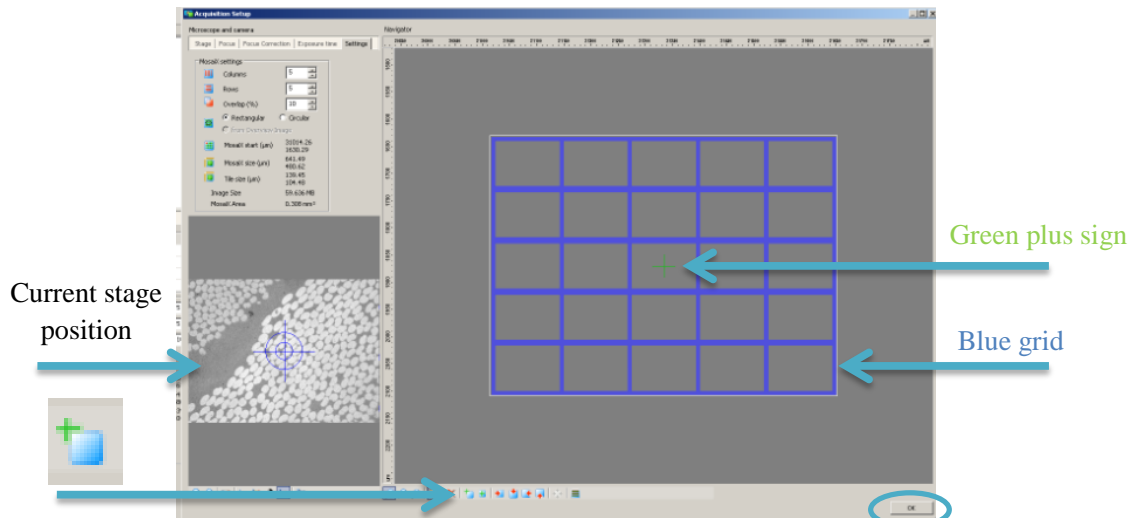


Figure 57. Current stage position relating to Setup grid.

- g. Select the **OK** button at the bottom-right of the window (Fig. 57). Then, select **Start** in the MosaiX Acquisition to start creating the image. It may require a few trials to find the correct starting point and number of rows and columns.



- h. **Stitch** together the image, which is the option below **Acquisition** for **MosaiX**. The final result should look like the image below (Fig. 58):

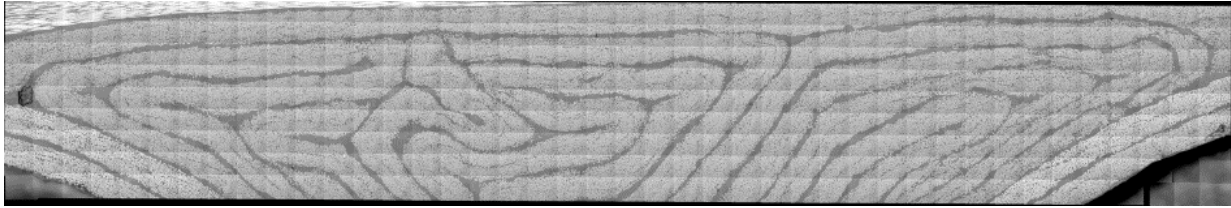


Figure 58. Top image of noodle after stitching.

- 28. Setting up the mosaic images to make the second (or bottom) half.
  - a. Now that the top image has been created, the image will be used as a guide to acquire the next set of images. First, identify the individual tiles that make up the entire image. In the last row of the image below, identify the two outermost tiles of the area of interest (Fig. 59).

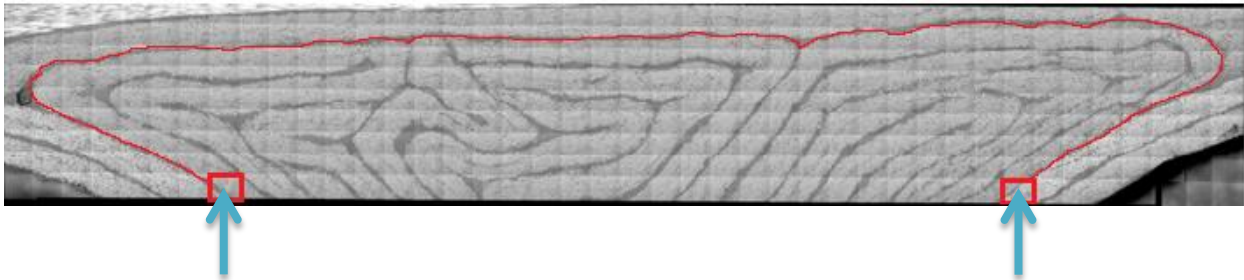


Figure 59. Outlined area of interest.

- b. These identified tiles (Fig. 59) represent the bounding tiles for the top of the next image to be collected. These tiles are both seven tiles in from the left and right side, so a total of 14 tiles should be deleted from the original number of columns. Therefore, the next image should have 28 columns.
- c. In the **MosaiX > Acquisition > Setup > Settings**, the live image needs to be moved to the next starting point. In order to do this, double click on the tile that was marked by the left arrow in the image above. The green plus sign should now be in the newly identified tile (Fig. 60). **Note:** By using this tile as a starting point, the initial tile will collect a row of tiles already collected in the previous image. This is necessary in order for the images to overlap and align later.

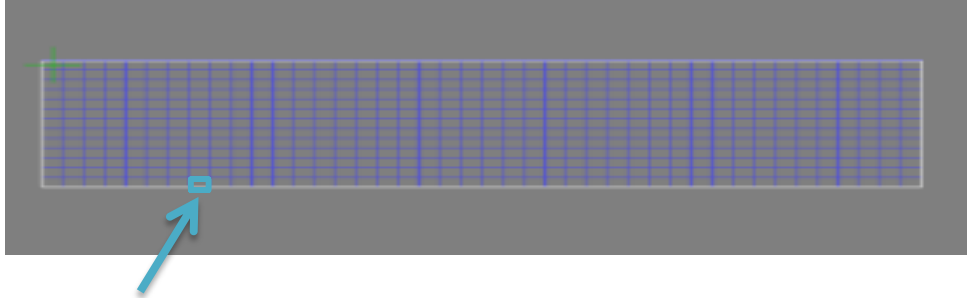



Figure 60. Acquisition Setup tile for next image.

- d. Press the button that looks like a **square with a green plus sign on the top-left corner** . Hovering over the icon will cause it to say “**Moves the MosaiX image to the current stage position.**” It is located below the grid space in the row of options (Fig. 57). This will make that specific tile the top left corner of the new image.
- e. Change the number of columns according to the explanation in **Step 28(b)**. Change the number of rows as required, remaining in the field of view. The next image in this example is a 28 X 5 mosaic (Fig. 61).

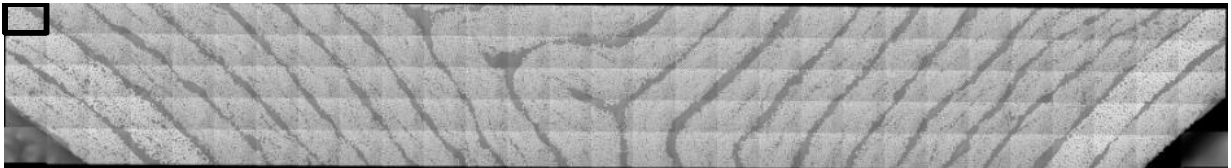


Figure 61. Second collected image of noodle.

- f. Continue **Steps 28(a–e)** until images have been collected of the entire noodle (Fig. 62). After collecting each one, **stitch** each respective image together. **Save** each image as a **.zvi file**. When choosing names for these files, include numbers so that the order of image collection is easily identifiable. Finally, save all of the files into one folder to make them easier to access later. See the images below that were created to form the whole noodle.

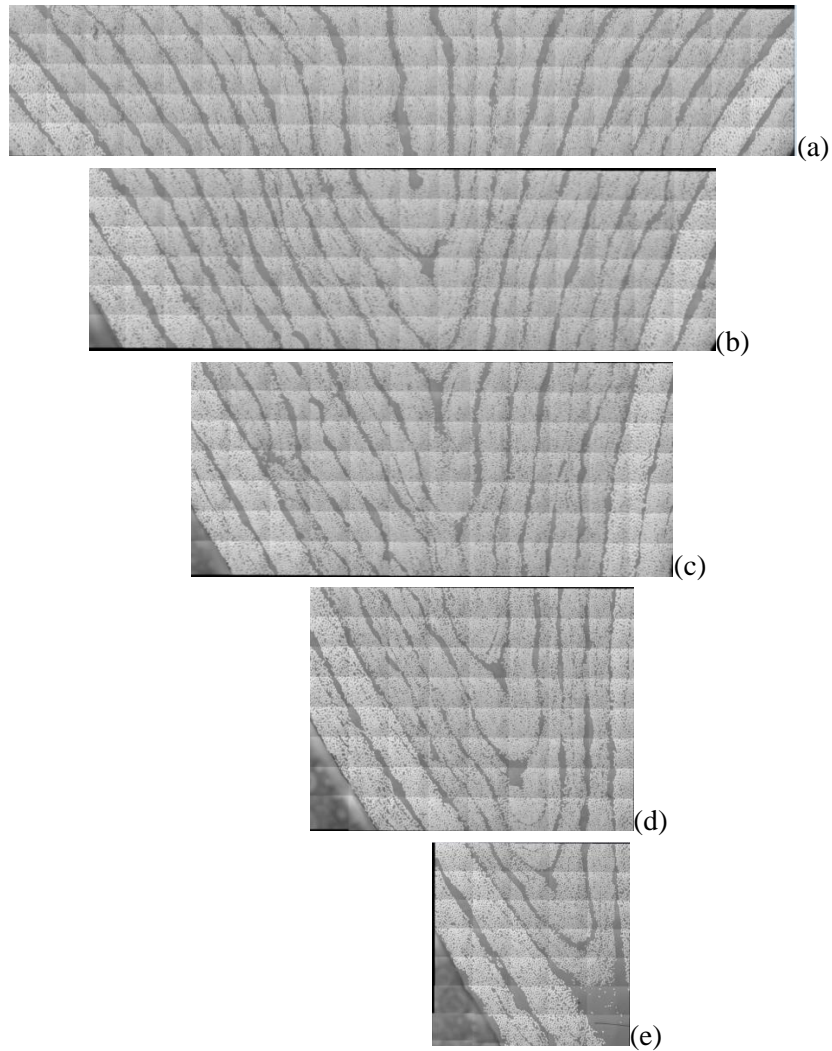


Figure 62. Third – seventh collected images of noodle a) section 1, b) section 2, c) section 3, d) section 4, and e) section 5.

29. Stitch the images together using **Panorama > Import from Files**.

- a. Above the **MosaiX** option is the **Panorama** option. Once expanded in the menu, choose the second option: **Import from Files** (Fig. 63).

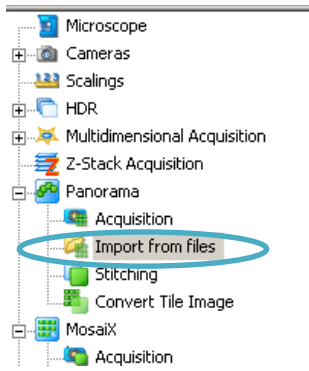



Figure 63. Panorama: Import from files option.

- b. Click the button with three dots  in the top-left of the window (Fig. 64) in order to open the folder that includes all of the .zvi files. The folders will start to open below (Fig. 65).

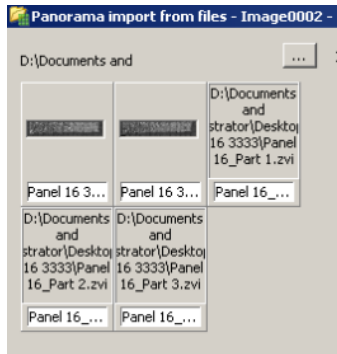


Figure 64. Open files to be imported for panorama.

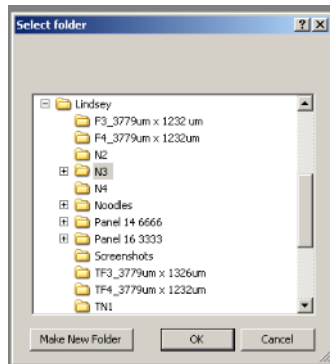


Figure 65. Select folder with images for panorama.

- c. Select the first image that was collected in **Step 27**. The image should appear in the workspace to the right (Fig. 66).

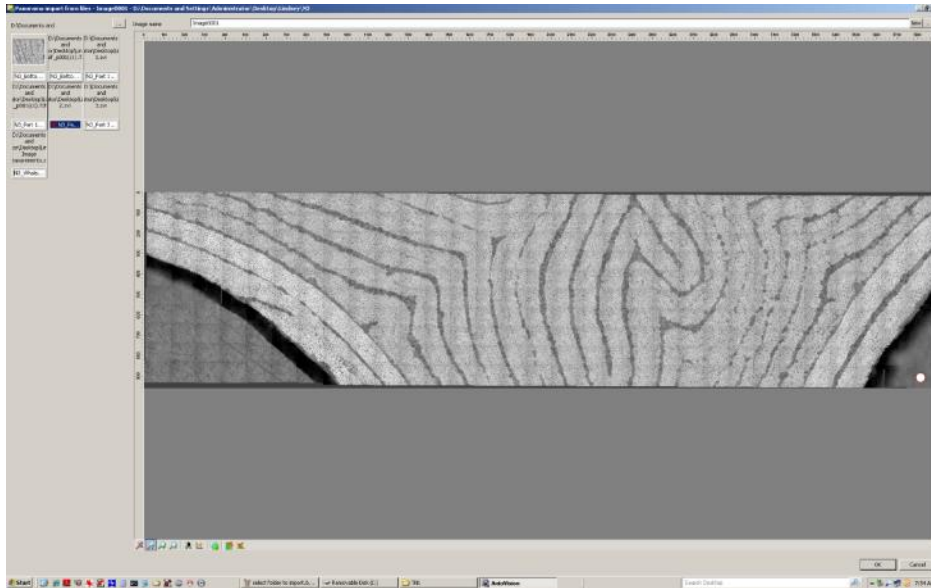


Figure 66. First image imported to make panorama.

- d. Select the second image that was collected in **Step 27**. The second image usually does not appear in the immediate field of view, so scroll down or across to find the second image in the workspace (Fig. 67).

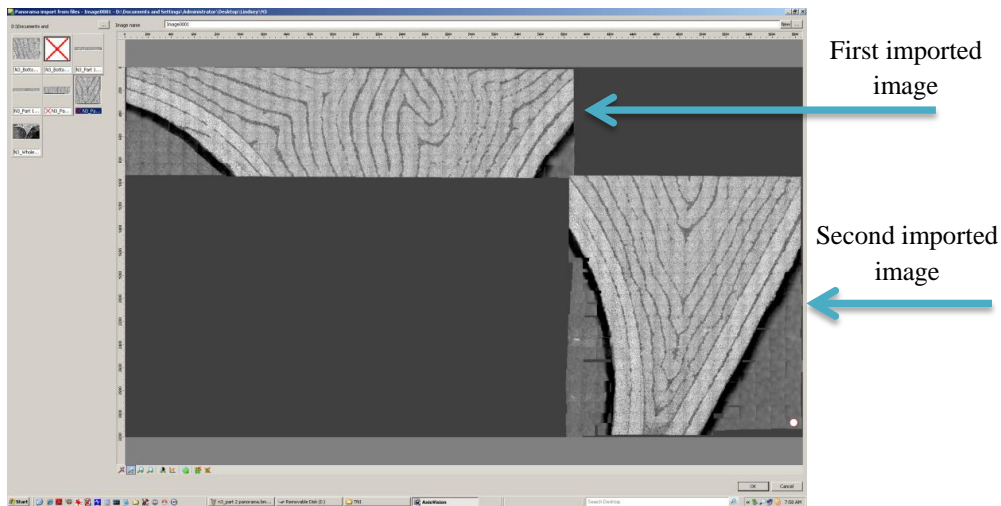


Figure 67. First and second images imported to make panorama.

- e. Delete any unnecessary tiles that are not a part of the area of interest. Hold down the **Ctrl** button as tiles are selected to be deleted (Fig. 68). Then, click on the button with **green tiles and a red X** (Fig. 68) that is located at the bottom of the page. This will delete the selected tiles.

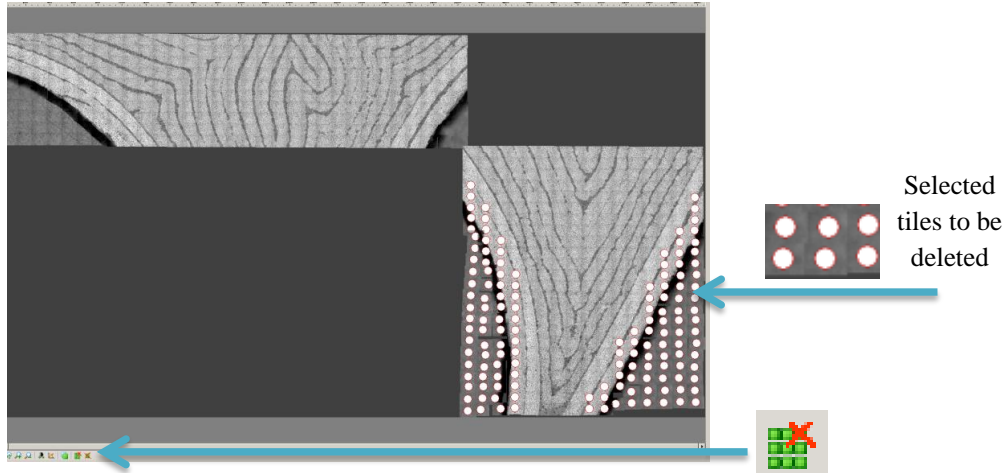


Figure 68. Selection of tiles for deletion.

- f. To move the second set of image tiles, hold down the **Ctrl** button and select each individual tile of the second image (Fig. 69). Do not move the tiles as they are being selected. If the tiles accidentally move while being selected, press **Ctrl + z** to undo.

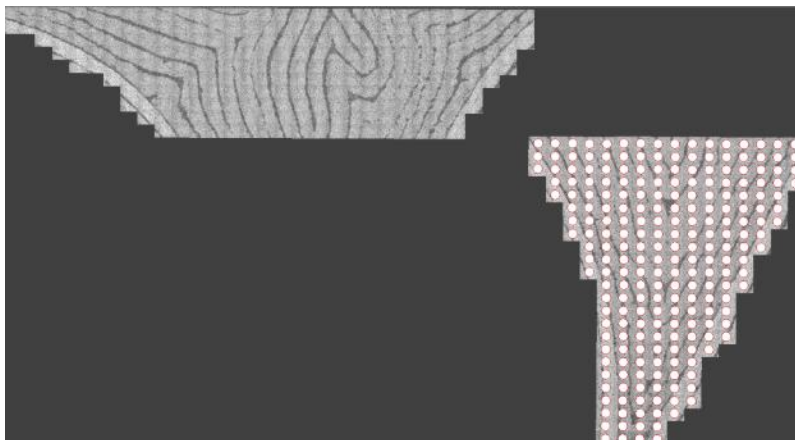


Figure 69. Selection second image for alignment.

- g. After all of the tiles are selected, move the collection of images towards the first image by clicking a part of the selected collection and dragging it towards the first set of images (Fig. 70). Align the images on top of each other by zooming in and watching the bottom of the first image collection overlap with the top of the second image collection (Fig. 71). This is why the previous note in **Step 28(c)** of overlapping the image collections is very important.

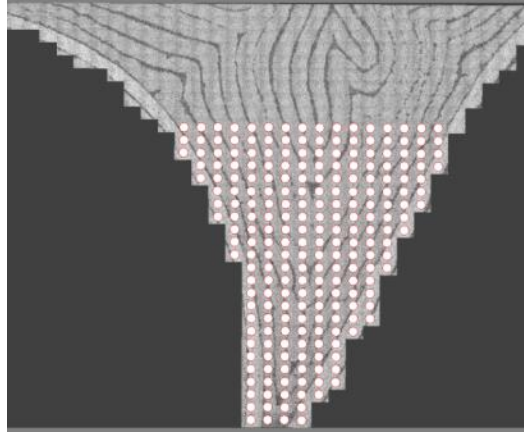


Figure 70. Second image alignment.

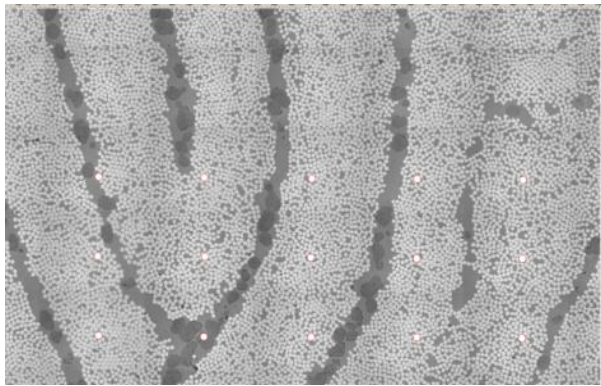


Figure 71. Second image alignment, zoomed in.

- h. Press **OK** at the bottom-right of the panorama workspace (Fig. 72). The image should now appear in a .zvi file format in the AxioVision workspace (Fig. 73). **Export** the panorama image as a .tif file. Place it on a removable disk or flash drive.

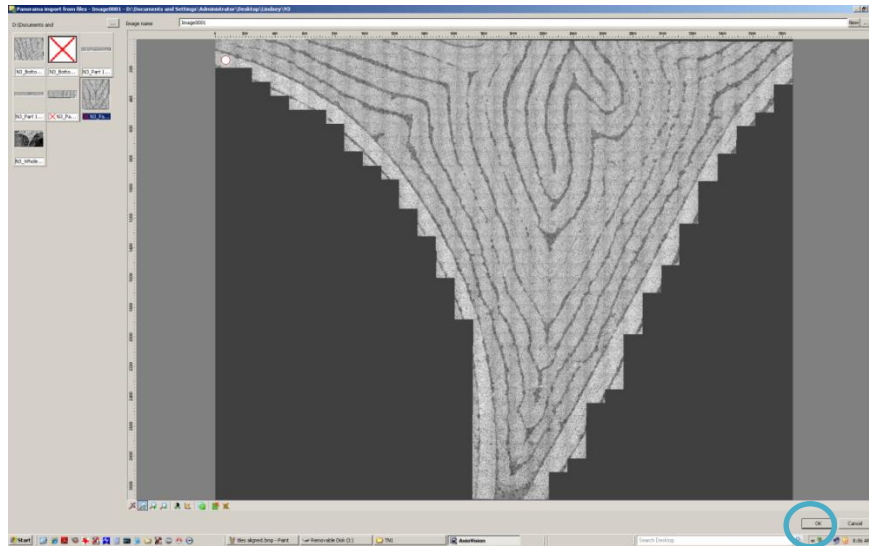


Figure 72. First and second noodle image combined in *panorama* workspace.

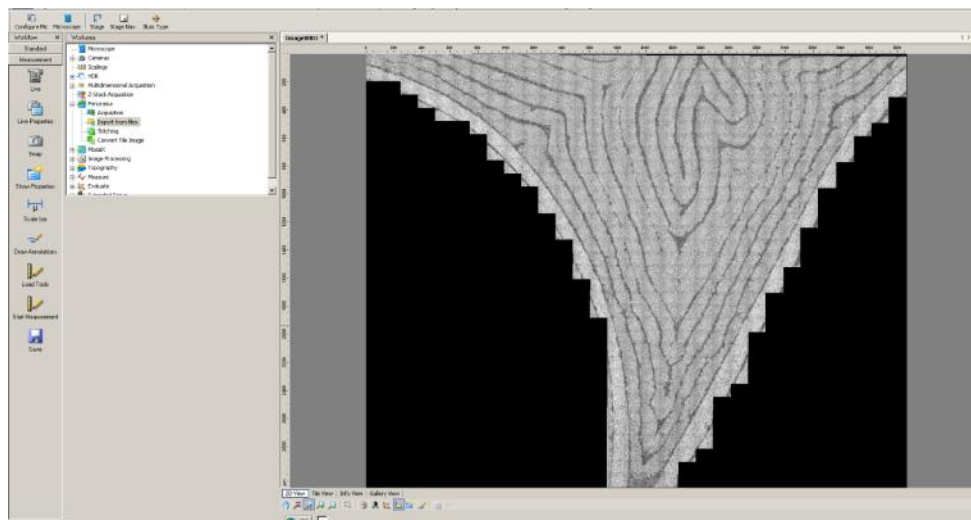


Figure 73. First and second noodle image combined in *AxioVision* workspace.

- i. Repeat this step for any additional images.
- j. **Note:** Combining the individual .zvi files into one large image will most likely exceed the 2GB limit on the computer. Therefore, two panorama images should be made (one of the **top half** and one of the **bottom half**). In the example in Figure 74, the seven files were split into three files for the top half and four files for the bottom half. Making two panorama images may not be needed if the image size of the area of interest is smaller than 2 GB.



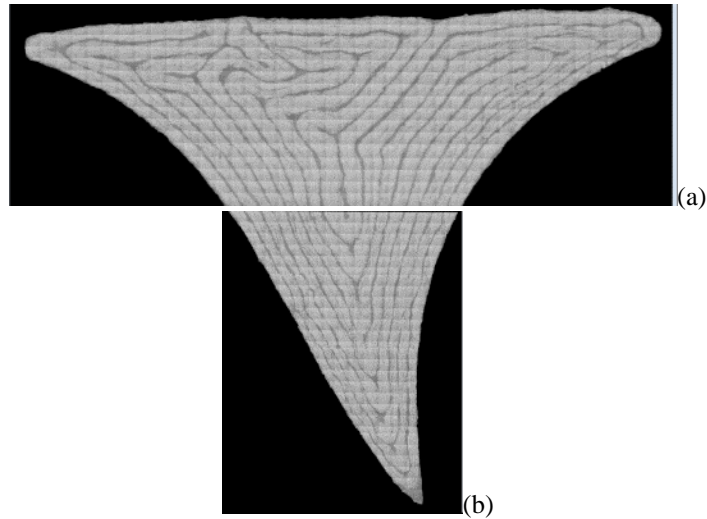



Figure 74. Outlined noodle using ImageJ Paintbrush tool a) upper portion and b) lower portion.

## 5.0 ImageJ Processing on Collected Noodle Images

30. After opening ImageJ, open the two images that were created in **Step 29**.
31. Double-click on the **Paintbrush Tool**  in the tool bar (Fig. 75). The **Brush Options** window will appear.

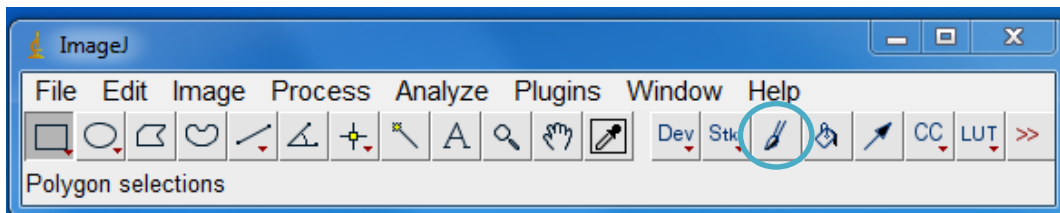


Figure 75. ImageJ Paintbrush tool location.

- a. Manually change the **Brush width** to **100** (Fig 76).
- b. Change the Color to **Black** (Fig. 76).

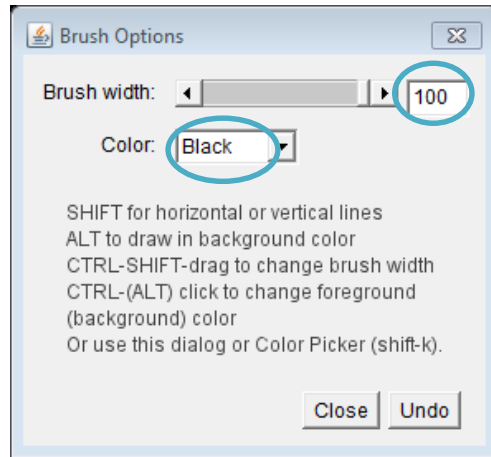




Figure 76. ImageJ Brush Options for Paintbrush tool.

32. Outline and define the fibers that are part of the area of interest.
  - a. Use the **Paintbrush Tool** in order to eliminate what is not a part of the area of interest.
  - b. **Note:** Use the **Magnifying Glass**  tool to zoom in and out with the **Paintbrush Tool** and clearly define/outline the area of interest.
  - c. Color in the rest of the unnecessary parts using a **Brush width** of **200**.
  - d. The final results should look like the two images shown before (Fig. 74a and Fig. 74b).
33. Eliminate the overlapping between the two images.
  - a. Use the **Paintbrush Tool** to make a straight line across the bottom of the first image and the top of the second image. Hold down **Shift** while using the tool in order to make straight horizontal lines.
  - b. This might take a few trial and error sessions to ensure that the correct amount of the overlapping noodle was removed.
  - c. **Note:** **Save** these individual images (Fig. 74) in order to find the total area of the noodle later.
34. Set the measurement scale from pixels to  $\mu\text{m}$ .
  - a. **Open** up the image created with a scale bar in **Step 26** (Fig. 53).
  - b. Use the **Straight Line Selection Tool**  in order to trace the known distance (Fig. 77).

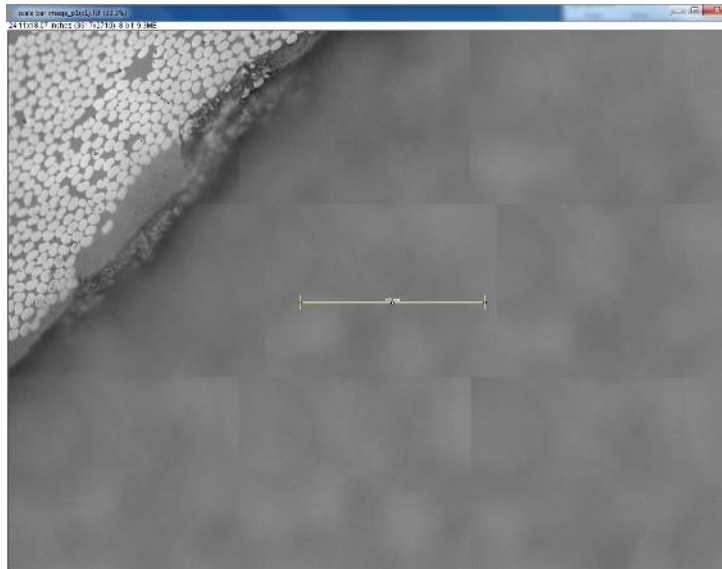


Figure 77. Traced scale bar of Figure 53 in ImageJ.

- c. **Analyze > Set Scale...**
- d. Enter in the **Known distance** (Fig. 78), which is **100 μm**.
- e. Change the **Unit of length** to “**micrometers**” (Fig. 78).
- f. Check the box that says **Global** (Fig. 78) in order to apply this to all images that will be manipulated.
- g. Press **OK** (Fig. 78).

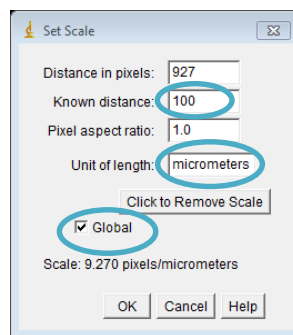


Figure 78. ImageJ Set Scale window.

- h. Now, at the top left of the current image, the dimensions should display # X # **micrometers** (Fig 79).

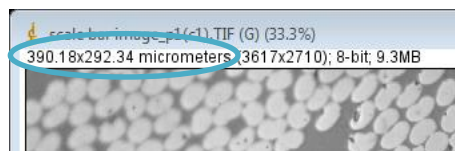


Figure 79. Image dimension change to micrometers.

- i. **Note:** After applying this scaling, and choosing to open a new image, a message may appear that looks like the image in Fig. 80. Exit out of the window in order to keep the same scaling.

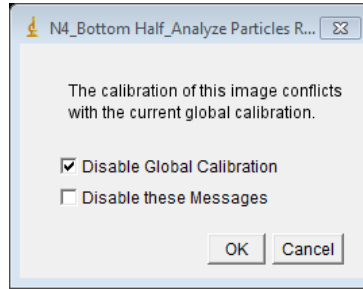


Figure 80. Global calibration warning message.

35. Perform **Steps 16–23** in order to complete the image processing.
  - a. Save the processed images (Fig. 81) as different files than the previous images (Fig. 76).

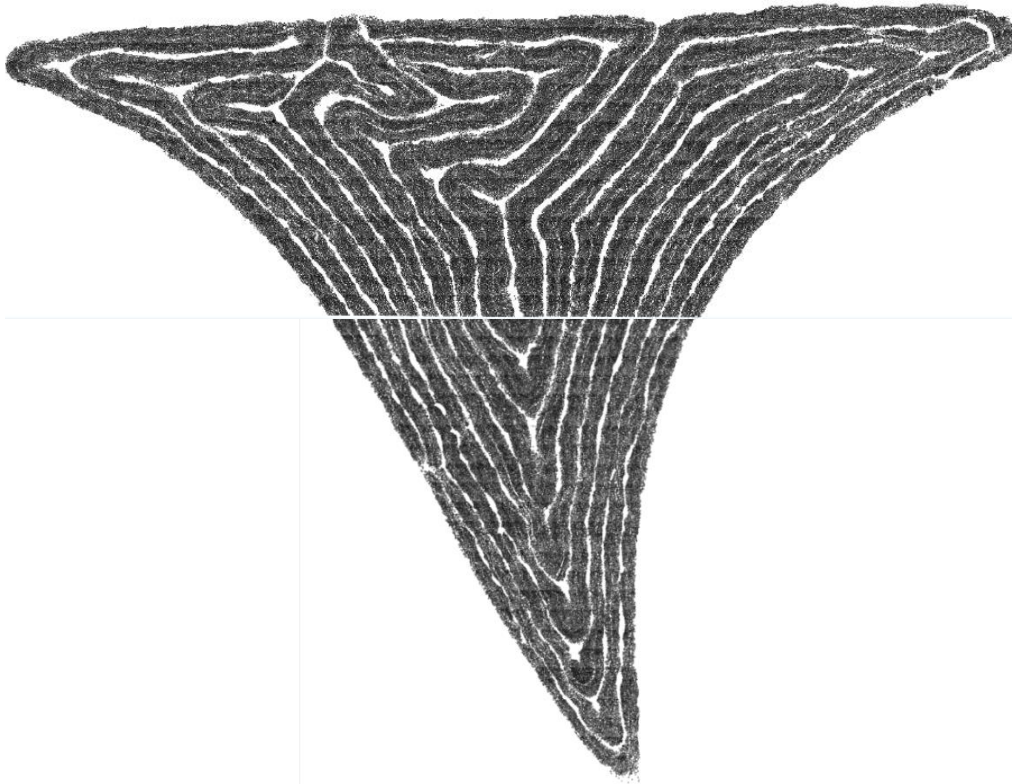



Figure 81. ImageJ processing for complete noodle images.

36. Determine the total area of the noodle.
  - a. Open the outlined images (Fig. 76) in ImageJ.

- b. Use the **Wand (tracing) tool**  (Fig. 82) in order to select the entire noodle area. This is best done by clicking on any part of the black portion outlining the noodle. Successful outlining can be seen when the entire area of interest has a yellow outline (Fig 83).

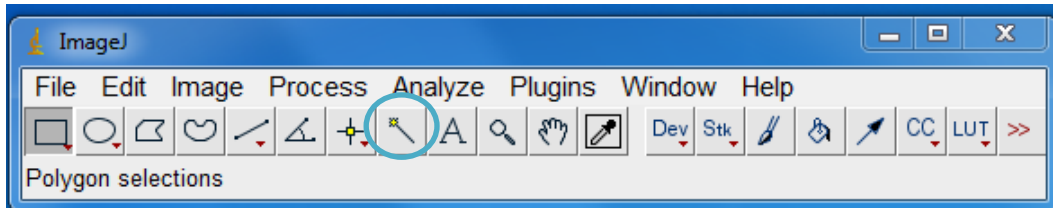


Figure 82. ImageJ Wand (tracing) tool location.

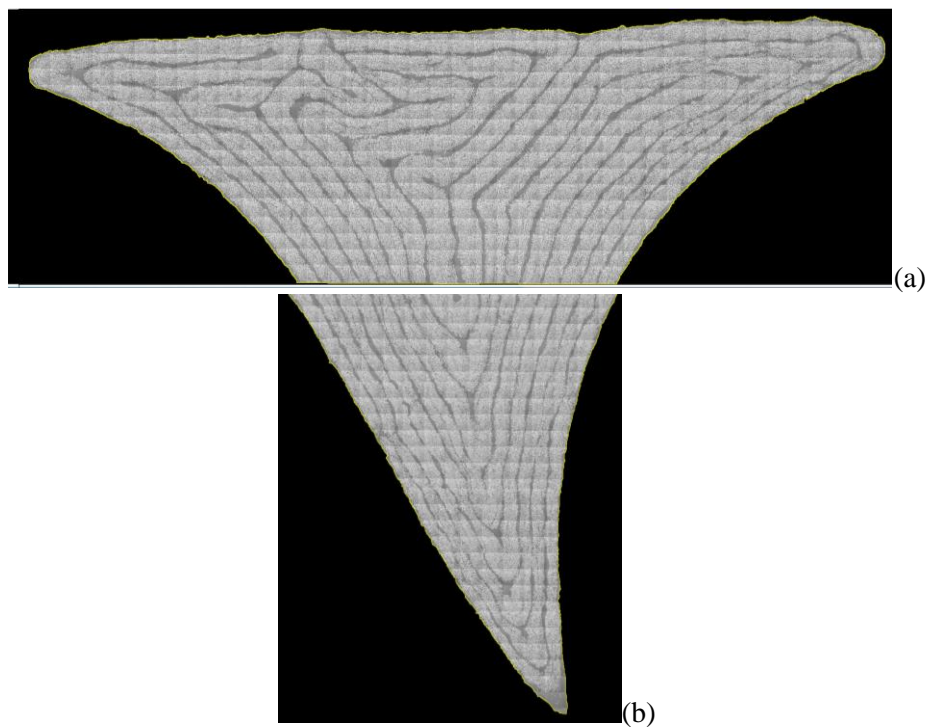


Figure 83. Noodle with yellow outline using tracing tool a) upper portion and b) lower portion.

- c. **Image > Adjust > Threshold...** Change the minimum threshold value to **1** and the maximum threshold value to **255** (Fig. 84). The area of interest should be entirely red (Fig. 85).

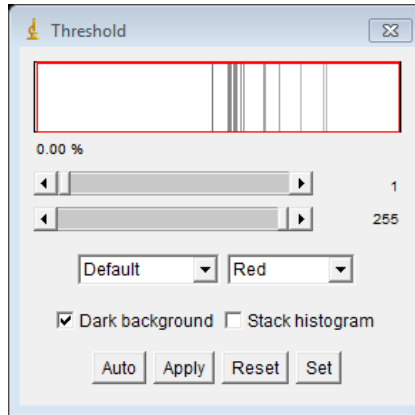


Figure 84. Threshold values set to 1 and 255.

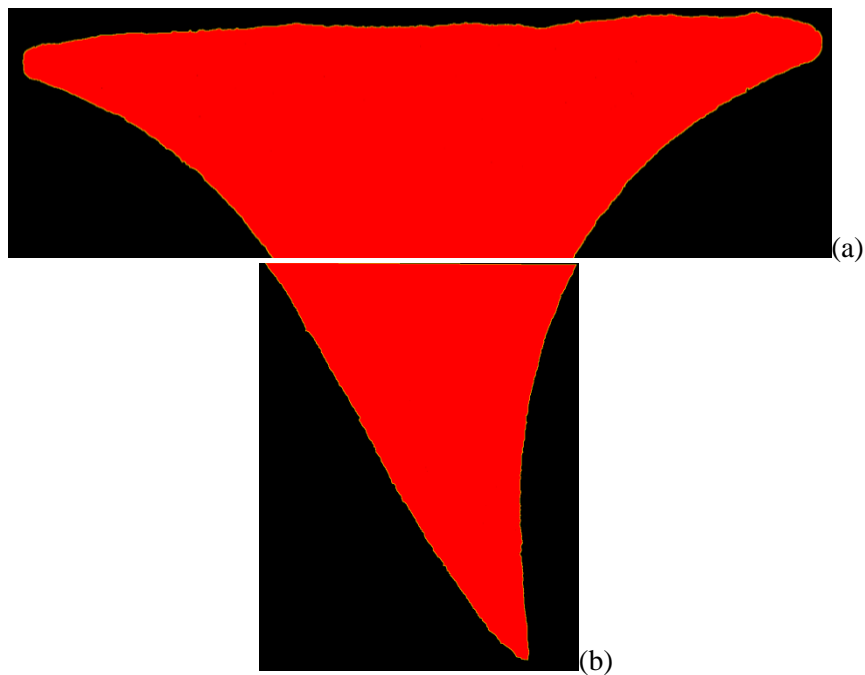


Figure 85. Entirely red thresholded noodle images a) upper portion and b) lower portion.

- d. **Analyze > Analyze Particles...** Add the total areas calculated by this function (Fig. 86). These two numbers are the only numbers needed from this step. **Note:** If the calculated areas in the summary table are close to zero or do not look correct, then redo the function after getting rid of the yellow outlines of the noodle. Note that the red thresholding should still be there.

Slice	Count	Total Area	Average Size	%Area	Mean
First Half_Whole Noodle_p001(c1).TIF	62	5007667.536	80768.831	59.266	49.630
Second Half_Whole Noodle_p001(c1).TIF	4	2207578.675	551894.669	43.744	156.781

Figure 86. Total area of the noodle.

37. Determine the area of fibers inside the noodle.
  - a. Open the processed images (Fig. 81).
  - b. **Analyze > Analyze Particles...** Add the total areas calculated by this function (Fig. 87). This will represent the total amount of fiber area inside the noodle.

Slice	Count	Total Area	Average Size	%Area	Mean
First Half_Whole Noodle_p001(c1)_ImageJ ready for analyze particles.tif	160968	2765524.903	17.181	32.730	0
Second Half_Whole Noodle_p001(c1)_ImageJ ready to analyze particles.tif	66803	1160253.116	17.368	22.991	0

Figure 87. Fiber area of the noodle.

38. Determine the fiber area fraction based on the numbers calculated in **Steps 36** and **37** (Figs. 86 and 87).

Noodle Part	Fiber Area	Total Area	Fiber Area Fraction
Top Half	2.76 mm <sup>2</sup>	5.01 mm <sup>2</sup>	0.5509
Bottom Half	1.16 mm <sup>2</sup>	2.21 mm <sup>2</sup>	0.5249
Total Noodle	3.92 mm <sup>2</sup>	7.22 mm <sup>2</sup>	0.5429

Figure 88. Fiber Area Fraction Calculations Table.

**REPORT DOCUMENTATION PAGE**

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<b>14. ABSTRACT</b> Fiber-reinforced composite structures have become more common in aerospace components due to their light weight and structural efficiency. In general, the strength and stiffness of a composite structure are directly related to the fiber volume fraction, which is defined as the fraction of fiber volume to total volume of the composite. The most common method to measure the fiber volume fraction is acid digestion, which is a useful method when the total weight of the composite, the fiber weight, and the total weight can easily be obtained. However, acid digestion is a destructive test, so the material will no longer be available for additional characterization. Acid digestion can also be difficult to machine out specific components of a composite structure with complex geometries. These disadvantages of acid digestion led the author to develop a method to calculate the fiber volume fraction. The developed method uses optical microscopy to calculate the fiber area fraction based on images of the cross section of the composite. The fiber area fraction and fiber volume fraction are understood to be the same, based on the assumption that the shape and size of the fibers are consistent in the depth of the composite. This tutorial explains the developed method for optically determining fiber area fraction performed at NASA Langley Research Center.					
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