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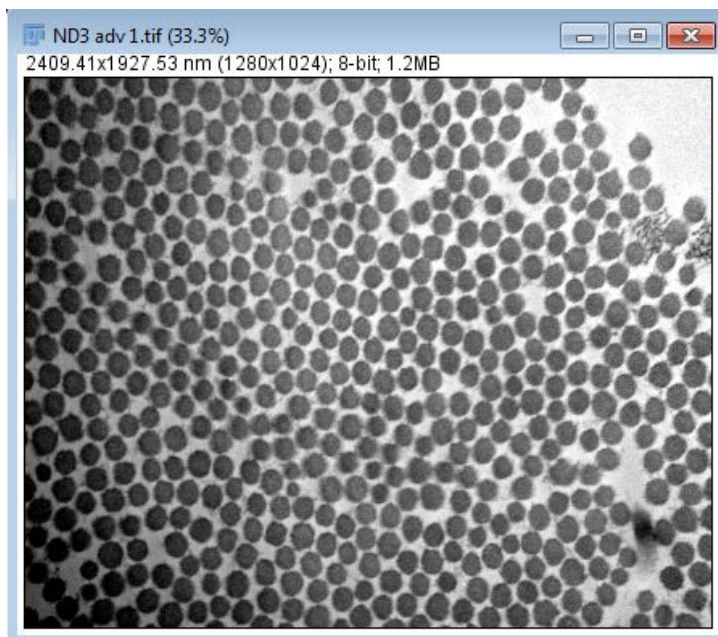
MICROSCOPY NEW ZEALAND INC. CONFERENCE WORKSHOP 2017

Methods for measuring collagen fibril diameter with Fiji

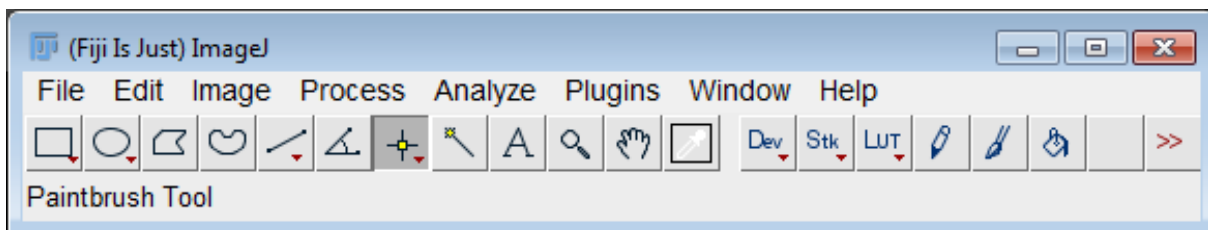
31 January 2017

Example 1 - Manual object counting using the ROI Manager and the Point Tool

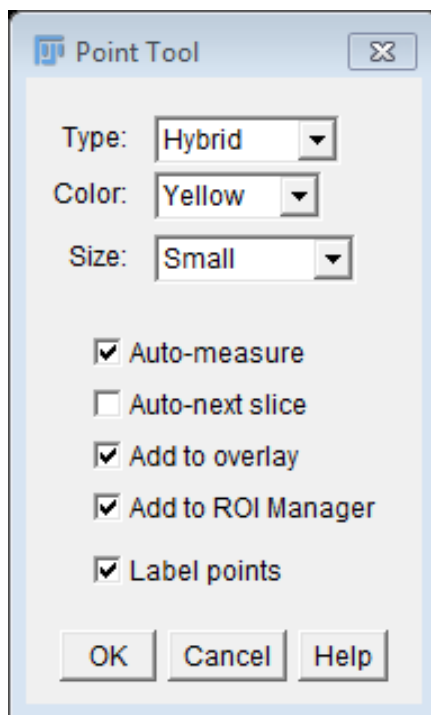
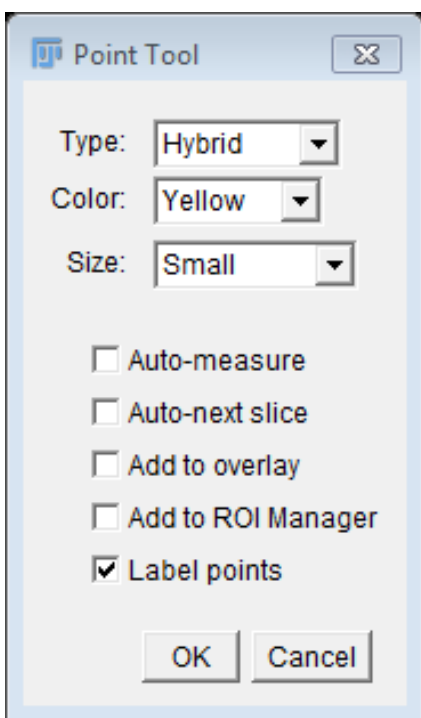
1. Open the image called **ND3 adv1.tif**;



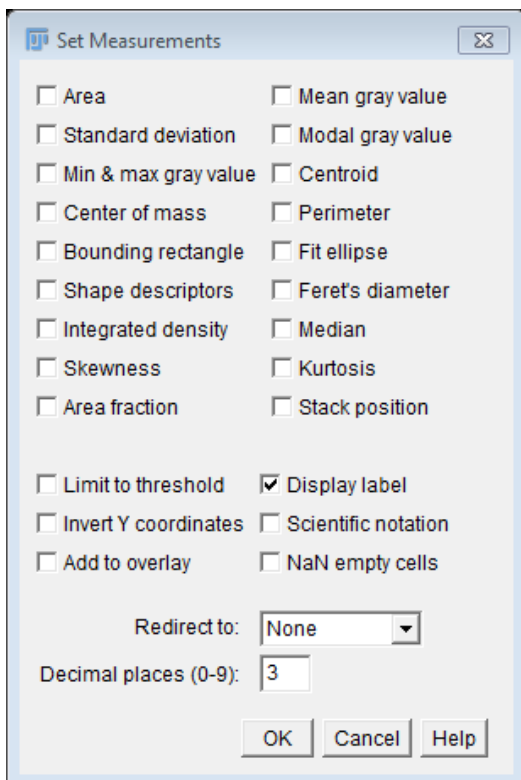
2. Select the **Point Tool** from the tool bar. Double-click on the tool to show the options;



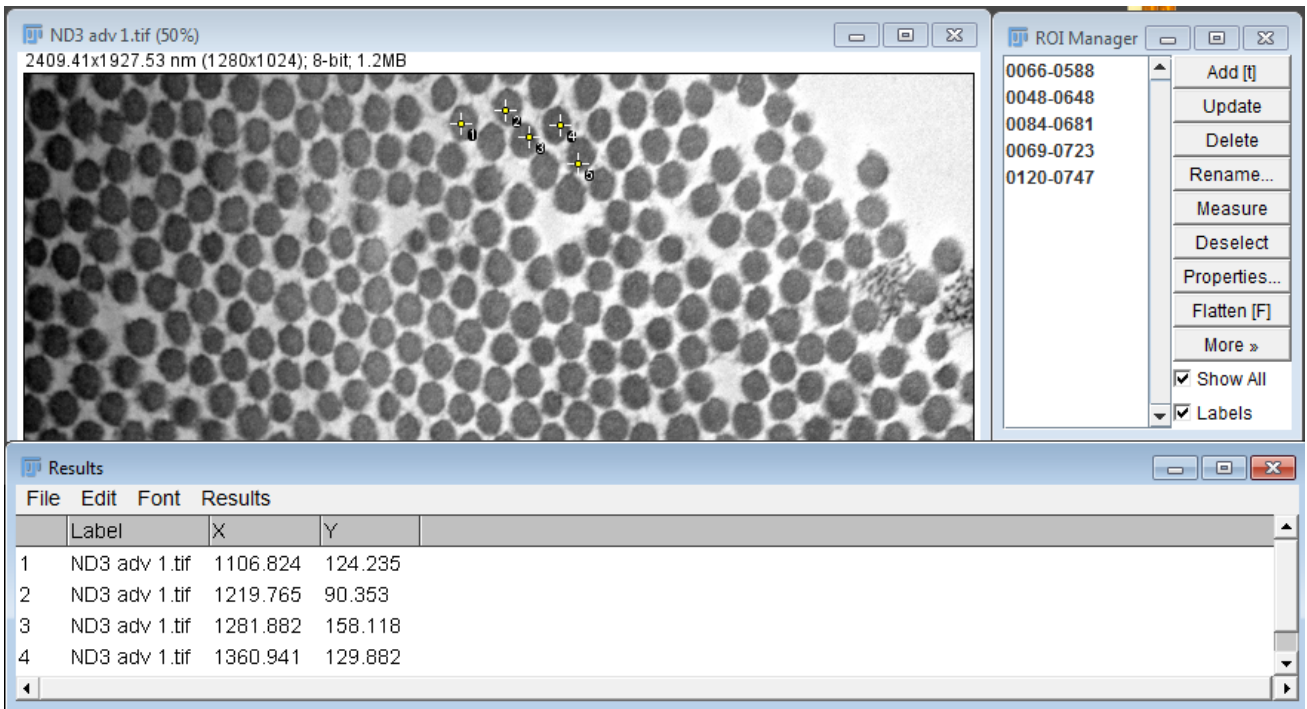
3. Select the options you want and click **OK**. You can choose to have the selections automatically entered into the **ROI Manager** or just as an overlay. Later, you can decide to send the selections to the **ROI Manager**.



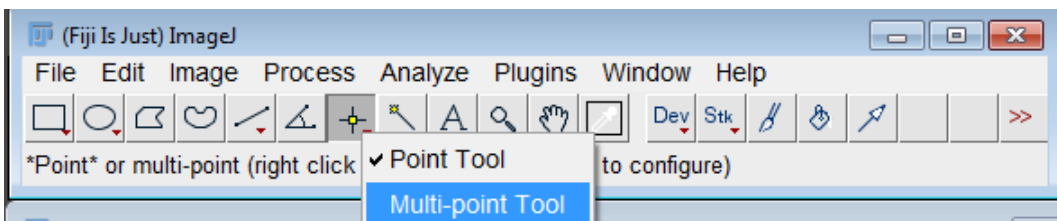
4. Go to **Analyze – Set Measurements** to select the parameters you want to measure. In this case, we only want the total number of points so you can turn all of the parameters off. Make sure you still have **Display Label** selected.



5. Now start clicking on the collagen fibres to count them. Note that each time you click the cross will remain at the location you clicked. It will also have the label attached. The **X Y coordinates** will be entered into the **Results** table for each point.

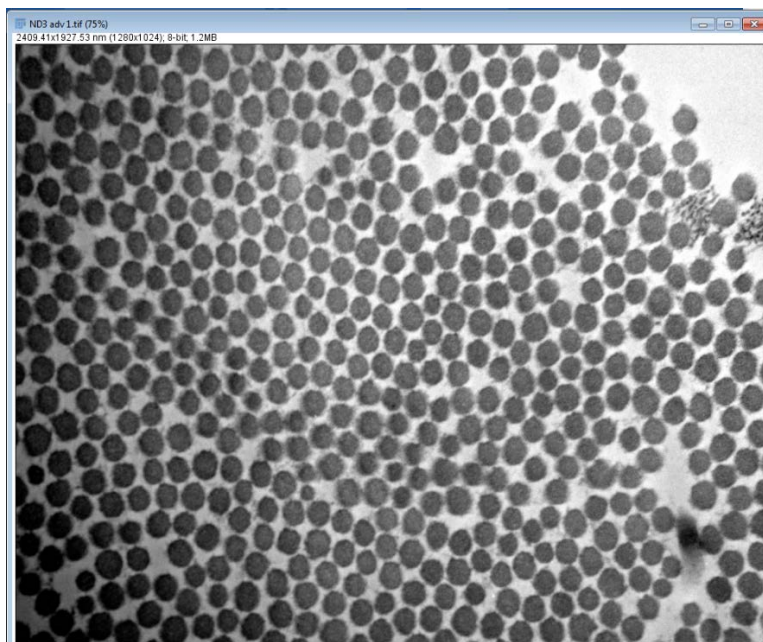


6. The **Results** table and **ROI set** can be saved. Close the **ROI Manager**, **Results** table and image.
7. **Multi Point Tool** – this is found under the **Point Tool**. It can be used for manual counting and labels each point as you click but doesn't enter any data into a **Results** table.

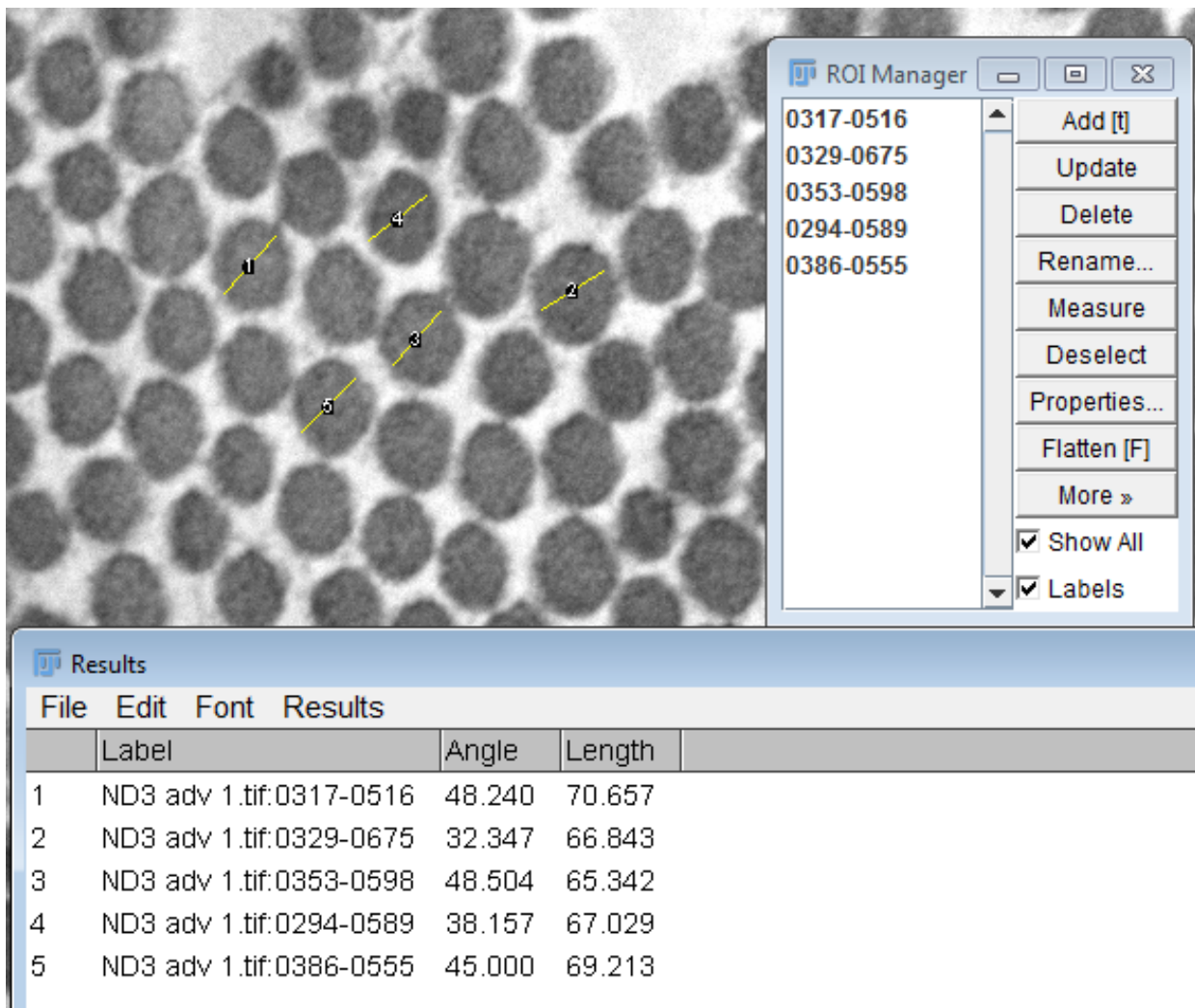


Example 2 - Manual measurements using the ROI Manager

1. Open the image called **ND3 adv1.tif**. Note that the image is already calibrated in nm.



2. Select the **Line Tool** from the Tool bar.
3. Open the **ROI Manager (Analyze – Tools – ROI Manager)**.
4. Go to **Analyze – Set Measurements** to select the measurements you want. In this case, we are interested in the diameter of each fibre (line measurement) so you only need to select **Display Label**.
5. Zoom up on the image using the **Magnifying Tool** so that you are looking at the image at 100%.
6. Draw a straight line across each fibre to measure the diameter. Click **Add** in the **ROI Manager** to add each line section to the **ROI Manager**. Turn on **Show All** (and Labels) so that you can see the fibres you have already added. When you have added all of the line selections you want, click **Measure** in the **ROI Manager** to get the measurements of all of the fibre diameters.



7. Take a note of the length measurements as we will use this information for the next example.

Example 3 - Automatic object counting using the BioVoxel Toolbox

Author – Jan Brocher

Available from: http://fiji.sc/BioVoxel_Toolbox

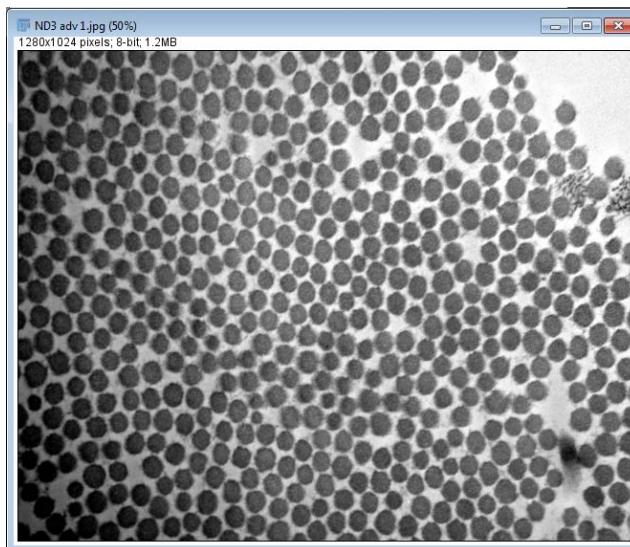
Installation instructions – from the website

BioVoxel Toolsets -Un-zip the BioVoxel_Toolset.zip file in the ImageJ/Fiji >[macros] >[toolset] folder. You will see 3 different toolsets (BioVoxel Toolbox, Montage Tools, Nearest Neighbor Indicator).

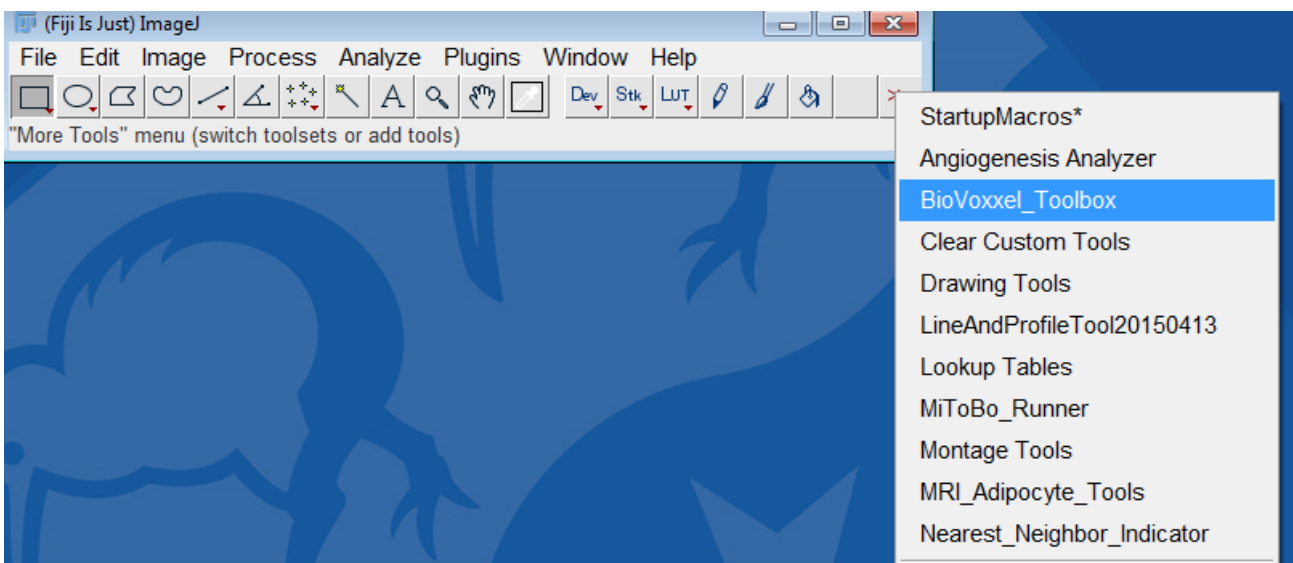
BioVoxel Plugins - The necessary **Plugins** are located in the following .jar-file and need to be placed in the ImageJ **Plugins** folder. After a restart (or >Help >Refresh Menus), the new “BioVoxel” menu will appear in the IJ plugins menu.

STEP 1 – Threshold Check

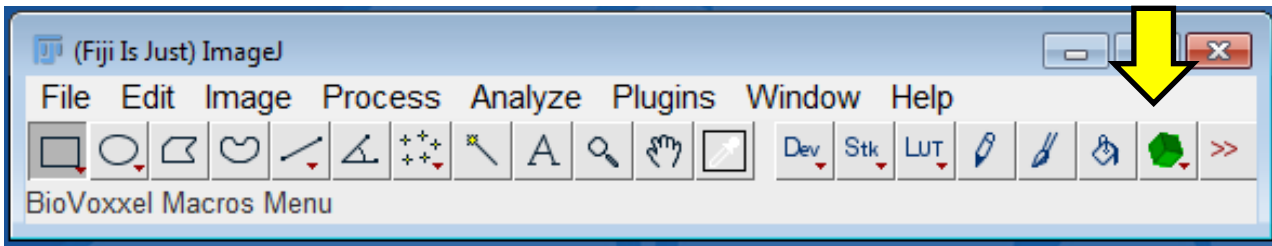
1. Open the image called **ND3 adv1.tif**;



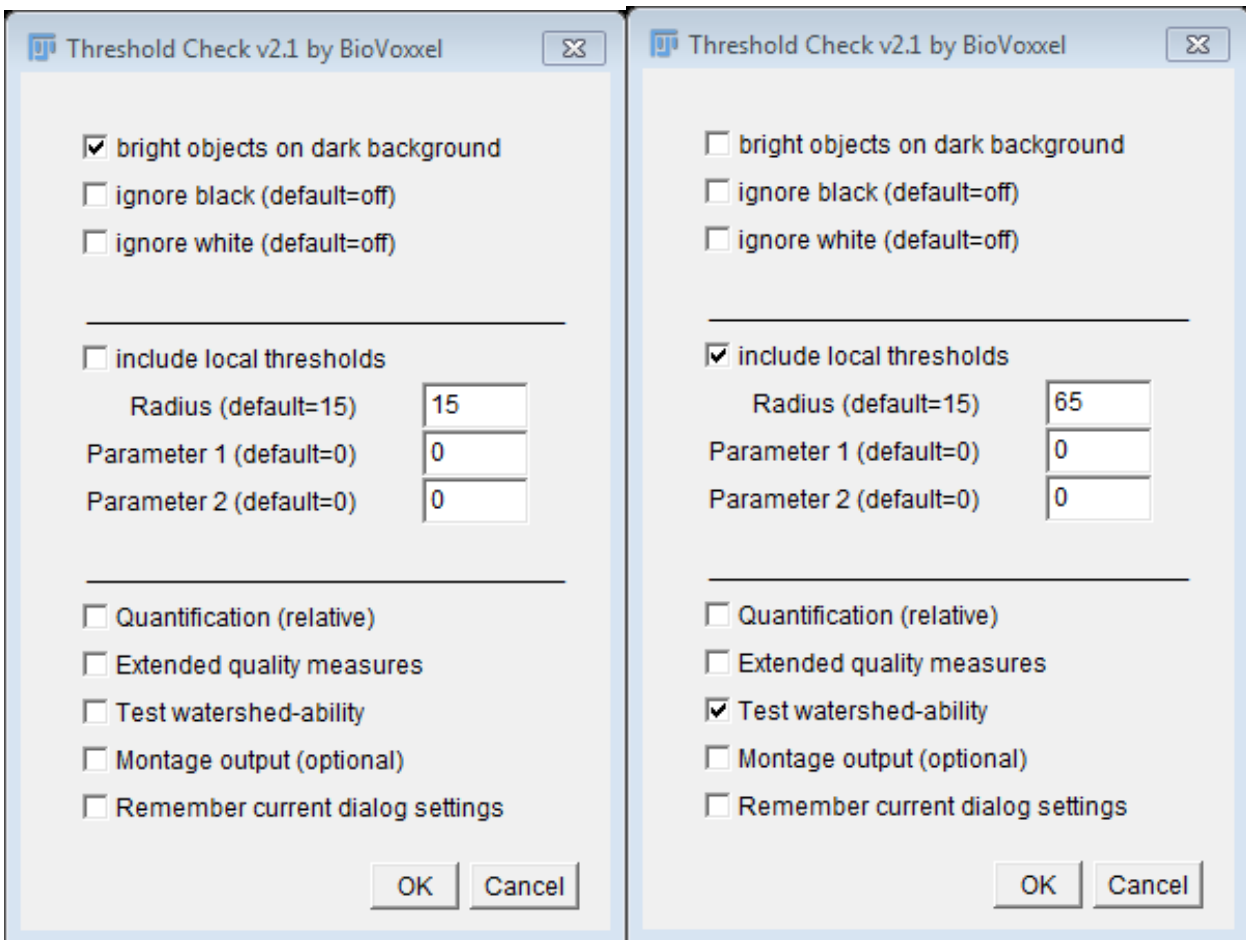
2. Click on the double arrowhead at the end of the **Tool Bar** to select the **BioVoxel Toolbox**;



3. A green cube icon will appear on the **Tool Bar**;

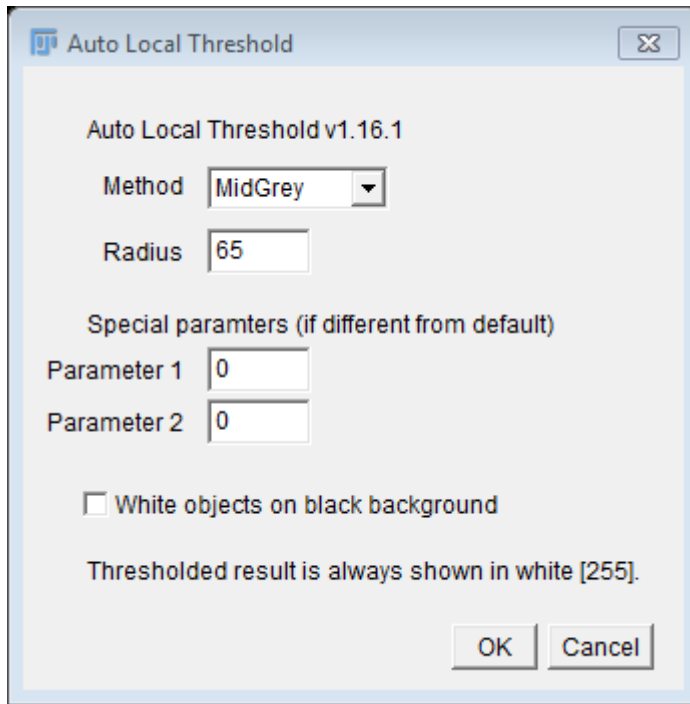


4. Click on the cube and select **Threshold Check**. Select the following options and click **OK**. The **Radius** value is based on the previous manual fibre diameter measurements.



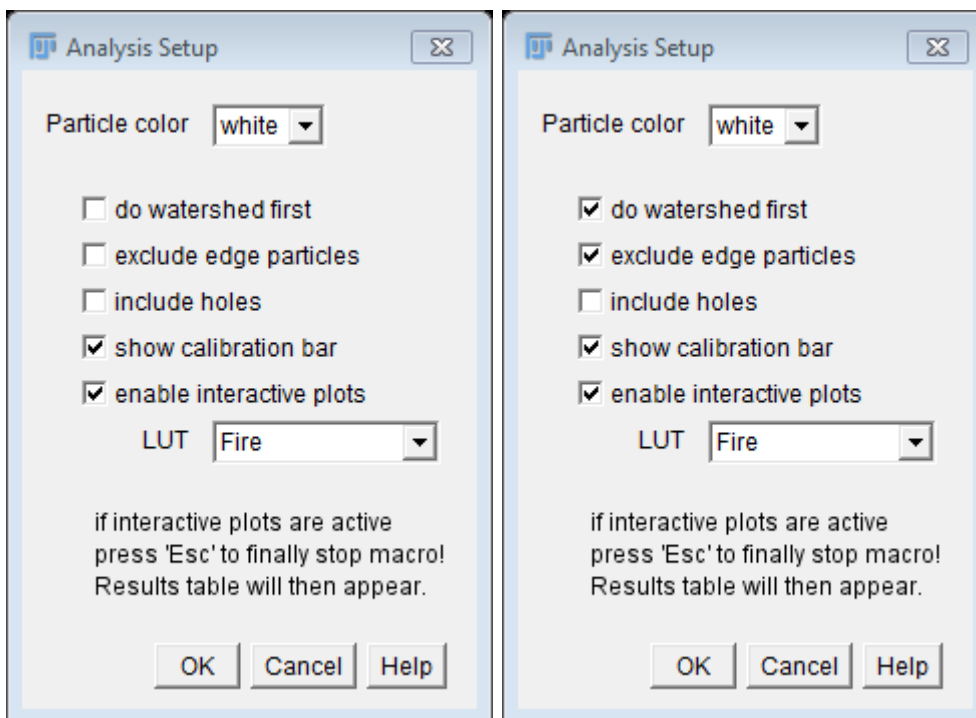
5. A colour-coded series of images will appear, one for each threshold method including watershed splitting. Look through the images and select the one that best represents the collagen fibres. The **Local Threshold** options look a little better than the Global. There are several that work well, including **Bernsen, Mid Grey, Otsu (Local)**.
6. Select the original image again and go to **Image – Adjust –Threshold** or **Image –Adjust –Auto Local Threshold**, select the method you want to use and apply to change the image to binary.

7. This time select only the method you want to use;



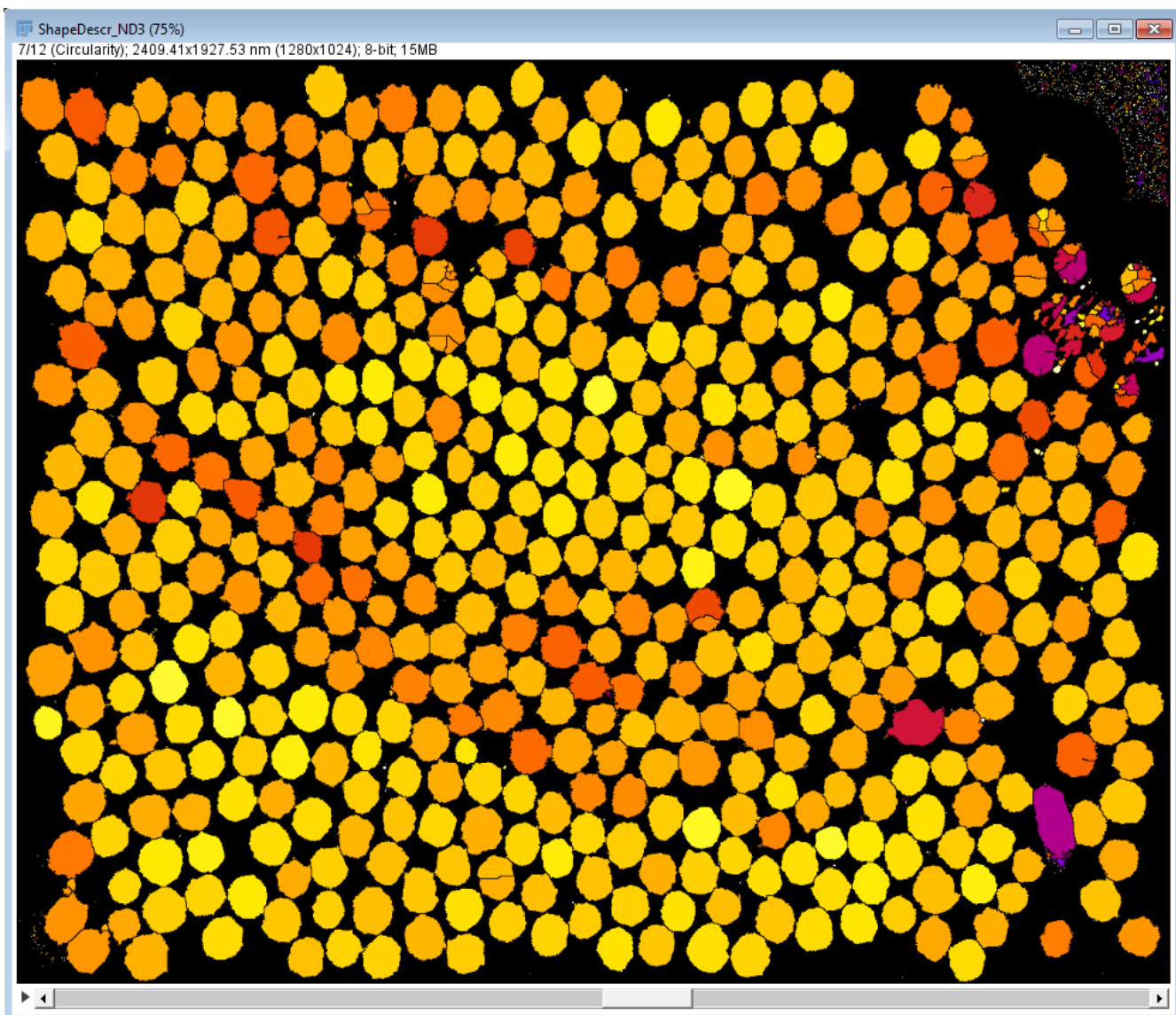
STEP 2 – Shape Descriptor Maps

1. Go back to the **BioVoxel Toolbox** and select **Shape Descriptor Maps**, select from the options shown and click **OK**.

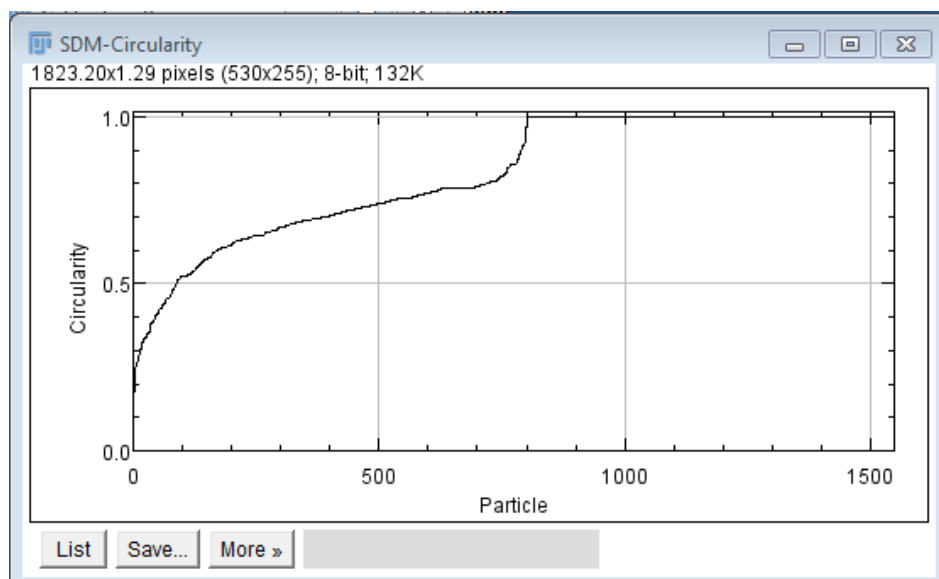


2. If you have selected enable interactive plots, then a plot of the parameter will appear if you click twice on a **Shape Descriptor Map**.

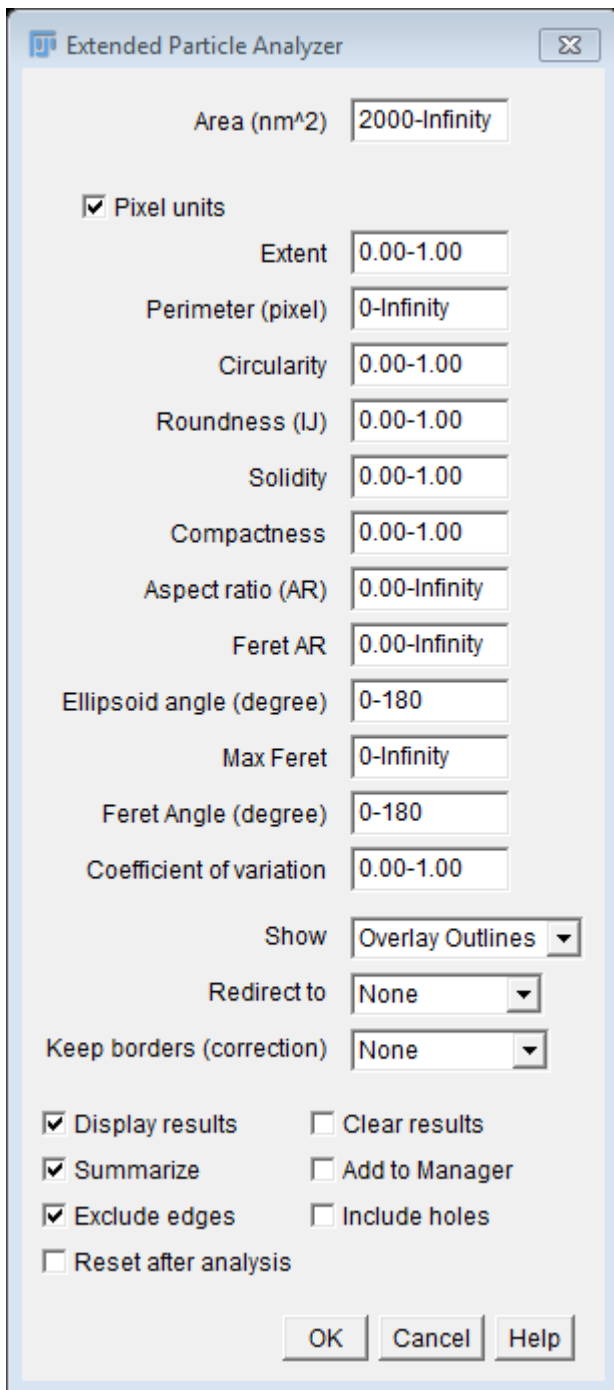
4. Limiting the analysis to circular objects is suitable for this image but perimeter and roundness would also be good.



5. Click twice on the image to get the plot;



6. Press the **Escape (Esc)** button to stop the macro. The **Results** table will appear. These are the results for every object measured. This information will help you to decide on how to do the particle analysis.
7. To get an accurate diameter measurement for these collagen fibrils, the shape should be very close to Circularity 1.0, i.e. a perfect circle as we're looking for a cross-section of the fibril. If you view the column for **Circularity**, you can see many of the values are 1.0 or similar.
8. Save the **Results** if you want to (you could also use the complete table if it's useful). Then close it and the Shape Descriptor Maps.
9. Go to the **BioVoxel Toolbox – Extended Particle Analyzer**. Note that it is similar to the built-in Particle Analyzer but has more options.



10. Enter values into the window (e.g. Circularity 0.70 – 1.0) and click **OK**.

Extended Particle Analyzer ☒

Area (nm²)

Pixel units

Extent

Perimeter (pixel)

Circularity

Roundness (IJ)

Solidity

Compactness

Aspect ratio (AR)

Feret AR

Ellipsoid angle (degree)

Max Feret

Feret Angle (degree)

Coefficient of variation

Show

Redirect to

Keep borders (correction)

Display results Clear results

Summarize Add to Manager

Exclude edges Include holes

Reset after analysis

Drawing of Mask of ND3 adv 1-1.tif (50%)
1280x1024 pixels; 8-bit; 1.2MB

ND3 adv 1.tif (50%)
2409.41x1927.53 nm (1280x1024); 8-bit; 1.2MB

ROI Mana...

- 0001-0026
- 0002-0034
- 0003-0035
- 0004-0046
- 0005-0048
- 0006-0052
- 0007-0069
- 0008-0068
- 0009-0091
- 0010-0089
- 0011-0092
- 0012-0096
- 0013-0098
- 0014-0105
- 0015-0105
- 0016-0107
- 0017-0114
- 0018-0115
- 0019-0123
- 0020-0136
- 0021-0148
- 0022-0158
- 0023-0161
- 0024-0176
- 0025-0172
- 0026-0188
- 0027-0188
- 0028-0195
- 0029-0195
- 0030-0203
- 0031-0209
- 0032-0210
- 0033-0225
- 0034-0239
- 0035-0239
- 0036-0236
- 0037-0254
- 0038-0250
- 0039-0262
- 0040-0268
- 0041-0277
- 0042-0282
- 0043-0280
- 0044-0280
- 0045-0295
- 0046-0294

Results

Label	Area	Mean	StdDev	Mode	Min	Max	X	Y	XM	YM	Perrim	BX	BY	Width	Height	Major	Minor	Angle	Circ.	Feret
259 Mask of ND3 adv 1-1.tif	1890	255	0	255	255	255	394.294	944.466	394.294	944.466	181.279	372	917	46	55	53.614	44.884	85.692	0.723	56.06
260 Mask of ND3 adv 1-1.tif	1904	255	0	255	255	255	163.296	943.906	163.296	943.906	177.966	138	918	50	51	50.215	48.278	76.516	0.755	52.63
261 Mask of ND3 adv 1-1.tif	1597	255	0	255	255	255	495.876	942.154	495.876	942.154	158.752	474	919	44	48	47.669	42.656	78.721	0.796	49.76
262 Mask of ND3 adv 1-1.tif	1225	255	0	255	255	255	784.666	939.764	784.666	939.764	146.167	764	919	40	41	40.279	38.723	93.079	0.721	43.35
263 Mask of ND3 adv 1-1.tif	1166	255	0	255	255	255	345.174	943.269	345.174	943.269	138.752	327	922	37	43	42.642	34.816	85.762	0.761	43.56
264 Mask of ND3 adv 1-1.tif	1365	255	0	255	255	255	734.180	945.067	734.180	945.067	146.510	714	922	39	46	45.702	38.028	93.517	0.799	47.12
265 Mask of ND3 adv 1-1.tif	1203	255	0	255	255	255	979.563	948.934	979.563	948.934	141.238	960	927	39	43	40.622	37.706	105.391	0.758	43.10
266 Mask of ND3 adv 1-1.tif	1698	255	0	255	255	255	841.902	954.517	841.902	954.517	170.066	819	928	46	53	50.714	42.631	97.485	0.738	54.57
267 Mask of ND3 adv 1-1.tif	1680	255	0	255	255	255	293.488	955.145	293.488	955.145	168.551	271	930	45	50	49.773	42.976	91.635	0.743	51.54
268 Mask of ND3 adv 1-1.tif	1426	255	0	255	255	255	544.176	956.701	544.176	956.701	154.409	523	933	43	47	45.116	40.244	68.758	0.752	47.38

