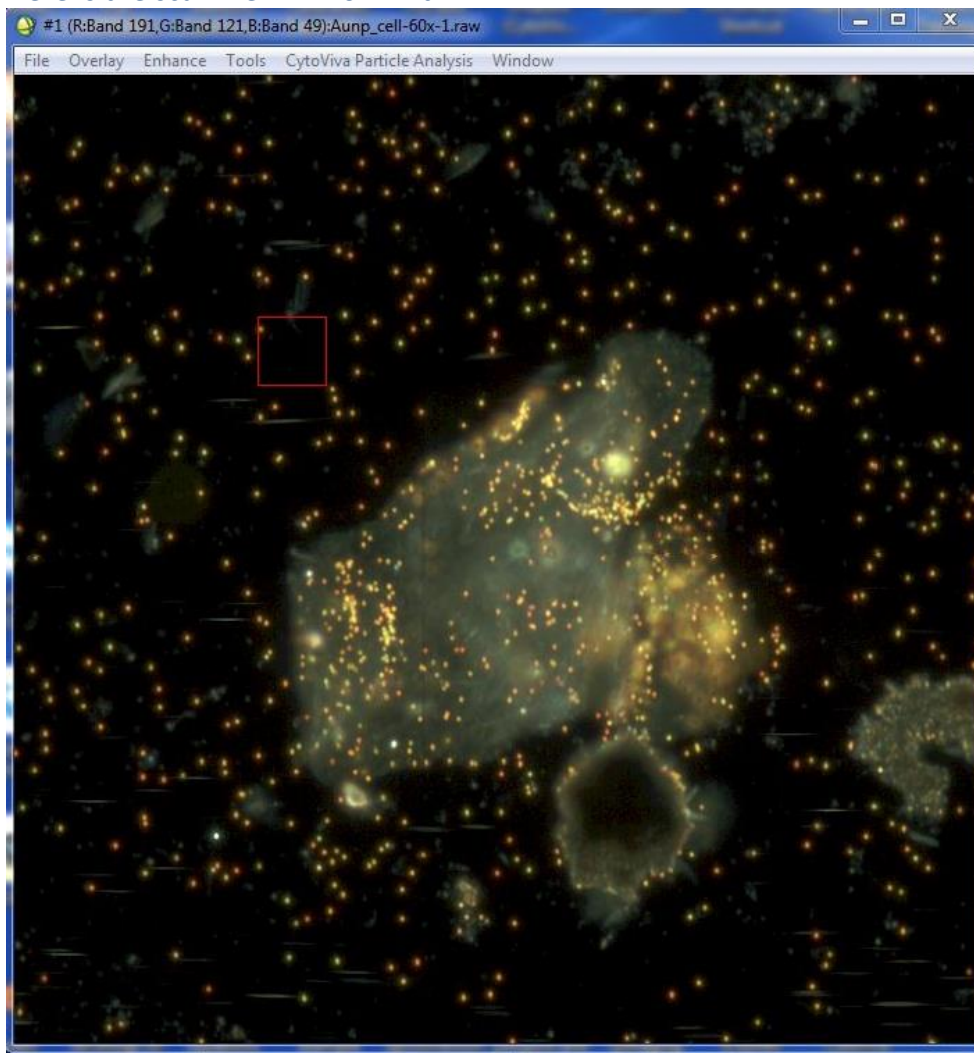


Creating a Mask in ENVI

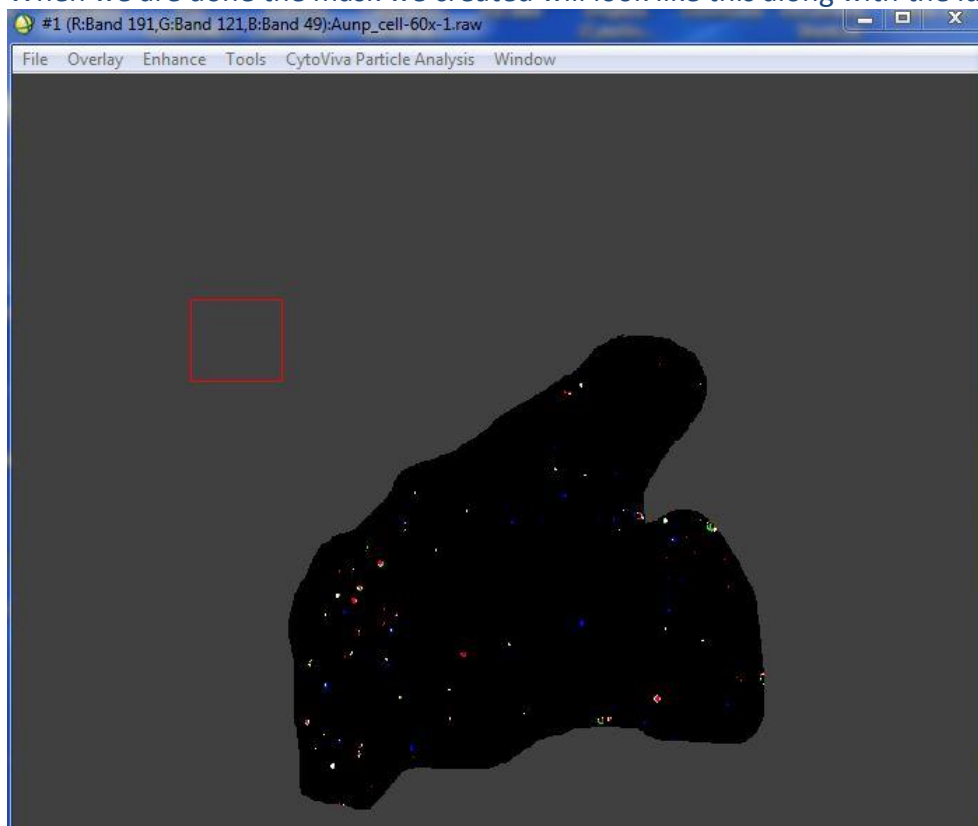
When mapping particles or materials of interest you may only want to see them and find their distribution within a certain area, like a cell or area of interest. This can be accomplished by creating what's called a "Mask" in ENVI.

In the example below we are going to only find gold nanoparticles within the area of a cell even though there are obviously particles outside the cell. What we will do is create a mask that will only show the cell and ignore the areas outside of the cell. Then we will use Spectral Angle Mapper to identify particles only within the masked area.

Here is the scan we will work with:

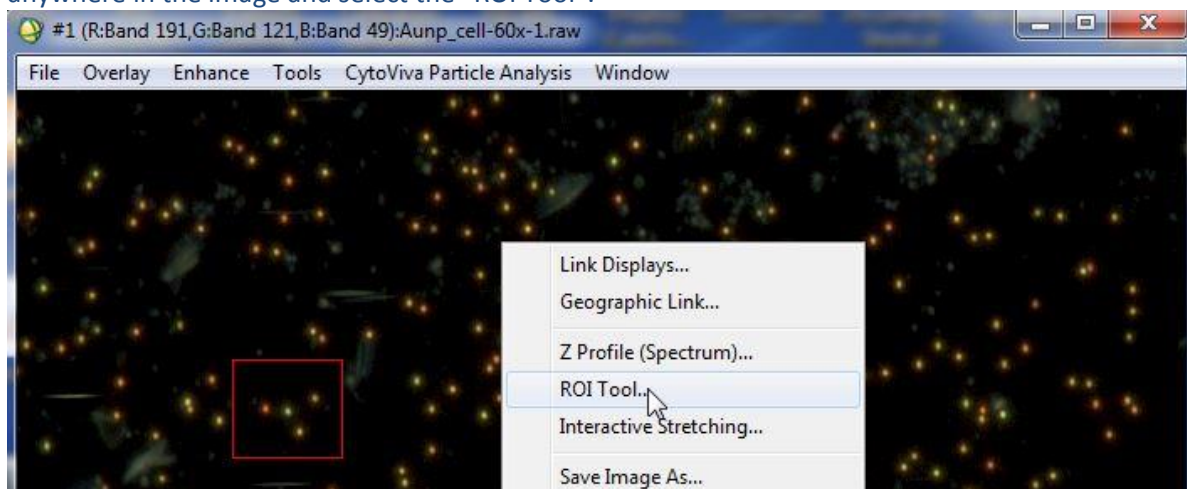


When we are done the mask we created will look like this along with the identified/mapped pixels:

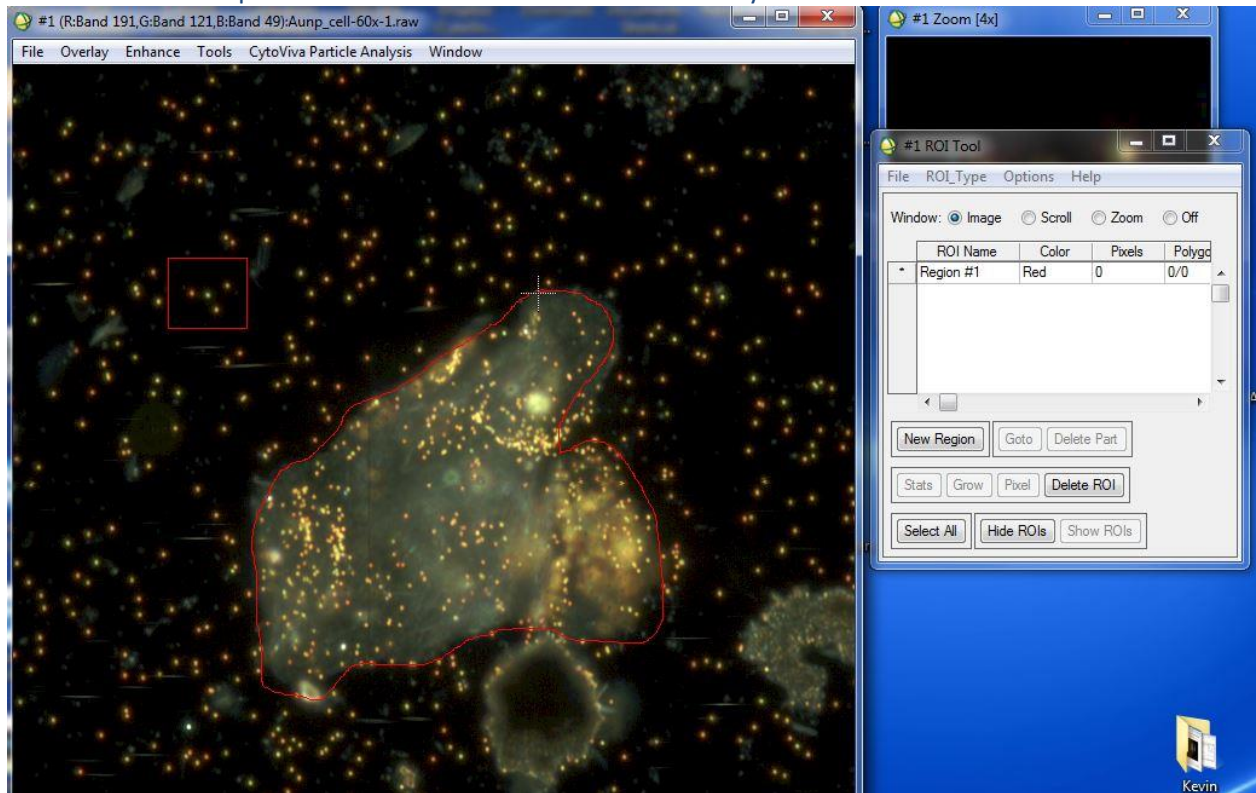


The Steps to create this mask are as follows:

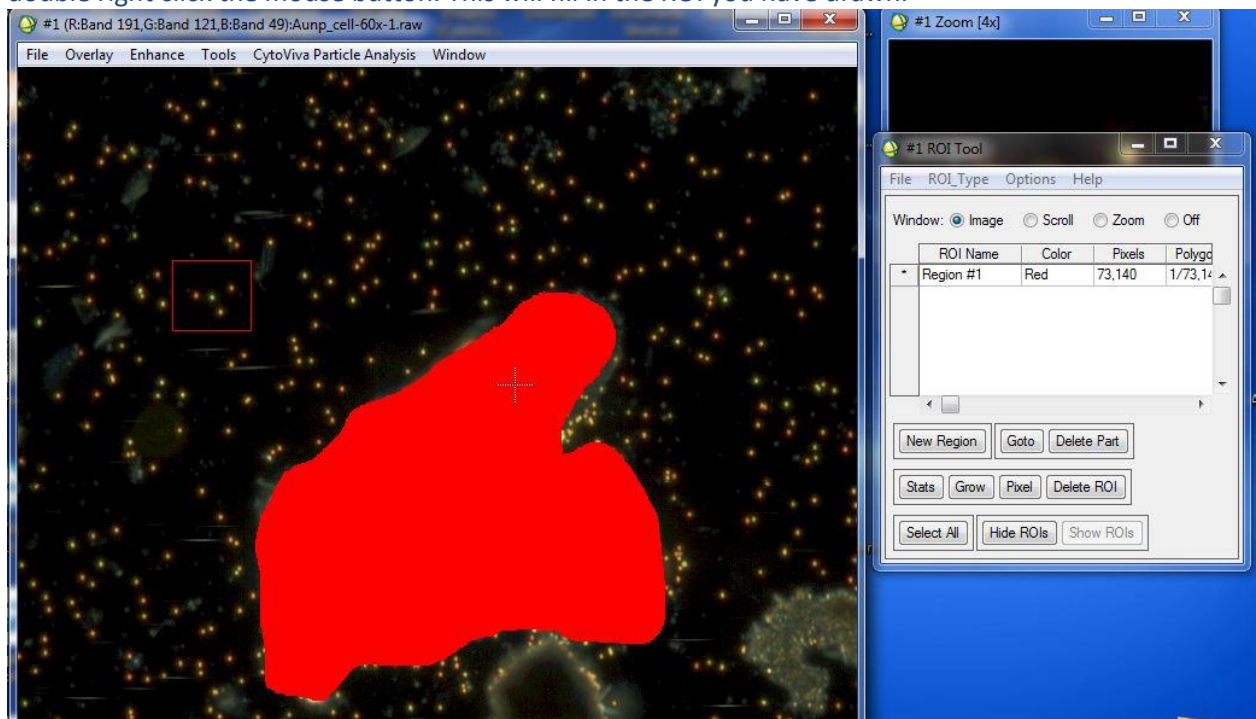
1. Start building the mask by first drawing a region of interest around the cell. Do this by right clicking anywhere in the image and select the "ROI Tool".



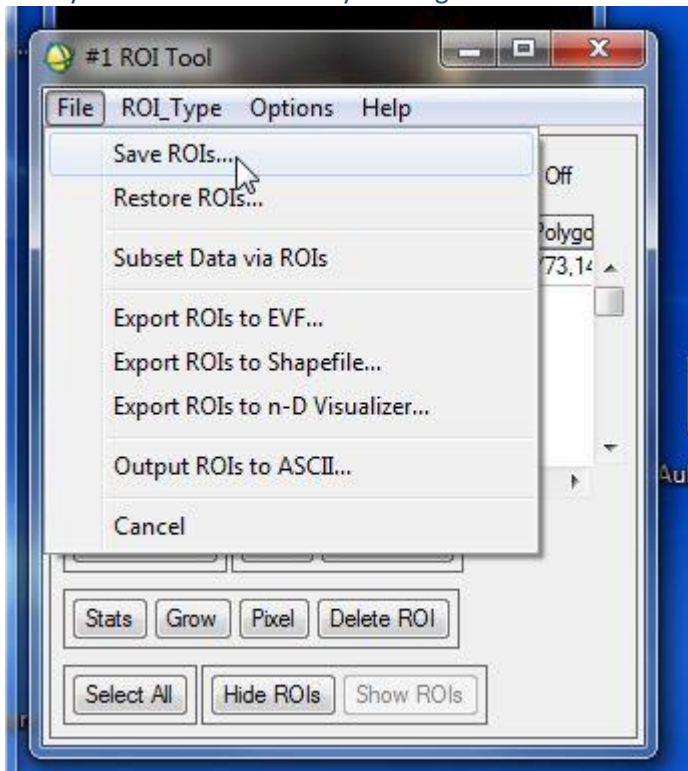
2. With the ROI tool open left click on the mouse and hold while you draw an area around the cells border.



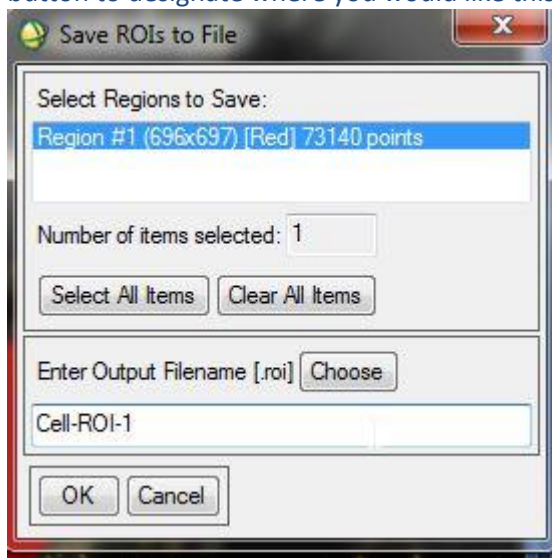
3. When you have completed drawing around the cells border release the left click button on the mouse and double right click the mouse button. This will fill in the ROI you have drawn.



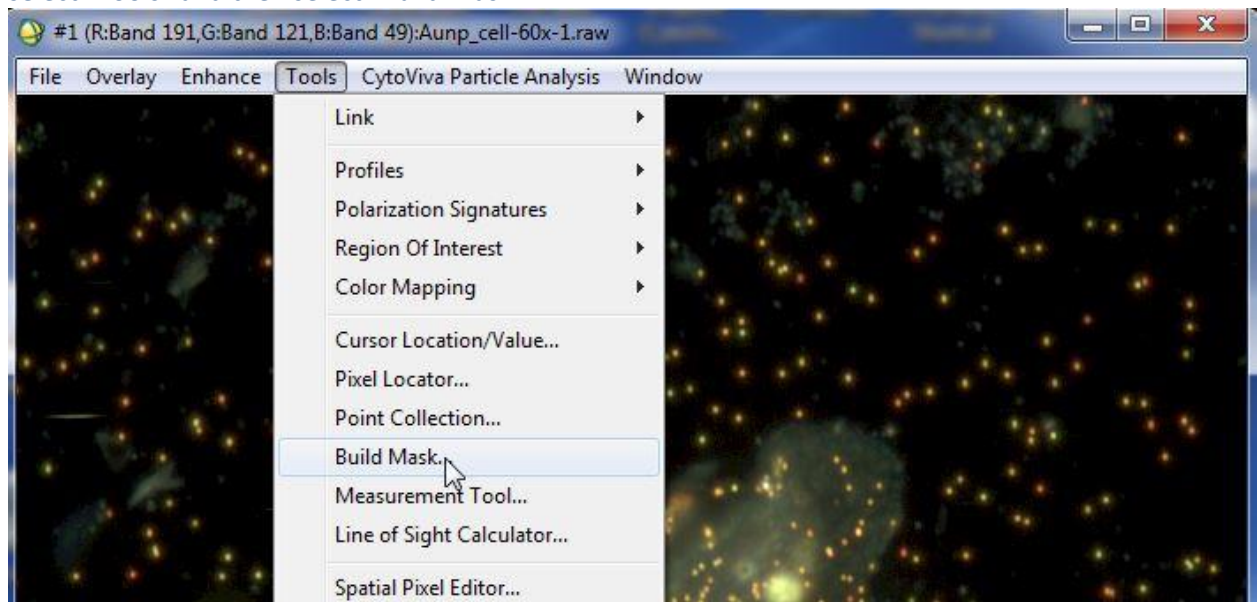
4. Now you will save this ROI by clicking on “File” and then “Save ROIs...” in the “ROI Tool” menu window.



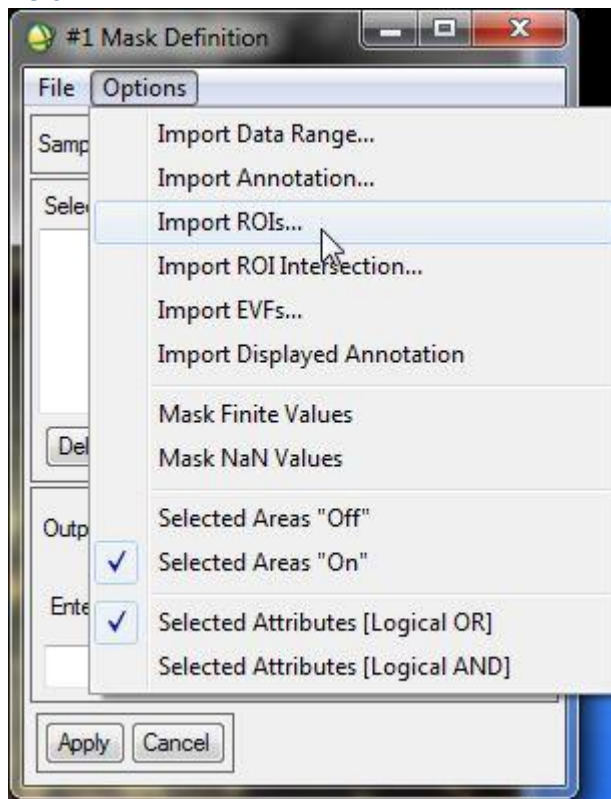
5. Then highlight the Region you have drawn and name it in the “Save ROIs to File” dialog. Click “Choose” button to designate where you would like this ROI to be saved. Then select the “OK” button to save it.



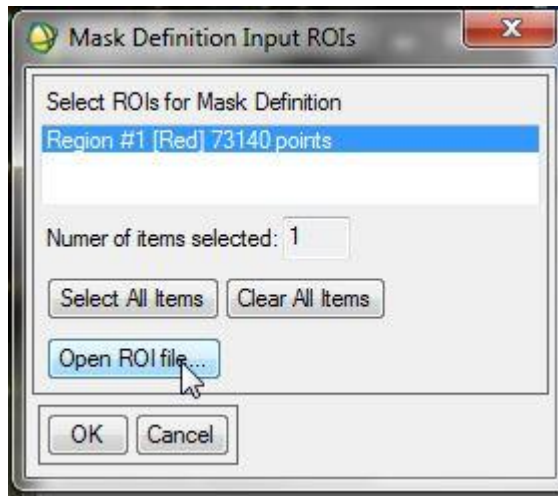
6. Now we will create the Mask from the ROI you have just created. In the main image window tool bar select “Tools” and then select “Build Mask”.



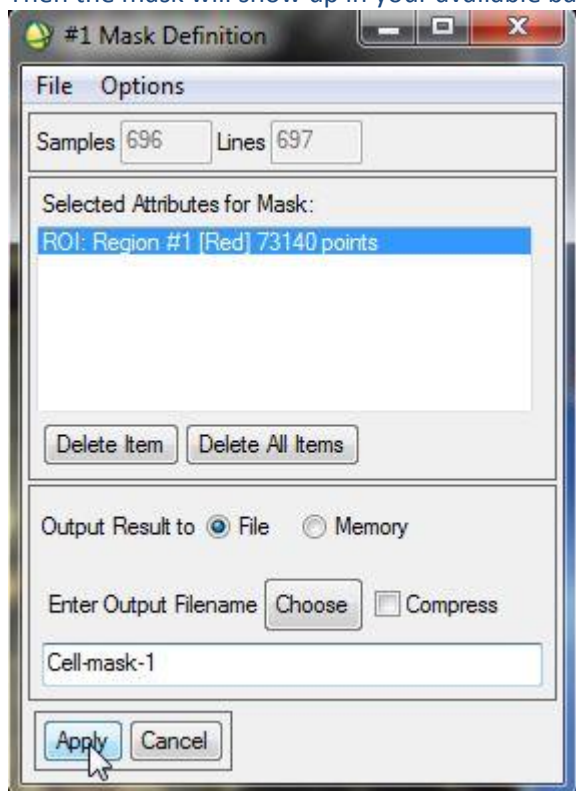
7. The “Mask Definitions” dialogue window will open. In this window select “Options” and then “Import ROIs...”.

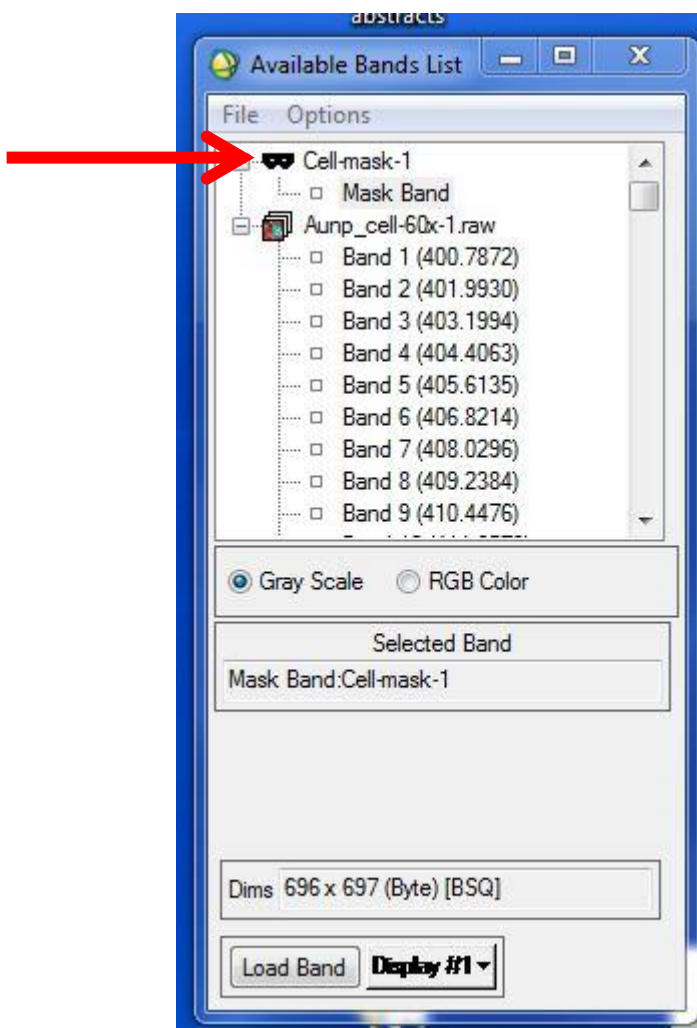


8. The “Mask Definition Input ROIs” dialogue window will open. Here you can either select the ROI you have just drawn because it is still technically open, or you can click the “Open ROI file..” button and navigate to ROI you have saved/named earlier in Step 5. Click “OK”.

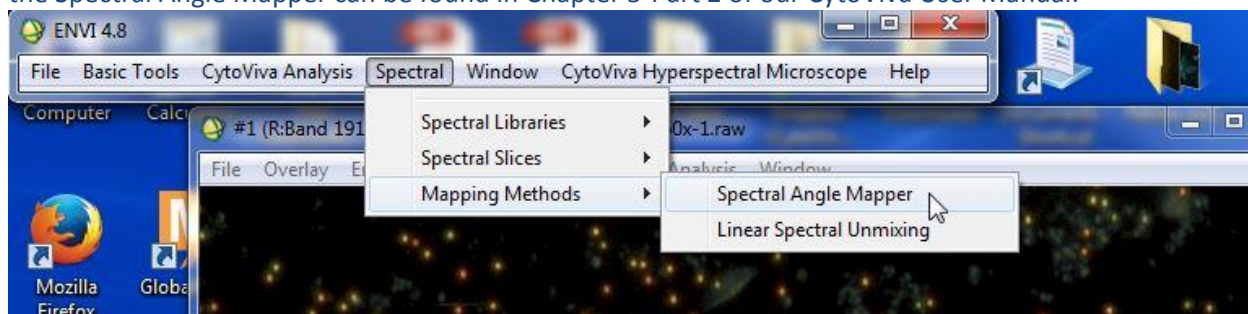


9. After clicking “OK” you will name your mask, choose where to save the file and click the “Apply” button. Then the mask will show up in your available bands list.

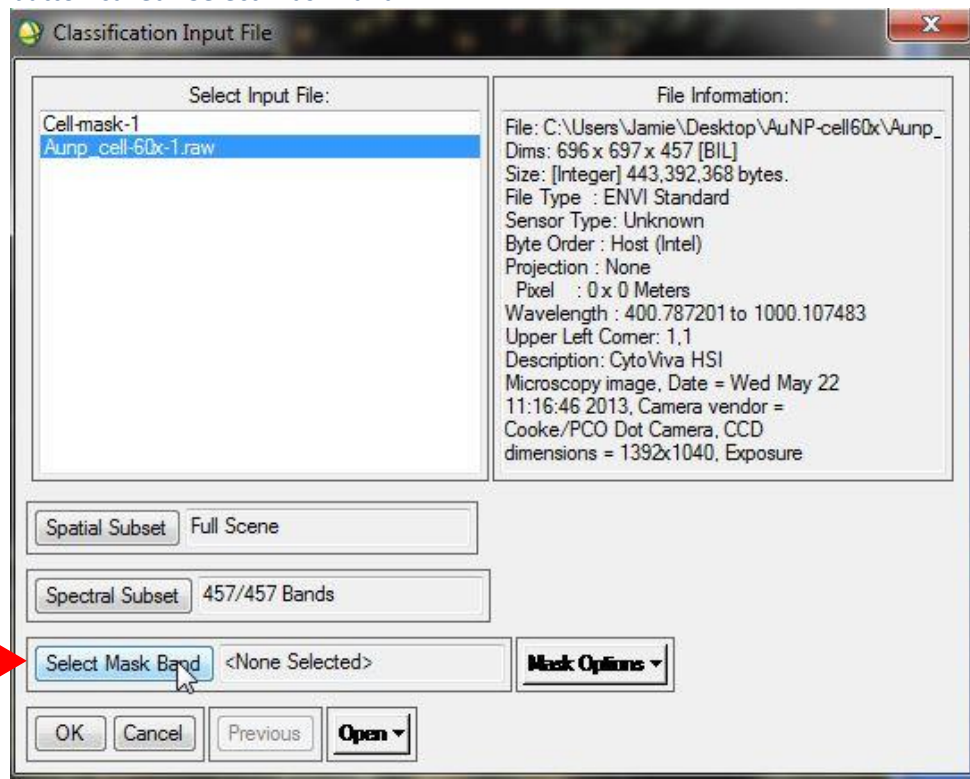




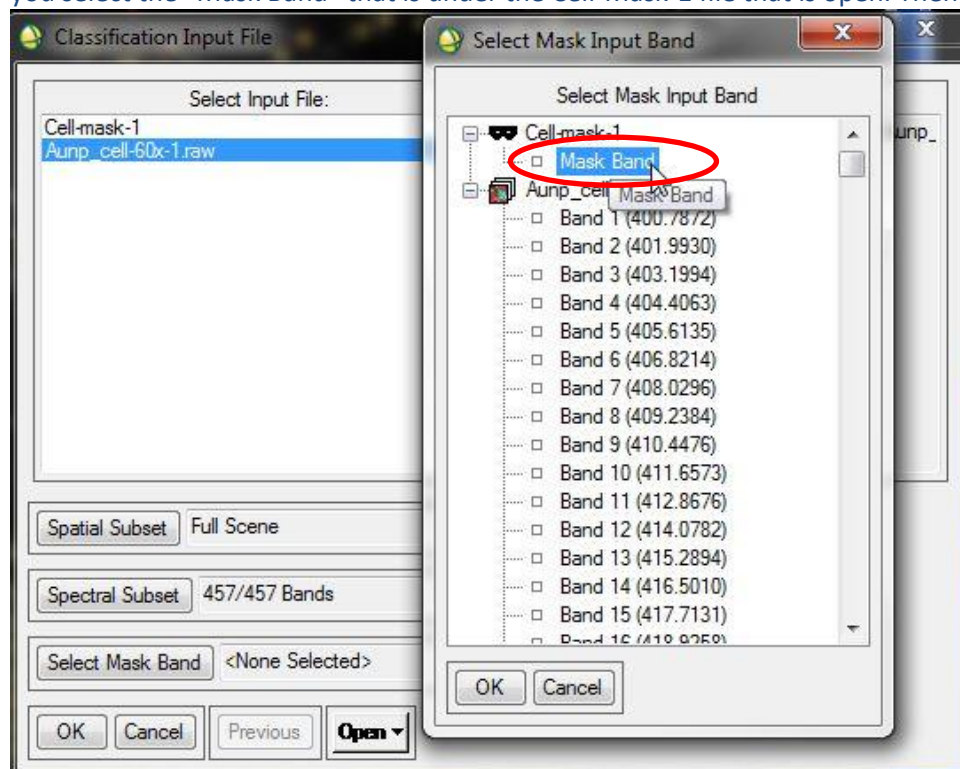
10. The next step will be to actually run your Spectral Angle Mapper (SAM) routine. In the main ENVI Tool bar go to “Spectral” and the “Mapping Methods” and choose “Spectral Angle Mapper”. Instructions on using the Spectral Angle Mapper can be found in Chapter 5-Part 2 of our CytoViva User Manual.



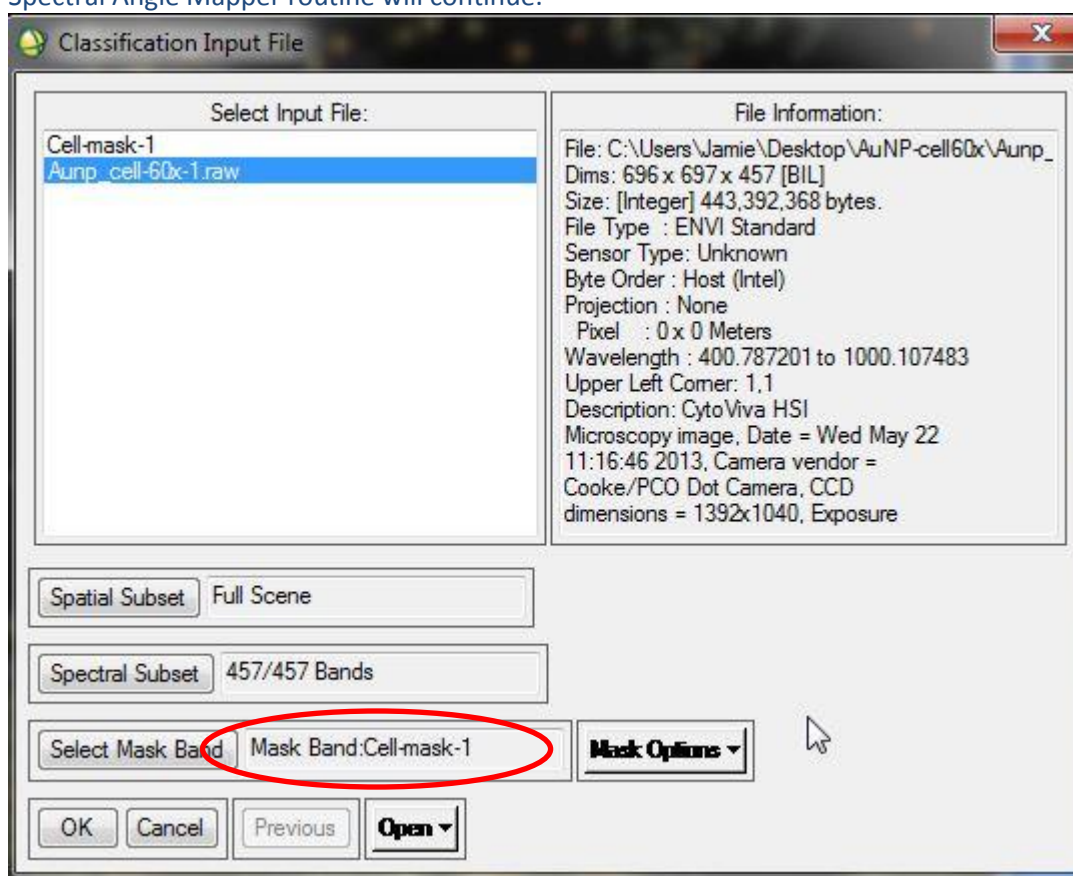
11. When the “Classification Input File” dialog opens you highlight the file you will run SAM on. In this case our file is called “Aunp-cell-60x-1”. You will also notice at the bottom of the dialogue window there is a button called “Select Mask Band”.



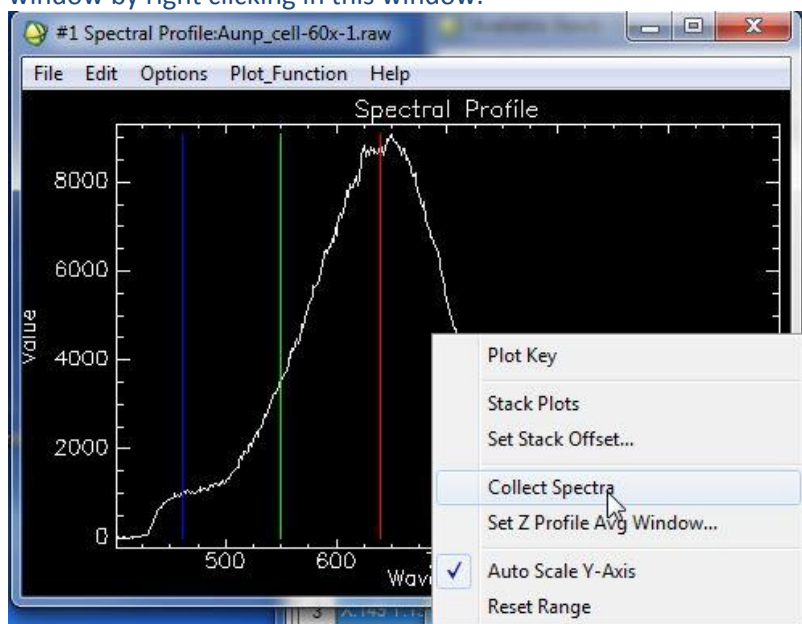
12. Click the “Select Mask Band” button and the “Select Mask Input Band” dialogue will appear. Make sure you select the “Mask Band” that is under the Cell-Mask-1 file that is open. Then Hit “OK”.



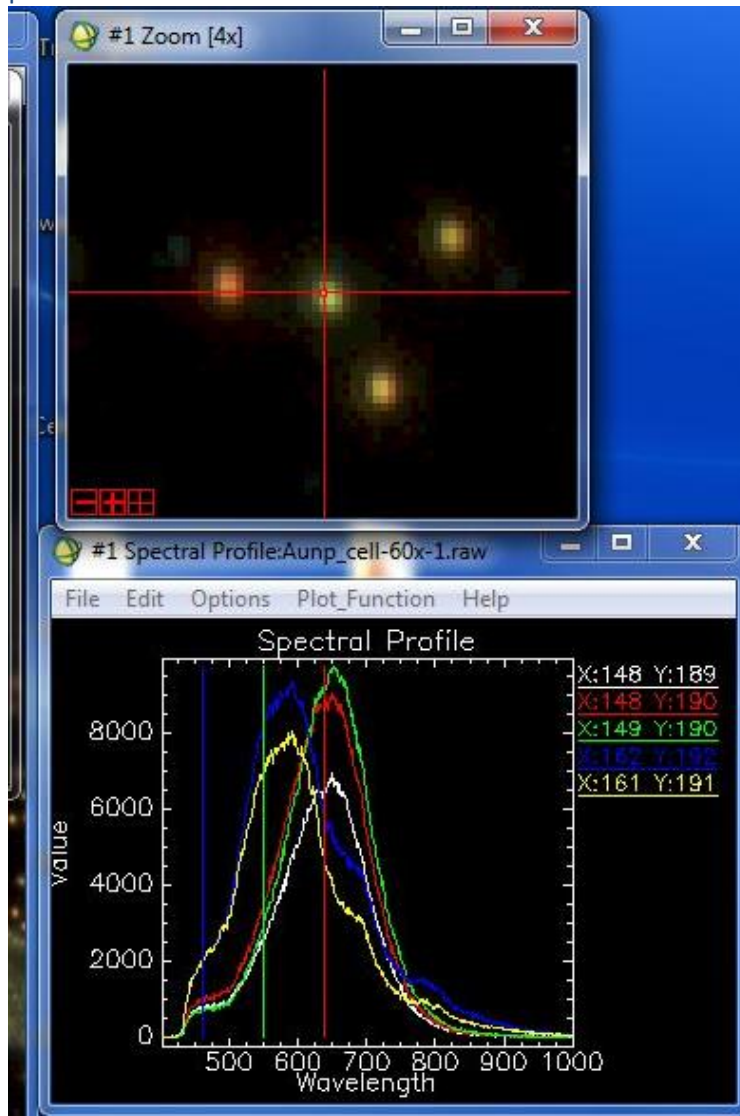
13. Now you will see the Mask Band show up in the “Select Mask Band” setting. Now click “OK” and the Spectral Angle Mapper routine will continue.



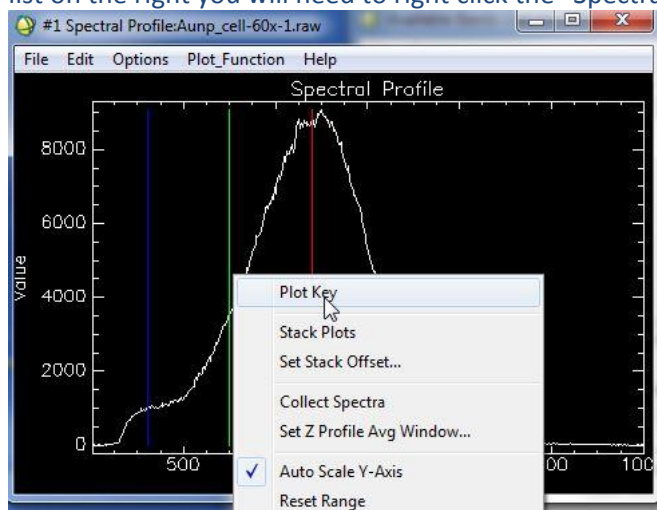
14. Next the “Endmember Collection: SAM” dialogue box opens. This is where we add spectra to be searched for by the SAM algorithm. For this example I have collected some spectra from a couple of particles outside the cell. I did this by using the “Collect Spectra” function accessed from the “Spectral Profile” window by right clicking in this window.



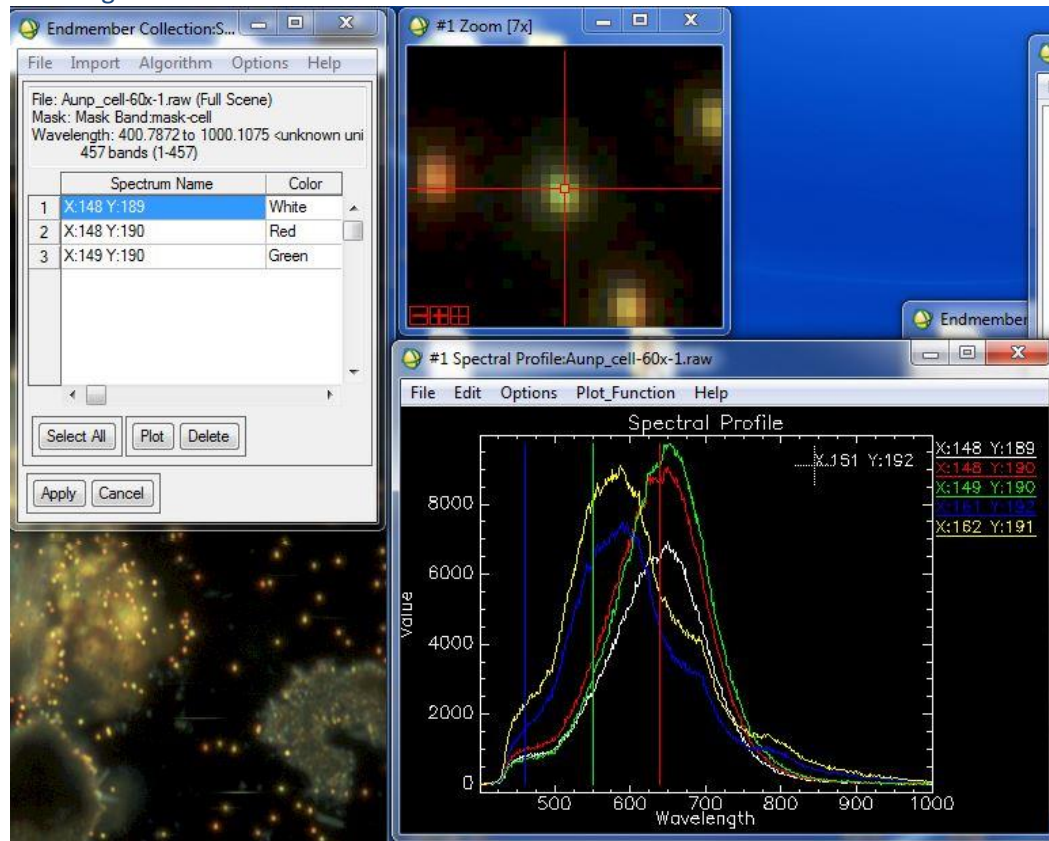
By clicking on individual pixels within particles you can now collect many spectra in your “Spectral Profile” plot.



15. Now we can add these collected spectra to our “Endmember Collection: SAM” dialogue box by holding down the left mouse button and dragging on them from the “Spectral Profile” window. To see the spectra list on the right you will need to right click the “Spectral Profile” window and select “Plot Key”.



Then click and drag the spectra in plot key list to the “Endmember Collection: SAM” window as seen in the image below.



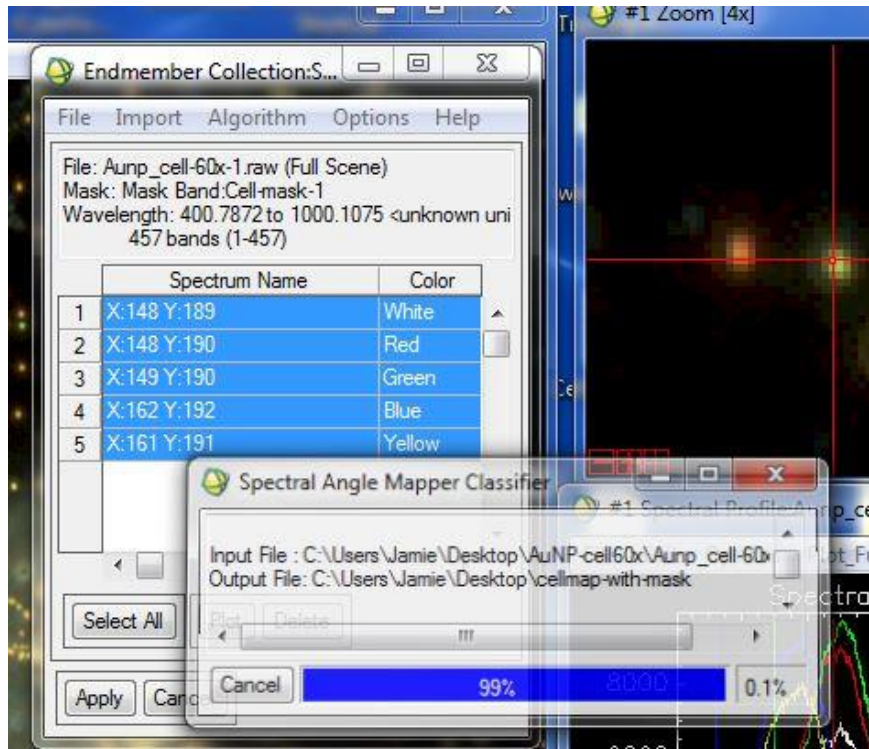
- When all the spectra have been added hit the “Apply” button and the “Spectral Angle Mapper Parameters” window will open. Here you will leave the “Maximum Angle (radians)” setting to 0.100. For “Enter Output Filename” name the file “cellmap-with-mask”, and for “Output Rule Images?” choose “No” by hitting the up and down arrows next to it.

The image shows the 'Spectral Angle Mapper Parameters' window. It contains the following settings:

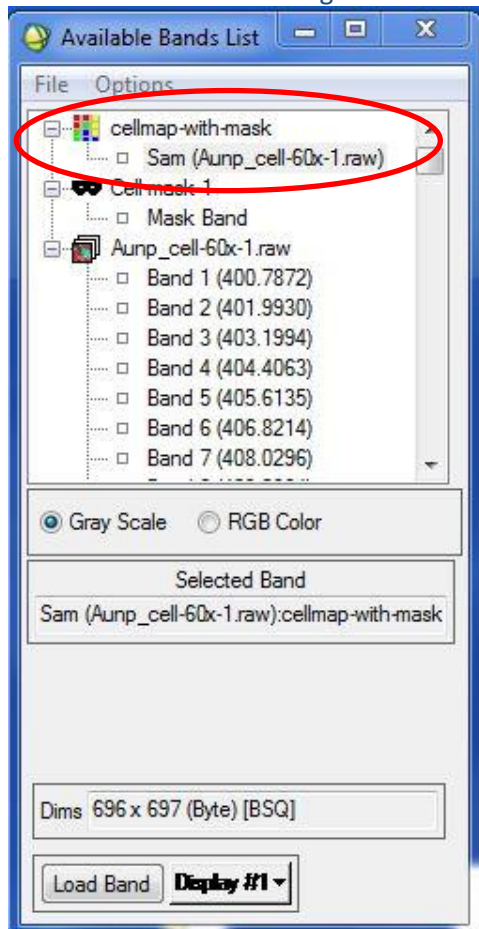
- Set Maximum Angle (radians):**
 - ☐ None
 - ☒ Single Value
 - ☐ Multiple Values
 - Maximum Angle (radians): 0.100
- Output Result to:**
 - ☒ File
 - ☐ Memory
- Enter Output Filename:**
 - Choose
 - Compress
 - cellmap-with-mask
- Output Rule Images ?** No

At the bottom are buttons for OK, Queue, Cancel, Help, and Preview.

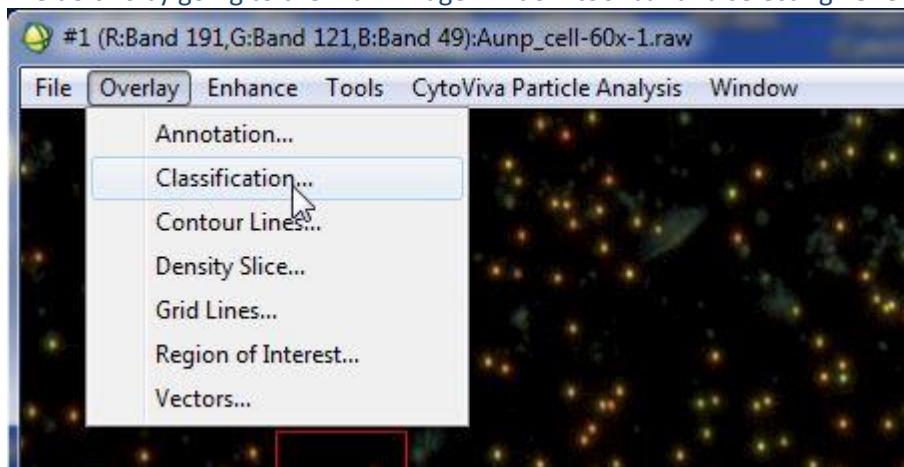
17. Select “OK” to run SAM.



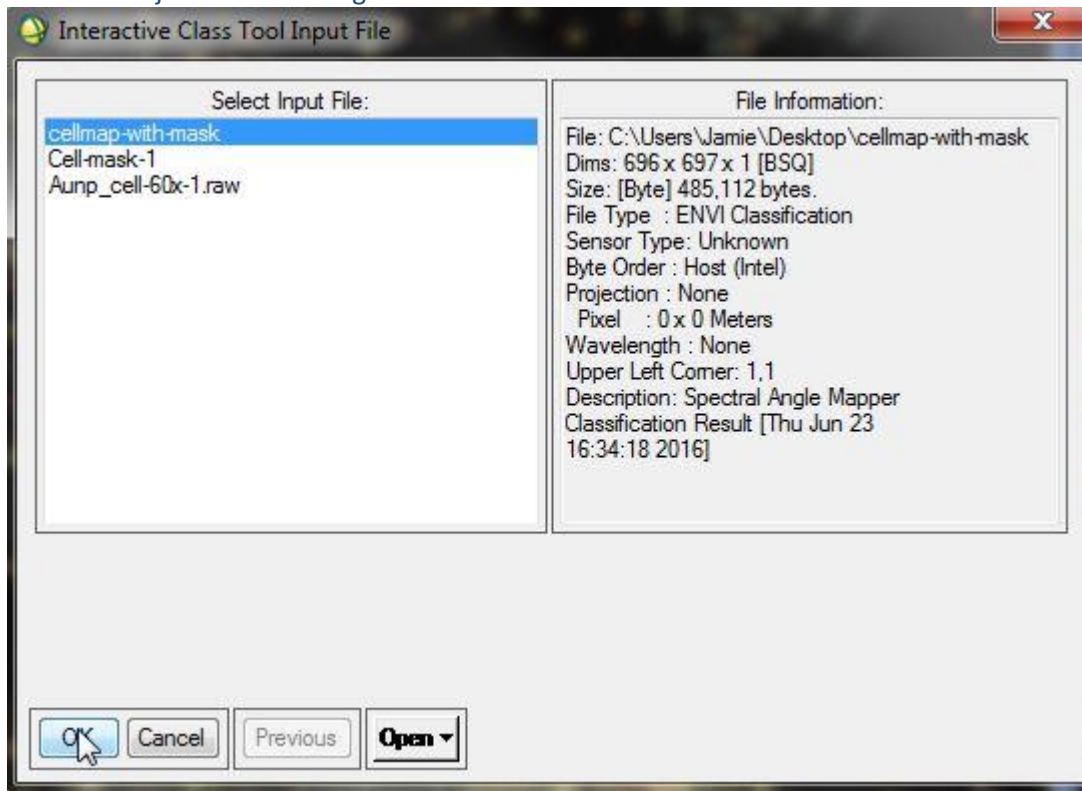
18. When SAM is done running the classification file it produces will show up in the “Available Bands List”.



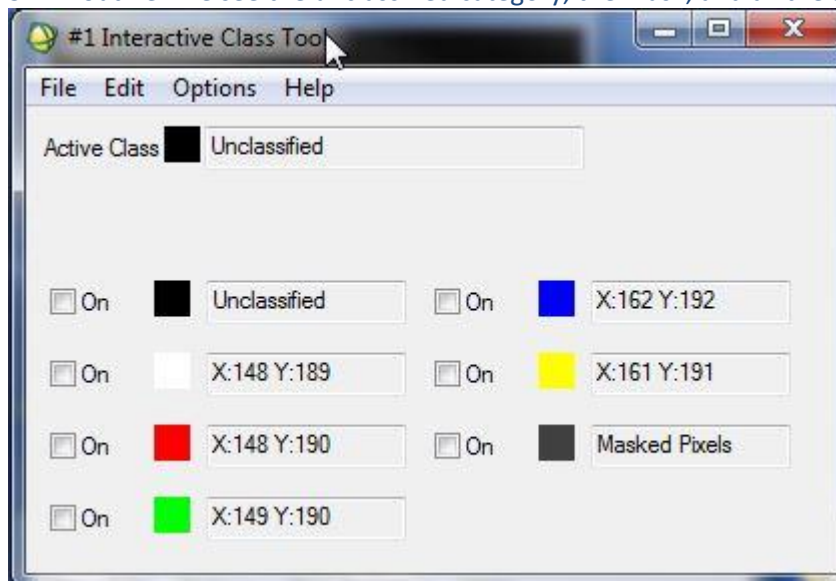
19. Now we will overlay the SAM file (it is technically a classification file) over the scan that includes the mask. We do this by going to the main image window tool bar and selecting “Overlay” and then “Classification”.



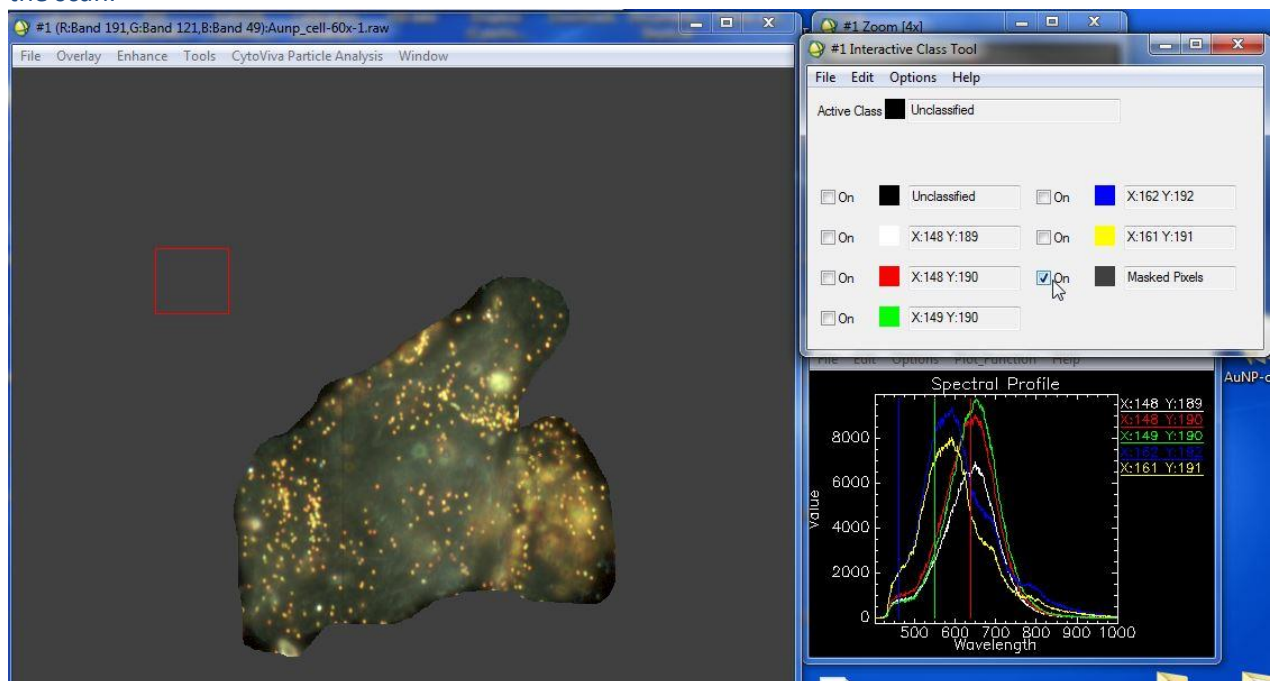
20. This opens the “Interactive Class Tool Input File” dialogue. Here we will select the “cellmap-with-mask” SAM file we just created using SAM and click the “OK”.



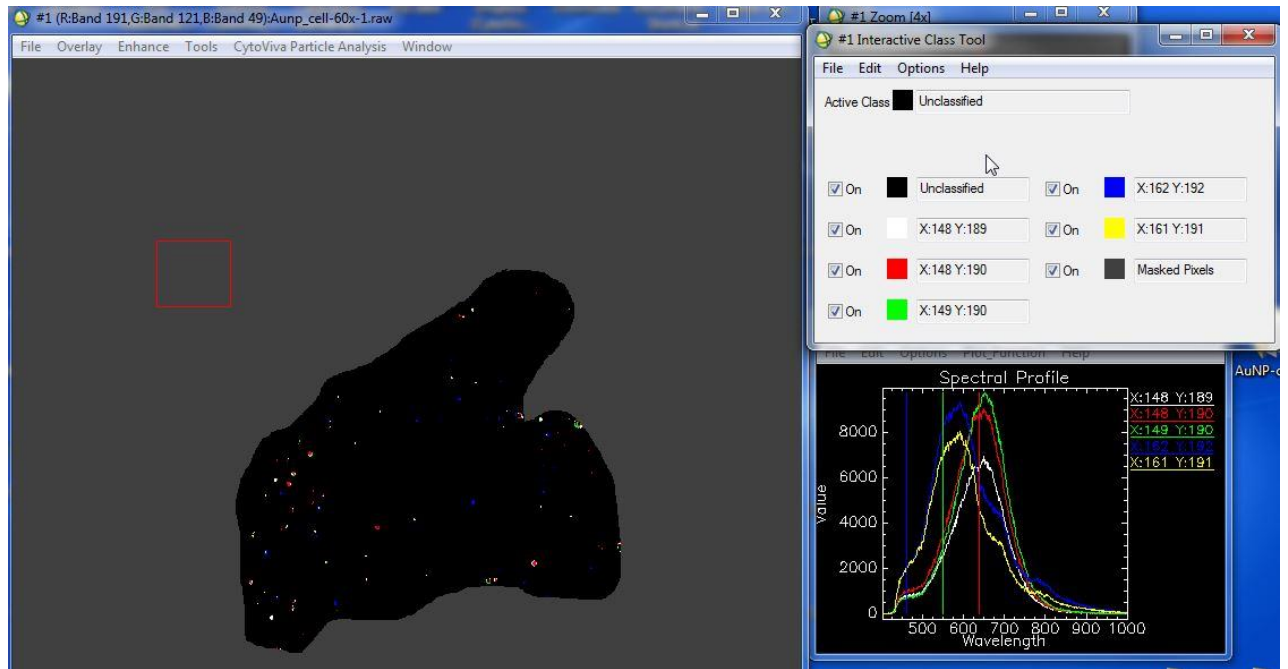
21. Now the “Interactive Class Tool” dialogue opens and we see all of the classified components from the SAM routine. We see the unclassified category, the mask, and all the spectra we chose.



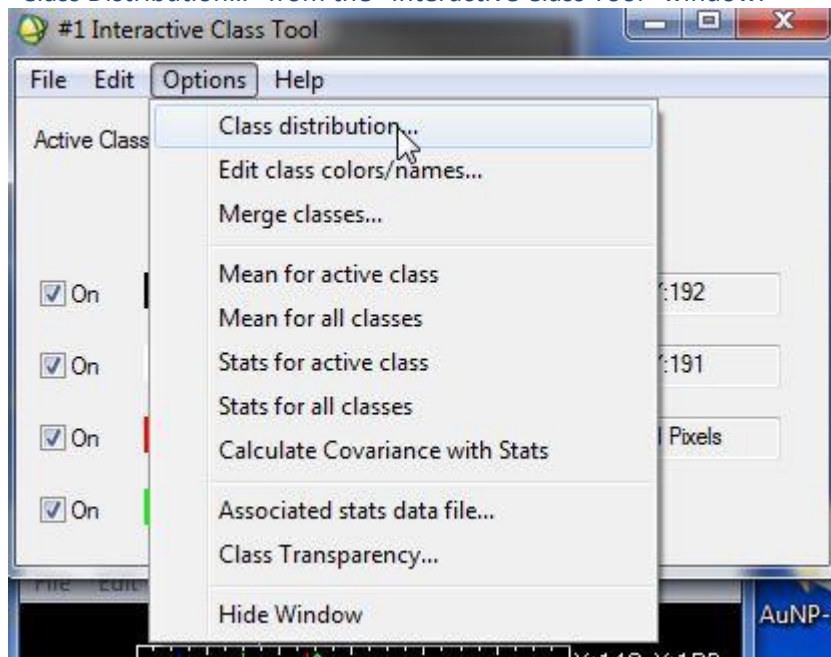
22. If we check the “On” box for just the masked pixels we will see the mask we have created overlayed onto the scan.



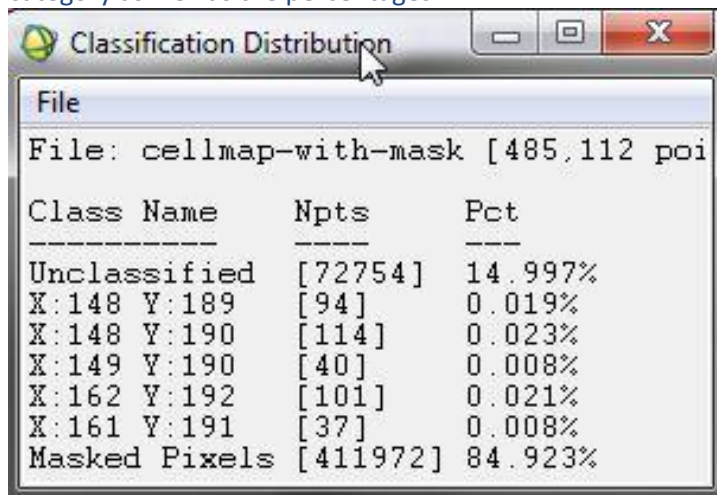
If we then click and turn on all the rest of the components we will see the classified spectra as different colors and the unclassified data as black.



23. To gain some more quantitative information we can compare the distributions of what is classified to what is not classified only within the bounds of the cell. We can see this by going to “Options” and then “Class Distribution...” from the “Interactive Class Tool” window.



24. Choosing this will open up the “Class Distribution” window. This shows the number of pixels for each category as well as the percentages.



File		
File: cellmap-with-mask [485,112 poi		
Class Name	Npts	Pct
Unclassified	[72754]	14.997%
X:148 Y:189	[94]	0.019%
X:148 Y:190	[114]	0.023%
X:149 Y:190	[40]	0.008%
X:162 Y:192	[101]	0.021%
X:161 Y:191	[37]	0.008%
Masked Pixels	[411972]	84.923%

Now we have to do a little calculation to see how many pixels were classified within the cells borders. To do this we will take the total # of pixels in the image which is shown in the first line.

For this scan there are a total of 485,112 pixels.

The Mask we created is 411,972 pixels

So the area of the cell is the difference: so $485,112 - 411,972 = 73,140$ pixels

So now we know the cells area is 73,140 pixels

Now we use this 73,140 as our 100% to calculate the percentage of mapped particle spectra in the cell.

For example spectra **x:162 y:192** contains 101 pixels within the masked area. So we divide the 101 classified pixels by the total pixel area of the cell to get the percentage of classified pixels for this spectra:

$$101 \text{ pixels of spectra } \mathbf{x:162 y:192} / 73,140 \text{ total cell area pixels} = .0013\%$$

This method can be used to calculate the total percentage within a defined area of mapped pixel area.