IMAGE J OVERLAYS

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3:54 PM

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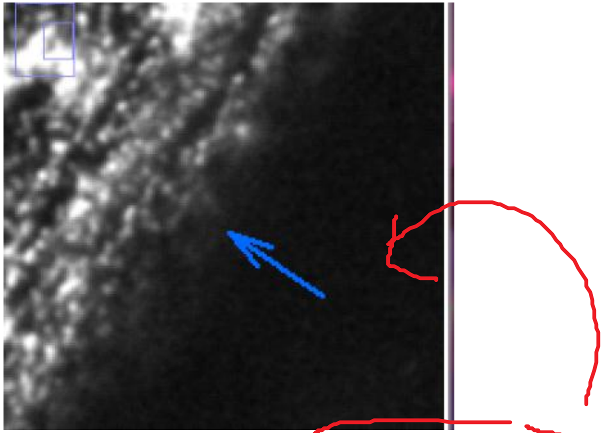
USING OVERLAYS TO DRAW THINGS ON IMAGES USING IMAGEJ

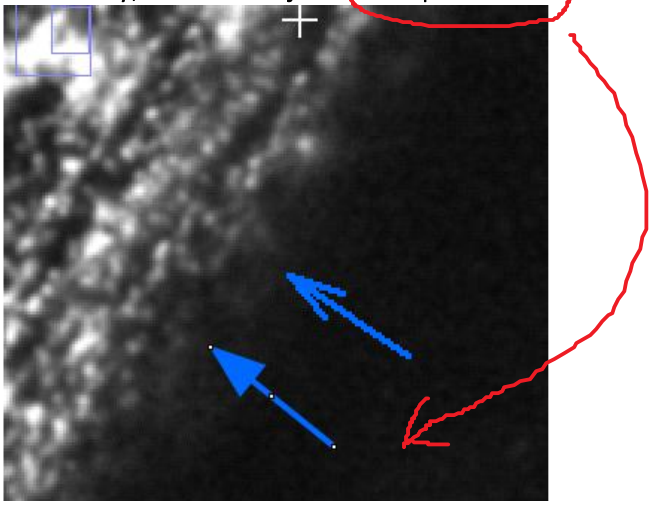
Image processing can be either destructive (i.e. the modified pixel value(s) are irreversibly changed), or non-destructive (i.e. the image will look different but the pixel values are unchanged). An example of destructive imaging using image j is drawing an object (e.g. arrow, box, etc) and hitting 'draw' (control+D). An example of non-destructive manipulation is adjusting brightness and contrast. We often need to point things out in our images and movies with arrows, boxes, and other shapes. This is best performed in imagej using overlays, which are non-destructive changes to an image.

ImageJ in particular, does a really bad job using the destructive 'draw' function:



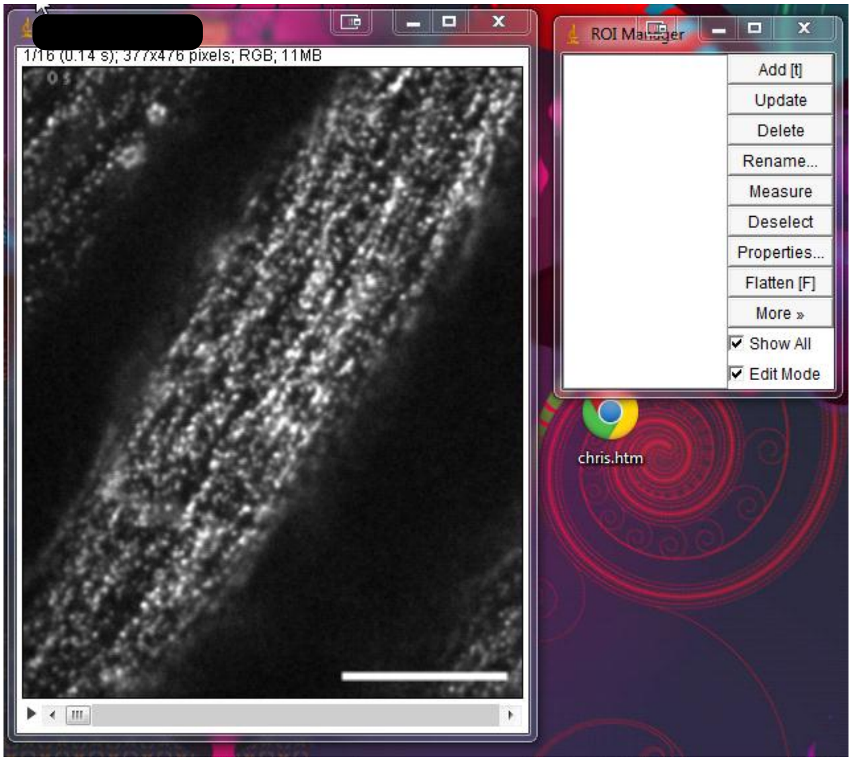
This arrow I drew on here is very pixelated, and if I were to track an object using this method, I would have to redraw the arrow from scratch for each timepoint, which results in a goofy arrow that changes angles and sizes each slice.

In contrast, imagej is made for overlays. Overlays can use the same exact object as many times as necessary, and the object is not pixelated:



TO ADD OVERLAYS TO TIME SERIES IN IMAGEJ:

1. Open t series and convert to RGB (image>>type>>RGB color)
2. Open ROI manager (analyze>>tools>>roi manager). In roi manager, make sure 'show all' and 'edit mode' are checked.



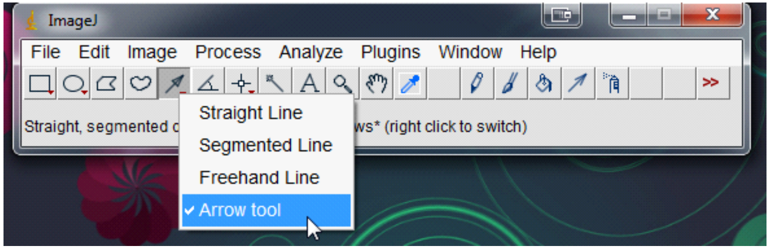
[redacted]

1/16 (0.14 s); 377x476 pixels; RGB; 11MB

☑ Show All

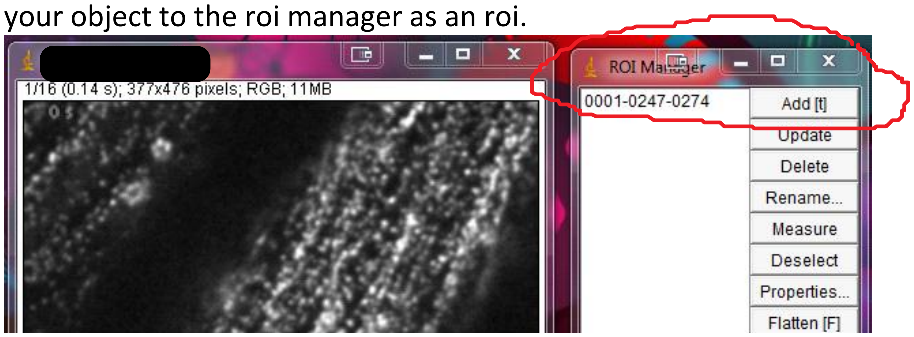
☑ Edit Mode

3. Select a drawing tool of any type (ex. Arrow, line, box etc.)



Straight, segmented o... ws* (right click to switch)

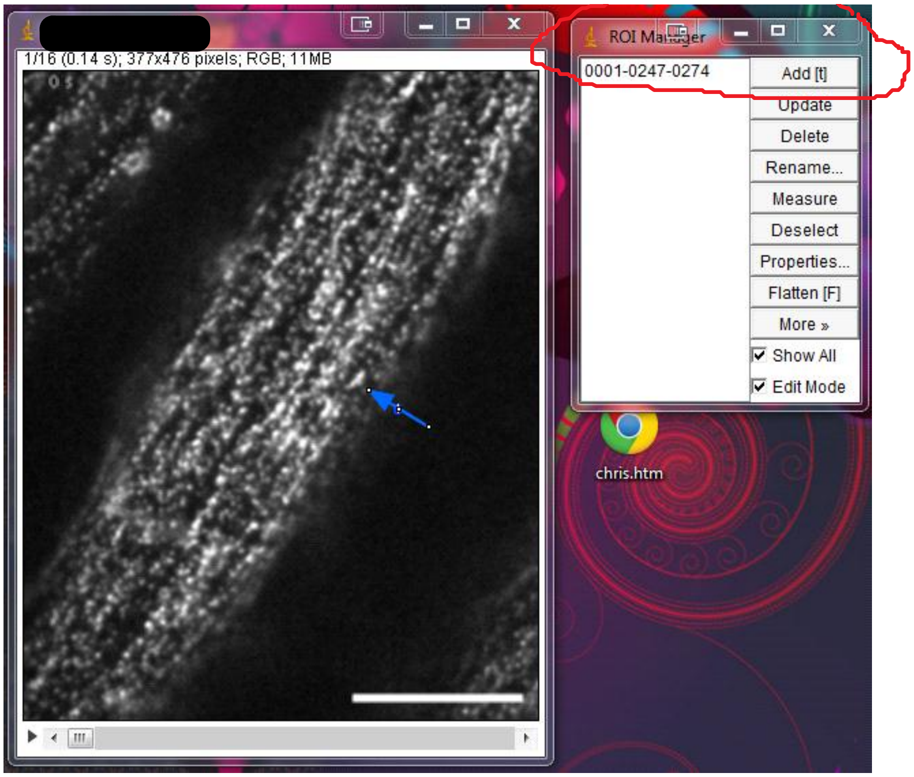
4. Go to first slice you wish to label, and draw your object where you want it, then hit 't'. This adds your object to the roi manager as an roi.



[redacted]

1/16 (0.14 s); 377x476 pixels; RGB; 11MB

0001-0247-0274

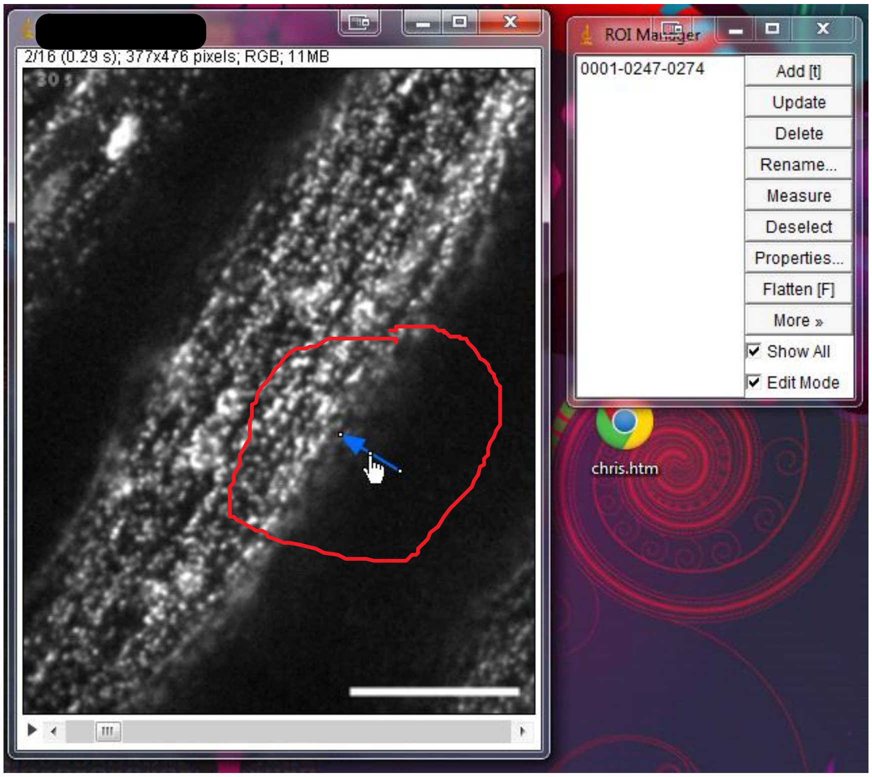


[redacted]

1/16 (0.14 s); 377x476 pixels; RGB; 11MB

0001-0247-0274

5. Now move to the next t slice (you can use the '>' key)
6. Grab the object and move it to its next position (you can grab the object as long as it has the little dots indicating it is selected)

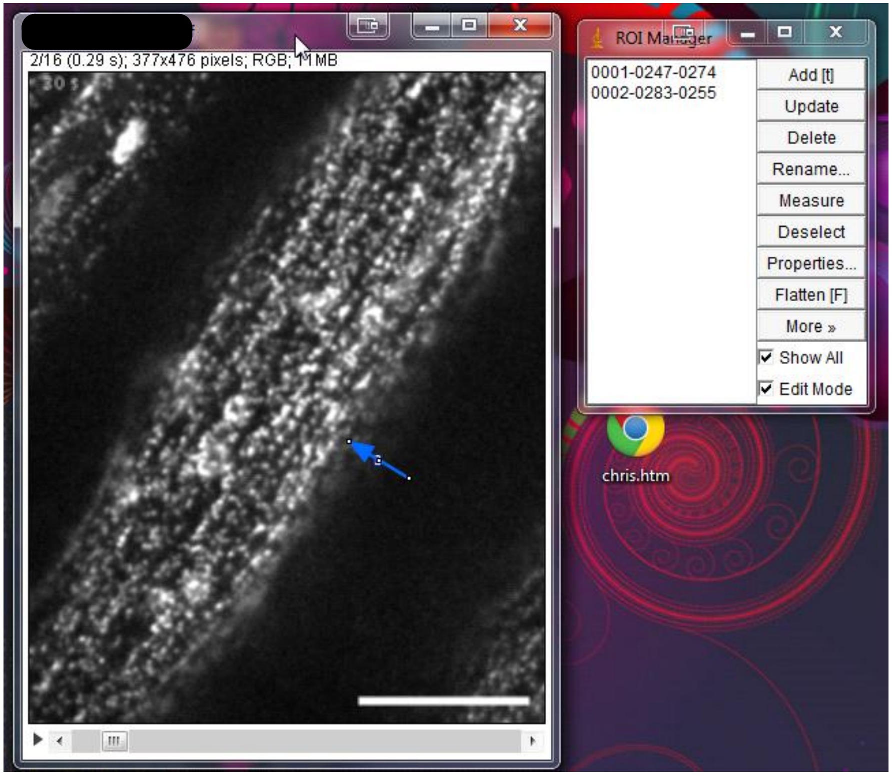


[redacted]

2/16 (0.29 s); 377x476 pixels; RGB; 11MB

0001-0247-0274

7. Again add selection to roi manager by hitting 't'



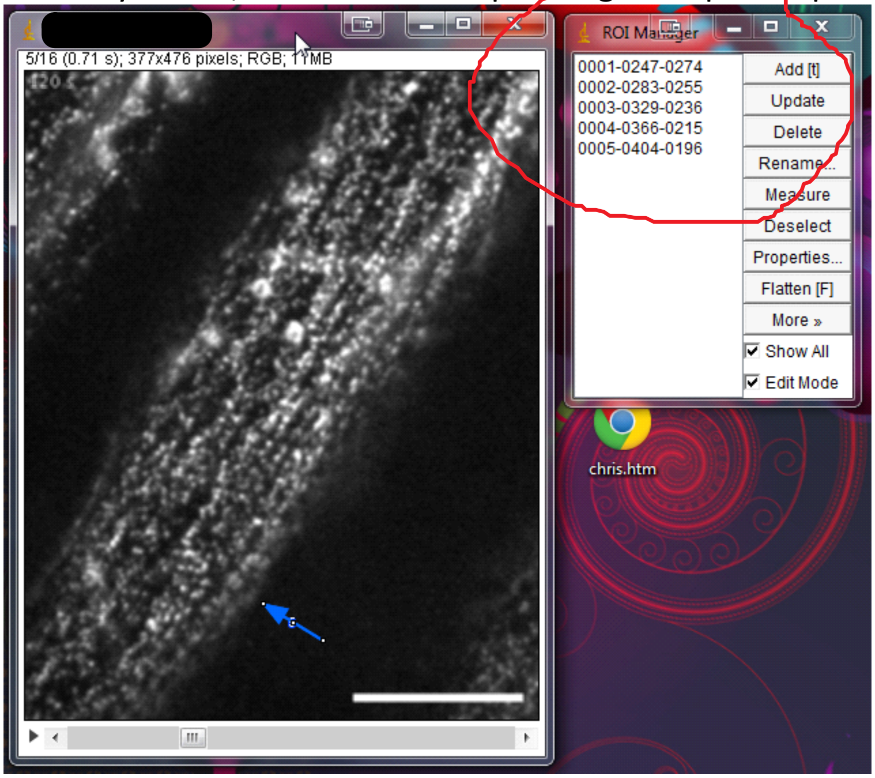
[redacted]

2/16 (0.29 s); 377x476 pixels; RGB; 11MB

ROI Manager

0001-0247-0274
0002-0283-0255

8. Now just repeat this for as many slices as you need. Here, I added a total of 5 ROIs from my arrow, each one corresponding to a specific position on a specific slice:



[redacted]

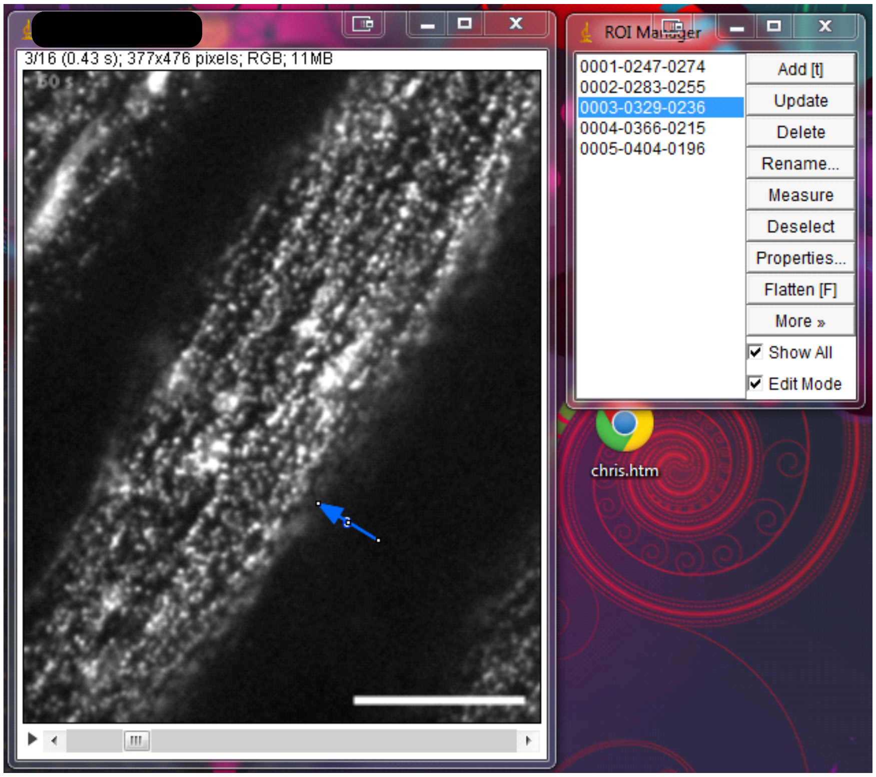
5/16 (0.71 s); 377x476 pixels; RGB; 11MB

ROI Manager

0001-0247-0274
0002-0283-0255
0003-0329-0236
0004-0366-0215
0005-0404-0196

9. Now, at this point, the objects are only ROIs, not overlays. So at this point, you can use the ROI manager to edit these ROIs as you like, such as change positions (just

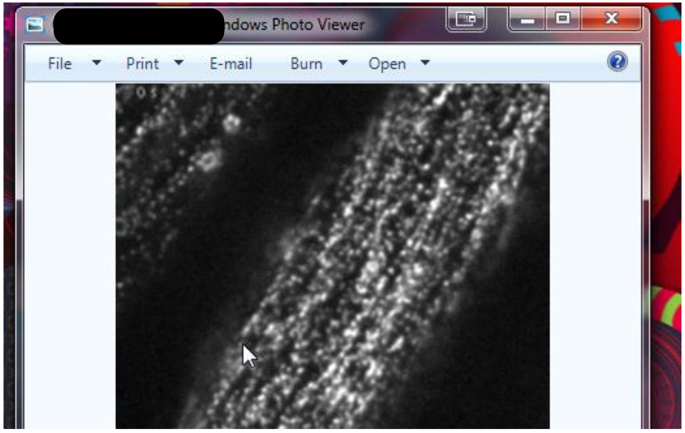
grab object, move, and hit 'update' on the ROI manager) or delete the objects ('delete' on the ROI manager). To select a given roi, just click its name in the roi manager, and it will go to the slice in the stack where that ROI is, and select it, so you can move it etc.

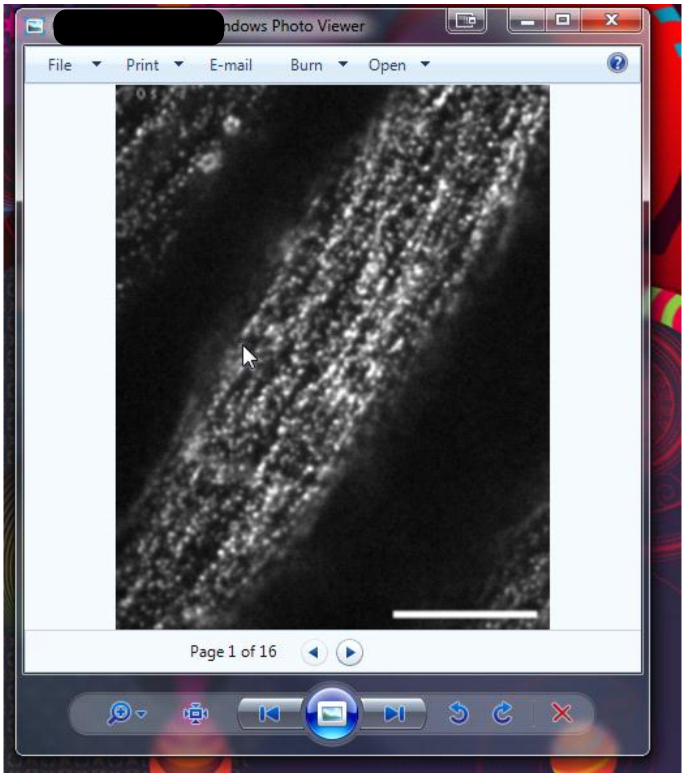

[redacted]

3/16 (0.43 s); 377x476 pixels; RGB; 11MB

0001-0247-0274
0002-0283-0255
0003-0329-0236
0004-0366-0215
0005-0404-0196

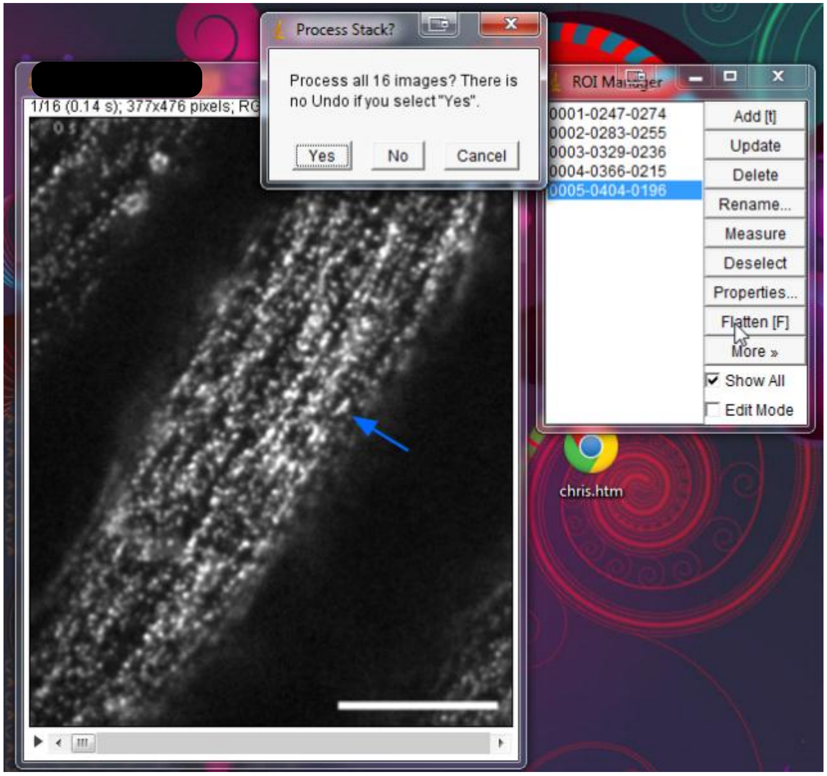
10. If you save the file at this point, these ROIs will be remembered, and can still be edited.
11. If you are happy with everything, you will now add your objects as overlays. To do this, just select any of your objects via the ROI manager. Then hit 'b' (or image>> overlay>>add selection), which adds the ROI as an overlay.
12. After adding overlays, if you save the stack, the overlays will be saved with it, so when you open it another time, they will be there.
13. These overlays are only seen by imagej, since they use imagej algorithms. For example, below I saved my example stack with the overlays, and then opened it using windows image viewer:


[redacted]



No arrows!

14. To get the objects permanently embedded within the image (i.e. destructive manipulation), all you do is go to the ROI manager, and select 'flatten'. If you check off 'edit mode' first, the little numbers on your objects will be hidden).



Process all 16 images? There is no Undo if you select "Yes".

0001-0247-0274
0002-0283-0255
0003-0329-0236
0004-0366-0215
0005-0404-0196

Say yes to process all, since you have a stack.

15. Now if I open with any program, the objects will be on the images:

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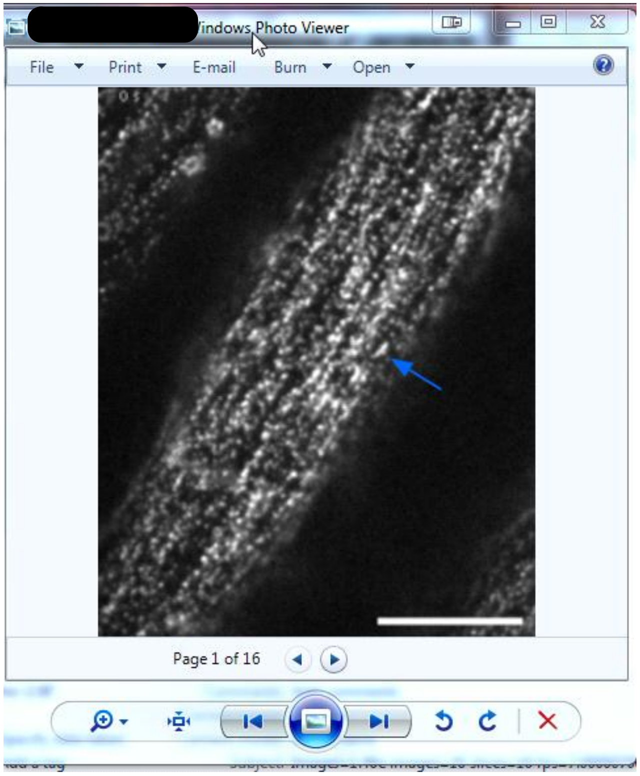
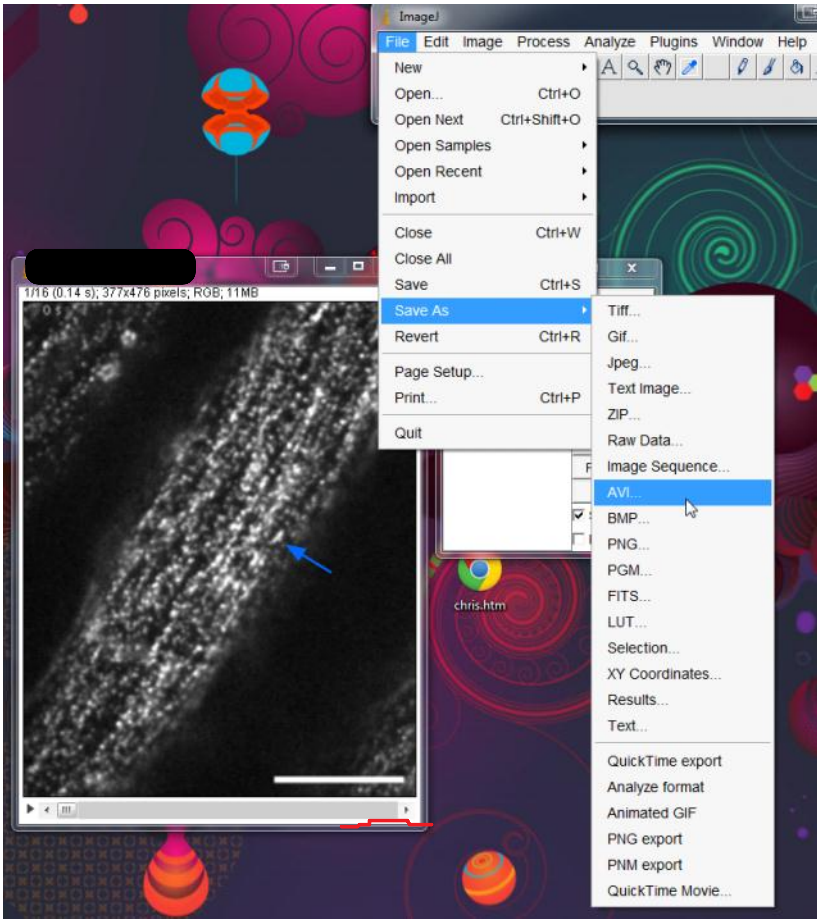


16. One flattened, the objects will be there in any format you save the image, which is nice, so you can do avi or mov or jpg etc files. Here, I save the stack as an avi (good for powerpoint etc):


[redacted]

THAT'S IT! THIS TOOK ME YEARS TO FIGURE OUT.