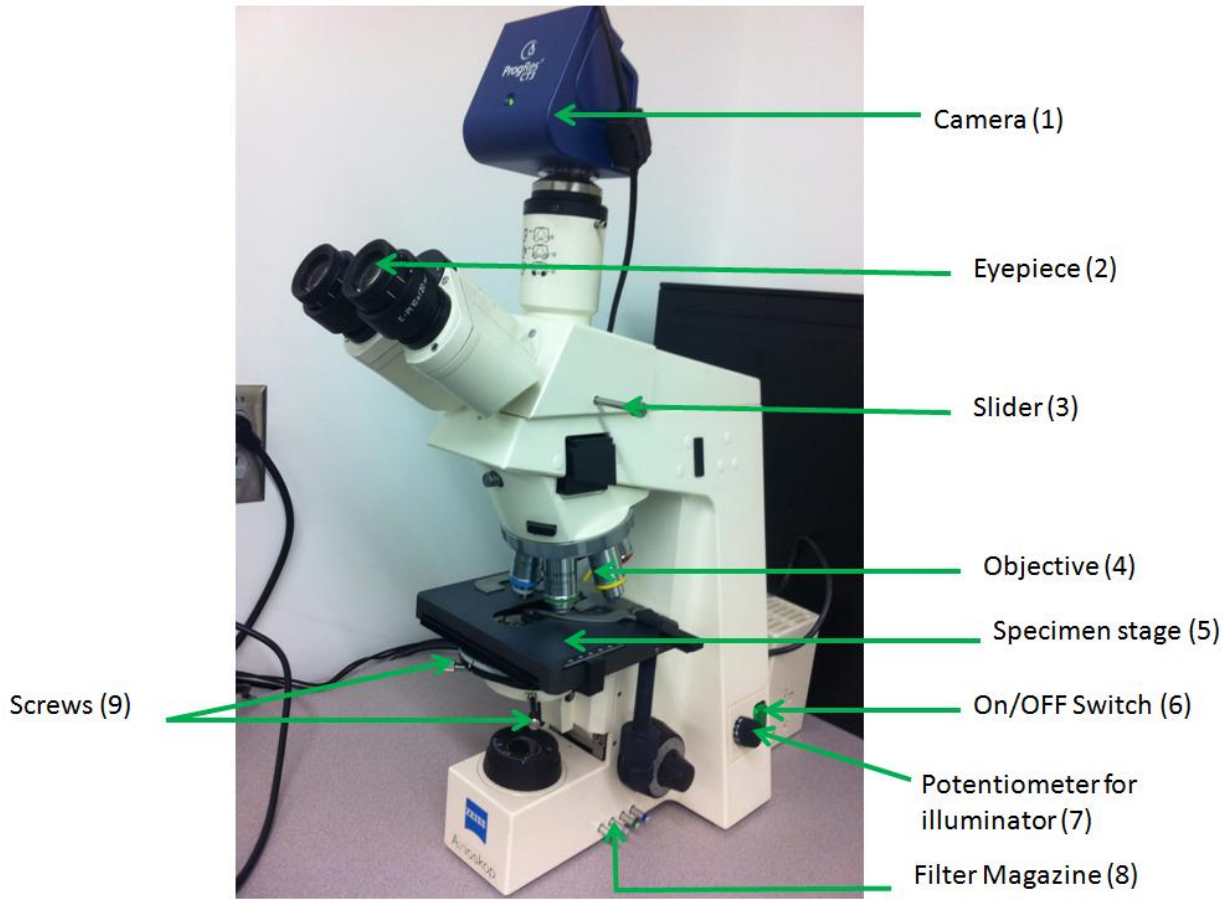
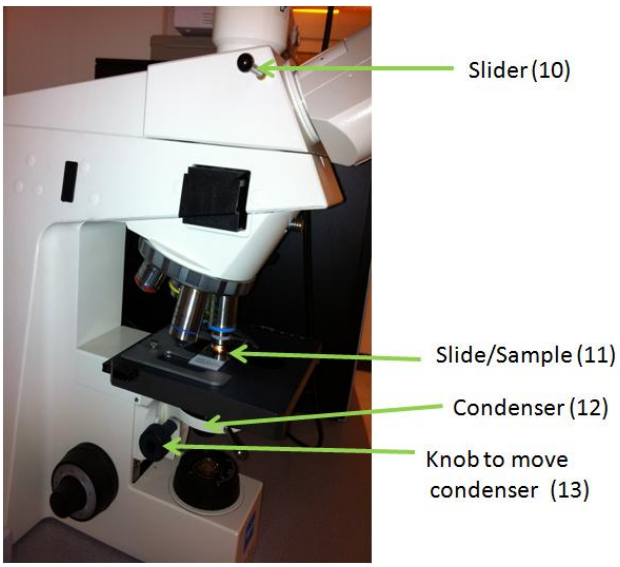
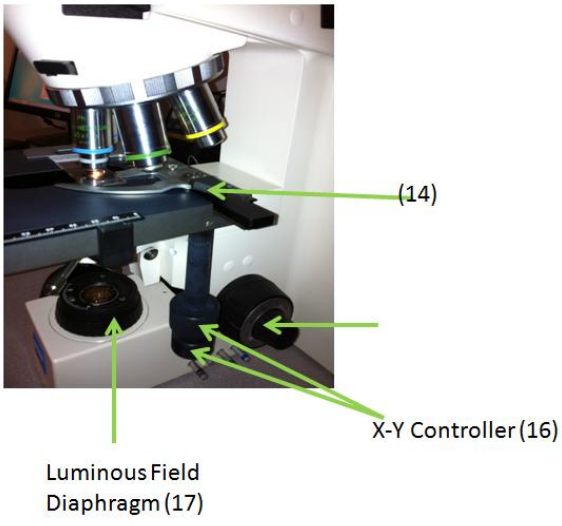
Lab 1

Description

We use Axioskop Zeiss microscope, PC and ProgRes CapturePro 2.8.8 to acquire images in this lab section. This microscope can capture image at magnifications 4X,10X, 20X, 40X and 60X







Your Task

1. Capture images at different magnifications and illuminations using ProgRes Capture Pro.
3. Use Fiji to do some basic image processing on them

Precautions:

1. Handle the specimen stage carefully in order to avoid contact between objective and the sample.
2. Change the magnification slowly avoiding the objective to hit the sample.
3. Donot try to acquire image from a wet sample. This can damage the objective.
4. Turn the x-y knobs and focusing knob slowly as they are sensitive.
5. If you need to clean the objectives inform the TA.

Acquisition of images

1. Turn on the source for lamp using the ON/OFF switch (6). Turn the potentiometer (7) to 10 Volts.

2. Make sure the diaphragm (17) is opened completely (in order to allow most light to fall on the sample/slide)

3. Place the microscopic slide/sample (11) on the specimen stage (on the thin glass surface) with the help of specimen holder. Use (14) to operate the slide holder.

4. Put the desired objective (4) above the slide. First start with smaller magnification like 20X.

5. To view through eyepiece:
   5.1 Push slider (3) inwards and pull slider (11) outwards.
   5.2 Adjust the eyepieces so that they fit your eye spacing by moving the eyepieces in and out until you see a complete image through both the eyepieces.

6. Use the x-y controller (16) for moving the specimen stage in x-y direction. Check through the eyepiece to make sure you see the required part of the slide.

7. If the image is not in focus then use coarse/fine controlling knob (9) to get a sharp image.

8. Switch to a higher magnification, 60X, by placing the corresponding objective above the sample.

9. Adjust the illumination by turning the potentiometer (7). Higher magnification requires more light.

10. Follow steps 6 and 7 to locate the desired region of interest. You want that part of the slide where the brown staining is much less and you can see few purple stained cells, basically you want to see a less cluttered part. You can check with the TA at this point.

11. You can now capture image using ProgRes Capture Pro.

Using ProgRes Capture Pro

12. Pull the slider (3) outwards (you should not be able to see through the eyepiece).

13. Open ProgRes CapturePro from Desktop. You will now be able to see the image of the sample on your PC screen.

14. Use XY controllers and focus controller to adjust the image if needed.

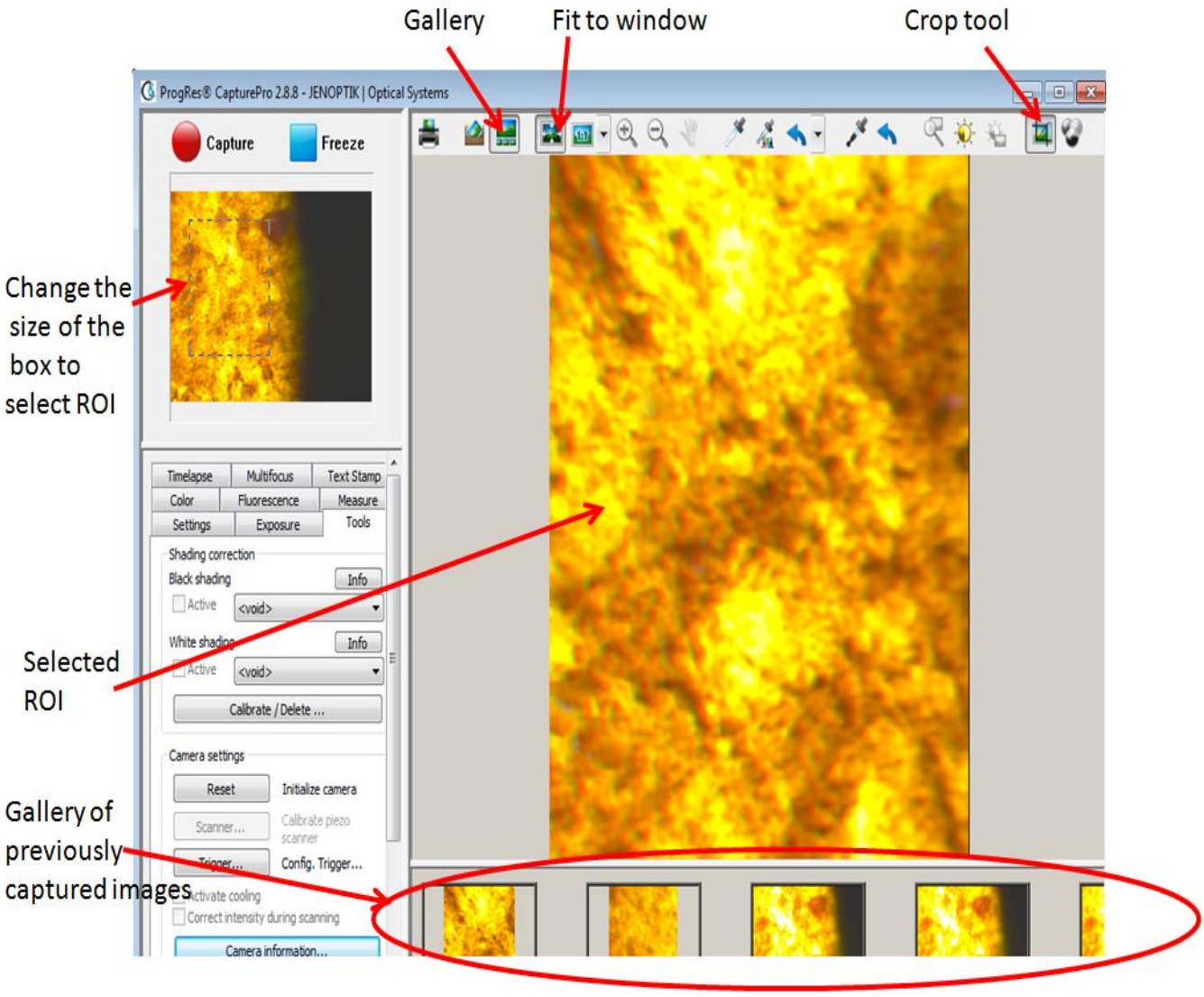
15. Use the crop tool to crop the desired viewing window.

16. Under settings select ‘Target Folder’ and ‘Image Format’. Capture image by clicking ‘Capture’.

17. Return to seeing live image by clicking on ‘Live’.

18. Keeping the magnification same collect images at atleast two different illumination levels.

19. Collect images at different magnification. When you switch from one magnification to the other adjust illumination and also the condenser position by using knob ( ).



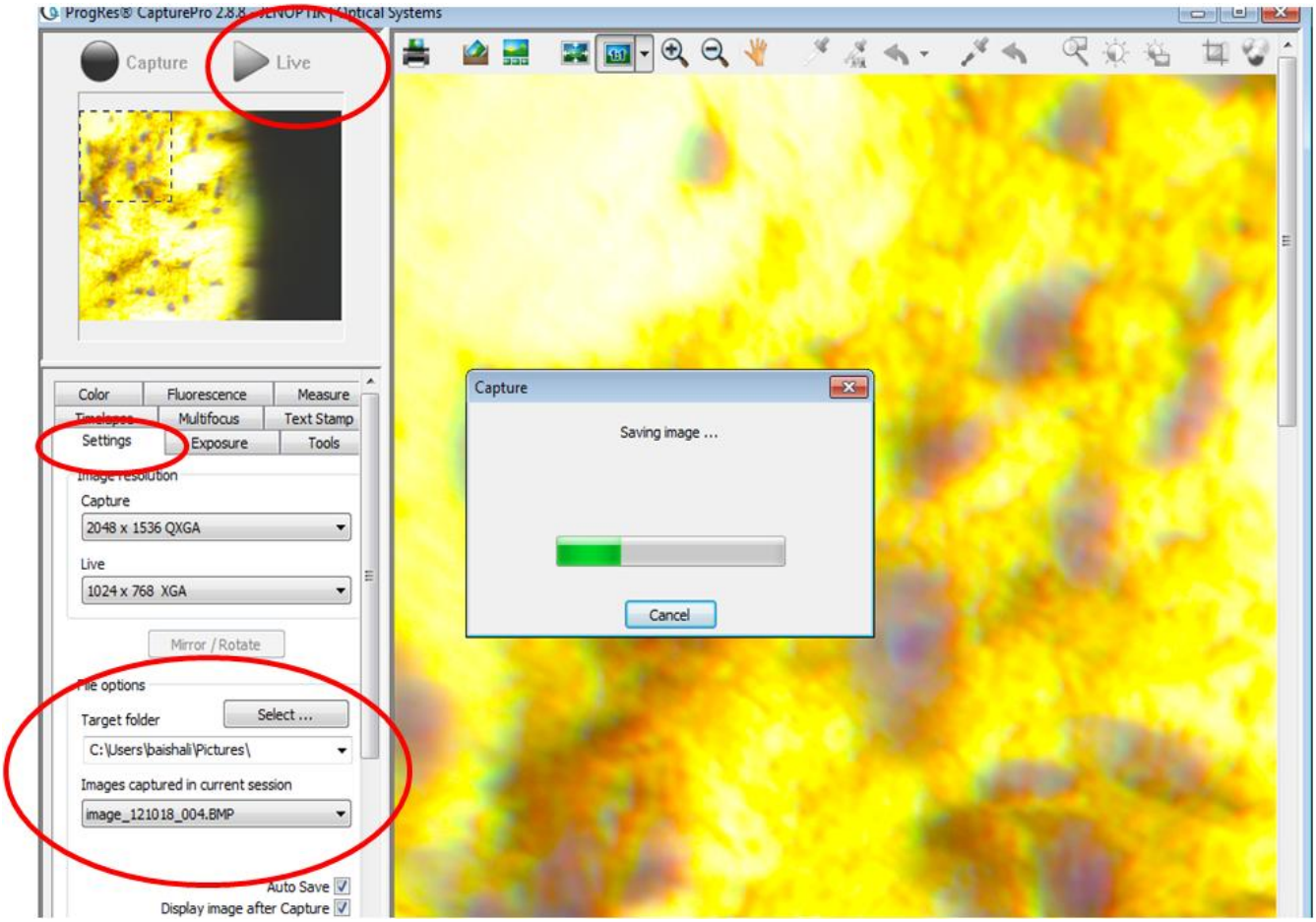
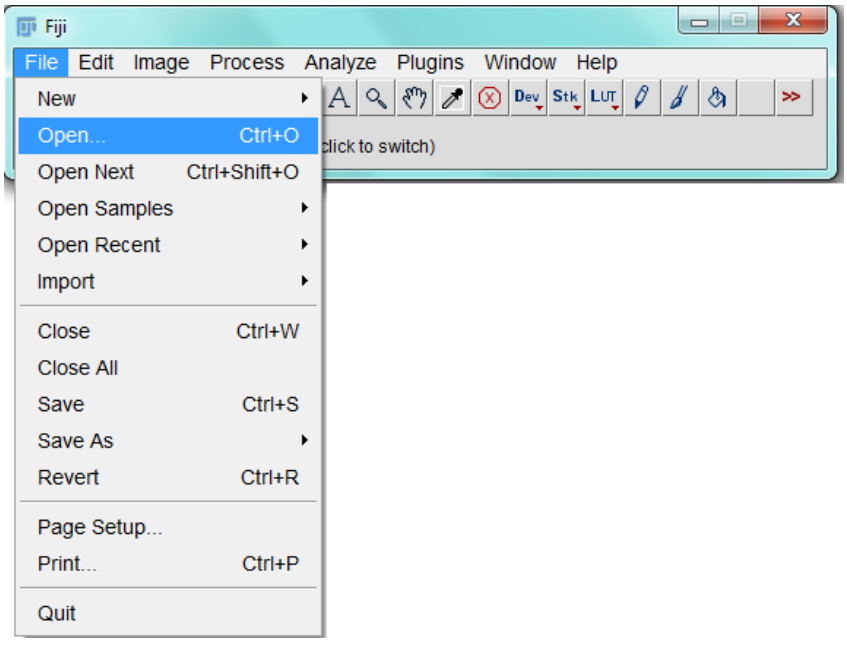
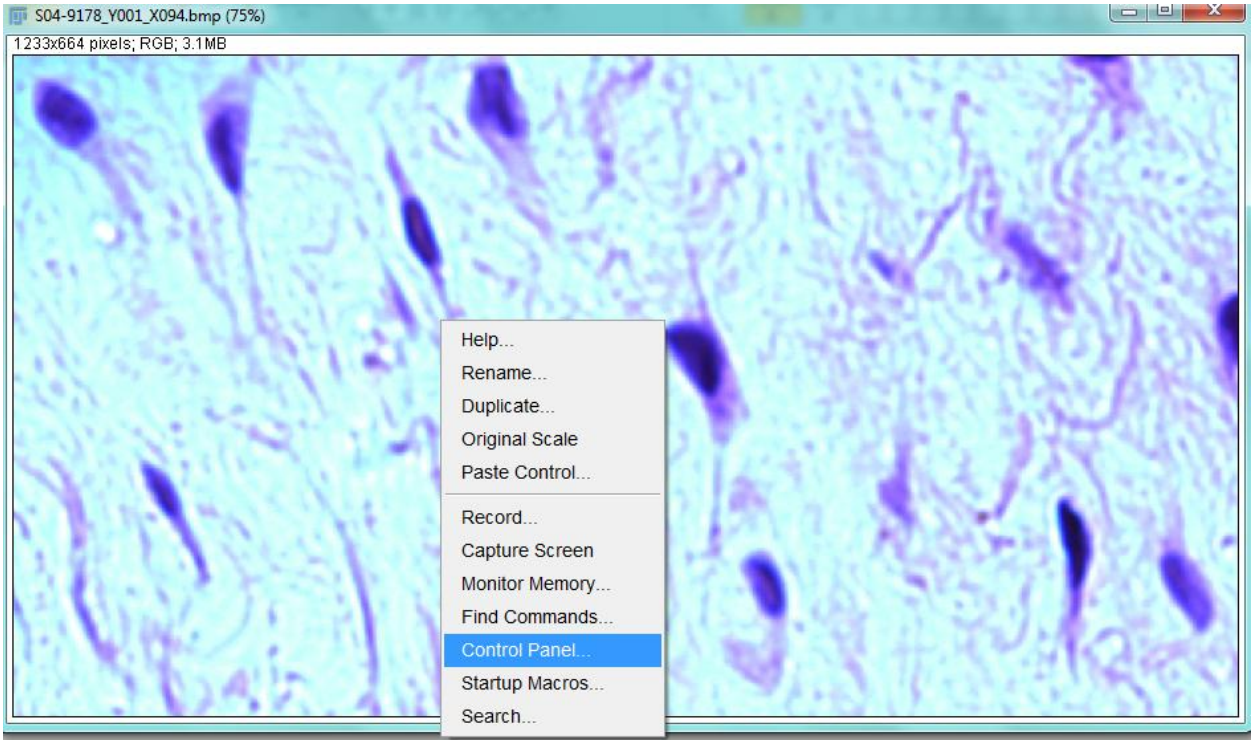


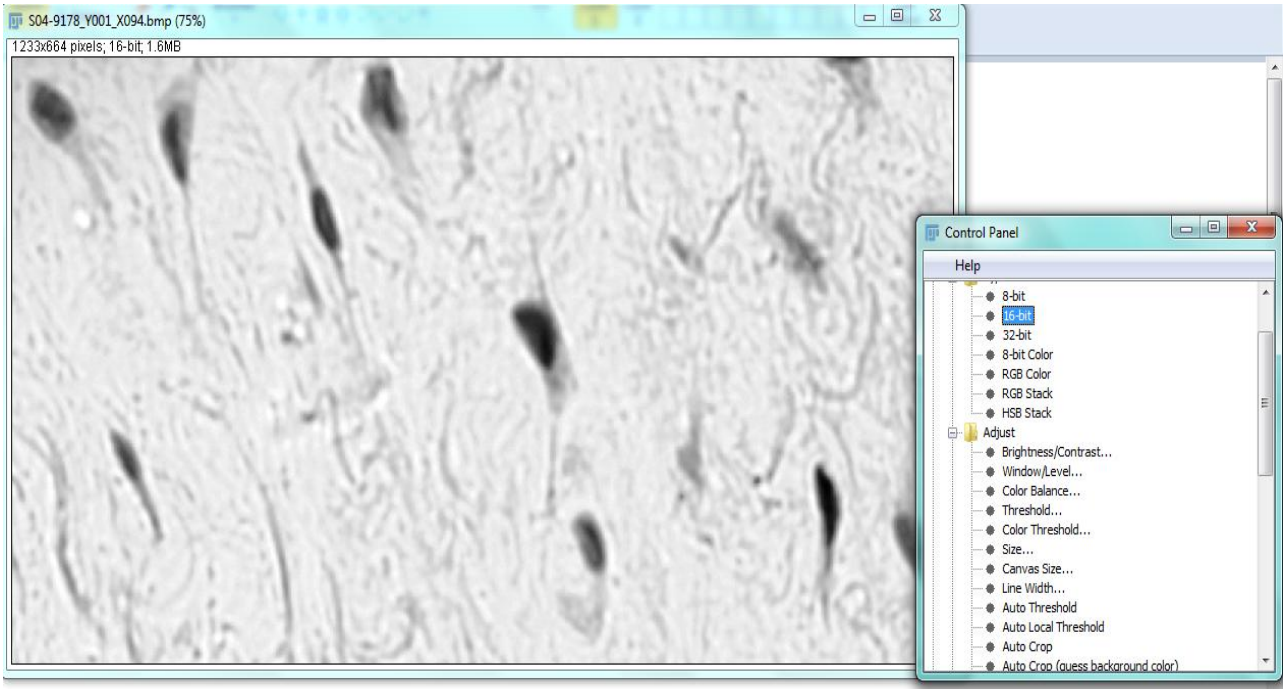
Image Processing on the acquired images

1. Open Fiji. Open Image (from directory where you saved image from microscope)

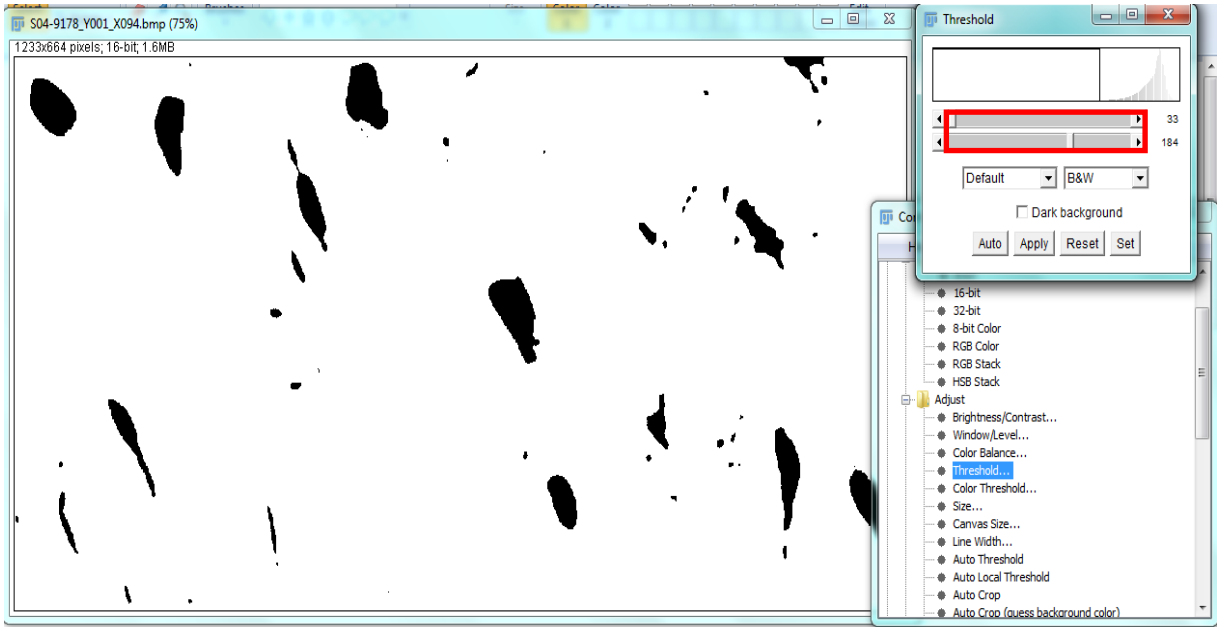


2. Right Click of mouse on image and select control Panel. Change to black and white (Image->Type 8-, 16-, or 32-bit)

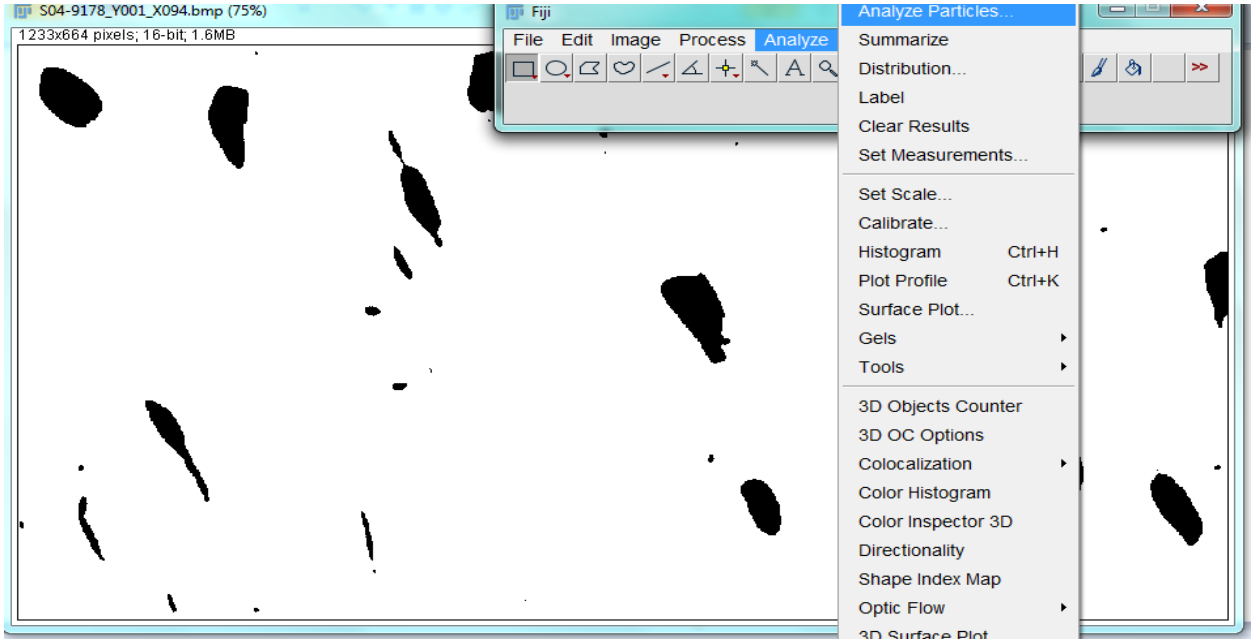


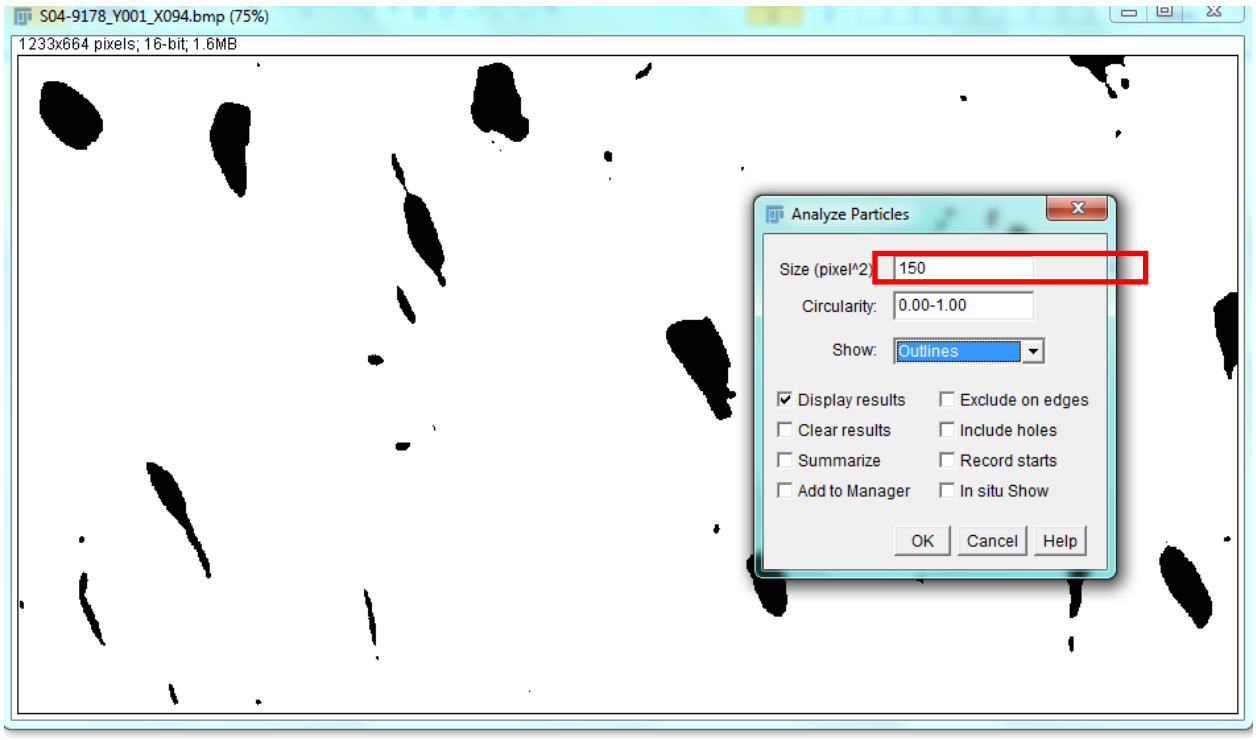


3. Adjust threshold (Adjust->Threshold) click “Set” and then “OK”



4. Analyze particles – select size -> will select parts of image with minimum pixel amount of ‘Size’, Show -> Outlines, Display Results (will produce a table)





Useful Links:

1. http://www.microscopy-news.com/download-center/Axioskop_40_Axioskop_40_FL_Routine_Microscope_e.pdf
2. http://labs.pbrc.edu/cellbiology/documents/AxioskopManual.pdf