**Rules for measuring sperm**

Usually we are collecting lengths and widths of the sperm head, midpiece and tail. Here are instructions to collect accurate lengths of all sperm components. First adjust scale (Cox lab microscope is 75 pixels/10um), you only need to do this once per image. To adjust scale, click on the Analyze toolbar, and drag down to set scale.

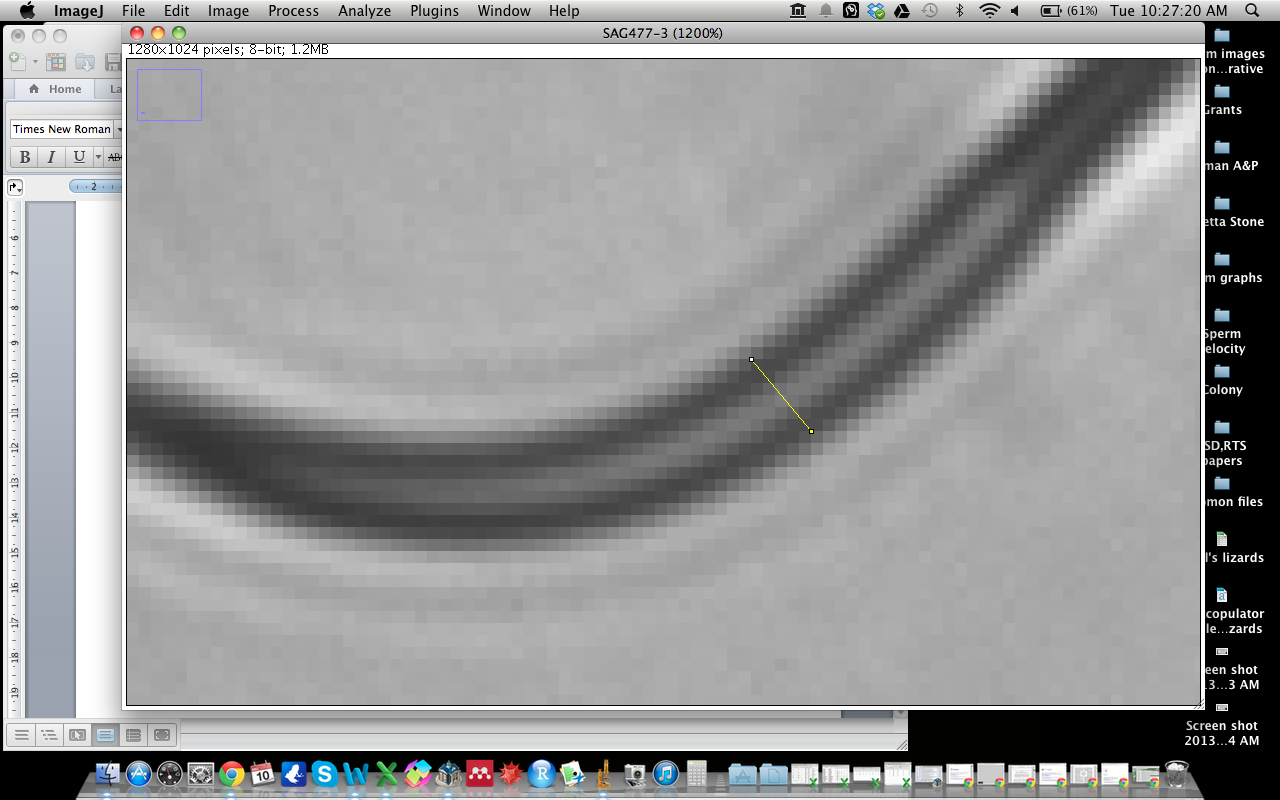
Head:

1. Make sure the head is intact and have not had an acrosome reaction. You can tell the difference between the two because reacted cells will have a blunt tip of the head (it will look more like a banana), and will have no recurve on the tip of the head.

2. Starting from the very tip of the head (where the shadow begins), use the segment tool to trace down the center of the cell ending at the beginning of the midpiece. To measure, click Command and M.



3. To measure the width of the head, go to the middle of the head, and zoom in close to find the outer edge of the head of the cell (defined as the last dark pixel on the boarder). Select that pixel and draw a line across the width of the head, ending on the outer edge of the other side of the cell.



Midpiece

1. The end of the head and the start of the midpiece can be a little blurry depending on the staining. Usually, there are 2-3 pixel that create a gradient between these sections, and you want to start your measurement in the middle of that section.

2. The end of the midpiece is a semi-circle. Draw the line to the end of the midpiece (occasionally, zooming out will help you define this spot, but usually the tail is thinner and lighter than the midpiece).

3. Measure the width with the midpiece with the same rules as the width of the head



Tail

1. Measure along the center of the tail all the way to the end. The end of the tail has a short section that lacks the fibrous sheath and is lighter and thinner. Make sure to include this section in the measurement, and if the tail is lacking this section it is likely broken and should be excluded.

