

**The Camera and computer are located in Rm846A, second drawer of the file cabinet.**

**The Camera and computer is all colour coded, so just plug everything in where the colours match up.**

**Attach the camera to the c mount, make sure that the camera lens is uncovered for the least amount as possible.**

**The camera sits on top of the microscope, where the cover sits. Make sure that the cover stays on as long as possible.**

**Starting off-** Taking pictures is really very simple!

Log onto the lap top. In order for you log on to work properly, your first log on has to occur while being connected to “lab data 4”, then it will recognize your name properly.

Double click on AxioVision Rel. 4.2 found on the desktop.

Click on “Live”, found in the tool bar. This opens the viewing window.  
Features available for “Live”:

Make sure to set “Live’ to “slow”. That can be found on the bottom of the live image window-there are three settings: Fast, Medium and Slow. Fast and Medium makes the image too pixilated.

Chose “Work Area”, located in toolbar, and pick AxioCam MRc5. This is only available once the camera has been plugged into the computer. This will bring up Adjust, Frame, General. More information on these features later on.

Set Top light to 2.

You can use an agar dish to set up the embryo. Using an agar dish with furrows cut into it, position the embryo. It is easiest to see the furrows if the bottom light is closed and just the top light is on. Reopen the bottom light once the embryos are in position.

Embryo is in position-Top light is at 2 and the bottom light is fully on and opened.

- 1) Focus and make sure Image Quality is on the highest setting. Image quality is under Work Area-second tab.
- 2) If the whole image is reddish, then click ‘Interactive’ and click on the background of the live image-should turn everything blue/gray.
- 3) If image is too dark, try increasing the exposure to somewhere between 90 and 115. Exposure is the first scale on the Work Area list.

- 4) Increase exposure so that the background is as white as possible without losing tissue.
- 5) If embryo is too pink, decrease top light. If you decrease top light you can usually increase the exposure a bit. **The trick is to find the balance between the top light and the exposure.**
- 6) If the embryo is too dark, increase top light just enough so that the tissue doesn't look pink.
- 7) Sometimes, too much exposure does cause the tissue to look pink, but just decreasing the top light a little and the exposure should fix it.
- 8) If the entire image looks to be the wrong colour, try balancing the colours by clicking on the coloured boxes found under the "adjust" tab of "Work Area".

Click 'snap' to take pictures. "Snap" is found in the toolbar and under acquisition. Close the "Live" window in order to view the pictures properly

### **Work Area; Options available:**

**Adjust:** Used to set exposure and to change the hue of the picture.  
I suggest to set exposure to anywhere between 90 and 115.

**Frame:** Quality of picture/ used to resize frame. Click on "refresh overview" and then by changing the size of the green box, you can crop "Live" images.

**General:** Used for setting "white balance". To do "white balance"- move the embryo out of the field of view, press white balance. It will clear up any marks in the background. However, 'white balance' isn't maintained while changing the exposure or the magnification. It also does not actually make the background white, it just makes it look uniform.

After you've set "white balance"- Click on the Adjust tab on the work area window and chose "Interactive". This will bring up a hand. Drag it over the live image and click anywhere over the background.

### **Live; Options Available.**

**Properties-** Min/Max, Linear, Best Fit} Contrast and Brightness. Use this in order to help get the best representation of the actual specimen. Can be used while viewing the "Live" image or after the picture is taken. Once you have selected the image you want, I usually take double and then check to see if 'Min/Max', 'Best fit', or 'Linear' give a more accurate result. Linear is almost always more accurate, so I suggest you do fiddle with those options. They can be found by "right click" on the image and choosing "Live Properties." The reason you need to take doubles of the same image is because once you start changing those properties, there is not way to revert back to the original. Better have an untouched copy just in case.

**Close after Snap-** Once a picture is taken it will automatically close the live window and bring up the picture.

**Spot meter;** used for **Measure:** I never use ‘measure’. I don’t find it reliable but if you want to use it, then chose ‘spot meter on’. This will give you the green flashing box that changes size and position. Place it over the part of the embryo that is important and click measure. It will change the exposure rate to suit the area within the box. But I always found that it either under/over exposes the picture and that it is easier to set the exposure manually.

Spot meter on also brings up the focus bar, but focusing manually once again, works better.

**To view the pictures:** Close ‘Live’. ‘Tile vertically’ is under ‘Windows’ and can be used to view all open pictures at once. I like this option because it allows me to compare many pictures at once so that I can pick the best one.

**Loading scale bars:** Click on Scalings –Load—C: (c drive) ---Scaling— and then pick the ones that apply. Scale Bars are only available for the top two image qualities.

**Scaling/Scale Bar-** Scaling is under measure and Scale bar is under annotation.

**Scaling:** Click to open. Select the correct magnification and image quality and then select the picture you want to apply the scaling to, and click ‘Apply’ on the scaling window. You should see the tool bar changing.

**Scale Bar:** Click on the picture where the scale bar is going to be applied. Scale bar in annotations. A little cross should appear on the picture and then just click and drag to place the scale bar.

**Scale Bar attribute:** Right click on picture and pick properties. Click on attributes and then click on scale bar. This is the only way to change the size of the scale bar or delete it.

**Open:** To open many images at the same time, click on the down arrow right next to the open button in the toolbar and choose Open Image. This is also found under File.

**Batch convert:** Under File. To select you file, pick the ellipsis in the right hand corner and then retrieve your file by highlighting and pressing OK. Your files will appear in one window and you can select which ones you want to convert. Then, to chose where the newly converted files are saved go to Options – Select—and then chose where the pictures are going to be placed. This is also where you can change the format to which the files are being converted. Press ok.

When you have selected the new file format and the domain under which it will be saved, press Convert.

**Tool Bar:** Right click and choose “Customize”. If you are going to be taking a lot of pictures then this option is worth it. You can replace icon which you don’t use in the toolbar with icons that you will commonly use, like “Scaling” or “Tile Vertically”. the

device the Axiocam recommends is to design “Work Flow”, but you don’t need a “Work Flow” if you just customize the toolbar. Plus Workflow takes up more space on the screen which could be better used for our pictures.

“Customize” also allows you to create keyboard shortcuts, under the keyboard tab and I also recommend doing this if you are going to be taking lots of pictures. Once you’ve set up keystroke shortcuts, the picture taking process is a faster.

**Save All:** Axiocam automatically gives each picture a name, “Axiocam M51” for the first one, “Axiocam M52” for the second and so on. Save all will systematically go through all the pictures in order and allow you to save each individually. It does not allow you to save all the picture under one common file, you must name each one separately. At the very least, this option allows you to save many images quickly.

**Close All:** Found under “Window”, very useful after viewing many images.