

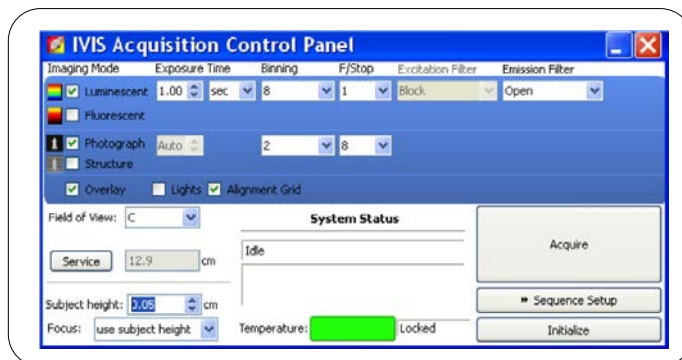
Acquisition of high resolution images.

Adjust settings for acquisition

Note: This quick reference guide is for those researchers who wish to perform analysis that requires high resolution including *in vitro studies* when one may want to discern aspects about cell layers, *ex vivo tissue imaging*, or *imaging of tissue slices*. You will not need this resolution in most *in vivo* studies.

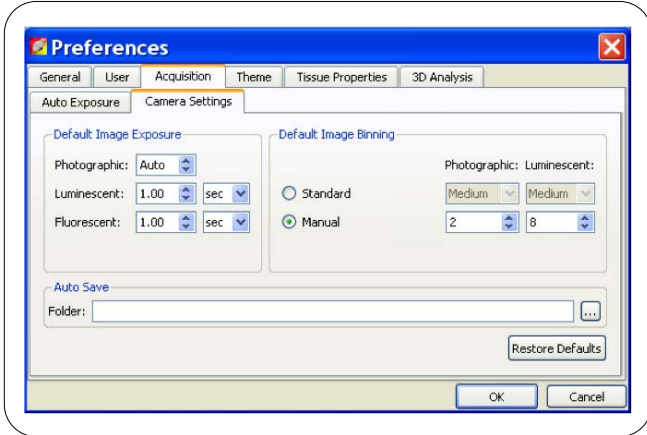
1. Go to FOV A

- Change subject height to approximate height of the subject. When choosing the subject height and then when focusing, remember that the IVIS® optical system is imaging the light that reaches the surface of your subject. Therefore you should be focusing on the surface where the light you are interested in seeing is leaving the subject.
- Acquire a photo at regular resolution to check positioning. Readjust the position of your subject as necessary.



2. Change to manual binning

- Go to **Edit > Preferences** then the **Acquisition** tab.
- Select **Manual** binning and Click **OK**



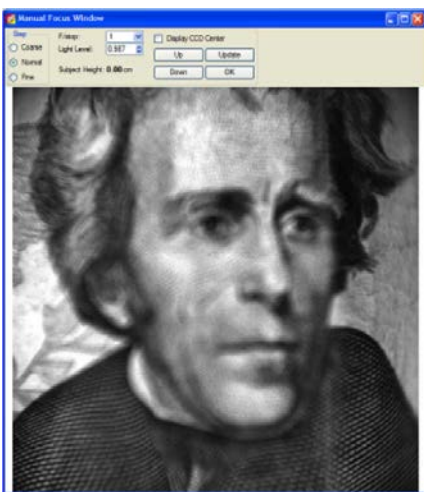
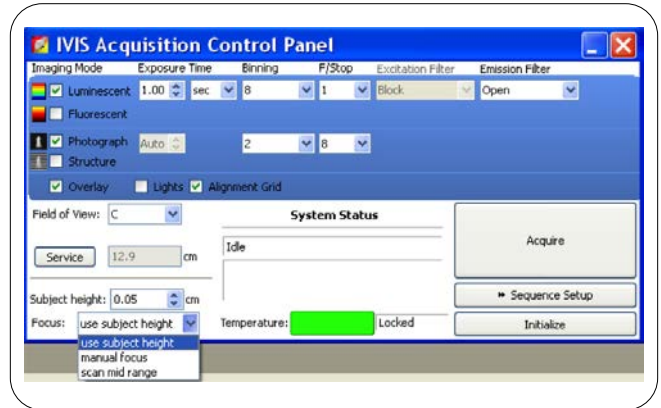
- Select **Manual Focus** from the **Focus** dropdown menu option on the **IVIS Acquisition Control Panel**.

Note: When setting manual focus check that the f-stop is set to f1 or f2 for best focusing (*default in manual focus is the F/stop setting for Luminescence in Control Panel*).

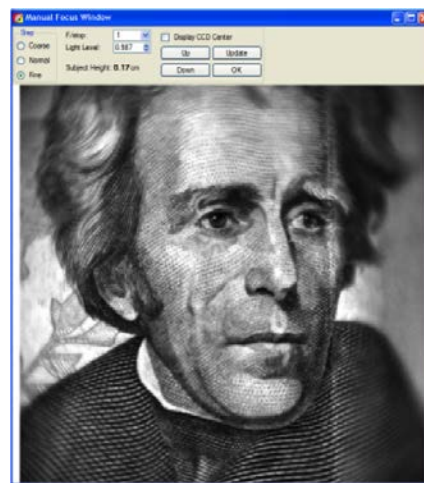
- Manual Focus Window Operation:

Focus is achieved by moving the stage up and down until you have visually achieved good focus. An overview of the different controls of the Manual Focus window:

- Up/Down buttons:** Move the stage and Living Image will automatically acquire a new image.
- Step Size:** Three different levels of how far the stage moves each time you hit Up/Down: Coarse, Normal, Fine. Use Coarse for larger stage movements if you are very far out of focus and Fine get your final stage height.
- Light Level:** If there is not enough light for good focus adjust this level up
- F/stop:** Smaller number f/stop has small depth of field. You will achieve best focus by using f/1 for determination.



Out of Focus



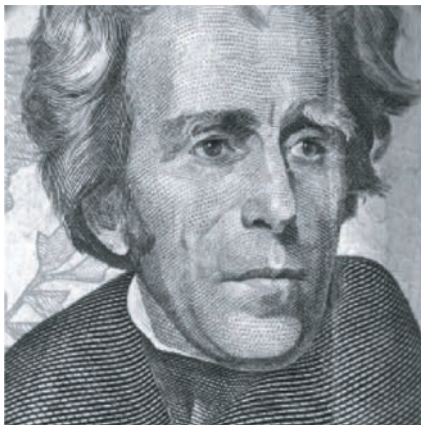
In Focus

Note: Notice difference in **Subject Height** in these two images. Focus on portion of image you are interested in imaging.

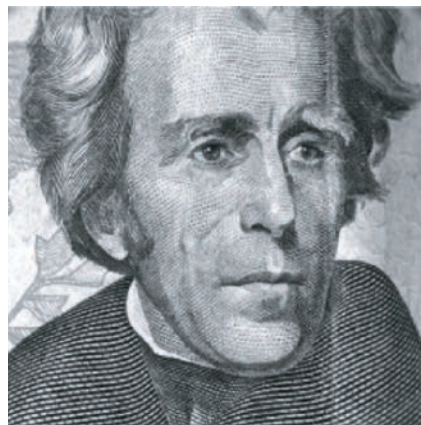
- Click **OK** when finished.

Acquire high resolution image

- Photo settings: Bin 1 or 2 f/stop 8
- Luminescent/Fluorescent: Bin 1 or 2. F/stop number depends on signal. If you have enough signal f/4 is good, if need more signal go to f/2 or f/1.
- For the small binning images the file sizes are much larger and readout time for image much longer (almost 1 minute). When looking at the entire image the resolution change from Bin 4 to bin 1 does not appear to be much. For example compare the \$20 bill looking at the full image:



Bin 1 no zoom:



Bin 4 no zoom:

If you are interested in a specific region of the image, then the added resolution can make a large difference. For example compare the different resolutions if we zoom on one of the eyes in the image using the zoom tools located in the Image Adjust portion of the Tool Palette:



Bin 1 zoom:



Bin 4 zoom:



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