



Spinning disk manual

Power-up protocol

1. Mercury Arc Lamp
2. Laser
 - a. Make the switch is set to stand-by (towards you) and the power dial is at minimum
 - b. Turn key to START and release
3. Power strip - controls filterwheel, z-stepper, camera, scanhead
4. Computer

The Mercury lamp should always be first-on and last-off, this prevents any electrical surges caused by ignition damaging other equipment on the same circuit.

The Microscope

- Binocular eye pieces – adjust inter-ocular distance
- Rotary port selector on right-hand side – eyepiece or SP (side port = to camera)
- Fluorescence shutter under objectives – rotates between open and closed
- Fluorescence filters in a wheel under the stage–
 - B (blue excitation for green fluorophores)
 - G (green excitation for red fluorophores)
 - White (for confocal)
- Stage – control xy position manually using hanging drive
- Focus knob – outer part is coarse; inner is fine focus.
- Z-stepper – this is attached to the right side focus knob. This controls the z-position allowing auto-focusing and 3D-imaging. Push inwards and tighten screw to engage. When engaged, use ONLY the controller knob on the box under the monitor for focusing.

Objectives

Position	Mag	NA	Oil?	Phase?
1	10x	0.3	DRY	no
2	40x	0.6	DRY	Ph2
3	40x	0.9	DRY	no
4	60x	1.2	WATER	no
5	63x	1.4	OIL	no
6	100x	1.35	OIL	no

Image acquisition using MetaMorph

When you have located and focused on your specimen manually, change the port selector to "SP" and the cube wheel to "white".. Remember that the field of view of the camera is a smaller than the field of view down the eye pieces so make sure your sample is centered.

Open the acquire dialog box

This can be opened from the task bar Acquire or from the dropdown menu, **Acquire> Acquire**

Things to set in the Acquire window:

- Set the display to "Autoscale" - the triangles on the image histogram show the scale min and max.
- The image gamma - the relationship between measured and displayed brightness. 1=linear
- Exposure Time - Type in value (or choose AutoExpose) mouse-over image to give pixel intensity values (between 0 and 4095)
- Full chip uses entire CCD camera, sub-arrays produce smaller files and may be faster
- Binning - combines CCD pixels to form n x n super pixels - increases sensitivity but reduces resolution
- Press Show Live to see the specimen through the camera - it may be necessary to focus or move the stage slightly
- Clicking the folder icon allows you to set whether each image starts in a new window or overwrites the previous image (handy for optimizing your image, be careful not to lose your data though)
- External shutter linked to camera should be [Current Shutter]
- Gain and offset for the camera under the Special tab - Start as 0 and 255 respectively

Press the Acquire button to capture the image

Saving images

Simply click save and name your file. Images will be saved as 12-bit TIFF images with all the original data. Not all programs will open these images. Photoshop will open the files as 16-bit images, you will need to adjust the levels ON A COPY of the file. ImageJ can open them also.

To save your images as 8-bit files (compatible with all programs)

- Press Copy to 8-bit button on the task bar
- Select the desired image from the "Image:" drop-down menu
- Press Copy and Save by going to **File >Save as** (make sure the file name is different otherwise you will overwrite the original)

Multidimensional acquisition

Open this dialog box for timelapse, multi-wavelength, and z-stacks for 3D acquisition.

Finishing and shutting down

- Lower the objectives and clean any oil/water objectives that you have used.
- Put the laser to minimum power and into standby
- If there is another user within one hour, log off and leave the system on. Otherwise power down -

1. Computer
2. Power strip
3. Laser
 - a. Put laser to minimum power and into stand-by
 - b. Turn key to OFF
4. Mercury Arc Lamp