

Microscope Protocol

1. For booking time to use the inverted or normal fluorescent microscopes in room 303, please go to - <http://www.my.calendars.net/iircscopes>.
- Include your name and lab in addition to the specific time booked.
2. To turn on Fluorescence, Turn on FluoArc (needs 2 min to warm up).
 - a. Write down bulb chronometer (how much time the fluorescent light bulb has been used).
 - b. Once you turn on the fluorescence, leave it on for at least 30min before you turn it off.
3. Turn on microscope and computer.
4. Change objective to what's desired. For picture taking, 63x is optimum.
5. Put on slide, add a drop immersion oil (for 63x objective).
6. Turn on HAL (halogen light) and find cell of interest. (Filter should be 7 or 8).
7. For fluorescence, turn off HAL and turn on FL. Change filter to respective colour:
 - a. 1- DAPI
 - b. 2- FITC
 - c. 3-GFP
 - d. 4-Texas Red/mCherry
 - e. 5-Cy3
 - f. 6-Cy5
 - g. 7 and 8 – bright field/Transmitted Light/Halogen
8. To take pictures, pull the metal bar to switch view from microscope to computer.
9. Turn on Axio Vision software on computer.
10. For single colour/fast checking, click on “live” and click on colour preset buttons. Adjust exposure and “Snap” to get pictures.

11. For multi-colour experiment, open “6-D Acquisition” or “Multidimensional Acquisition”.
 - a. Under “Experiment” tab, press “Load” to load experiment (All channels).
 - b. Go to “C” Tab and right click to de-select channels/colours not wanted.
 - c. Left click on the channels/colours to set exposure time. Use “Measure” to find optimum exposure time.
 - d. After setting exposure time for each channel, press “start” at bottom right to take pictures. (Note: un-check Z-stack, default is ON)
 - e. To look at each channel/colour individually, click on channel number at bottom left or window.
 - f. To change brightness/contrast, turn off colour, right click on image, go to “Properties”.
12. To save pictures, go to “File”, then “Save”. (Always save as Axio vision Image (.zvi) first before saving as TIFF or JPEG to preserve all details)
13. To save as TIFF or JPEG, Go to “File”, then “export”.
14. Turn off Microscope, FluoArc and AxioVision software (Computer should be left on)
15. Turn off Axio Vision (Do not save “All Channels.Zvx”, only save your own images)
16. Wipe Objectives with Kimwipes and Zeiss Lens Cleaner.
17. Record Bulb Chronometer and amount of time spent on microscope.

Angela and Vijender from the Hmama Lab – Rm 436 – x68494 – are experienced users. Please go to them first with any questions or concerns.