BIOLOGICAL IMAGING DEVELOPMENT COLAB

UCSF University of California San Francisco

Zeiss Axio Imager 2: Apotome

Fluorescent Microscope

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Contents

1 Introduction

The Apotome is an upright fluorescent microscope with an objective turret that houses four lenses:

Magnification	NA	Immersion
5x	0.16	Air
10x	0.3	Air
20x	0.8	Air
40x	1.3	Oil

Please sign up for time using the MyCores microscope scheduler.

2 Microscope Start-up

Note: This microscope is sensitive to the order in which you turn things on. The software may not properly communicate with the microscope if it is not turned on in the proper order.

1. Turn on Lamp.

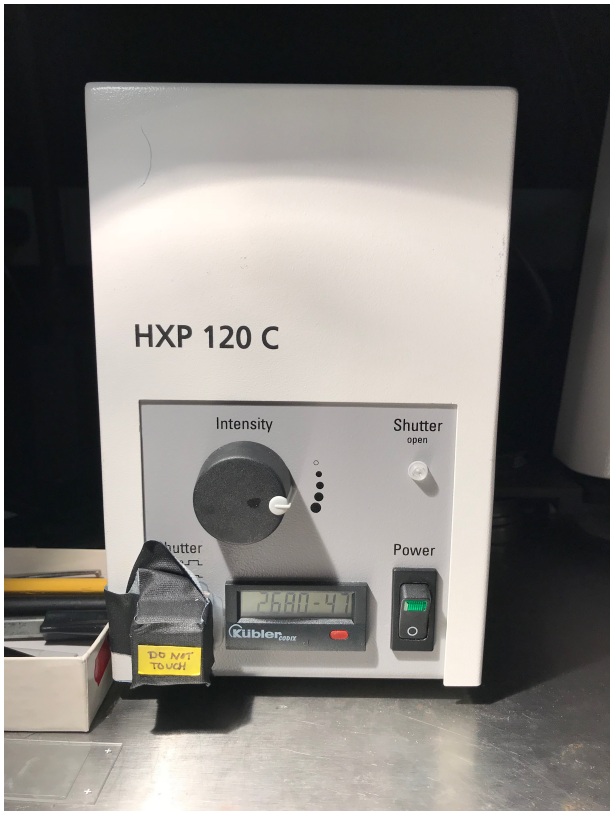

Figure 1: The image above shows the power box for the lamp and shutter.

Note: This is a halogen lamp and it must be on for at least 20 mins before turning it off again. DO NOT TOUCH the shutter button on the lamp. The shutter button should be OUT in order to communicate properly with the microscope.

1. Turn on the power strip.This will power up peripherals so they won't have to be turned on individually.
2. Turn on microscope base.
3. Turn on computer.
4. Use your UCSF login to log in to the computer.
5. Check if the microscope is communicating with the lamp:
   (a) Select any filter set on the touch pad (e.g. DAPI) and press 'On' button for the RL Illumination (reflected light).
   (b) Check to see if the microscope responds. You should see excitation light illuminating your sample.
   (c) If the microscope is unresponsive, cycle the power to the scope by pressing the On/Off button on the left-rear side of the microscope base. Repeat steps (a) and (b).
   (d) If the microscope responds without problems, continue with the start-up and start the microscope software, Axiovision.

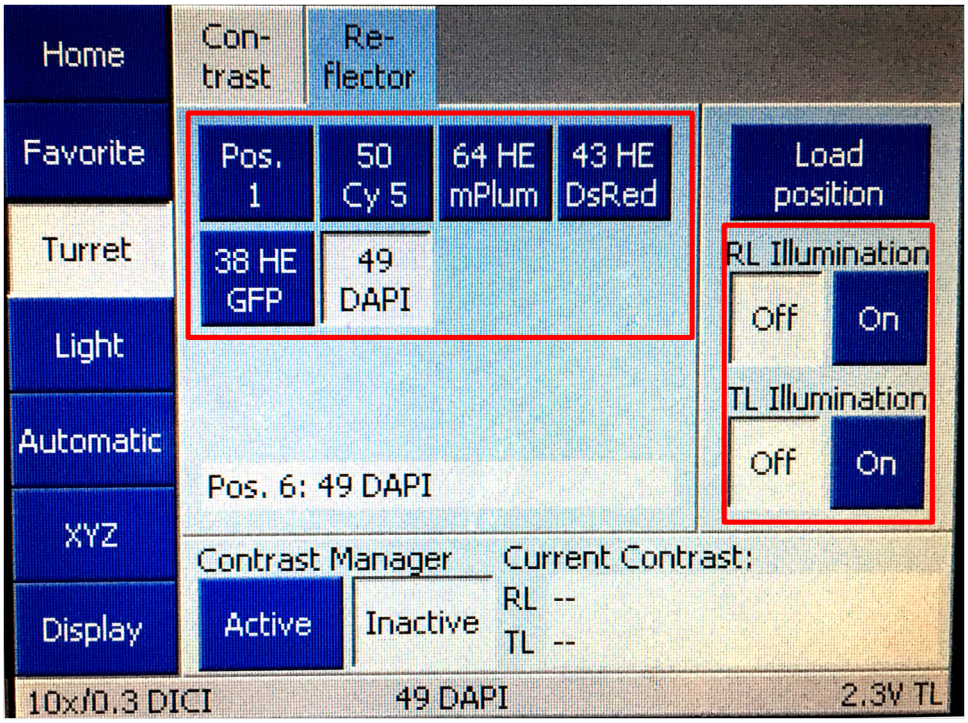

Figure 2: The image above shows the microscope touch screen. The touch screen can be used to select different filters and settings.

3 Software Start-up

1. Open AxioVision Software.

Figure 3: The image above shows the AxioVision icon.

Note: Do not use the microscope or press buttons during this process, or the software may not load properly.

2. Select an appropriate filter set, turn 'On' the RL Illumination and focus your sample though the eyes. (Sliding mirror to the 'In' position). Selecting a filter set and turning the RL Illumination 'On' can be done either on the microscope touch screen or in the software, Figure 4.

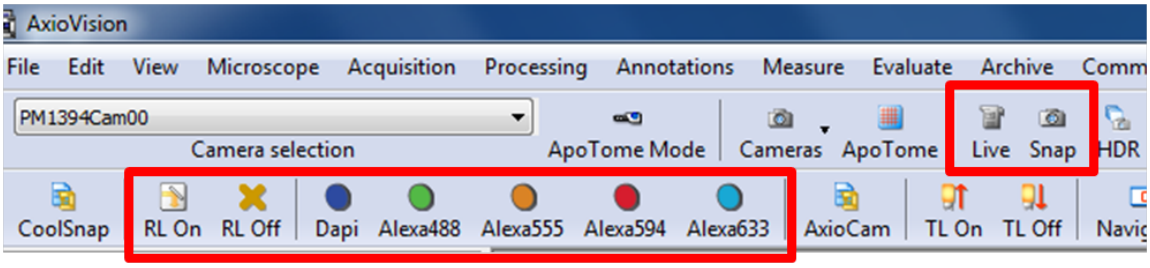

Figure 4: The image above shows the location of the filter sets, lamp, Live mode and Snap button.

4 Multi-Dimensional Acquisition

4.1 Channels Tab

1. Right click to activate or deactivate the channels. Right clicking to deactivate will put an "X" over the channel, indicating it is deactivated. Right clicking a deactivated channel (a channel that has an X on it) will activate it.

2. Select channel with Left click. Click "Measure" in order to find optimal exposure time. Once in focus, remeasure for accurate exposure time, or use manual tools to properly adjust exposure.

3. Click 'Start' to acquire image.

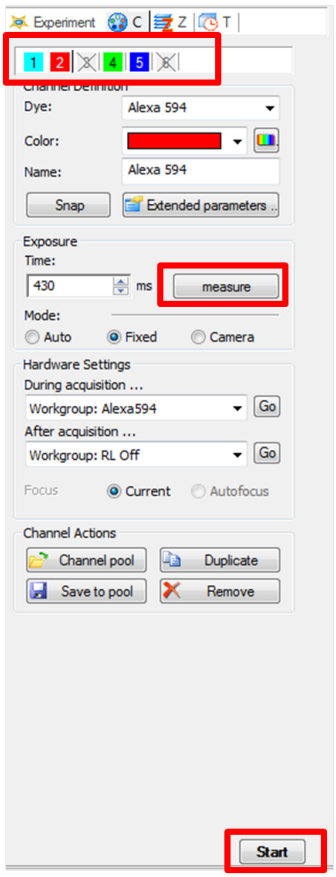

Figure 5: The image above shows the multi-dimensional acquisition settings window.Here, users can set the channels, exposure, and take an image.

4.2 Z Tab

1. Set the desired number of steps, and slice distance.

2. Click Ellipses next to start and stop to set upper and lower limits.
   Note: If " All Channels Per Slice" is unchecked, the software will switch filters for each line of the image scanned instead of switching filters for each stack. In addition, setting a small Z stack near focus will allow you to pick the image with the best focus out of the stack.

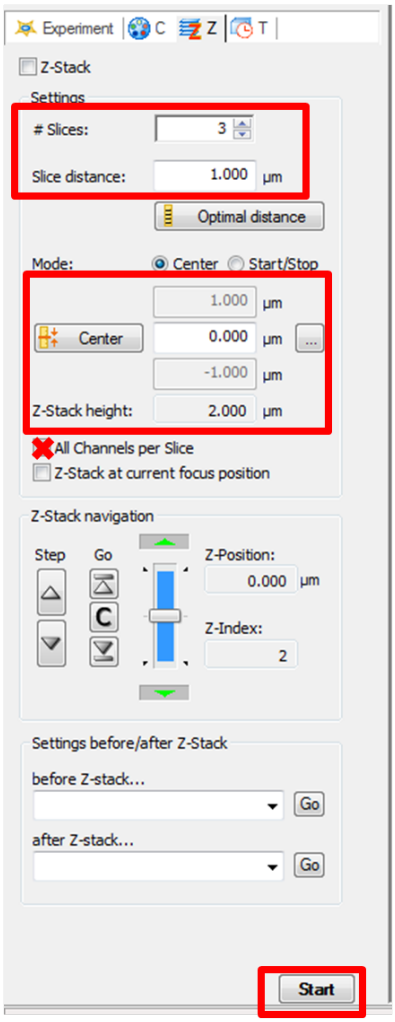

Figure 6: The image above shows the Z-stack window. Here, users can set the number of slices, slice distance, and stack limits.

4.3 Apotome

1. Push in the Apotome module. This will automatically start Apotome mode.
2. Click 'Settings' in Apotome properites window. Note: The window should say "Calibrated." If it does not say "Calibrated", try restarting the software. If this doesn't work, contact the BIDC.
3. Set Acquisition Mode to Optical Sectioning.
4. Use the visible grid (grid lines will be visible in live view).
5. Choose the Medium filter(this filter correlates to how much out of focus tissue is not imaged. For example, if set to 'High', less tissue is left in. If the filter is set to 'Low', more tissue is left in.
6. Choose desired frame average.
7. Click 'Start' to acquire image.

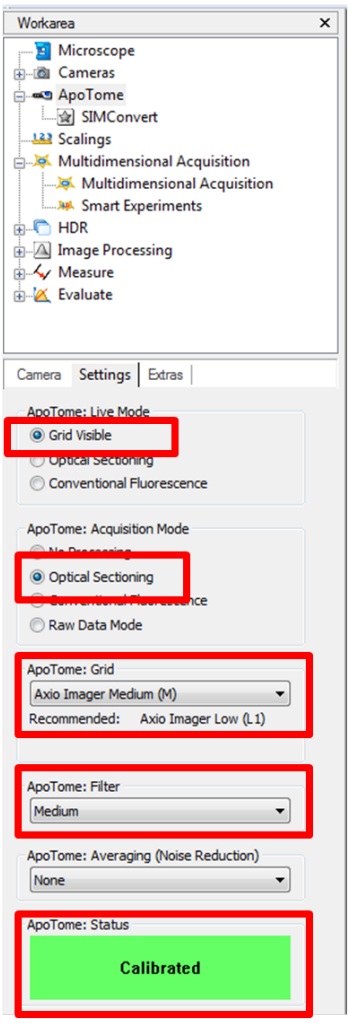

Figure 7: The image above shows the Apotome work area window. Here, users can set Apotome acquisition settings.

5 File Export

1. Click 'File' then 'Export.'
2. Save as ZVI file
   Note: A ZVI file is an older proprietary Zeiss file.

6 Shutting Down

1. If used during imaging, clean the oil objective. To clean oil objective, click button on side of scope to drop stage. Clean slide with Kim-Wipe, clean lens with lens paper (NO KIM-WIPES ON LENSES).

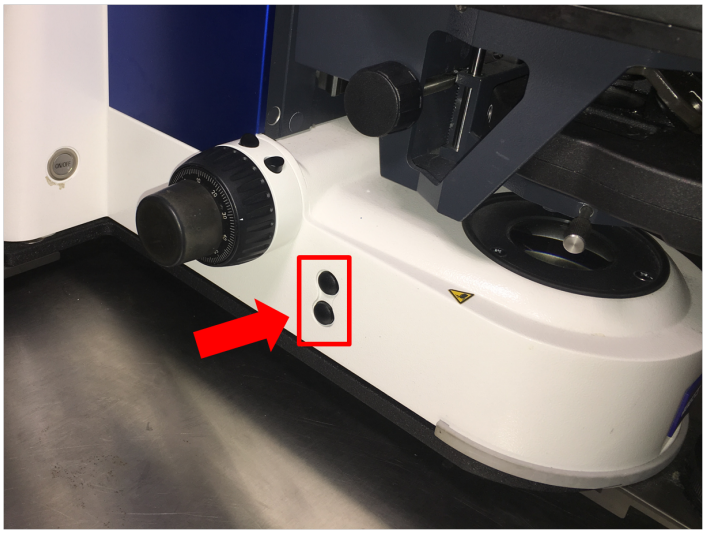

Figure 8: The image above shows the buttons that will raise and lower the stage.

2. Save files onto external hard drive. DO NOT save images on C drive (or save temporarily and move over directly after imaging).
3. Exit software.
4. Shut down computer.
5. Shut off power switch.
6. Shut off light source.

7 Troubleshooting

1. If you cannot see your sample through the eyepieces, check the beamsplitter lever located at the top of the microscope below the CoolSnap camera. This lever should be pushed in to see your sample with the eyepieces, and pulled out in order to see your sample with the camera.
2. If the light is dim through the lens to your sample, check the aperture located on the Apotome arm (it's the dial facing you).
3. Users should NOT TOUCH the shutter button on the lamp source. The shutter button should be OUT in order to communicate properly with the microscope. If the shutter button gets pressed, turn off scope body and lamp source, and software. Toggle shutter button OUT, turn on lamp source, turn on microscope body, and start software again.

Contact the BIDC

The BIDC office is located in Medical Sciences Building Room S1109.
The BIDC office phone number is 415-476-4550.
If you need immediate assistance and no one is available in the office, or it is after business hours, please call the **BIDC Hotline** at 415-745-2432.