ApoTome Usage Guide

To turn on:

All users must create a reservation on the Google calendar for **every** use, even if you are only using it for a short period of time.

1. Sign into the log book and remove microscope cover.
2. Turn on the power strip.
3. If you will be using fluorescence, turn on X-Cite power supply for the fluorescent lamp.
4. Turn on the microscope by pressing the power button on the left side of the base of the microscope.
5. Wait for the microscope to complete its startup process and then turn on the computer (the power button is on the top left corner of the computer case).
6. Log in and start the Axiovision software.

To turn off:

1. Click "Load position" on the microscope screen to lower the stage and remove your microscope slide.
2. Remove oil from 63x objective if you used it.
3. Click the upper left button ( ) to return the stage to its working position.
4. Save all unsaved files that you want to keep.
5. Close Axiovision software
    a. If a window appears that says "Your experiment has changed" and asks whether you want to save your experiment, click "No." If you click "Yes," default settings, such as exposure times for the channels, channel pseudocoloring, and z-stack settings will be overwritten with the changes you made during this session.
    b. If there are unsaved files open, a window will appear that asks if you want to save those files. If you have already saved the files you want to keep, click "Uncheck all" and then "Ok."
6. Transfer your files to a server or to a portable hard drive.

    Do not leave the only copy of your files on the local hard drive. We will be periodically upgrading and doing other maintenance on the computer and your files may be deleted without warning.
7. Turn off the computer.
8. Once the computer has **completely** shut down, turn off the power strip and the X-cite fluorescent bulb power supply.
9. Sign out of the log book and replace microscope cover.

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To use the camera and ApoTome:

1. To use the camera, you will need to have the Multidimensional Acquisition panel open. If it is not open already, go to Acquisition → Multidimensional Acquisition → Multidimensional Acquisition.
2. To use the ApoTome, you will need to have the ApoTome panel open. If it is not open already, go to Acquisition → ApoTome (**Not** ApoTome Mode) → ApoTome dialog.
3. Click on the Experiment tab in the Multidimensional Acquisition window. In this tab, you input the settings you want to use for this session.
    a. Click "Load" and chose the experiment that most closely matches the settings you want to use.

       Note: "Experiments" are sets of commonly used default settings. For example, one Experiment might be set up to use the DAPI and GFP only while another is set up to use all five: DAPI, GFP, Alexa 555 and Alexa 633 and brightfield.

    b. If you would like all your images to have a common base name, put that name in the "Image name" box.

    c. The "Settings for Experiment" section controls what happens before and after an image is acquired. These settings are loaded automatically when you Load an experiment (Step 3a), so they usually do not need to be changed. For most applications, you want nothing to happen before you acquire an image (the pull-down menu is blank) and you want the fluorescent light to turn off after you acquire the image (the pull-down menu says Workgroup: RL OFF)
4. Click on the C tab. In this tab, you set exposure times for each channel.
    a. Left click on a channel to chose it; right click on a channel to deactivate it.

       Select the channel you want to set the exposure time for and click measure to bring up a live window with an exposure-time slider.

       Important: Do not move the slider immediately after opening the window. The software will select an appropriate exposure time within 3-5 seconds after the window opens. Usually this is pretty good, but you can change it afterwards if you want. **Moving the slider while the computer is still calculating its recommendation often results in computer crashes!!**

    b. Repeat for every channel.
5. If you want to take a z-stack, click on the Z tab. If not, click on the Z tab anyway just to make sure the Z-stack check box is not checked.
    a. Click the Start/Stop radio button under "Mode."

    b. Click the grey square with the three dots located right under the words "Start/stop" to set the starting position of the z-stack. Use the focus knob on the microscope to find the desired position and click "Ok."

    c. Click the next grey square down and repeat the rest of step 5b to set the stop position.

d. Set the slice # and/or slice distance you want. Click “Optimal distance” to get the recommended slice thickness for the objective you are using.

6. Click start to acquire the image.