Zeiss Axioplan 2e imaging Deconvolution Microscope

Tips for Use and Troubleshooting

Menu:

Powering-up the system

-Flip the switch on the powerstrip by your feet to the "On" position. This will power up the monitors, microscope power source, and the microscope.
-Now turn on the computer.

Igniting Fluorescent Light Source

-Flip the green button on the mercury light source to "On" position.
-Depress the red "ignition" button for several seconds and release. You should see the "percent power touchpad" light up and show "100%".
NOTE: The mercury lamp must be on at least 45 minutes before turning off, and the lamp housing must be cool to the touch before re-igniting. In order to keep track of mercury lamp usage, please write the date, time in which the lamp was ignited, time when lamp was turned off (if applicable), and your name.

-The Xenon lamp has no warm-up restrictions before shutting down

NOTE: The mercury and xenon lamps cover different UV wavelengths. If your using the mercury lamp and cannot visualize your fluorochrome, you may want to try using the xenon to determine if the wavenlength of your fluorochrome lies in the valley of the mercury wavelength.

Checklist of system settings

-Because the software retains the information (including camera and lightpath settings) from the pervious user, it's best to go over a sort of mental checklist before beginning.

always lower the stage to load and remove your slide

Make sure the lightpath and camera settings correspond For example: Top port or "TP" corresponds to the High-resolution color camera, and Back port or "BP" corresponds to the Medium-resolution monochrome camera. The lightpath can be chosen either by the software, or manually on the microscope. Choosing the camera must be done through the software by clicking on the small blue icon in the top right hand corner of the program

Light Manager

The Light manager turns itself off after using any multi-channel function. In order to go from fluorescent light to transmitted (phase contrast), the light manager will have to be re-activated with the following steps:

check the light manager box
turn on the "internal shutter"
switch the reflector to the "Empty" position

make sure to listen to the microscope, you should be able to hear the reflector turret, the condensers and filters all moving.

Using the Multi-Channel Function

-To psuedo-color fluorescent images, you must use the monochrome camera.
-After selecting the MR camera, click on "Multi-Dimensional Acquisition" on the left-hand side of the menu
-Click on the "Color" tab

from here you will be able choose which filters you want to use to visualize your fluorochrome (click here for information on filters)

right-click on the number tab to activate or deactivate a filter

when the tab corresponding to the filter you want appears, left-click to bring up it's properties (remember, exposure time will be left-over from the last user -- to change the exposure, click "measure")

-Make sure the "Z-stack" is off if you are planning on taking a single image
-Click start at the bottom of the menu

Using the high-resolution color camera

-when doing brightfield work, it's important to complete a white reference for each objective. This needs to be done with every objective with which you plan on taking pictures. However, this only needs to be done ONCE for each objective.

For instance, if you want to take a picture using the 10x, and then take a picture with a 20x, both lenses need to be white referenced. But, if you change slides and again take pictures using the 10- and 20x, they DO NOT need to be white referenced again. The best way to white reference so that no lingering "ghosts" appear is to lower the stage, take off the slide, and click the "white reference" button. This will mitigate any possible mistakes while white referencing. If there are still too many spots, most likely the lens is dirty. If it is not cleaned well after using oil, crystals will form. Clean the lens thoroughly with Lens Cleaner.

-if your brightfield image is too dark, rotate the brightfield neutral density filters

Powering Down

-Remove your slide from the stage and remember to raise the stage to the upright position
-Clean any lenses used with oil with lens paper and lens cleaner provided
-If you moved any cameras, move them back to their original position
-Check the Sign-up page on the internet at the workstation computer to see if anyone is using the microscope after you are. If they are, leave everything on. If it is going to be 30 minutes to 2 hours, turn down the percent power to 15%. If it is going to be over 2 hours, turn off the mercury lamp.
- Shut down the computer
-Turn off the power strip
-Turn off the mercury lamp light source
-Cover the microscope
-Close the door firmly behind you

A Note on stage position and parfocality

-Parfocality allows you to change the lenses and remain close to the focal point of your sample. For instance, if you are using the 10x lens, and are in focus, you can switch to the 63x and be very close to the focal point. There are two caveats, however: 1) switch lenses at 10x or above (this does not work with when starting from 1.25x) 2) the stage must be raised to the work position when the microscope is turned off in order to retain the settings in the microscope

Lenses:

When you want to use a higher-mag oil lens, simply click the lens. The stage will automatically lower and a warning screen will pop up reminding you to place oil on the

specimen. When oil has been put on the slide, simply press "Ready" and the stage will rise to the work position.

Remember to clean any oil lens you used by first wiping free of oil, and then cleaning with liquid Lens Cleaner provided. If it is not cleaned well, the residual oil will crystallize on the lens.

Resetting the upper limit of the stage*:*

If for some reason the stage's upper limit has been set to a position lower than the focus plane of your sample, you will hear a series of beeps as you try to focus, alerting you that the upper limit has been reached.

Solution: In order to reset the upper limit, hold the "UP arrow" for the stage controls until you hear a beep, release briefly and press and hold down while focusing to the proper plane and continue focusing below your sample (moving the stage up) just a little and release. Over-compensating the focus will give you a greater range of resolving power when larger objectives are swung into place.

Clean-up:
Cleaning the lenses completely will prolong the life of the equipment and prevent any crystals from the accumulation of oil

First, wipe all the lenses free of oil with the lens paper provided

Next, dab a generous amount of "Lens Cleaner" on fresh lens paper and wipe the lenses thoroughly in a circular motion.