Live Imaging System User guide

Zeiss Axio Observer.Z1 with Apotome 2

AVOID THESE DAMAGING EVENTS

- Oil dripping down objective lenses. Use oil sparingly. Gently wipe the top and sides of objective when you are finished. Use lens paper.
- Bumping objective lenses with the stage Insert or your slide. Avoid any rough handling of any part of the instrument.
- Nail polish or other substances contacting dry objectives or on microscope components (e.g. Oil on Dry lenses)

Video tutorial of similar instrument: https://www.youtube.com/watch?v=jrWoszi0HGw&t=1s

Fig 1 Microscope stand "on" button.

Startup

If live imaging, warm up system ~1 hour before experiment.

CO2 can be turned on just prior to placing cells at stage.

If heating, turn on:

1. Air curtain (hot air) : "X Unit XL" blue button under touch screen, "incubation".
2. Water bottle heater (sleeve), "none" or Label "X", under touch screen, "incubation".
3. And optionally: heated cover, and heated dish holder.

Turn on:

1. Power strip.
2. Microscope stand, gray button, left side (**Fig 1**).
3. Wait for the Zeiss touch screen **(Fig 3**) to boot up then, turn on computer with PC power button.
4. Heaters, if needed.
5. Login to "user-1" account. Password needed.
6. Double click Zen Blue software (**Fig 2**).

Fig 2. Zen Blue Icon

If Zen software won't run (e.g. gives MTB connection errors),

1. Close Zen and double click the MTB Config icon on the desktop. If this works, then Zen should now work.
2. If this doesn't work: turn off and on microscope (gray button).
3. If this doesn't work, turn off and on the entire system.

Prepping the system for use

Use the Zeiss touchscreen (**Fig 3**)

Press ''Microscope'', Press ''Incubation''.

The row of blue squares corresponds to the row of inputs in the back panel (**Fig 4)** of the Zeiss Heater/ CO2 Module. The blue square controls will change if the cable inputs in the back panel have changed. Typically, the blue square will control:

H Unit XL = Hot Air incubation enclosure temperature
[Label X] = Stage insert heating
[Label Y] = CO2 humidity/bottle heater sleeve
[Label …]
CO2 Small V = % CO2

Warm the system to 37°C ~1 hour prior to experiment by Turning on "X Unit XL" (Hot Air), and CO2 humidity/bottle heater sleeve

CO2 is not needed until just prior to imaging.

Heating of stage insert holder and lid is generally not needed.

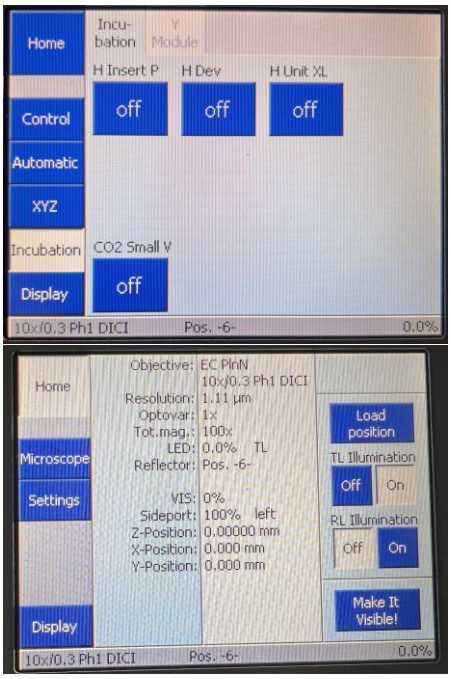

Fig 3. Zeiss touchscreen

Make sure the CO2 tubing is not clogged, and CO2 is reaching the chamber. The CO2 sensor is inside the white module not the chamber itself. The CO2 humidifier/heater bottle often gets clogged at the outlet with rust / debris.

Fig 4. Back panel heater connections

<u>CO2 covers and heated stage Inserts (Fig 5)</u>

There are currently CO2 covers for 1. multi-well plates and 2. dishes/chambers. Blue tubing needs to be connected to the cover as CO2 enters through this.

1. <u>Multi-Well plates.</u> No heating element. Fits standard multi-well plates with bottom stage insert.
    a. I have rigged this to work with Nuc Lab-Tek dishes (ask me). Additional solutions are in the works.
2. <u>Dishes and Chambers</u> (35 mm dishes and possibly other chambers). Heavy dish holder stage insert and cover. There are heating elements and connections for cover and stage insert but heating these may not be necessary and have given errors and can overheat.

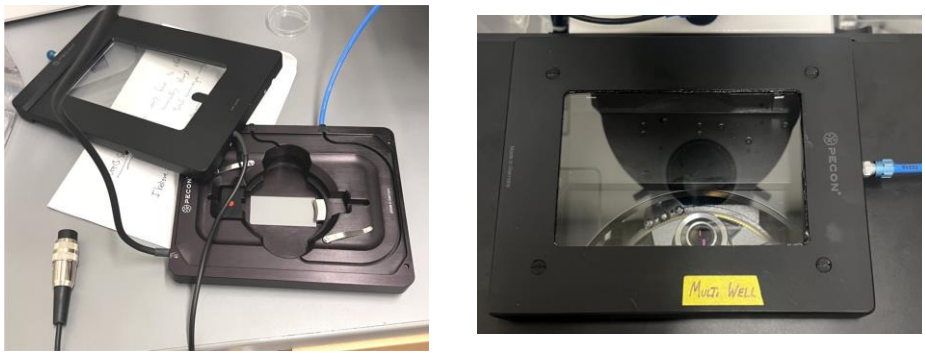

Fig 5. CO2 covers and stage inserts. Left: Dish insert and cover. Right: Multi-well cover. Black covers are required for CO2. CO2 enters the covers thru blue tubing at metal nut as shown (right).

Inspect the water level and water clarity of the CO2 humidity/bottle heater sleeve (Fig 6). Water should be free of contamination and between the two marks. If not, clean and fill with de-ionized water. This water bottle perfuses your cells with 5% CO2 and humidity. It needs to be heated and flowing.

Fig 6. CO2 bottle heater sleeve / humidity aerator.

Bringing Your Sample to the Microscope

Be very careful lowering the condenser arm and opening/closing all the plexiglass latches and moving the covers. The covers are fragile. The tubing and wires can easily be pinched.

<u>CO2 Regulator</u>

Start CO2 flow from the tank **(Fig 7**): open the gray screw valve on top of the tank. This will pressurize the right gauge. It should read above 500 psi (inner numbers), meaning the tank contains adequate CO2. Open the lower left brass valve. This sends air to the unit and your chamber. The left gauge should read between 5 and 10 psi (inner numbers). This level may decrease as CO2 flows. Adjust the flow regulator (large black knob) to keep consistent CO2. When experiment is over, close the tank valve and the brass outflow valve. CO2%, heating, and humidity reading can be seen on the Zeiss touchscreen and Zen software. Nota bene: **CO2 sensor is within the white unit not the chamber. Verify flow into the chamber.**

Fig 7. CO2 regulator, main valve (gray), and tank

ZEN BLUE Software

Click Zen Blue desktop icon (**Fig 2**).
Make sure stage area is clear and click "Calibrate now" to calibrate the stage.
Locate tab (left) controls the 'by-eye' use of the microscope,
Acquisition Tab sets up the experiment,
Processing Tab is used for post processing.
Analysis Tab is for quantitation.

At the top, the most recent **imaging settings** will be seen. Some of these settings may not work with the Apotome2 (described below). You may want to change settings and save your new settings with a new name.

Various additional modules are available (check them as needed).

Z-stack: For Z-series
Tiles: Specify stage locations for acquisition.
Time series: Time-lapse imaging.
For example, you could setup 12 tiles, to be imaged for 24 hours, and take one frame every 30 minutes.
Autosave: Automatically save your images with a specified file name and folder. Without this you may lose your data if system crashes.

Fig 8. Channels Module. Set intensity and exposure time

To Setup an Acquisition

Select the objective within the Acquisition Tab, if you set these in Locate tab, it may change in Acquisition.
Available lenses: 5X, 10x, 20x long distance (collar), 20X, 63x Oil, 63X water.
Select Fluorescence channels in the Channels Module (**Fig 8**) (select/deselect desired fluorescence filters
Available filters: DAPI, CFP, EGFP, DsRed, Cy5, Brightfield.
Set the lamp intensity. The X-Cite LED Lamp can be set from 0-100%.
Set the exposure (lower portion) and other settings of each channel, either manually or automatically, using "set exposure".
For live fluorescent Imaging, avoid strong excitation light. It is less toxic to use longer exposures with dimmer excitation. The X-Cite 120LED (intensity set/viewed by the dial or software slider) is less toxic than mercury lamps but still damaging.
For Brightfield, use the Intensity Slider to control the lamp rather than the dial/wheel on the microscope stand. The software will not remember the dial settings.
''Set exposure'' will auto adjust exposures for you.
Histogram (button in center area, left side) and range indicator (button in center area, toward the bottom) can help with selecting optimal exposures.

Set camera to Bin=2 for sensitive live fluorescence imaging. Binning of 2 = 2x2 averaging of your pixels, is much brighter than bin=1. This is set in imaging setup.

Apotome "Z" Sectioning

Pull Apotome 2 unit out of the light path 1 click if you want standard fluorescence (**Fig 9).** Apotome slicing takes ~5 images for each Z-section, usually not ideal live imaging. The Apotome 2 is an optical component that physically

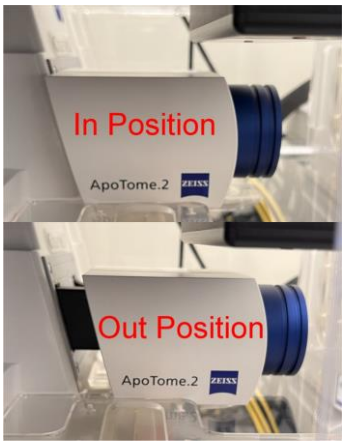

Fig 9. Apotome 2

slides into the excitation light path and provides a structured illumination, grid projection that allows removal out-focus light. The benefit is a **confocal-like Z-slice.** Trade-offs include a slower acquisition (more images taken), and photodamage, more complicated acquisition, may require calibration. **It does work nicely when set properly.**
To use: make sure the unit is in the light path (**Fig 9**) and check "enable" in software module. There are 3 settings which capture variable amounts of extra images. You can image with the Apotome in place but with Apotome effect disabled (unchecked), but you may see grid lines in your image.

Saving Apotome images (Fig 10)

for opening with other software: In lower right, go to ApoTome tab > set the settings e.g. enable correction, local bleaching (Fourier filter does not seem to help > Create image. This will create the Confocal-like image that can be saved by: right click > save selected as (or file > Save As CZI). Export as TIF may not work, **I recommend saving as CZI.** Saving images without "creating image" will give an image stack grid lines (raw data). You cannot Apotome section the Bright Field channel (transmitted light), but you can perform multi-channel apotome sectioning and include the BF channel and save the standard bright field image. To calibrate the Apotome grids, see separate instructions.

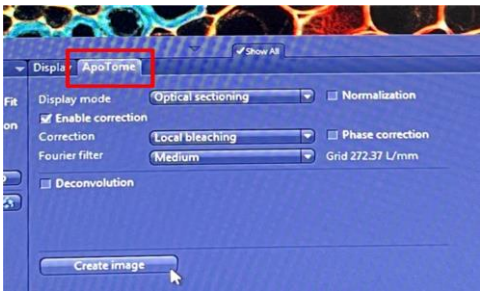

Fig 10. Save Apotome Images.

Tile Scanning module (Fig 11)

Check the Tile Scan box (left area, upper right).
This accesses a complicated menu (Fig 11). There is a Tile Regions section and a Positions section. Delete (right click, delete) any previous positions to clear it. To set positions like Leica's Navigator, you can use Advanced Setup.

Options > Split Scenes into Separate Files will cause your images to be saved as individual tiles. If this is unchecked, your images may be merged into a single image (depending on how you have acquired). A Zeiss "Scene" is a tiled region.

Setup a new position by clicking the "+" button in the Positions section (delete by right click, delete). You can name the positions if desired by double-clicking. To change a position to current position, right-click and ''Set current X/Y/Z for selected position''. Clicking with the mouse during live imaging will center the field at that position (very handy feature). You can use the **Tile Regions section** to for instance, capture 5 x 5 grid of tiles, or to define a tile scan by marking the edges, as in LAS-X navigator.

The stage will acquire positions in the order set in this module, NOT THE WAY YOU SET UP THE POSITIONS. This is important for determining what position is what location later. Pay careful attention to these options.

Fig. 11 Tile Scan menu

Time Series Module

Use the slider or type values for: experiment duration (minutes/hours/days), the unit of time, interval of acquisition. You can also set up pausing. The minimum time for acquisition (move stage, change channels, acquire) may not be correctly indicated in the software.

Autofocus Module

Autofocusing is generally required for long term imaging but can give errors (fail to find focus) depending on the settings. Autofocusing with the Bright field channel is ideal, but settings can be tricky. Autofocusing the fluorescent channels will increase photoxicity.
You can select the “reference channel” used for autofocusing (click Focus Ref. in channels module).
In the Software Autofocus module, I have had the best luck with Auto, default, and smart, default for fluorescence focusing. if you are doing tile scans, **check “Use Focus surface Z values defined by Tiles Setup”** In the focus strategy module.

Autosave' module

Recommended for long term imaging. This will periodically save your experiment while acquisition is ongoing, so that if something happens, you will not lose your data.
Do not save files to the C:\ drive. These will be deleted.
Use the D:\ Pl Name\YOUR NAME to save your experiments.
'Automatic Sub-Folder'', will cause Zen to create a new folder, or you can specify manually.

Experiment Information module

Use this to review your experiment and estimated size of the files to be generated. Make sure the hard drive of the computer (D:\) has enough free space.

To acquire your experiment, click ''Start experiment'' (upper left).

Check your experiment during acquisition, especially the first 2 hours.

Temperature changes and other movement may alter focus and require X-Y-Z adjustments. Use sliders to view acquired images. Check the incubation panel (**Fig 12**) for proper temperature and CO2.
If you need to adjust the positions during acquisition:
Press ''Pause experiment'' (not ''Stop'' which will terminate experiment). “Live” image to see your cells, and in ''Tiles'' module, double-click positions (goes to position), focus image, Right-click, and click ''Set Current.”

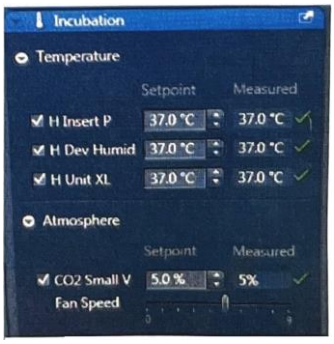

Fig 12. Incubation Module

Shut down

- Stop experiment and close (X-out) Zen software.
- Clean area, remove your sample.
- Save your files.
  - Back up your files to multiple drives and/or the cloud.
  - Data older than 2 weeks may be deleted. Please delete unnecessary files from the computer after they have been backed up.
- Turn off all 4 environmental units on the Incubation touchscreen, turn off all units (H insert P, H Dev, H Unit XL, CO2 small V).
- Close the CO2 tank by turning the main gray valve to closed position. Close the small brass valve by turning it.
- Gently wipe any immersion liquid (oil or water) off lenses.
- Set the objective to 10x and lower focus (Load Position).
- Close all doors and top sliders to prevent dust accumulation.
- Shut down the computer.
- Turn off the power strip.

John W Murray, PhD
jwm2175|cumc|columbia|edu
2024-11-12