

STANDARD OPERATING PROCEDURE

Zeiss Live Cell Imaging System

PURPOSE

This document describes the operational setup and procedures for Zeiss Live Cell Imaging System Axiovert 200M (Zeiss microscope). This document offers users, particularly beginners, a brief step-by-step instruction on how to take images using the Zeiss microscope with its acquisition software Zen v2.6.

The Zeiss microscope is a fully motorised inverted wide-field epi-fluorescence microscope equipped with a temperature-controlled CO₂ incubator for live cell imaging applications. It comes with standard filters for fluorescence (DAPI, FITC, TRITC, Cy5) and BF/Phase imaging options. The acquisition software has been upgraded to Zen in 2019.

SCOPE

This procedure applies to single-channel image acquisition, termed as “locate” in Zen. This document covers starting up the system, preparing the system, image acquisition, saving data, and shutting down the system.

Users are to refer to the full manual provided by the vendor for more detailed operational instructions and other advanced applications such as multi-channel acquisition, time course, multi-area imaging, etc. as well as data processing & analysis workflows.

SAFETY

The operator must exercise caution during loading or removing of the slide from the stage, never put fingers into the sample loading area while the stage is moving or initialising.

In addition, safe operation of the instrument also requires the awareness of other risks e.g. glass cuts, exposure to lasers and UV light, mercury lamp explosion, fatigue and gesture discomfort/injury etc. Refer to the latest Safe Work Procedure applicable to the microscope that can be found on the Westmead Imaging internet site, WIMR-SWP-WHS-GEN, “Using a fluorescence or laser scanning microscope”:
<https://sydneyuni.atlassian.net/wiki/spaces/WIF/pages/768016621/Manuals+Protocols>.

Acquisition of samples on the Zeiss microscope must only occur after the approval of an associated project in PPMS. Any projects to be run on the Zeiss microscope involving hazardous chemicals must have appropriate approval. OGTR requirements for safe work in a PC2 laboratory apply.

TRAINING / COMPETENCIES

All personnel require training prior to independent operation of the instrument. Training is conducted by facility staff with competency demonstration necessary before authorisation.

Competency is assessed via demonstration of independent instrument operation, in conjunction with verbal explanation of all aspects of operation and troubleshooting common faults. After training is carried out, a training competency quiz is to be completed and passed before instrument access is authorised by imaging staff. All instrument operation is to be conducted by trained operators.

EQUIPMENT & SUPPLIES

The Zeiss Live Cell Imaging system is located in J2.12C, Level 2 of WIMR.

PROCEDURE

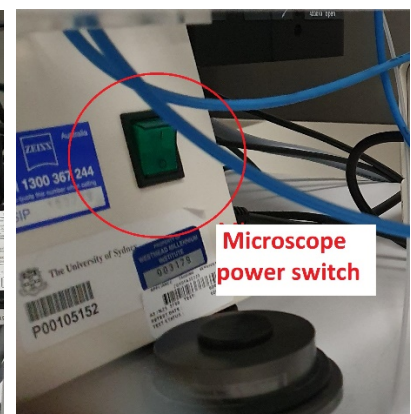
1. Start up

- 1.1 Turn on the computer. Log in (WIMR active directory login).
- 1.2 Turn on the system
- 1.2.1 For **fixed samples** (CO2 supply and temperature control are not needed), turn on the following components/parts (no particular order):

- Mercury lamp burner (fluorescence imaging only. Skip it if BF only)

Safety notes:

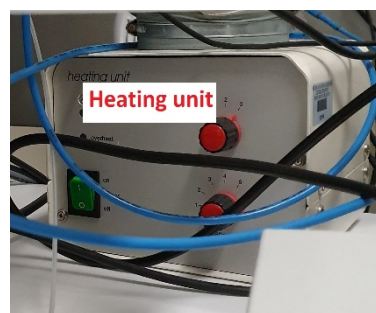
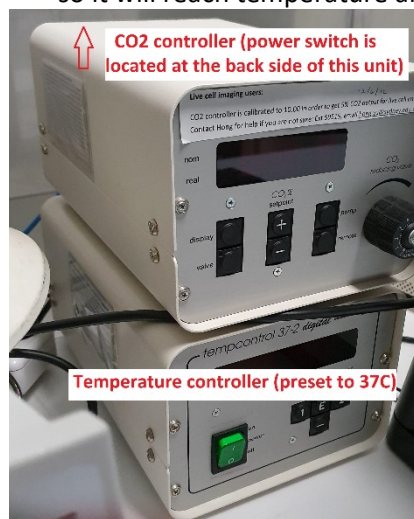
- a. Hg lamp needs minimal 15 min stabilisation time after turned on and 15 min cooling off time before being turned back on after switching off.
 - b. We apply a 30 min interval in deciding whether users are to turn off the Hg lamp before next booked user takes it over.
- Stage controller for the motorised stage
 - Microscope (this will also turn on the camera)



- 1.2.2 For **live samples**, turn on additional components for CO2 supply and temperature control

- CO2 controller and temperature controller
- Heating unit (to blow the heated air into the incubator)
- CO2 gas valve (no need to fully open the valve, just a little bit) and adjust the regulator knob (anti-clockwise to open) till gas pressure reaches the marked sign as shown in the right photo

Note: warm up the weather station at least 2 hours in advance to stabilise the temperature to 37°C as well as the CO2 levels. Do not forget to place the appropriate oil (if applicable) in the incubator so it will reach temperature also.



2. Open up Zen

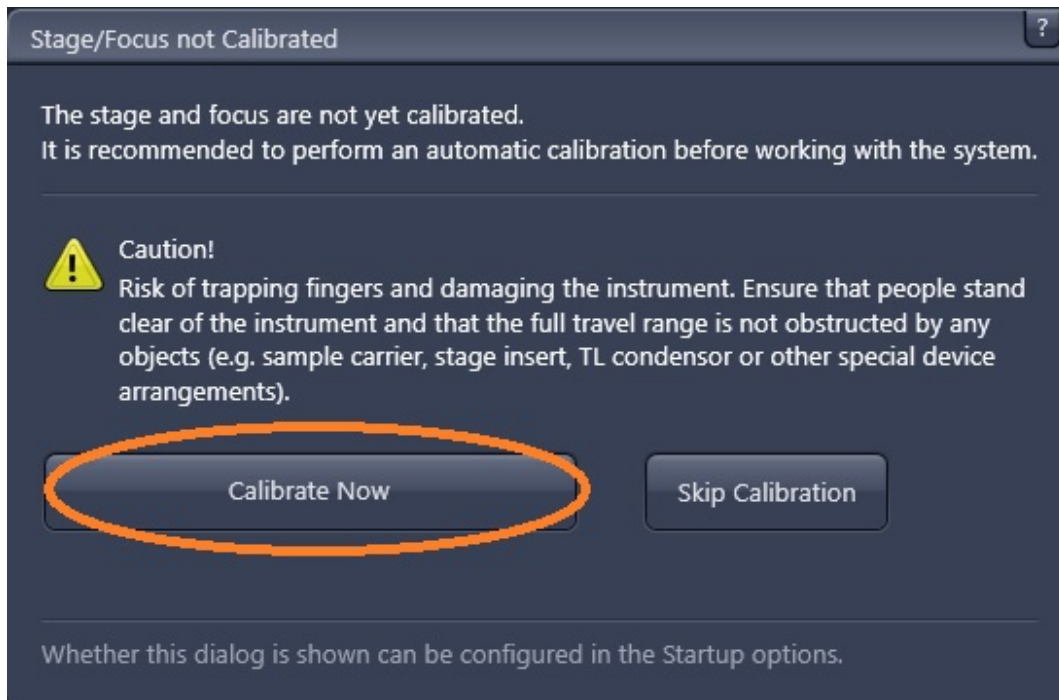
2.1 After the system has started up, double click the **[ZEN]** icon on the desktop to start up the software.



2.2 Then click on “Zen pro” to open the acquisition software

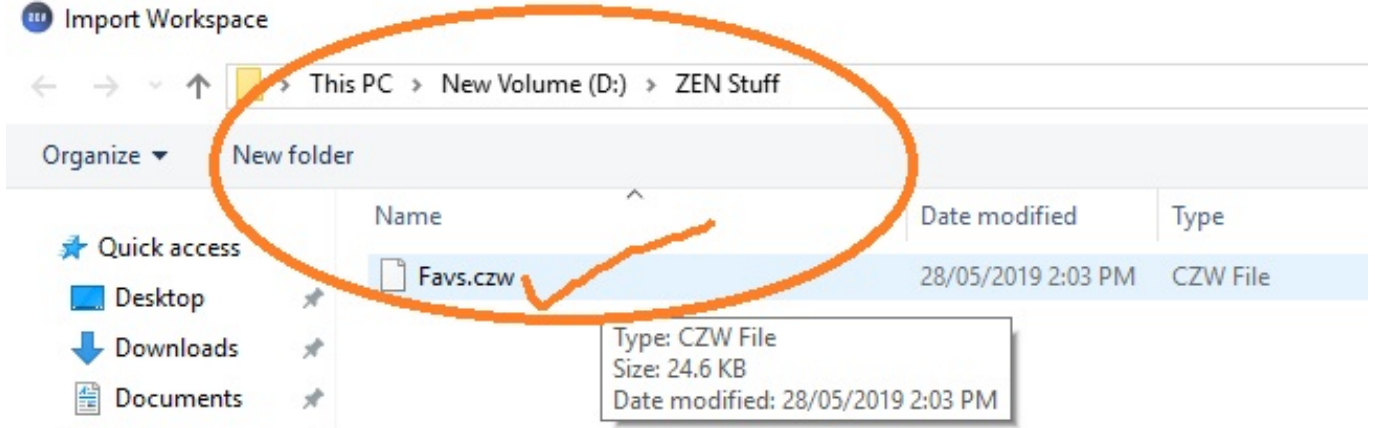
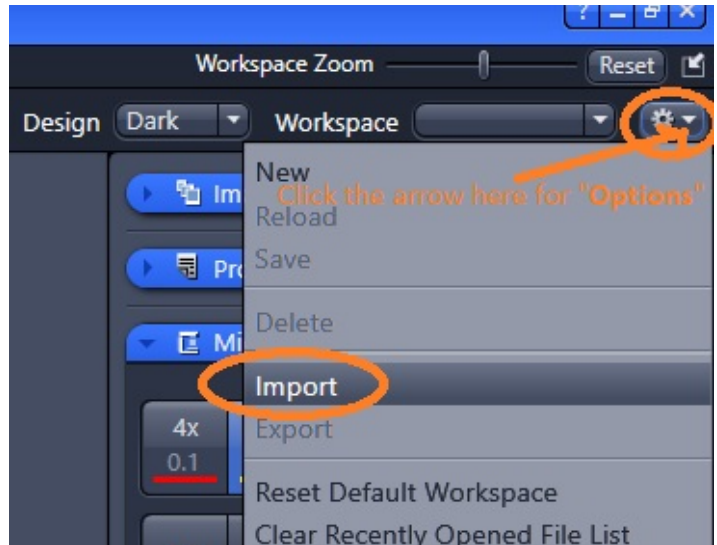
3. Prepare the system and settings

3.1 Calibrate the stage in Zen



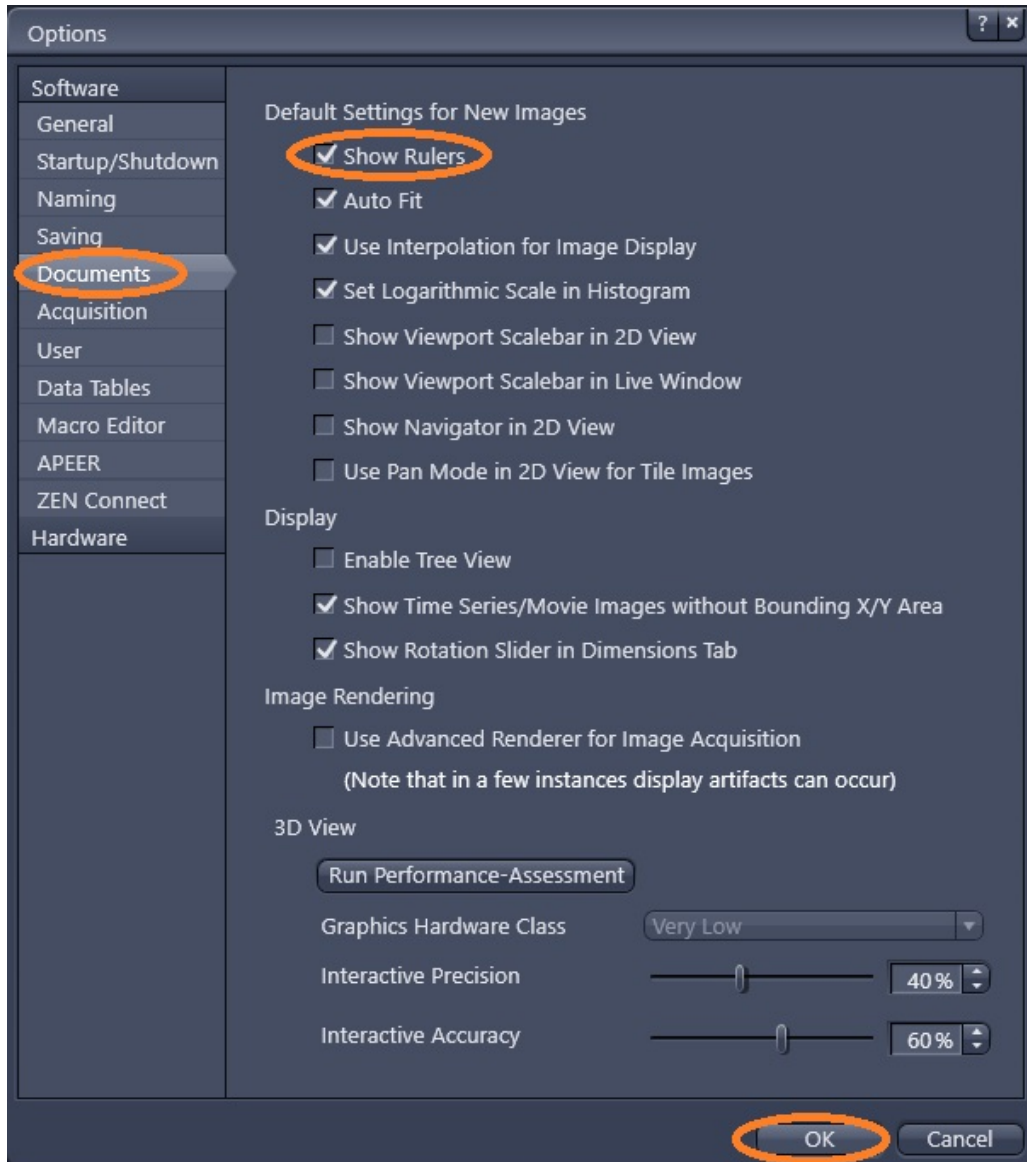
3.2 If you are a 1st time user follow the below steps (skip if you are a returning user):

3.2.1 Import pre-set favourite settings “Favs.czw”

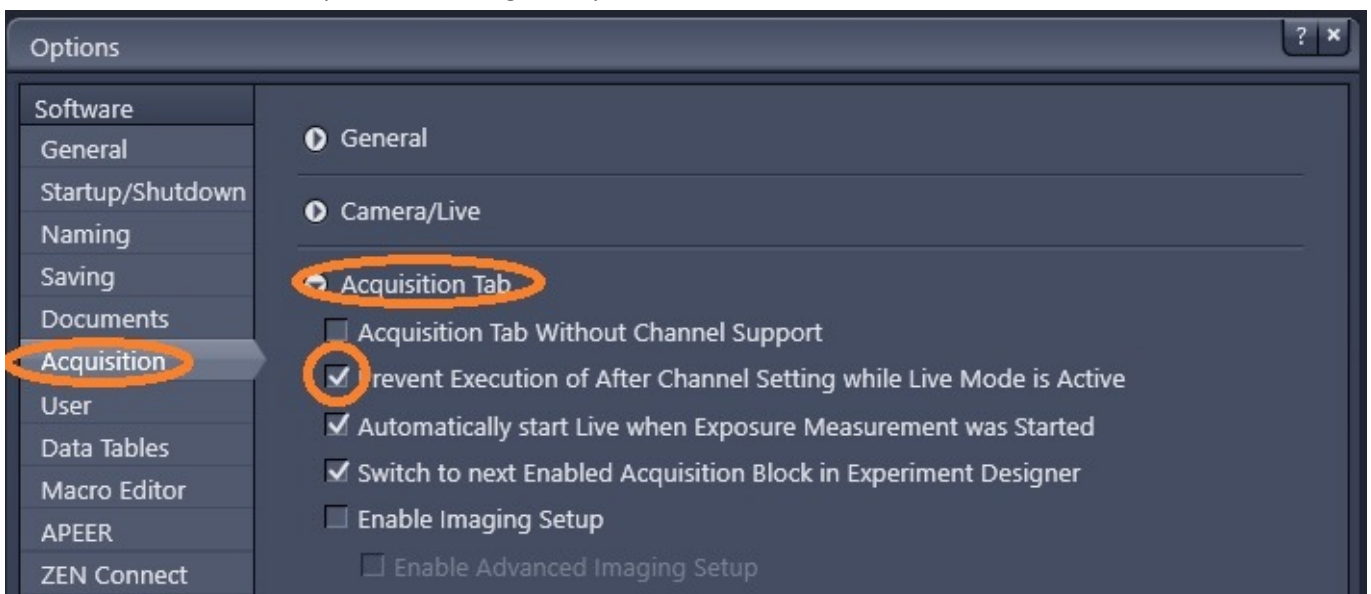


3.2.2 Get the settings ready as shown: “Tools” (main menu)—“Options”

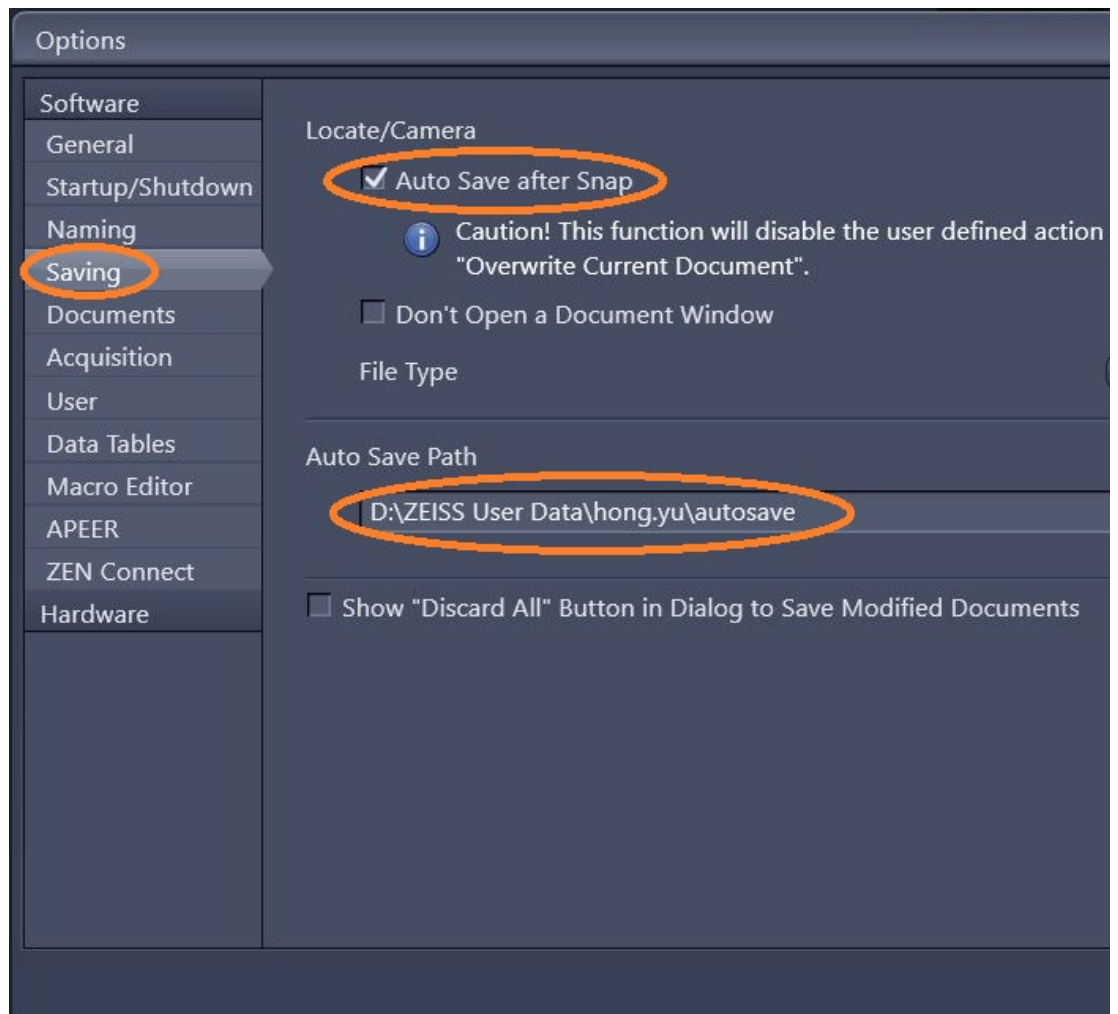
- Go to “Document”, tick items as shown below, click “ok”



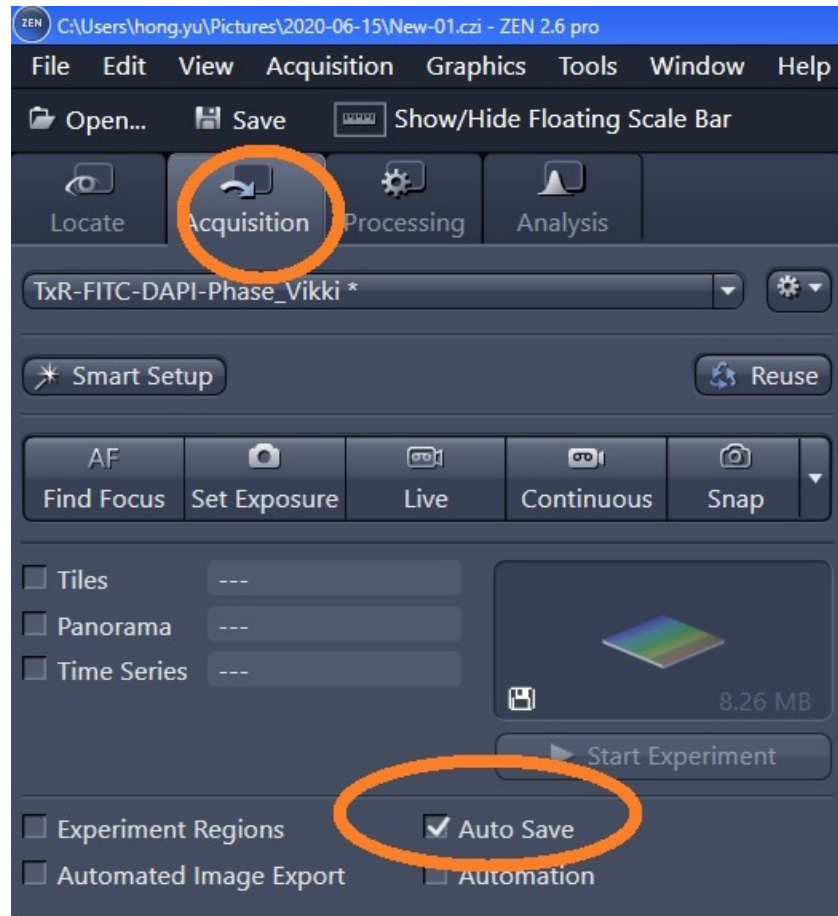
- Go to “Acquisition” settings ready, tick items as shown below, click “Ok”



- Set up autosave: go to “Saving”, tick “Auto save after snap”. Make sure to save your data to the designated folder on the local computer first: D:/Zeiss Users Data/“your_folder”. click “ok”



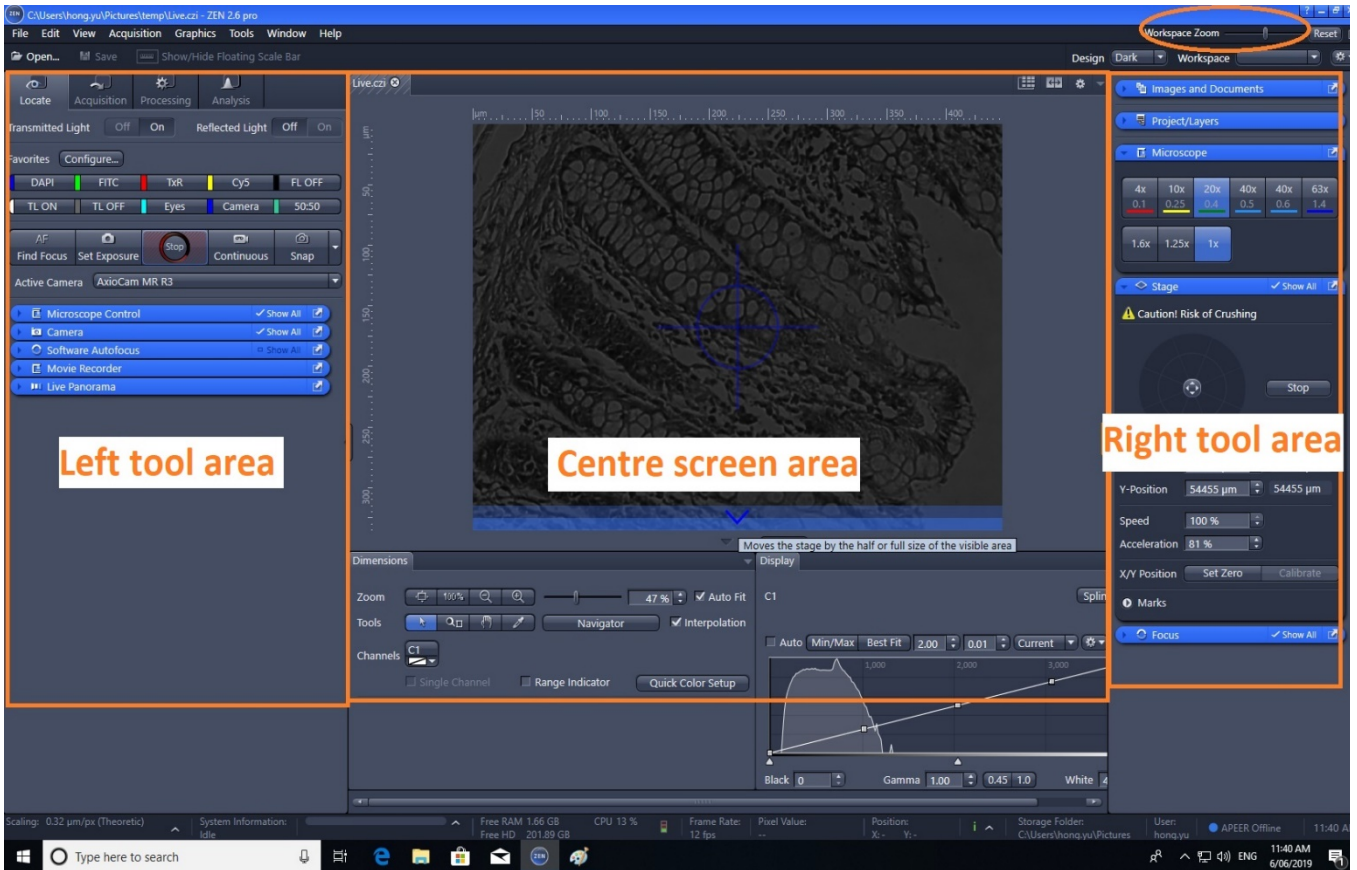
Note: if you are running “Acquisition” (not “Locate”), ensure “autosave is selected as shown below.



Data management policy: all data must be saved here (kept for 7 days before auto deleting: (D:) \ Zeiss Users Data \ “your folder”. Your data will be transferred to the server instantly: Scientific Platforms \ Cell Imaging \ Zeiss Live Cell Imaging \ “your folder” (kept for 14 days before auto deleting).

4. Image acquisition with Zen

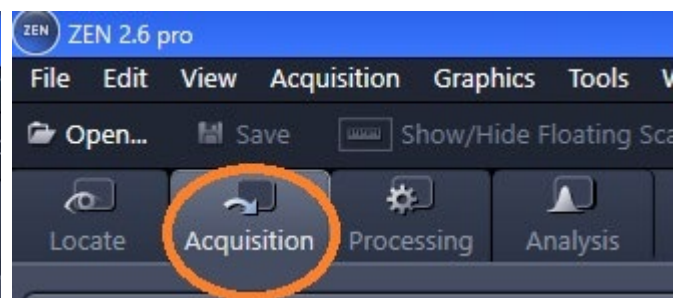
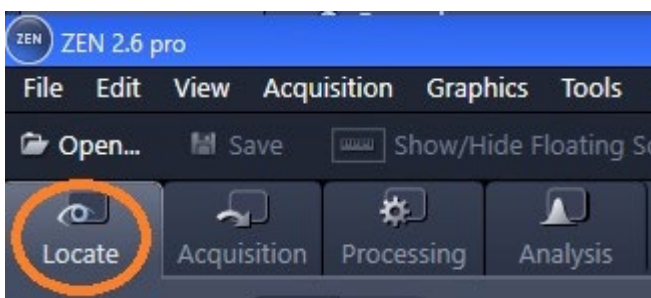
4.1 Lay out of Zen



4.2 Two important terms: Locate and Acquisition

“Locate”: where you run single dimensional acquisition, i.e. single channel imaging

“Acquisition”: where you run multi-dimensional acquisition, i.e. multiple channel imaging, multi areas, time course, Z stacking, etc.

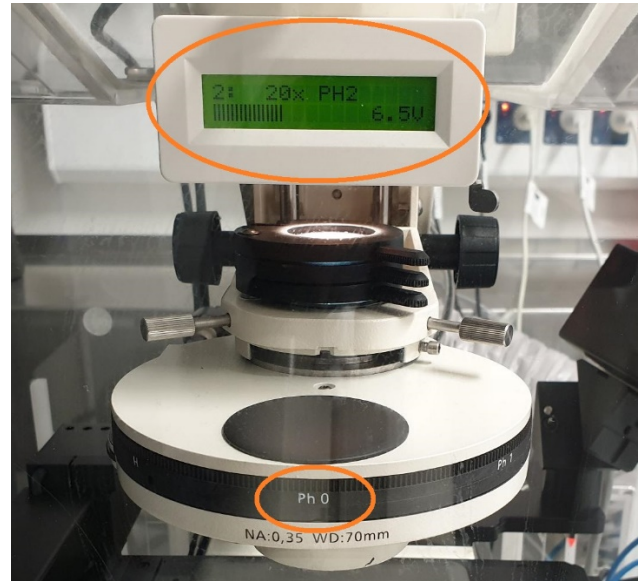
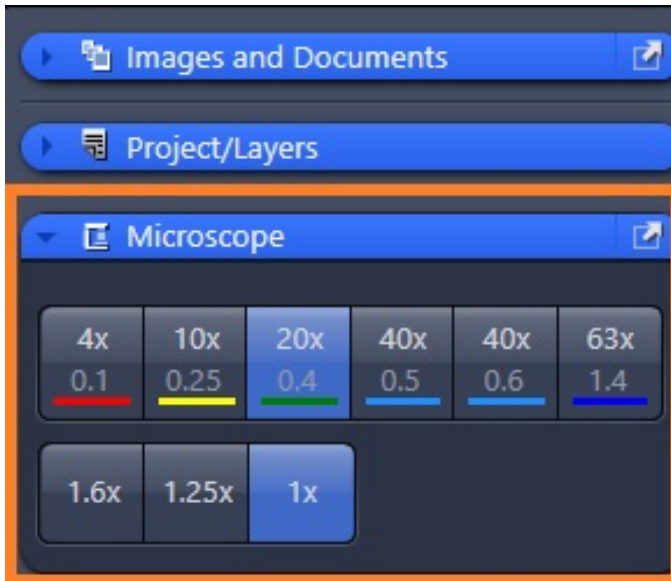


4.3 Single dimensional acquisition (“Locate”)

4.3.1 Click “Locate” tab from the Left tool area

4.3.2 Select a lens from the right tool area

Note: If you need phase contrast for BF imaging, make sure the condense position match up with the lens phase requirement. Otherwise, stay in “Ph 0” for non-phase contrast BF imaging (see below screenshot)



4.3.3 Set up the microscope in Zen

- Click “Eyes” to observe under eyepieces
- Click “TL ON” to open the transmission light shutter or one of the fluorescence filters (DAPI, FITC, or TxR) for fluo illumination



4.3.4 Now place your sample to the stage and focus under the microscope

- Click “Eyes” to observe under eyepieces
- Use the right stage insert to accommodate your sample carrier
- Click “TL ON” and check Kohler Illumination (refer to: <https://sydneyuni.atlassian.net/wiki/spaces/WIF/pages/765397549/Tips+Tricks>).
- Check Fluorescence staining with eye observation under eyepieces

4.3.5 Acquire an image

- Click “Camera” in Zen
- Click “Live” to view live image on the monitor
- Click “Set exposure” and “snap” to take a photo or select “auto expose” or manually adjust exposure time in the camera settings as shown in the below screenshot

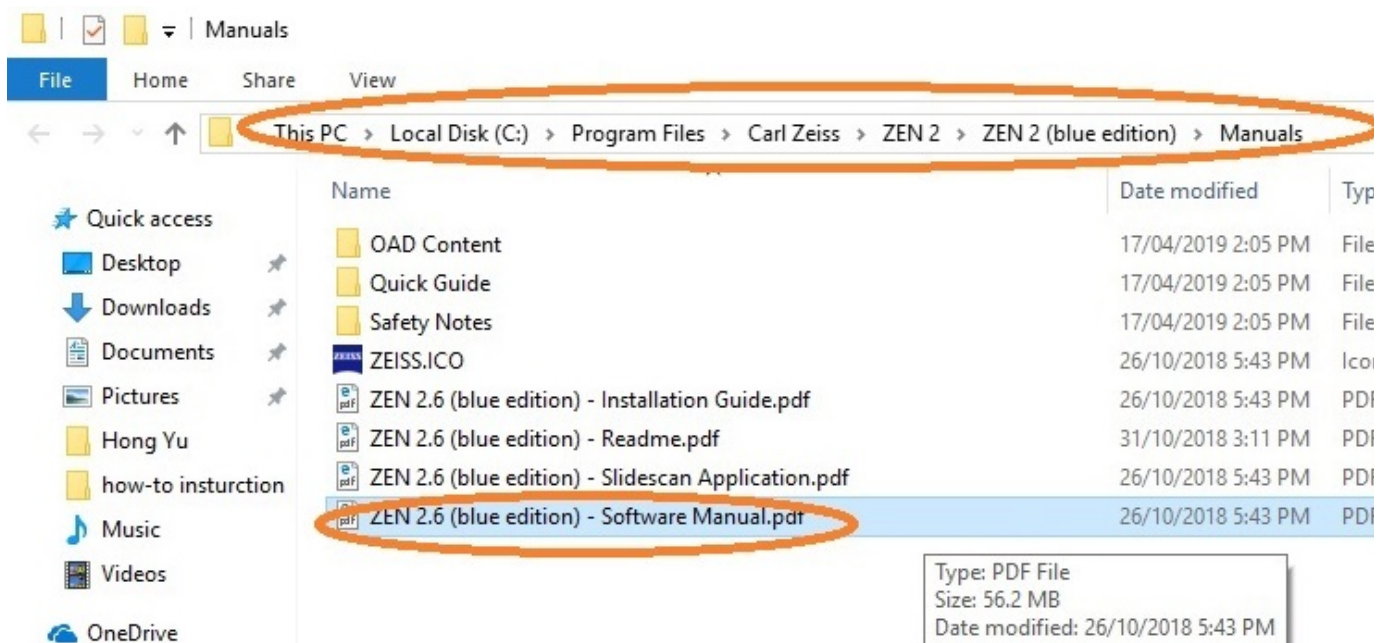


5. Shut down

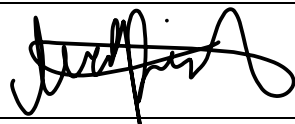
- 5.1 Close software and shut down the computer
- 5.2 Remove your slides from the
- 5.3 Turn off the fluorescence lamp
- 5.4 Turn off the microscope
- 5.5 Turn off the stage controller
- 5.6 Clean the bench
- 5.7 Log your usage in the provided sheet
- 5.8 Leave the room tidy

REFERENCES

The full e-manual provided by the instrument vendor is available on the computer that runs the Zeiss microscope: C:\Program Files\Carl Zeiss\ZEN\ZEN2 (blue edition)\Manuals. This manual is also published on the Imaging Website: <https://sydneyuni.atlassian.net/wiki/spaces/WIF/pages/768016621/Manuals+Protocols>.



DOCUMENT CONTROL

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REVISION HISTORY

Revision Date	Version No.	Amended by:	Change