Zeiss AxioZOOM.V16

User manual

Cell Imaging & Cytometry

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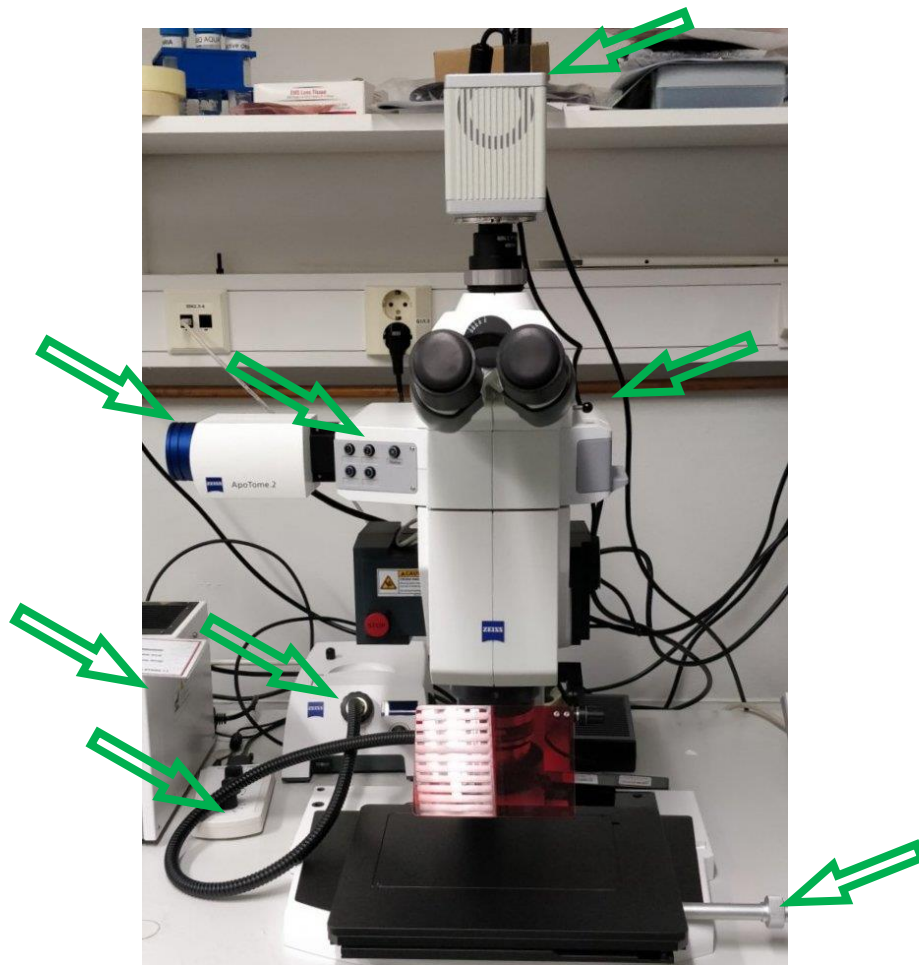*Turku Bioscience 5th floor, room 5077*

Contents

1. Information

Maintenance of this microscope is organized by the Cell Imaging Core.

- **Only authorized users may use the CIC instruments.**

- **Users are kindly asked to report any malfunction or problem to the CIC personnel.**

- **The user is responsible for removing their data from the hard drive and should do so immediately.**

2. Before Imaging

Clean your slides

Clean the remaining salt and mounting medium off the coverslip. Dirty coverslip compromises the image quality. You can use ethanol to aid the cleaning. Do not use lens tissue. Do this beforehand in your own lab as it is impractical to use microscopy time for cleaning.

Check the environment

Switch on the lights and check if the microscope environment is tidy. If there are oil spills or other issues, please inform CIC personnel (microscopy@bioscience.fi)

Cancellation

Cancellation should be done at least 24 hours prior the reservation starts. However, if you suddenly cannot use your time, <u>inform the next user and cancel your reservation</u>.
If you are the last user of the day and cannot come, you are responsible for the instrument shutdown.

Unsure?

If you feel that you need support, please contact CIC personnel.

3. Starting up the instrument

Switch on the camera, ApoTome.2, EMS3, required light sources and the computer
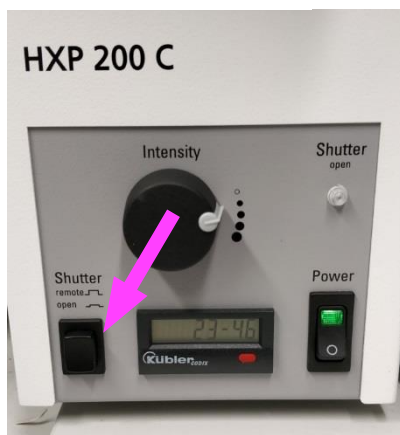
1. Switch on the camera, ApoTome.2 and EMS3 via the distributing box

2. Switch on required light sources

HXP 200 C for fluorescence, CL9000 LED for the upright light (collar), VisiLED MC 100 for transmitted light

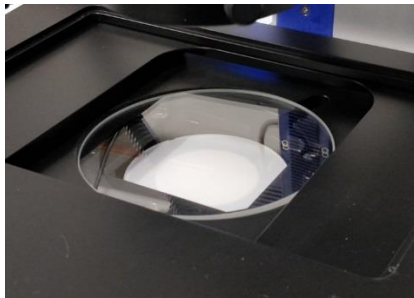**HXP 200 C**   **CL9000 LED**   **VisiLED MC 100**

3. Wait for 30 seconds

4. Switch on the computer

5. Double-click the ZEN icon

6. Start the ZEN Pro software.

4. Sample visualization

Place the sample below the objective, open the light path, activate the light source, and focus to the sample

1. If you need transmitted brightfield light (VisiLED MC1000), remove the black stage cover and place a glass disk into the holder. This light source is not controllable by the softrware.

2. Place the sample below the objective
3. Push the light path lever gently in

Lever in: sample visualization
Lever out: image capture

4. Choose and activate the correct light source.
   -Choose filter cube 4.
   -CL9000: turn the black knob clockwise for good illuminaton.
   -VisiLED MC1000: turn the lowest knob clockwise for good illumination. Use the two upper knobs for contrast enhancement.

1. DAPI (G365, 445/50)
2. GFP (470/40, 525/50)
3. Alexa 647 (640/30, 690/50)/Alexa568
4. ApoTome/Brightfield

Fluorescence shutter circled

5. Zoom and focus to localize the sample

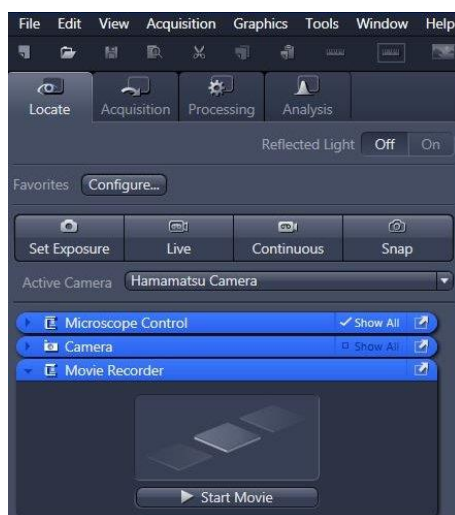| The small scrolls are the fine focus |

| Moving joystick in left-right direction zooms out and in, respectively

Moving joystick forward-backward focuses up and down, respectively |

6. Image acquisition

1.1 Single image acquisition

1. Pull the light path lever gently out
2. Click Live in ZEN
3. Adjust exposure by moving the Exposure Time slider under the Camera menu, or alternatively click Self Exposure
4. Click Snap to capture an image
5. Save the image in D:\userdata\group folder

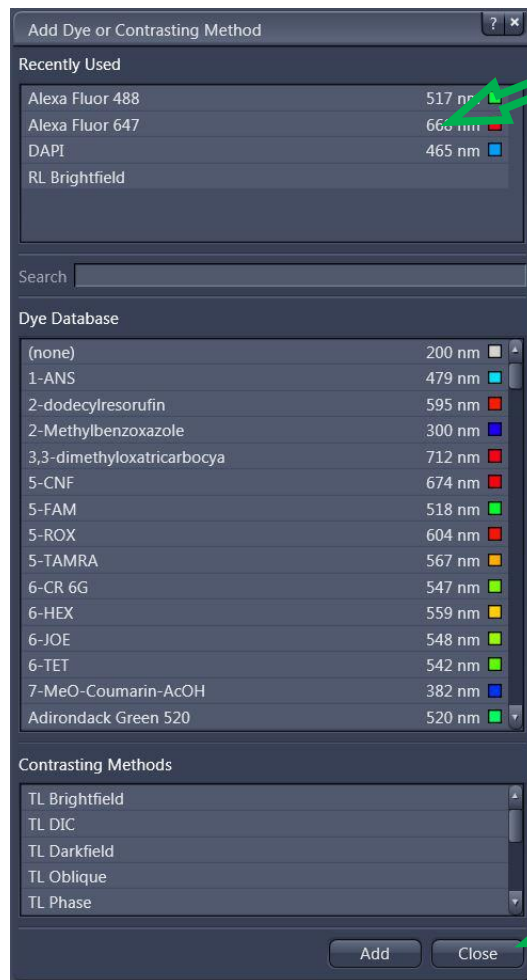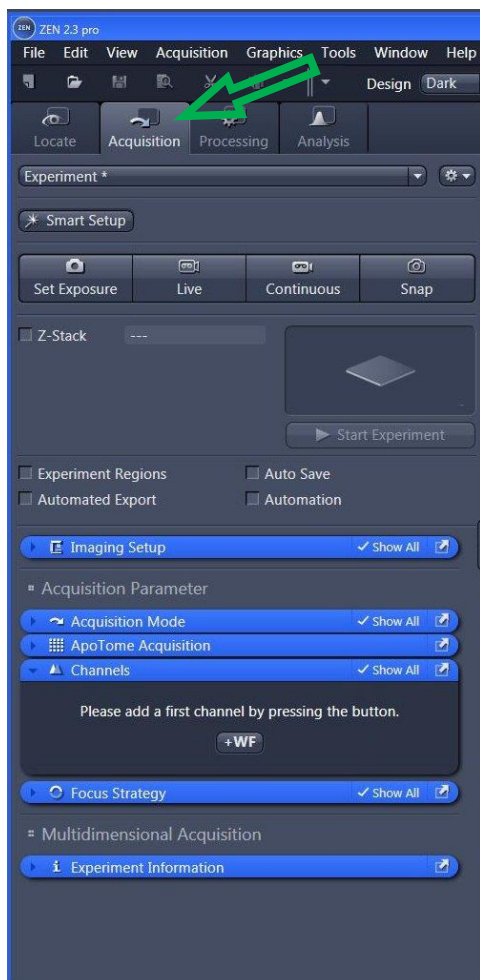| Live: live image
Snap: capture an image

Camera: adjust exposure time

Movie Recorder: capture a fast time-lapse |

1.2 Fast time-lapse image capture

Images are captured with camera's fastest burst mode. The user cannot modify speed.
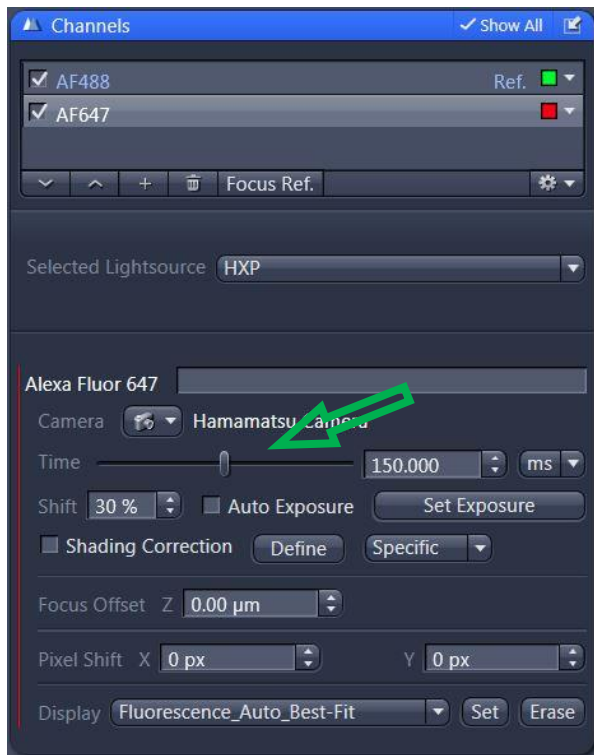
1. Open the Movie Recorder window
2. Click Start Movie
3. If you want to pause data acquisition to continue it later, click Pause
4. If you want to stop data acquisition, click Stop.
5. Save the image sequence to D:\userdata\group folder

1.3 Multichannel, Z-stack & ApoTome

1. Choose Acquisition
2. Open Channels and add the channels (labels) you need, fluorescence and/or brightfield. Double-click the dye or click once and click Add
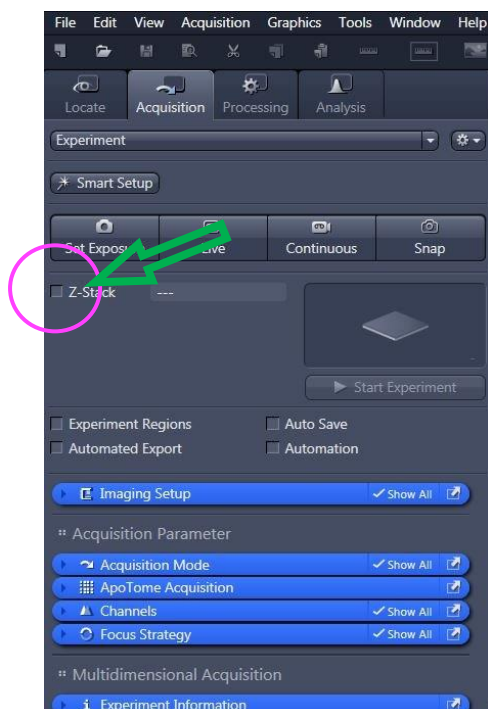3. Finish the selection(s) by clicking Close

4. Adjust exposure time for each channel
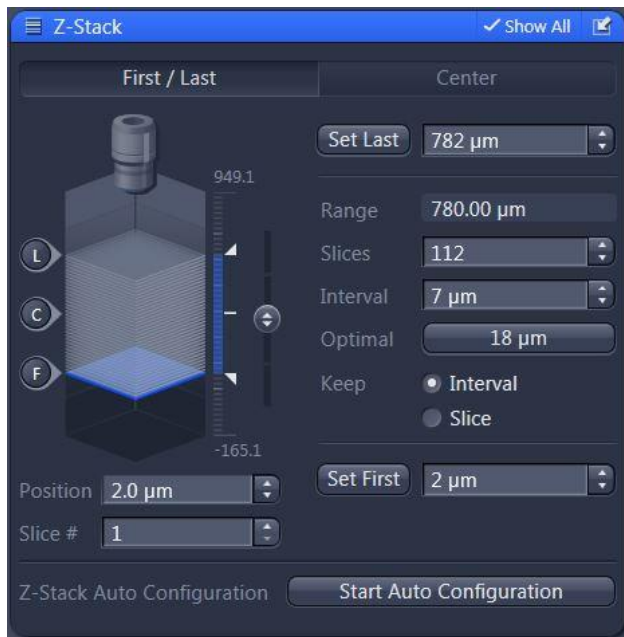5. In Live mode, you can toggle between channels by selecting them in the Channels window

Camera: Hamamatsu b/w camera

Time: Exposure time

6. Click Snap to take a multichannel image. All ticked channels will be imaged.

7. Choose Z-stack to capture a 3D volume.

8. In Live mode, focus down. Click Set First. Then, focus up and click Set Last. To select an optimal image interval, click the tab beside Optimal.
9. To image the stack, click Start Experiment.

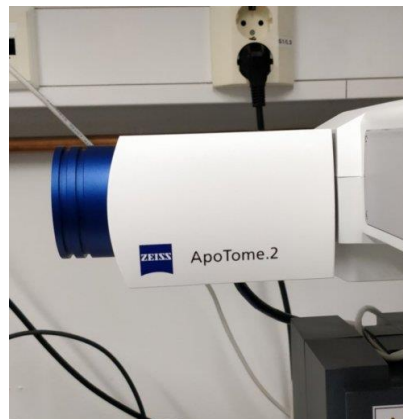| Set Last: the uppermost point |
| --- |
| Range: the depth of the stack
Slices: amount of images |
| Optimal: recommended imaging interval |
| Set First: the lowest point |

10. ApoTome image acquisition
11. Push the ApoTome arm softly in. The Enable ApoTome is automatically activated. collecting 5 Phase Images is a standard recommendation.

12. Define the stack as in normal z-stack acquisition. Click Start Experiment.

Shutting down the instrument

If the next user arrives within an hour

1. Clean the stage
2. Close all shutters
3. Take your data with you

If the next user comes after one hour, or if you are the last user of the day
1. Clean the stage
2. Close the Zen software
3. Shut down the computer
4. Switch off all light sources
5. Switch off the instrument and camera
6. Cover the microscope with the blue cover