

Updated in January 2023

Apotome Fluorescence Microscope



Make/Model:

- ZEISS Axio Imager Z1 (2009)

Features:

- Microscope: Axio Imager Z1 (motorized version with high-precision Z drive) with 6 objective lens-10x, 20x, 40x, 40x Oil, 63x Oil and 100x Oil. DIC and DF.
- Light sources: 1) **LED** modules (Colibri): **365**, **470**, **505** and **590nm**; 2) **UV** light source Illuminator HXP120.
- Filter sets (Colibri): **38HE** (GFP, CFP), **43HE** (Alexa 545, mTangerine, dTomato, mStrawberry, DsRed, Nile Red, Rhodamine, Propidium Iodide, etc.), **46HE** (YFP, Calcein, Fluorescein, mNeonGreen, Venus, etc.), **47** (CFP), **49** (DAPI, BFP), **50** (Cy5, Alexa 647), **62HE** (multi line for DAPI/GFP/hcRed), **64HE** (mCherry, mPlum, mRFP), and Analy DIC TransLight, plus other options. For a complete filter features, please visit <https://www.micro-shop.zeiss.com/en/de/shop/filterAssistant/filtersets/>.
- Cameras: 1) Digital CCD: AxioCam MRm (black/white, 1.4MP); 2) DinoEye (color, 1.2MP)
- Imaging software: AxioVision 4.8; DinoCapture2
- ApoTome slider and control box
- **Heating stage** (adjustable RT-60°C)
- Application modules: 3D Z-stacks; time course; **Extended Focus**; AutoMeasure etc.

Applications:

- ApoTome is an innovative slider module (structured illumination) for fluorescence microscopy. With a flexible choice of LEDs and filters, the microscope can produce high quality 2D and 3D digital images for fixed or living cells or tissues.

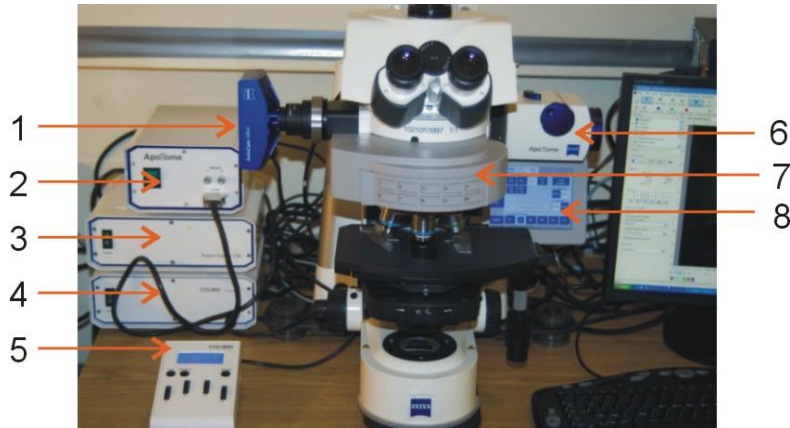
INSTRUCTION

Before you start, please note:

- This scope was designed for fluorescence microscopy with a wide range of choice of emitters and reflectors. With the additions of ApoTome apparatus and powerful AxioVision software, flexible fluorescence imaging for 2D, 3D (Z-stacks), time-series and multi-channels becomes feasible.
- Most components of the microscope are motorized and can be operated manually (knobs, switches, buttons), by small touch screen (TFT display) or by Axiovision (computer screen). However, computer-driven operations are strongly recommended.
- You are supposed to be familiar with the ordinary microscope operations including mounting and focusing your specimen, adjusting lens. Special care should be taken when you use oil objective lens (40X, 63X and 100X).
- Upon your experiment, you can use one or a combination of the following light sources: transmitted light (HAL100 halogen illuminator) or reflected lights (including single wavelength LEDs, and a metal halide white light Illuminator HXP120). Remember: transmitted light and LEDs can be switched on/off at your will, but HXP120 needs 20 minutes' interval. For comparisons among LED, HXP and HBO light sources, please click [here](#).
- *Software practice is the key to enhance your image quality!*

Operating Steps:

1. Turn on the power switches in the following sequence: HXP120 (only if you need), Colibri control unit, ApoTome (only if you need), microscope power supply, microscope toggle, and computer. Shutdown sequence will be the opposite.



- | | |
|-----------------------|--------------------------|
| 1. Camera | 5. Colibri control panel |
| 2. ApoTome power | 6. ApoTome slider |
| 3. Colibri controller | 7. Reflector turret |
| 4. Microscope power | 8. TFT display |

- Enter the Windows by logging on your username and password, then click the "Axiovision Rel. 4.8" icon to open the software.
- Load your specimen slide onto the stage, select appropriate objective lens and focus the point of interest.
 - Change lens can be controlled by touch screen, Axiovision or manually.
 - Don't switch from oil lens to dry lens. If it happens, remember to clean the dry lens!
 - Make sure the transmitted light shutter is "Open" by checking microscope, touch screen or Axiovision.

2. To obtain single bright field (BF), DIC or PH image (using transmitted light).

- Click "Workarea" to open the microscope/camera/processing window. Under the tab "Microscope", click the tab "Transmitted Light" and make sure the light is "On" with appropriate voltage.
- Select appropriate condenser turret manually or on touch screen. Focus the specimen, and adjust slider if required, via eyepieces to get best imaging effect.
- Click "Cam side port" and select the 100% light to the side.
- Click "Live", a window will pop up with the rough image of your specimen.
- Click "Property" to open a histogram, and adjust the quality of the image by playing with "Min/Max", "BestFit" and "Gamma" etc.
- Click "Snap" to capture the image.
- Click "Save" to save your image. Always save as ".zvi" format.
- To edit the images, click "Scale bar" etc. to label, measure or change colors etc.

3. To obtain single fluorescent image (using LED modules).

- Select the appropriate LED module and **filter set** (<https://www.micro-shop.zeiss.com/en/de/shop/filterAssistant/filtersets/>) based on the excitation/emission property of your dye/compound.
 - There are 4 LED modules available: 365, 470, (505) and 590nm; and 8 filter sets: **38HE** (GFP, CFP), **43HE** (Alexa 545, mTangerine, dTomato, mStrawberry, DsRed, Nile Red, Rhodamine, Propidium Iodide, etc.), **46HE** (YFP, Calcein, Fluorescein, mNeonGreen, Venus, etc.), **47** (CFP), **49** (DAPI, BFP), **50** (Cy5, Alexa 647), **62HE** (multi line for DAPI/GFP/hcRed), **64HE** (mCherry, mPlum, mRFP), plus an Analy DIC TransLight.
 - Under the tab "Microscope", click the tab "Reflected Light".
 - Under the "LED" list, click "desired LED "On" or press the Colibri control panel wheel.
 - Under the "Reflector" list to select reflector (filter set).
 - Make sure the FL (reflected light) shutter is "On" and TL "Off".
 - Following above procedures to acquire and save images.

4. To obtain single fluorescence image (using HXP120).

- Turn on the light by switching on the power and pushing the Shutter button IN on the HXP120 unit. If this light source has just been turned off, please wait at least 20 minutes before you start it again!
- Click "Open" External Shutter under the "Colibri", or press "Ext"/"Shutter" on Colibri control panel.
- Choose appropriate filter under the "Reflector" list.
- Then following same procedures as for LEDs to acquire and save images.

5. To obtain multiple images or multi-channel images (transmitted light and/or fluorescence) in the format of 2D, Z-stack or time-lapse.

- Click "6D Acquisition" button; or under "Workarea", click "Multidimensional Acquisition".
- Click "Experiment" tab, "Load" factory default or the experiment you have saved previously.
- Click "C" tab, set the channels you want to use.
 - There are five default channels (Green, Blue, Red, BF and DIC). Right-clicking the number icon to remove, left-clicking to add.
 - You can easily arrange the order of the channel by click-dragging the number icon.
 - If you want to add a new channel to the channel pool, click "Extended parameters", "Channel pool", and then "Add to channel". However, the maximum capacity of multi-acquisition is 5 channels. Remember to correctly select the channel settings for your new channel (e.g. exposure mode at "fixed"). If some settings are not available for some dyes, ask Guosheng to help.
- Then click "Measure" to optimize the exposure time for EACH individual channel. "OK" to accept the measurement.
- Click "Start" at the bottom to acquire multiple images.

- Following common steps to save and edit the images.
- You can set "Z-stack" or "Time Series" for each channel at your will by clicking the tab and following the on-screen instructions.

6. To obtain single or multiple images with Apotome.

- ApoTome power supply must be on when you start the microscope.
- First, following the same procedures as described above to acquire ordinary single image.
- Then, carefully push the Apotome slider into the microscope.
- Click "Apotome" icon on the left-hand menu. You can check if the grid (structured illumination in ApoTome) was calibrated with your lens and dye used.
- Click "Live" to acquire the image and snap/process as normal operations.
 - You will see the dark raster on the live image.
 - The light intensity usually decreases at ApoTome mode. Adjust as required.
 - You should use ApoTome slider under reflected light sources and at any acquisition mode such as 2-D, Z-stack or single/multiple channel(s).
 - The grid should be calibrated in each lens/filter combination. If not yet, please calibrate the grid focus (NOT Phase) now according to the [instructions](#).
- The ApoTome slider should be off after use. To switch off ApoTome, just pull out the slider one step (hear a click-sound).

7. To obtain image with Extended Focus module.

- Obtain normal Z-stack images under multi-dimensional mode and save. Select "Extended focus" under tab "Processing" to open the saved file. It will convert the Z-stack images to a single sharp image.
- Or acquire the extended focus image by "Extended Focus" module in "Workarea". Obtain the "live" image, manually adjust different focal plane and meantime click the "start" button. The images will automatically fuse to single extended focus format.

8. "AutoMeasure" module for image analysis after acquisition either from confocal or apotome microscopes. Please see tutorial at YouTube:

<http://www.youtube.com/watch?v=hr73-1-bavs>.