

AxioVision Version 4.2

How to Use This Guide

This guide has been prepared by Imaging Associates to help you get started with your ApoTome imaging system. For a description of the full functionality please refer to the manual or help file accompanying the system.

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1. Calibrating the ApoTome

In order to use the ApoTome it must be calibrated for use with different objectives and fluorochromes. Without the correct calibration optical sectioning will not be successful. It is not necessary to calibrate the ApoTome before each use however it must be carried out if image quality deteriorates and we recommend that calibration is checked each time the mercury bulb is changed.

This brief guide takes you through Phase Calibration and Grid Calibration required for optimal ApoTome use. The ApoTome is supplied with:

- 2 ApoTome grids (for high and low magnification)
- An ApoTome calibration reflector block
- A mirrored slide
- A sample slide which fluoresces in a broad range suitable for most common applications. If you cannot visualise the fluorescence with your filter sets then you will need to prepare a very well labelled slide containing the specific fluorochromes you are working with.

Remove the ApoTome slider from the microscope. Using the tweezers supplied, insert the correct grid for the required objective and microscope type into the ApoTome slider (see Table 1 below – PL and PH are low and high grids for an Axioplan microscope whilst VL and VH are low and high grids for an Axiocvert microscope). The grid must be inserted with the labelling of the grid clearly visible.

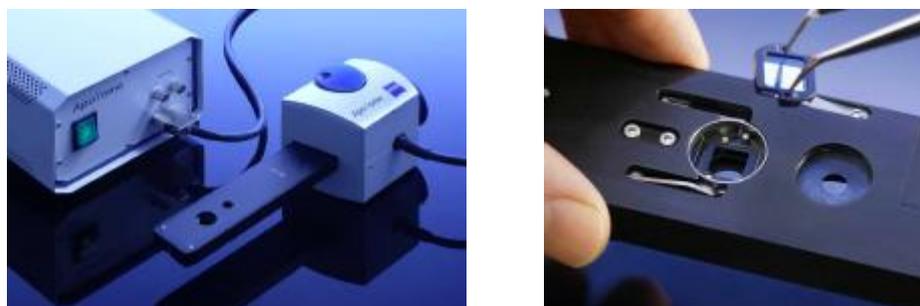


Table 1: Objective list and appropriate Grid

Objective	Low Grid PL/VL	High Grid: PH/VH
Plan-Neofluar 10x/0.3	P	O
C-Apochromat 10x/0.45W	P	O
Plan-Apochromat 10x/0.45	P	O
Plan-Apochromat 20x/0.75	P	O
Plan-Neofluar 25x/0.8 Imm Korr.	P	O
Plan-Neofluar 40x/0.75	O	P
C-Apochromat 40x/1.2W	P	O
Plan-Neofluar 40x/1.3 Oil	P	O
C-Apochromat 63x/1.2 W	O	P
Plan-Neofluar 63x/1.25 Oil	O	P
Plan-Apochromat 63x/1.4 Oil	O	P
Plan-Neofluar 100x/1.3 Oil*	O	P
Plan-Apochromat 100x/1.4 Oil	O	P

* Not recommended for use with DAPI (UV excitation)

Ensure that the ApoTome calibration block is in position – this is vitally important as it is a UV blocking neutral density filter enabling safe visualization. Using the mirrored slide supplied, focus on the centre of the cross and adjust the microscope to send 100% of the light to the camera.

Phase Calibration

1. Start AxioVision.
2. In a live image window, focus on the cross of the slide, measure the exposure time required and adjust it using the sliders should it be required.
Note: Over-exposure should be avoided when using the ApoTome as over-exposure can not be imaged correctly by the processing algorithms.
3. Select the *ApoTome Mode* (in *Acquisition* menu). Then select the *ApoTome* menu and *Phase Calibration* (again in *Acquisition* menu) to start the calibration wizard.

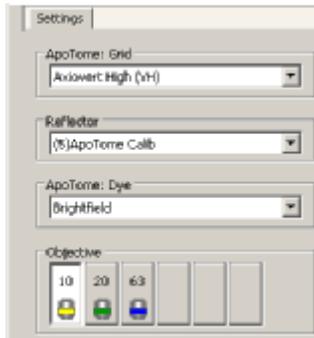


Figure 1

4. Using the calibration wizard settings page (Figure 1), identify which grid has been inserted into the ApoTome, set the reflector to the ApoTome calibration block position, set the ApoTome dye to brightfield and select an objective. Click *Next* to move onto the next page.
5. It is critical for the correct function of the ApoTome that the grid lines of the ApoTome are exactly horizontal (Figure 2). It should not be necessary to re-adjust the camera unless it has been moved or replaced. If necessary the camera can be rotated to align the grid image by releasing the screws holding the camera mount and rotating it until the lines are horizontal.

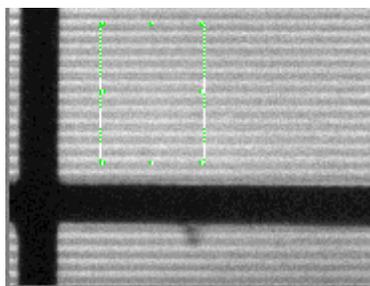


Figure 2

6. Ensuring that the cross on the slide is in focus (not the grid). Use the mouse to adjust the size of the region of interest to cover at least 10 black lines (see previous image). Click on the *Next* button.

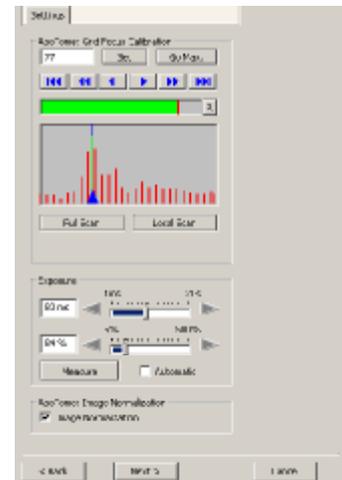


Figure 3

7. In step 3 of the ApoTome calibration, the grid contrast is being maximized to bring the grid into focus with image plane (Figure 3). This can be carried out manually however better results are usually obtained using automated scans to identify the maximal contrast intensities as seen in the graph. Click on the *Full Scan* button and allow the scan to finish and repeat using the *Local Scan* button. Click on the *Next* button.

8. During this step, the calibration setup selects the phase value with the minimum contrast between grid lines. Click on *Full Scan* and then *Local Scan* and select *Finish*. (Figures 4 and 5).



Figure 4

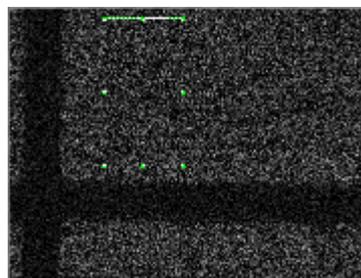


Figure 5

Grid Focus Calibration

This must be carried out for each objective and fluorochrome to be used with the ApoTome.

Remove the mirrored slide and replace with a suitably labelled sample slide (a sample slide which fluoresces in a broad range suitable for most common applications is supplied) and ensure it is in focus.

1. Select the *ApoTome* and *Grid Focus Calibration* (in *Acquisition* menu).
2. Using the calibration wizard settings (Figure 6), identify which grid type has been inserted into the ApoTome. Set the reflector to the relevant position for the particular fluorochrome, set the ApoTome dye to the same fluorochrome and select an objective. Click *Next* to continue. Additional fluorochromes can be added as in Figure 7, if required.

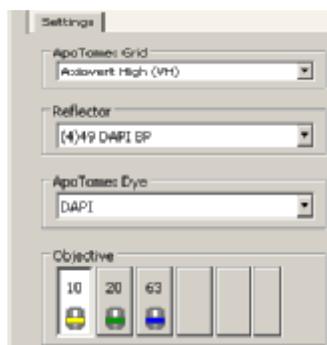


Figure 6

3. Ensuring that the live image of the sample is in focus not the grid and adjust the camera exposure if required. Over-exposure must be avoided when performing acquisition and calibration using the ApoTome. Adjust the size of the region of interest to cover at least 10 grid lines (see previous image) and ensure that the region selected has good contrast between the gridlines and the sample. Click on the *Next* button.

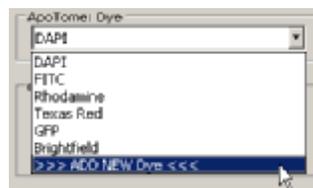


Figure 7

4. In step 3 of the Grid Focus Calibration, the grid contrast is being maximised. This step can be carried out manually however better results are usually obtained using the automated scans to identify the maximal contrast intensities as seen in the graph. Click on the *Full Scan* button and allow the scan to finish and then use the *Local Scan* button (see Figure 8 and 9). Click on the *Finish* button.

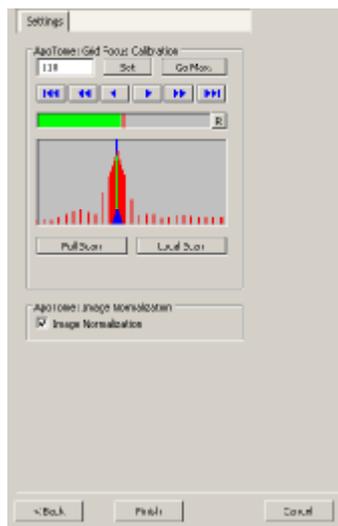


Figure 8

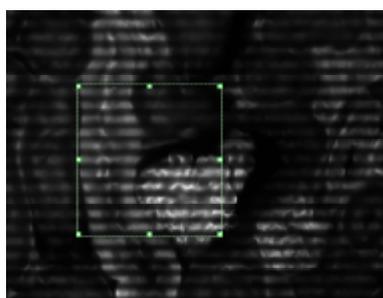


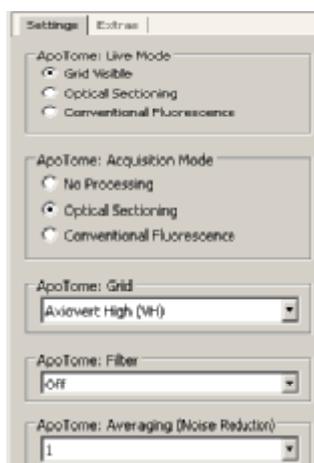
Figure 9

5. You will be asked whether you wish to calibrate another setting. Click *Yes* to repeat until all the required objectives and fluorochromes have been calibrated. Click *No* to exit setup.

2. Image Capture using the ApoTome

1. In the *Acquisition* menu select *ApoTome Mode*.
2. Again in the *Acquisition* menu select the *ApoTome* menu and the *ApoTome Dialog* box. Open a live image window and focus on your sample.
3. The *ApoTome Dialog* box gives the following options for live and acquisition modes (Figure 10).
 - Grid Visible – no processing applied - the grid is present in the view allowing faster update.
 - Optical Sectioning – ApoTome in use.
 - Conventional Fluorescence – simulates conventional fluorescence.

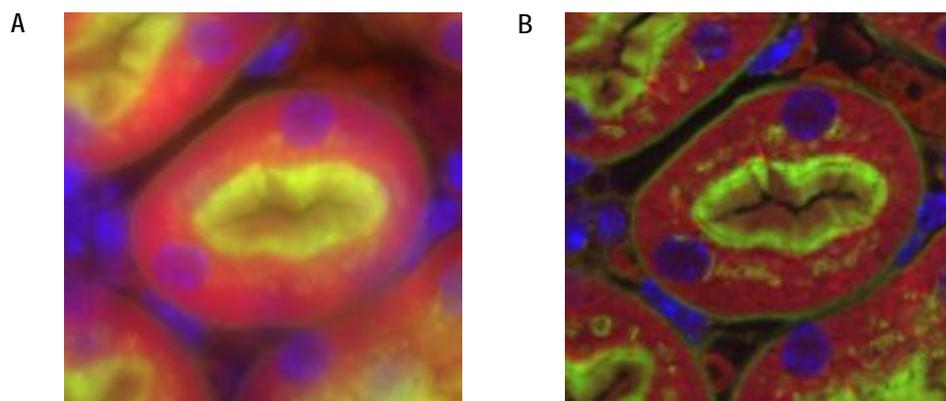
The recommended configuration for viewing live images is to leave the grid visible.



4. In the *ApoTome Dialog* box you can alter the level of filtering that is used to remove any grid lines. In optimal imaging situations the grid will be completely removed by selecting the correct *ApoTome filter* setting. In some cases no filtering is necessary.
5. *ApoTome: Averaging (Noise Reduction)*: use this to average between 2 to 5 images. This will lengthen the acquisition time but increase the image quality.
6. Using your usual *Multidimensional Acquisition* setup (see *AxioVision Takeoff Guide*, Chapter 12), measure the exposure time for each fluorochrome and focus if necessary and start the image capture.

Figure 10

In order to check effective optical sectioning it is often a good idea to compare a sectioned image against a conventional fluorescence image, as below.



Mouse Kidney section - 16 μ m section thick. A) Image captured using conventional fluorescence B) Image captured with ApoTome.

IMPORTANT!

The ApoTome should never be switched off when in the active position. Always remove the ApoTome slider out to the resting (inactive) position and then switch off the power supply.