

A simple explanation of how the Apotome III works

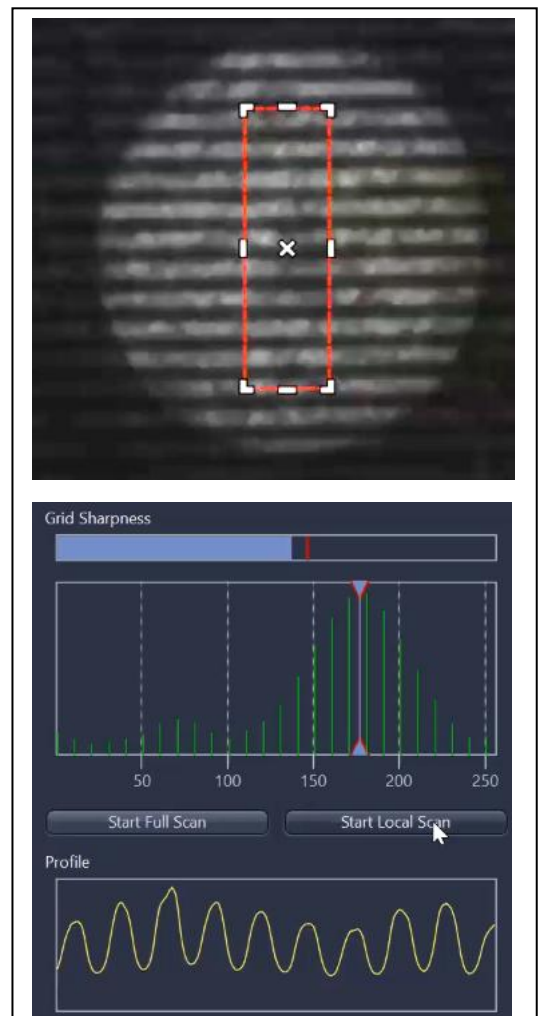
“Light from outside the focal plane needs to be suppressed to extract the in-focus image information. Optical sectioning using structured illumination allows you to efficiently minimize out-of-focus light to create crisp images and 3D renderings. Apotome 3 uses a grid to generate a pattern of intensity differences. If out-of-focus light is present at a certain region of the sample, the grid becomes invisible. After the fluorescence of a grid position is acquired, the grid moves to the next position. A true optical section with higher contrast and resolution is calculated. No matter which magnification you are using the Apotome 3 automatically places the optimum grid in the beam path.”ⁱ

Calibrating the Apotome III grid

To ensure that the Apotome works well for your samples and gives the best images possible, it is necessary to calibrate the grid at the magnification that you intend to take your images at. You will also need to calibrate it for all the wavelengths that you want to use. Fortunately, this calibration does not take long to do.

NOTE: *If your Apotome images seem to have stripes or lines in them, running this procedure will ensure that the problem does not occur with images captured after the calibration.*

1. Find, focus, and set up the filters & exposure times for your sample using the objective lens that you plan to capture the images with.
2. If you happen to have a nuclear stain like DAPI, this is a good example and it's easy to see the grid in the nucleus. Once you are familiar with the calibration, you do not need to start with DAPI.
3. In ZEN Blue, go to the very top text-based menu item **ACQUISITION | Apotome Focus Calibration Wizard**
4. Pick the channel that you want to calibrate. Then click **NEXT>** down in the lower left.
5. The red box can and should be moved to a location that is over an area of your sample that is in focus (you can use the fine focus knob on the TFT) and you can see the grid in the sample's fluorescence in the image.
6. You might need to use the **Set Exposure** button on the left to ensure that the image is bright enough (or not too bright).
7. Once the sample has a proper exposure time and is in focus, press **NEXT>**
8. In the third window, press the **Start Full Scan** button. The grid sharpness graph starts filling from left to right. Once that has completed, then press the **Start Local Scan** button to fine tune the grid alignment.



9. Then you can press **FINISH**. You will be asked if you want to calibrate another channel. Continue on until you have calibrated all the channels that you intend to use.
10. That's it!

ⁱ Zeiss - <https://www.zeiss.com/microscopy/en/products/light-microscopes/widefield-microscopes/apotome-3.html>

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