

Microscopy QuickTip – Tiling on Confocal 5

Version 1.0 for ZEN 2012 SP1 (8.1.6.484). © Darren J. Paul & The University of Queensland, 11 Jul. 16

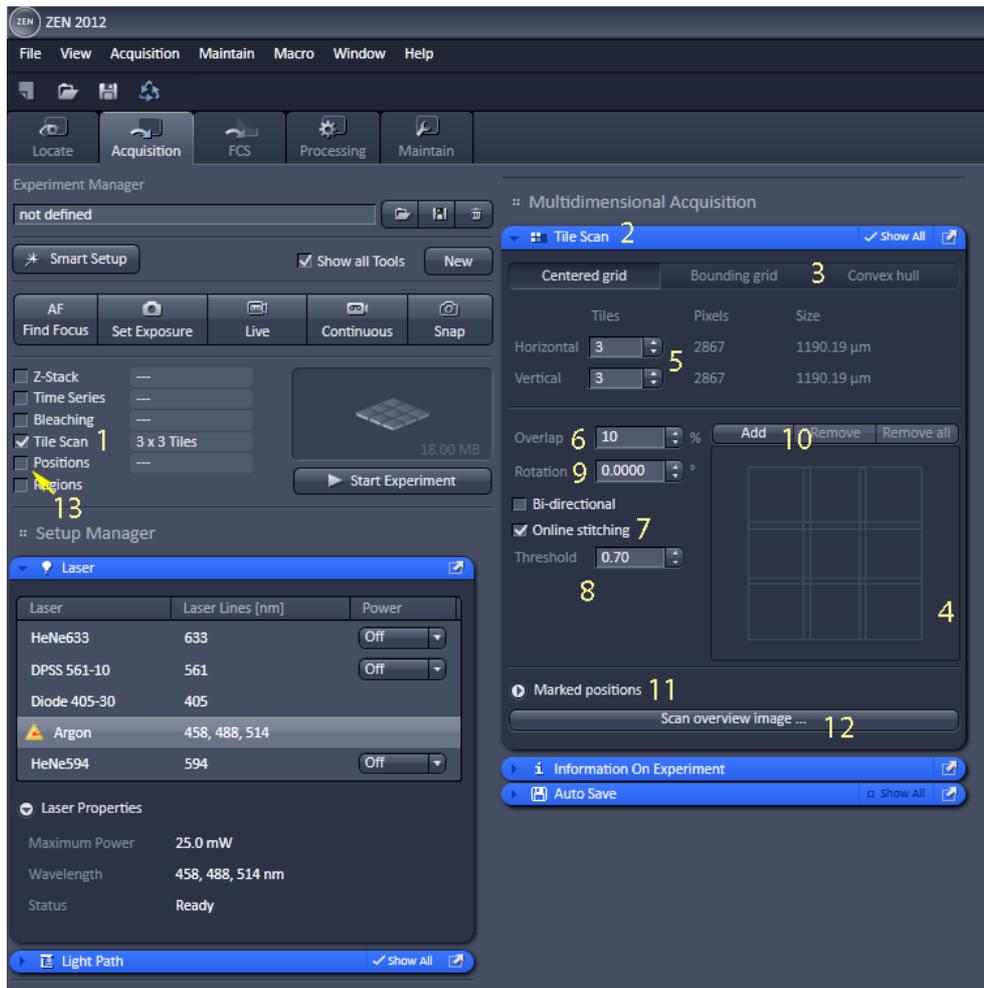
The ZEN microscope control software on the Zeiss LSM 710 BiG microscope (Confocal Five) incorporates an image stitching module capable of seamlessly joining adjacent images into a panoramic tile. This is useful for obtaining a comparatively large area, low magnification overview of large or distributed specimens, or for increasing the field of view on samples usually captured at higher magnification, such that an entire organ (for example) may be imaged.

Set up is quite straightforward. After configuring and optimizing a capture as normal, select Tile Scan (1) and the Tile Scan panel will open (2).

Three methods of configuring the tiling are possible; **Centred**, **Bounding Grid**, and **Convex Hull** (3).

Centred will establish a tiled capture around and including the present stage position. **Bounding Grid** will produce a rectangular tile encompassing all the positions marked, the space between them, and an approx. 0.5 field surrounding buffer. **Convex Hull** will capture a tile of marked positions similar to Bounding Grid, but with the minimum number of tiles. It will thus omit corners of the encompassing grid if these do not incorporate a marked point.

The arrangement of the individual images within the tile is indicated (4).



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The number of images within a tile is specified by x and y axis selector boxes (5). The area to be scanned is also displayed in both pixels and μm . The former is dependent on the frame size selected for a single image, the number of images and image overlap, and the latter on magnification, zoom (if selected), scan area, number of images, and image overlap. There may be up to 100 frames (images) within a tile, so if capturing z-slices or multiple colour channels, reduce the area to be scanned accordingly.

Specifying an area of overlap (6) between images to be tiled aids automatic alignment and stitching. A value of 10% usually works well, though Zeiss recommend 15 to 20%. Stitching itself is activated by checking the option “Online Stitching” (7). A threshold value (8) will be automatically assigned if stitching is active and in general, will be found to perform well. The threshold value represents the degree of rigour the software will employ in lining up overlapping features. While it may seem that a higher value would necessarily be better this is not the case; too high or too low a value for the configuration options selected will produce a substandard result. Adjust with caution.

The X-Y axes of the microscope stage and the confocal scanning mirrors are typically well aligned, and the stitching software can generally cope with minor errors. However, if objects do not align precisely across tiles, rotational correction (9) of the scanning axis may be necessary. The value required will typically be very small (note, the adjustment is in 10^{-4} ° increments). Adjust methodically, and with caution.

Location points for use with the **Bounding Grid** and **Convex Hull** are defined by moving the microscope stage to the required location and clicking the “Add” button (10). The X, Y and Z positions of defined points may be displayed by clicking the arrow next to the “Marked positions” tag (11). A preview encompassing marked points can be set up under “Scan overview image” (12). Note that these points are not the same as those defined via the usual Positions dialog (13).

Note Well:

- If tiling at one position only, there is no need to define a location point. The tile, however configured, will be centred at the current stage position.
- If **Centred** tiles at multiple positions are required, locations for these may be defined in EITHER the **Tile Scan** panel’s point list (as above) OR in the more usual **Positions** panel (13), BUT NOT BOTH.
- **Bounding Grid** and **Convex Hull** MUST be defined under the **Tile Scan** panel’s point list. It is important to ensure the Positions (13) module is not also enabled. If both point lists are active, location errors will occur.