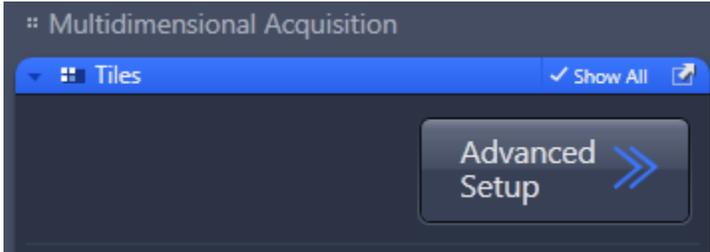
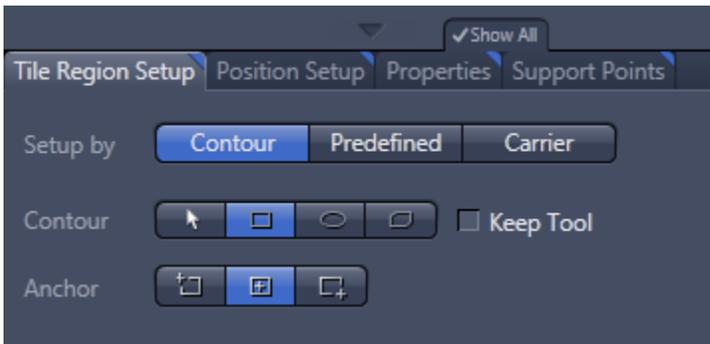


Axio Observer Advanced Tile Scan Setup

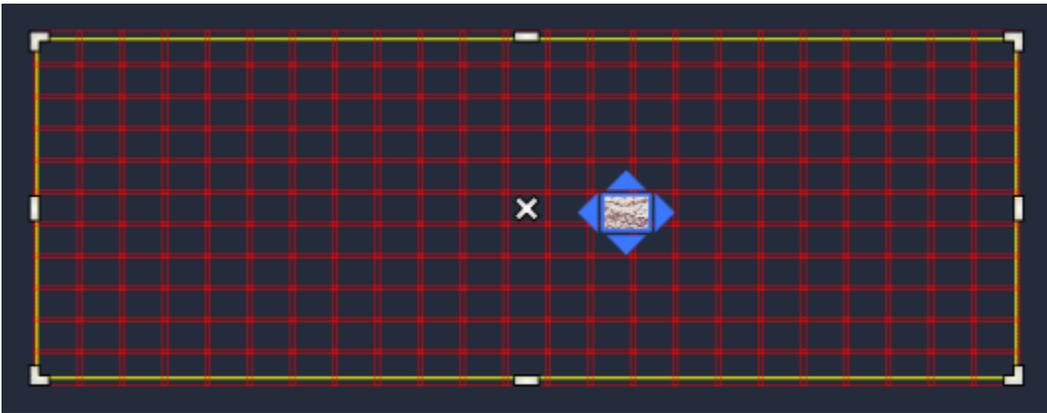
- Set up channel exposure etc. as normal...
- Select **Advanced Setup** within the **Tiles** tab.



- Go to the **Tile Region Setup** tab as below



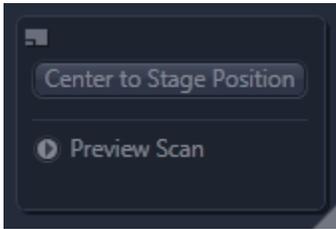
- Select **Setup by Contour** and use one of the contour tools to define the region eg. rectangle.
- Draw around the area you think your sample is in, then you can adjust it...



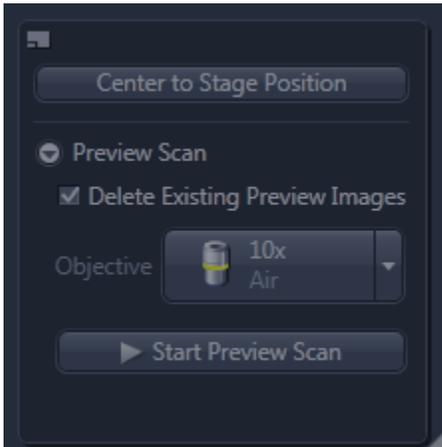
- You can then view tiles within the grid by double-clicking on them...



- Click on the **Preview Scan** button...



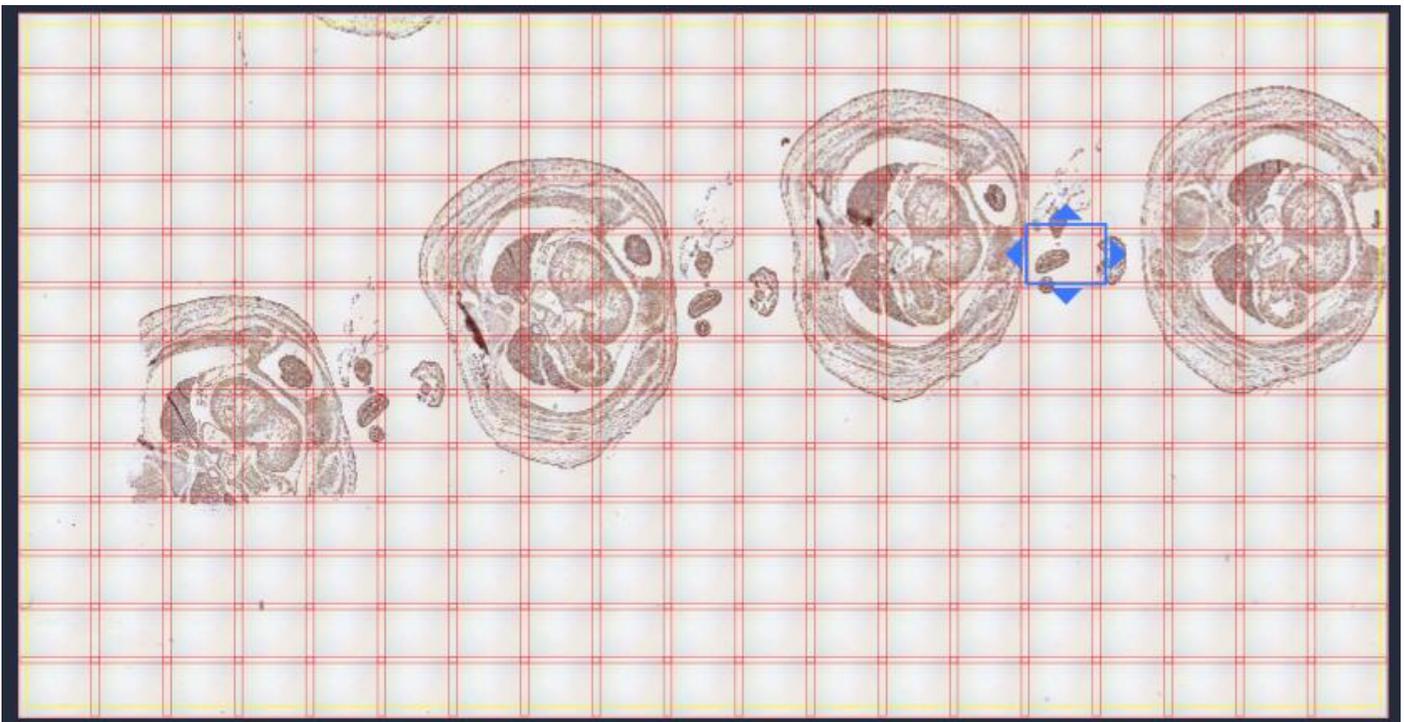
- Once expanded it looks like this...



- You may wish to select a lower magnification to make it easier to Preview the regions contained within the selected areas. To do this, Click on the Objective button...

*When fluorescence imaging, the live image will display signal from whatever fluorescence channel is selected. If all channels are ticked, then all channels will be used for the preview scans, and for the main tile scan, once set up. To Preview scan in only one channel, untick the channels you don't want, and then re-tick them once ready to do the main scan.

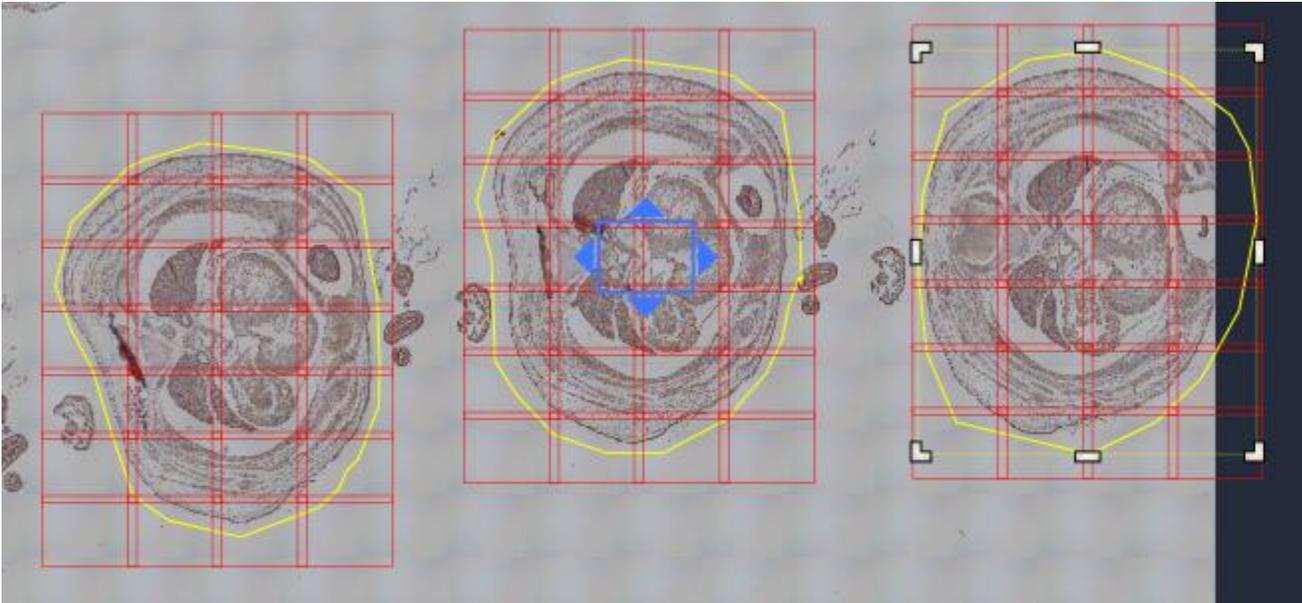
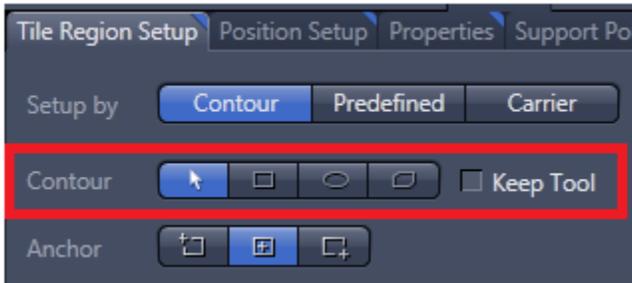
- Once you're happy with your setup, click **Start Preview Scan**.



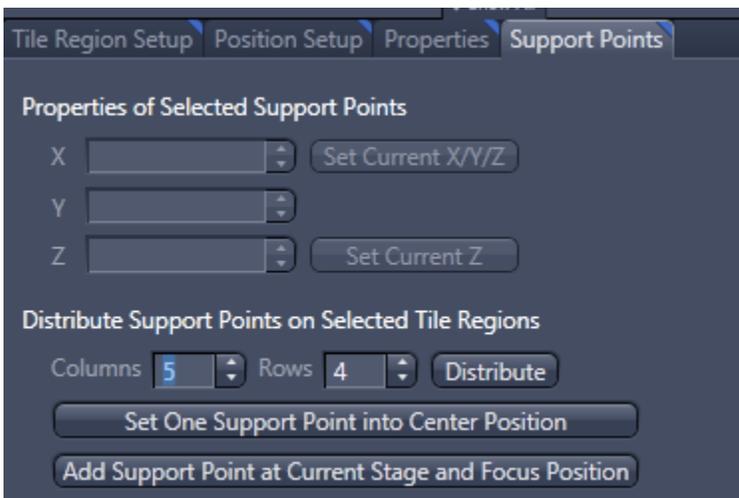
- You can delete the **Preview Grid** and make smaller, more specific grids for tiling as desired. If you think you will

need it again, do not delete it and go to the instructions at the end of this sheet.

- Select specific regions to be tiled. You can draw as many as you like and they will each be set up as individual tile regions...



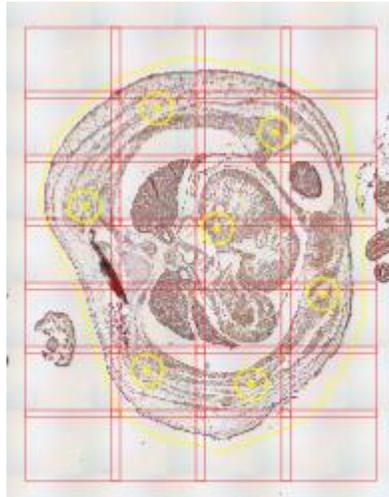
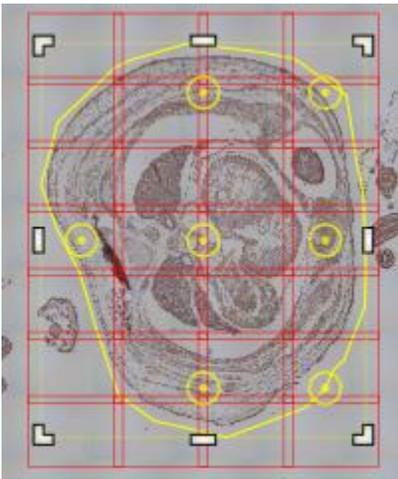
- To setup a **focus surface map** (a map of focal points) for your tile regions, go to the **support points** tab (support points = focal points for each tile).



- To distribute the support points equally across the tiles, select the number of columns and rows that you need and click distribute. Do this for each individual tile region...

Distribute Support Points on Selected Tile Regions

Columns Rows



- You can then click on each support point and move it to an optimal location eg. to the center or corner of a tile; repeat for each tile created, as desired.
- If you want to add additional support points without deleting the ones you already have, double click on the tile you would like a new support point and click on the + sign under Tiles > Focus Surface.

Focus Surface

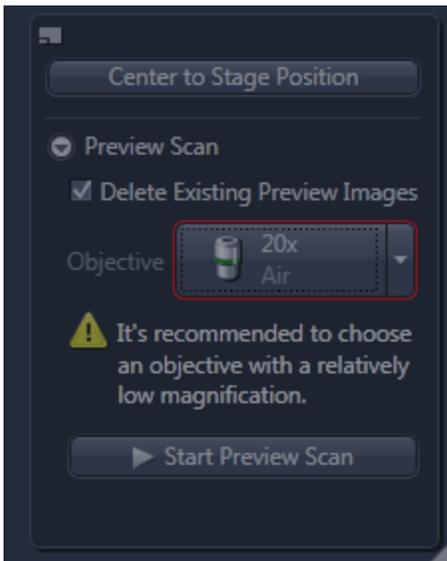
Local (per Tile Region)

Support Points of Selected Tile Region: TR2

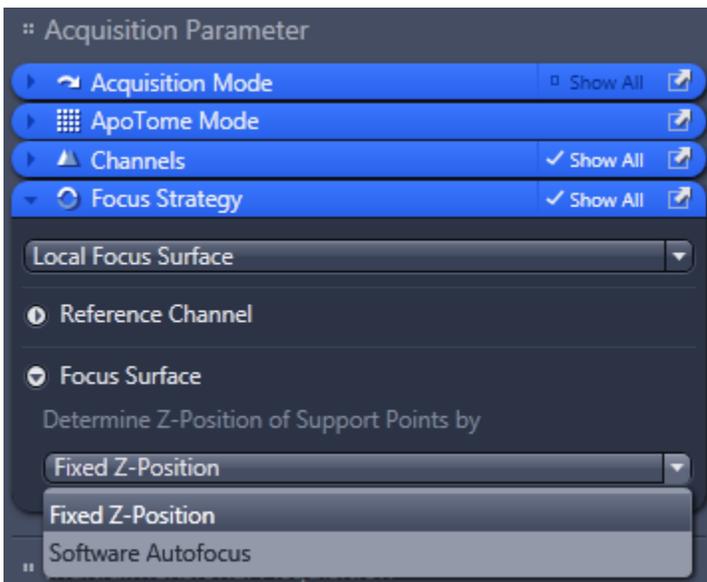
	X (μm)	Y (μm)	Z (μm)	
⊙	16628.4	2025.9	-39.1	
⊙	18235.9	2351.7	-39.1	
⊙	15645.5	3360.7	-39.1	
⊙	17443.0	3659.4	-39.1	
⊙	18887.5	4555.4	-39.1	
⊙	16492.7	5591.6	-39.1	
⊙	17910.0	5781.7	-39.1	

Interpolation Degree

- You can still click and drag these new support points around as before.
- Now select the magnification you want to scan at eg. X20



- Now set the focal positions for each support point in the focus surface map.
- Select **Focus Strategy** on the side under Acquisition.



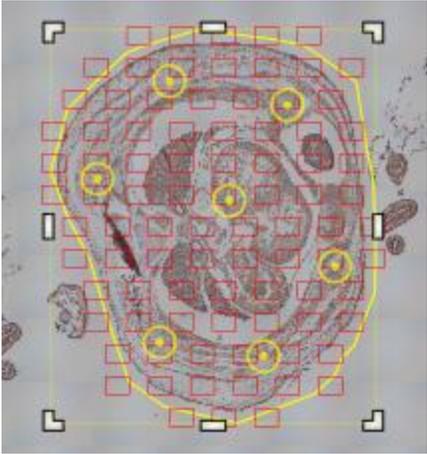
- Under here you want to select **Local Focus Surface** as our strategy.
- Under **Focus Surface**, you can choose how you want the Z position located:

Fixed Z-Position - in this setting, the focus for each support point is set *manually*, this is still fast and instructions follow.

Software Autofocus - With this option, when you click **Start Experiment**, the software will go to each support point and run the autofocus to determine the optimal focus position. *This may not be optimal for your specimen, as it will look for the best contrast as the best focus.

Fixed Z-Position Setup:

- Select the **Live** mode in the acquisition tab.
- Select the tile region you want to set the focus for, and then go in to **Tiles** and **Focus Surface** and you will see the list of support points for that tile region.



Focus Surface

Local (per Tile Region)

Support Points of Selected Tile Region: TR2

	X (μm)	Y (μm)	Z (μm)	
⊙	16628.4	2025.9	-39.1	
⊙	18235.9	2351.7	-39.1	
⊙	15645.5	3360.7	-39.1	
⊙	17443.0	3659.4	-39.1	
⊙	18887.5	4555.4	-39.1	
⊙	16492.7	5591.6	-39.1	
⊙	17910.0	5781.7	-39.1	

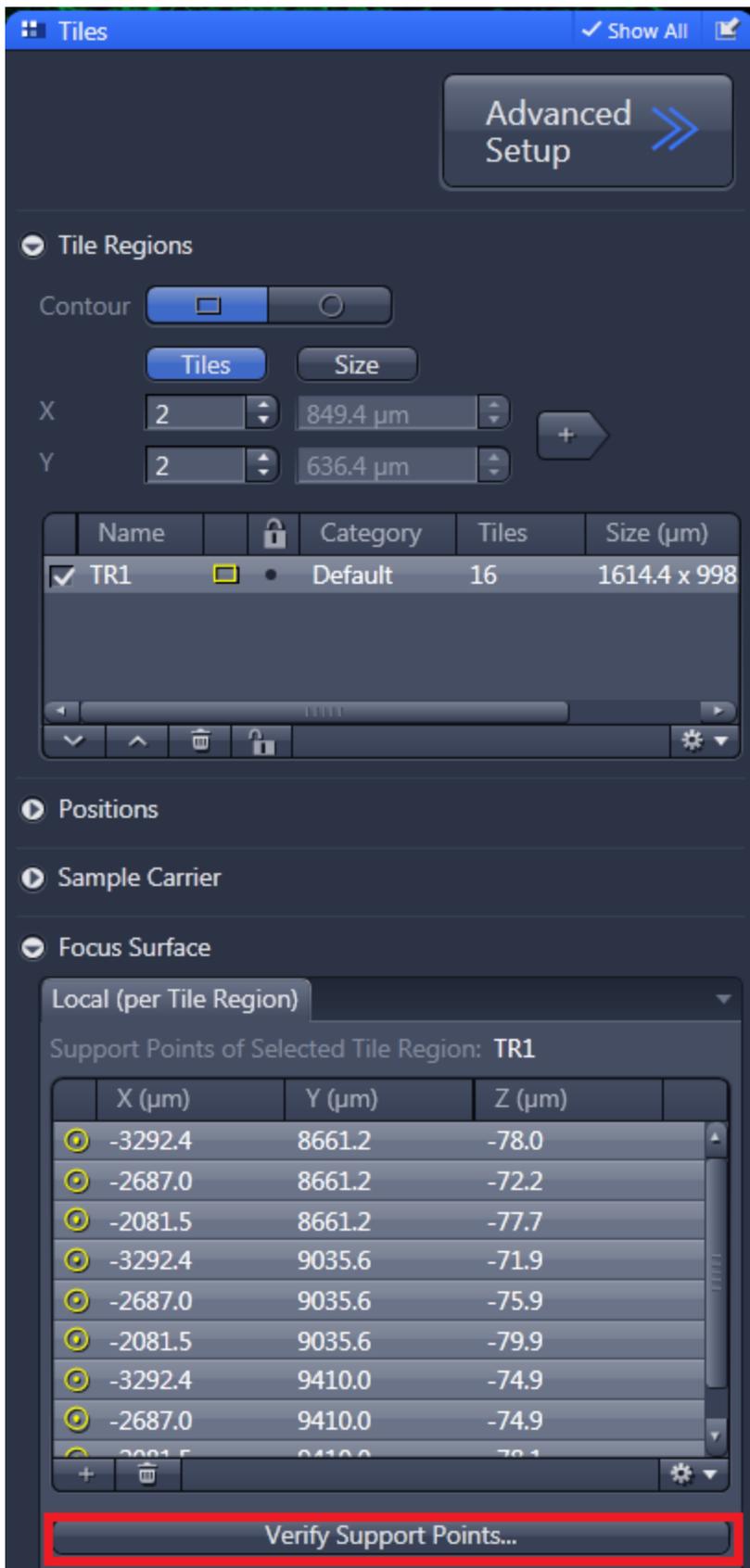
+

Verify Support Points...

Interpolation Degree

1 - Tilted Plane (at least 4 support points)

Click on **Verify Support Points...**



- Now click **Move to Current Position**, focus manually, and then click **Set Current Z**
- Click **Move to Next Point**, and repeat the process until the focus has been set for all Support Points.

Verify Local Support Points

	X (μm)	Y (μm)	Z (μm)	Tile Region
✓	-3292.4	8661.2	-78.0	TR1
✓	-2687.0	8661.2	-72.2	TR1
✓	-2081.5	8661.2	-77.7	TR1
✓	-3292.4	9035.6	-71.9	TR1
✓	-2687.0	9035.6	-75.9	TR1
✓	-2081.5	9035.6	-79.9	TR1
✓	-3292.4	9410.0	-74.9	TR1
✓	-2687.0	9410.0	-74.9	TR1
✓	-2081.5	9410.0	-78.1	TR1

— 

Move to Current Point

 Current Stage X/Y Position \neq Current Point

Set Current Z

Run Autofocus

Include Z when Moving to Points

Move to Next Point

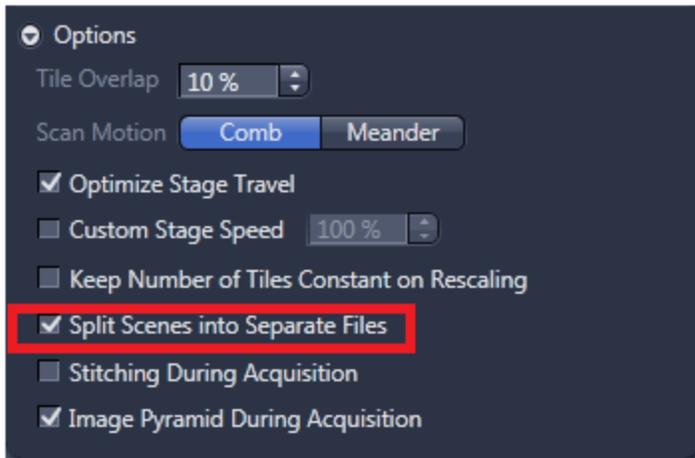
Move to Next and Run Autofocus

Automatically Determine all Remaining by Autofocus

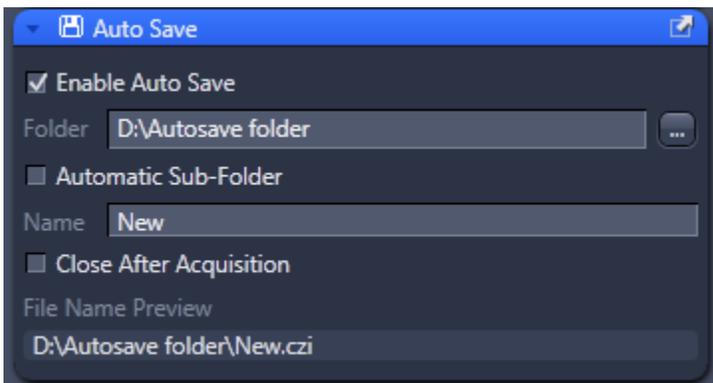
 All points have been verified.

Close

*If you are capturing more than one tile in the experiment, you may wish to select **Split Scenes into Separate Files**. This means you will get one file for the whole experiment, as well as individual files for each tile region captured. This is useful particularly if you have very large tile regions, as it reduces the size of individual files.

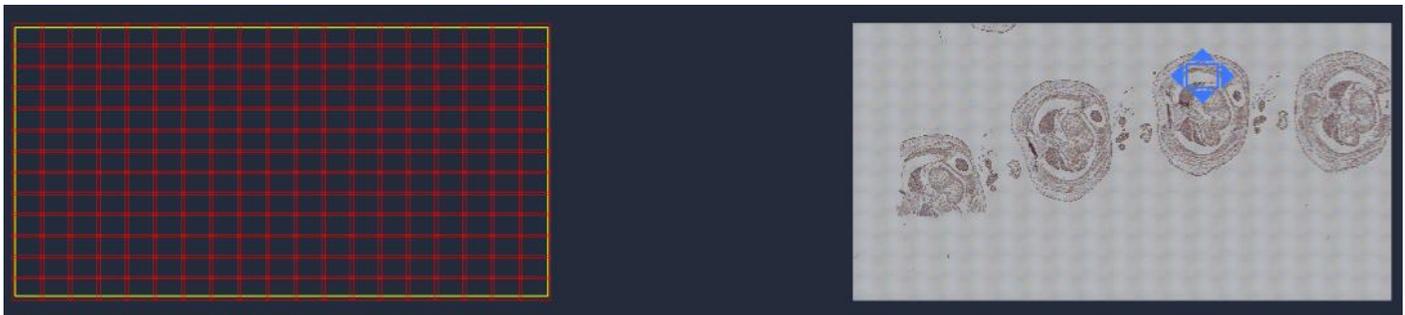


- Finally, select Enable **Autosave** under acquisition, and click **Start Experiment**.



How to store your grid:

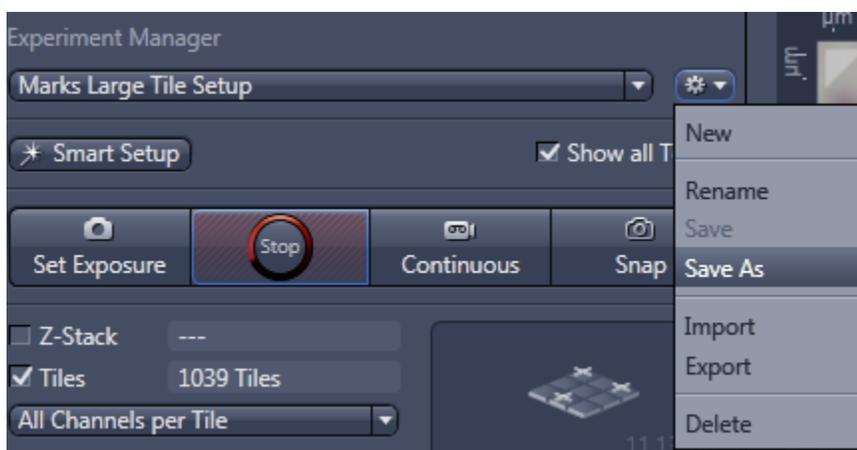
- If you want to keep the large Preview Grid for your next experiment, what to do is grab it (click and hold), and move it away from your current slide as below...



- Once you have moved it, in order to make sure you do not scan it at a higher magnification, untick the **Tile Region** line under the tiles tab under Acquisition... The grid will disappear but it will reappear when ticked again.



- Now you can save your experiment template. Click on the settings wheel, and select **Save As...**



- When using your saved experiment in the future, move your slide to the center under the objective, and then you can grab your old Preview Grid and move it so that the blue live window is in the center of your Preview Grid, then you can do a new Preview Scan...

