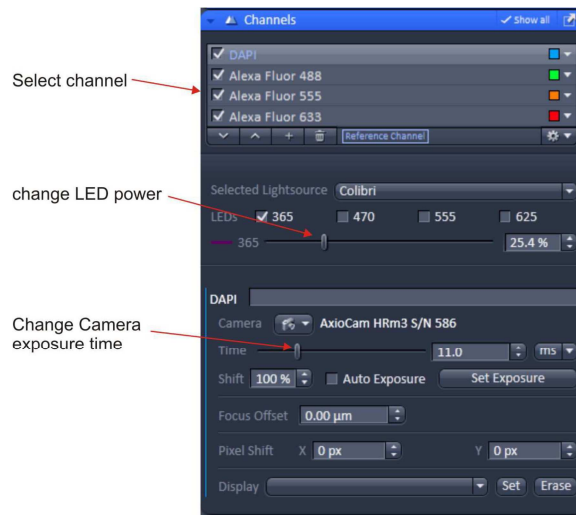# Multi position high power acquisition from low power preview in ZEN

<u>Low power preview (x10 or x20 objective)</u>

Under the "Acquisition" tab:

- Select channels for preview image (tick marks) on "Channels" window
- Click "Live", focus sample and set the levels for each <u>preview</u> channel to provide a balanced image

- Select "Tiles" to open tile tab
- Add a 2x2 tile ("+" symbol)
- Click "Advanced Setup"
- Run Preview Scan (control – on lower right of image window)

Adjust tile region

- Click "Live"
- Double click on screen to move stage to areas of interest
- Click on orange/yellow box – changes to yellow box with side and corner adjusters
- Expand to cover region of interest (no. of tiles will increase automatically (red frames on screen)
- Re-run preview scan to get final preview image

Add positions

- On lower window (below Image)
- Select Positions tab
- Click on the + symbol and keep tool
- On the preview image select positions of interest (these will appear in the "Tiles" window under "Tile Regions and Positions")

Change to 63x oil lens

- Move sample away from lens using joystick
- Change to 63x oil but **do not** bring up into focus yet
- Add immersion oil to the lens and move lens back to the approximate position on slide
- Then select the "Done" button on the keypad to bring it into focus
- Select position one (by double clicking on it in the "Tile Regions and Positions" window) to move the stage to that position
- Click "Live", focus sample and setup the levels for each of the required channels

Autofocus

- Select "Focus Strategy" window (options will depend on what other functions are selected eg Z stack and Tile)
- Select "Software Autofocus" and set the required "reference channel"
- To test strategy **de-select the tile** and **all but one** position and run experiment
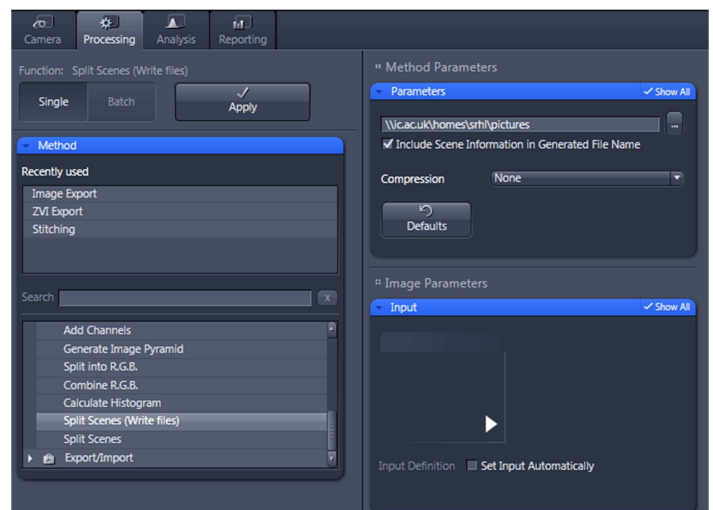
Image Capture

- De-select the **tile region** from the "Tile" window or it will image tile as well as the positions
- Click "Start Experiment"
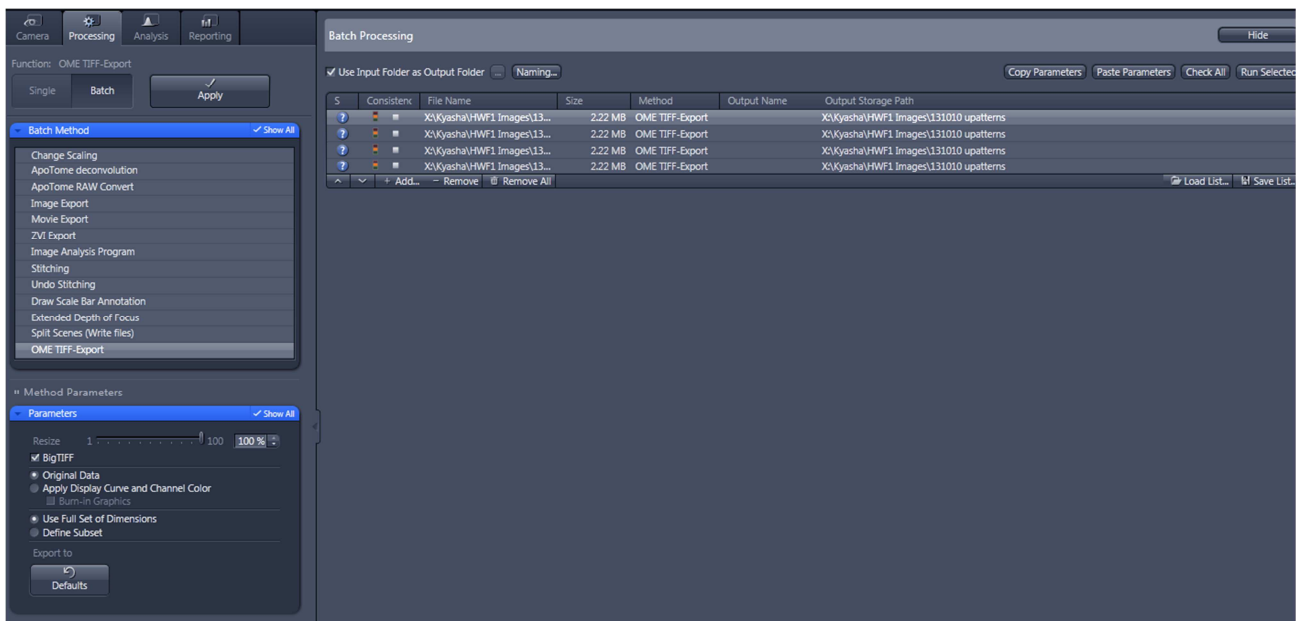- Save file

To split the scenes for analysis

Under processing

- Select "Utilities"
- Then "Split Scenes (write files)"
- Under "Method Paraemeters – Parameters", change save location, tick "Include Scene Information" and set compression none
- Under "Image Paraemeters – Input" select input image
- Click "Apply"

To batch export (use ome.tiff format)

- Under processing - select "Batch"
- Select "Ome TIFF-Export"
- Under "Method Parameters – Parameters", select options (for BigTIFF – see appendix)
- On the "Batch Processing" window click add to add files for export
- Check details are correct
- Click "Apply"

Appendix

What is BigTIFF?
The TIFF file format uses 32bit offsets and, as such, is limited to 4 gigabytes. This has been quite sufficient for many years. Today however, there is a need for a good multi-purpose open image file format that can handle huge images, or very large collections of images, breaking the 4 gig boundary.
There is currently an on-going attempt to launch a new variant of TIFF, called BigTIFF that closely resembles TIFF, but uses 64bit offsets instead. The benefits of closely resembling TIFF are huge. For instance, existing TIFF libraries can quite easily extend their support for TIFF to also include this new variant. Documentation needs are minimal. All the much appreciated properties of a file format that has been around and has been extended for more than a decade are inherited. All properly known tags are being reused, all supported bit depths and data types remain valid. The arbitrary number of 'extra channels', the tiling and striping schemes, the multitude of compression schemes, and the private tag scheme, that made TIFF very useful in pre-press as well as for storing scientific data, and many other applications, all remain intact. Yet, the offset bit depth changes, and BigTIFF files are no longer restrained by the 4 gigabyte limitation from which classic TIFF suffers.