Widefield Tiling method

Start Zen – run Zen Pro – Calibrate stage if asked

Locate (Locate tab)

- Select X5 objective – load the slide onto the stage and using Locate Tab, find sample eg. using PH or Dapi buttons on

Acquisition (Acquisition tab)

- Open previous **raw** – data image (capture settings) using "file" – "open"
- Click on it and select re-use (bottom centre of screen)

Channels window:

- Uncheck all but one channel for preview (eg PH or Dapi)
- Select preview channel (eg PH or Dapi)
- Go live and focus the sample on screen

Tile window

- Either delete any old tile and position definitions and add a new tile definition (eg 3x3 tile) using the + symbol
- Or you can reuse an old tile definition by moving it in the preview window
- Click advanced setup to open the preview window

Preview Window

- Run the preview – start preview (bottom right of window)
- Adjust tile region. (go Live - Double click to move frame – single click inside region to change shape/sixe of region)
- Re-run preview if required
- Select objective for capture (eg x10 or x20)
- Scroll out to see whole of preview and adjust tile region or draw new tile region if required – freehand tool under image window under tile regions
- Re-check fluorescent channels required for capture in the Channels window
- Select channel for focussing
- Double click on centre of tile – go live and focus
- Right click on tile definition in tile window and set z focus (first option)
- Create focus map if required (select a position on screen – go live and focus – add **focus surface** position on tile window (plus symbol) – add more focus positions)
- Check focus strategy is on Local surface focus
- Run Start Experiment
- Save raw image

Stitching

- Go to processing tab
- Select stitching under method
- Select image in Input
- In parameters set new output and fuse tiles
- Press apply at top

- Save fused image