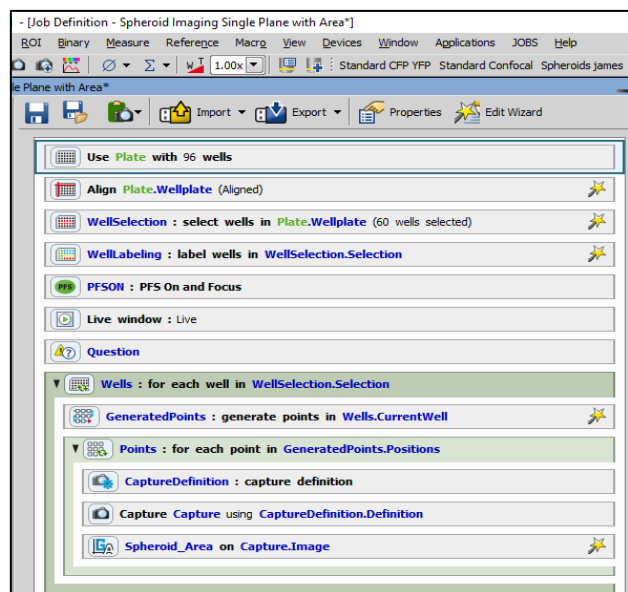
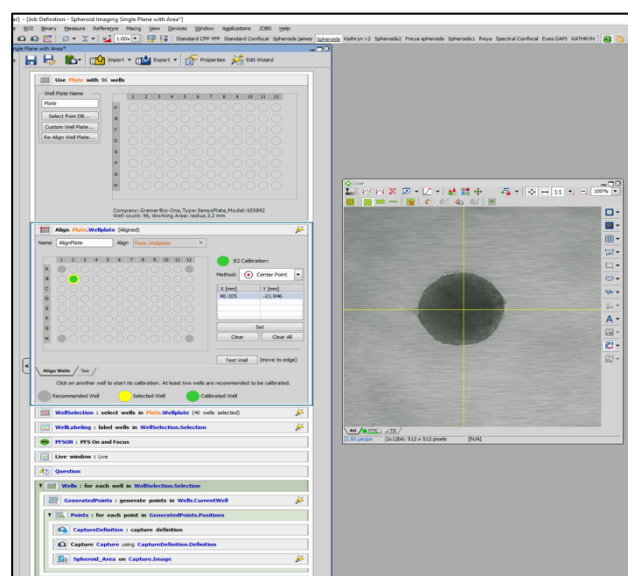


Imaging spheroids using the bespoke JOB

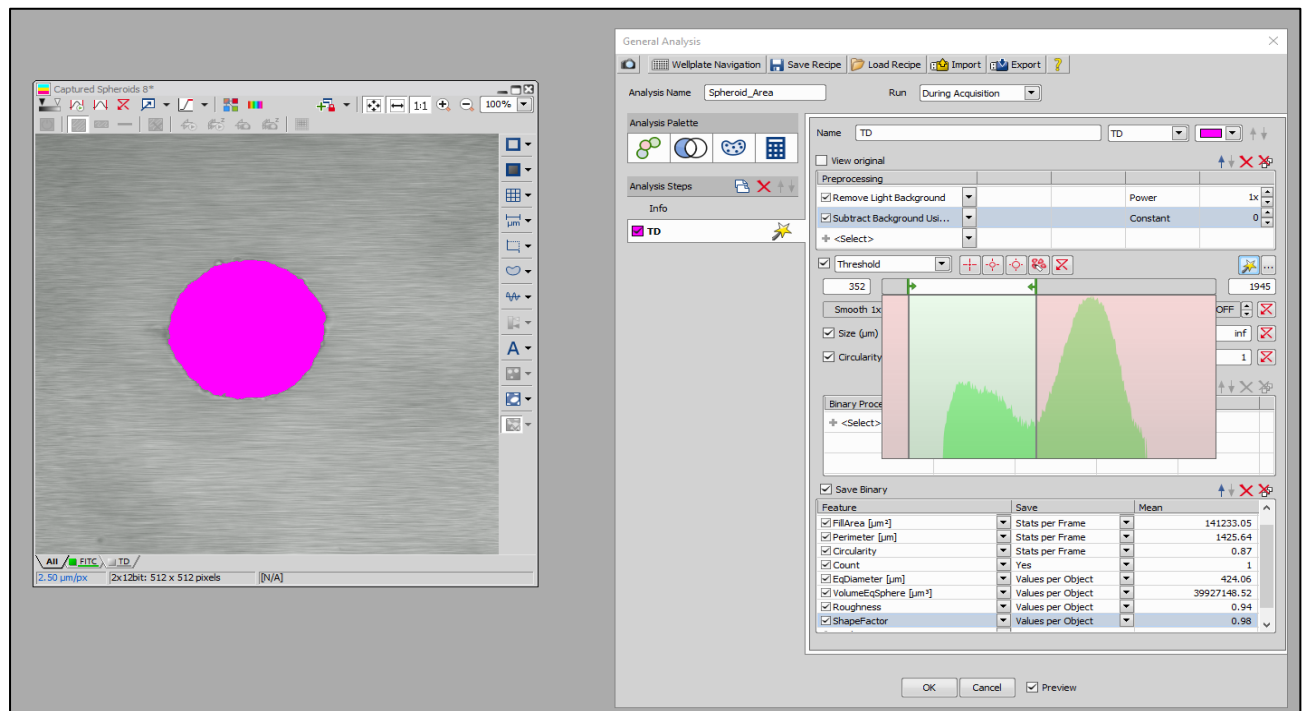
1. Import the JOB file (*Spheroid_Imaging_Single_Plane_with_Area.bin*) into the **software** by clicking import and then...
2. To open the JOB by going to file, JOBS
3. Double click on the JOB to open
4. Set up and focus the confocal on a single well using the 10x lens so that the spheroid in this well can be seen clearly. Note, the fluorescence bulb does not need to be switched on as the programme uses brightfield light only. Tip: these imaging settings can be saved for ease of use
5. Select the plate used from the 'Use plate with 96 wells' tab.



6. The 'Align plate.wellplate' tab is used to align the plate in relation to where the spheroids sit within a well. If the spheroids are in relatively the same place in each well, only one well will need to be aligned. Focus on a spheroid in a well, use the align plate tab to click on the well in focus, and move the yellow grid to show the position of the spheroid in the well. If necessary, multiple wells can be aligned per plate. This will need to be redone for each plate imaged.



7. The wells to be imaged should be highlighted in the 'WellSelection' tab
8. If necessary, the wells can be labelled in the 'WellLabelling' tab
9. The JOB works more effectively if the confocal Perfect Focus is available to use. This is specified to turn on and be in use on the 'PFSON' tab – this tab should not need to be altered
10. In order to set the threshold for imaging, a still image of a spheroid in a well needs to be taken. Following this, double click on the 'Spheroid_Area on Capture_Image' tab to open a box, and adjust the arrows in the threshold, size and circularity tabs as required. Click OK when complete.



11. The play button at the bottom right-hand side of the JOB can now be pressed. This will open a dialogue box in which every tab will have to be viewed order to press the next play button and continue on to the next step. Press play.
12. A dialogue box will open to act as a reminder to set the focus on a spheroid. Once complete press 'OK' and the imaging will begin.
13. Upon completion, a plate map showing an image of each spheroid in the well will appear. Using this view it is easy to compare spheroids to look for anything that will cause obvious outliers (e.g. empty wells as the result of losing spheroids when media changing). In this example the outer wells of the plate contain no spheroids.

