Batch Processing Single Image Tutorial

In many experiments, it is sufficient to measure the steady-state redox potential from a snap-shot image after incubation in the treatment for a fixed period of time, rather than analyse a full time-series. This dramatically increases the number of treatments that can be investigated, and reduces the amount of processing required per treatment. Nevertheless, each image still has to be processed though the same sequence of steps, including background subtraction, auto-fluorescence correction, ratioing with masking, calibration and colour-coded display. In this case the same processing parameter settings will be applied to all images, so the interface can be simplified to include only the parameters that are unique to that particular image. These include the background signal, the auto-fluorescence correction, any channel alignment needed, and the ROIs for analysis.

# Step 1: Set-up the batch processing program

1. Open the batch processing program from the menu bar and navigate to the image folder using the **Dir** button.
2. Select the following three files and press the right arrow to load them:
   1. At\_cotyledon\_batch\_DTT
   2. At\_cotyledon\_batch\_H2O2
   3. At\_cotyledon\_batch\_control
3. You will automatically be prompted to load an appropriate database with the processing parameters. Load the **RRA\_basic\_database** and click **OK**

# Step 2: Running an analysis

1. Select **batch-roGFP2** from the dropdown menu to set the processing parameters for the first image.
2. Click on **Back** to set the background.
3. There is no real auto-fluorescence or channel mis-alignment in these images, so we only have to choose some ROIs for the measurement.
4. Once the ROIs have been selected, press **Test** to run through the analysis on this image.
5. The table on the left shows the results for each ROI, the graph shows a scatter plot of the signals from each channel, along with a linear regression fitted to the data. The results panel gives the overall statistics for the whole image and the regression line.
6. If a **transect** ROI has been chosen, the intensity and ratio along the transect is shown in the transect panel. Individual ROIs can be deleted or all of them cleared if required.
7. All the parameters and the ROIs are automatically saved in a folder called ‘*Processing*’ and are automatically recalled if the same image is processed again.
8. Repeat this sequence for the other two images and compare the results for the DTT treatment to drive to a reduced state with H2O2 to oxidise the probe.

# Step 3: Running the batch

1. Once the processing is established, all files to be processed are transferred to the **Accepted** list box.
2. Clicking on **Process** will now run the complete analysis on each image and save the output as a series of images and in a single Excel spreadsheet.
3. Full details of the function of all the control can be found in the manual.