

# Supplementary Material 1

## a Setting up

(i) Click “Analysis” tab on top of the main screen and upload the macro file (Supplementary Material 3). Select “Multi Channel” configuration. **(b)**

(ii) Select the image data with “With “1-Select Images to Measure” function. Assign Texas Red and YFP to corresponding channels.

Image treatment

(iii) Determine the Texas Red threshold so that the resulting images represent Texas Red and white light images in all frames by using “3-Adjust Threshold”. Check each frame to verify the threshold. **(c)**

(iv) Fine tune the threshold by removing dust. This can be done using “Discard Detail - Open” and “Combine Black Detail – Close” functions in the “4-Binary Processing Pre-filter.”**(d)**

(v) Determine the YFP threshold. Adjust the threshold for YFP at “3-Adjust Threshold” function within “YFP” tab. Check each frame to verify the threshold. **(c)**

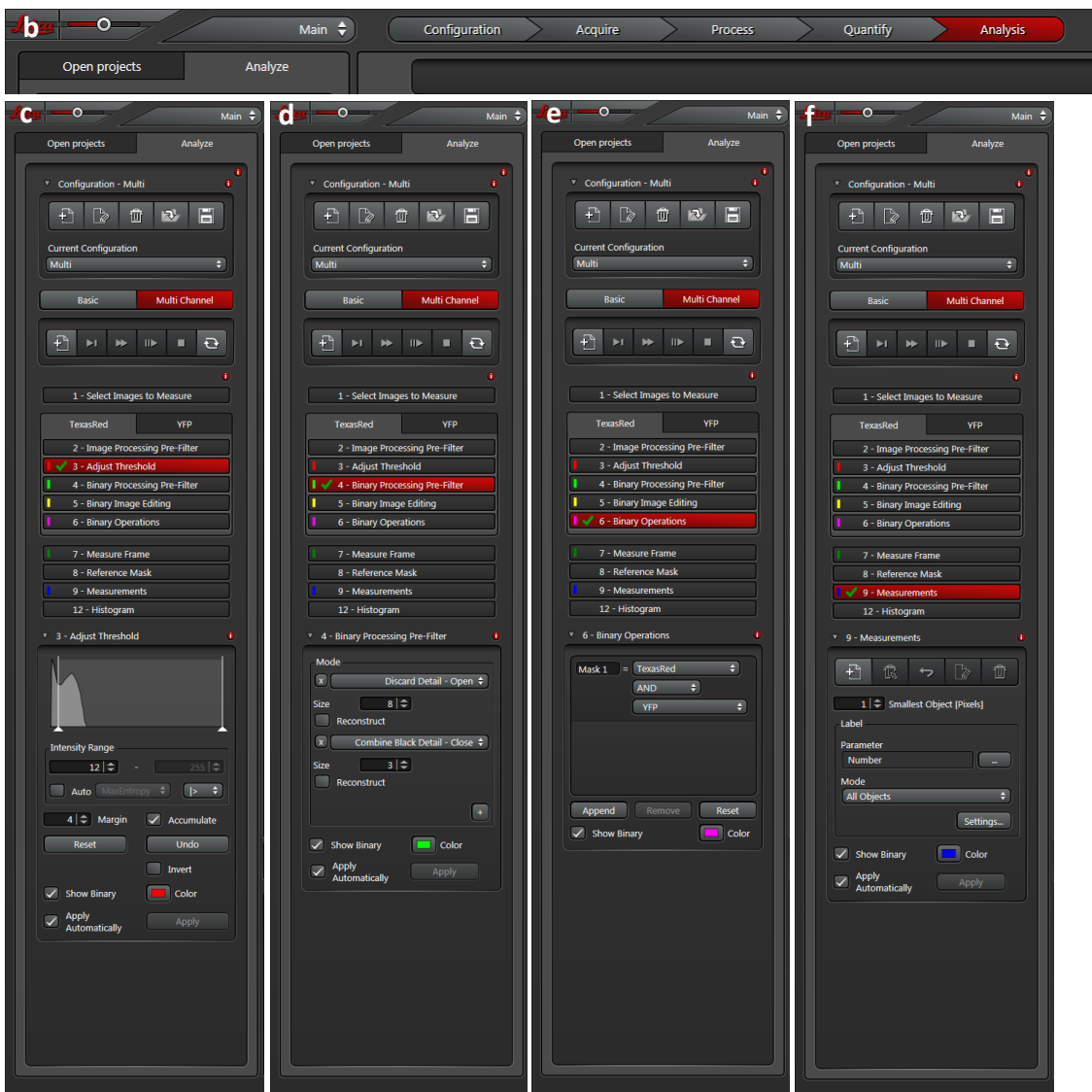
(vi) Merge autofluorescence and YFP images by going to the Texas Red tab and click “6 – Binary Operation.” If the merged pictures of Texas Red and YFP are not shown, go back to “7-Measure Frame” and include object(s) in the frame. **(e)**

Measurement

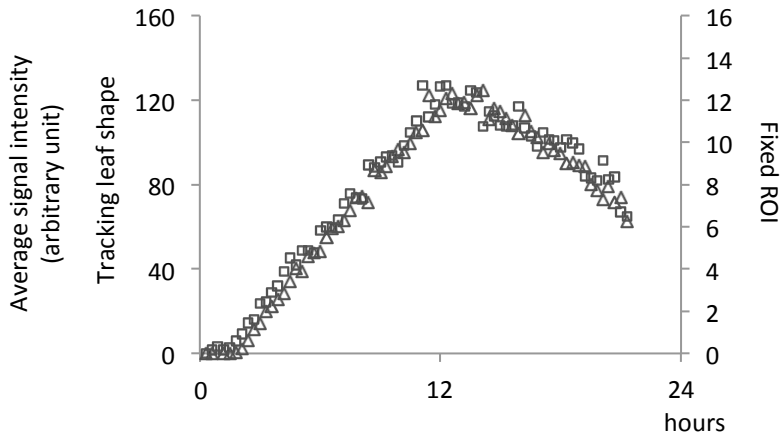
(vii) Measure the sum of the YFP signal intensity. The sum of YFP intensities will be displayed under “Image Measurement.” **(f)**

(viii) To calculate the average YFP signal intensity per plant, measure the Texas Red area by defining both channels as Texas Red. Use identical thresholds as were used in the YFP measurement for both channels. The area of autofluorescence will be shown under the “Image Measurement” tab.

Mean YFP signal intensity is calculated by dividing the sum of YFP signal intensity by the area of the Texas Red region.



## Supplementary Material 2



# Supplementary Material 3

