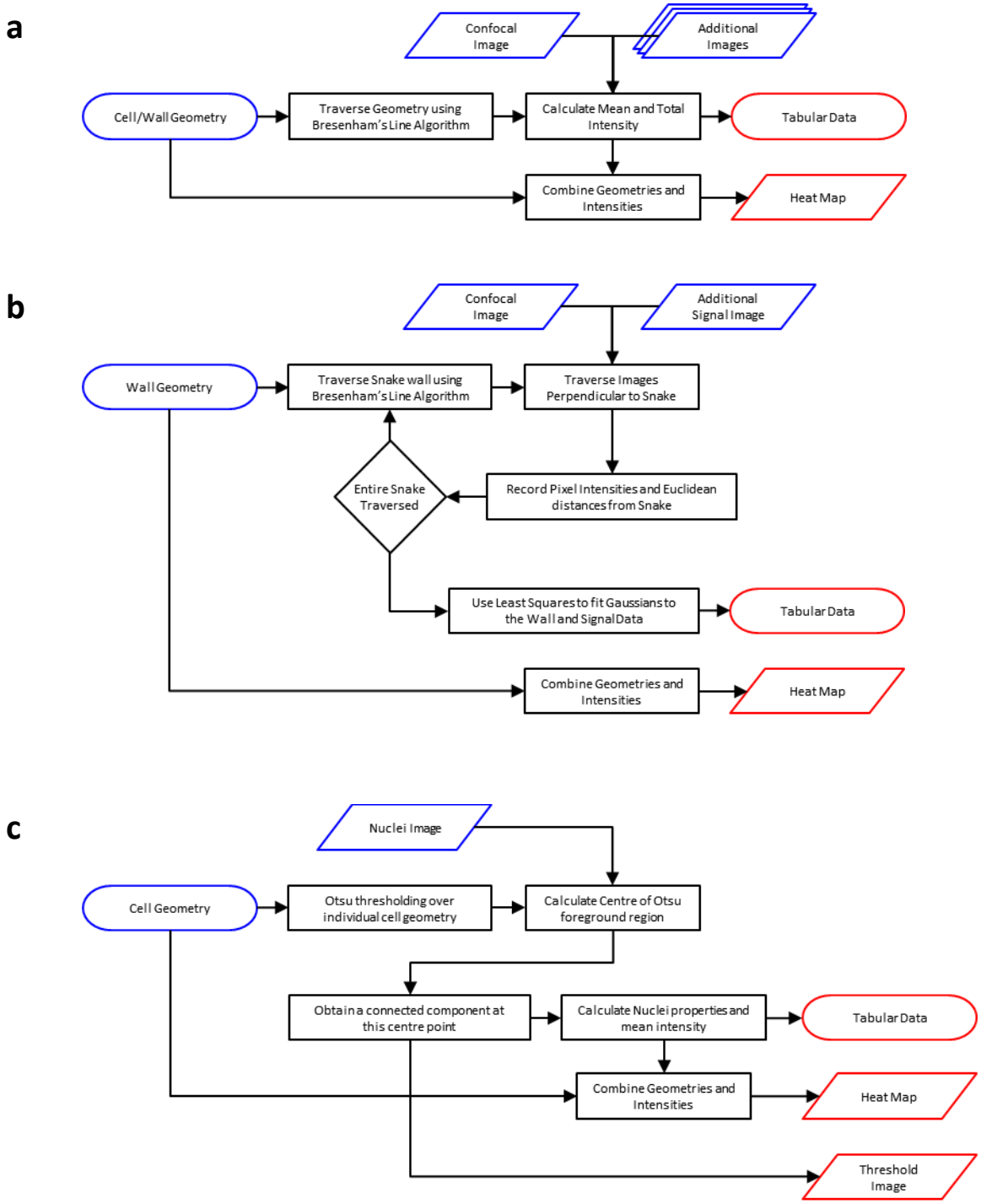
Supplemental Figure 1.

Flow diagrams for each existing CellSet plugin

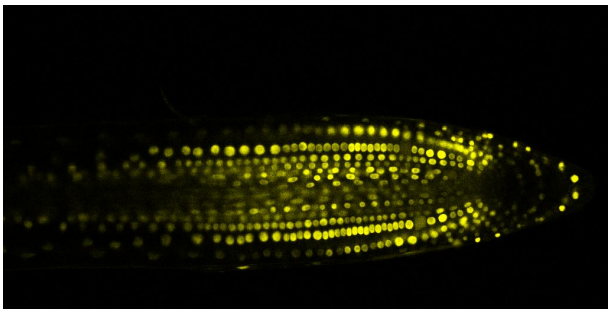


(a) Wall intensity Plugin. (b) Protein localisation plugin. (c) Nuclei analysis plugin. Blue: Input from CellSet. Black: Plugin logic. Red: Output to CellSet.

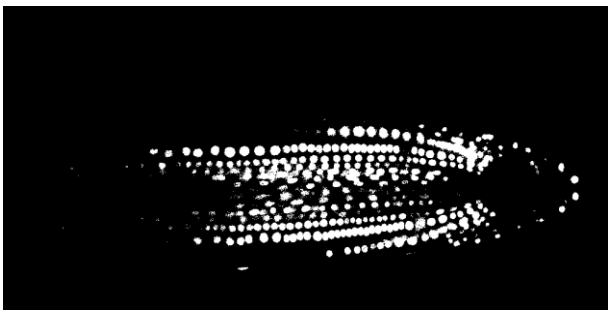
Supplemental Figure 2.

Evaluation of local Otsu thresholding against global Otsu thresholding

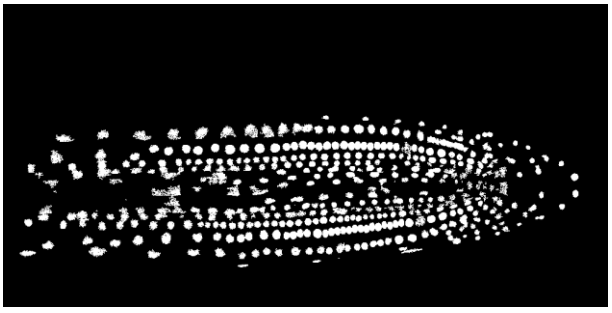
a



b



c



d

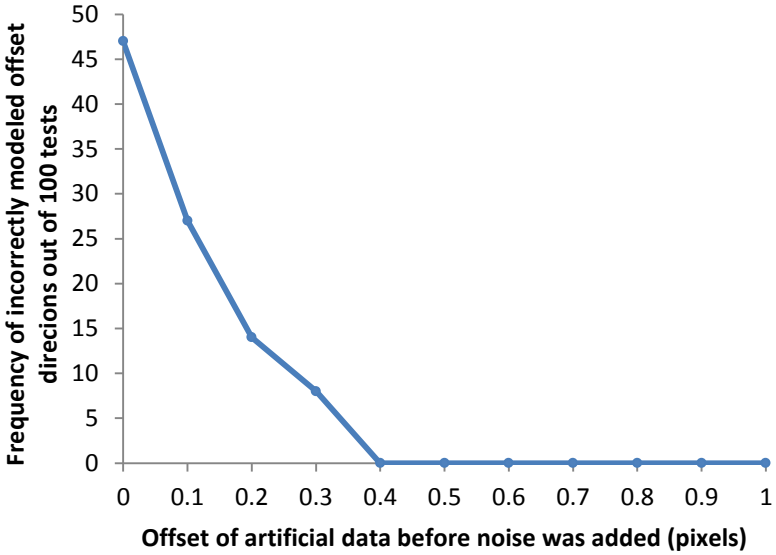
Cell Type	Global Detection	Per-Cell Detection	Cell Count	Global %	Per-Cell %
Epidermis	37	61	62	60%	98%
Cortex	67	76	76	88%	100%
Endodermis	63	80	80	79%	100%
Pericycle	25	56	69	36%	81%
Rootcap	24	38	44	55%	86%
Columella	5	22	24	21%	92%
All	221	333	355	62%	94%

(a) Image channel showing a root expressing a nuclear-localised reporter. (b) A standard image-wide Otsu thresholding approach; note the poor segmentation at the darker end of the root. (c) CellSeT plugin thresholding run independently on each cell, followed by connected component noise removal; note improved segmentation compared to (c). (d) A quantitative comparison of the detection rate of both approaches (Global versus CellSeT's per-cell detection). It can be seen that utilising cell geometry in detecting nuclei can produce significant improvements in detection rate.

Supplemental Figure 3.

Quantitative analysis of the CellSeT protein localisation plugin using artificial data

a



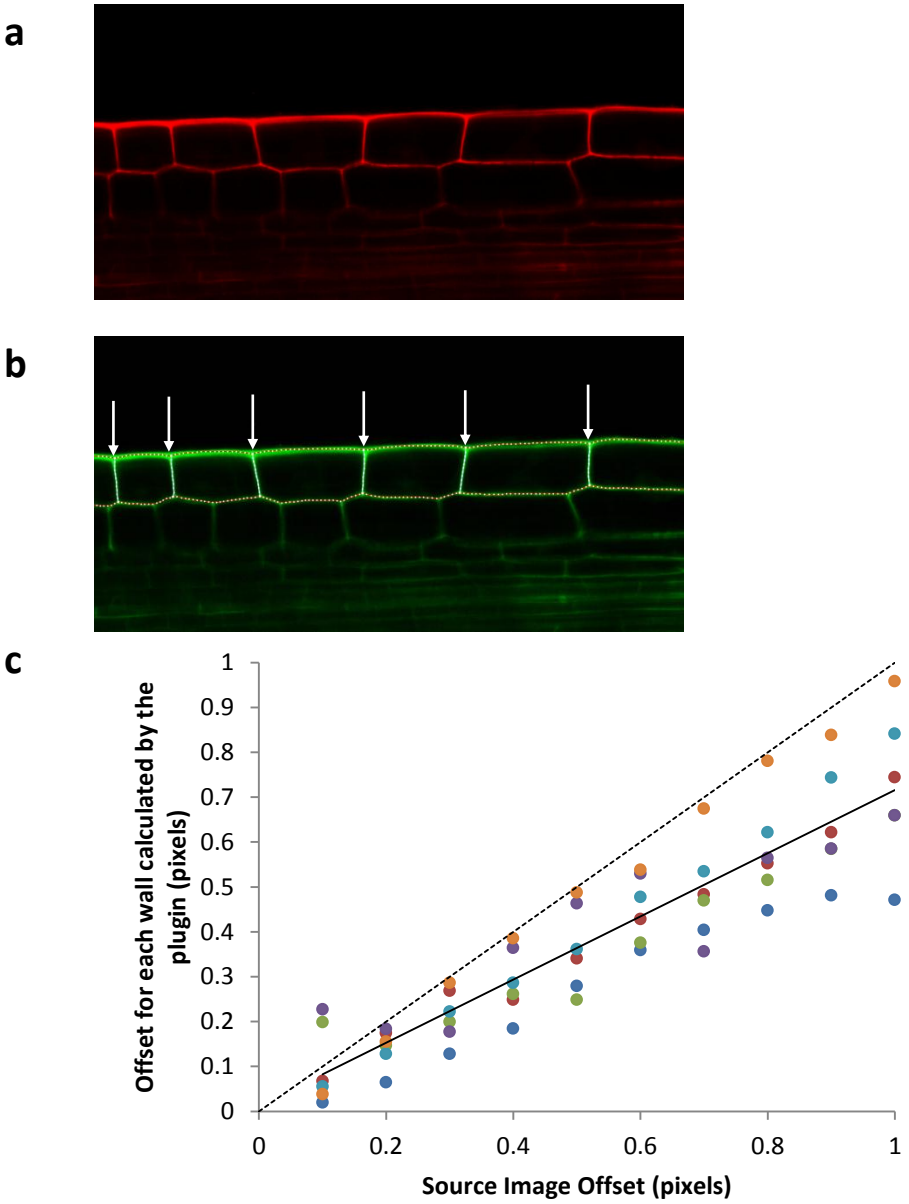
b

Noise Component	Noise Type	Frequency
Sensor Noise	Gaussian s.d. 15	100% of measurements
Biological Noise	Gaussian s.d. 20	12% of measurements
Outlier Noise	Additive noise	10% of measurements

(a) The frequency of incorrectly determined offset direction for artificial data sets of varying offset magnitudes. Artificial data was generated using noise components present in confocal data (b). Each offset size was tested 100 times, and an incorrect result recorded if the fitted Gaussian distribution incorrectly matched the offset of the ground truth. At an offset of 0.1, 27 of 100 tests failed. (b) the nature of the noise components added to the artificial data in (a).

Supplemental Figure 4.

Quantitative analysis of the CellSet protein localisation plugin



(a) The source image used to analyse the protein localisation plugin. The protein channel was simulated by duplicating this image and offsetting the channel by a sub-pixel amount, from 0.1 to 1.0 pixels. This meant that for each wall the true offset of the signal channel was known, providing a reliable ground truth. (b) The segmented image with the six highlighted test walls marked using arrows. (c) Graph showing the offset calculated by the plugin for each wall against the true offset produced by offsetting the signal channel. Each wall is represented by a different colour. In every case the plugin has successfully identified a positive offset, showing that it can reliably determine whether a protein is on the left, or right, of a wall. The offset distance is often underestimated (note the dashed line representing a perfect offset and the solid line representing the average offset calculated by the plug-in); this is caused by lost data as the signal channel begins to moves outside of the range of pixels sampled by the plugin. However, it still provides a useable sub-pixel estimate.