Computer visualisation and modelling of plant morphogenesis: Optical flow analysis of time-lapse confocal microscopy images.

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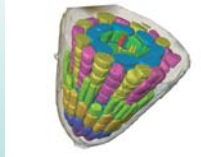
Background

- The dynamics of cell growth and expansion in roots underlies root function and morphogenesis.
- This project aims to develop tools to visualise these processes in Arabidopsis roots by analysing confocal microscope images.
- Techniques are being adapted from computer vision & statistical modelling, geotechnical engineering, root biophysics, molecular and cell biology.
- A computational framework is being developed for interfacing with future developments and studies in cell biology.



Cells in the Arabidopsis root meristem (Escience1 project, J. Haseloff)

Longitudinal section through an Arabidopsis root showing developmental zones superimposed on an epidermal cell layer (Escience1 project, J. Haseloff; image by E. Truernit)

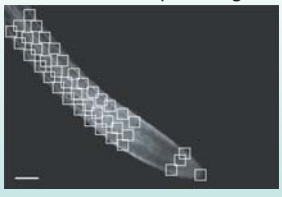
Particle image velocimetry - Methodology

- Particle image velocimetry (P.I.V.) has been used for inital analysis of root growth. P.I.V. tracks the displacement of patches of pixels between images.

2-D confocal images of growing roots over time captured at 2 min. intervals.

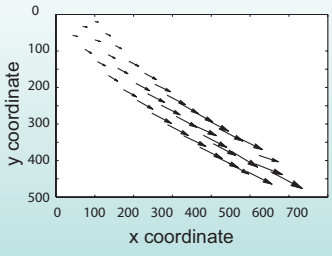
Fluorescent marker - 35S ER-GFP - green fluorescent protein targeted the endoplasmic reticulum

P.I.V. tracks the displacement of pixel patches between successive timelapse images



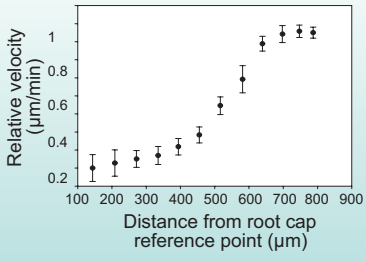
Three rows of patches (each 20 x 20 pixels) manually placed along root length.

P.I.V. output as vector plot of patch displacement



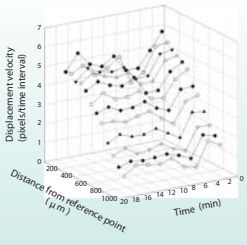
Length of arrows indicates relative displacement (axes changed).

Growth data for individual root calculated from the P.I.V. output

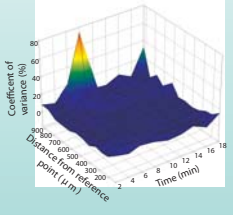


Patch velocity relative to a reference point in root cap. Averages across the 3 patch columns. Bars = St.err.

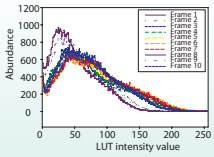
Particle image velocimetry - Root growth and P.I.V. output analysis



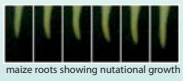
P.I.V. analysis shows large temporal variation in patch velocity between sets of images.



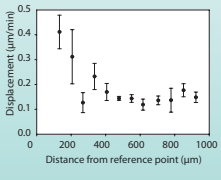
Large variation in velocities across the root axis can indicate errors in patch tracking.



Tracking of patches using P.I.V. can be affected by changes in image intensity.



maize roots showing nutational growth

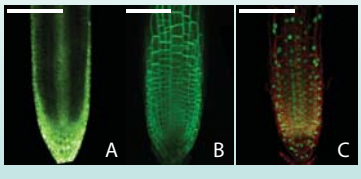


Nutation is the spiralling growth habit of roots. The graph shows the maximal amplitudes of movement orthogonal to the main growth axis.

Similar movements to these occur in the 3rd dimension. This results in cellular features moving perpendicular to the imaging focal plain. Such movements create difficulties for 2D analyses, but can be addressed in 3D.

Movement of cell contents - Images used for analysis were 35S ER-GFP (endoplasmic reticulum (ER) targeted GFP).

ER moves rapidly throughout the cell cytoplasm. This movement results in changes of image intensity and noise within the image analysis. Alternative flourescent markers are more promising for growth analyses and cell identification.



A - Current 35S ER-GFP (green) green fluorescent protein targeted to the endoplasmic reticulum
B - 35S LTI6b-eGFP (green)- GFP targeted to the plasma membrane
C - 35S H2B-YFP (green) - YFP targeted to the cell nucleus counterstained with Plasma membrane stain Fm 4-64 (red)

Conclusions and Future work

- P.I.V. is a useful tool for measuring root growth in the confocal images.
- We are optimising P.I.V. for probing changes in cell growth in roots subjected to environmental perturbations.
- We will visualise cell expansion in 4-D using dual-labelling and fluorescent cell reporters.
- We will track cell location, shape, and size as a function of time (i.e. in 4-D).

Please see accompanying poster - Computer visualisation and modelling of plant morphogenesis: in-vivo segmentation and tracking of cells using confocal microscopy. by Timothy J. Roberts et. al.

Acknowledgements

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