4D dynamics of auxin signalling pattern during lateral root development

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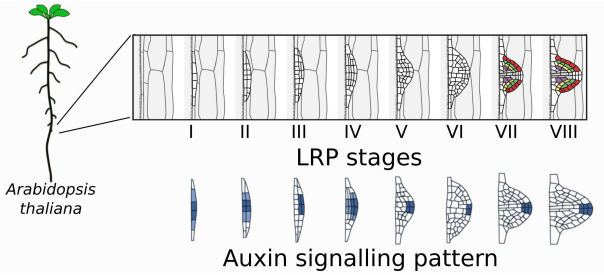
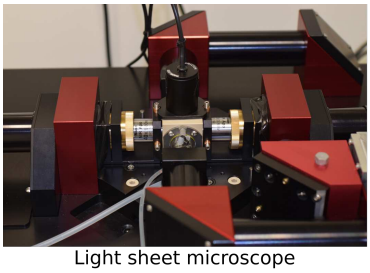
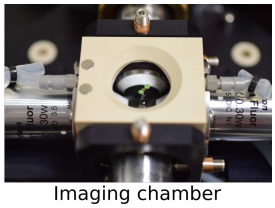


Images from Péret et al. 2009, Péret et al., 2012 and Ristova et al. 2014

Summary

During lateral root development, the pattern of auxin signaling is dynamic and polymorph. As the primordium develops, auxin signaling transitions from a gradient to the fountain like pattern seen in the root apical meristem. Whereas this has been described, we currently lack precise and high resolution of this dynamic on live specimen.
To study the 4D dynamics of the auxin signalling pattern, we use DR5v2 reporter, and do live imaging with light sheet microscopy. To probe its robustness, we use IR laser ablation. A precise 4D depiction of auxin signaling pattern will be instrumental to establish dynamics models.


Light sheet microscope


Imaging chamber


Seedlings 2 and 14 days post imaging

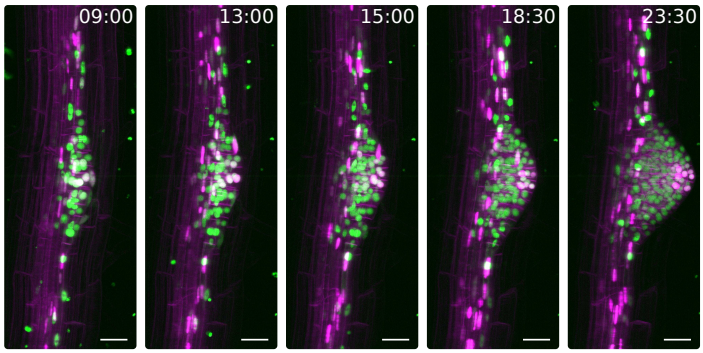

Seedlings extruded from glass capillaries

Light sheet microscopy: imaging in physiological conditions

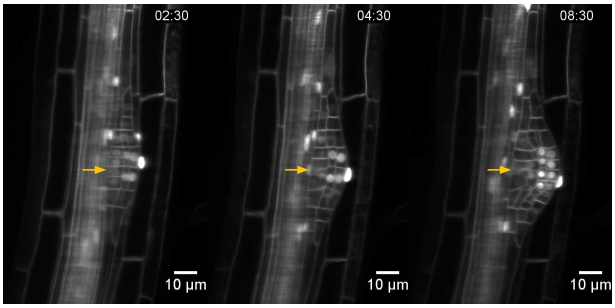
We use a light sheet microscope with two illumination lenses (10X) and two detection lenses (40X). The imaging chamber is filled with liquid half MS and closed by a lid containing LEDs.
Seeds are sown in glass capillaries filled with 1/2 MS Phytagel. 4-5 days old seedlings are extruded from capillaries and imaged over 30-40h, with one stack acquired every 30 minutes.
Imaging do not stop growth, as seedlings transferred on soil continue their development.

4D dynamics

We use the DR5v2 auxin signalling reporter together with the PIP1;4 plasma membrane reporter, the GATA23 nuclear reporter for lateral root primordia, and the RPS5A nuclear reporter for actively dividing cells. Movies are analysed first qualitatively, then quantitatively using Fiji plugins such as MaMuT and the 3D Suite, as well as homemade R scripts.



Maximum intensity projection of a few stacks from a light sheet timelapse. Time in hours. Scale bar = 25 µm.
pUB10::PIP1;4-3xmGFP pGATA23::H2B- 3xmCherry x DR5v2- 3xYFP pRPS5A::dtTomato-NLS



Orange arrow points at ablation site, before the cell collapses (t=2h30) and after it has collapsed.

Robustness

Our light sheet microscope is equipped with an infrared laser ablation system that allow us to make local (one cell mininum) and targeted damage to the primordia.