Quantifying cell polarities in confocal images using 3D wall meshes

A computational geometry approach to the estimation of cell-interface and tissue-level polarities in 3D stacks of co-imaged fluorescent transmembrane carriers and cell-wall markers

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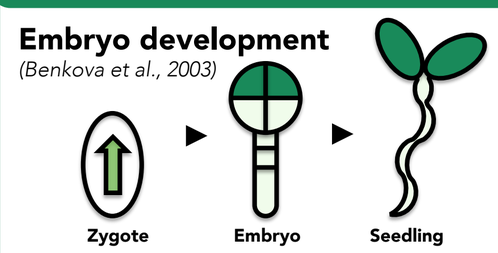
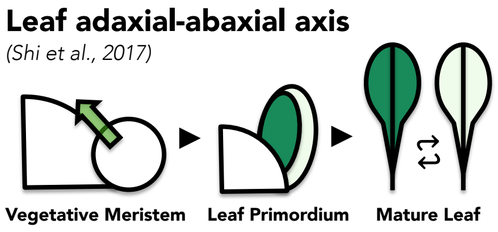
Polarized auxin transport is a key process for the patterning of multicellular plant tissues

Embryo development
(Benkova et al., 2003)



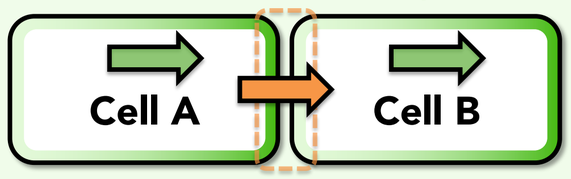
Leaf adaxial-abaxial axis
(Shi et al., 2017)



Cell polarity and hormonal signaling

Intra-cellular polarization (PIN proteins)

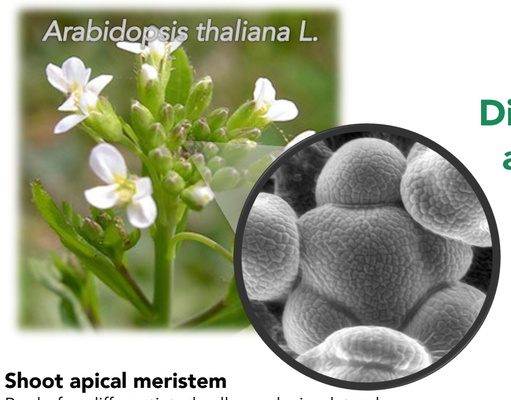
Differential concentration at the plasma membrane



Differential efflux to intercellular compartment

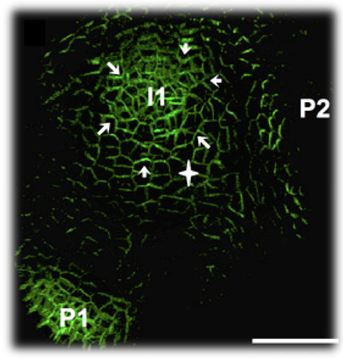
Local net flow of molecular signal (Auxin)

Organogenesis at the SAM



Shoot apical meristem
Pool of undifferentiated cells producing lateral organs

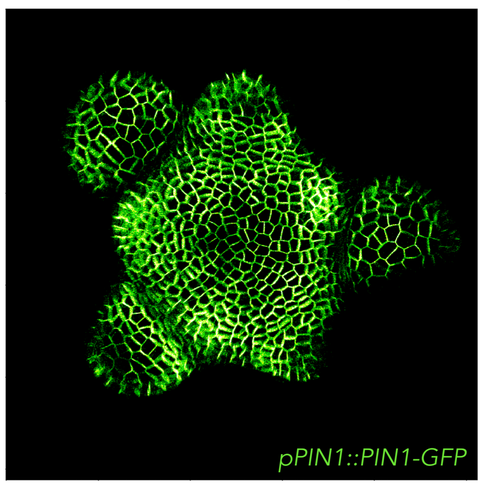
Convergent PIN1 cell polarities
▼
Directional flow of auxin molecules
▼
Local peak of signal
▼
Organ initiation at tissue scale



(Bayer et al., 2008)

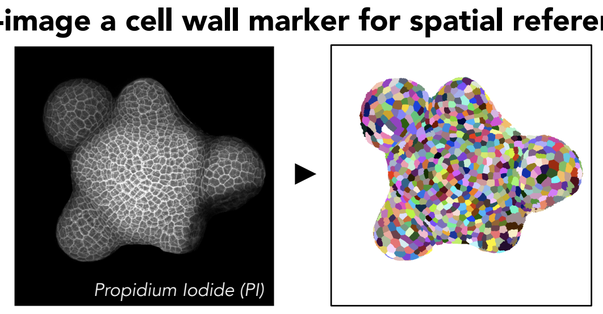
How to quantify PIN1 polarities in the SAM at cellular level?

Live-imaging of SAMs expressing fluorescent PIN1 proteins using confocal microscopy



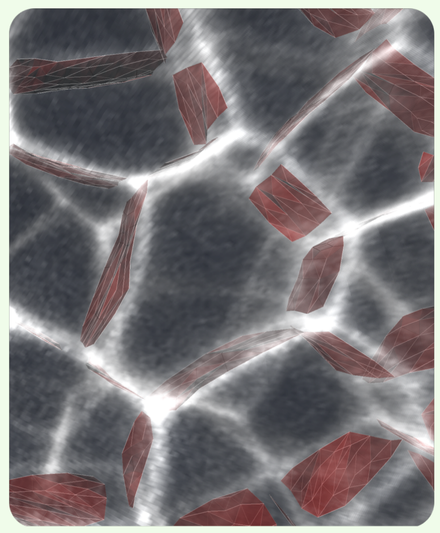
- Resolution cannot separate cell membranes (0.2µm)
- Crescent caused by gradient often used as a clue
- Hard to know which cell the signal belongs to

Co-image a cell wall marker for spatial reference



3D image segmentation to identify cell walls

Extraction of cell wall triangular meshes from 3D segmented image



Labelled 3D confocal image stacks (0.2x0.2x0.6µm voxels)

- 3D seeded watershed segmentation *(Fernandez et al., 2010)*

Compute a mesh for each anticlinal L1 cell interface

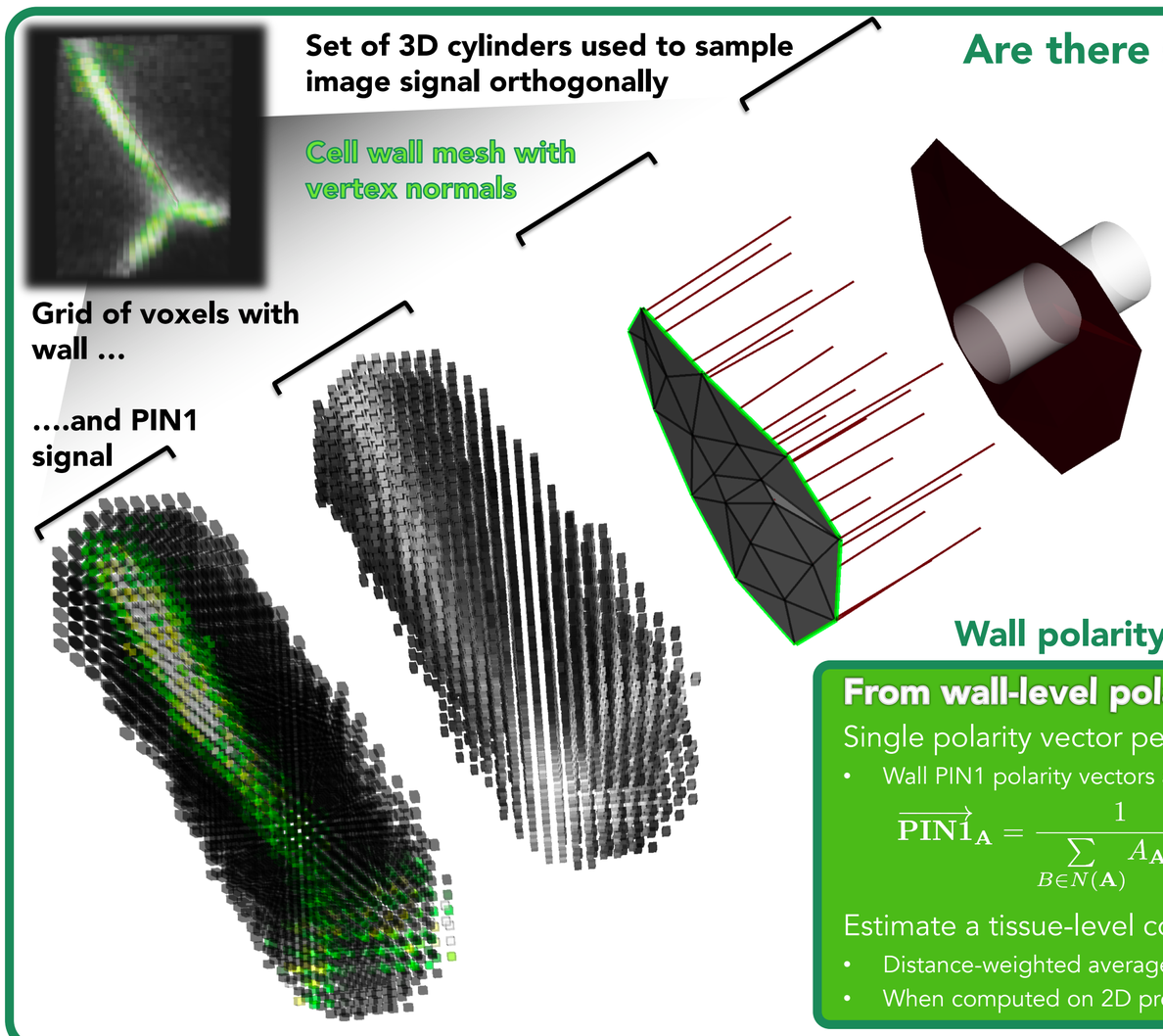
- Marching cubes on every L1 cell (contact with background label)
- For each pair of neighbor cells, keep only common vertices
- Use the triangle subset of the left cell in the label pair
- Apply quadric decimation and isotropic remeshing on wall mesh

Regular surface meshes within the image grid

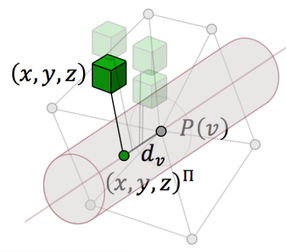
Estimate normal at each vertex of every wall mesh

- Oriented left to right according to cell label pair

Use wall marker information to quantify accurately PIN1 levels within each cell and estimate local polarity

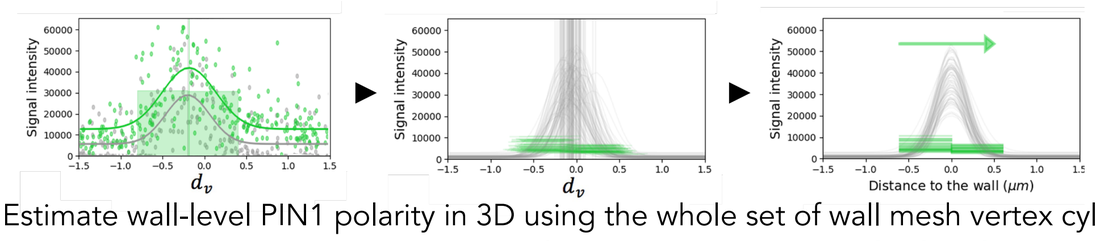


Are there significantly different PIN1 levels between sides of a cell wall?



At each vertex, 1D projection of image signals on a locally normal axis

- For each vertex **v** of the wall mesh (except on contour), cylinder centered on $\mathbf{P_v}$
- **Signed abscissa d_v** of the orthogonal projection of voxel on the normal vector $\vec{n}_v$
- **Distance r_v** of the voxel center to its projection up to cylinder radius $r_v < r_C$=1µm
- Keep only voxels on the 1D axis up to half-cylinder height : $|d_v| < d_C$=1.5µm
- **Wall abscissa d_0** estimated using a Gaussian approximation of membrane signal



Estimate wall-level PIN1 polarity in 3D using the whole set of wall mesh vertex cylinders

- Compute the average intensity on each side of the wall using close voxels : $|d_v - d_0| < d_{max}$=0.6µm
- Statistical test on the two PIN1 intensity distributions to decide if they have different means : $p_{A\blacktriangleright B} = \begin{cases} 1 & / \ 0.5 \\ 0 & \\ -1 & / -0.5 \end{cases}$

Wall polarity vector : $\overrightarrow{\mathrm{PIN1}}_{A\blacktriangleright B} = p_{A\blacktriangleright B} \left|\mathrm{PIN1}_{A\blacktriangleright B} - \mathrm{PIN1}_{A\blacktriangleleft B}\right| \vec{n}_{A\blacktriangleright B}$

From wall-level polarities to cell PIN1 polarity and towards tissue-scale polarity maps

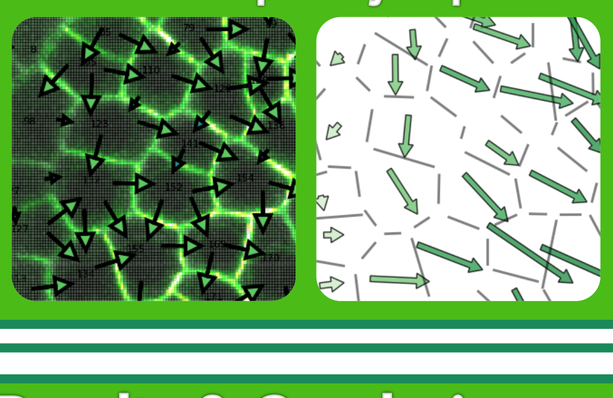
Single polarity vector per cell : weighted average (area) of wall vectors

- Wall PIN1 polarity vectors are the same for cells (A ► B) or (B ► A)

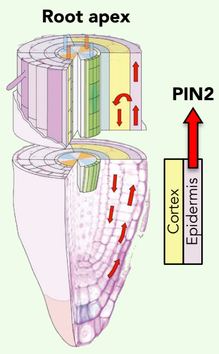
$$\overrightarrow{\mathrm{PIN1}}_A = \frac{1}{\sum_{B \in N(A)} A_{A\blacktriangleright B}} \sum_{B \in N(A)} A_{A\blacktriangleright B} \overrightarrow{\mathrm{PIN1}}_{A\blacktriangleright B}$$

Estimate a tissue-level continuous vector-field of local PIN1 polarity

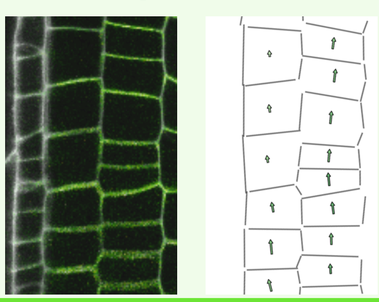
- Distance-weighted average of cell polarity vectors in any point of the space
- When computed on 2D projected L1 plane, allows to derive PIN1 convergence maps



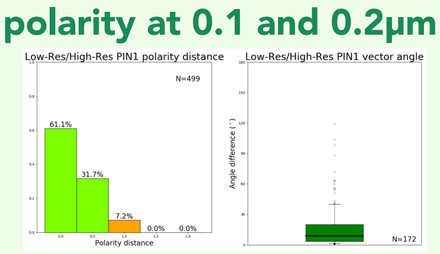
Correct PIN2 polarity in root epidermal cells



(Grunewald et al., 2007)



Consistent SAM PIN1 polarity at 0.1 and 0.2µm

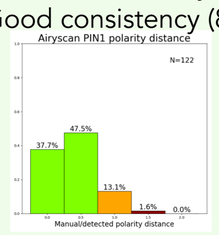


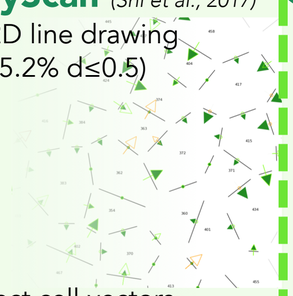
- Mean wall polarity distance: 0.23
- Mean cell PIN1 vector angle: 16°

Comparison to super-resolution techniques : AiryScan *(Shi et al., 2017)*

Manual estimation by 2D line drawing

- Good consistency (85.2% d≤0.5)





- Local differences affect cell vectors

Results & Conclusions

Highly consistent estimation of polarities

Provides reproducible cell-level PIN1 polarity measures

- Same tissue imaged at different resolutions
- Comparable to (manual) methods with super-resolution
- Several individual SAMs show similar patterns (N=16)

A new tool for tissue scale polarity analysis

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ANR
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