



Can U-Net replace CLV3? Machine learning for the identification of biological landmarks in Shoot Apical Meristem images

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Can U-Net replace CLV3?

Machine learning for the identification of biological landmarks in Shoot Apical Meristem images

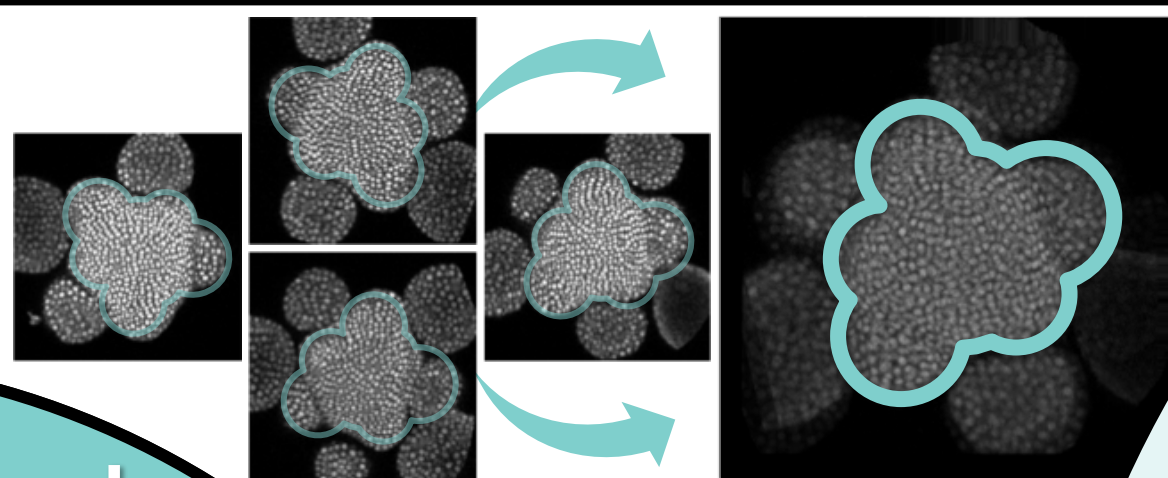
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The meristem : a strikingly regular plant organ

Stationary dynamical system + constant divergence angle

The regularity of the phyllotaxis allows to align & superimpose a population of SAMs with a limited uncertainty



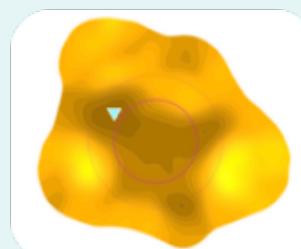
Landmark-based Alignment of SAMs

Map a natural cylindrical coordinate system on experimental SAM data

Detect landmarks using biological markers



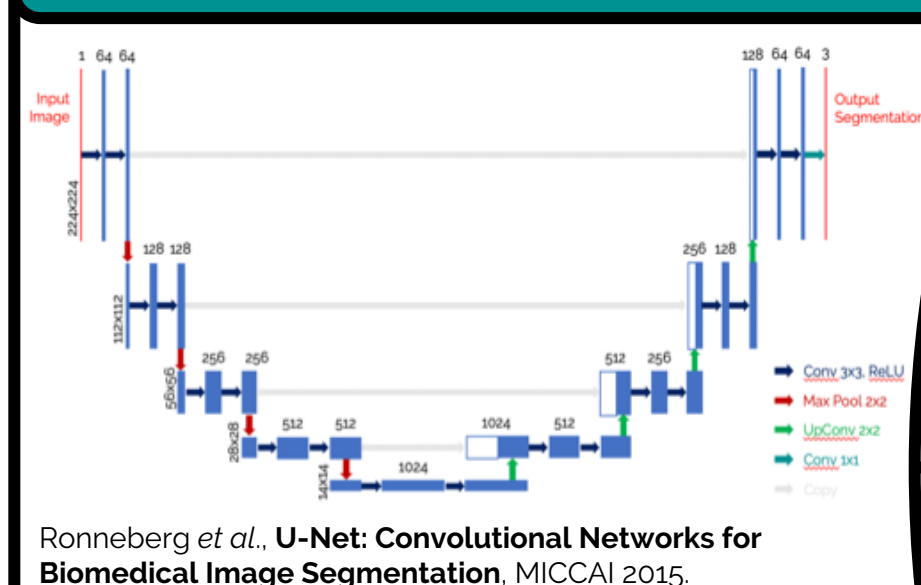
- CZ center, origin of the system
Center of the CLV3 domain
- Main vertical axis, z unit vector
Optimal CZ rotational symmetry axis
- Direction of the last primordium P_0 , $\theta=0$
Global maximum of Auxin level
- Orientation of the phyllotactic spiral
(Expert) manual input



Possible to use curvature to determine P_0 on a centered SAM (using reference data) but **CLV3 is required** for the CZ center

Galvan-Ampudia, Cerutti *et al.*, Temporal integration of auxin information for the regulation of patterning, eLife 2020.

U-Net CNN Model



Ronneberg *et al.*, U-Net: Convolutional Networks for Biomedical Image Segmentation, MICCAI 2015.

More U-Net?

CZ U-Net 3D

Same CNN on 3D images

- 224 x 224 x 56 (~1µm)
- 1 less level in U-Net depth

More parameters, similar results
Possibility to handle tilted images

Graph U-Net on surface mesh

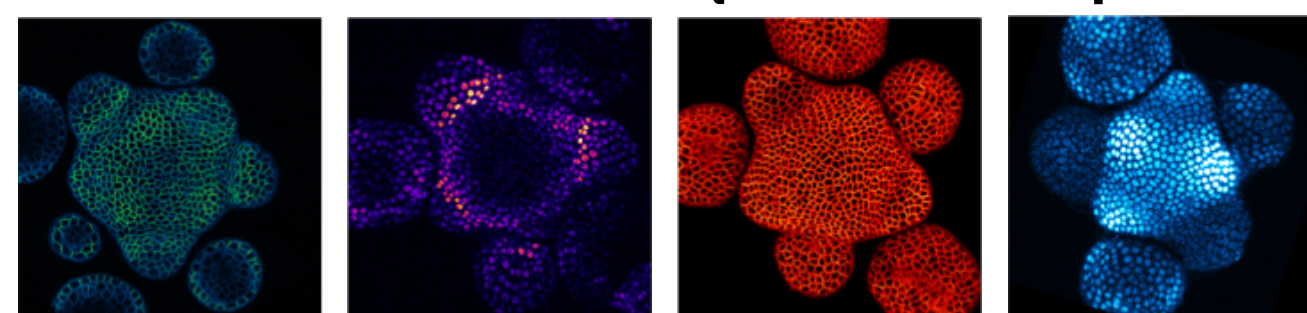
GCNN using graph Conv/Pool layers

- Nodes (x,y,z) + mesh connectivity
- Binary CZ belonging prediction

Preliminary, no satisfactory results

Towards the automated construction of SAM atlas

Aggregate quantitative data from various (WT) SAMs onto a unique 3D+t template



(neglecting non-linear inter-SAM deformations)

Cylindrical coordinate system

Rigid mapping to a common 3D template

+

Organ based temporal indexation

Requires precise positioning of the system center

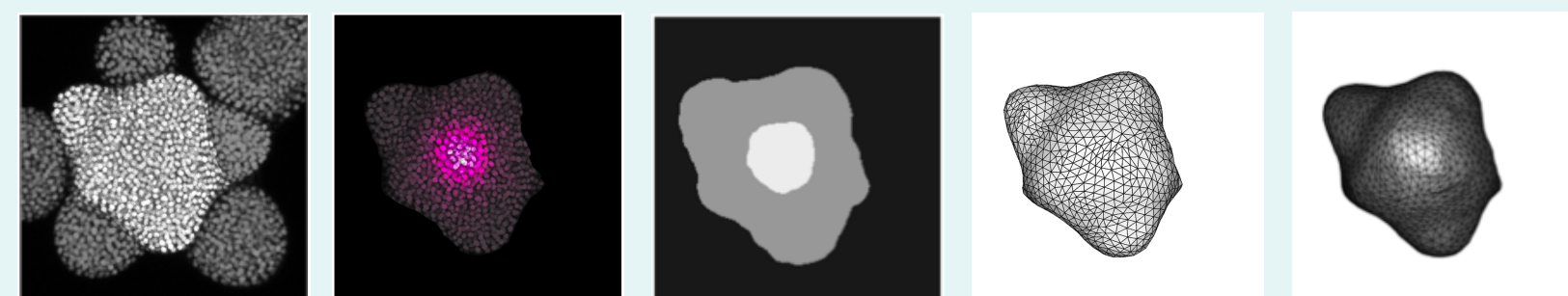
Interest of CZ U-Net 2D

- Allow to pinpoint the center with high confidence:
- Without having to fuse plants with CLV3 reporter
- Re-using suitable existing data (without CLV3)

ML Problem

Predict the SAM Central Zone

Previous work provides a rich 3D image dataset with both CZ location (CLV3) and geometrical information (from nuclei/PI)



Is SAM geometry enough to accurately predict biologically relevant zones (CZ)?

Test **Machine Learning approaches** trained on (geometry + CZ) data

- Using 3D or 2D image intensity to predict belonging to the central zone
Semantic segmentation problem: voxel/pixel classification
- Using geometrical descriptors on a surface mesh to predict CLV3 or CZ
Supervised classification problem on vertices

- Using 3D surfaces as geometric graphs
Node classification problem

Use the same metric for all approaches :

Distance of the predicted CZ center to the actual CLV3 domain center

Surface vertex classification

3D mesh of the SAM surface

Geom. features + measured CZ data

- principal curvatures
- contour distance
- closeness centrality
- betweenness
- rotational symmetry

- CLV3 level
- CZ (binary)

Train classifier models to **predict CZ** with SAM-level cross-validation

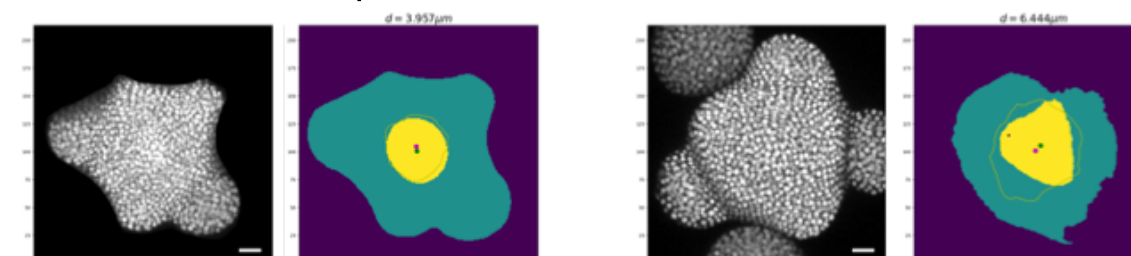
Best classifier: **Logistic Regression**, **88.2% (balanced) accuracy** on Test

How robust is CZ U-Net?

• To growth conditions ? to mutants ?

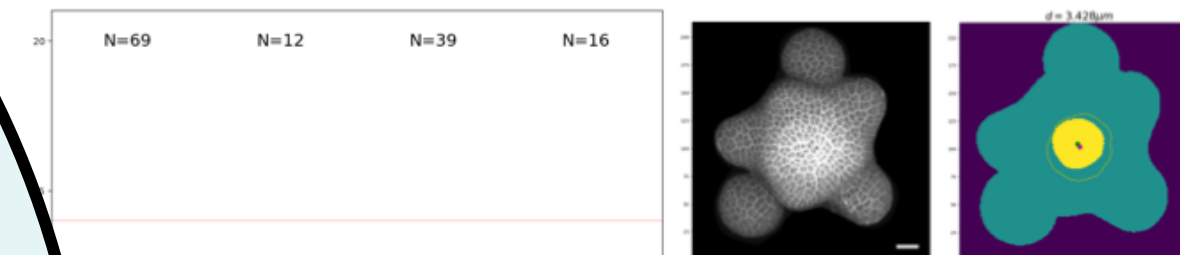
SD growth condition
alters SAM shape

Yucca mutants exhibit
phyllotaxis defects



• To the type of geometry marker ?

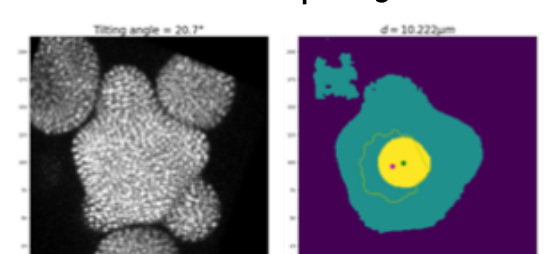
PI wall staining instead of nuclei targeted marker



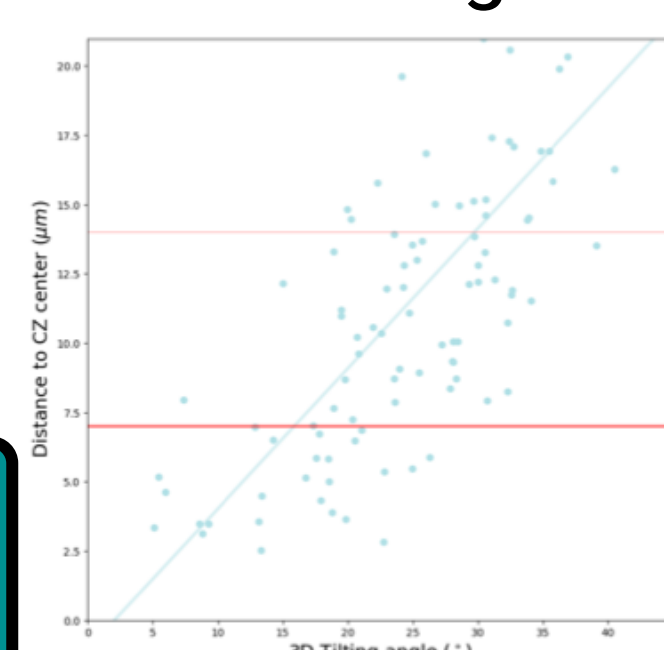
Sensitive to **major**
shape changes only
Surprisingly good on
an **unseen marker!**

• To 3D tilting of SAM in the image ?

Random 3D rotation
before 2D projection



Performs reasonably
well up to a **15° tilting**



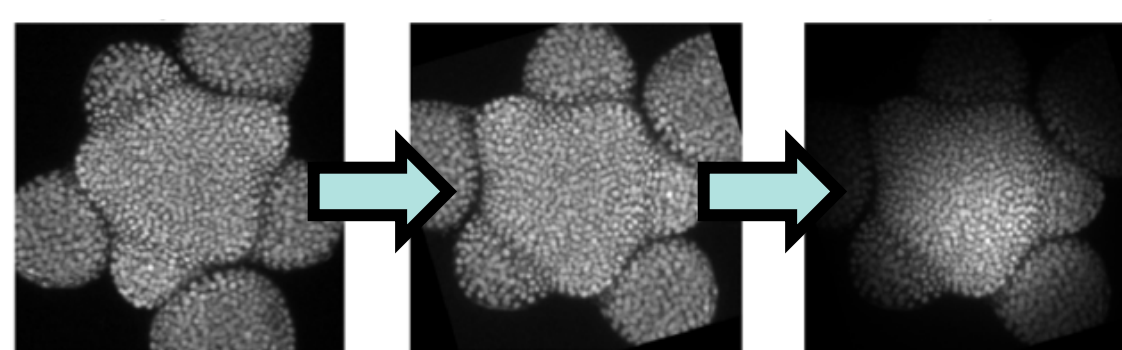
CZ U-Net 2D Convolutional network on 2D projected SAM images

3-class semantic segmentation: Background, SAM, CZ

Training

CNN input data: **2D images**

- Maximum intensity projection
- Downsampling to 224 x 224 (~1µm)
- Rescaled (1%-99%) 8-bit intensity



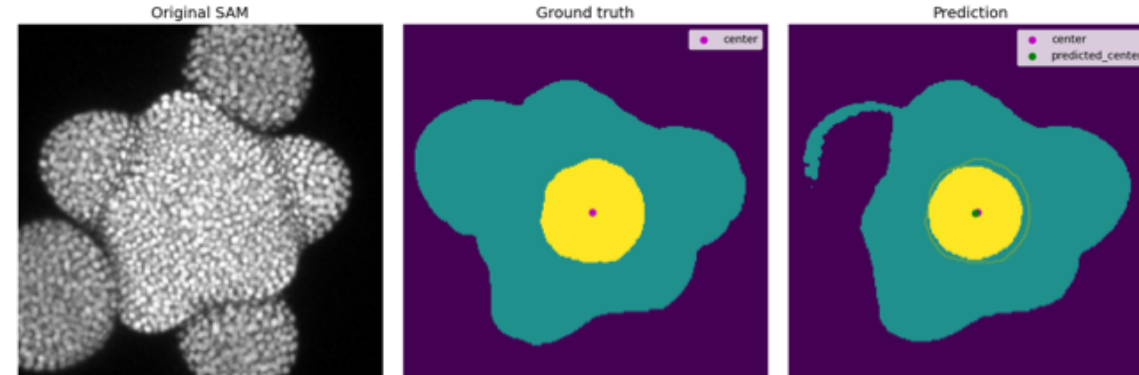
Training dataset: **23 SAM series**

- 3 time points (0h, 5h, 10h)
- Input: nuclei-targeted marker
- Labels: thresholded CLV3 2D map

Data augmentation for U-Net training

- With/without manual organ cropping
- Random 2D rigid transformation
- Random 2D Gaussian intensity factor

Validating

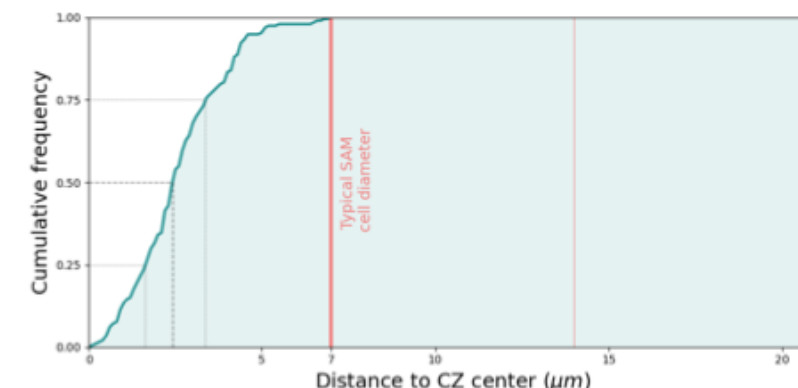


CNN output data: **2D predictions**

- Softmax probabilities (size 3 vector)
- Assign each pixel max probable class

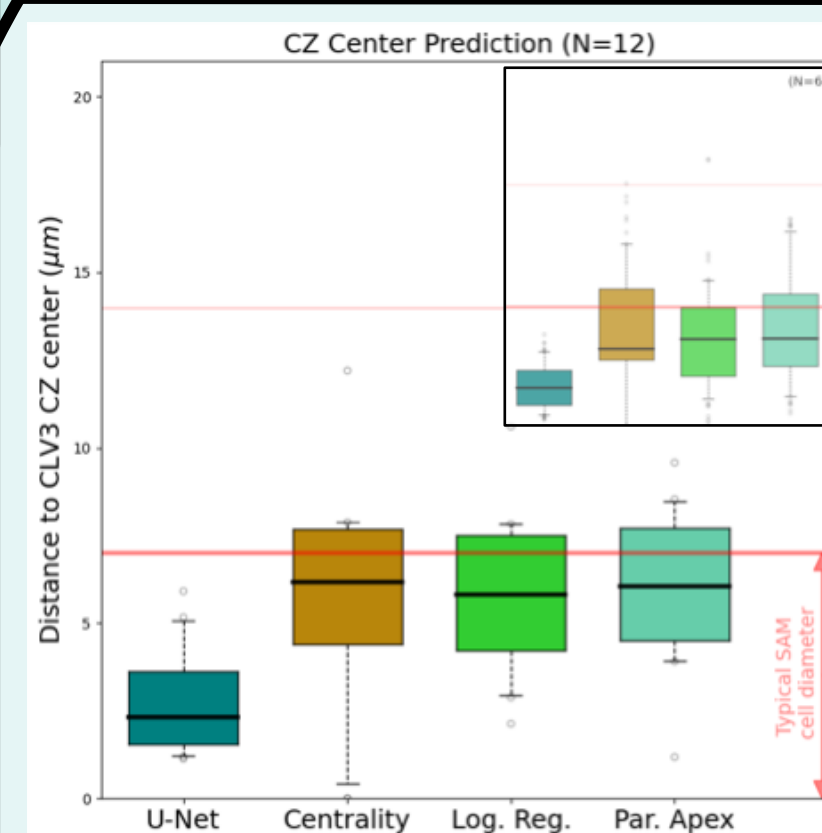
Cross-validation approach on SAMs

- 80% / 20% ratio on individuals
- Using cross-entropy loss on **Train**
- **96.6% accuracy** on both **Train** and **Test**



CZ center predicted with an error of less than 1-cell on the validation dataset

Method evaluation Comparison to other CZ predictions



Evaluation of 12 SAM images, independent from the Train dataset.

- **CZ center is reliably predicted by U-Net with a low error**
- **Outperforms other (3D, rotation invariant) ML method**
- **Outperforms geometric modelling approach**
- The interest of geom. features is limited

Ready to be tested!

Compare methods on the same individual SAMs

- Pre-trained CZ U-Net 2D
- Center vertex on surface mesh
- Logistic Regression prediction
- Apex of paraboloid surface fit

Reimplemented from (Åhl *et al.*)

- Triangle mesh of SAM surface
- Min. curvature watershed seg.
- Least squares fit of paraboloid

Åhl *et al.*, High-Throughput 3D Phenotyping of Plant Shoot Apical Meristems from Tissue-Resolution Data, Frontiers in Plant Science, 2022.