



HAL
open science

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

Anthony Scriven, Carlos Galvan-Ampudia, Guillaume Cerutti

► To cite this version:

Anthony Scriven, Carlos Galvan-Ampudia, Guillaume Cerutti. Can U-Net replace CLV3? Machine learning for the identification of biological landmarks in Shoot Apical Meristem images. From genes to plant architecture: the shoot apical meristem in all its states, Nov 2022, Poitiers, France. hal-03892383

HAL Id: hal-03892383

<https://hal.science/hal-03892383v1>

Submitted on 9 Dec 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Can U-Net replace CLV3?

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

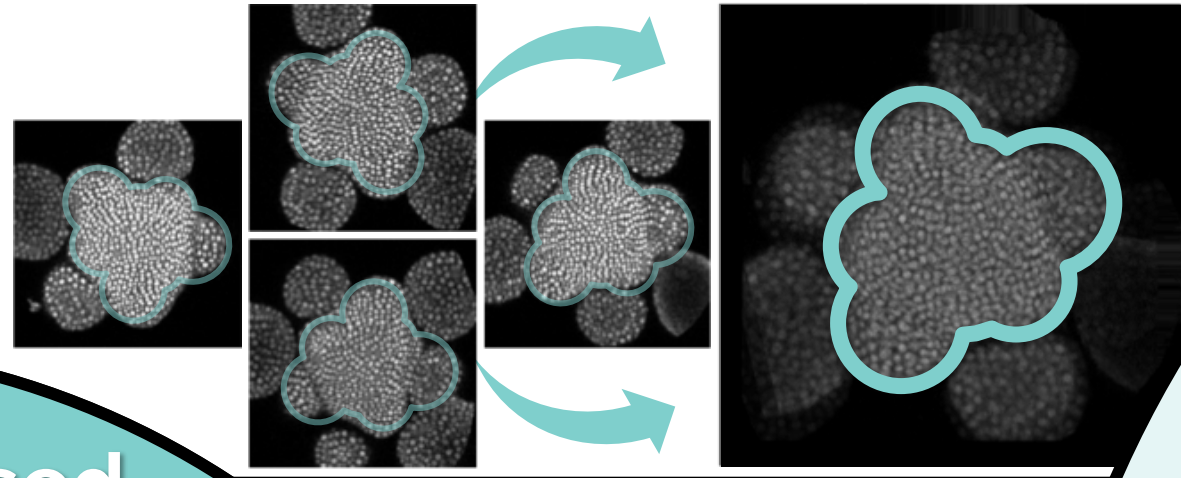
ANTHONY SCRIVEN, CARLOS GALVAN-AMPUDIA, GUILLAUME CERUTTI*

Laboratory for Reproduction and Development of Plants, Université de Lyon, ENS de Lyon, INRAE, Inria, CNRS, Lyon, France.

The meristem : a strikingly regular plant organ

Stationary dynamical system + constant divergence angle

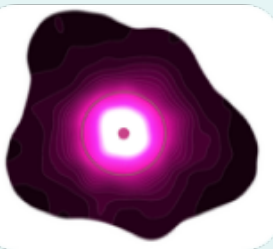
The regularity of the phyllotaxis allows to align & superimpose a population of SAMs with a limited uncertainty



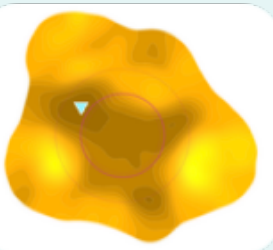
Landmark-based Alignment of SAMs

Map a natural cylindrical coordinate system on experimental SAM data

Detect landmarks using biological markers



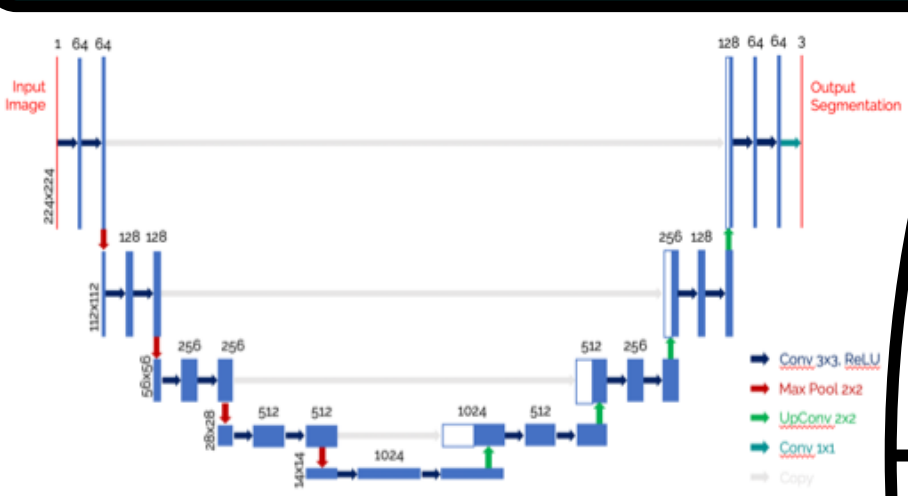
- CZ center, origin of the system
Center of the CLV3 domain
- Main vertical axis, z unit vector
Optimal CZ rotational symmetry axis
- Direction of the last primordium P_0 , $\theta=0$
Global maximum of Auxin level
- Orientation of the phyllotactic spiral
(Expert) manual input



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

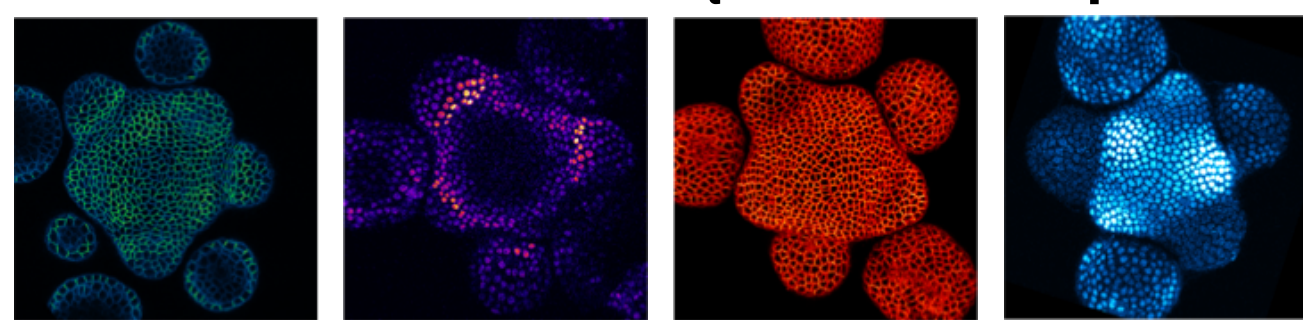
Graph U-Net on surface mesh

- 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

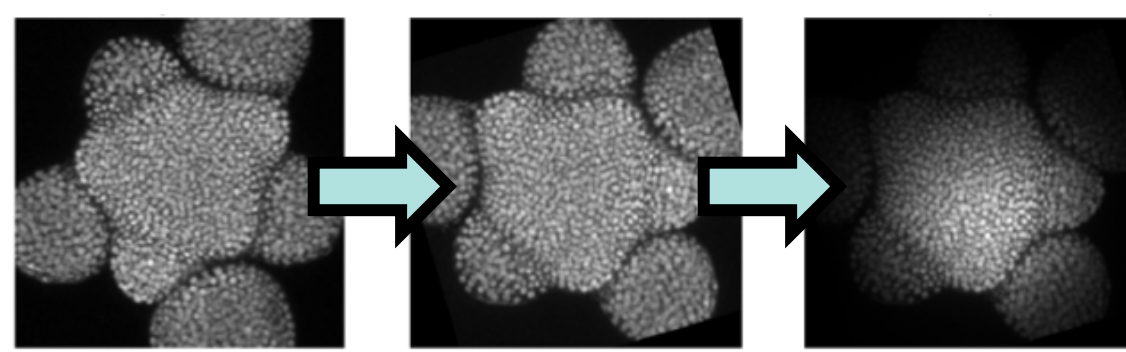
CZ U-Net 2D Convolutional network on 2D projected SAM images

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



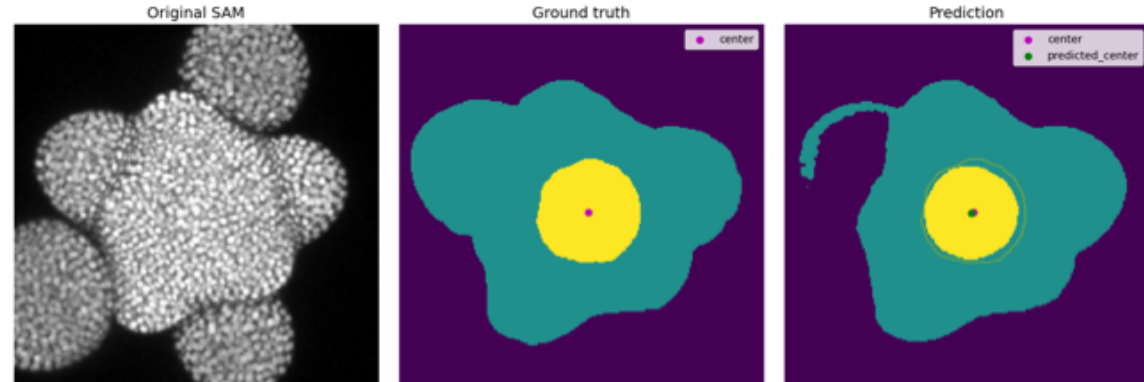
Training dataset: 23 SAM series

- 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

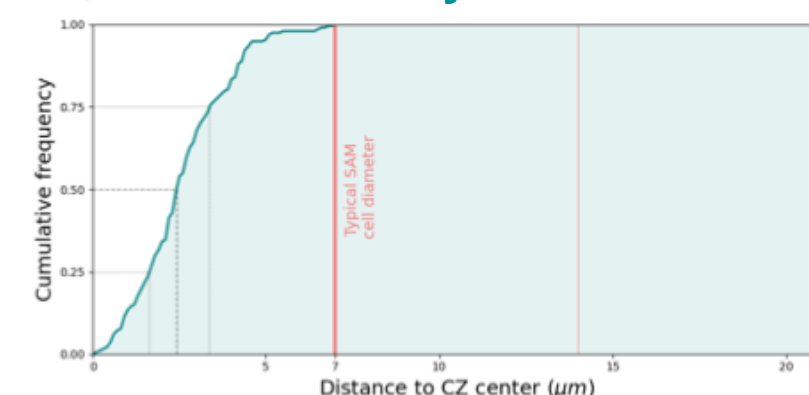


CNN output data: 2D predictions

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

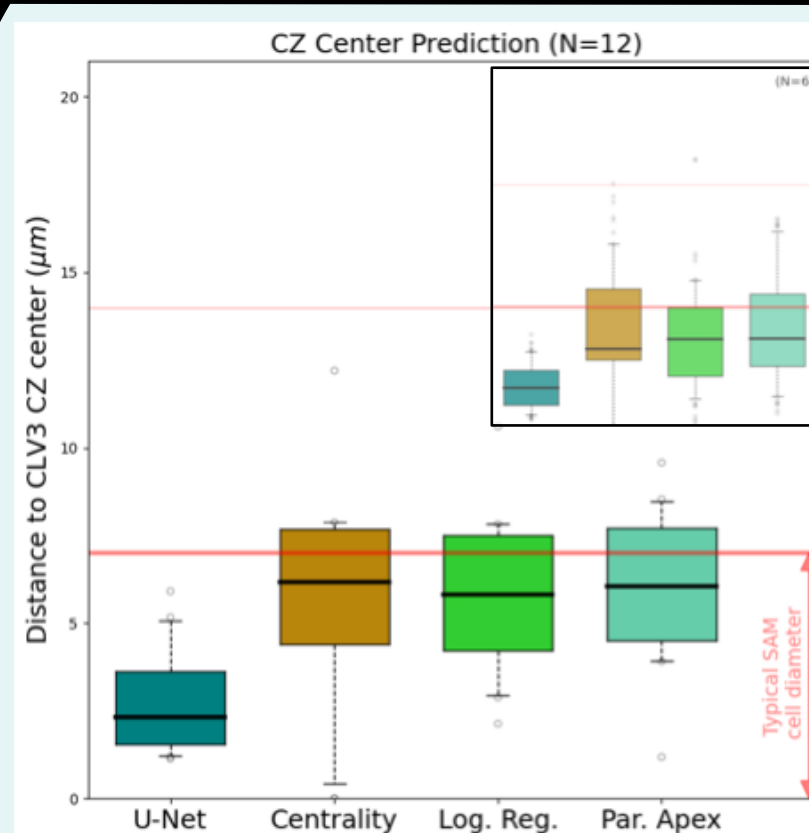
Cross-validation approach on SAMs

- 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



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.

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!

Surface vertex classification

3D mesh of the SAM surface

- Geom. features + measured CZ data
- principal curvatures
- contour distance
- closeness centrality
- betweenness
- rotational symmetry

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

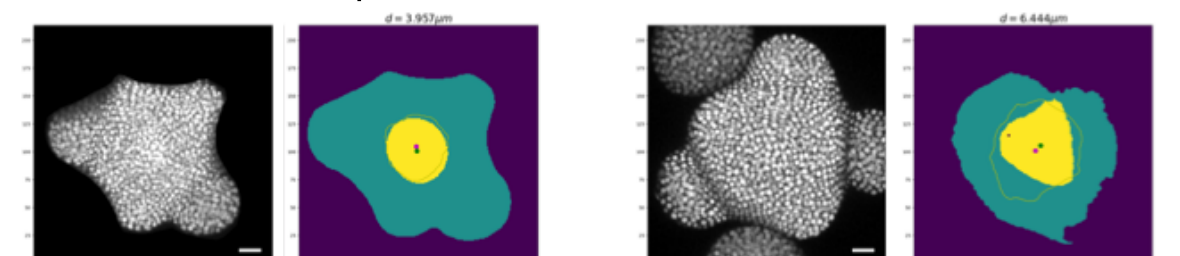
Best classifier: **Logistic Regression, 88.2% (balanced) accuracy** on Test

How robust is CZ U-Net?

• To growth conditions ? to mutants ?

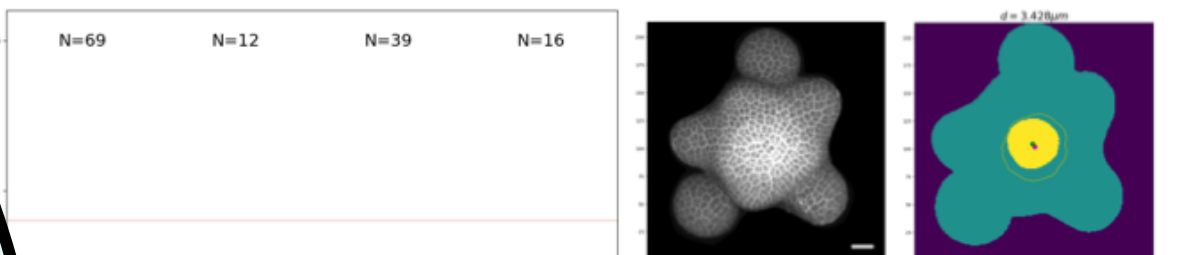
SD growth condition alters SAM shape

Yucca mutants exhibit phyllotaxis defects



• To the type of geometry marker ?

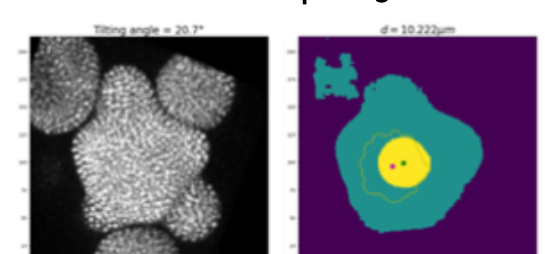
PI wall staining instead of nuclei targeted marker



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

