CEMPS

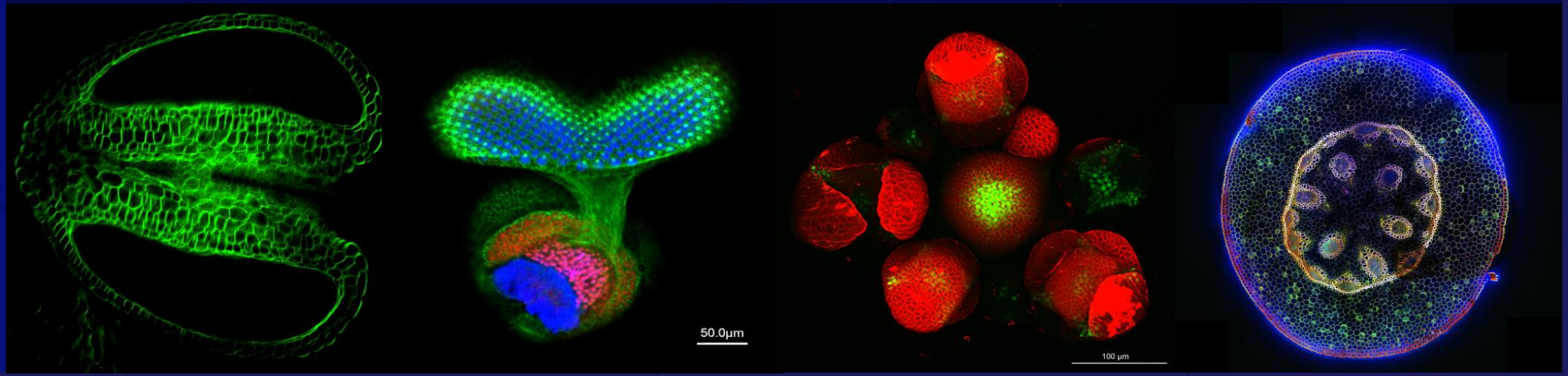
中国科学院分子植物科学卓越创新中心 | 植物生理生态研究所

CAS Center for Excellence in Molecular Plant Sciences | Institute of Plant Physiology and Ecology

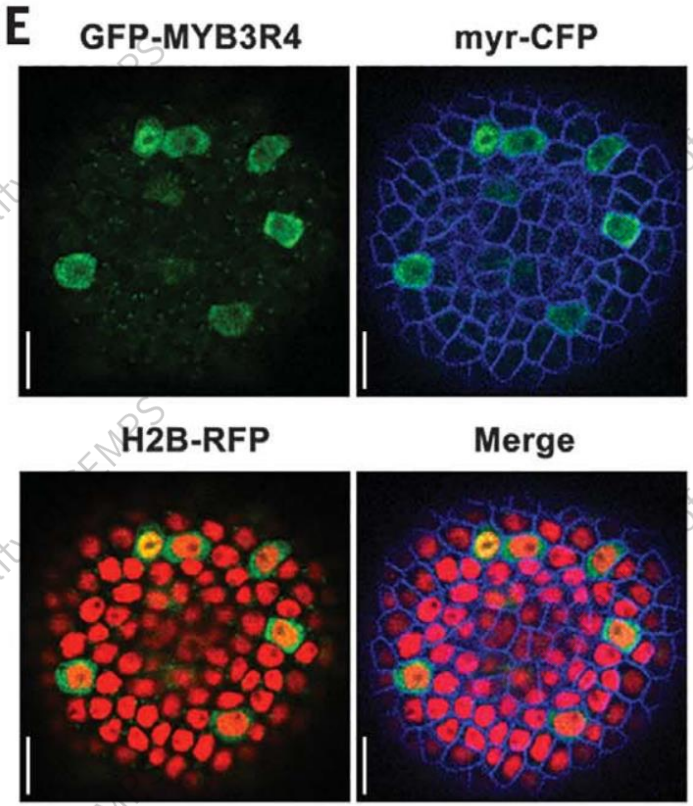
See the details in vivo

the application of micro-imaging in plant science

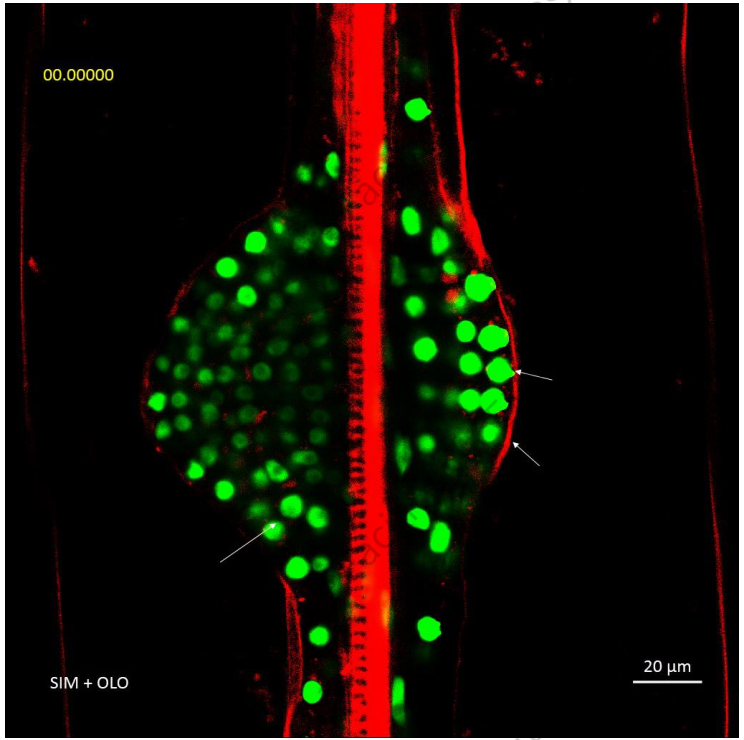
Wenjuan Cai / Core Facility of CEMPS / June 9th, 2021



Importance of micro-imaging: see the details in vivo

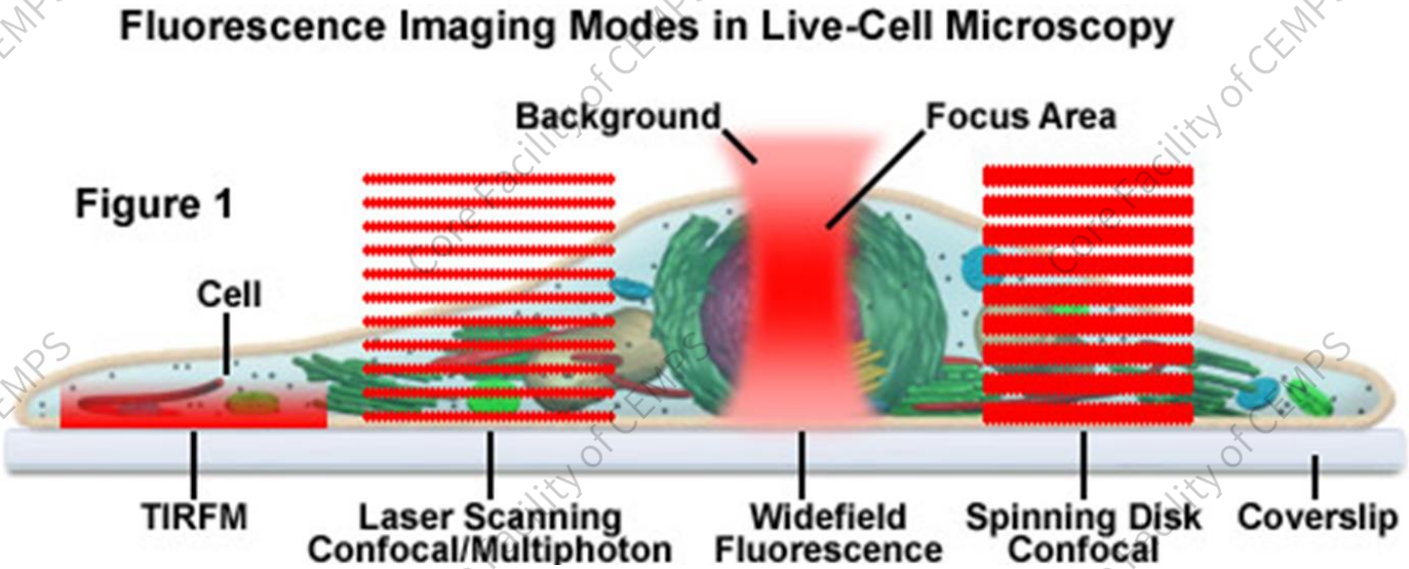


Yang WB. et al., Science. 2021



Zhang TQ. et al., The Plant Cell. 2017

Different micro-imaging systems: z-axis resolution



全内反射显微镜　激光扫描共聚焦/双光子显微镜　宽场显微镜　转盘共聚焦

Outline

- Laser scanning confocal
- Spinning disk confocal
- TIRF system
- Others: Two-photon/Lightsheet/Super-resolution

Laser Scanning Confocal-
Classical and universal

2D/3D imaging

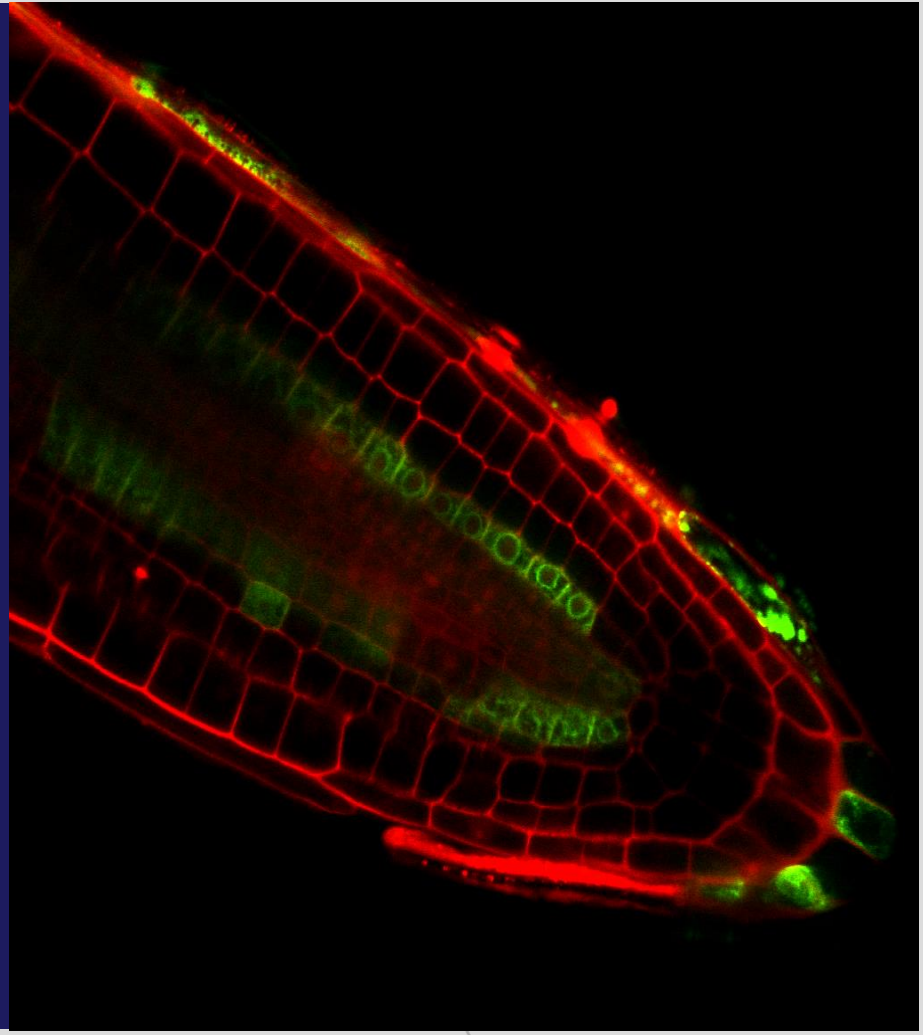
Colocalization

FRAP

FRET

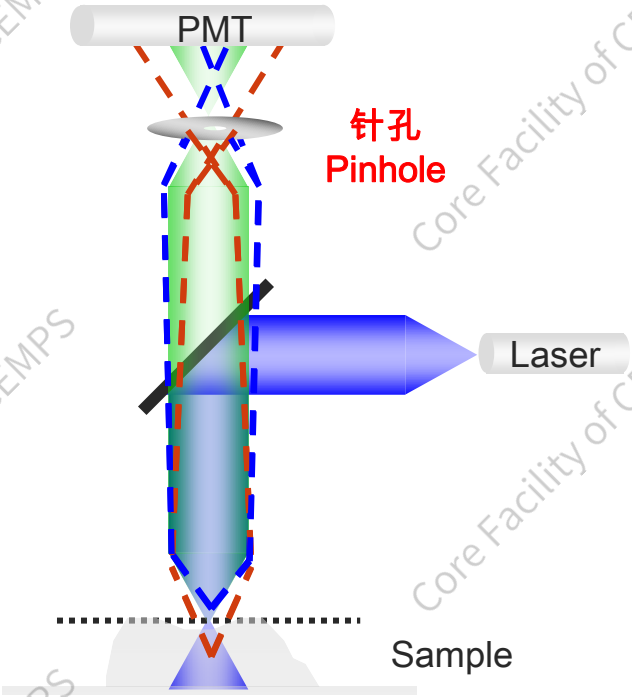
Spectral unmixing

…



Laser Scanning Confocal

Keywords: pinhole

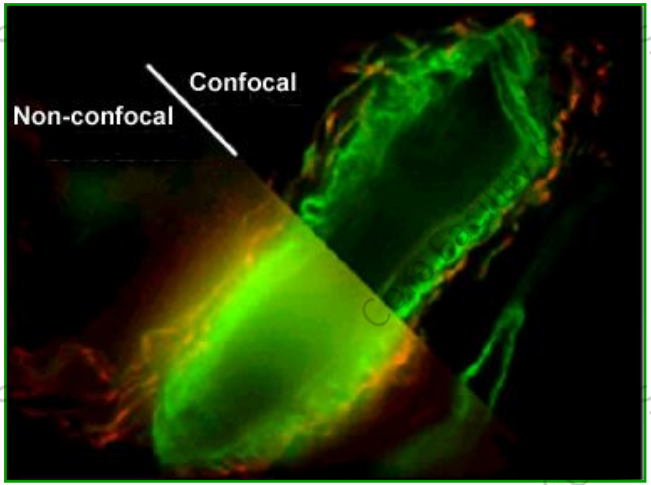


Pinhole: nice z-axis resolution

Scanner + detector: images with point scanning

Smart photon manipulation: **ZOOM** and **FRAP**

Spectral imaging: autofluorecense and spectral unmixing.

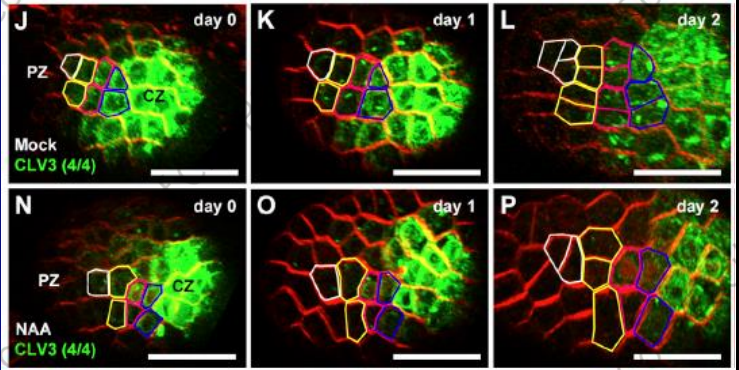


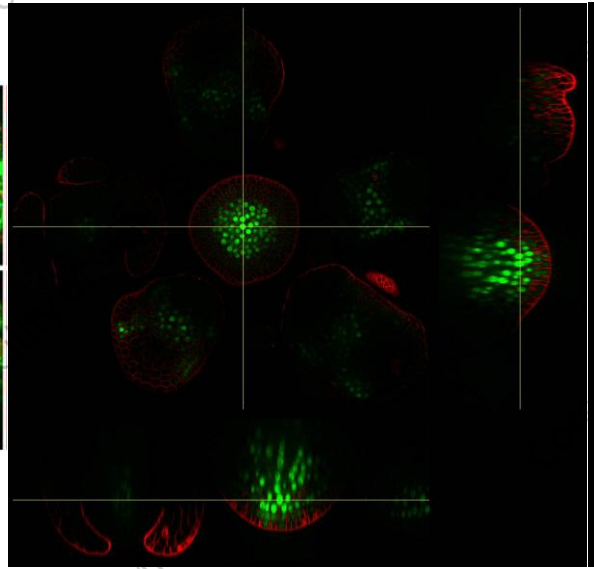
2D imaging of the interested focus

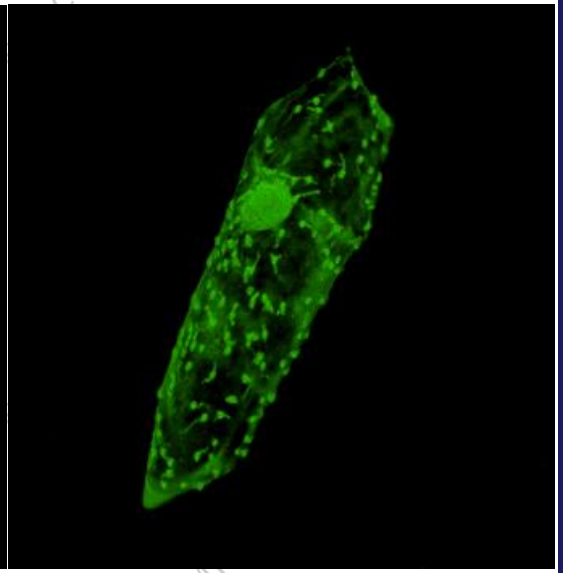
3D reconstruction: z-stacks and reconstruction

Attention:
no overexposure
no crosstalk

Auxin negatively regulate SAM size



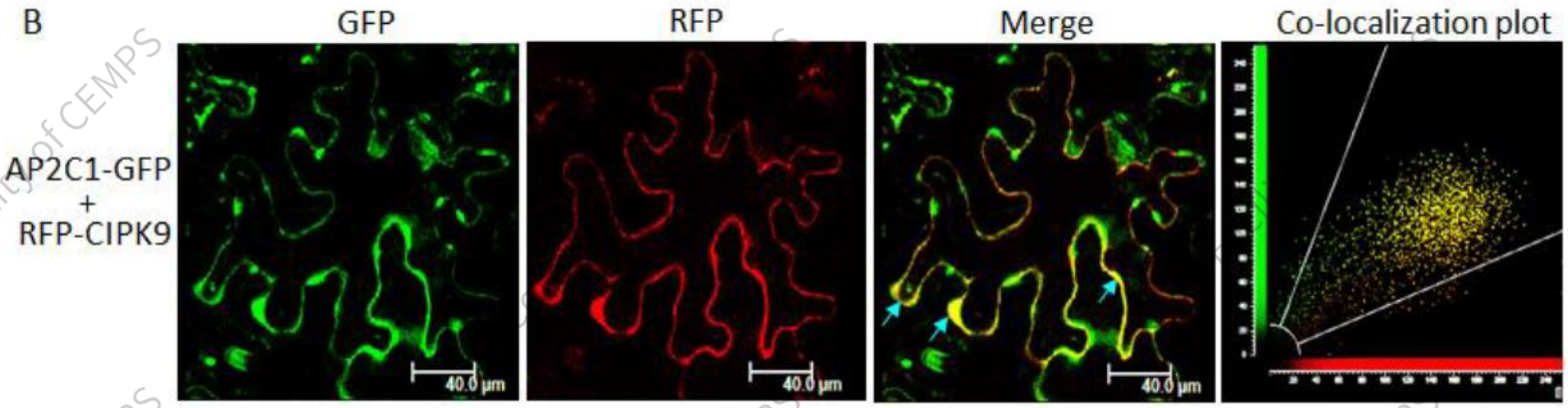


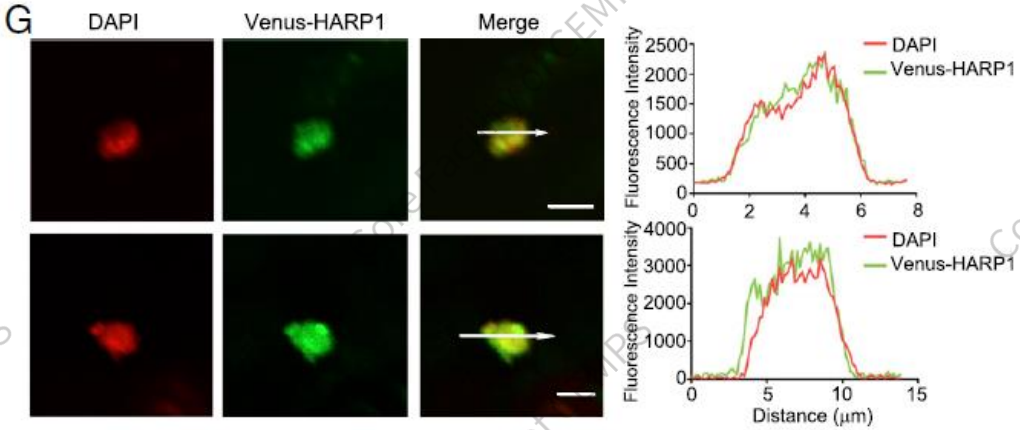


Shi BH et al., Developmental Cell. 2018

Colocalization analysis

Pearson's value/Manderson's value/Line profile



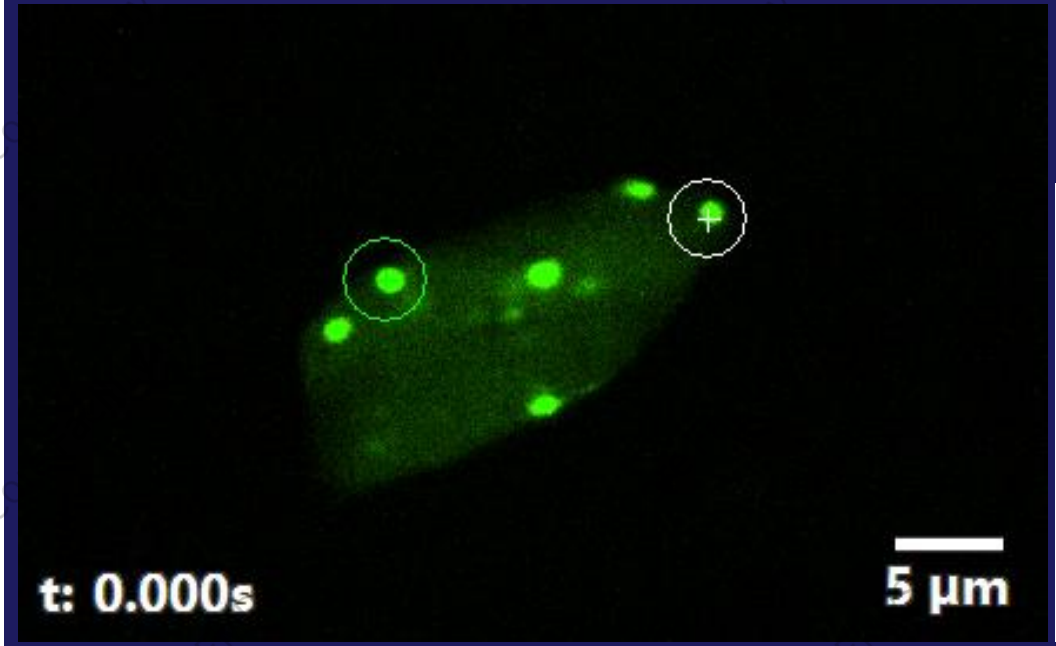


With the right images,
it is easy to quantify the
fluoresence signals.

Singh A et al., J Exp Bot. 2018
Chen CY et al., PNAS. 2019

FRAP: Fluorescence Recovery After Bleaching

to study the molecular kinetics



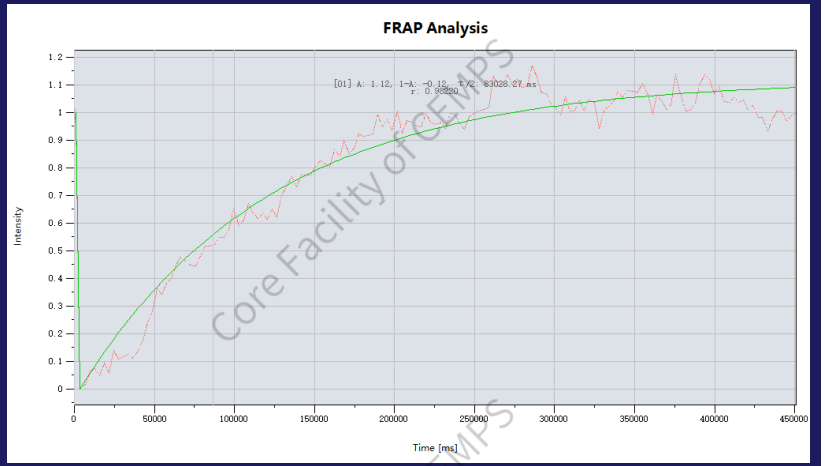
Sample: nucleus of Arabidopsis root cells

5 cycles—bleach with 488 laser—150 cycles with 3s interval.

Total duration time: 7min30s

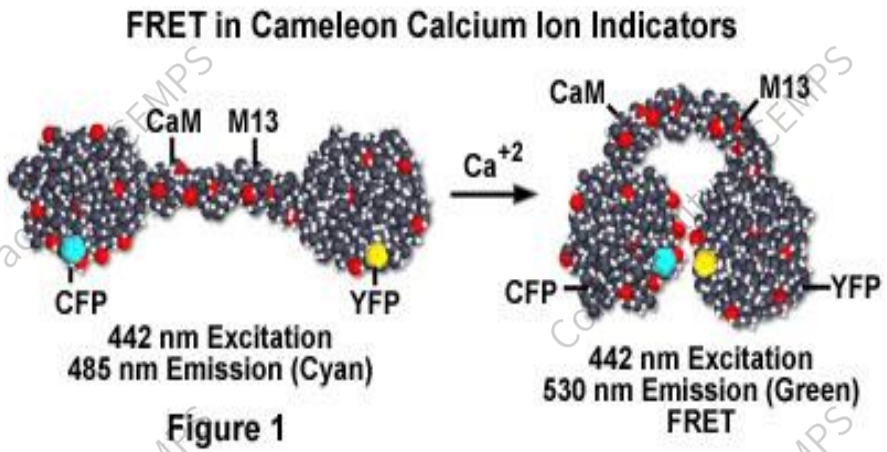
White ROI: bleaching area,

Green ROI: no-bleaching area as a control.



FRET: Förster Resonance Energy Transfer

- ✓ 分子内结构变化(蛋白质、DNA构象等);
- ✓ 分子间相互作用(酶活力、离子通道结构变化等);
- ✓ 离子浓度测量(通过构建FRET蛋白，测量Ca^{2+}浓度, PH值, cAMP等);
- ✓ 通过FRET效率(E_{FRET})可计算供体和受体之间的距离(r)。



Cameleon YC3.6— Ca^{2+} indicator

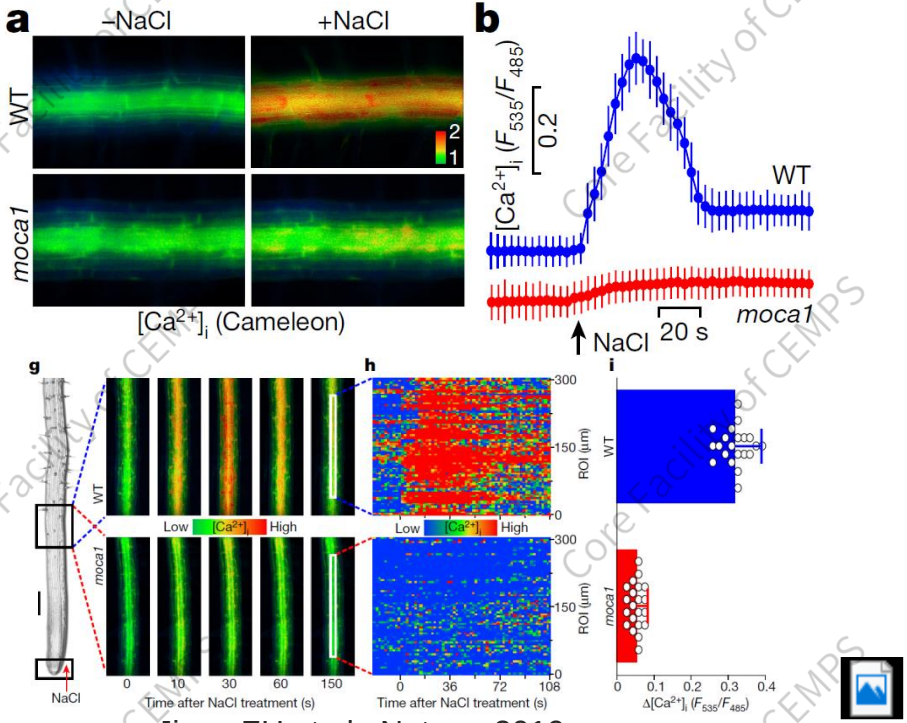
- CFP
- CaM
- M13
- YFP

When CaM combined with Ca^{2+}，the protein complex will fold and make CFP close enough to YFP. Then the FRET occur and YFP signal will be brighter.

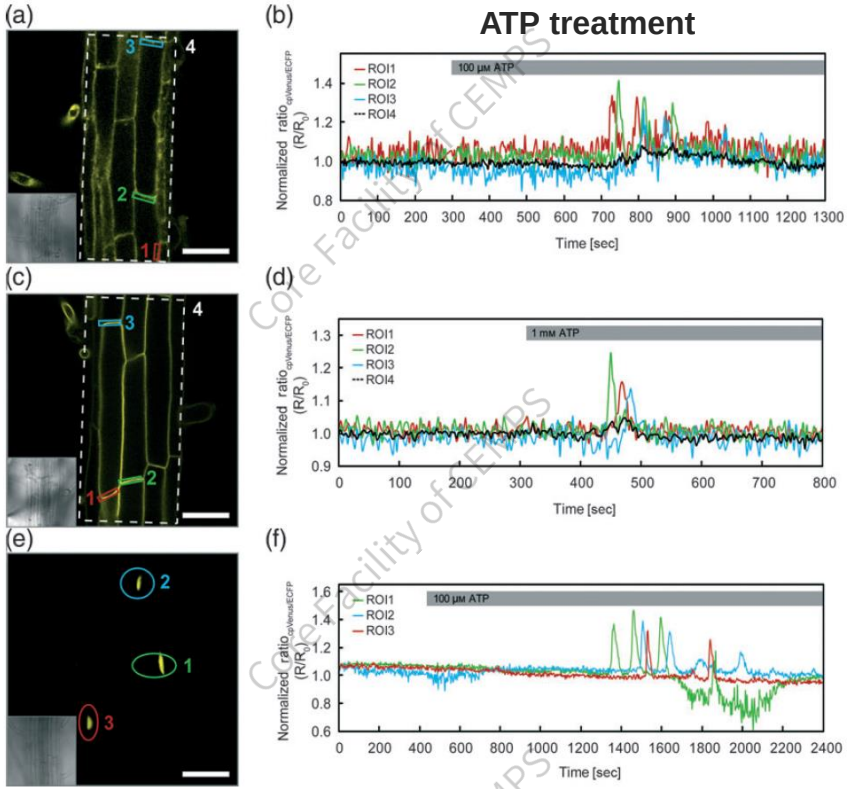
Application note of YC3.6

High temporal and spatial resolution of calcium dynamics with CLSM

NaCl-induced increases in [Ca2+]i were lower in *moca1* roots



Jiang ZH et al., Nature, 2019

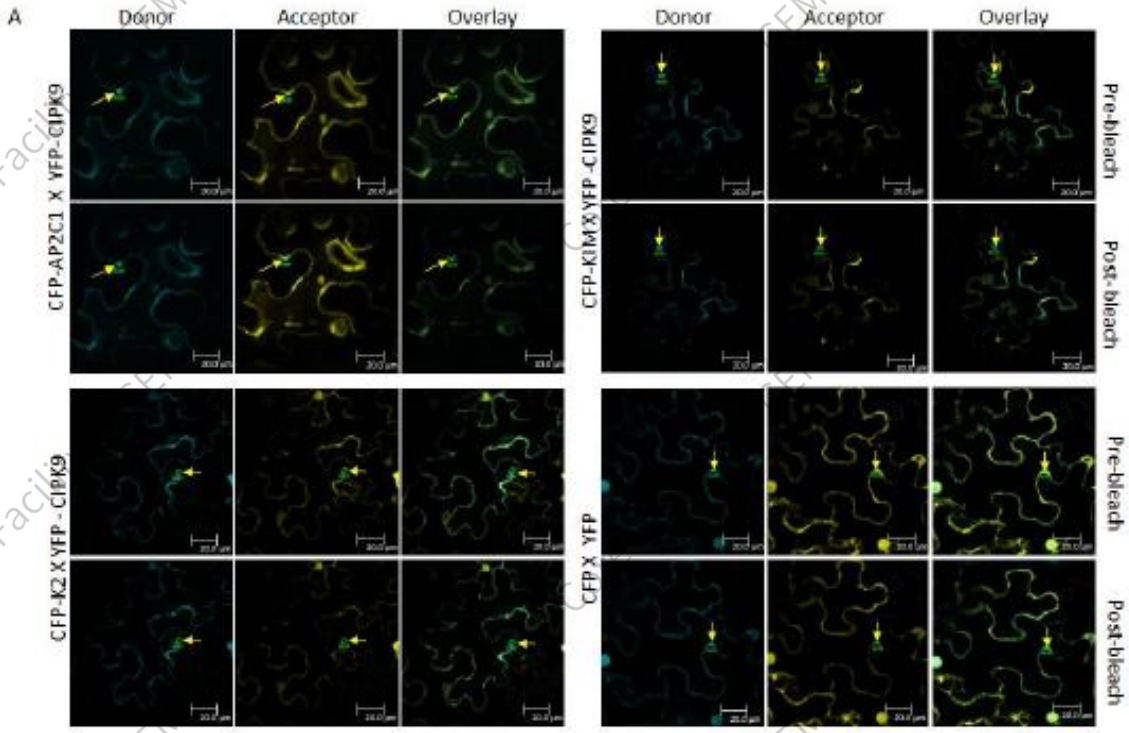


tpj_4780_sm_movies3 plant journal2012.mp4

Krebs M et al., The Plant Journal, 2012

Application note of protein interaction with FRET

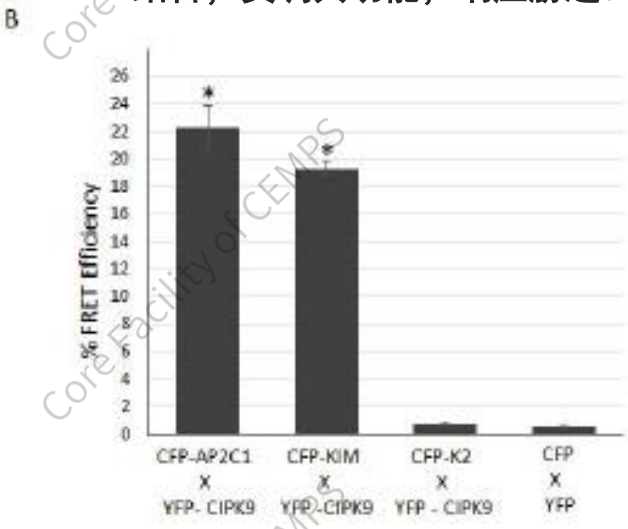
Interaction of CIPK9 and AP2C1 by FRET analysis



Singh A et al., J Exp Bot. 2018

acceptor bleaching: easy to use
sensitized emission: most strict method
ratio method: easy but tough to repeat

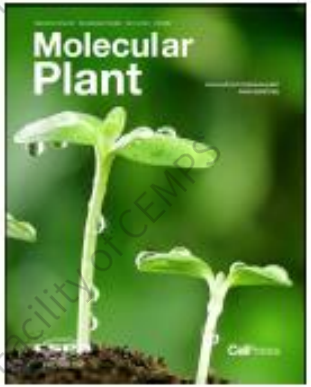
低钾胁迫条件下，磷酸酶AP2C1可与CIPK9结合，负调其功能，响应胁迫。

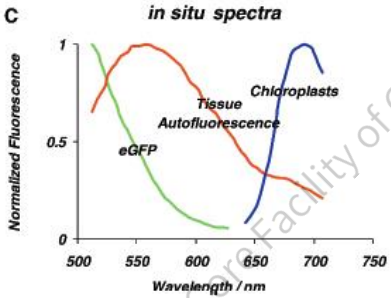


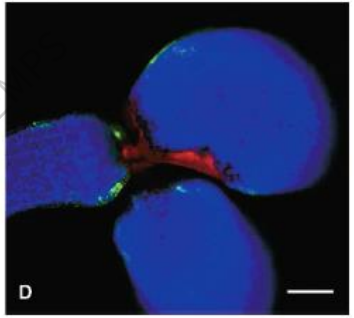
Spectrum: autofluorescence and spectral unmixing

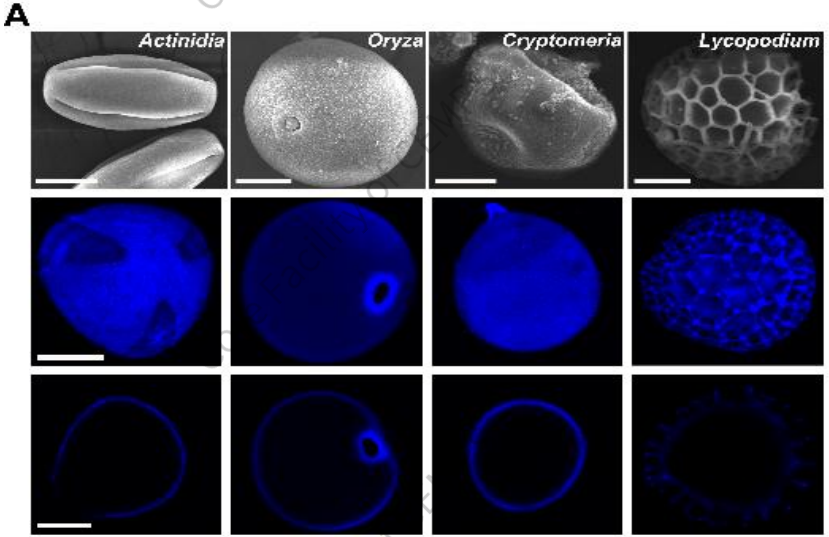
Phenylpropanoid Derivatives Are Essential Components of Sporopollenin in Vascular Plants

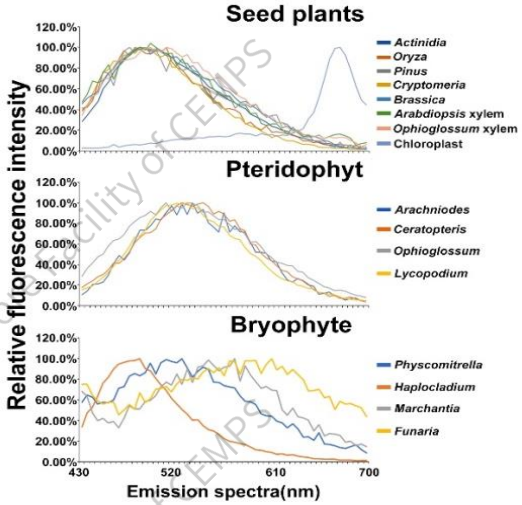
Jing-Shi Xue, Baocai Zhang, HuaDong Zhan, Yong-Lin Lv, Xin-Lei Jia, TianHua Wang, Nai-Ying Yang, Yu-Xia Lou, Zai-Bao Zhang, Wen-Jing Hu, Jinshan Gui, Jianguo Cao, Ping Xu, Yihua Zhou, Jin-feng Hu, Laigeng Li, Zhong-Nan Yang











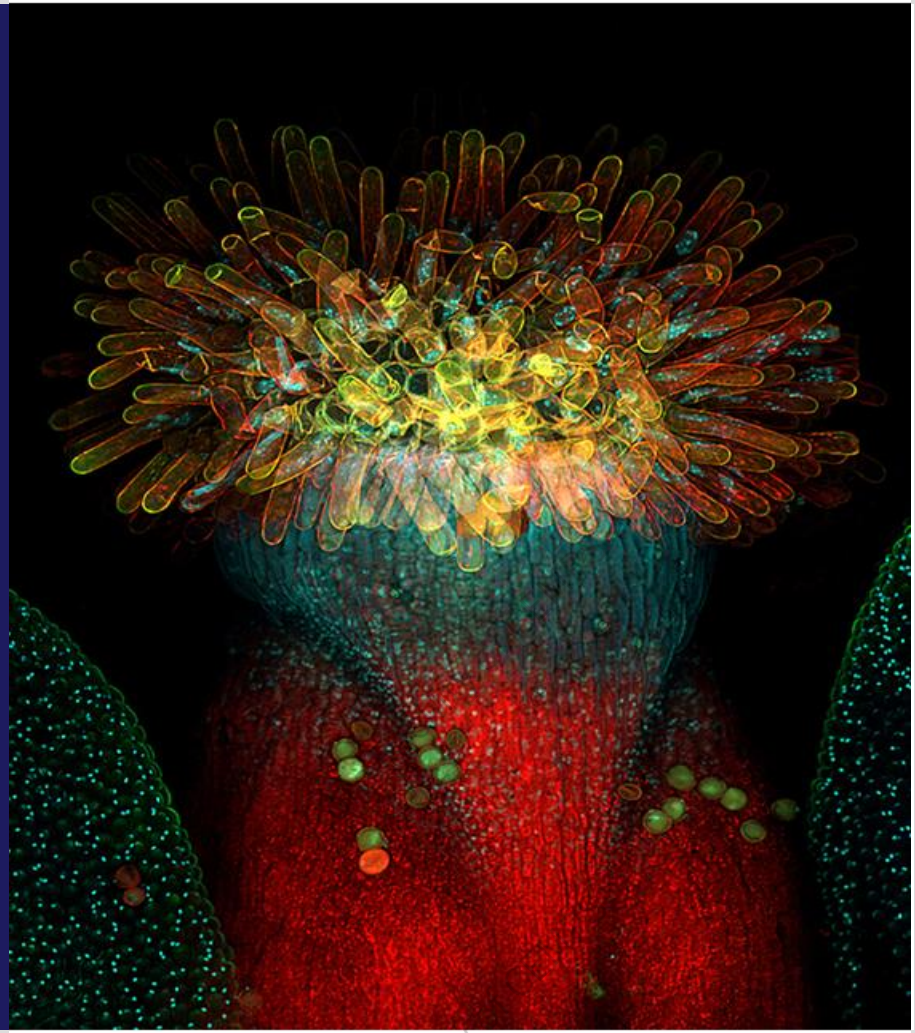
TIRF system
– cell surface/membrane

Only focus on the signals at cell surface

Highest z-axis resolution

Sensitive: nice for weak signal

Typical application: Autophagy/vesicle tracking/membrane kinetics…



TIRF application: single-molecule imaging

Single-molecule imaging shows ZAR1 oligomerization at cell surface

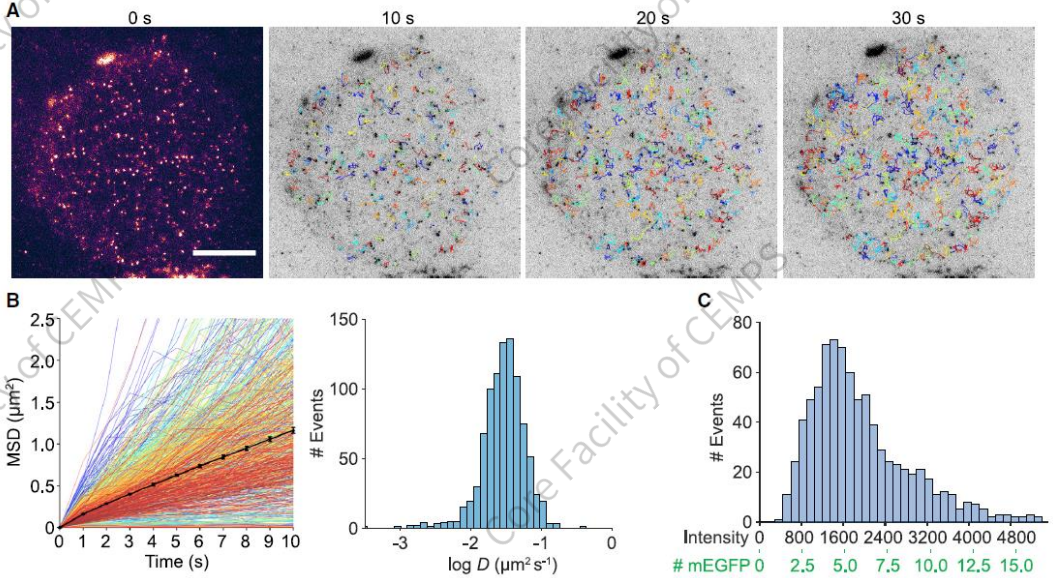


Figure 4. Single-molecule imaging shows ZAR1 oligomerization at cell surface

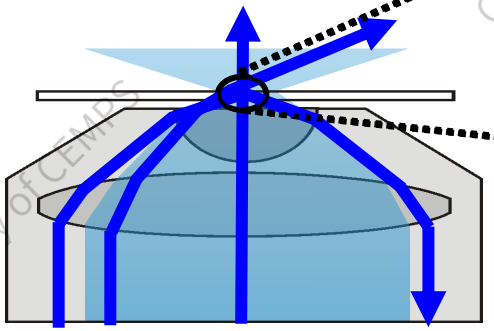
(A) Live-cell TIRF imaging of the bottom surface of protoplasts co-expressing ZAR1-mEGFP

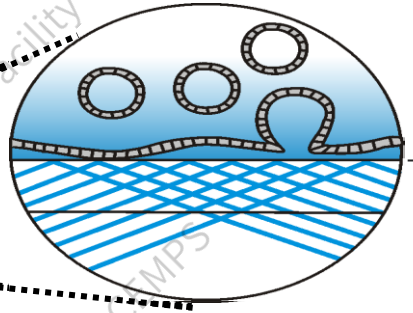
Keywords:
monomeric EGFP
TIRF imaging
Spots tracking
MSD analysis

周lab视频

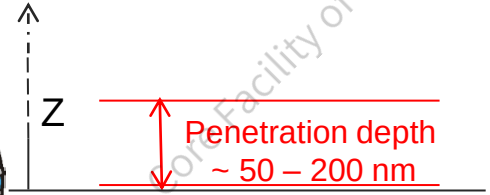
Bi GZ et al., Cell, 2021

What is TIRF? Why only cell surface were imaged?





intensity decays exponentially in Z direction



TIRF

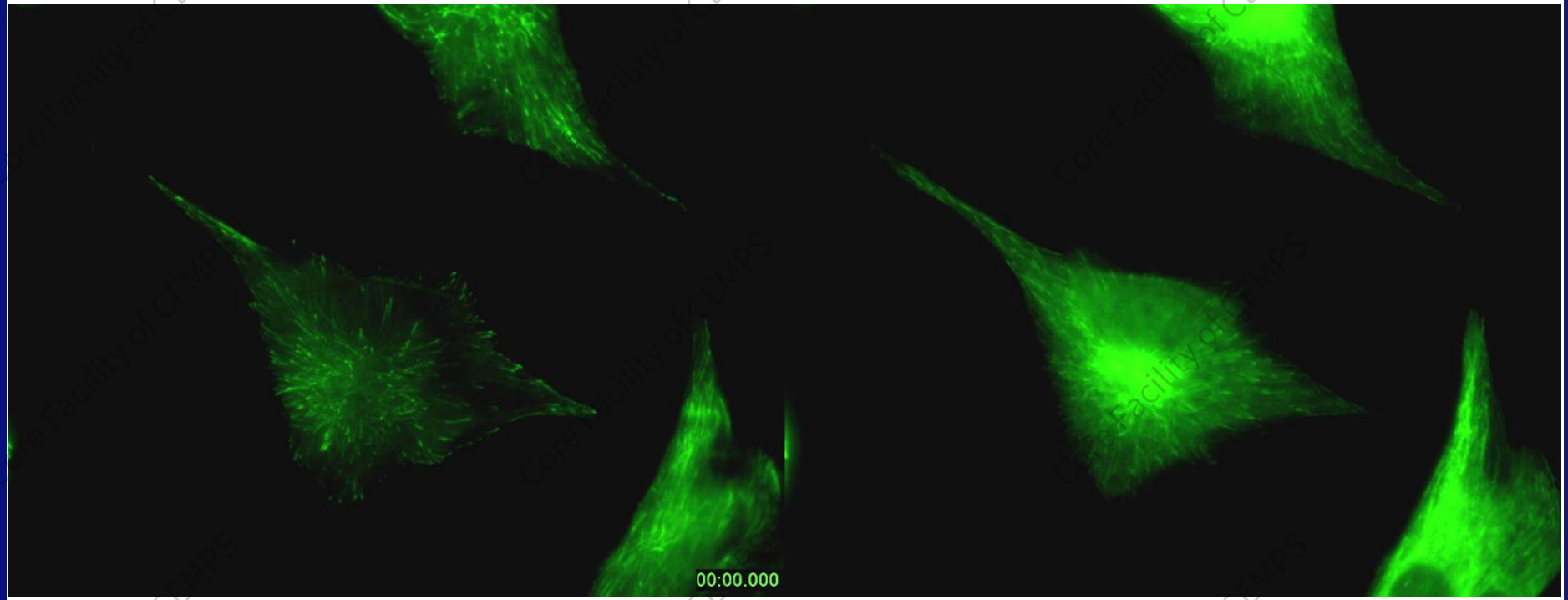
$$n_1 \sin \theta_1 = n_2 \sin \theta_2$$

$$d_P = \frac{\lambda}{4\pi \sqrt{n_1^2 \sin^2(\theta_1) - n_2^2}}$$

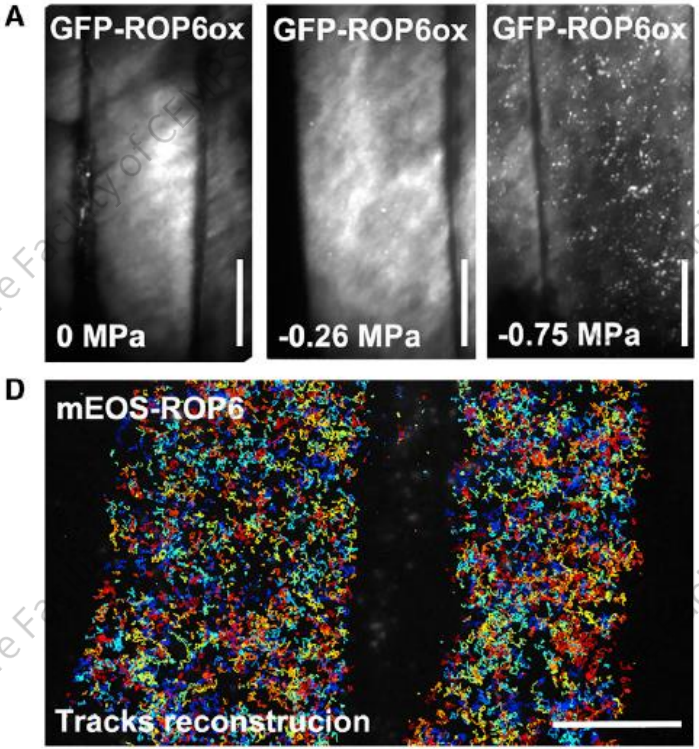
Figure 4. Single-molecule imaging shows ZAR1 oligomerization at cell surface

(A) Live-cell TIRF imaging of the bottom surface of protoplasts co-expressing ZAR1-mEGFP

TIRF vs Widefield

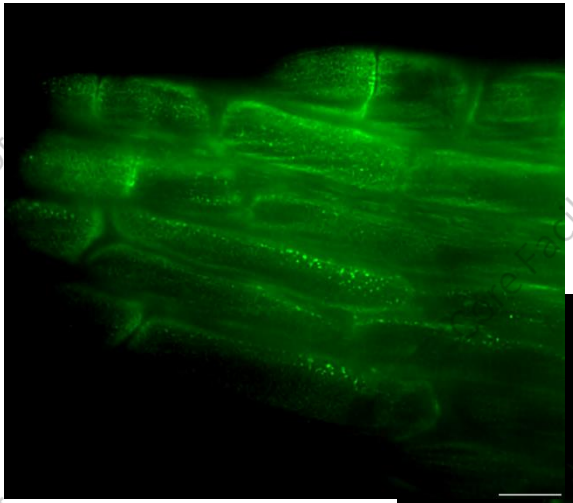


TIRF application in plant science: vesicles/spots at the cell surface



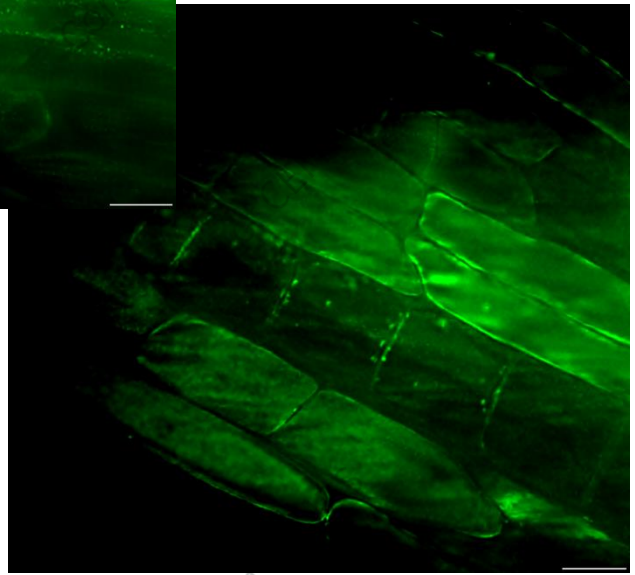
GFP-ROP6 appeared in diffraction-limited spots at the cell surface after osmotic treatment

Smokvarska M et al., Current Biology, 2020



488 TIRF imaging

Arabidopsis root cell surface



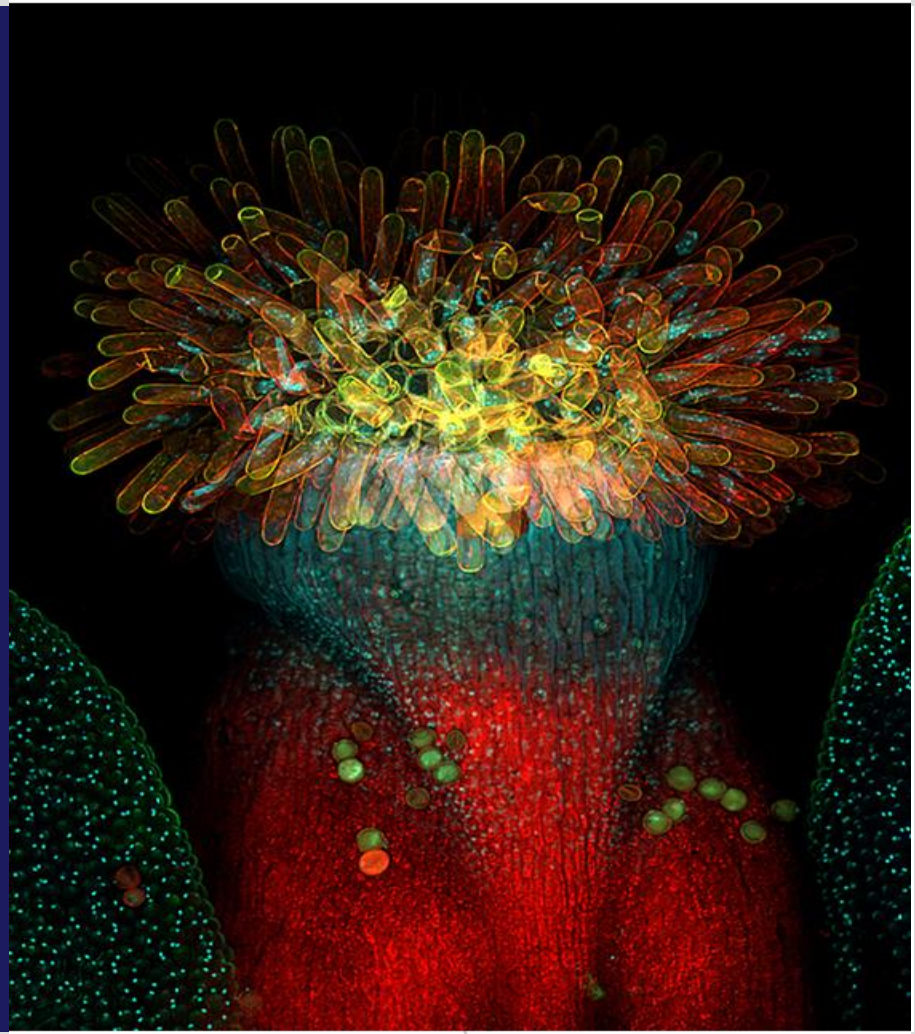
Spinning disk confocal – fast confocal

Low photon-toxicity: long-term imaging

Fast imaging speed: ms

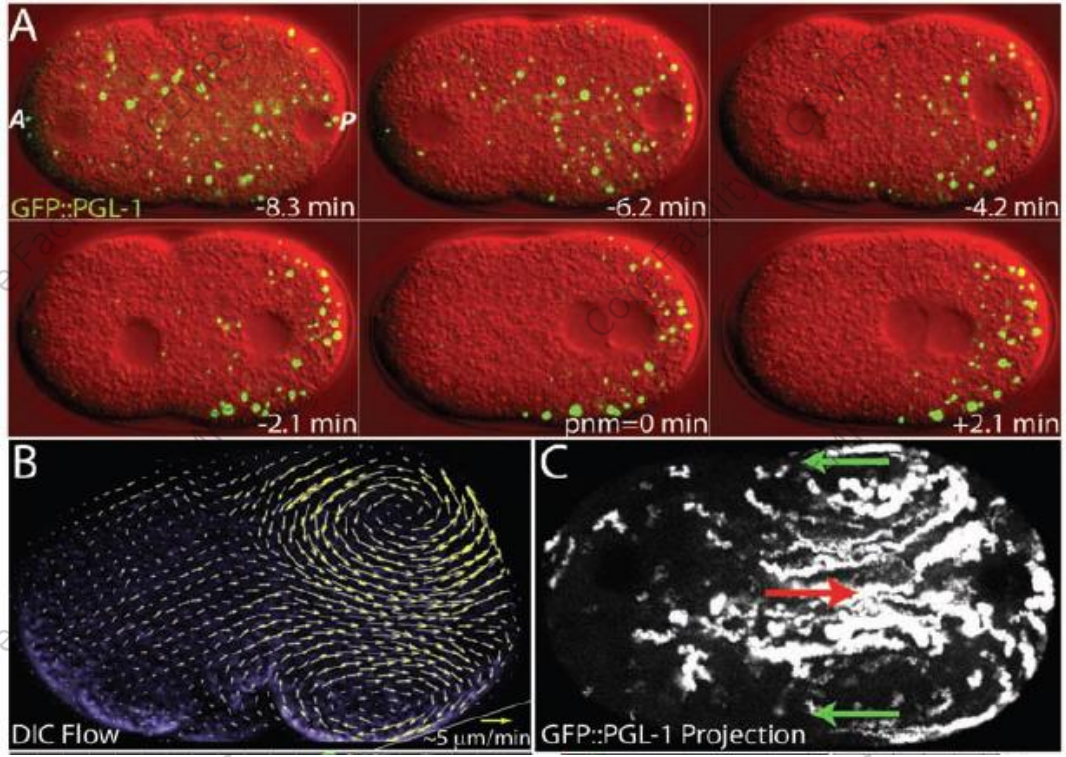
Sensitive: weak signal detection

LLPS/autophagy/calcium spikes etc…



Application：LLPS-related research

Spinning disk confocal data



1172046s3.mov

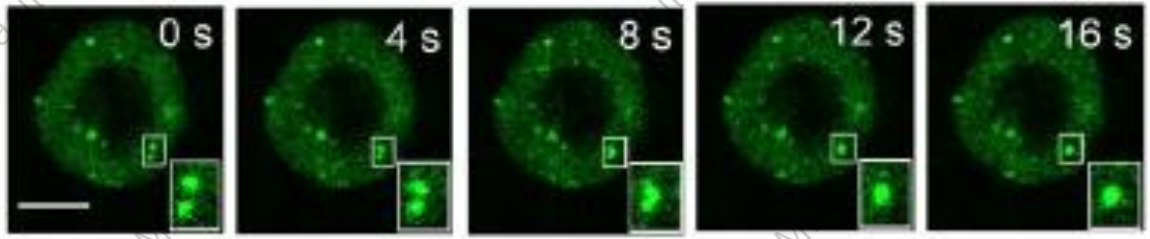
aax9554s2.mov

Clifford P. Brangwynne and Anthony A. Hyman reported LLPS (Liquid-Liquid Phase Separation) for the first time in 2009.

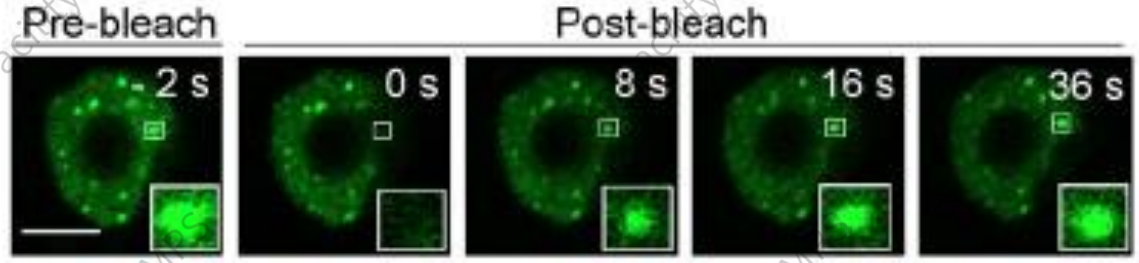
Application in plant science

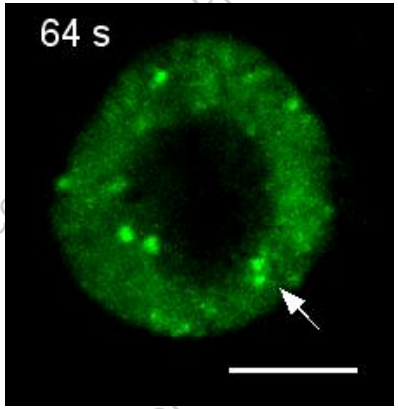
EMB1579 control transcription and pre-mRNA splicing through phase seperation

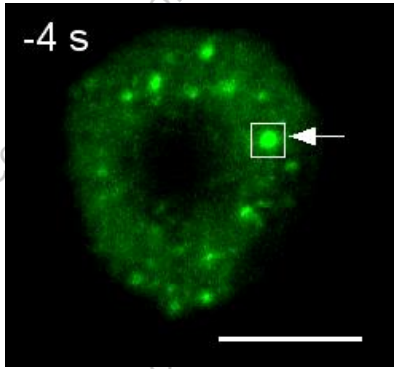
LLPS in vivo



FRAP in vivo

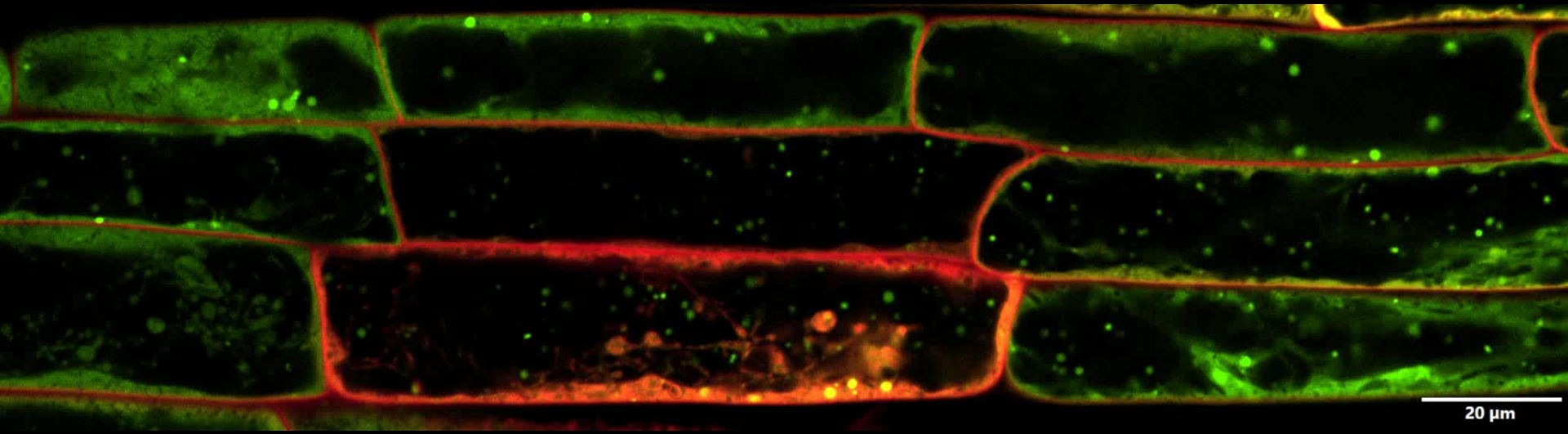






Zhang Y et al., PLOS BIOLOGY, 2020

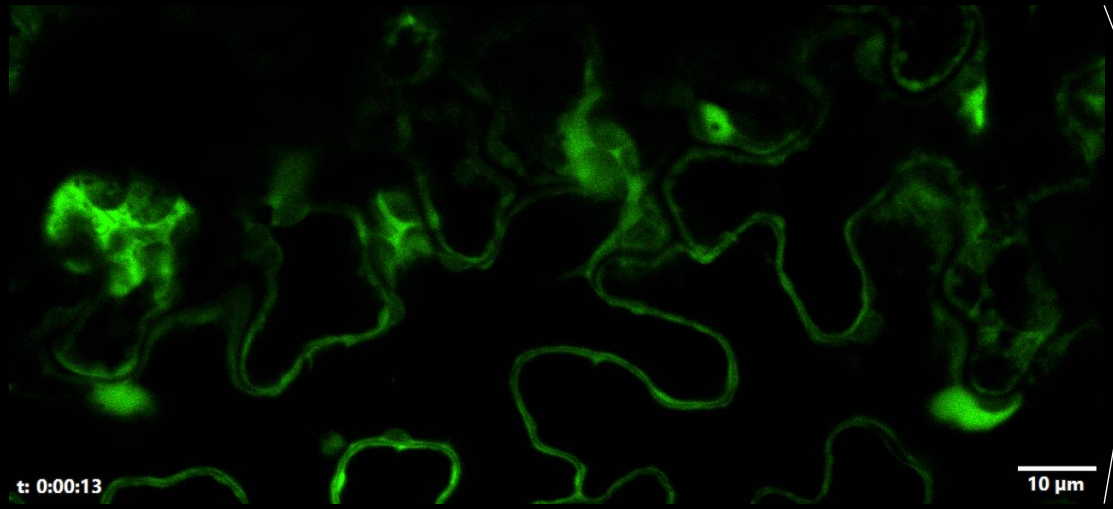
Autophagy vesicles tracking in arabidopsis root cells

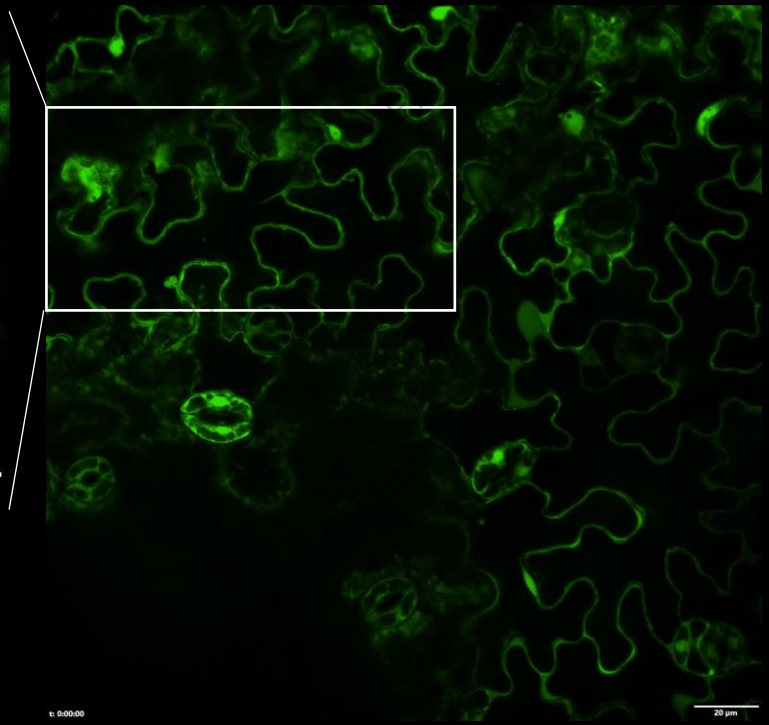


1 fps for 90s with spinning disk confocal

Green: autophagy marker
Red: FM4-64

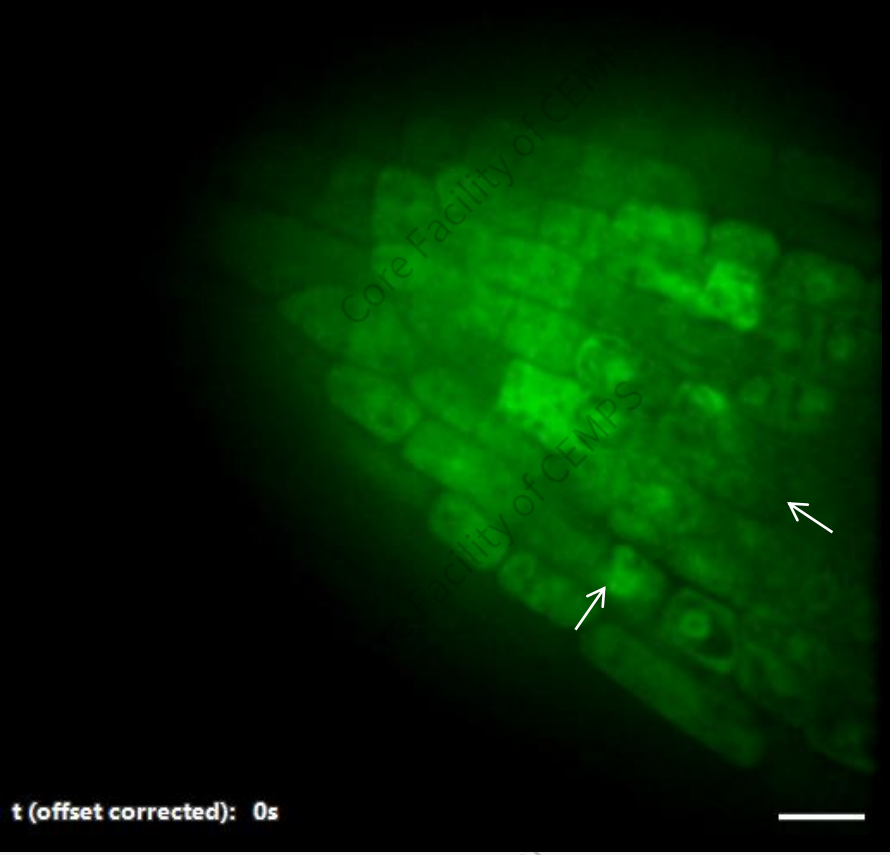
Vesicles tracking in arabidopsis leaf cells





1 fps for 90s with spinning disk confocal
Green: autophagy marker

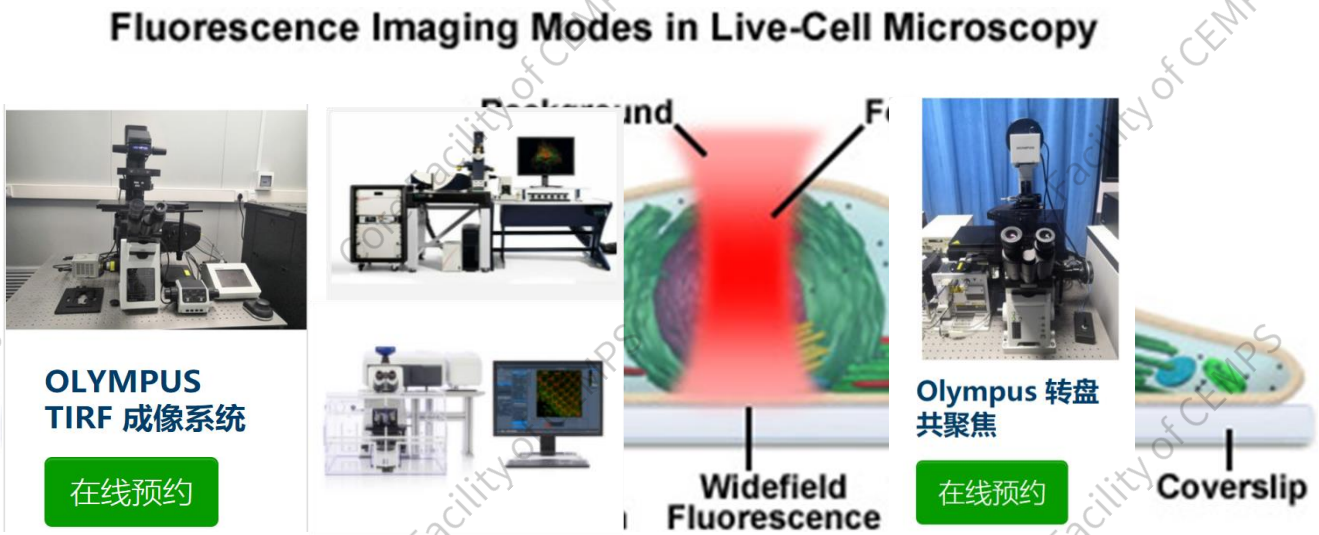
Calcium spiking in arabidopsis root cells



0.5 fps for 310s with spinning disk confocal

Green: Calcium marker

Different micro-imaging systems: z-resolution



全内反射显微镜　激光扫描共聚焦/双光子显微镜　宽场显微镜　转盘共聚焦

Summary

- Laser scanning confocal: classical technique and fit for most samples, most smart system.
  Pinhole: optical sectioning, nice for 3D structure
  Scanning: nice for ZOOM, FRAP and FRET
  Spectrum: nice for multi-color, FRET, spectral scanning and unmixing

- TIRF system: very thin excitation region, highest z-resulution
  only excite the cell surface/membrane signal, clear particle/vesicle imaging

- Spinning disk confocal: fast, low photon-toxicity
  Fast imaging: calcium spiking/autophagy/LLPS
  EMCCD: nice for very weak signal
  Low photon-toxicity

Other important techniques

Two-photon system: deep and live

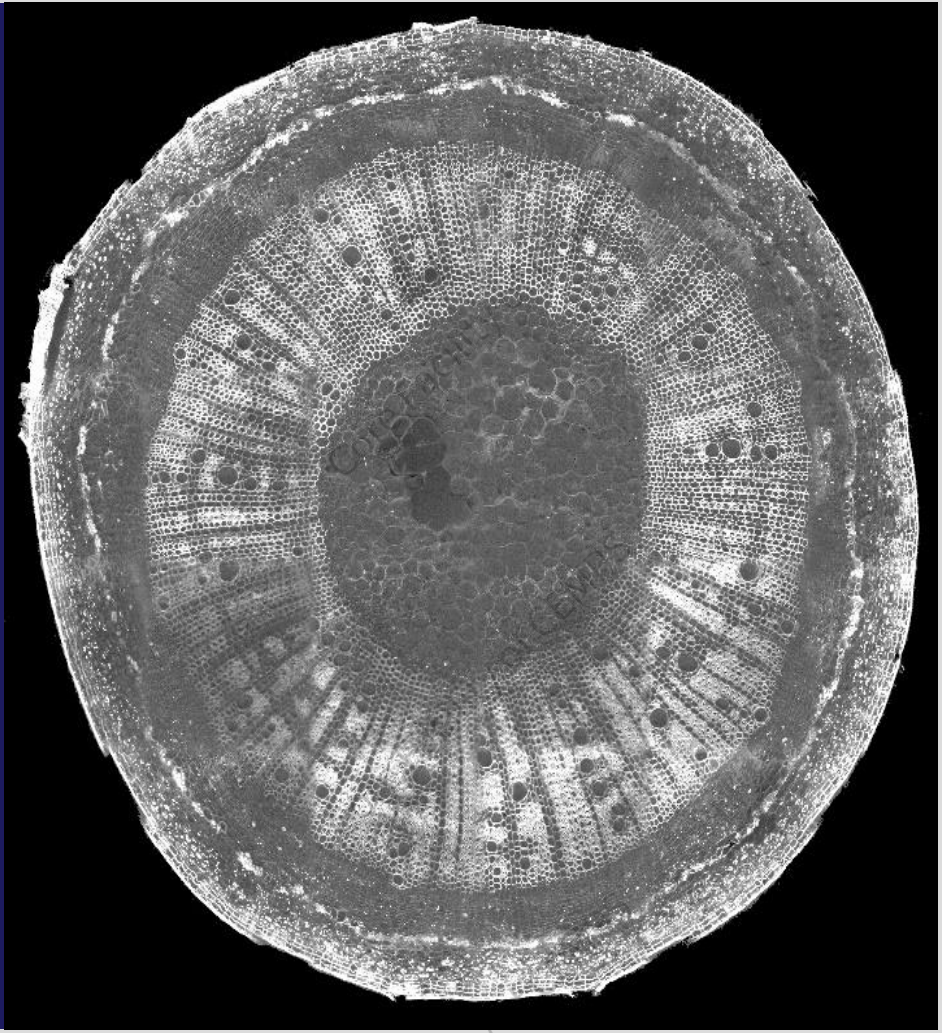
Lightsheet: deep

FLIM imaging: another dimension

Super resolution: nice xy resolution

SRS imaging: without label

…



Hardware:
3 sets of Laser Scanning Confocal：Leica SP8/Zeiss LSM880/Olympus FV1000
1 set of Spinning disk Confocal: Andor spinning disk
1 set of TIRF system: Olympus dual-line TIRF
3 sets of super-resolution systems: Zeiss LSM880 Airyscan /GE OMX/ Leica SP8 STED

1 set of Leica Stellaris5 (Confocal combine with lightsheet)

Software for image processing and analysis:
iMaris: convenient for 3D image data
FIJI: strong tool for 2D images and usual 3D data processing, free

Thanks for Your Time and Attention!

分子植物中心
公共技术服务中心

Welcome for questions through wechat/email/phone…!