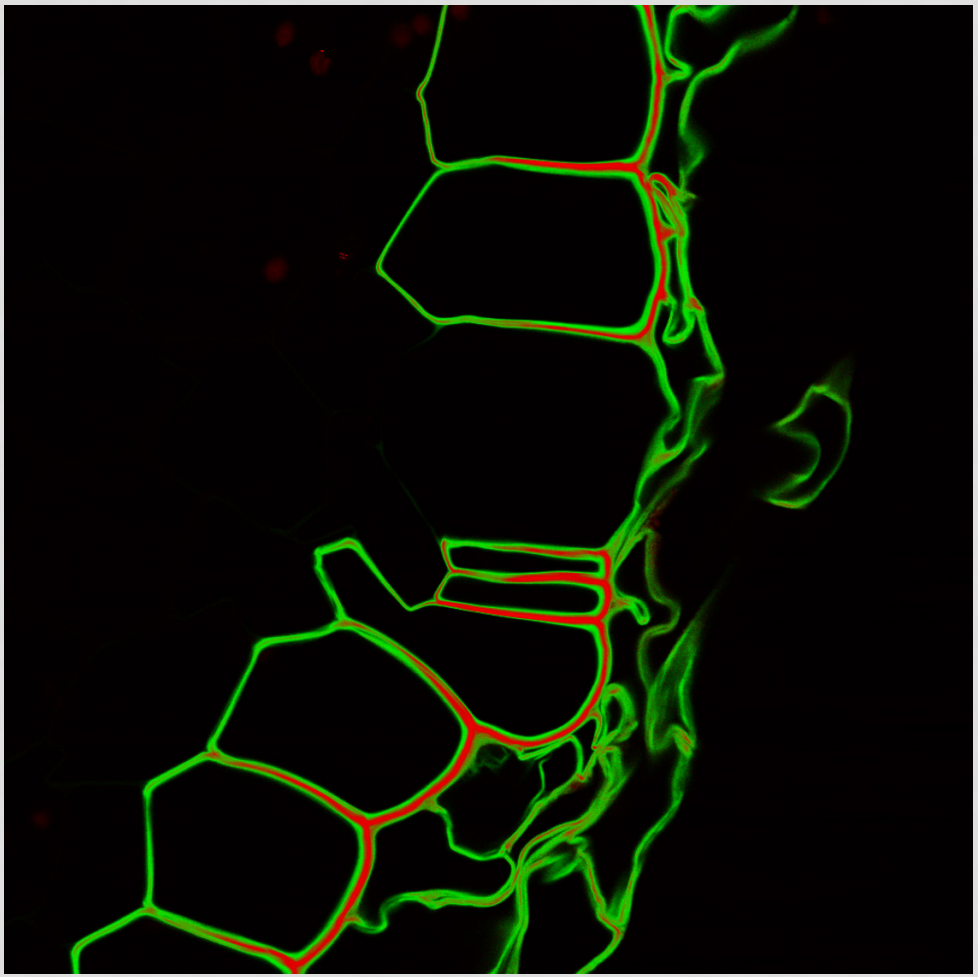
# VISUALISATION 3D

MICROSCOPIE À FEUILLE DE LUMIÈRE ET AUTRES TECHNOLOGIES
ETAT DE L'ART

Journées Scientifiques et Techniques du RµI Toulouse 25-27 novembre 2015

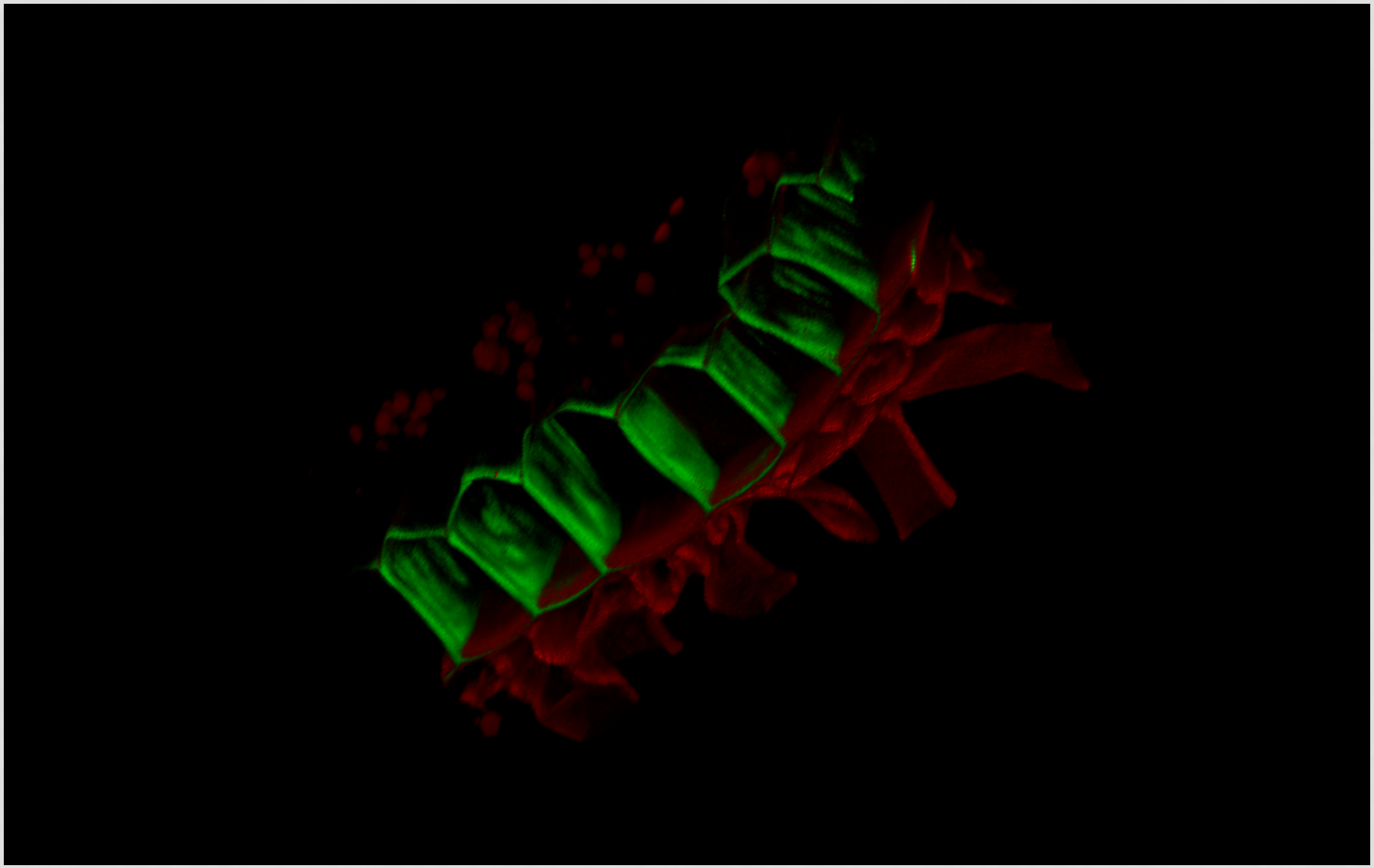
Geneviève Conéjéro

# COUPE OPTIQUE, MULTIPHOTON



Coupe transversale racine vanille, image spectrale, visualisation de 2 composés pariétaux en fausses couleurs, S Koyyappurath, Cirad

# DE LA 2D À LA 3D: UNE PLUS-VALUE



Coupe transversale racine vanille, image spectrale, S Koyyappurath, Cirad

# VISUALISATION 3D EN MICROSCOPIE DE FLUORESCENCE
## POURQUOI, COMMENT?

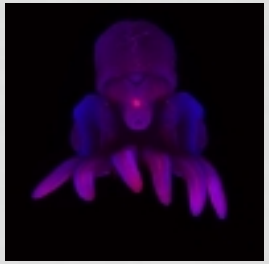
- **Méthodes en microscopie de fluorescence**
- Epifluorescence → Déconvolution
- Lumière structurée
- Microscope Confocal
- Microscope Multiphoton
- Microscope à feuille de lumière: Light-Sheet
- Serial Block Face Imaging

# LIGHT SHEET: MÉTHODE DE L'ANNÉE 2014 (NATURE METHODS)

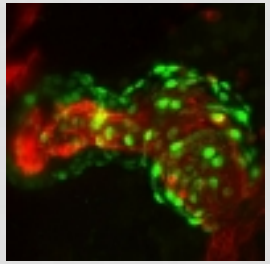
- technologie unique pour imager des organismes *in vivo* sur de longues périodes, en 3D/4D, sans photo-dommages, avec une bonne résolution XYZ

**Guide to light-sheet microscopy for adventurous biologists**
**EG Reynaud et al, Nature methods, janvier 2015**

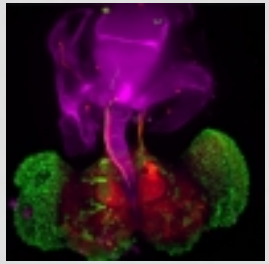
*« Ten years of development in light-sheet microscopy have led to spectacular demonstrations of its capabilities. The technology is reday to assist biologists in tackling scientific problems, but are biologists ready to it? »*



E Edsinger et D S Rokhsar
Okinawa



M Weber, J Huisken,
Dresden



A A Bohra, K V Raghavan
Bangalore

http://www.zeiss.fr/microscopy/fr_fr/produits/systemes-d-imagerie/lightsheet-z-1.html

# LIGHT-SHEET FLUORESCENCE MICROSCOPY

- Conception: groupe **Ernst Stelzer** (EMBL, 2004)
- Présentation de la microscopie Light Sheet par E Stelzer:
- https://www.youtube.com/watch?v=IteywF6wKu8
- http://www.physikalischebiologie.de/downloads

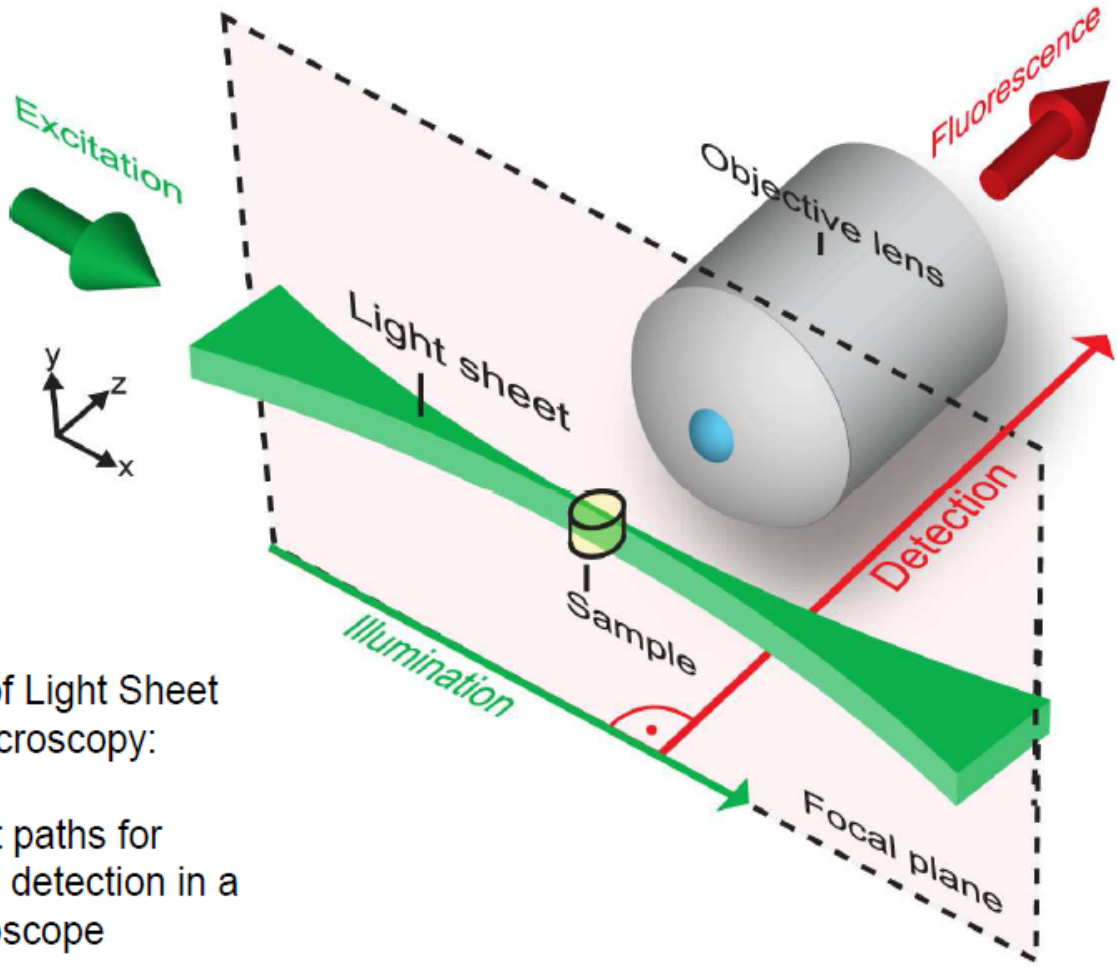
- Photobleaching limité, faible phototoxicité, haute résolution en 3D, time-lapse (jusqu'à plusieurs jours), vitesse d'acquisition élevée.
- Basée sur une technologie centenaire pour imager les colloïdes
- Renaissance de la technologie depuis une dizaine d'années: nécessaire collaboration entre domaines technologiques
- physique, instrumentation, biologie, informatique

- Cellules et embryons peuvent supporter jusqu'à qlqs $\mu J/\mu m^2$
- Au-delà, viabilité affectée.
- Peu de microscopes peuvent imager à un aussi faible niveau d'énergie, mais c'est le cas du light-sheet.

**Principe de la microscopie à feuille de lumière**

-La feuille de lumière est obtenue grâce à une lentille cylindrique ou un balayage laser

- La détection est ORTHOGONALE à l'illumination



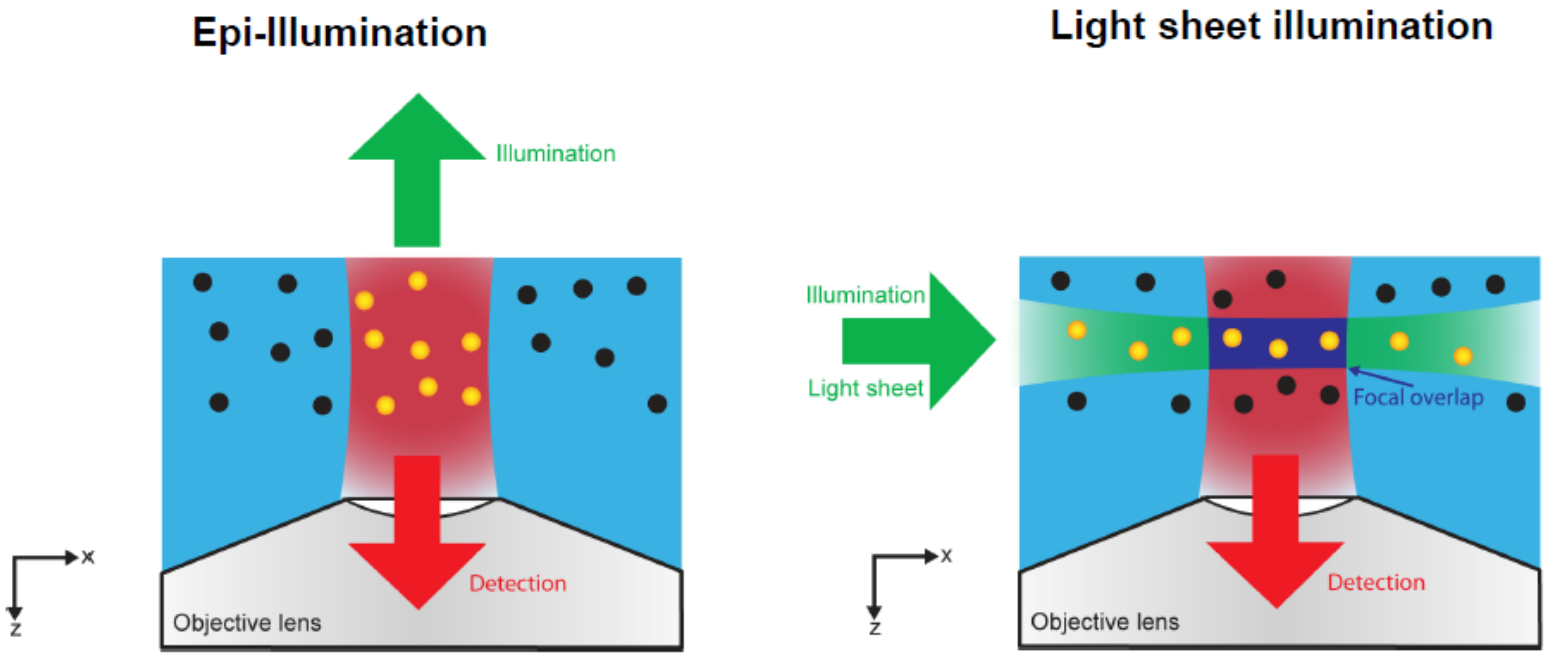

The **Principle** of Light Sheet Fluoresence Microscopy:

Orthogonal light paths for Illumination and detection in a horizontal microscope

Figure from the PhD thesis of Jörg Ritter (2011), University of Bonn, Germany)

# Light Sheet: SPIM

## Selective plane illumination microscopy




- Inherent optical sectioning capability of the illumination method
- No excitation of out-of-focus fluorescence

Figure from the PhD thesis of Jörg Ritter (2011), University of Bonn, Germany)

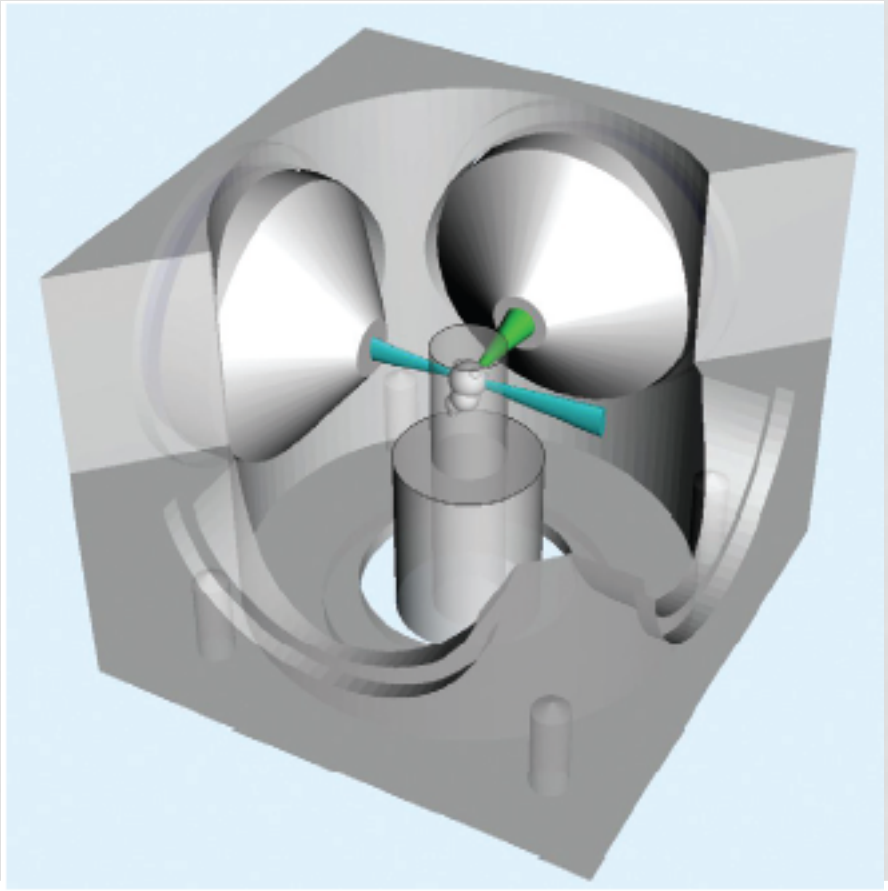
# ILLUMINATION/DÉTECTION

- LASER VISIBLES: 488, 561, 633 nm etc…
- LASER PULSE 2 PHOTONS infra-rouge

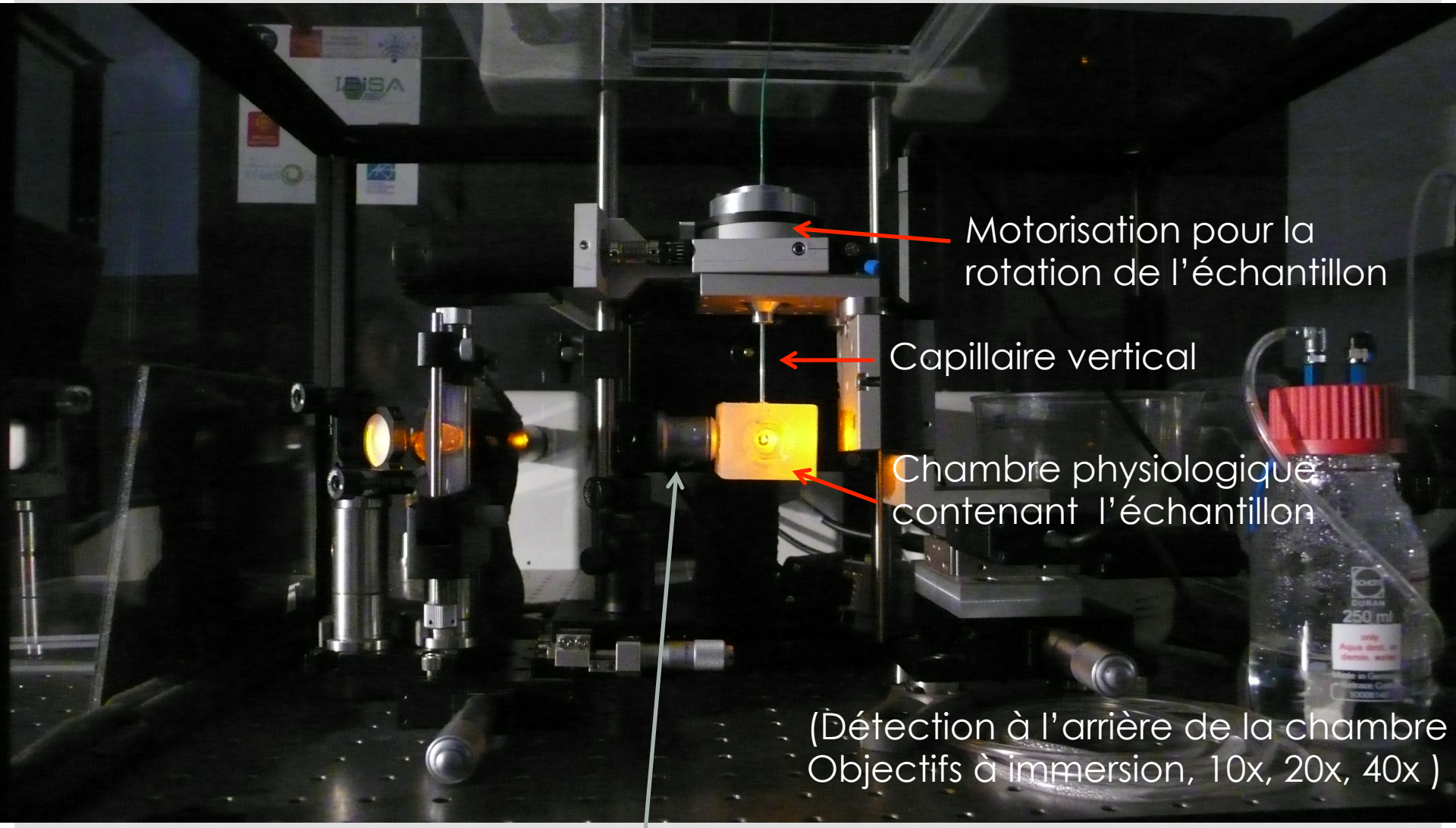
- Rotation échantillon
- motorisée
- → Single view, dual view
- ……Multiview

- Cameras CCD ou sCMOS
- 10 à 100 images/s

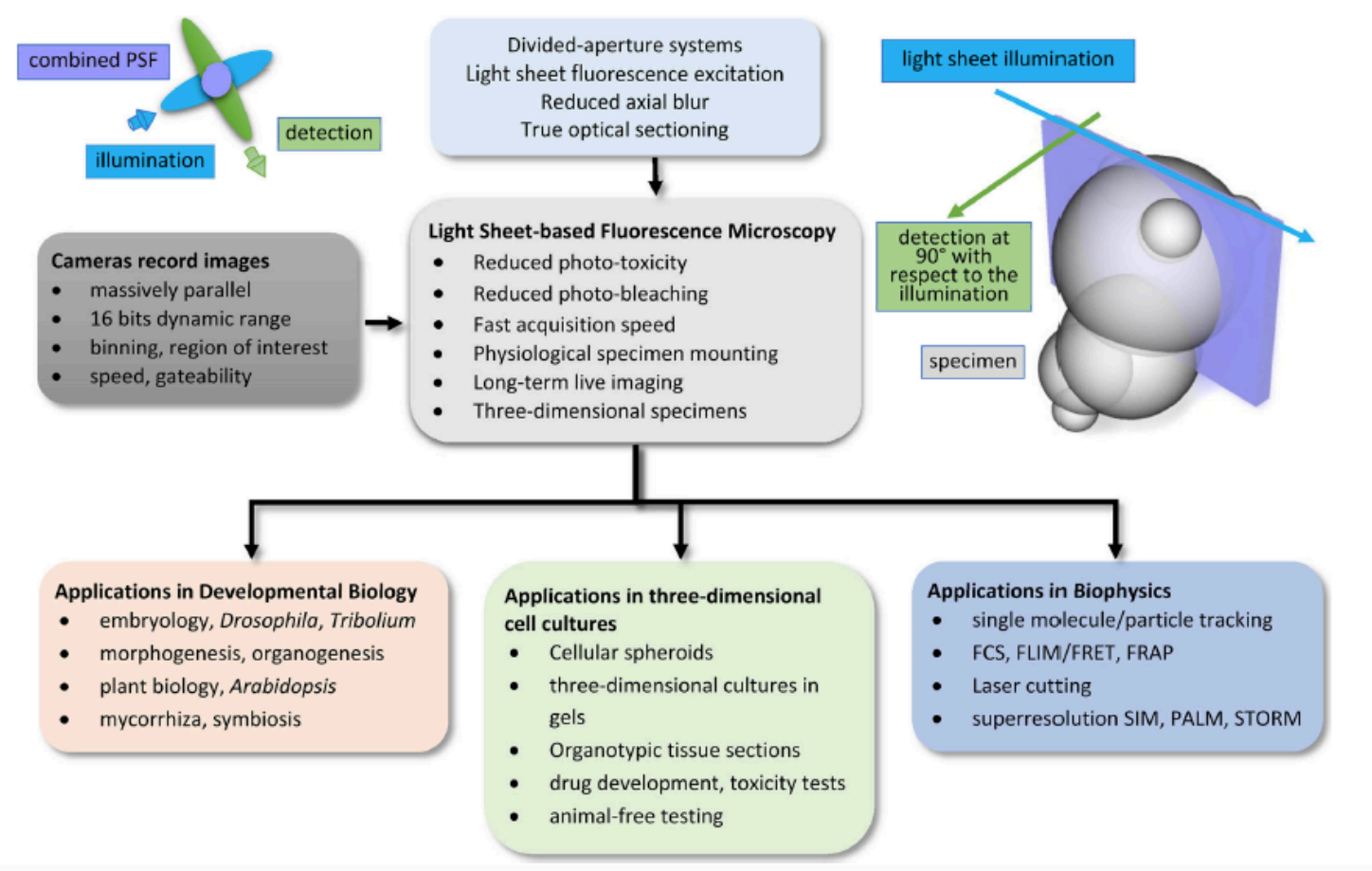
- Plusieurs couleurs



SPIM plateau d'imagerie ITAV, Toulouse



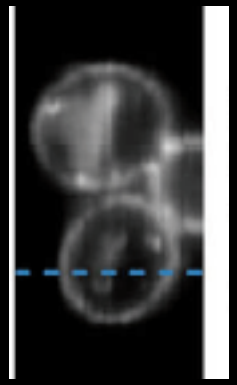
# CHAMPS D'APPLICATIONS DU LIGHT SHEET




Pampaloni, Chang & Stelzer, Cell Tissue Res, 2015

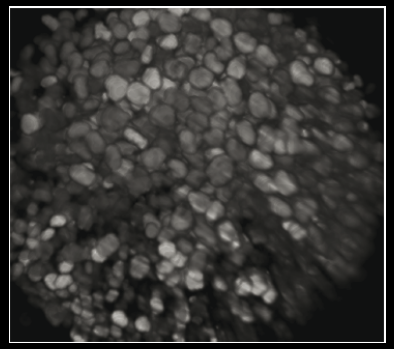
# Champs d'applications: quelques exmples

## Molecular & cellular biology



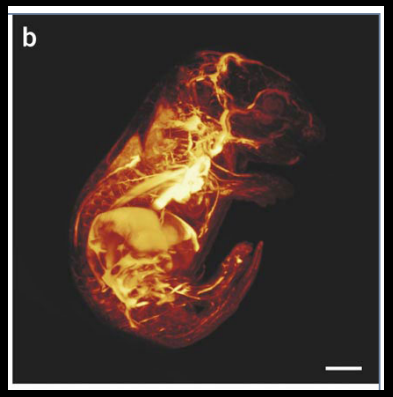
Capoulade & *al.*, 2011

## Cancer biology



Lorenzo & *al.*, 2011
Spheroid

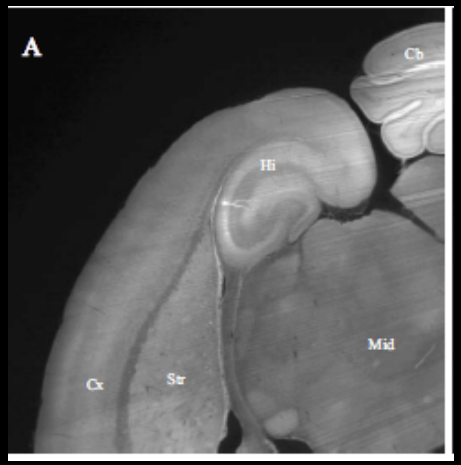
## Neuroscience

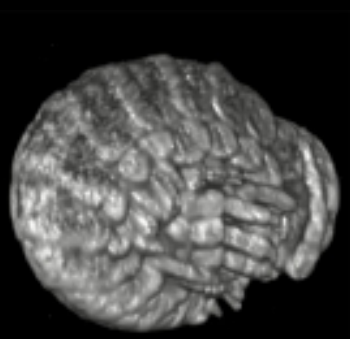



Dodt & *al.*, 2007

## Anatomical studies

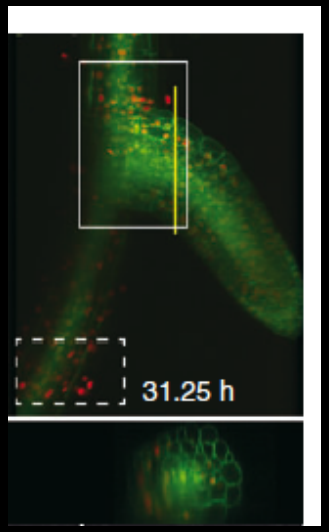
Mertz & al,2012,
mouse brain



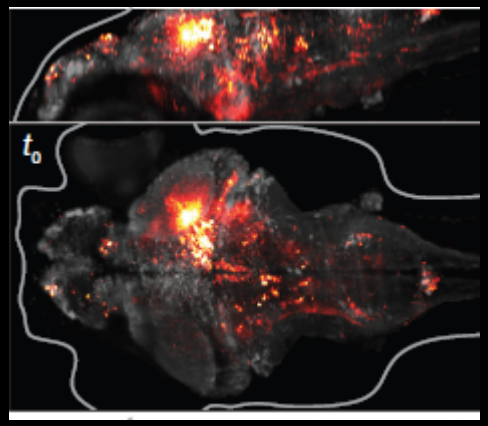
Marine amphipod
Parhyale hawaiensis
Zeiss website

## Plant biology

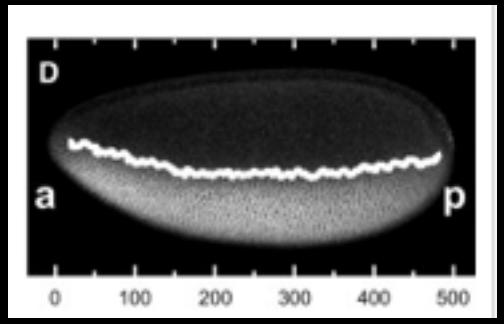



Maizel & *al.*, 2011

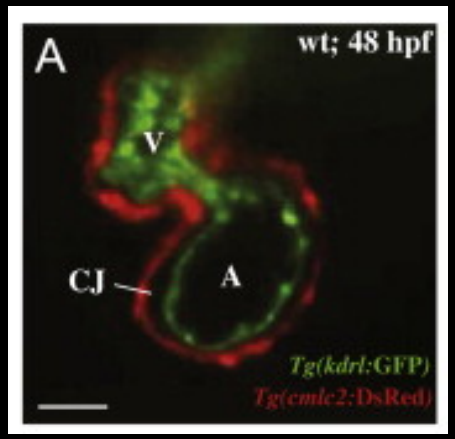
## Whole-brain functional imaging




## Developmental biology




Reeves & *al.*, 2012




Mellman & *al.*, 2012

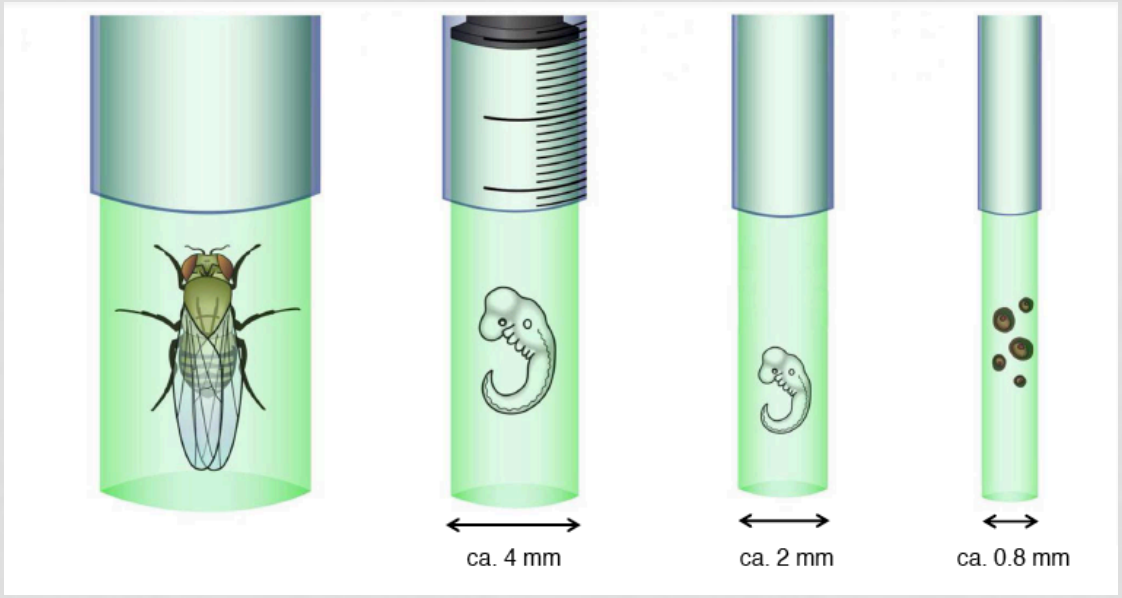
# PRÉPARATION DES ECHANTILLONS

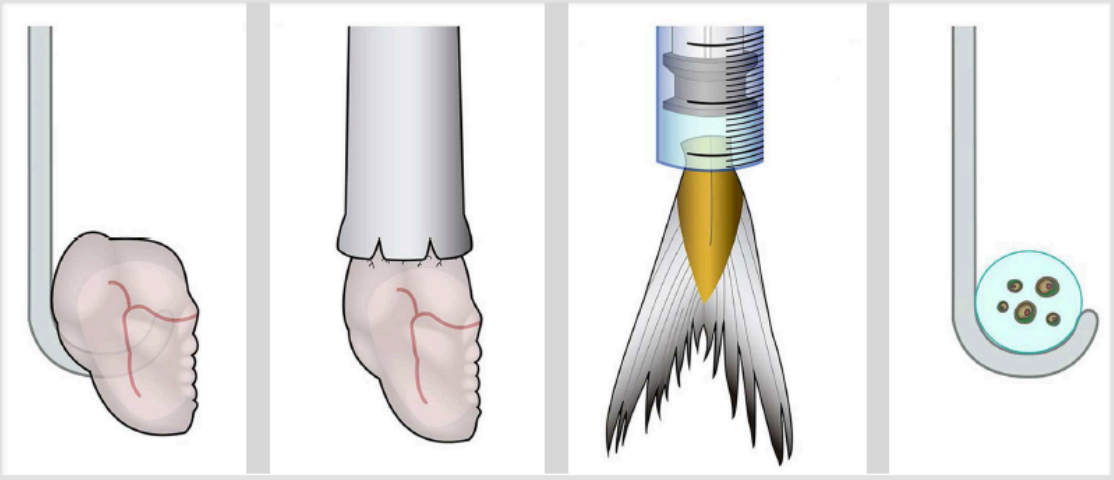
- Echantillon idéal pour la microscopie
- = FIN, PLAT, TRANSPARENT….

- En microscopie LightSheet, la préparation des échantillons est en soi un projet de recherche:
- **Publication de nouvelles techniques de montage des échantillons (chambre, capillaire, milieu de montage)**

- **Exemple: 5 protocoles pour le montage des échantillons**
- **« embedded, clipped, enclosed, flat and flow-through »**
- **http://www.zeiss.fr/microscopy/fr_fr/produits/systemes-d-imagerie/lightsheet-z-1.html#telechargements**

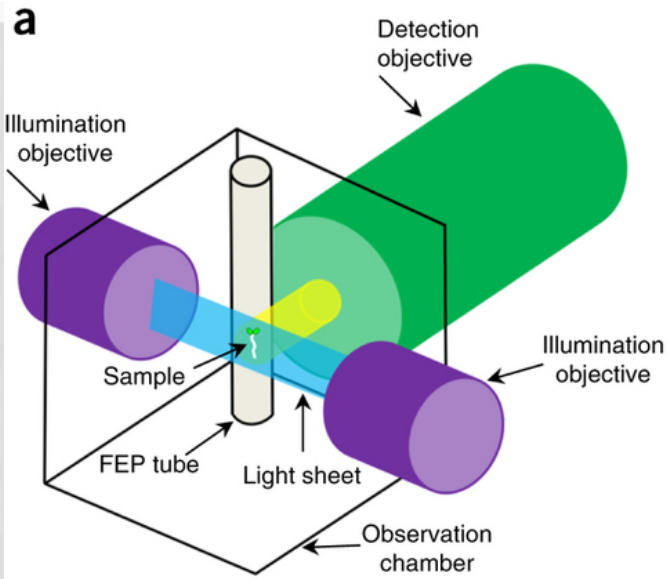
# QUELQUES EXEMPLES DE PRÉPARATION



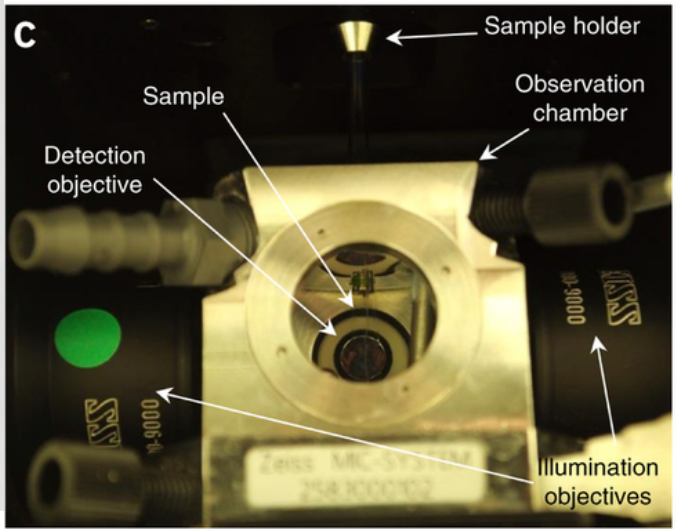



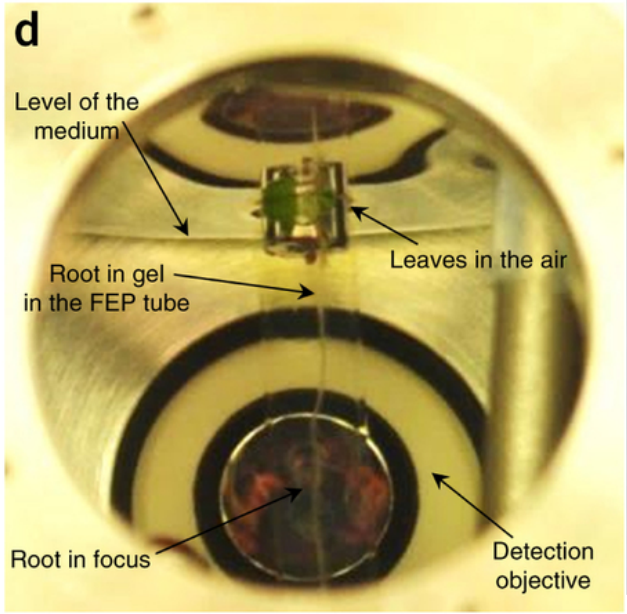
# EXEMPLE PRÉPARATION PLANTE

- Ovecka et al.,
- 2015
- Nature Methods

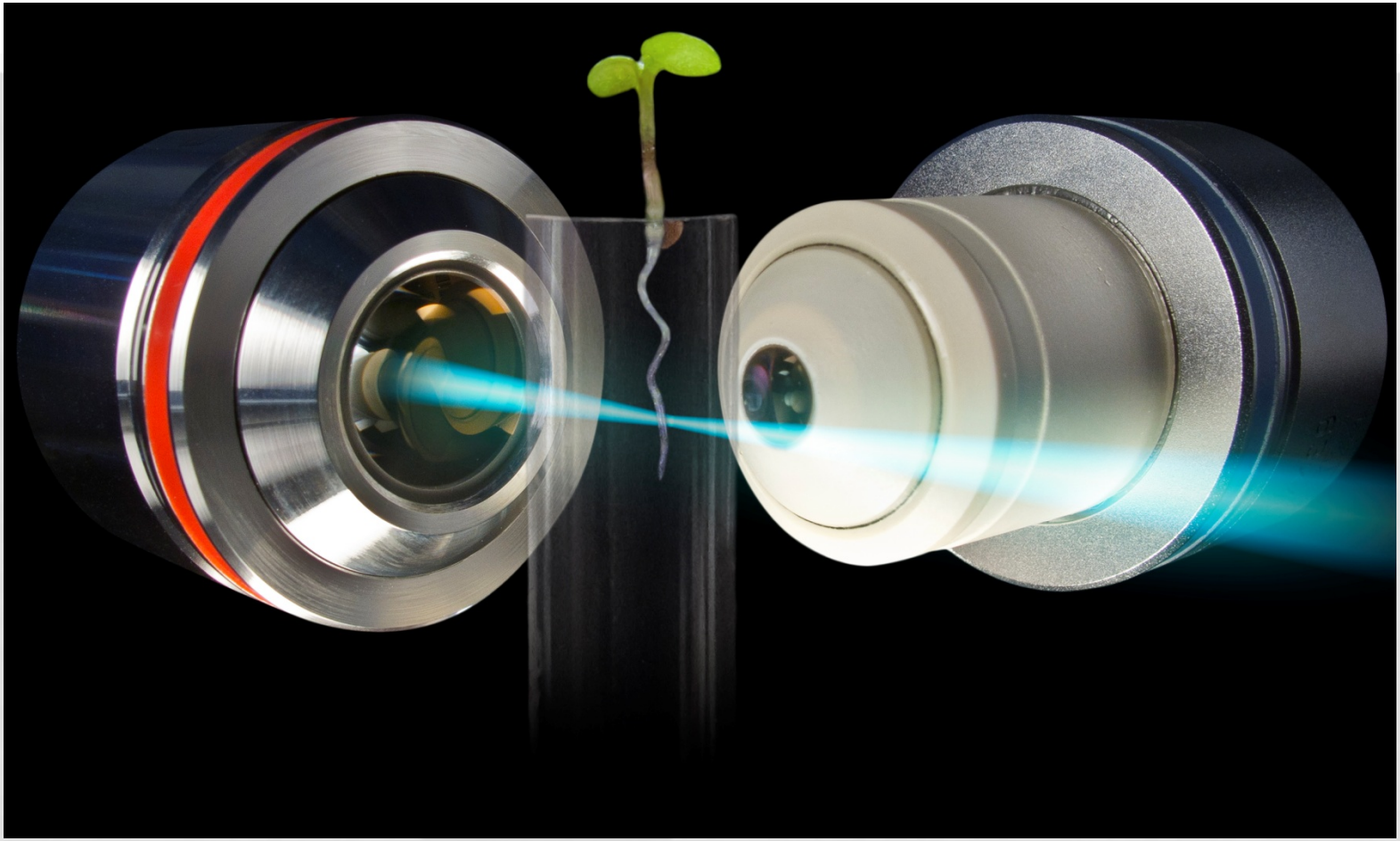






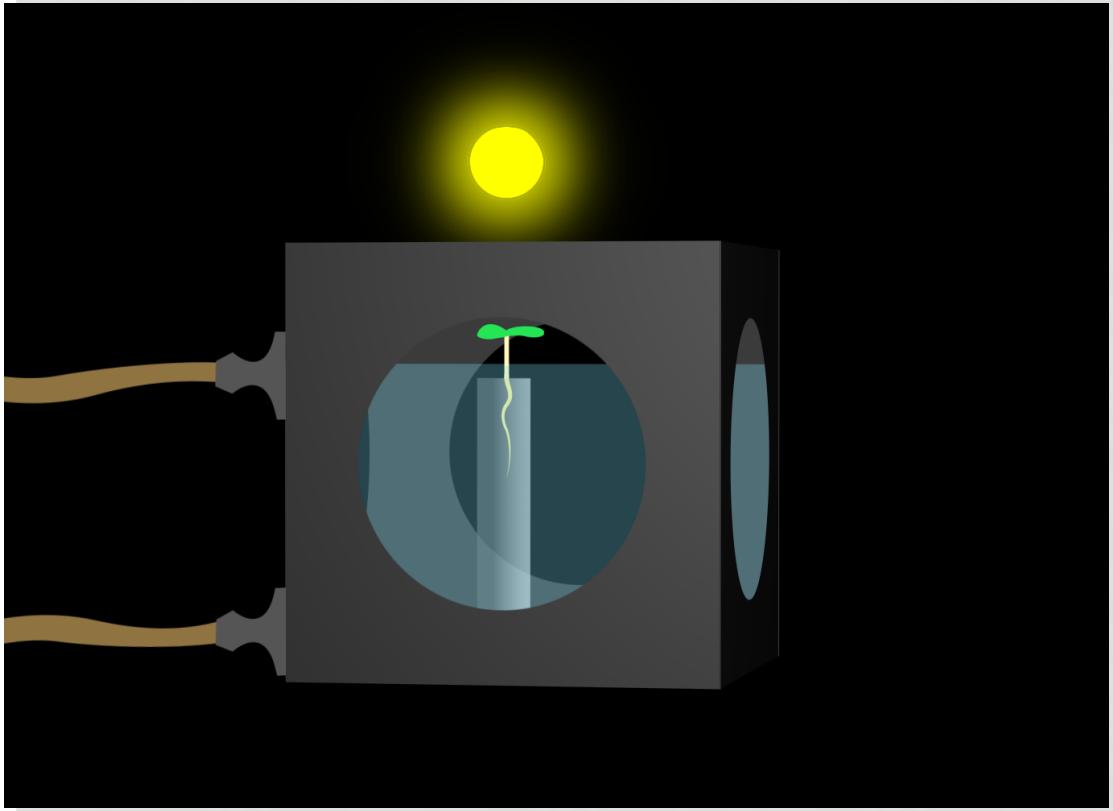
# ARABIDOPSIS IN DSLM



Sena G, Frentz Z, Birnbaum KD, Leibler S (2011) Quantitation of cellular dynamics in growing Arabidopsis roots with light sheet microscopy. *PloS One*.

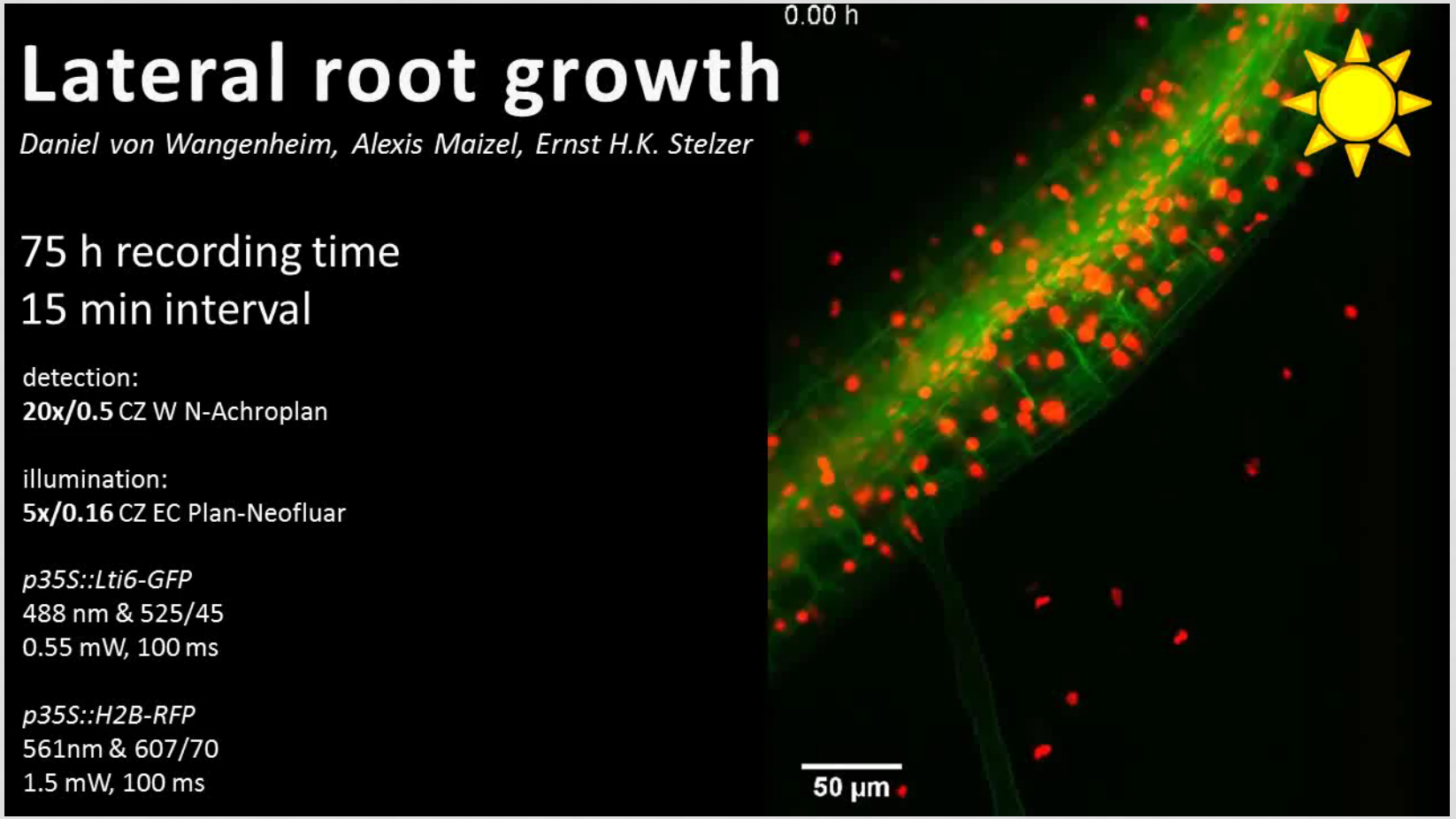
Maizel A, von Wangenheim D, Federici F, Haseloff J, Stelzer EHK (2011) High-resolution live imaging of plant growth in near physiological bright conditions using light sheet fluorescence microscopy. *Plant J*.

# CONDITIONS OF GROWTH IN THE MICROSCOPE CHAMBER



- plant grows in upright orientation
- leafs are in the air while roots are in the medium
- shuttered artificial sun light maintains diurnal cycle
- perfusion system provides fresh medium

# RACINE ARABIDOPSIS

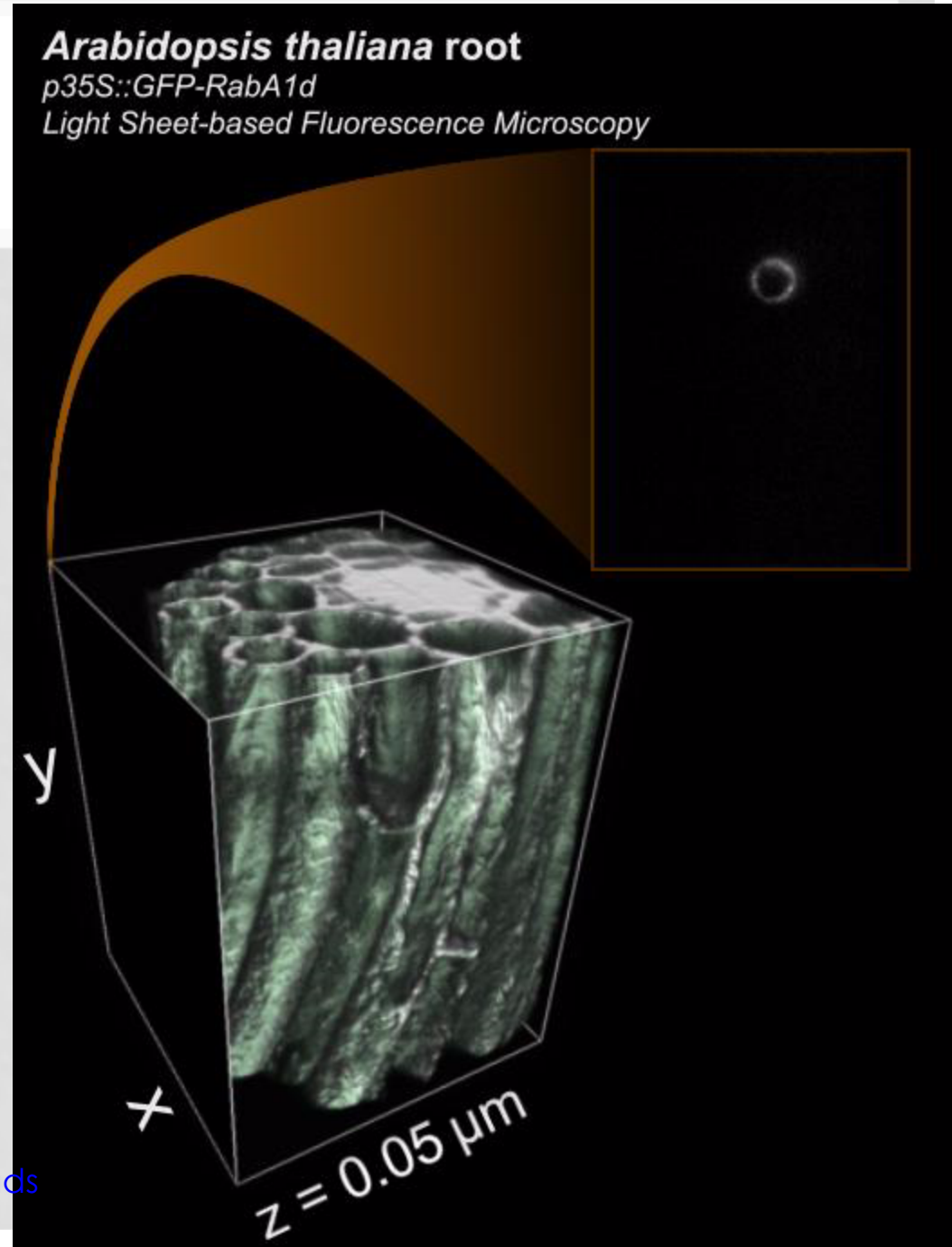


# LIVE MICROSCOPY OF FAST MOVING ENDOSOMES IN THE ROOT

1 Stack
2100 planes
0.05 µm spacing
9.6 fps

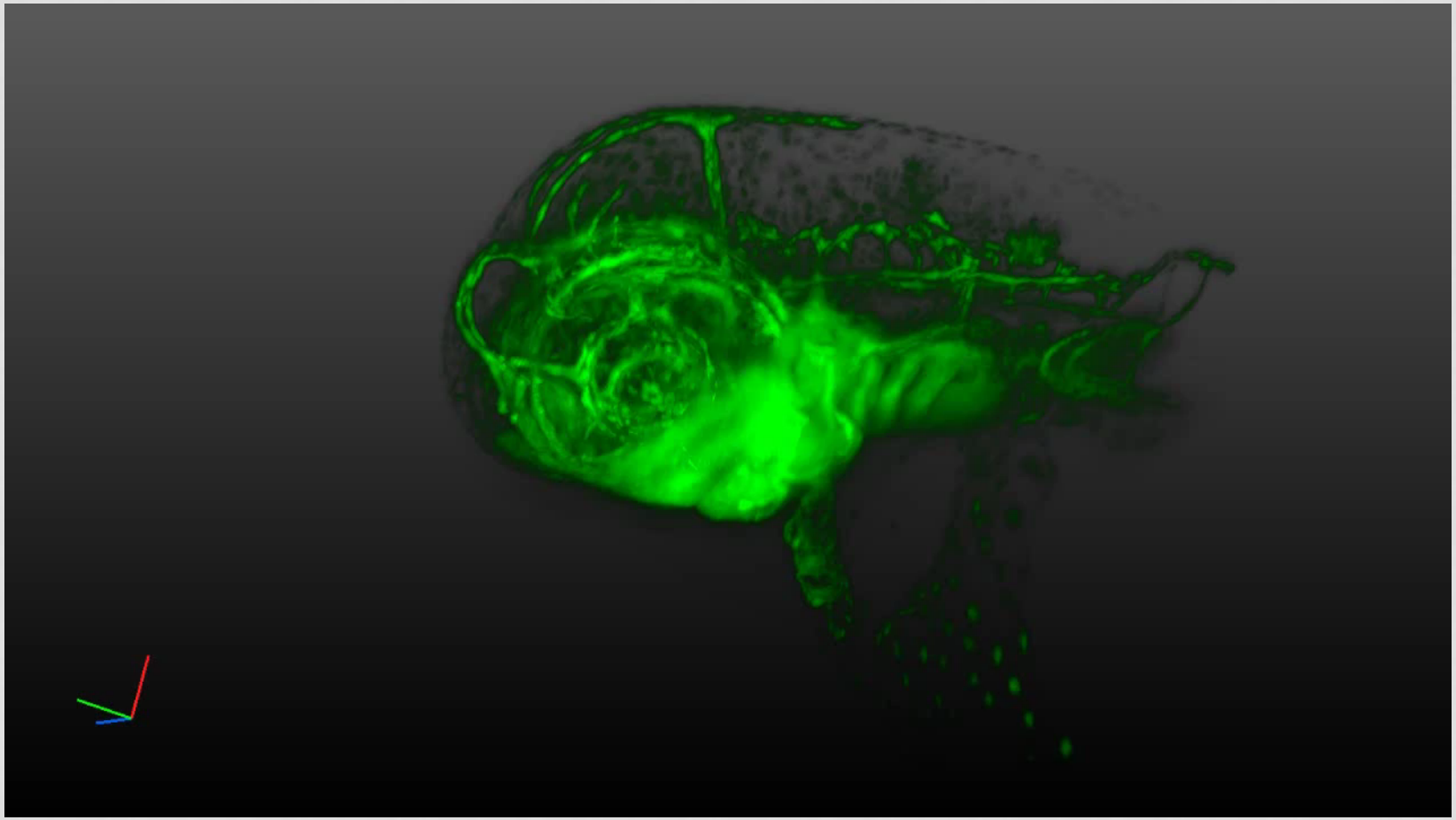
detection:
**63x**/1.0 CZ W N Apochromat
illumination:
**5x/0.16** CZ EC Plan-Neofluar

*p35S::GFP-RabA1d*
488 & 525/50
1 mW, 50 ms

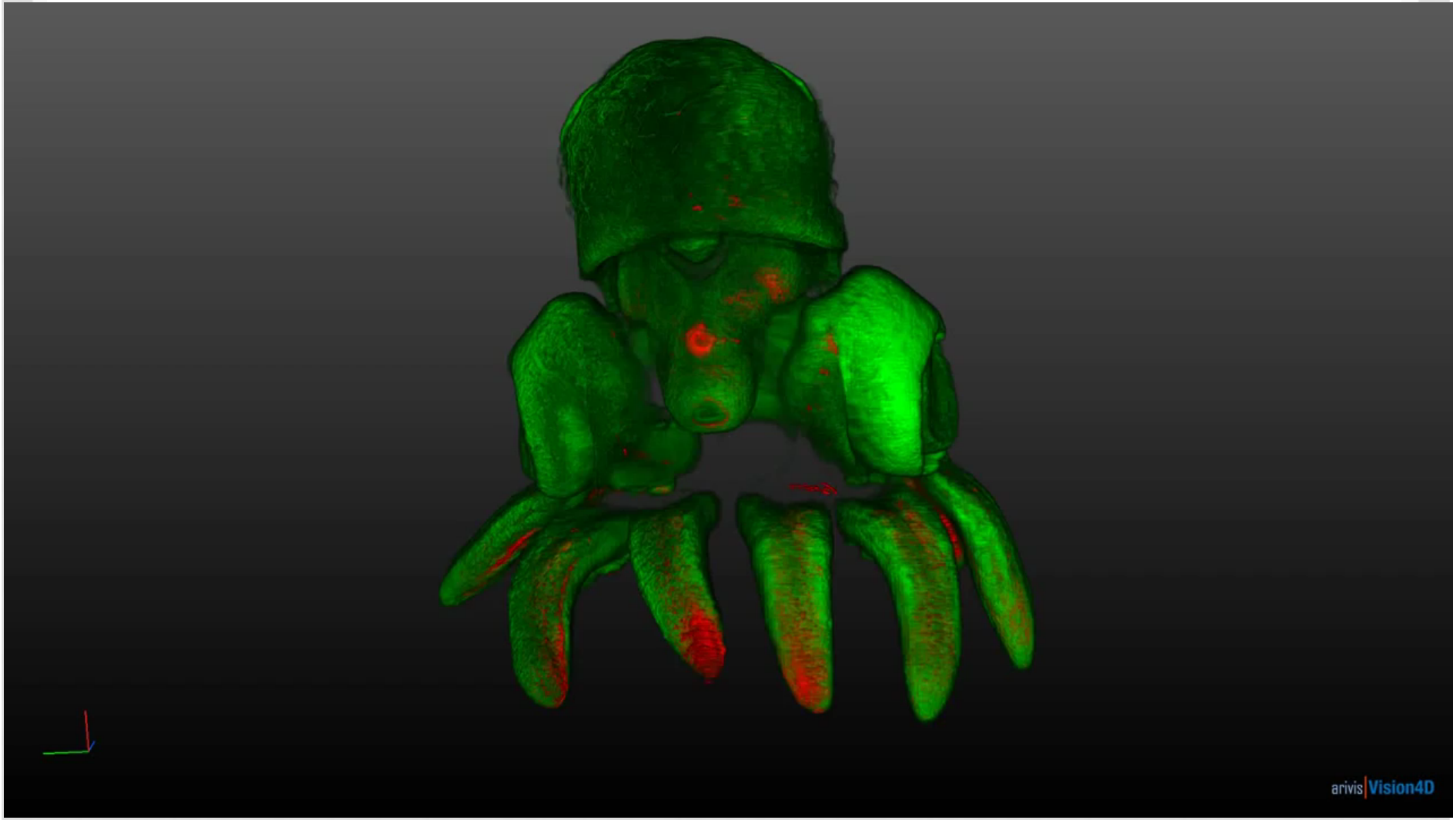



http://www.physikalischebiologie.de/downloads

# EMBRYON ZEBRAFISH



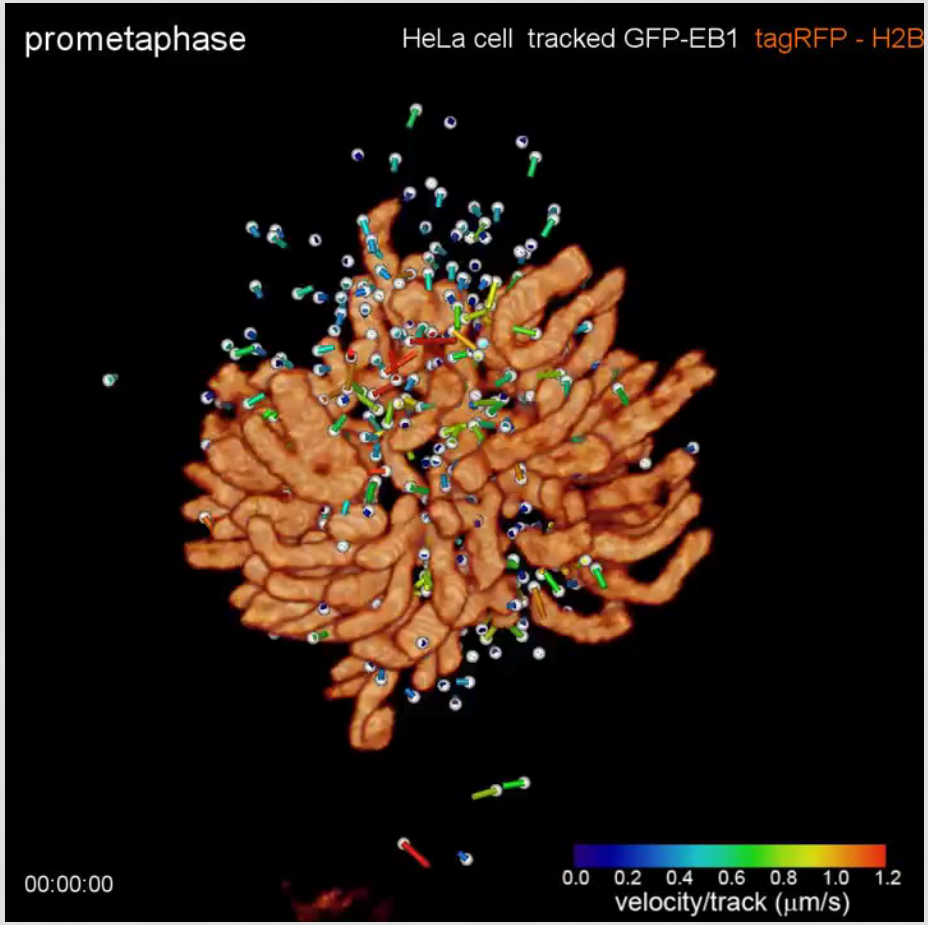
https://www.youtube.com/watch?v=YX8eKv-osrk




Octopus bimaculoides, age approx 1 month: Light sheet Z1 Zeiss
Eric Edsinger & Daniel S. Rokhsar, Okinawa Institute of Science and Technology
3D-Volume Rendering with arivis Vision4D  Zeiss website
https://www.youtube.com/watch?v=Ep4dxiAeEUg

# LLS: LATTICE LIGHT-SHEET

Eric Betzig: Light sheet + lumière structurée + Bessel beam



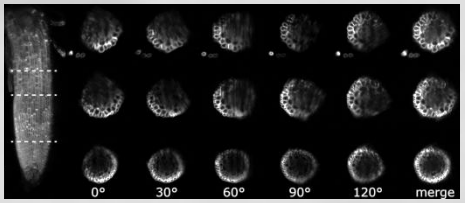

https://vimeo.com/109402304

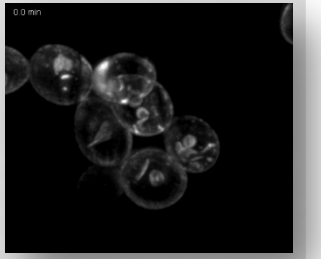
# LSFM TECHNICAL SUMMARY

http://www.physikalischebiologie.de/downloads

LSFM penetrates large specimens relatively well

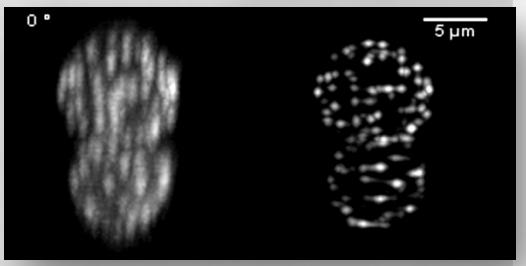
LSFM illuminates only the single plane in focus and causes less photo bleaching and less photo toxicity



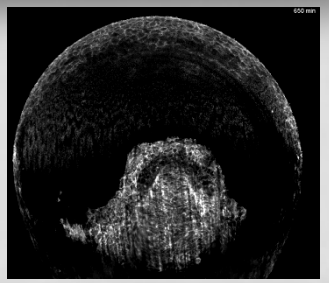
Multi-view LSFM compensates artifacts

Multi-view LSFM provides an isotropic resolution



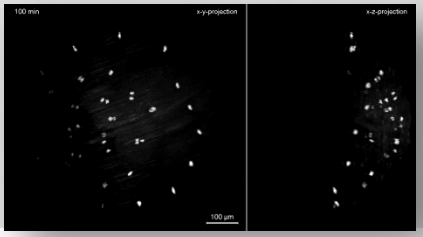

LSFM-SI estimates the background contribution and improves contrast and resolution

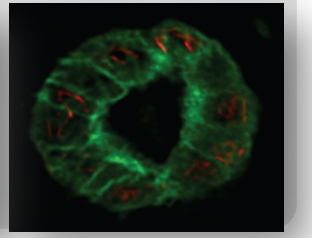
LSFM exposes specimen to one to four orders of magnitude less energy than wide-field and confocal fluorescence microscopes



LSFM easily generate more than twenty frames per second of images with several million pixels and a dynamic range well above 10bit

LSFM provides a solid basis for modern approaches to cell and developmental biology.





LSFM can be combined with many other techniques.

# LSFM PERFORMANCE AND MEMORY AND SPEED REQUIREMENTS

| | Range | Typical | $\sum$ & $\prod$ |
|---|---|---|---|
| Dynamic range | 6 … 16 bit | 12 bit/pixel | 2 Byte/voxel |
| Single image | 256 … 4096 pixel | 2500 x 2000 pixel | 10 MB/image |
| Channels | 1 … 4 dye | 2 channel | 20 MB/spectrum |
| Image stack | 10 … 500 plane | 200 plane/stack | 4 GB/stack |
| Multiple views | 2 … 18 direction | 4 angle/stack | 16 GB/view |
| Time points | 1 … 2000/specimen | 1000 /specimen | 16 TB/specimen/ day |
| Instruments | 1 … 10 | 6 | 72 TB/lab day |
| Specimens | 1 … 10000 | 2000 | 32 PB/experiment |

http://www.physikalischebiologie.de/downloads

# VERY BIG DATA

- Des fichiers images de plusieurs centaines de Go à
- plusieurs To

- Quelles solutions d'analyse, de transfert (plusieurs heures à plusieurs jours) et de stockage des données?
- Quel format d'images?
- Nowadays (plug-ins Fiji)
- BigDataViewer (BDV) (plug-ins Fiji)
- CATMAID: Collaborative Analyse Toolkit Massive Amount Image Data

- Visualisation, management, partage et analyse, stockage
- OMERO: https://www.openmicroscopy.org/site
- Work Group Data management France BIO Imaging

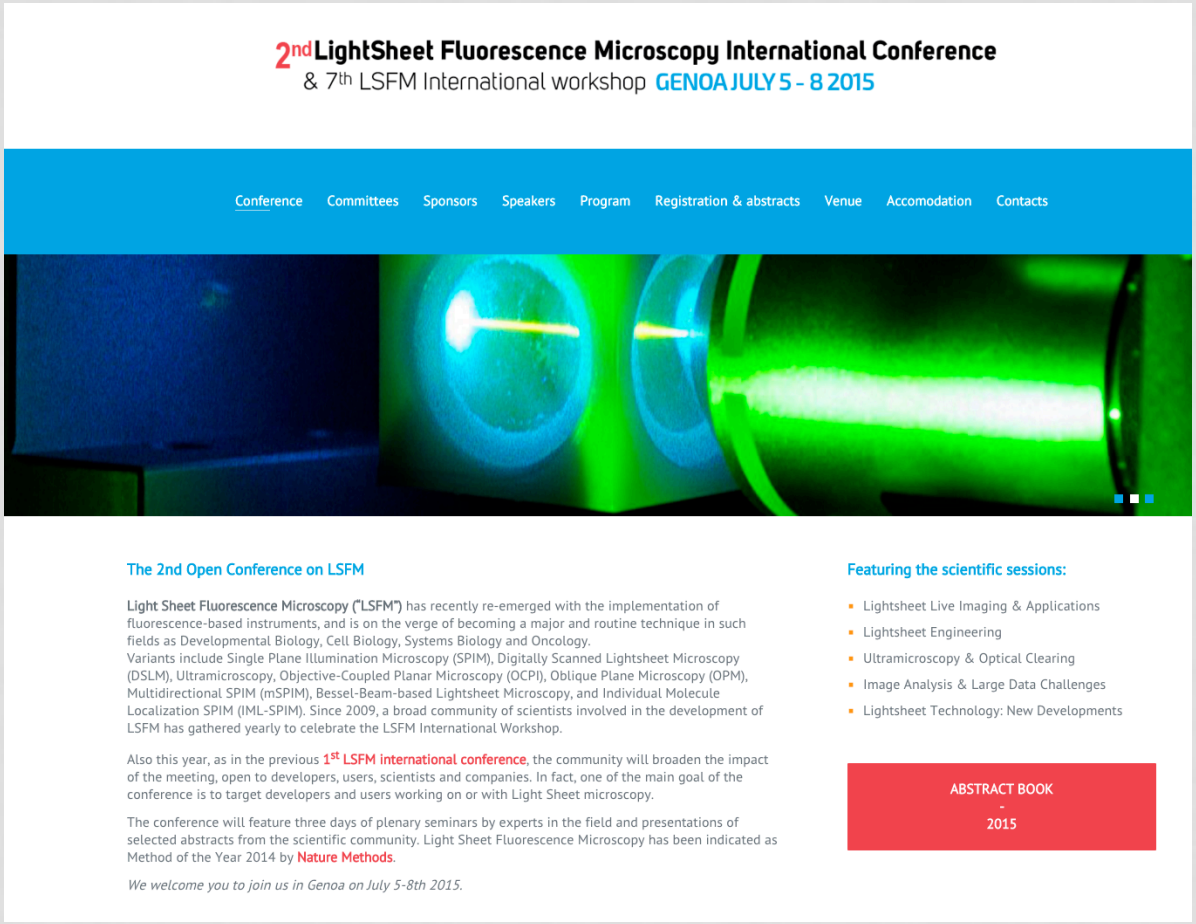
# UN DÉFI POUR LES BIOLOGISTES
## (AVENTUREUX)

- **Acheter un système commercial** (production récente)
- Light-sheet Z1 Zeiss (set-up horizontal)
- Ultramicroscope LaVision Bio tec (set-up vertical)
- Leica TCS SP8 DLS (s'adapte sur SP8)
- Applied Scientific Instrumentation (iSPIM, diSPIM)

- **Collaborer avec un labo « light-sheet »**
- ITAV Toulouse
- ICFO barcelone
- EMBL Heidelberg

- **Construire son propre système**
- Communauté scientifique importante
- Deux plates-formes: OPEN SPIM (µManager/Fiji) et OpenSpimMicroscopy
- DIY « Do-it-yourself »!

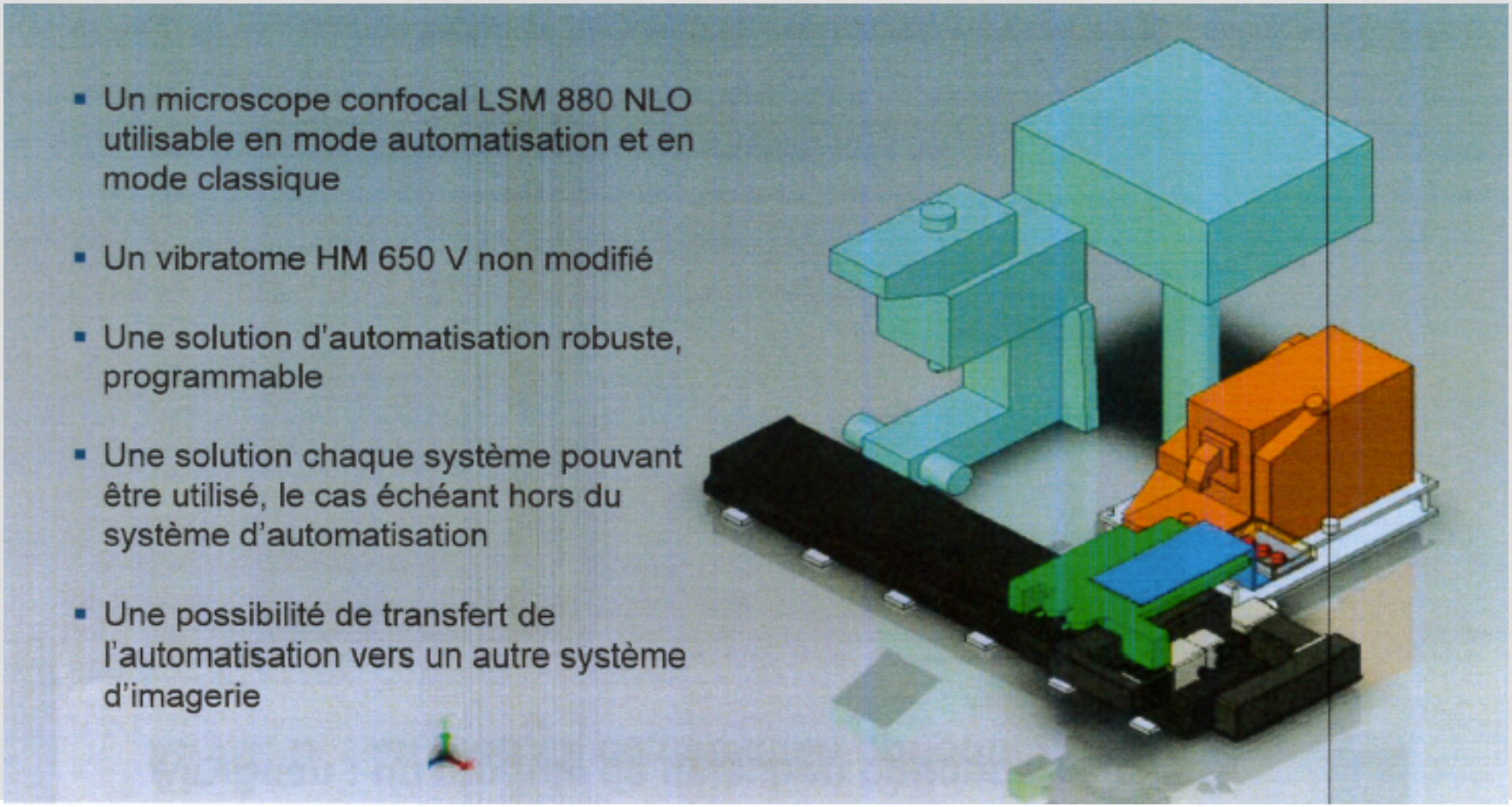
# LIGHTSHEET FLUORESCENCE MICROSCOPY INTERNATIONAL CONFERENCE



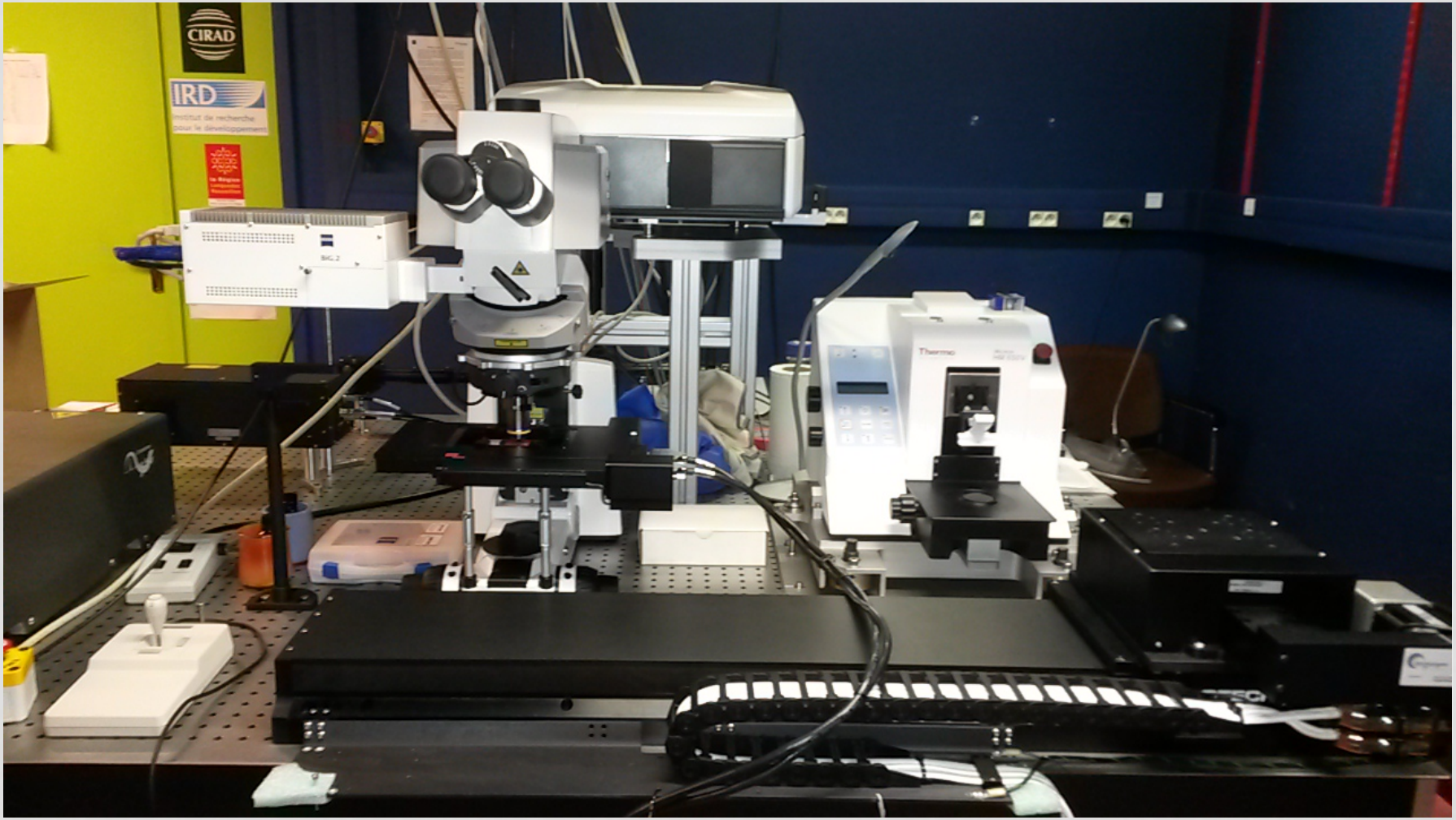

www.lsfm2015.org

# ET POUR LES ÉCHANTILLONS ÉPAIS DENSES, GRANDS??

- Serial Block face imaging



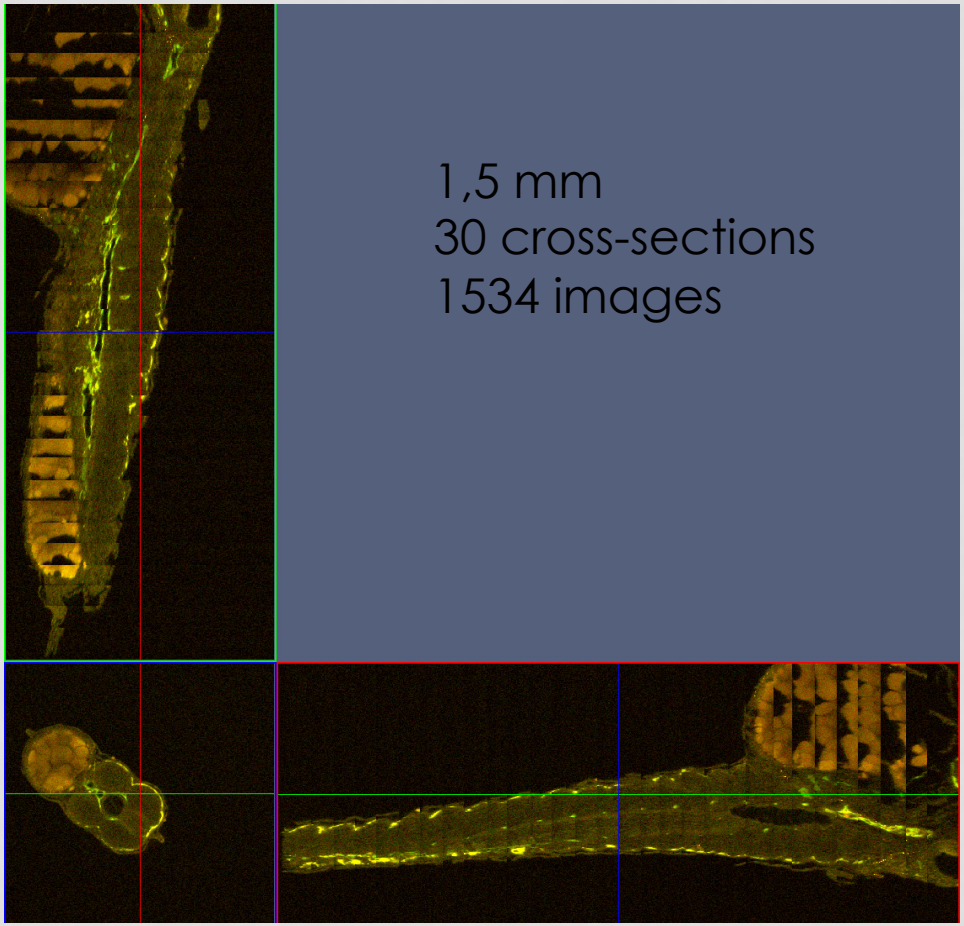
# COMBINED SYSTEM CONFOCAL/MULTIPHOTON MICROSCOPE NLO 880 ZEISS AND VIBRATOME



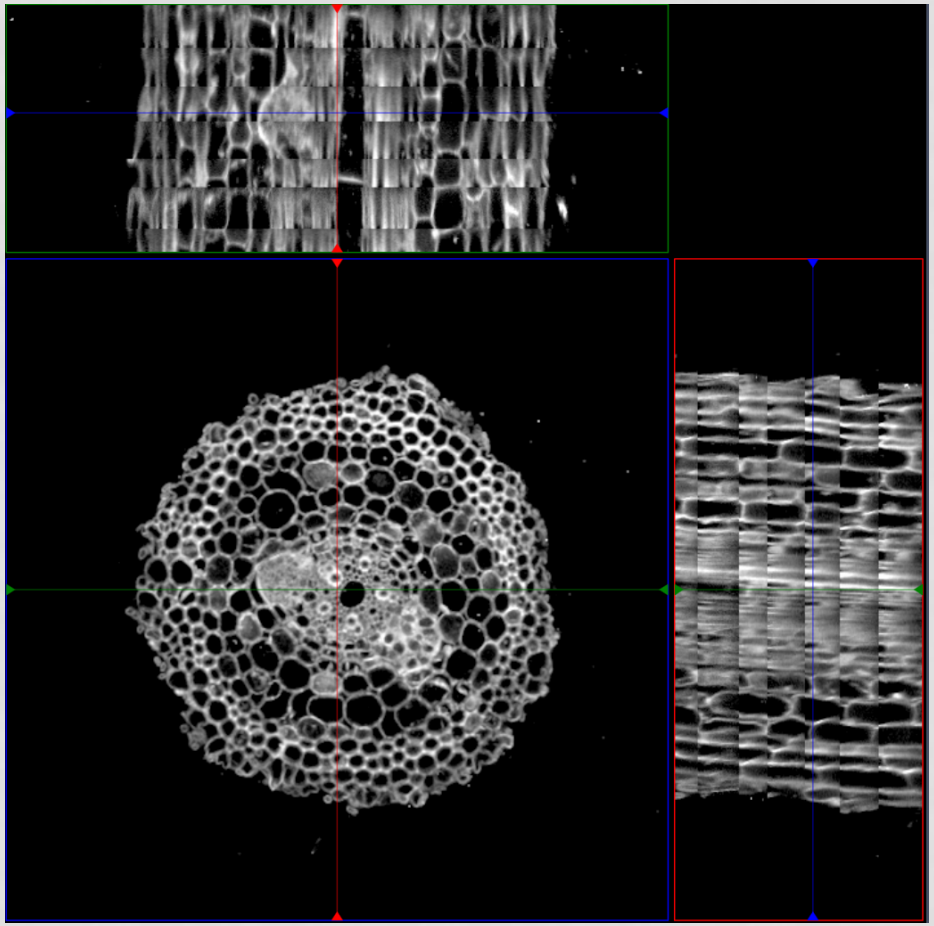

AUTOMATED TISSUES SECTION AND IMAGES ACQUISITION OF THICK SAMPLES

**HIGH THROUGHPUT CELLULAR IMAGING**

# FIXED EMBRYO ZEBRAFISH



# AUTOFLUORESCENCE: RACINE DE RIZ



Multiphoton, 740 nm,

# DES QUESTIONS? GDR MIV, RTMFM, PSEN

# *MERCI AUX BIOLOGISTES AVENTUREUX ET AUX PHYSICIENS AVERTIS*


- Marc Lartaud, JL Verdeil, Houssam Hajjoul, Carine Alcon
- (INRA CIRAD Montpellier, MRI-PHIV)

- Pablo Loza, Jordi Andilla, ICFO, Barcelone: SPIM 2 P

- Corinne Lorenzo ITAV Toulouse: atelier SPIM Mifobio 2012

  Zeiss: atelier SPIM Mifobio 2014, essais Light Sheet Z1 ( E Elias)

- Serial Block Face Imaging Zeiss (Noël Converset)


INRA SCIENCE & IMPACT cnrs cirad PHIV Plate-forme d'Histocytologie et d'Imagerie cellulaire Végétale MRI