



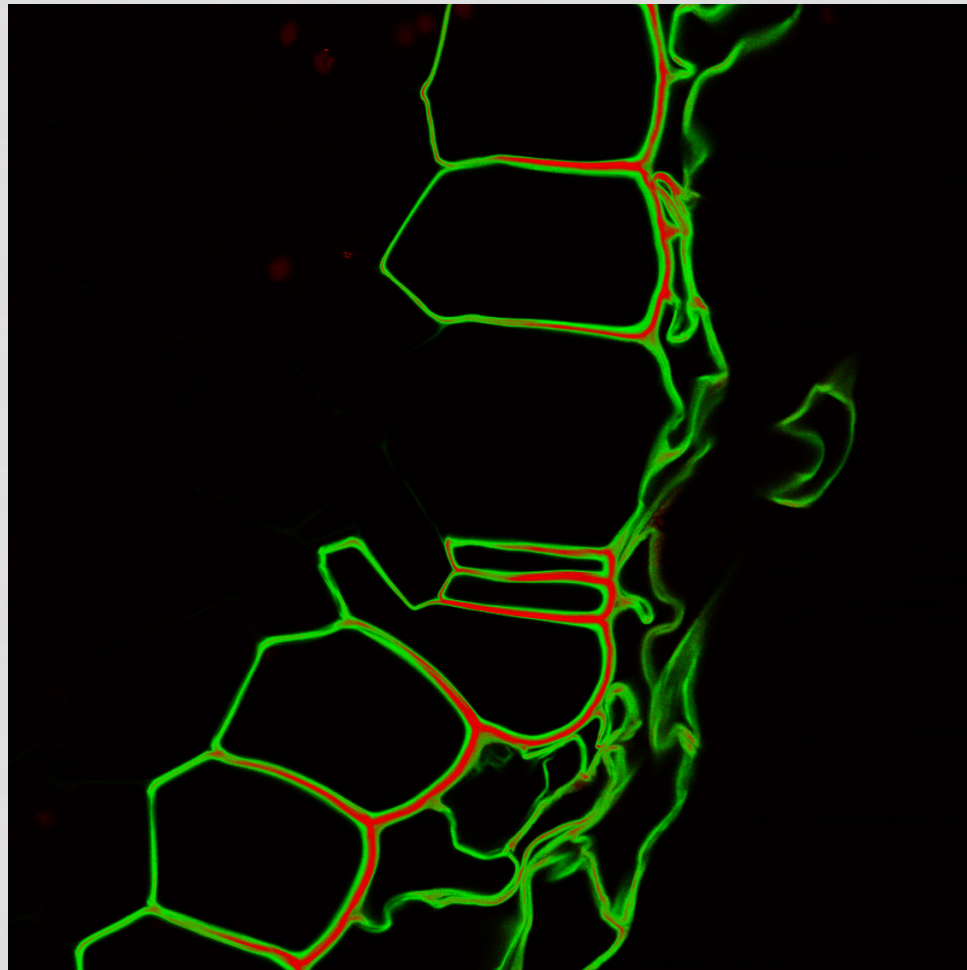
VISUALISATION 3D

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

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

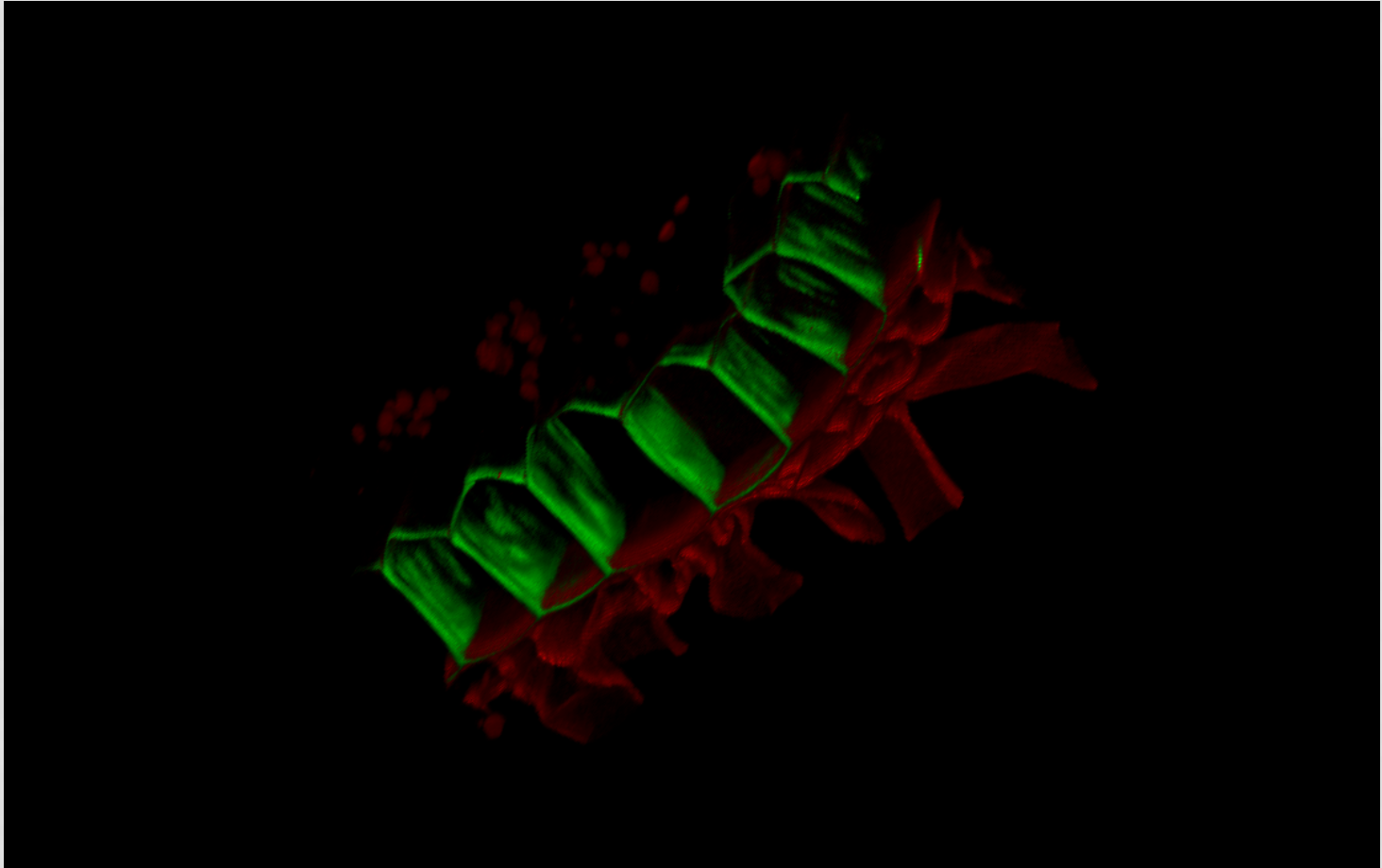
Geneviève Conéjéro

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 photodommages, avec une bonne résolution XYZ

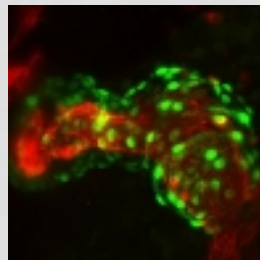
Guide to light-sheet microscopy for adventurous biologists

EG Reynaud et al, Nature methods, janvier 2015

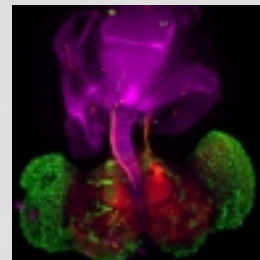
« Ten years of development in light-sheet microscopy have led to spectacular demonstrations of its capabilities. The technology is ready to assist biologists in tackling scientific problems, but are biologists ready to it? »



E Edsinger et D S Rokhsar
Okinawa



M Weber, J Huisken,
Dresden



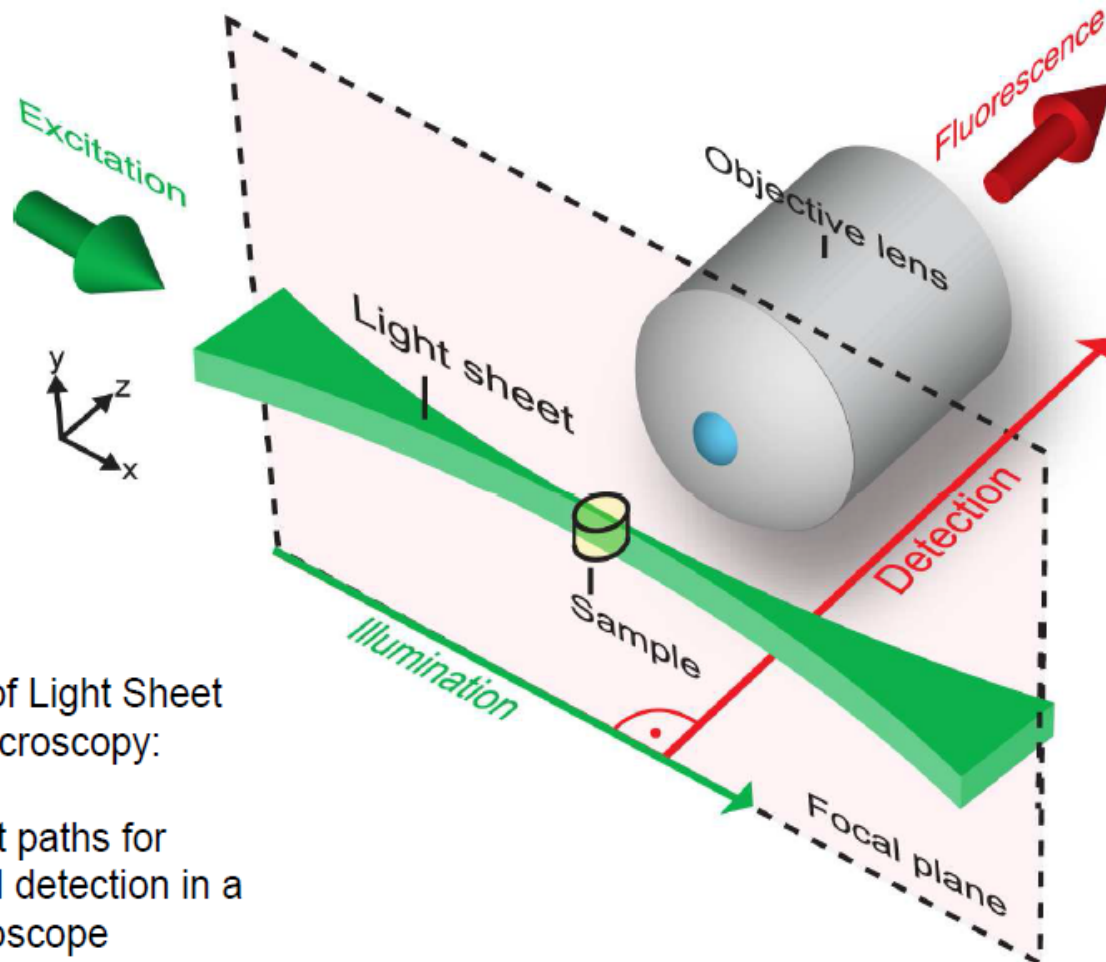
A A Bohra, K V Raghavan
Bangalore

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=ltewwF6wKu8>
- <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 $\mu\text{J}/\mu\text{m}^2$
- 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

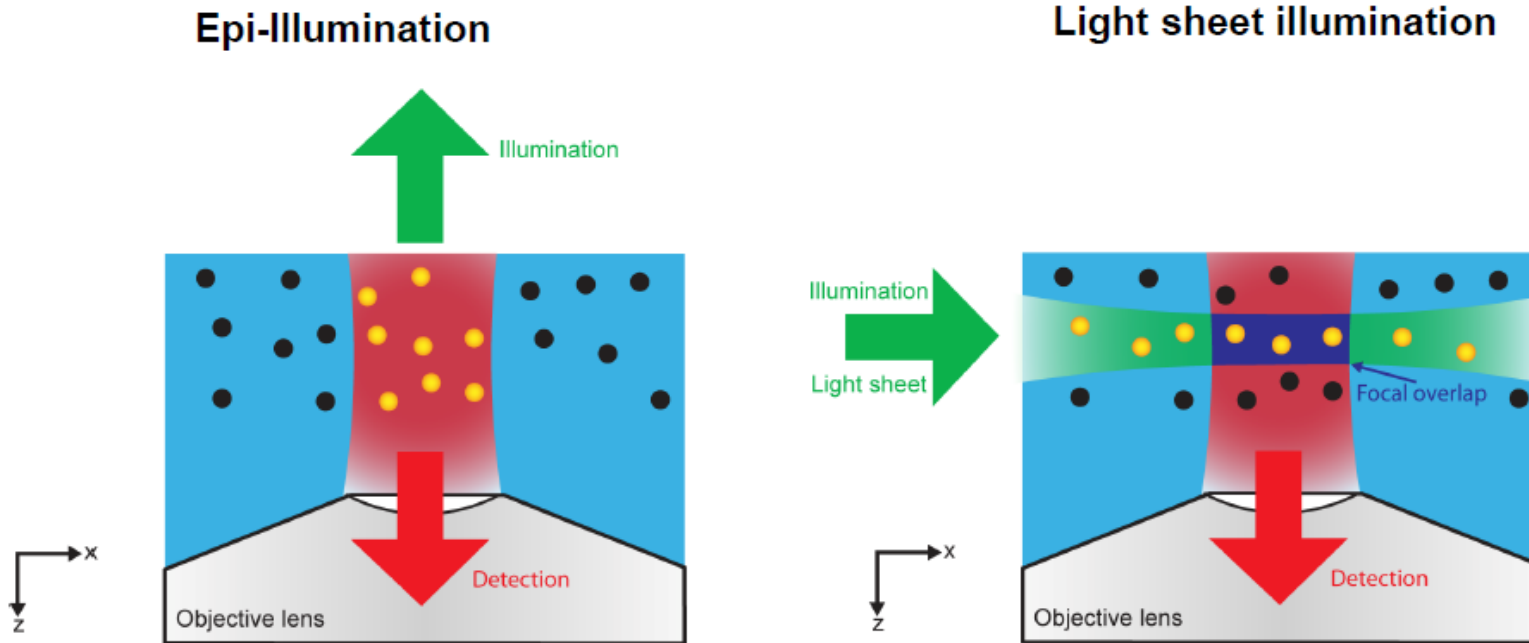


The **Principle** of Light Sheet Fluorescence Microscopy:

Orthogonal light paths for Illumination and detection in a horizontal microscope

Light Sheet: SPIM

Selective plane illumination microscopy

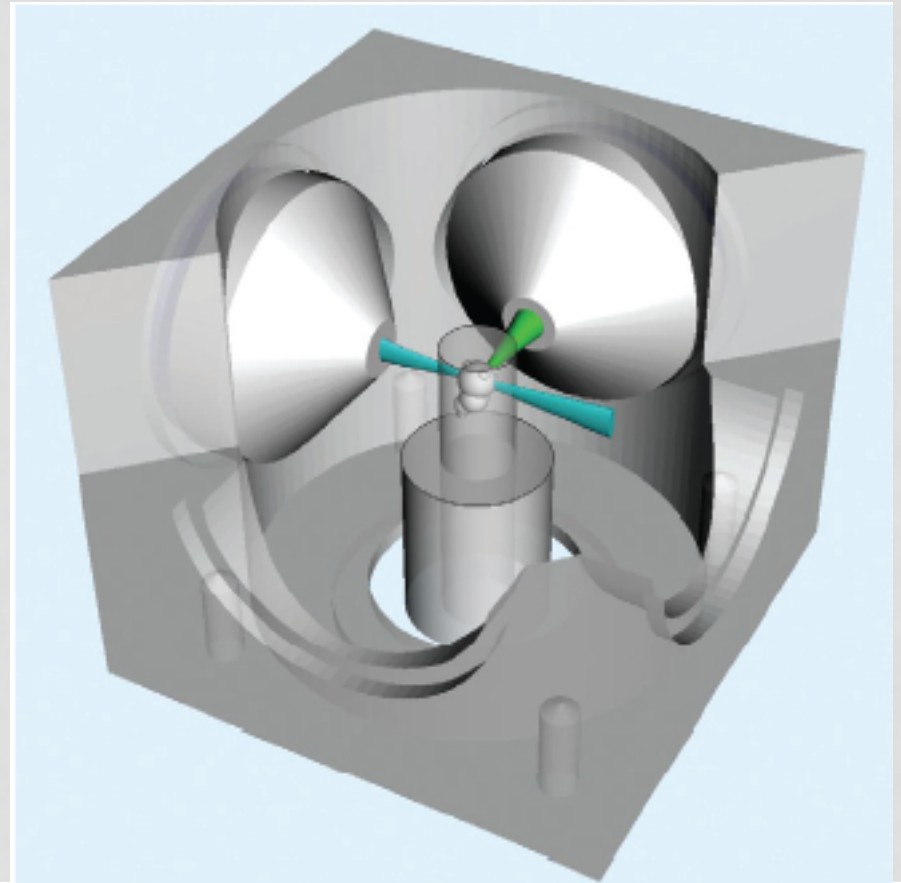


- Inherent optical sectioning capability of the illumination method
- No excitation of out-of-focus fluorescence

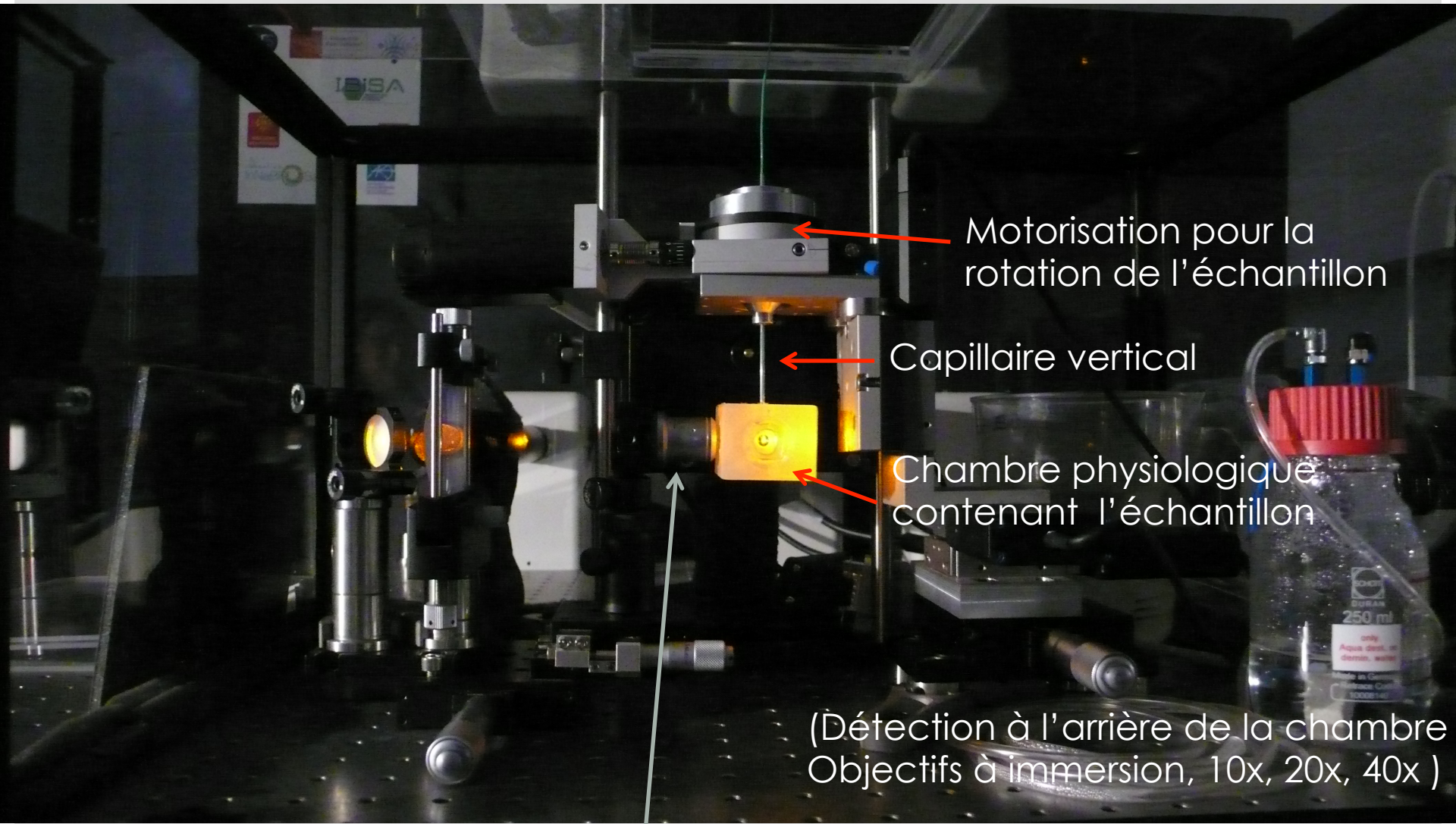
Figure from the PhD thesis of Jörg Ritter (2011),
University of Bonn, Germany)

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



Motorisation pour la rotation de l'échantillon

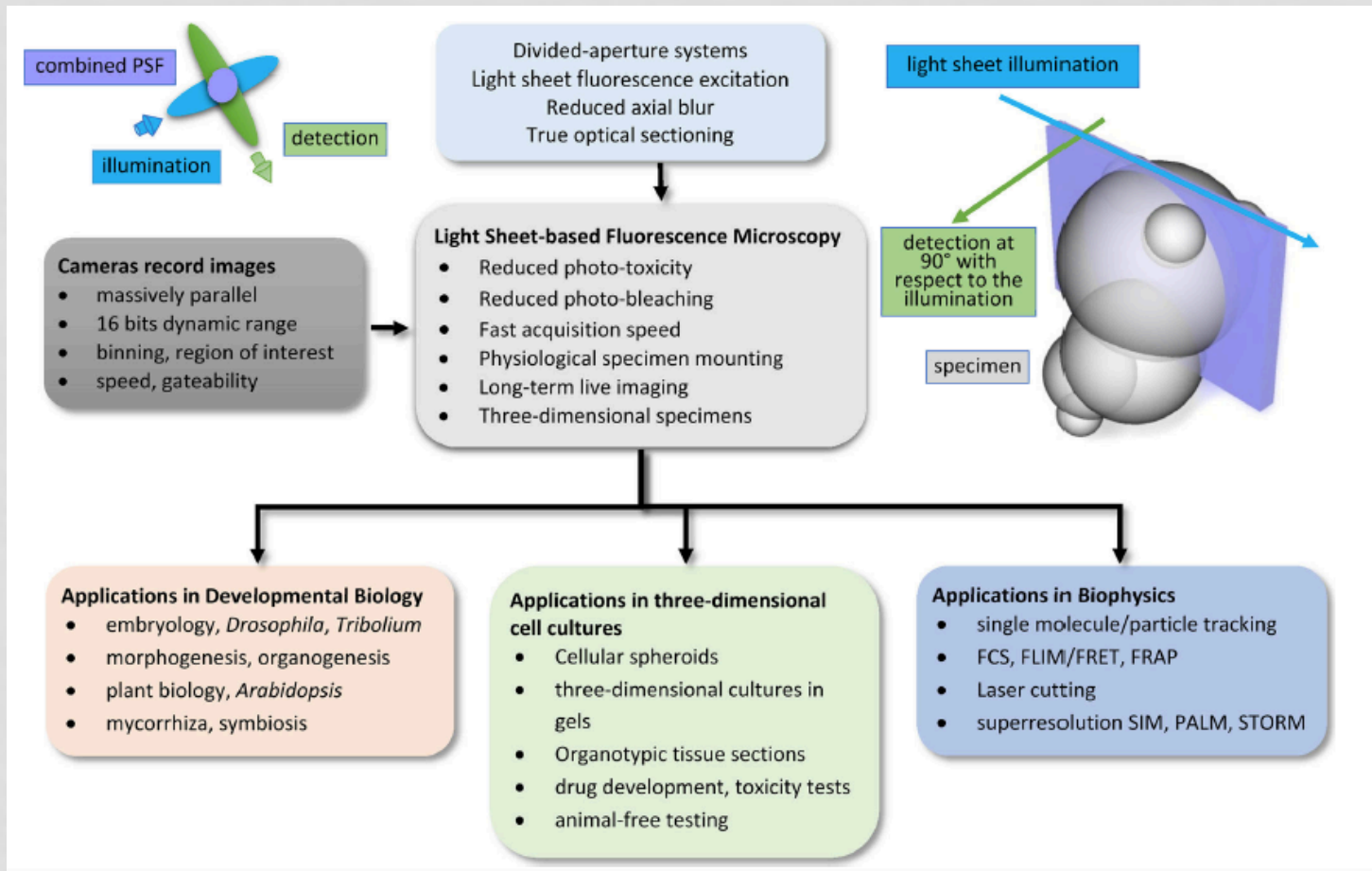
Capillaire vertical

Chambre physiologique contenant l'échantillon

(Détection à l'arrière de la chambre
Objectifs à immersion, 10x, 20x, 40x)

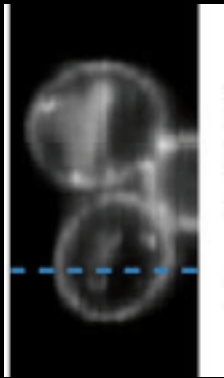
Illumination (excitation) horizontale (10x, NA 0,25)

CHAMPS D'APPLICATIONS DU LIGHT SHEET



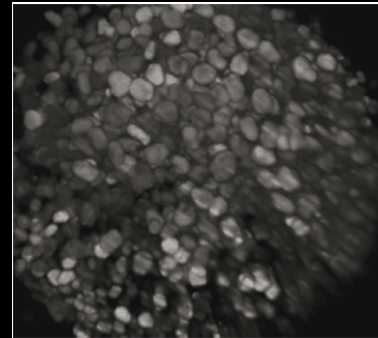
Champs d'applications: quelques exemples

Molecular & cellular biology



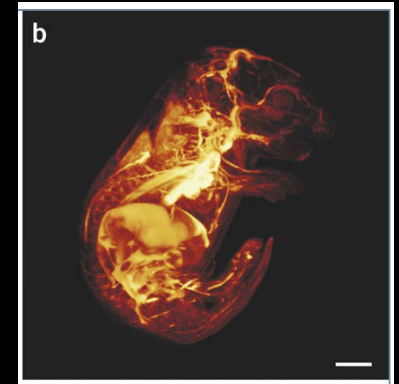
Capoulade & al., 2011

Cancer biology



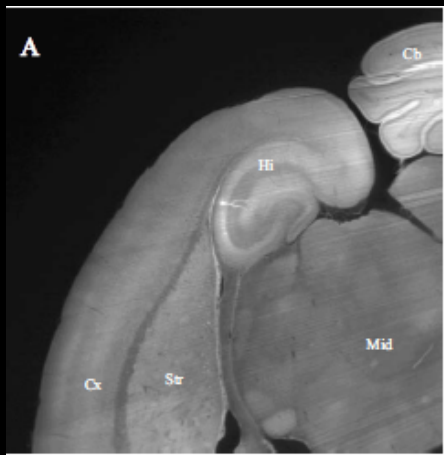
Lorenzo & al., 2011
Spheroid

Neuroscience

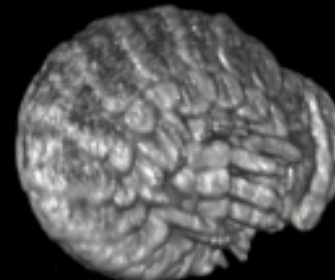


Dotd & al., 2007

Anatomical studies

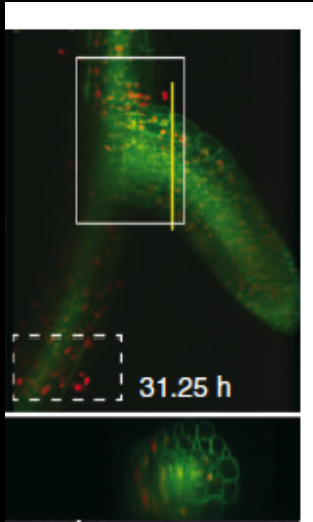


Mertz & al, 2012,
mouse brain



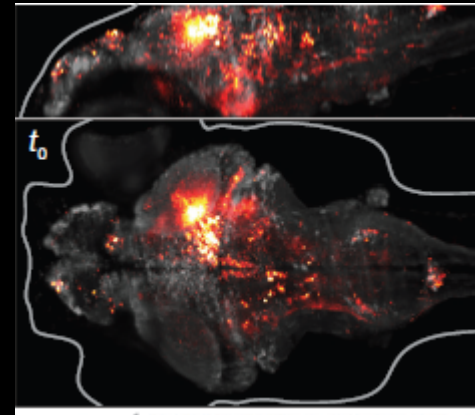
Marine amphipod
Parhyale hawaiiensis
Zeiss website

Plant biology

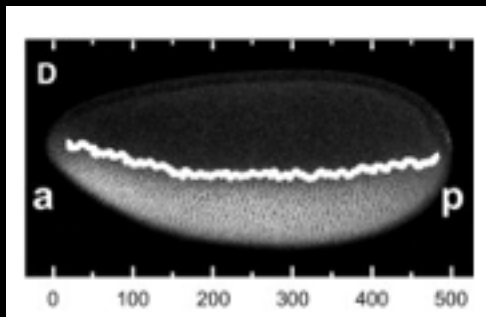


Maizel & *al.*, 2011

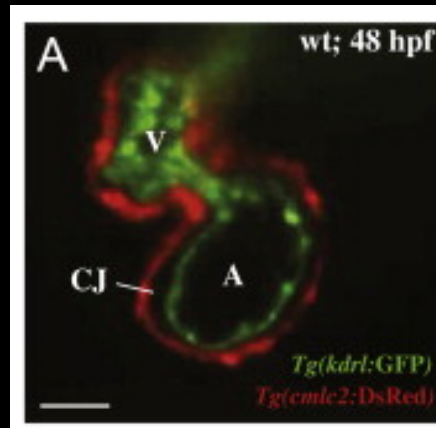
Whole-brain functional imaging



Developmental biology



Reeves & *al.*, 2012

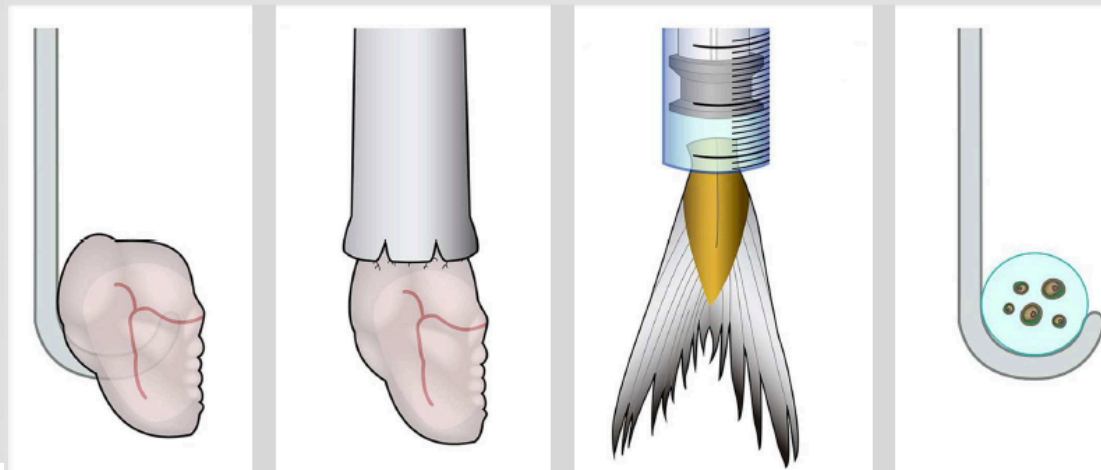
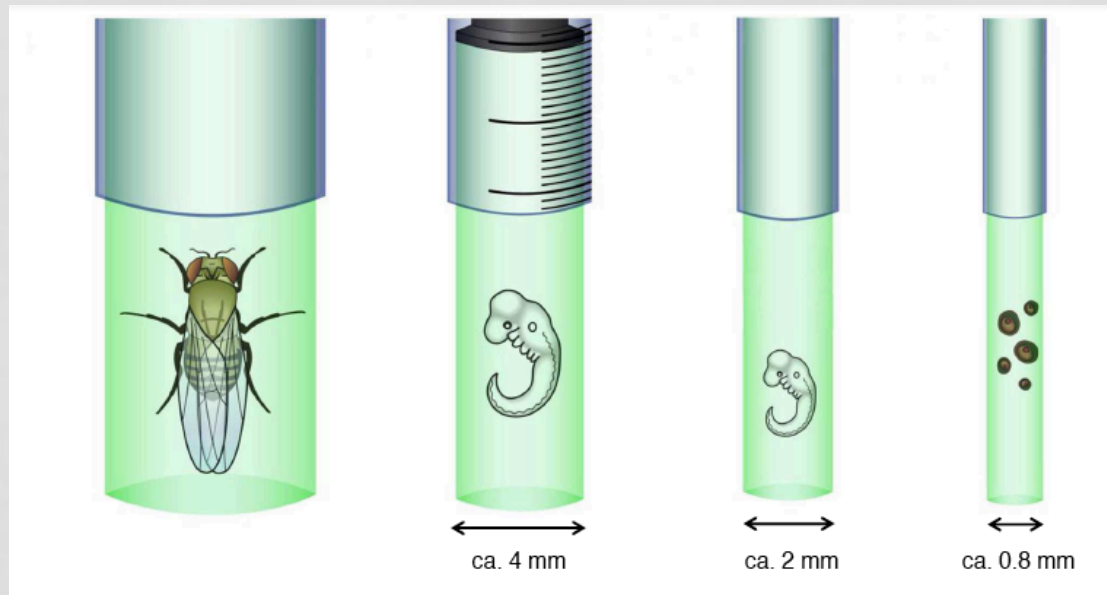


Mellman & *al.*, 2012

PRÉPARATION DES ECHANTILLONS

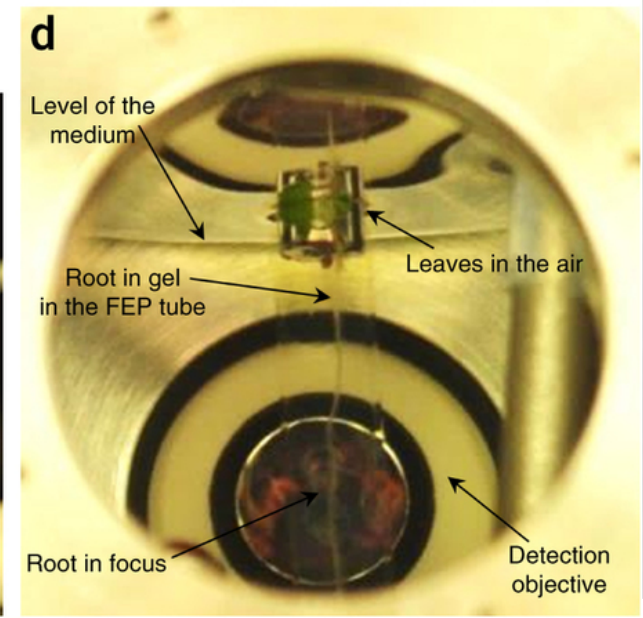
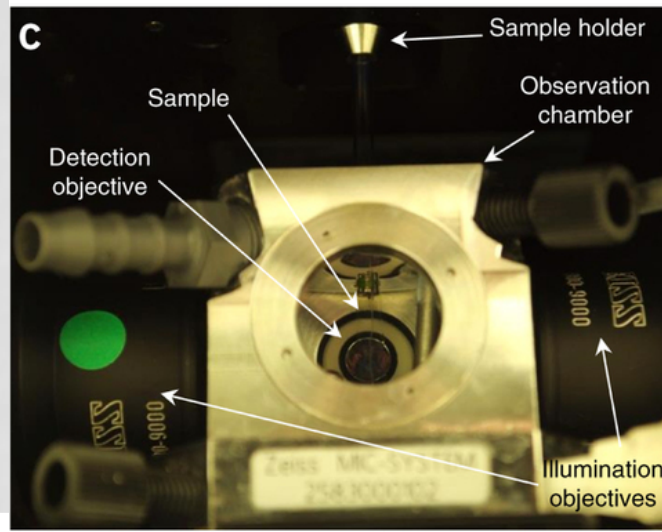
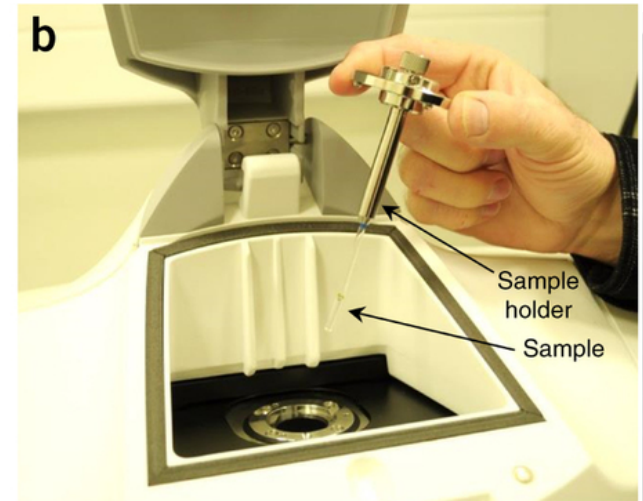
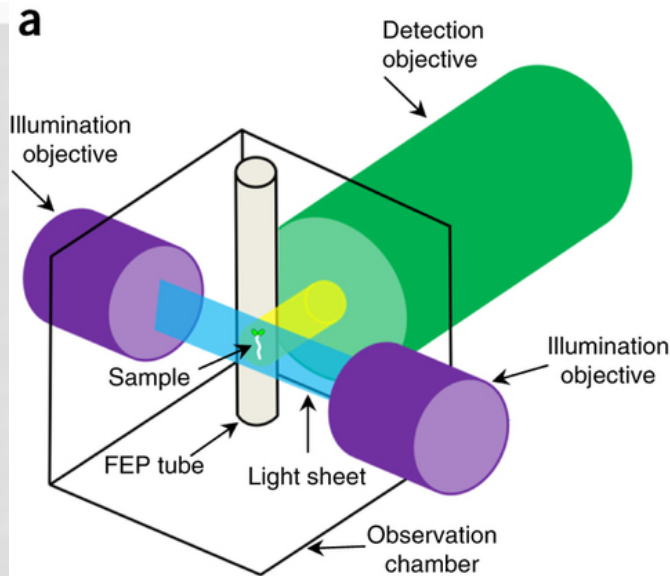
- Echantillon idéal pour la microscopie
- = FIN, PLAT, TRANSPARENT....
- En microscopie LightSheet, la préparation des échantillons est en soi un projet de recherche:
- **Publication de nouvelles techniques de montage des échantillons (chambre, capillaire, milieu de montage)**
- **Exemple: 5 protocoles 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

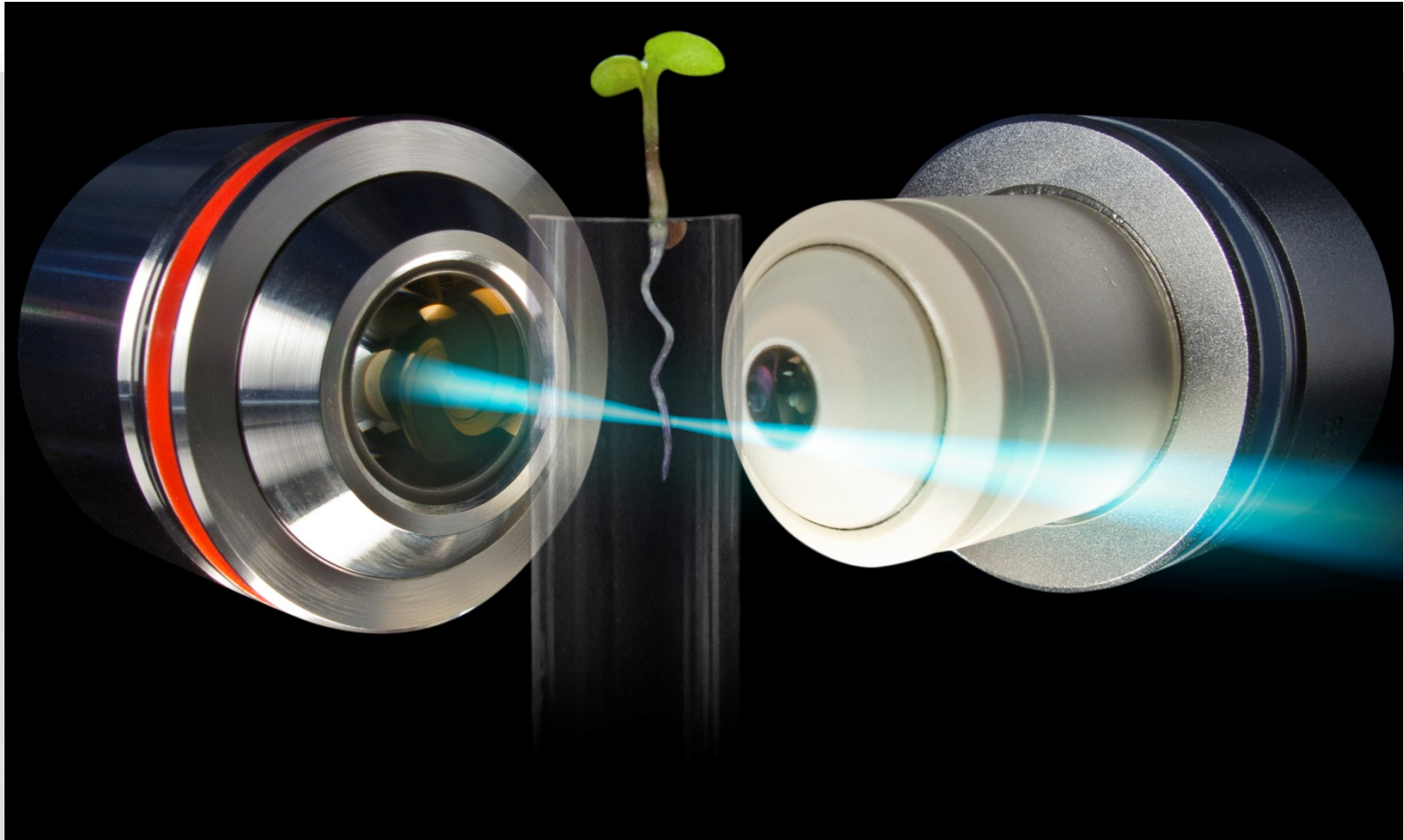


EXEMPLE PRÉPARATION PLANTE

- Ovecka et al.,
- 2015
- Nature Methods



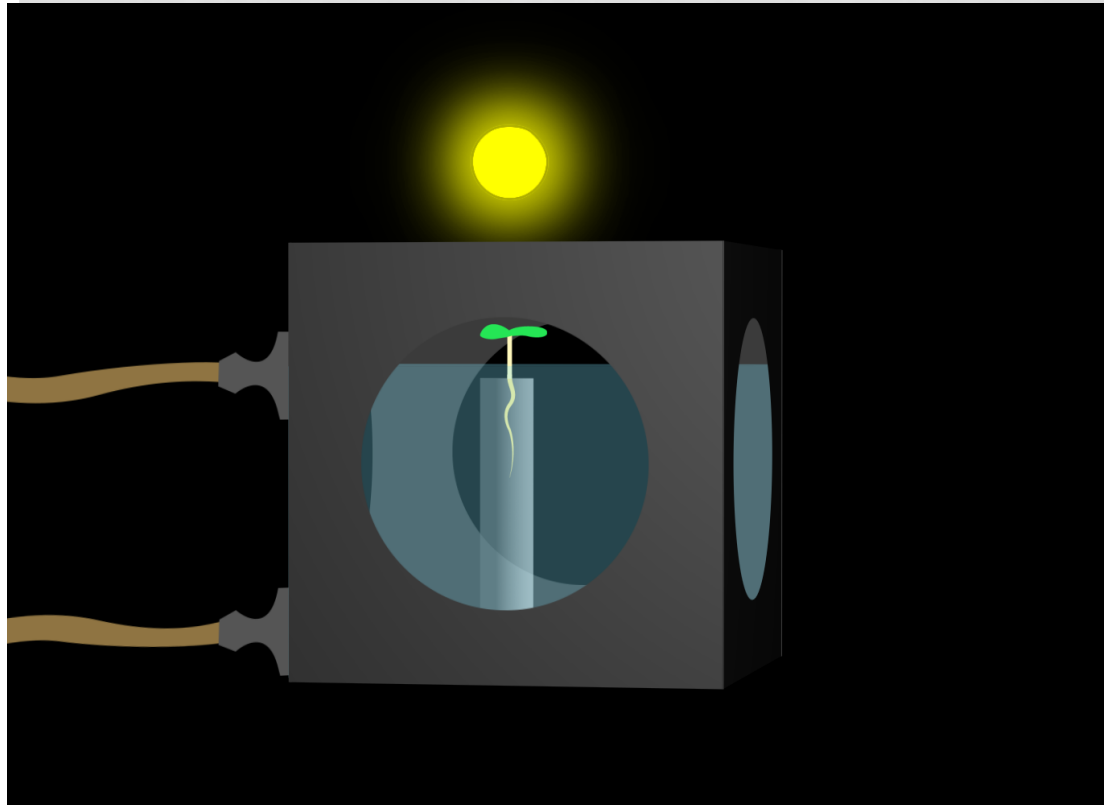
ARABIDOPSIS IN DSLM



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

Lateral root growth

Daniel von Wangenheim, Alexis Maizel, Ernst H.K. Stelzer

75 h recording time

15 min interval

detection:

20x/0.5 CZ W N-Achroplan

illumination:

5x/0.16 CZ EC Plan-Neofluar

p35S::Lti6-GFP

488 nm & 525/45

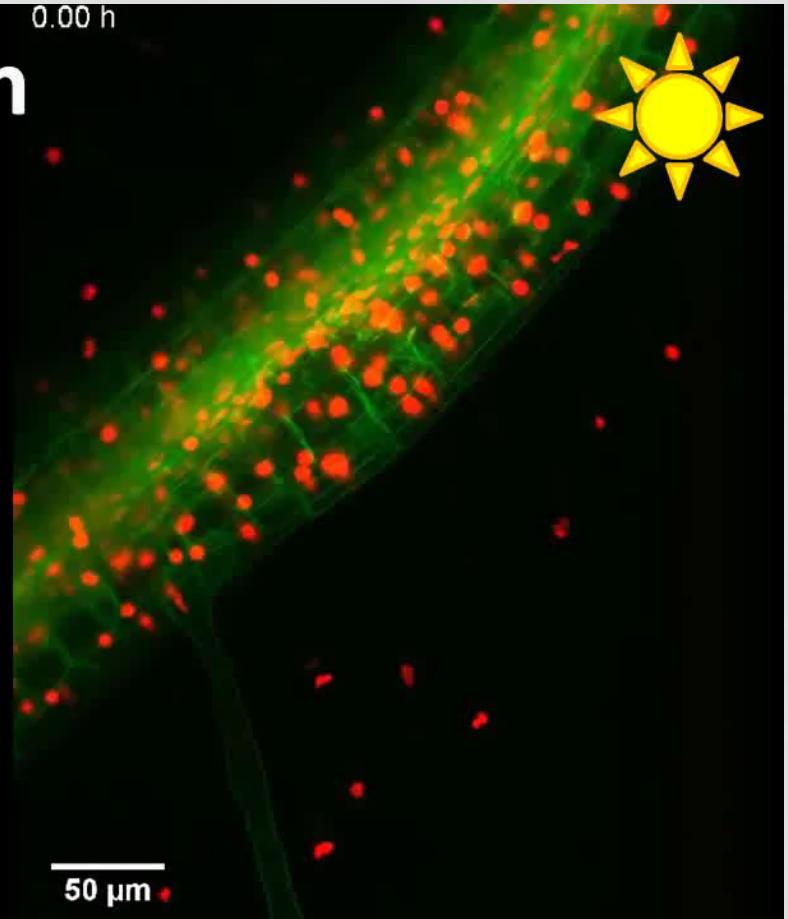
0.55 mW, 100 ms

p35S::H2B-RFP

561nm & 607/70

1.5 mW, 100 ms

0.00 h



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

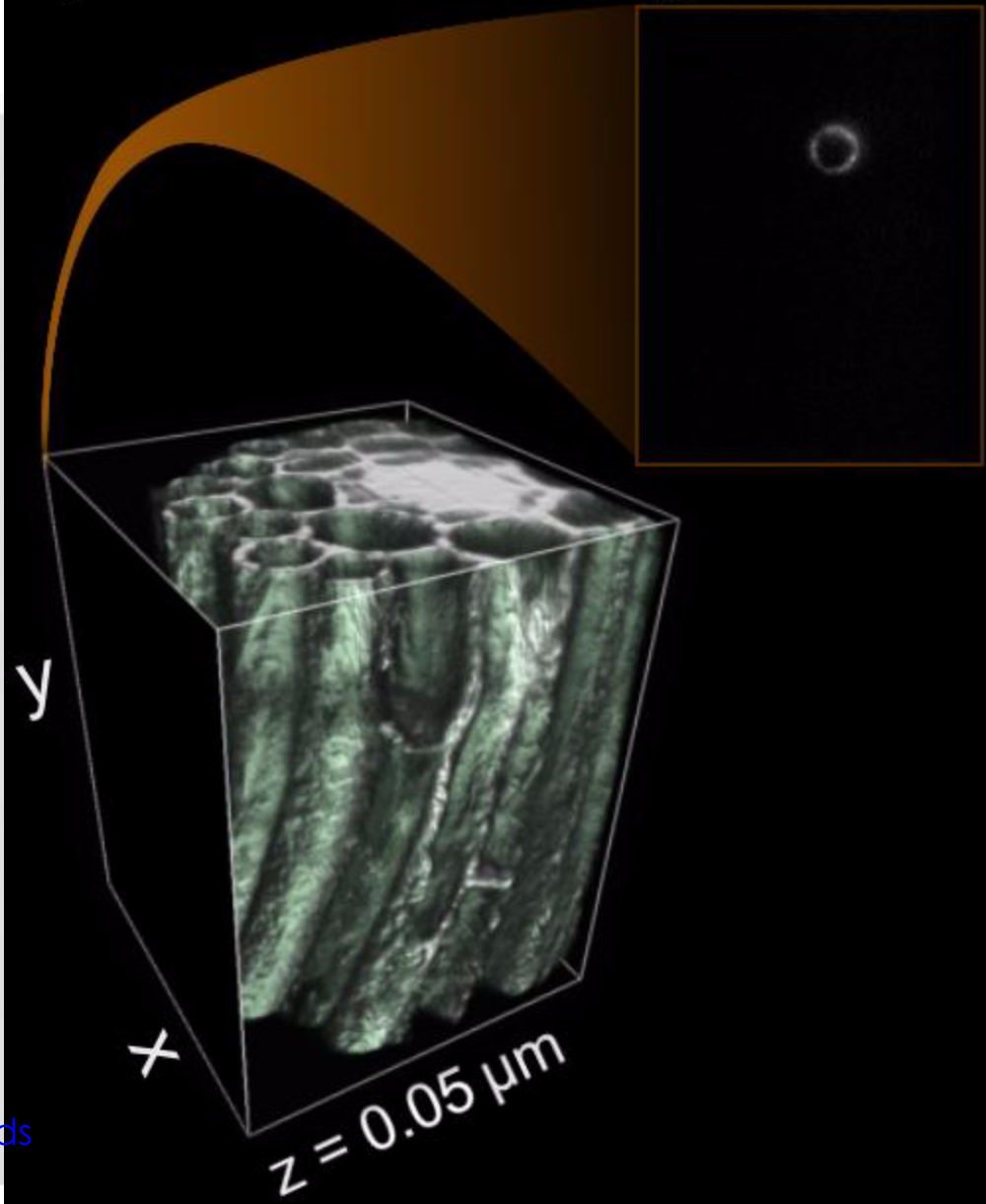
1 mW, 50 ms

<http://www.physikalischebiologie.de/downloads>

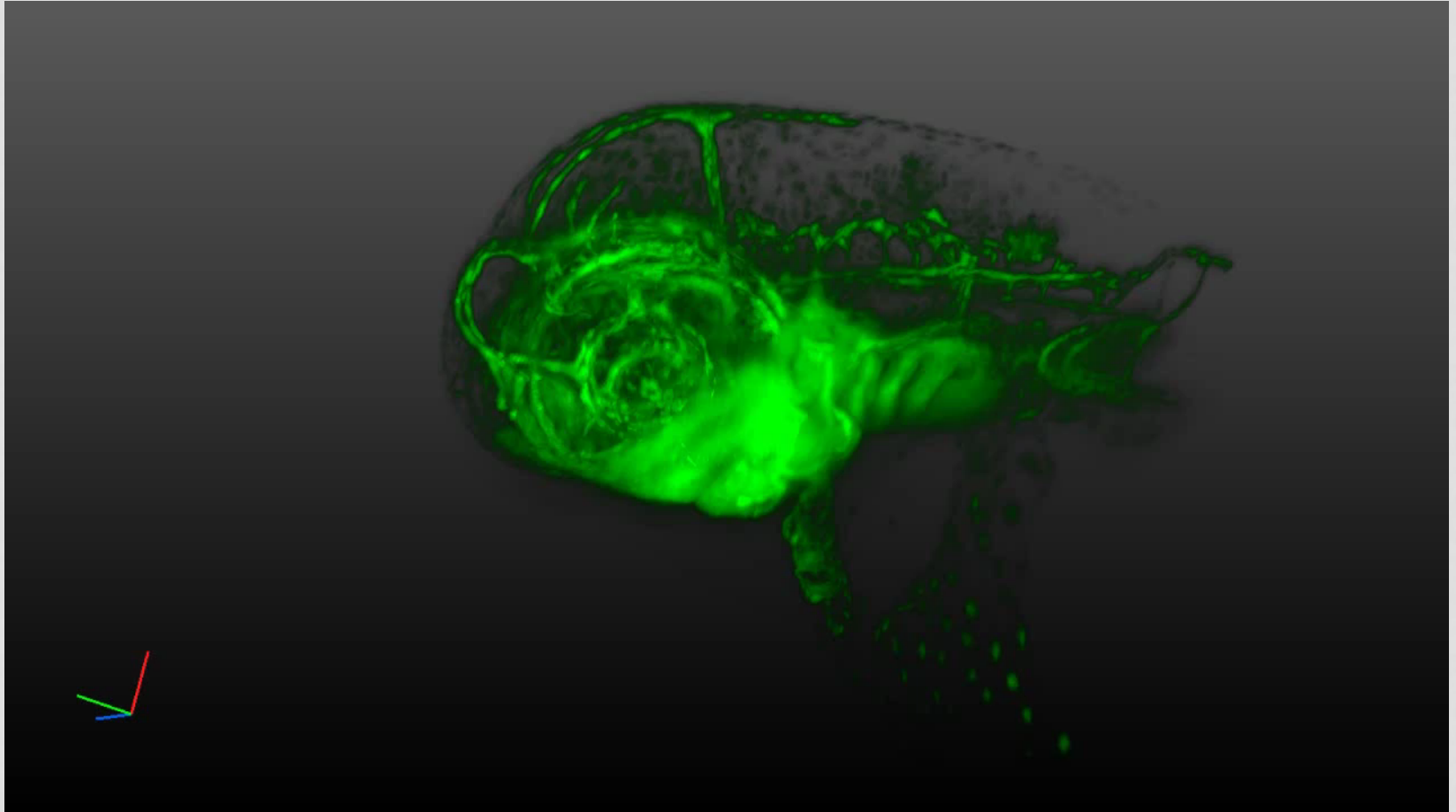
***Arabidopsis thaliana* root**

p35S::GFP-RabA1d

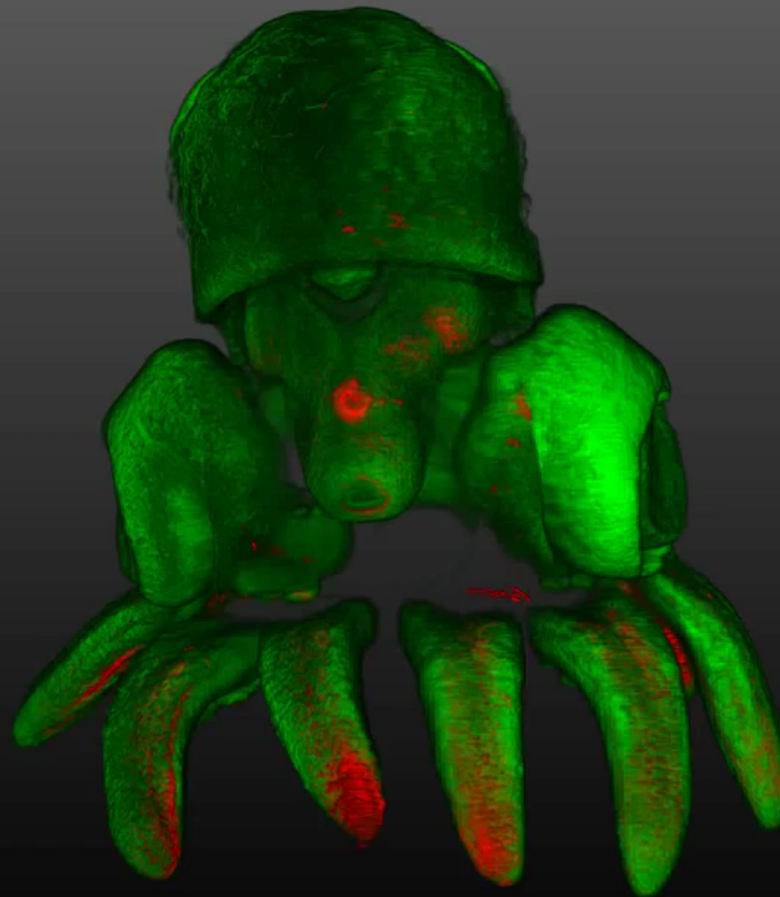
Light Sheet-based Fluorescence Microscopy



EMBRYON ZEBRAFISH



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

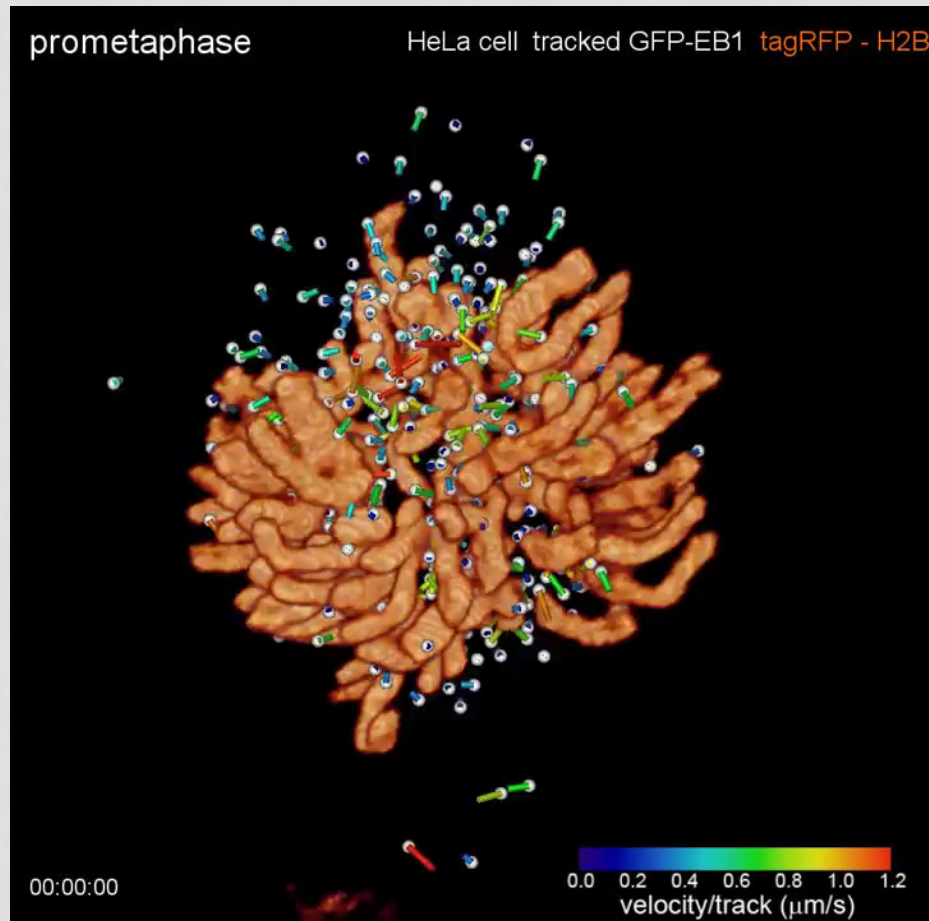


Octopus bimaculoides, age approx 1 month: Light sheet Z1 Zeiss
Eric Edsinger & Daniel S. Rokhsar, Okinawa Institute of Science and Technology
3D-Volume Rendering with arivis Vision4D Zeiss website
<https://www.youtube.com/watch?v=Ep4dxiAeEUg>

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

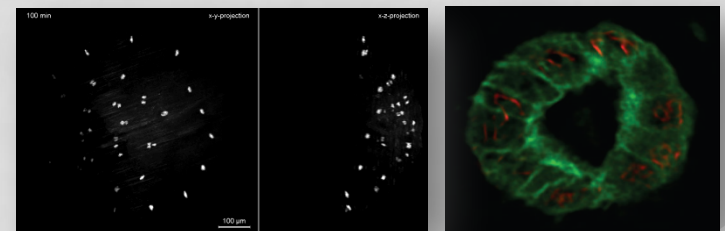
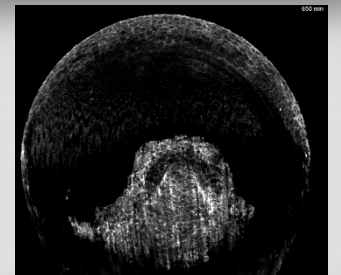
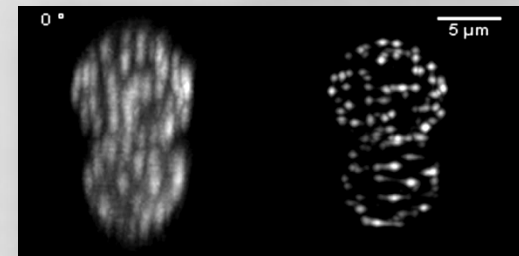
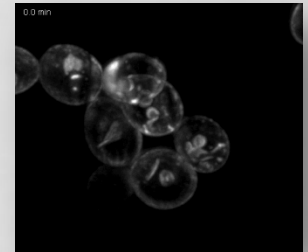
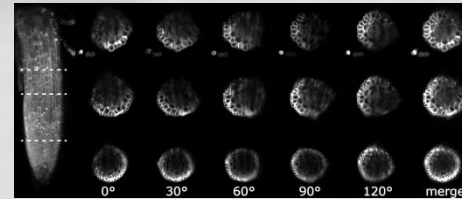
LSFM-SI estimates the background contribution and improves contrast and resolution

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	Σ & Π
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

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

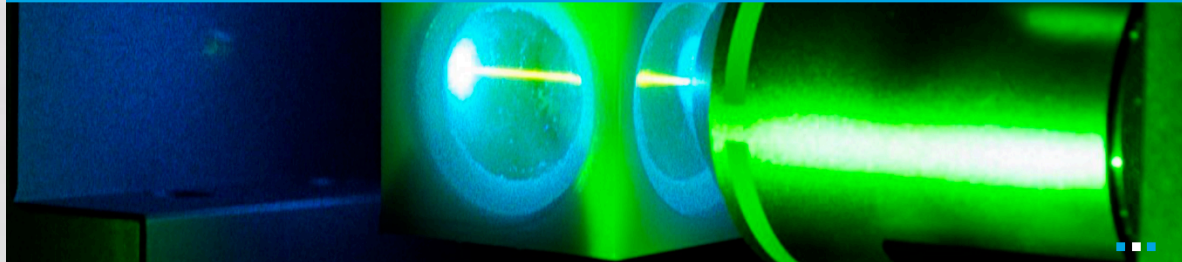
(AVENTUREUX)

- **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 (μ Manager/Fiji) et OpenSpimMicroscopy
 - **DIY « Do-it-yourself »!**

LIGHTSHEET FLUORESCENCE MICROSCOPY INTERNATIONAL CONFERENCE

2nd LightSheet Fluorescence Microscopy International Conference
& 7th LSFM International workshop **GENOA JULY 5 - 8 2015**

[Conference](#) [Committees](#) [Sponsors](#) [Speakers](#) [Program](#) [Registration & abstracts](#) [Venue](#) [Accommodation](#) [Contacts](#)



The 2nd Open Conference on LSFM

Light Sheet Fluorescence Microscopy (LSFM) has recently re-emerged with the implementation of fluorescence-based instruments, and is on the verge of becoming a major and routine technique in such fields as Developmental Biology, Cell Biology, Systems Biology and Oncology. Variants include Single Plane Illumination Microscopy (SPIM), Digitally Scanned Lightsheet Microscopy (DSLIM), Ultramicroscopy, Objective-Coupled Planar Microscopy (OCPM), Oblique Plane Microscopy (OPM), Multidirectional SPIM (mSPIM), Bessel-Beam-based Lightsheet Microscopy, and Individual Molecule Localization SPIM (IML-SPIM). Since 2009, a broad community of scientists involved in the development of LSFM has gathered yearly to celebrate the LSFM International Workshop.

Also this year, as in the previous **1st LSFM international conference**, the community will broaden the impact of the meeting, open to developers, users, scientists and companies. In fact, one of the main goals of the conference is to target developers and users working on or with Light Sheet microscopy.

The conference will feature three days of plenary seminars by experts in the field and presentations of selected abstracts from the scientific community. Light Sheet Fluorescence Microscopy has been indicated as Method of the Year 2014 by **Nature Methods**.

We welcome you to join us in Genoa on July 5-8th 2015.

Featuring the scientific sessions:

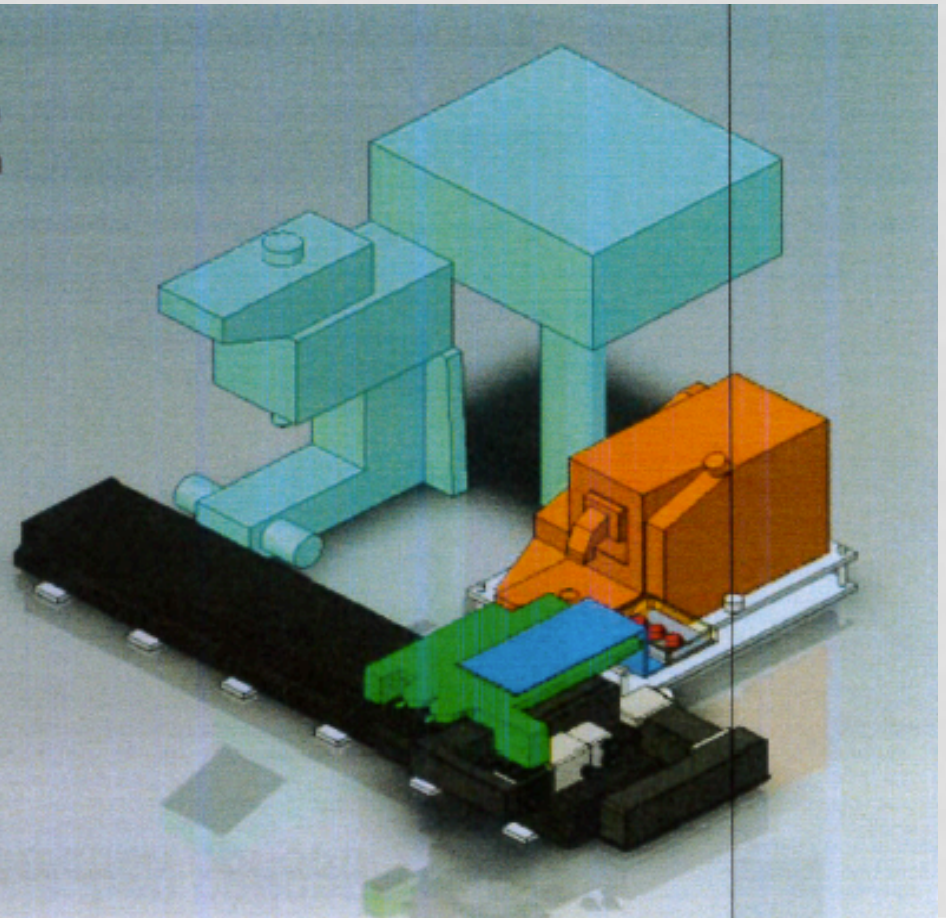
- Lightsheet Live Imaging & Applications
- Lightsheet Engineering
- Ultramicroscopy & Optical Clearing
- Image Analysis & Large Data Challenges
- Lightsheet Technology: New Developments

ABSTRACT BOOK
-
2015

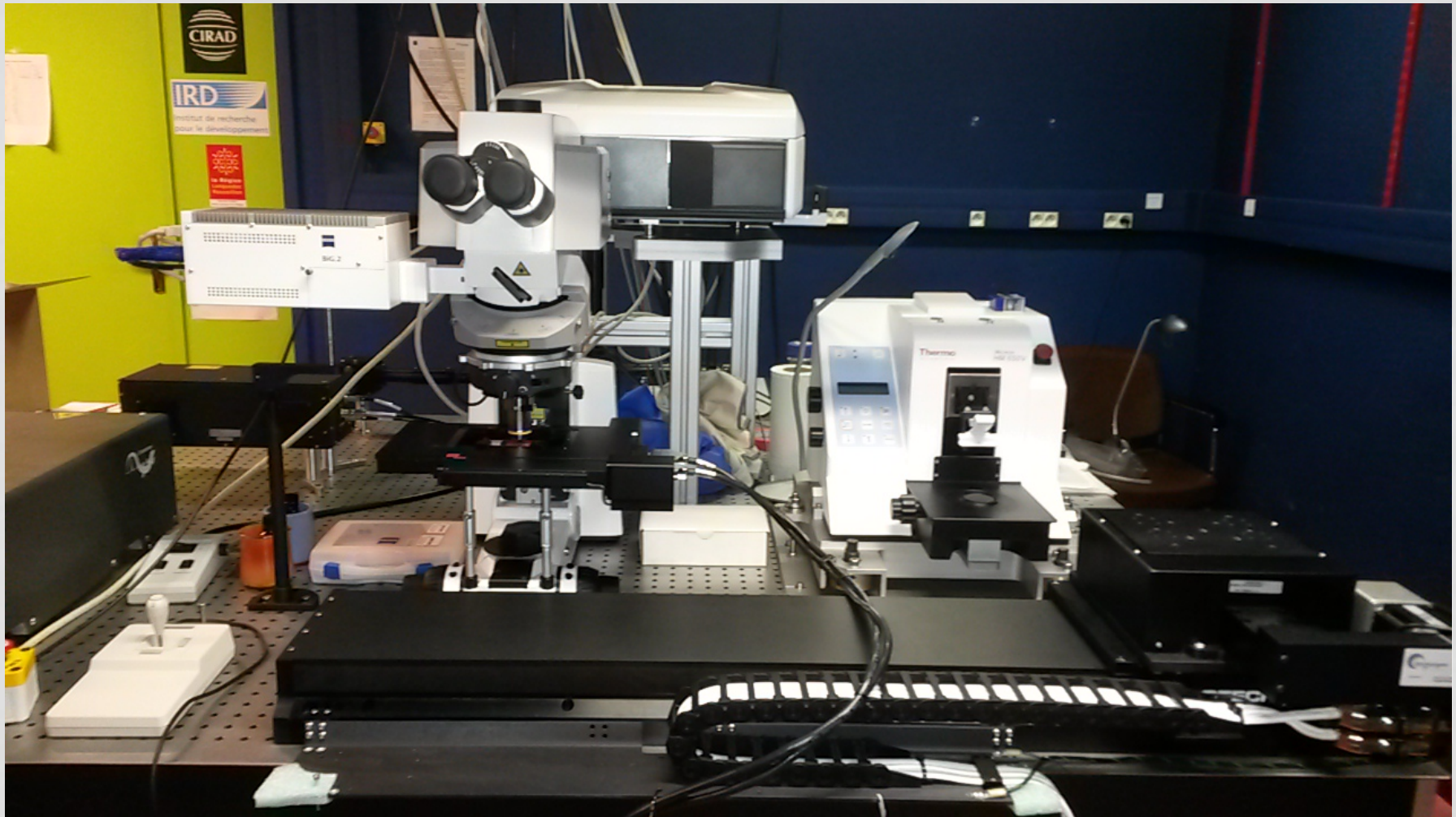
ET POUR LES ÉCHANTILLONS ÉPAIS DENSES, GRANDS??

- Serial Block face imaging

- Un microscope confocal LSM 880 NLO utilisable en mode automatisation et en mode classique
- Un vibratome HM 650 V non modifié
- Une solution d'automatisation robuste, programmable
- Une solution chaque système pouvant être utilisé, le cas échéant hors du système d'automatisation
- Une possibilité de transfert de l'automatisation vers un autre système d'imagerie



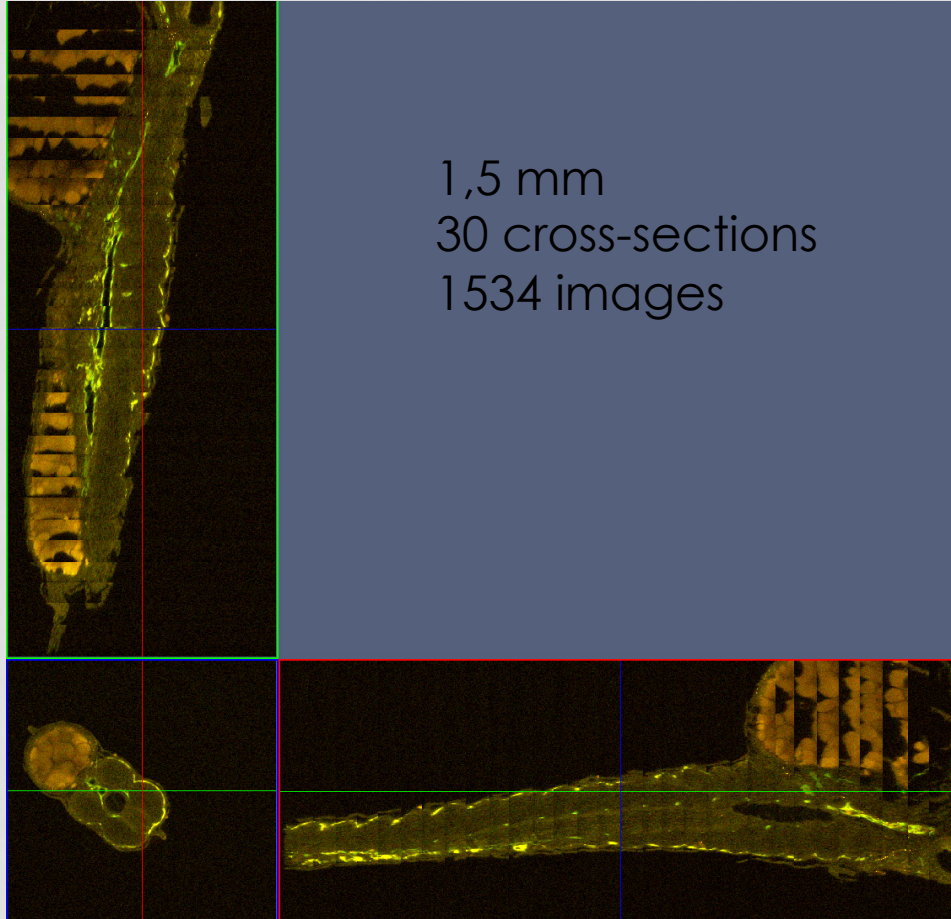
COMBINED SYSTEM CONFOCAL/MULTIPHOTON MICROSCOPE NLO 880 ZEISS AND VIBRATOME



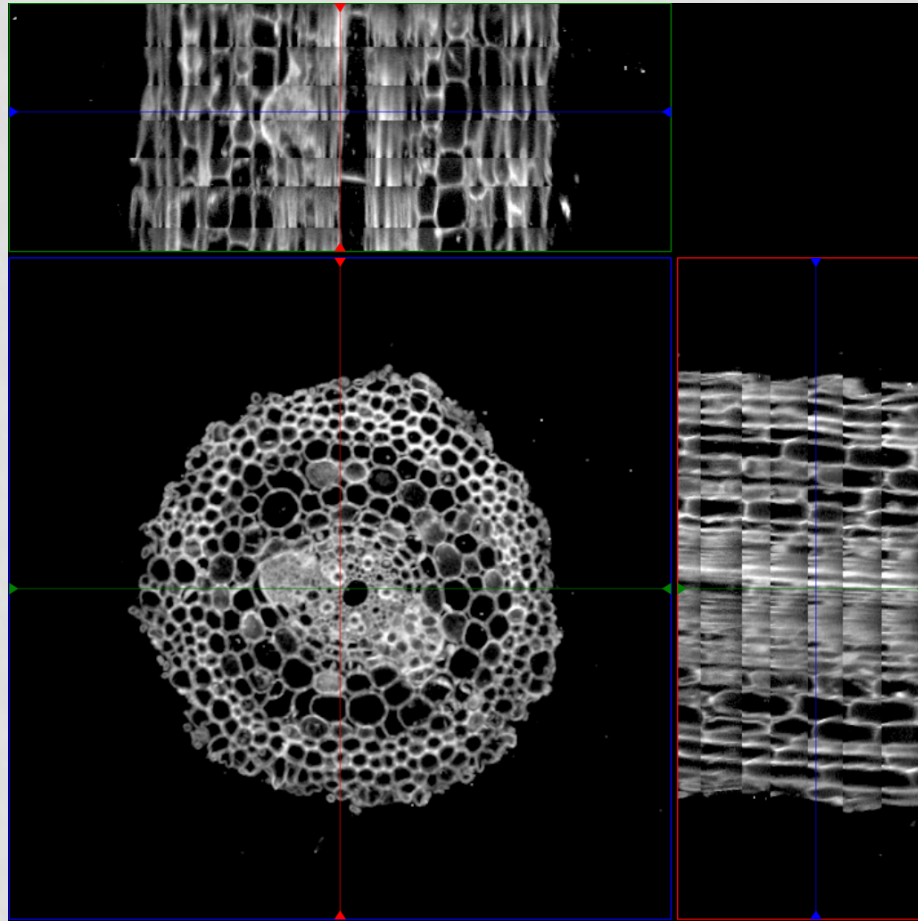
AUTOMATED TISSUES SECTION AND IMAGES ACQUISITION
OF THICK SAMPLES

HIGH THROUGHPUT CELLULAR IMAGING

FIXED EMBRYO ZEBRAFISH




AUTOFLUORESCENCE: RACINE DE RIZ



Multiphoton, 740 nm,

DES QUESTIONS? GDR MIV, RTMFM, PSEN



Perdu ? Besoin d'aide ?

Des ingénieurs et chercheurs au service de la communauté scientifique,
privé comme publique.

TROUVER UN EXPERT ! >

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)

