Imaging Biology in Context in Organs & Tissues:

Lightsheet Imaging

Lecture 13: Advanced Microscopy Course
14th Nov 2019
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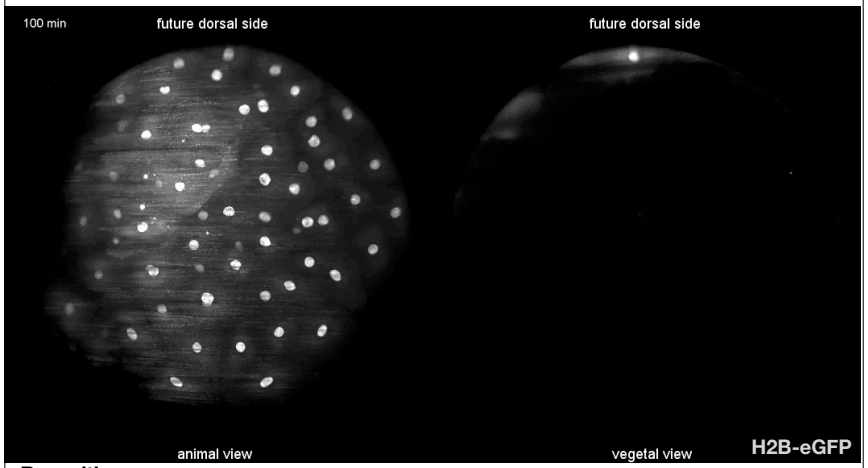
University of Oxford

Department of PHYSIOLOGY, ANATOMY & GENETICS
Defining Excellence
Medical Sciences Division

Micron Oxford

Why use lightsheet imaging?

Live Samples



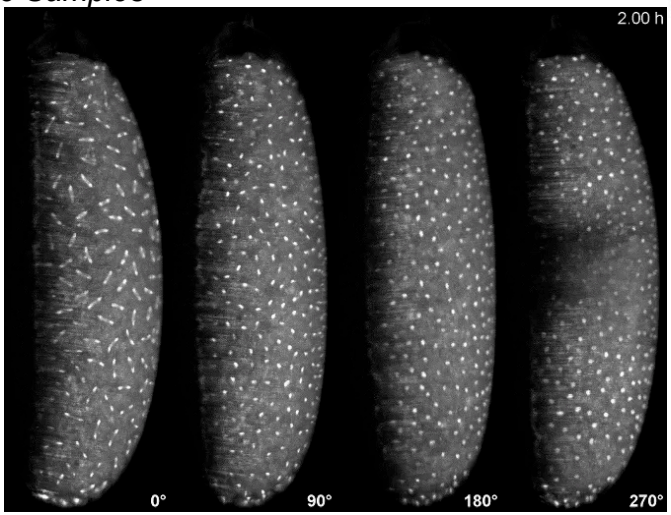
Resoultion
lateral 300 nm
Axial 1000 nm

16,000 cells

Keller et al., (2008) Science, 322

Why use lightsheet imaging?

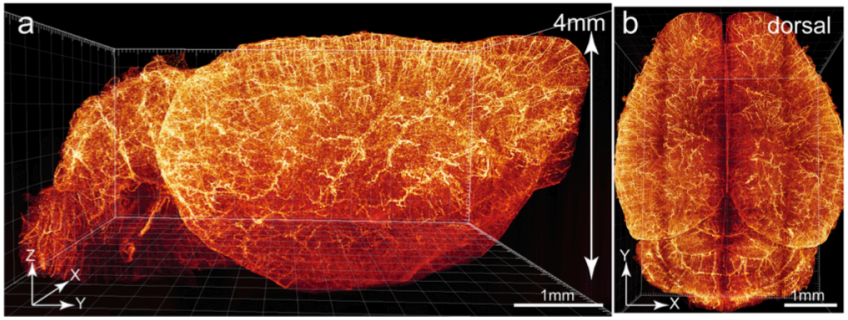
Live Samples



Keller et al., (2010) Nat Meth.

Why use lightsheet imaging?

Large Cleared Samples

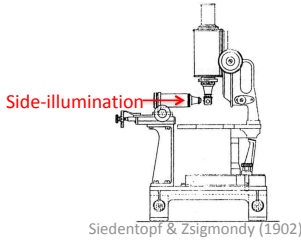


Jing et al., 2018 Cell Res.

Lightsheet microscopy

Background

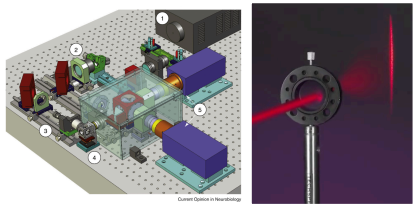
Ultramicroscopy, 1902
bright-field microscopy



Siedentopf & Zsigmondy (1902)

Sunlight projected through a **slit-aperture** to observe gold particles

Lightsheet, 1990's
flourescence microscopy
Ernst Stelzer

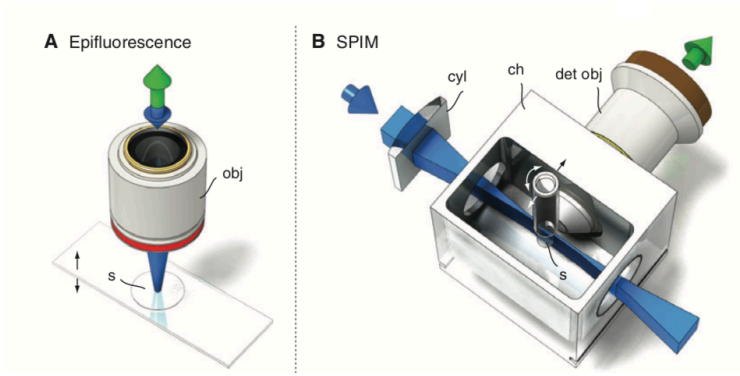


Keller & Stelzer (2008)

Lightsheet formed by a cylindrical lens scanned through a **selected plane** of the sample

Lightsheet microscopy

Background



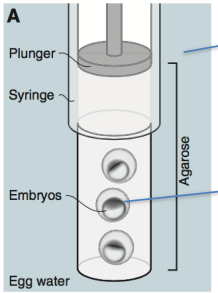
Upright Design

Horizontal Design

Huisken and Stainer (2009)

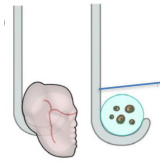
Lightsheet microscopy

Sample Mounting



Fluid filled specimen chamber (Water/PBS/Medium)
Normally try to match refractive index sample mounting – liquid (water RI 1.33)

Sample immobilised in hydrogel (e.g. agarose) and suspended from motorised stage.

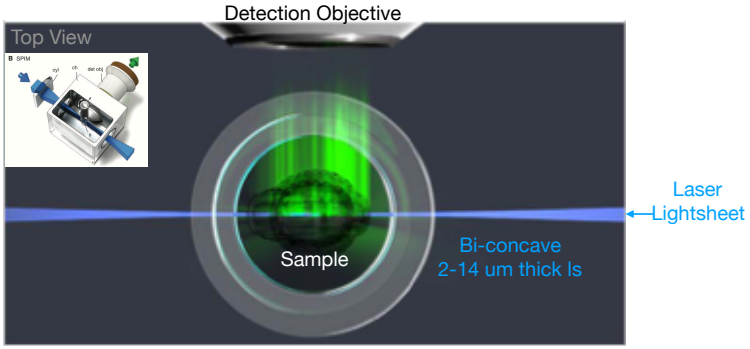


Sample hooked in place & suspended from motorised stage

Huisken & Stainer 2009 Dev 136:1963-1975

Lightsheet microscopy

Selective Plane Illumination



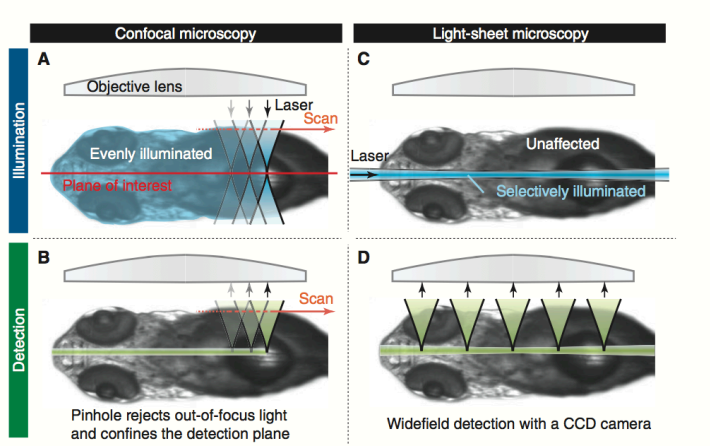
1) **Single plane** of sample - illuminated by laser lightsheet

2) Fluorescent Emission - detected by orthogonally positioned lens

3) Each plane of sample is sequentially exposed to the lightsheet
= **3D volume of the sample imaged**

Lightsheet microscopy

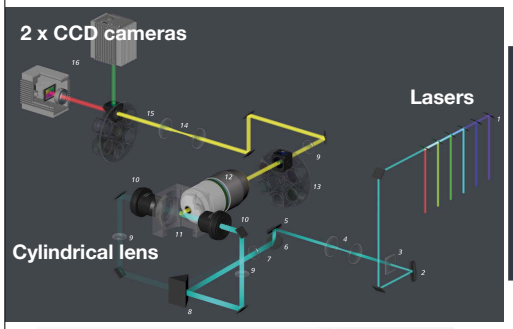
Faster & less damaging

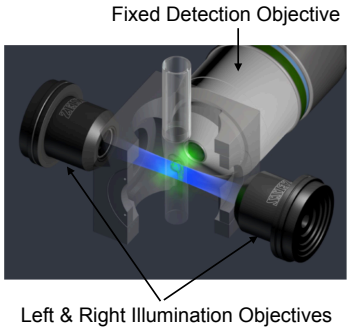


Huisken and Stainer (2009)

Lightsheet microscopy

Dual-Side Illumination





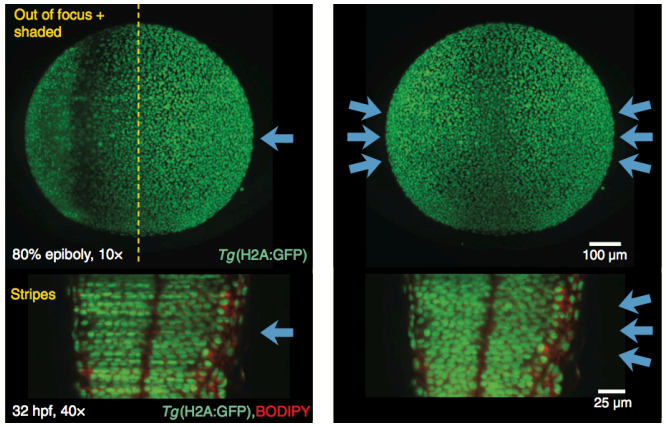
5x/0.16 EC Plan-Neofluar; WD = 5.6mm
20x/1.0 W Plan Apo; WD = 2.4mm (water immersion)
63x/1.0 W Plan Apo; WD = 2.1mm (water immersion)

Lightsheet microscopy

Illumination issues

Single-side
Collimated illumination

Dual-side
Multidirectional illumination

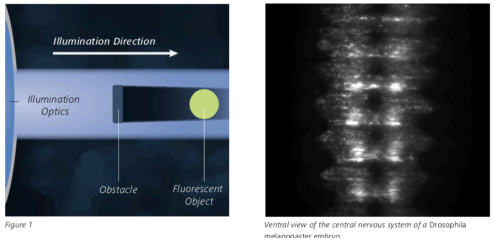


Huisken & Stainer 2009 Dev 136:1963-1975

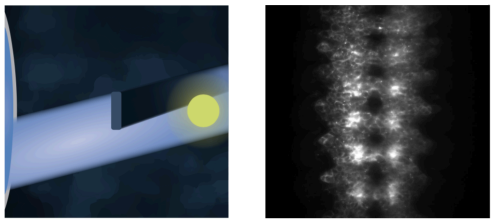
Lightsheet microscopy

Illumination issues

Without Pivot scanner



With Pivot scanner

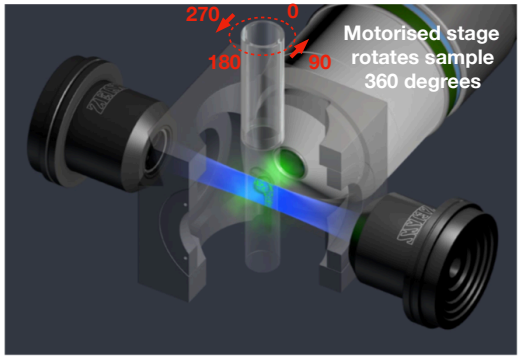


Zeiss

Lightsheet microscopy

Multi-view Imaging

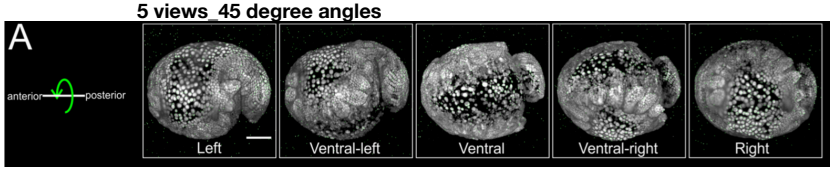
Sample rotation



- The sample can easily be imaged from multiple view angles
- Post-processing is required to form a single data set
- This is improves axial resolution – especially important for large samples

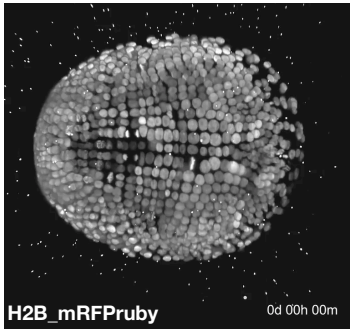
Lightsheet microscopy

5 views_45 degree angles



Parhyale hawaiensis

All 5 views merged into 1 data-data-set



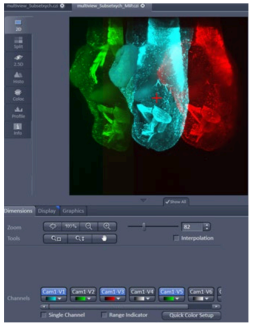
- ~0.2 um diamete fluorescent "fiducial" beads are embedded in the agarose
- By matching up these points, each of the z-stack volumes can be transformed to the same co-ordinate space & merged
- Beads = "microspheres"
  - Blue (365/430 nm)
  - Green (505/515 nm)
  - Orange (560/580 nm)
  - Red (575/600 nm)
  - Dark red (660/680 nm)

Wolff et al., (2018) Elife: 7

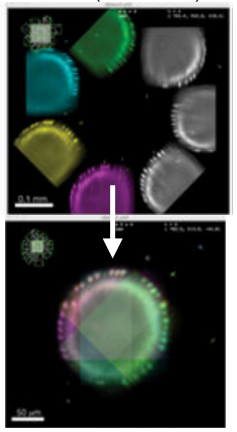
Lightsheet microscopy

Multi-view Reconstruction

Zen
Zeiss Software

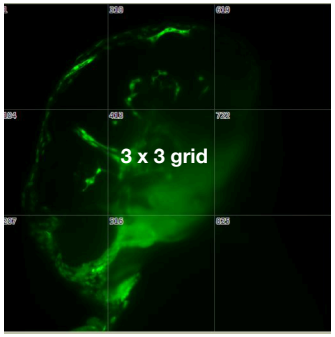


Mutliview Reconstruction
Preibisch (MDC Berlin)



Tiling

Arivis 4D Zeiss Edition



Lightsheet microscopy

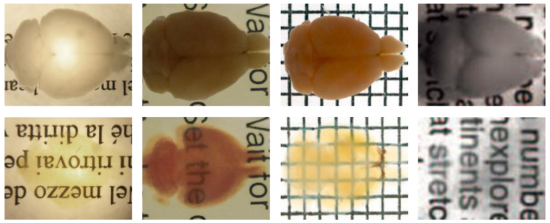
Large Fixed Samples will require clearing

Ethanol
+ BABB

3DISCO

CUBIC

CLARITY
FocusClear



Silverstri et al., 2016 J. Biomed Optics

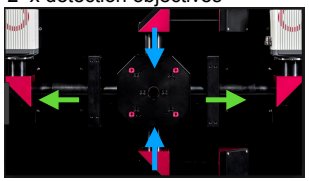
- Multiple Methods exist -e.g. DISCO, PEGASOS, CLARITY, FLUO CLEAR
- Requires empirical testing to ID the best for each sample type
- Protocols can takes days/weeks depending on the sample

Lightsheet microscopy

Commercial Versions

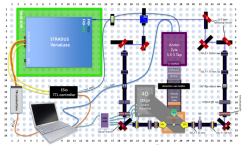
Zeiss Z1 Lightsheet
released 2013

Luxendo (Bruker) Lightsheet
released 2015
2 x detection objectives



Bespoke Versions

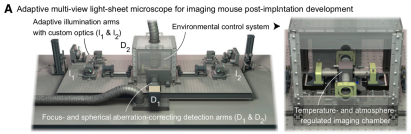
OpenSPIM.org
Step-by-step guide to build your own



Pitrone et al. 2013
Girstmair et al., 2016

$50k

Adaptive multiview microscope
Keller lab (Janelia Research Campus)

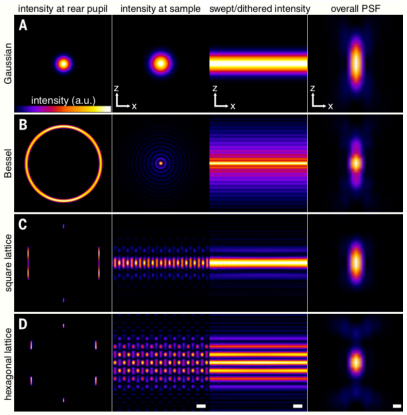


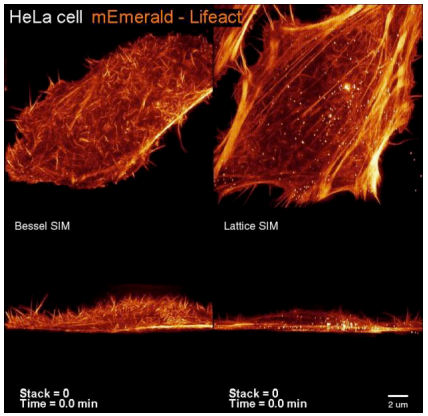
McDole et al., 2018

Lightsheet microscopy

High Resolution LS Microscopy

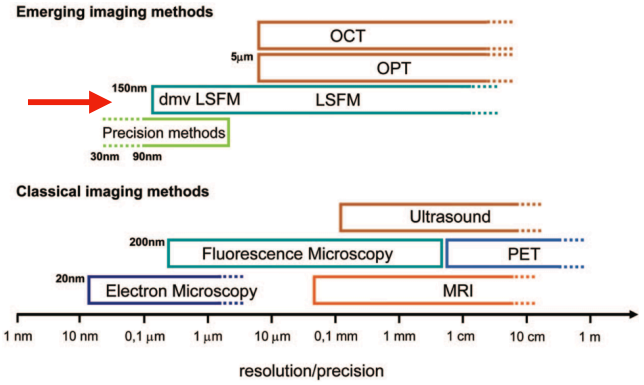
Lattice Lightsheet





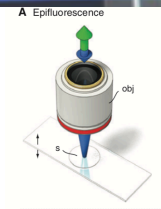
Chen et al., (2014) Sci. 346

Lightsheet microscopy

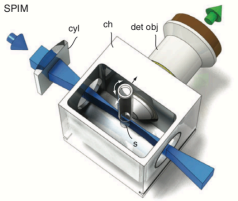


dmv LSFM = deconvolve multiview lightsheet fluorescence microscopy

Lightsheet microscopy



VS



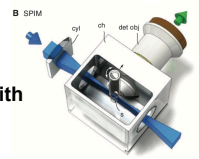
Whole depth of sample illuminated	One focal plane illuminated at a time
Pin-hole reduces out of focus light	No pin-hole (deconvolution post-processing maybe required)
Easy to mount samples	Sample mounting requires optimisation
Slow acquisition (mins/z-stack)	Fast acquisition (secs/z-stack)
Single view imaging	Multiview imaging
Additional modules exist (FRAP/Semi-Super-resolution/Laser ablation)	Requires bespoke equipment/microscope

Lightsheet microscopy

Challenges

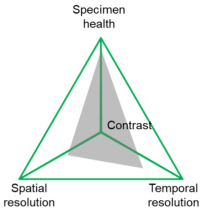
Fixed samples

- Optimisation of clearing **with** IHC/ Fluorescent marker
- Mounting of samples
- Reconstruction of image volumes from multi-view angles



Live imaging

- Mounting of live samples
- Optimising imaging parameters

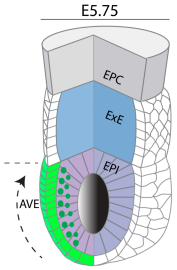


- Post-processing large amounts of data
  -TBs of data!

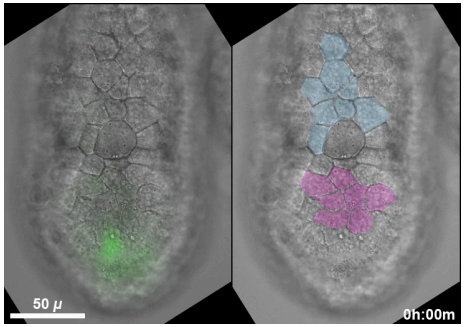
Lightsheet microscopy

Ligthsheet case study

Mouse embryo



Confocal imaging & DIC



Trichas et al., 2012

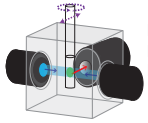
Q- How are Cell Movements Co-ordinated Tissue-wide in VE?

- AVE Migration takes place over 3-5 hours
- E5.5 embryos are highly light sensitive
- Conventional imaging could only capture a sub-set of the embryo

Lightsheet microscopy

Ligthsheet Imaging

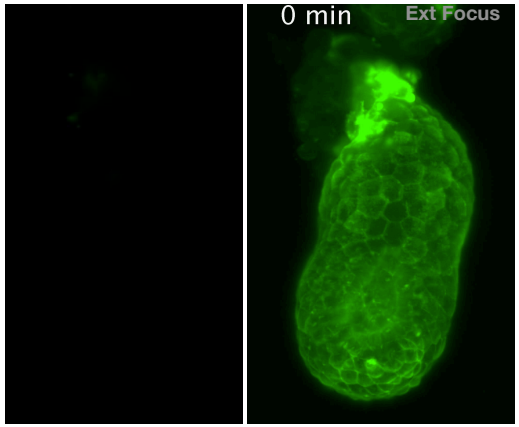
Zeiss Z1 Lightsheet



Fast acquisition
Low phototoxicity
Multi-view angles

2 full z-stacks (2um step)
2 x view angles (0,180)
Every 5 mins
10 hours

Timelapse Datasets



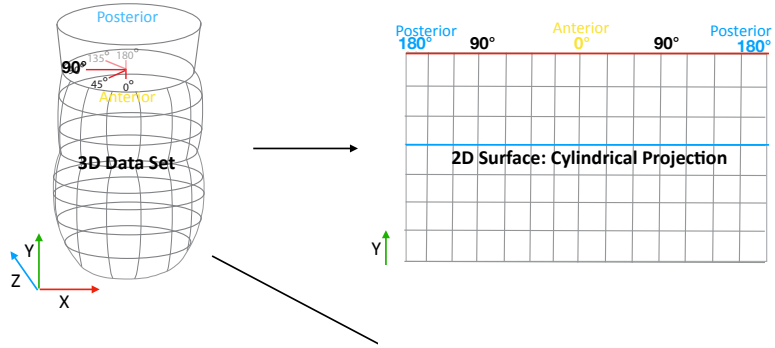
Lightsheet imaging enabled visualisation of all cells in a single embryo
Challenge = Large Data Sets

Lightsheet microscopy

Can we present VE SPIM data in a more visually accessible manner?

Data size 0.5 TB

100 MB



Extract only surface data & re-project it to 2D

rell

Lightsheet microscopy

Surface Data Projections

2 Colour

LS Data

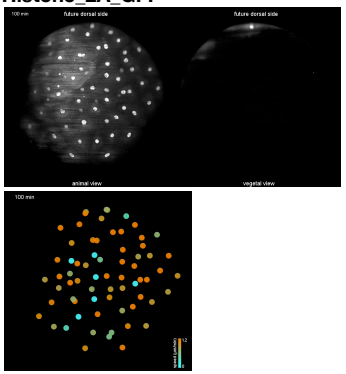
Augmented

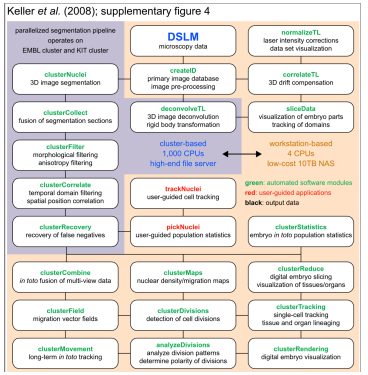
Tracking

Lightsheet microscopy

Extracting Quantitative Data - Cell tracking

Histone_2A_GFP





Automated tracking software

Imaris **MaMut -Massive Multi-view tracking**

Arivis **RACE -Real-time accurate cell-shape extractor**

Lightsheet microscopy

Summary

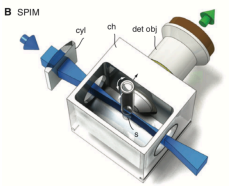
Selective plane fluorescence imaging

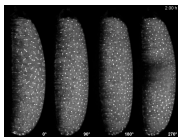
Optically sections samples

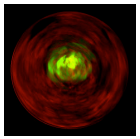
Combines fast acquisition & low photo-toxicity

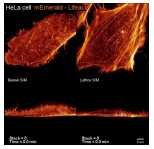
Enables multiview imaging

An emerging technology - we are in the 1st generation of commercial microscopes









Lightsheet microscopy

Any Questions?