Light sheet microscopy
- Fast and gentle 3D imaging

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Imaging = conversation

- limited photon-budget (photobleaching)
- Doesn`t like light and fluorescence (photo-toxicity)
Light sheet microscopy

Wide field microscope
+ illumination
  • lateral
  • by a sheet of light
Confocal microscopy

Light sheet

Fast imaging

Confocal microscopy

Light-sheet microscopy

A

Objective lens

Laser

Scan

Evenly illuminated

Plane of interest

B

Pinhole rejects out-of-focus light and confines the detection plane

Low photo-stress

- 300 v < WF
- 5000 v < LSM

High SNR

SNR 100v > than WF

C

Laser

Unaffected

Selectively illuminated

D

Widefield detection with a CCD camera

= WF

100 > LSM
McDole et al., 2018

Ms embryo, E 6.5 – E 8.5
The Light-sheet microscope

Lasers

fiber
collimator
iris
cylindrical lens
chamber
objective
light sheet
sample
filter wheel
tube lens
CCD camera
“Build the microscope around the sample”

Jan Huisken
The light-sheets
Light-sheet and spatial resolution

- Lateral resolution = Wide Field + high contrast
- Axial resolution depends on ratio Sheet/DOP

2x / NA 0.5

Sheet < DOP: 
↑ Resolution

Sheet > DOP:
= Axial Resolution

↑↑↑ contrast

20 x / NA 1

Depth of focus (DOP)
Playing with Gaussian sheets

Focused Gaussian beam

- NA of illumination lens determines the sheet thickness
- The thinner in the center = the thicker on the borders

- Image borders can be blurry
- Need to adapt the sheet thickness to the sample
- Stripes can happen
The light-sheet journey

Sample prep → Sample mounting → Imaging → Storage → Data analysis

Before
The brain is not a world, it is a world of a number of unmet, uncorrelated, and great stretch of unknown territory.

After CLARITY
The brain is not a world, it is a world of a number of unmet, uncorrelated, and great stretch of unknown territory.

![Image of a brain sample](image1)

![Image of a light sheet](image2)

![Graph showing C-Fos(+) Neurons](image3)
Gentle and creative sample mounting

**Images and Text:**
- **Agarose Beaker**
- **Simax glass**

**Figure 1:** Illustration of the steps to seal the Simax tube that will contain the non-compatable chemicals. The cut barrel of 1 ml syringe is sealed with silicone as well as the Simax tube. Then, the sample coated with AIRM is mounted in the tube and covered with fresh AIRM. Finally, both parts are mounted together.
Light-sheet @ The Crick

Luxendo-Bruker
MuVi-SPIM
Virtual (scanned) light-sheet

The MuVi-SPIM Light Sheet

Dual light-sheet

Light sheet diameter 2.5 – 5.5 µm
Rolling shutter system

- **water**
- **Agar + fluo beads**

**AREA mode**

**Rolling shutter**

Ovaries (Silvana G)
Multicolor imaging with the MuVi Spim

Filters present in

WL width | Band
---|---
440 | 44
479 | 44
525.5 | 57
534 | 70
649 | 302
545 | 38
665 | 270
630.5 | 41
703.5 | 193
728 | 144
MuVi-SPIM Imaging

- Both left and right side are illuminated
- Simultaneous detection of front and back side of the sample
- Subcellular resolution
MultiView Acquisition

Z Fish embryo (Andrew E)
8 views @ 45 deg
Resolution
Lateral = 300 nm
Axial = 800 nm

Egg chamber (Georgina F)

Organoids (Jorge A)
Which sample (and how)

- Max sample size = 1 x 1 x 3 mm
- Water based media (no clearing !!)
- Live / Fixed

Glass capillary, ID:
- 1 mm
- 0.7 mm

FEP tubes (RI 1.33)
Dros. Embryo (Maxime T)

Egg chamber (Georgina F)
DATA WORKFLOW

- Control MuVI-SPIM
- Store data (1 day)
- Quick analysis

10 Gb/s
(real: 600 MB/s)

InfinyBand*
(40-50 Gb/s)

wCAMP000
384 GB RAM

Cluster

- Analysis

Lab PC

Remote Desktop

- Analysis

WORKSTATIONS
(CALM)

- Analysis

CAMP

- Store data (months)
DATA PROCESSING

- 2D Visualization
- Processing
- Stitching

- Conversion
- Opposite fusion
- Multiview/rotation fusion

FIJI
- Load H5
- BigData Tools
- BigSticher

H5 file
- FUSED/resized

Imaris master

BDV

Elastik

IMARIS

• 3D Visualization

BigSticher

H5 file

FUSED/resized

Imaris

• 9.2.1

BDV

•
Luxendo MuVi-SPIM

“Gentle & live imaging”

Samples
- Small (< 1 mm)
- Live (or fixed)

- Embryos (Zebrafish, Drosophila, Ms),
- Organoids/spheroids
- Blastocyst
- Embryonic bodies
- C Elegans?

- Cells tracking during development
- Test drug effect on live sample
- Understand 3D morphology of fragile sample
- Ca2+ imaging
- Optogenetics / photostimulation *

- Cleared samples
- Fluo intensity studies
- 2D cell cultures
- Confetti mice / spectral umixing

CO2/O2
Lavision Ultramicroscope II

Zoom: 0.63-6.3 x
2x / NA 0.5

Sample

Chamber: 120 ml

6 Sheets: 5 – 12 um

Lens protection
More even illumination (dynamic focus)

Sample size: 1 cm x 1 cm x 3 cm
Laser lines and emission filters on the Lavision

488  561  638  705  785
Autofluorescence

AlexaFluor 488
Ex 488; Em 525/50

AlexaFluor 568
Ex 561; Em 595/40

AlexaFluor 647
Ex 640; Em 680/30

Scattering

Absorption

Renier et al, Cell 2014
Example of projects using the Lavision UMII

Lavision test @ the Crick

ECi Clearing

Ms Bone (Stefania DB)
Lavision
Ultramicroscope II

“Cellular question in large samples “

Samples
• Fixed and large (1-10 mm)
• Cleared (BABB, DBE, Eci,..)

- Vasculature, lungs alveoli,..
- Find few cells in big organ
- Tracing sparsely labelled neurons

- Spine density
- Count cells very densely packed
- Live imaging
- Cell colocalization

• Ms brains
• Ms embryos
• Bones
• Adult Drosophila
Summary - Light Sheets @ The Crick

- Young
- New
- Flexible

- Low photo-bleaching/-toxicity
- Fast
- Gentle mounting

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Graph showing a comparison between MuVI and UM II based on size in millimeters and centimeters, with live and fixed samples indicated.
The light-sheet supporters
What`s for you

• Join the CALMUSER@LISTS.CRICK.AC.UK

• Book training (PPMS)
  ❖ Muvi-SPIM
  ❖ Lavision UM *

• Write to me / Deborah Aubyn (CALM)

• Pop up at the facility (SW312)

• Join the Wednesday Imaging HelpDesk
What`s next

- X-Clarity (Labtech):
  - Seminar: **Wed 21\textsuperscript{st} November**
  - Demo: **3-7 December**
- Intro to 2P microscopy (Rocco) **Wed 28\textsuperscript{th} 3 pm**
- Luxendo Workshop: **13-15 February 2019**
- Open SPIM afternoon
- Survey x users

- Any Q or suggestion?