Challenges in Light Sheet Microscopy?

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Summary

- Young and confusing
- Energy, time and space
- Sample preparation
- Image processing
- Data Deluge
- Conclusion
The acronym war...

LS: Light Sheet or **LISH**
UM: Ultramicroscope
OPFOS: Orthogonal Plane Fluorescence Optical Sectioning
LSP: Light Scanning Photomicrography
SPIM: Selective (or Single) Plane Illumination Microscopy
TLSM: Thin laser light sheet microscope
DSLM: Digital Scanned Laser Light Sheet Microscope
LSFM: Light Sheet based Fluorescence Microscopy (very close to LSM???)
OCPI: Objective Coupled Planar Illumination microscopy
OPM: Oblique Plane Microscopy
TSLIM: Thin-Sheet Laser Imaging Microscope
LSBM: Light-Sheet based Microscopy
LISM: Light Sheet Microscopy
**Light Sheet Illumination Microscopy (LSIM)**

...  
**Planar Illumination Microscopy (PIM)**, Azimuthal microscopy

But Light sheet microscopy is more often used...
Single Plane Illumination Microscopy

Light sheet illumination:
optical sectioning
no damage outside light sheet
very low laser power

Detection with regular lens:
focal plane overlaps light sheet
water immersion or air lens
variety of NA & magnification

Sample mounted e.g. in agarose:
translation & rotation
in medium / buffer
physiological conditions

Chamber:
aqueous medium
minimized aberrations
temperature controllable
perfusion (environment)

Huisken, Swoger, Del Bene, Wittbrodt, Stelzer, Science 2004
Single Plane Illumination Microscopy

<table>
<thead>
<tr>
<th>Advantage</th>
<th>Description</th>
<th>Attribute</th>
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<tr>
<td>Shorter time intervals</td>
<td>Record images more often</td>
<td>5 fps instead of 1 fps</td>
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<td>Longer observation periods</td>
<td>Record images for very long periods of time</td>
<td>Days instead of hours</td>
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<td>More images per stack</td>
<td>Improve z-sampling</td>
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<td>Higher signal to noise ratio</td>
<td>Cameras provide excellent dynamic range</td>
<td>12 - 14 bits instead of 3 - 5 bits</td>
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<td>Improved resolution</td>
<td>Resolution determined by NA not statistics</td>
<td>Isotropic resolution 200 nm</td>
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<tr>
<td>Excellent deconvolution</td>
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<td>Isotropic resolution 150 nm</td>
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<tr>
<td>Multiple views</td>
<td>Observe specimen not only from top</td>
<td>Depending on specimen properties 2 – 18 stacks</td>
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<td>Life time imaging</td>
<td>Take advantage of high dynamic range</td>
<td>Record more frequencies</td>
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<td></td>
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<td>Distinguish more lifetimes</td>
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SPIM and friends

SPIM and friends
SPIM and friends
Light Sheet Illumination Techniques

- **FRAP** (Fluorescence Recovery After Photobleaching)
- Laser Nanosurgery
- **FLIM** (Fluorescence Life-Time Imaging)
- Structured illumination
- **STED** (Stimulated Emission Depletion microscopy)
- **FCS** (Fluorescence correlation spectroscopy) and **FCCS**
- STORM, PALM...
- Adaptive optics
- Cell biology applications up to weeks
The Open SPIM to travel with..
<table>
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<th>Sample/Model Organism</th>
<th>Technique/ LSFM implementation</th>
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<td>Fahrback et al, 2010</td>
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<td>Structured illumination</td>
<td>Mouse cochlea</td>
<td>sTSLIM</td>
<td>Schroter et al, 2011</td>
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<td>Light Sheet Characteristics</td>
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<td>SPIM</td>
<td>Ritter et al, 2008</td>
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<td>C. elegans</td>
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<td>Olarte et al, 2012</td>
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<td>Image View fusion</td>
<td>live sea urchin embryo, live Danio rerioembryo</td>
<td>LSFM</td>
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<td>Engelbrecht et al, 2007</td>
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<td>mRNA nuclear export</td>
<td>Chironomus tentans Salivary Glands</td>
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<td>Siebrasse et al, 2012</td>
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<td>Heterochromatin dynamics</td>
<td>MCDK cells, Drosophila melanogaster</td>
<td>LSFM (FCS)</td>
<td>Capoulade, 2011</td>
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<td>Drosophila melanogaster</td>
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<td>Ejsmont et al, 2009</td>
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<td>Marine microbiology</td>
<td>Various bacteria, protozoa etc.</td>
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<td>Fuchs et al, 2002</td>
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<td><strong>Cell Biology</strong></td>
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<td>Adaptive optics to improve imaging performance</td>
<td>Tumour spheroids</td>
<td>waoSPIM</td>
<td>Jorand et al, 2012</td>
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<td>Intracellular imaging</td>
<td>Mammalian cell organelles</td>
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<td>Zanacchi et al, 2011</td>
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<td>Maizel et al, 2011</td>
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<td>Drosophila embryo</td>
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<td>Huisken et al, 2004</td>
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<td>Danio rerio</td>
<td>mSPIM</td>
<td>Kaufmann et al, 2012</td>
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<td>Cell identity lineaging and neurodevelopmental imaging</td>
<td>Caenorhabditis elegans</td>
<td>iSPIM</td>
<td>Wu et al, 2011</td>
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<td>Drosophila melanogaster</td>
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<td>Kalinka et al, 2010</td>
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<td>3D reconstruction of inner ear</td>
<td>Cavia porcellus</td>
<td>OPFOS; LSFM</td>
<td>Hofman et al, 2009</td>
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<td>Brain in vivo imaging</td>
<td>Microspheres</td>
<td>miniSPIM</td>
<td>Engelbrecht et al, 2010</td>
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<td>3D reconstruction for morphological analysis</td>
<td>Bast's valve</td>
<td>OPFOS</td>
<td>Hofman et al, 2007</td>
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<td>Scan of whole brain</td>
<td>Mouse brain</td>
<td>LSFM</td>
<td>Mertz and Kim, 2010</td>
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<td>Neural network imaging</td>
<td>Mouse brain</td>
<td>Ultramicroscope</td>
<td>Dodt et al, 2007</td>
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<td>Sectioning of thick tissues</td>
<td>Mouse cochlea/zebrafish inner ear, brain/ rat brain</td>
<td>TSPLIM</td>
<td>Santi et al, 2009</td>
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<td>Imaging neuronal activity</td>
<td>Mouse vomeronasal cells</td>
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<td>Mouse</td>
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<td>Klohs et al, 2008</td>
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<td>Optical sectioning</td>
<td>Meriones unguiculatus cochlea, Hippocampus reidi head, Xenopus laevis</td>
<td>OPFOS</td>
<td>Buytaert et al, 2012</td>
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<td><strong>Large organism general biology</strong></td>
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<td>Whole organism 3D reconstruction</td>
<td>Ormia ochracea; Emblemamosoma audixtrix</td>
<td>LSP</td>
<td>Huber et al, 2001</td>
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<td>Whole organism 3D reconstruction</td>
<td>Drosophila melanogaster</td>
<td>Ultramicroscope</td>
<td>Jahrling et al, 2010</td>
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<td>Imaging copepod gut contents</td>
<td>Calanus pacificus</td>
<td>PLIF</td>
<td>Jaffe et al, 2009</td>
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Light Sheet Illumination

- Architecture (upright, azimuthal...)
- Illumination (lens, scanner, Bessel beams...)
- LISH angle (90 degrees...)
- Add-ons (nanosurgery, adaptive optics...)
- Camera numbers
- Drivers and computers
Light Sheet Use

- Single plane (FCS...)
- Single stack
- Multiview stacks
- + Time
Ultramicroscope

Whole brain imaging at cellular resolution
3D high speed imaging without bleaching
High throughput phenotype screening

LAVISION BIOTECH

H. Siedentopf and R. Zsigmondy,
Light Sheet-based Fluorescence Microscope for long-term live imaging

- Turn-key
- Compact
- Laser Safe
Sample preparation

• The sample is a 3D object
• Different sizes and types of samples
• Mounting techniques
  – Embedded samples
  – Hanging samples
  – Flat samples
  – Enclosed samples
• Cell biology applications
The sample is a 3D object

- Glass support
- Fixed orientation
- Fixative
- Mounting media
- Pressure…

Illumination

Detection

- Support from above
- Rotation
- Fixative
- Objective
- Wet environment (stability…)
- Light sheet (penetration)
- Objective (obstacle…)
- Hanging mounting (gravity…)

LSFM specificities
Different sizes

A: Very large object
B: Large object
C: Medium size object
D: Small object
Different sizes and types of samples

- 2.5X, 5X, 10X, 20X, 40X, 63X, 100X
- Large samples (mm)
  - *Mus musculus* (brain)
  - *Anopheles Gambiae*...
- Mid range samples (μm)
  - *Drosophila melanogaster* ovaries
  - Cell clusters, cysts...
- Small samples (μm to nm...)
  - *Saccharomyces cerevisae*
  - Single microtubules
A few more points…

• **Media** (Air, water, PBS, oil…)

• **Fixed or alive** (Temperature, pH, drugs, anesthesia…)

• **Labelling** (Dyes, diffusion, penetration, washing…)

• **Clearing** (Penetration…)

• **Accessibility** (Size ratio (chamber, objective)…)

• **Time** (Stability, movements…)
Mounting techniques

EMBEDDED

HANGING

FLAT

ENCLOSED

Side View

Top View
Embedded samples
d. melanogaster  pupa mounting

- dissection
- self alignment
- dissected organs, embryos…
Gelling agent related problems

- **Optical properties**
  - light sheet penetration
  - detection limitations
  - aberrations

- **Physical properties**
  - melting temperature
  - gelling temperature
  - air drying
  - gelling stability

- **Gel properties**
  - Ashes
  - Structure
  - Diffusion

- **Preparation**
  - purity
  - air content
  - homogeneity
Embedded samples

- *S. cerevisiae*
- Copepod
- *D. melanogaster* head
- *D. melanogaster* ovary
- *c. elegans* gut
- Cell balls
- Cell cysts
- Spheroids
- Fish embryos...

Hanging samples

- Stability (rotation...)
- Contact
- Accessibility
- Live samples?
Flat samples

- Accessibility
- Rotation
- Contact
Flat samples

Planchon et al., 2011
Enclosed samples
Enclosed samples

MDCK cells cultured for 10 days in matrigel, inside an agarose beaker within Matrigel (37°C, 5% CO₂)
Enclosed samples

A. thaliana
- development and growth
- stability
Enclosed samples

Kaufmann, A et al, Development 139, 3242-3247 (2010)
Enclosed samples

- Diffusion
- Accessibility
- Rotation
- Assays

Keller et al, Nat. Meth, 2007
Cell biology applications

- Three dimensional growth conditions
- Support (scaffolds, matrices)
- Protocols
- Imaging fixed and live cells
- 3D culture chamber
SPIM-compatible perfusion chamber
Environmental control of the 3D culture

- CO₂ 5% / air
- Culture media
- Peristaltic pump
- Tight flexible foil
- Probe holder
- SPIM cell culture chamber
- Media inlet
- Media outlet
- Heating block 37°C
- Waste
Image processing

- Stacks...
- Multiple stacks...
- Channels
- Time, gravity and movement
- Data deluge
- Registration
- Fusion
- Add-ons
- Artefacts (stripes, blurring)
Image processing

A

B

C

D

E

F

G

Optical axis

Illumination

Light sheet

Image processing
1. Signal Degradation
2. Limited Overlap
3. Varying orientations of the optical sections
4. Development of the specimen
5. Scaling introduced by refractive index change
Opaque/complex sample
Rotation to access ROIs
Compensate absorption
Combination allows reconstruction of details invisible in single stack, but require huge amounts of data (up to several hundred GByte) and heavy processing
A Drosophila experiment...

- Multi-View acquisitions
- Multi-Channel acquisitions
- Time-lapse acquisitions
  - Preview
  - Compression?
- Acquisition speed is very high over 24 hours
  - 5-50 fps @ 1 Megapixel (soon 4-8 Megapixel)
- 0.5-4 TB raw data per experiment
  - 100s of terabytes of data

Welcome to Big Data Land!!!!
Multi-View

Nuclei in red
Membranes in cyan

Jan Huisken
Bioessays 34: 406–411
Examples of recombination
SPI mage fusion

Single view  orthog. view  2-view fusion  4-views  8-views

"real" space

Fourier transform

Phase space

PSF: small = high resolution

OTF: large = high resolution

Swoger, Huisken & Stelzer, Opt Lett, 2003
Verveer, …, Stelzer, Nature Methods, April 2007
Swoger, … & Stelzer, Opt Expr, 2007
SPI mage processing: pollen

Auto fluorescence of a Paper Mulberry pollen

Slices from 3D data sets

Images are ~ 20 μm square

Raw, single view
Deconvolved, single view

Fusion, 2 views
Fusion, 3 views
Fusion, 6 views
Fusion, 18 views

Fusion, 18 views (no deconvolution)

Figure by J. Swoger.

Swoger, Huisken & Stelzer, 2003
Swoger, ... & Stelzer, Opt Expr, 2007
A real example (single view)

SPIM, auto-fluorescence, 5x NA
0.25 air lens, 488LP filter
Multi-view SPIM
Multi-view SPIM
Multi-view SPIM

Record image stack

Illumination

Record image stack
Multi-view SPIM

Record image stack

Illumination
Pre-processing
Image registration
Multi-view image alignment
Registration fusion

Intensity based
- No embedding necessary
- Sample independent
- Typically slow
- Hard to cope with developing samples
- Result hard to verify automatically

Bead based
- Very fast
- Sample independent
- Easy use with developing samples
- Automatic verification
- Embedding in rigid medium

Segmentation based
- Potentially fast
- Automatic verification possible
- No embedding necessary
- Staining dependent
- Hard to cope with developing samples

P. Shaw et al., in Biophysical Journal 55, 1989.
R. Heintzmann et al., in Analytical Cellular Pathology 20, 2002
R. Heintzmann et al., in Journal of Microscopy 206, 2002
J. Swoger et al., in Optics Express 15, 2007.


P. Keller et al., in Science, 2008..
P. Keller et al., in Nature
S. Preibisch et al., in IEEE ISBI, 2008.
Bead based Registration Framework

What to remember...

- Which LISH do you have?
- Which sample do you want to image?
- Can you mount it?
- Plan your data flow!!!
- Plan your image processing!!! (scale it down first!!!)
- Enjoy!!!