Processing Multi-View Selective Plane Illumination Microscopy Data

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The SPIM
(Selective Plane Illumination Microscope)


Optical Sectioning
Fast
High SNR
Sample Rotation
Low Bleaching
Light Sheet Illumination
Isotropic Resolution
CCD Camera
In toto Live Sample Imaging
(Drawing adapted from Huisken et. al.)
Registration - A lot of Problems

1. Signal Degradation

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2. Limited Overlap

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3. Varying orientations of the optical sections

Registration - A lot of Problems

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2. Limited Overlap
3. Varying orientations of the optical sections
4. Development of the specimen

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5. Scaling introduced by refractive index change

Registration - A lot of Problems

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
6. Size of the datasets (up to terrabyte range)

Compensate for incomplete sample coverage

Achieve in toto imaging

Potential Registration Approaches

Intensity based

- No embedding necessary
- Sample independent

- Typically slow
- Hard to cope with developing samples
- Result hard to verify automatically

S. Preibisch et al., in *IEEE ISBI*, 2008.
S. Preibisch et al., in *SPIE Medical Imaging 2008*. 
Potential Registration Approaches

**Intensity based**
- No embedding necessary
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**Bead based**
- Very fast
- Sample independent
- Easy use with developing samples
- Automatic verification
- Embedding in rigid medium

R. Heintzmann et al., in *Analytical Cellular Pathology* 20, 2000.
R. Heintzmann et al., in *Journal of Microscopy* 206, 2002.
J. Swoger et al., in *Optics Express* 15, 2007.
S. Preibisch et al., in *IEEE ISBI*, 2008.
S. Preibisch et al., in *SPIE Medical Imaging 2008*.

Potential Registration Approaches

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- Very fast
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- Automatic verification
- Embedding in rigid medium

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

W. Wein et al., in *proceedings of MICCAI*, 2007.
S. Preibisch et al., in *IEEE ISBI*, 2008.
S. Preibisch et al., in *SPIE Medical Imaging 2008*.

S. Preibisch, S. Saalfeld T. Rohlffing, P. Tomancak, in *SPIE Medical Imaging 2009*.
Bead based Registration Framework

Bead based Registration Framework


Incorporate Beads
Bead based Registration Framework


Incorporate Beads

Local Descriptor Matching
Bead based Registration Framework


Incorporate Beads

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Incorporate Beads

Local Descriptor Matching

Robust Outlier Removal (3d-affine)

View 0°

View 45°
Nuclei based Registration

Drosophila
20x/0.5NA

Correspondences  
122

Average Error  
1,37 px

Computation Time  
112 seconds
Nuclei based Registration

RANSAC

Drosophila
20x/0.5NA

C. elegans
100x/1.0NA

Correspondences
122
156

Average Error
1,37 px
1,80 px

Computation Time
112 seconds
97 seconds
Nuclei based Registration

RANSAC

Drosophila
20x/0.5NA

C. elegans
100x/1.0NA

Zebrafish 6dpf
20x/0.5NA

Correspondences
122
156
92

Average Error
1,37 px
1,80 px
2,97 px

Computation Time
112 seconds
97 seconds
110 seconds
Nuclei based Registration

Global Optimization

Iteration 0 (???.?? px)
Zebrafish 48 hours post fertilization
Zebrafish 48 hours post fertilization
Multi-View Deconvolution

C. Elegans embryo
4-cell stage
Ph-GFP Lipid binding domain

Angle 0  Angle 60  Angle 120  Angle 180  Angle 240  Angle 300
Multi-View Deconvolution

C. Elegans embryo
4-cell stage
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Angle 0
Angle 60
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Content based Fusion

Multi-View Deconvolution

<table>
<thead>
<tr>
<th>Angle 0</th>
<th>Angle 60</th>
<th>Angle 120</th>
<th>Angle 180</th>
<th>Angle 240</th>
<th>Angle 300</th>
</tr>
</thead>
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Deconvolution makes use of

**Multiple Observations of the same Scene**

Content based Fusion

Multi-View Deconvolution

U. Kržič et al., submitted.
Bead-based time-lapse registration

One machine with 8 cores, 64 GB RAM

12 hours for 249 time points
Implementation in ImgLib

- Open-Source + Plugins available through Fiji
- **ImgLib** – Generic Image Processing in Java
- Dimension-, storage- and datatype independent
- Generic, more algorithm-like programming
- Framework underlying the new NIH-funded ImageJ2

What are we doing now with SPIM?
Fluorescent reporters

- Bicoid (patterning)
- Polo Kinase (mitotic activity)
- Senseless (peripheral nervous system)
- Cysteine String Proteine (membrane protein)
- Neuronal Synaptobrevin (neurotransmitter release)

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What is new, what should be stored?

• 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 Megapixels)
    • 0,5-4 TB raw data per experiment
  – 100s of terabytes of data
• Good news: Data is usually accessed sequentially
What is new, what should be stored?

• Registration
  – Rotation axis
  – Rotation angle (if known)
  – At least affine transformation models for each view
  – Higher-order transformation models?
  – Point detections for each view?
  – Exemplary overlays to visualize registration quality
  – Keep the original stacks!
What is new, what should be stored?

• Fusion
  – Illumination direction (modeling of light)
  – Light sheet type (gauss, bessel, single/multi-photon)
  – Light sheet thickness
  – Embedding medium
  – Point spread functions of each view
  – Point spread function is variant
  – Fused output image + preview (3d renderings?)
What is the future of SPIM?

• Many research groups build their own SPIM microscopes or derivatives

• Own conference series

• Focus starts to turn from microscope development into normal application

• There is going to be a commercial microscope by Zeiss at some point

• Great chance if OMERO supports such image data – currently it is more or less a mess to deal with those amounts of data
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http://pacific.mpi-cbg.de/