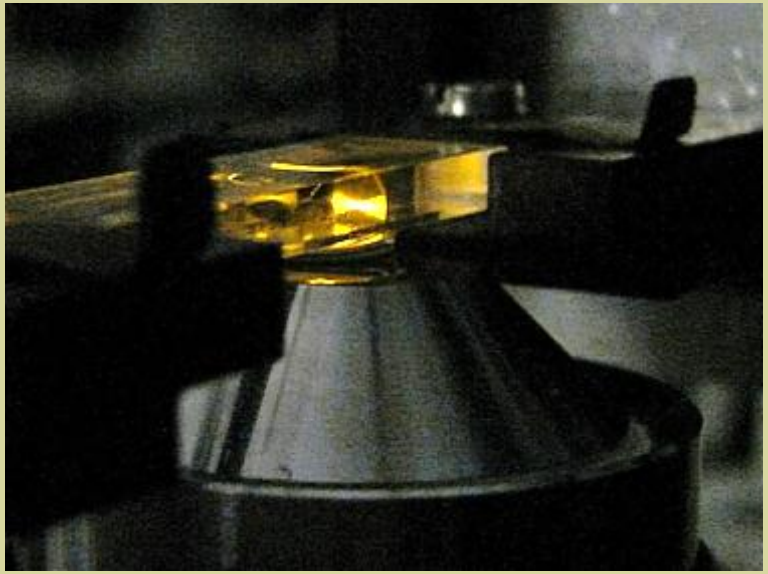
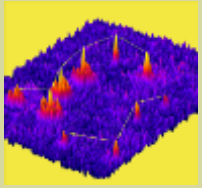
Course: Optics, forces & development

In vivo 3D-microscopy for the analysis of cell behaviour in developing embryos

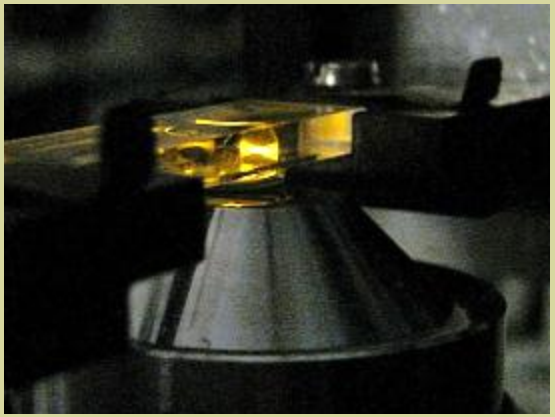


Applications of light sheet microscopy



Jan-Hendrik Spille
Institute for Physical and Theoretical Chemistry
Rheinische Friedrich Wilhelms-Universität Bonn

universitätbonn

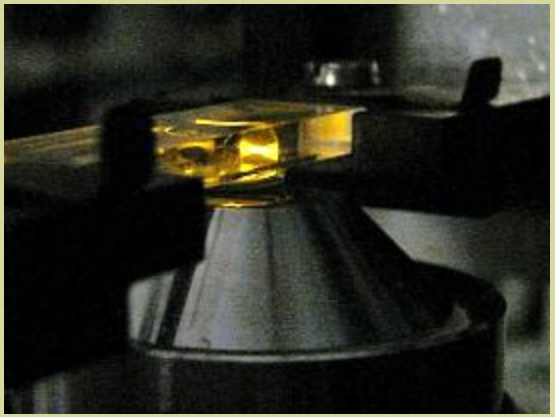


Applications of light sheet microscopy

- **Basics:**
  - Why use a light sheet microscope?
  - Fundamental optics
  - State of the art instruments and latest developments
  - Commercial instruments
  - Data processing

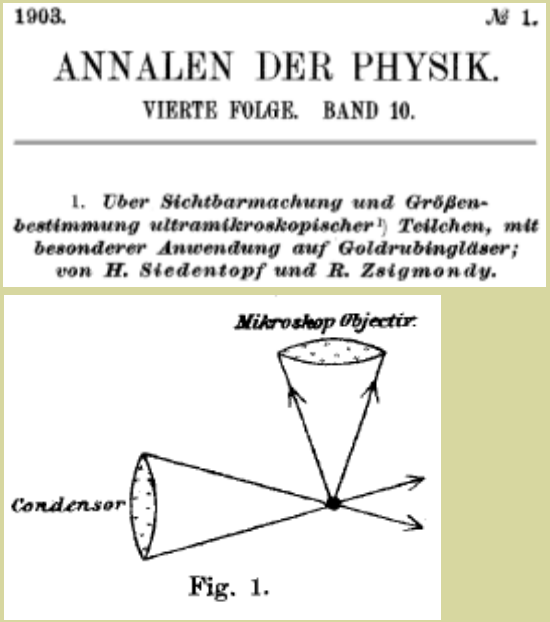
- **Applications:**
  - Cleared specimen: Neurons in the mouse brain
  - Classics: Zebrafish and Drosophila embryogenesis
  - High speed imaging
  - Superresolution and single molecule imaging

- **Literature**



Light sheet basics

- Why use a light sheet microscope?
- Fundamental optics
- State of the art instruments and latest developments
- Commercial instruments
- Data processing


1903. № 1.

ANNALEN DER PHYSIK.

VIERTE FOLGE. BAND 10.

1. *Über Sichtbarmachung und Größenbestimmung ultramikroskopischer*[1] *Teilchen, mit besonderer Anwendung auf Goldrubingläser;*
von H. Siedentopf und R. Zsigmondy.

Fig. 1.

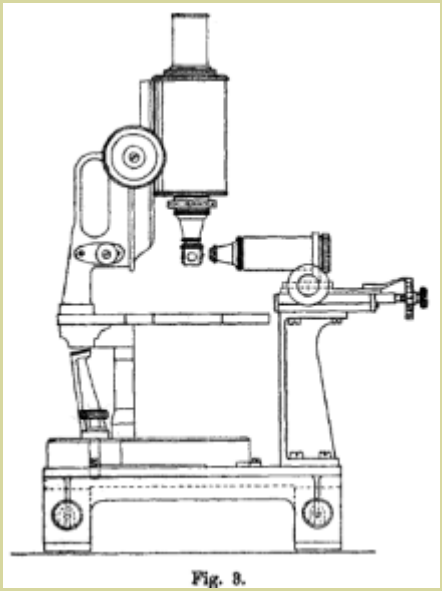
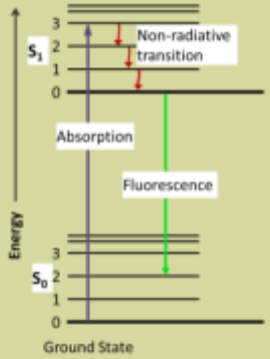


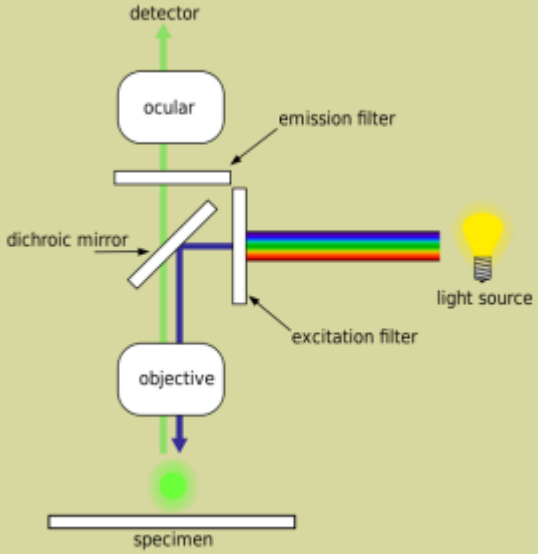
Fig. 3.

[Siedentopf, Zsigmondy, Annalen der Physik 4(10), 1903.]

1903 – ultramicroscopy:

- „ultramicroscopy“ of sub-wavelength gold particles
- diffraction limited imaging
- illumination: sun light
- detection: eye



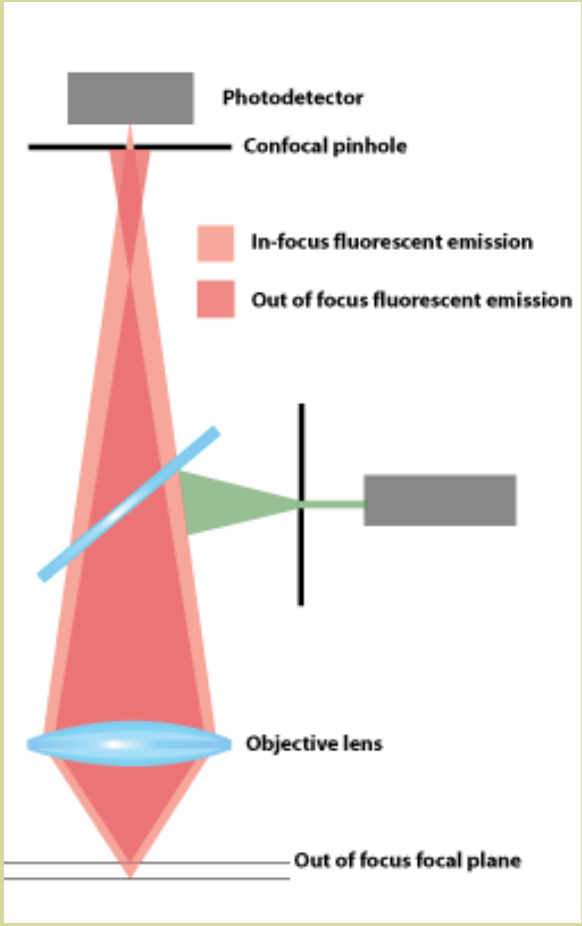


[http://en.wikipedia.org/wiki/File:FluorescenceFilters_2008-09-28.svg]
[http://en.wikipedia.org/wiki/File:Jablonski_Diagram_of_Fluorescence_Only.png]

wide-field fluorescence microscopy:

- homogeneous illumination of the specimen
- fluorescence excitation
- filtered detection of emitted light

confocal widefield (pinhole open)



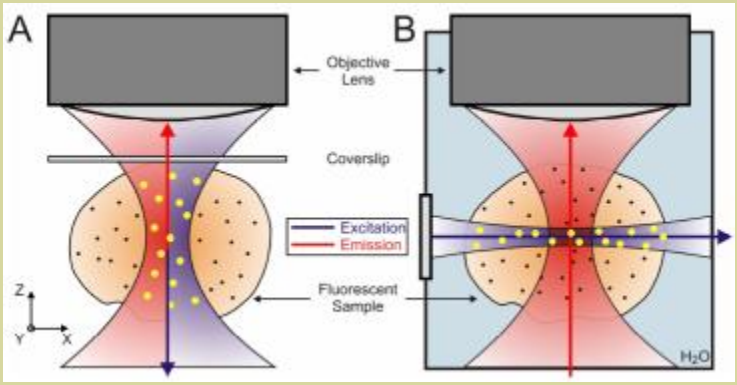
confocal microscopy:

- illumination with focused laser beam
- detection through confocal pinhole
- filtered detection of emitted light
- out-of-focus light rejected
- point-scanning

[http://www.scienceinyoureyes.com/uploads/pics/Confocal-microscope-new1_02.gif]
[http://en.wikipedia.org/wiki/Confocal_laser_scanning_microscopy]

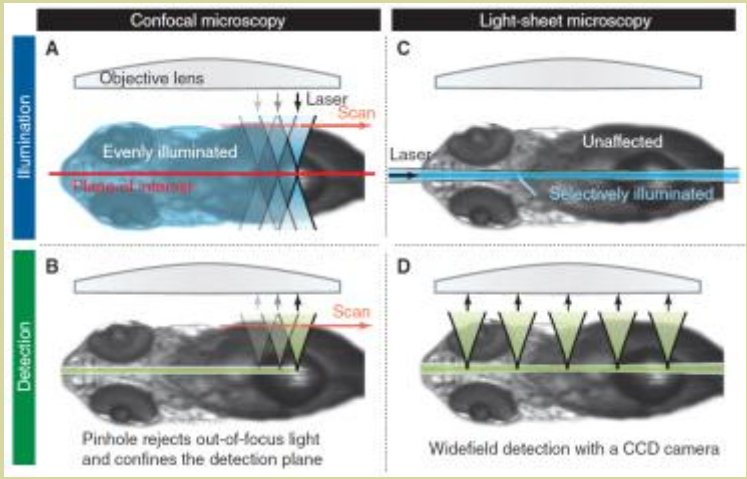
light sheet microscopy:

- illumination orthogonal to detection
- optical sectioning by sheet illumination
- wide-field detection



epi illumination light sheet illumination

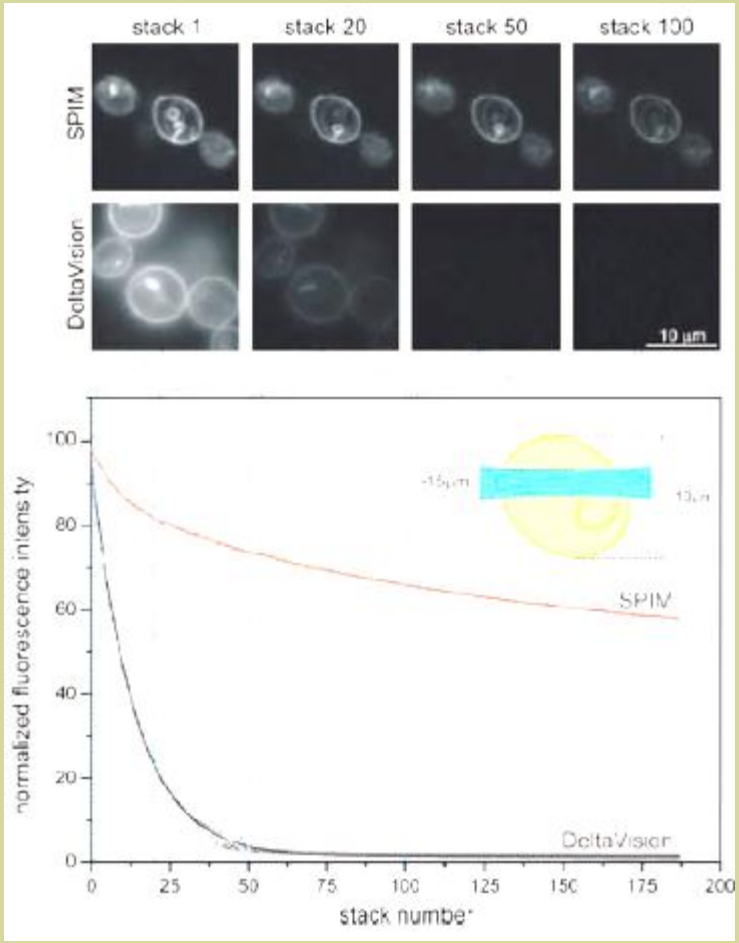
- fast image acquisition
- efficient use of photon budget:
  - lower toxicity
  - less bleaching



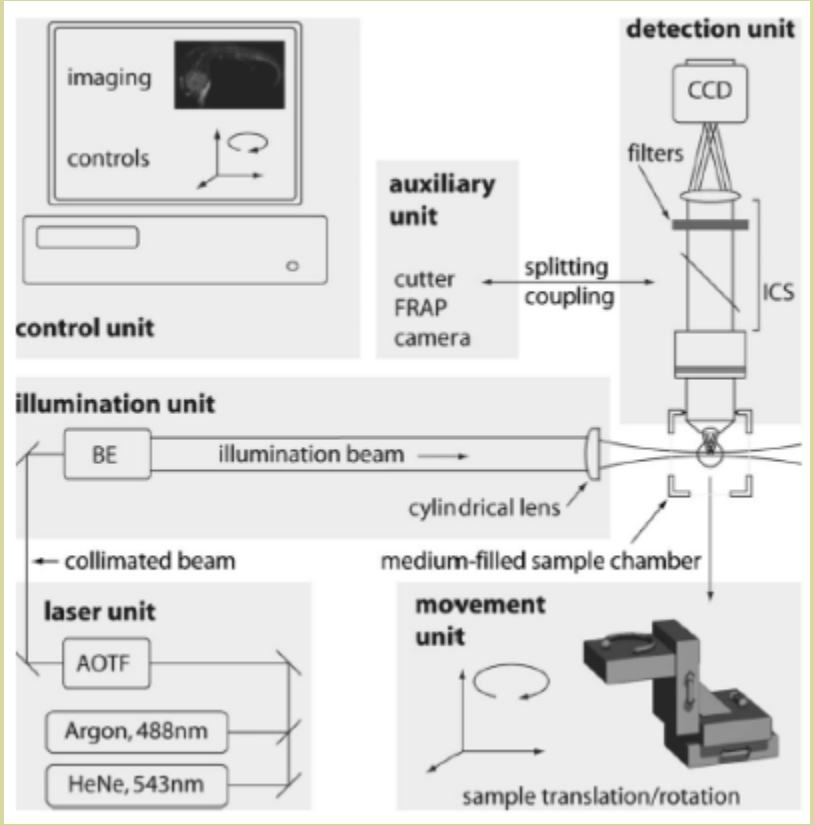
[Ritter et al., Optics Express 16(10), 2008.]
[Huisken, Stainier, Development 136, 2009.]

photon efficiency:

- SPIM generates photons in focal plane
- less photons „wasted“ in out-of-focus layers
- lower illumination power for similar signal-to-noise ratio due to background suppression
- bleaching reduced by ~ factor n, where n = number of slices in stack



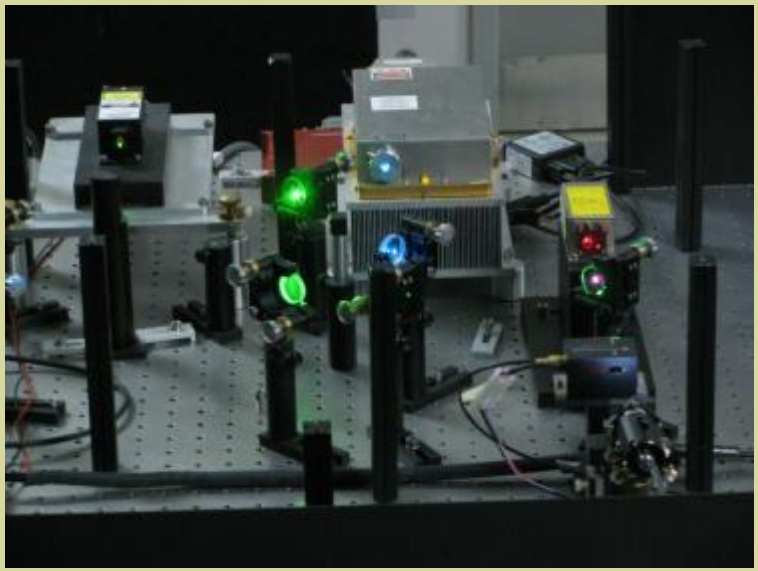
[Reynaud et al., HFSP Journal 2(5), 2008.]

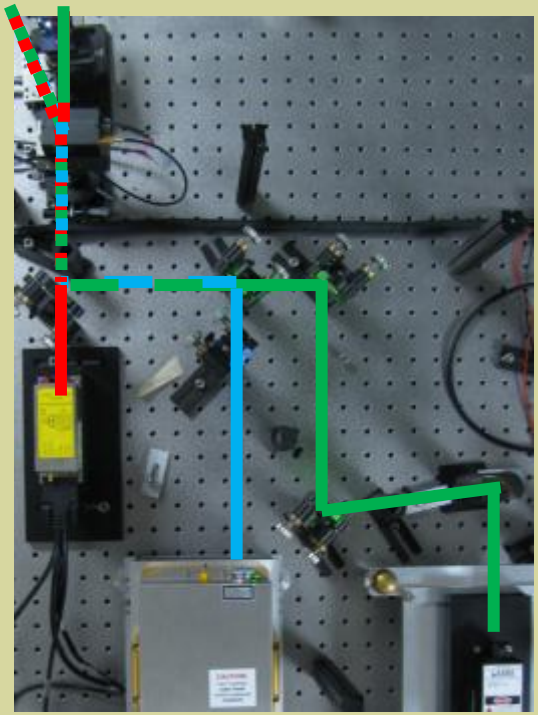


[Greger et al., Rev. Sci. Instrum. 78 , 2007.]

building an LSFM:

- illumination control
- light sheet formation
- sample mounting
- fluorescence detection
- data storage and evaluation





illumination control:

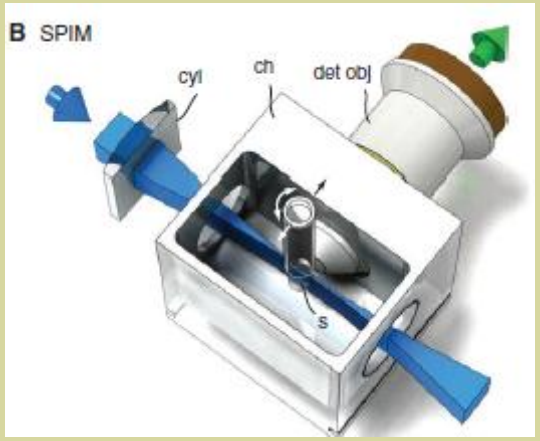
- combine various laser lines
- select line and intensity via AOTF (µs)
- blank during camera read out phase

light sheet formation:

- basic – cylindrical lens

xz: cylindrical lens focusing elliptical beam

xy: collimated beam passing a glass surface

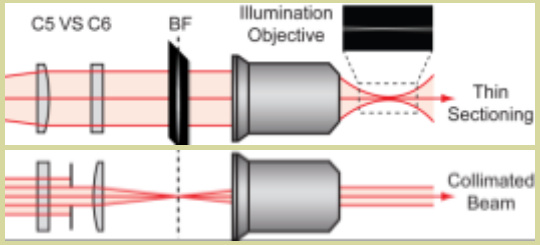


[Huisken, Stainier, Development 136, 2009.]

- advanced – illumination objective

xz: beam focused into specimen chamber

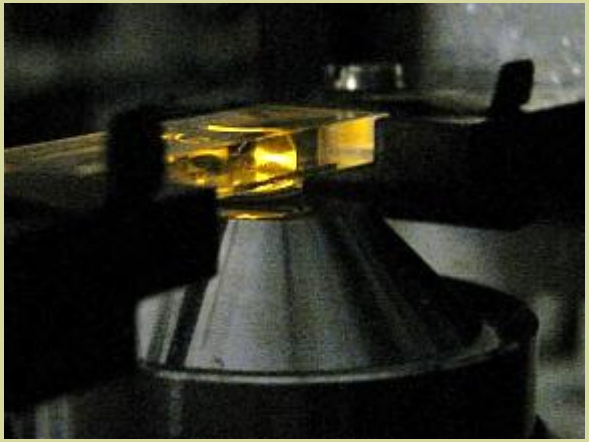
xy: cylindrical lens focusing into back focal plane

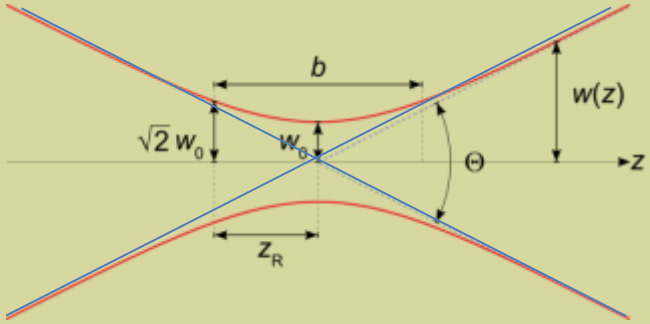


[Ritter et al., Biomedical Optics Express 2(1), 2010.]

light sheet characterization:

Gaussian beam parameters	
waist	w_0
angle of divergence	Θ
Rayleigh length	x_R
field of view	$b = 2*x_R$
refractive index	n
wavelength	λ_0





$w(x) = w_0 * \mathrm{sqrt}(1 + (x / x_R)^2)$

$x_R = \pi w^2_0 / n \lambda_0$

$w(x_R) = w_0 * \mathrm{sqrt}(2)$

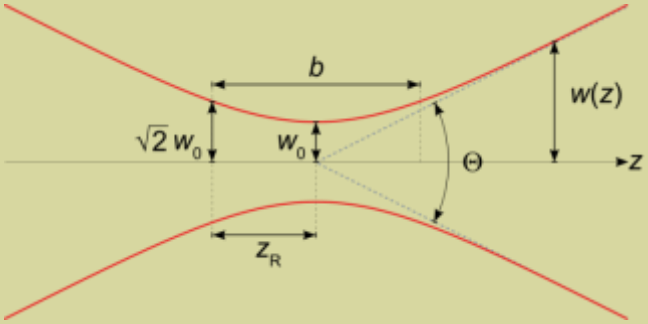
λ_0 [nm]	w_0 [µm]	x_R [µm]	b [µm]
488	1.5	10.9	21.8
640	1.5	8.3	16.6
488	6.0	174.3	348.6
640	6.0	132.9	265.8

[http://en.wikipedia.org/wiki/Gaussian_beam]

light sheet characterization:

Gaussian beam parameters

waist	w_0
angle of divergence	Θ
Rayleigh length	x_R
field of view	$b = 2*x_R$
refractive index	n
wavelength	λ_0



$w(x) = w_0 * \mathrm{sqrt}(1 + (x / x_R)^2)$

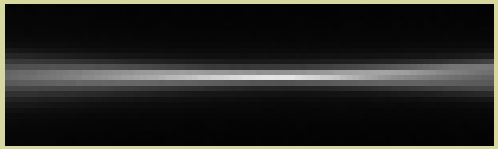
$x_R = \pi w^2_0 / n \lambda_0$

$w(x_R) = w_0 * \mathrm{sqrt}(2)$

	488 nm	532 nm	640 nm
wy (FWHM) [µm]	99,5	94,2	101,7

„width“

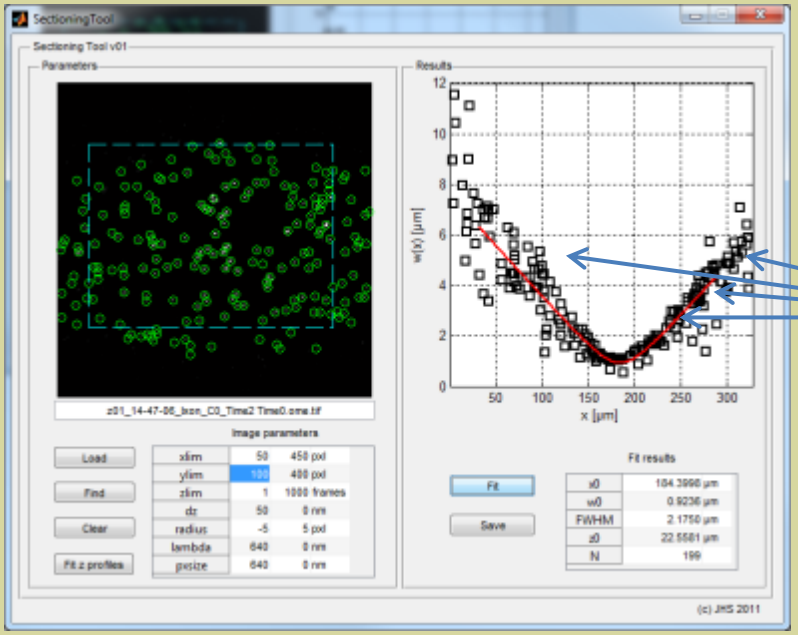
top view

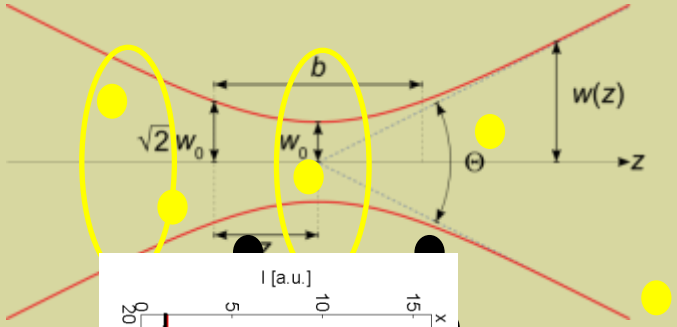


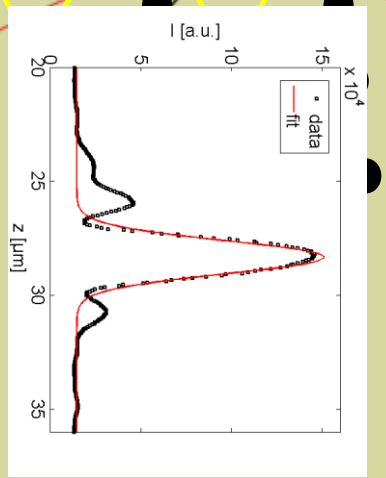
regular beam / side view

λ_0 [nm]	w_0 [µm]	x_R [µm]	b [µm]
488	1.5	10.9	21.8
640	1.5	8.3	16.6
488	6.0	174.3	348.6
640	6.0	132.9	265.8

light sheet characterization:

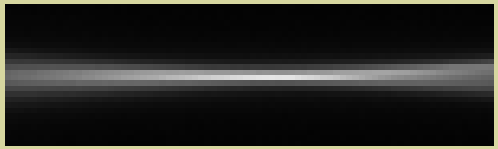






top view

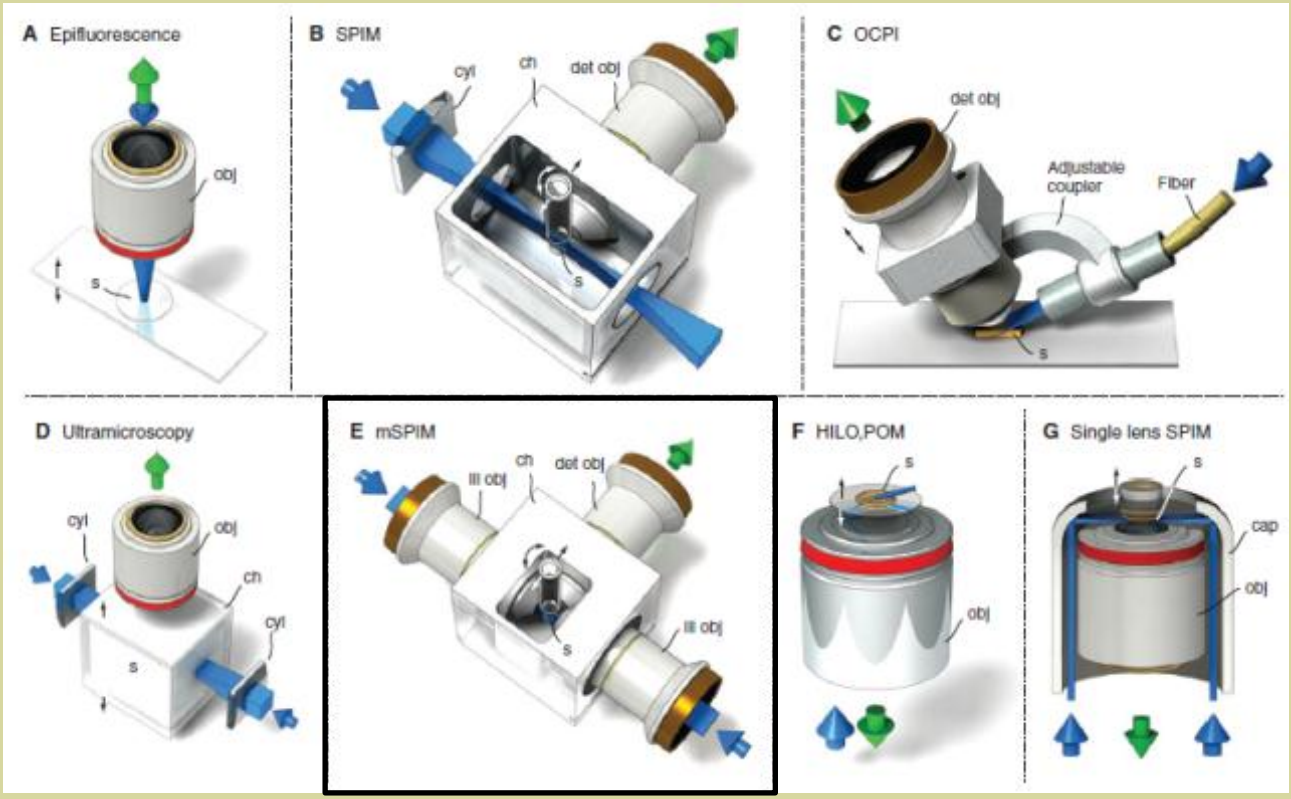
Fluorescent dye in solution

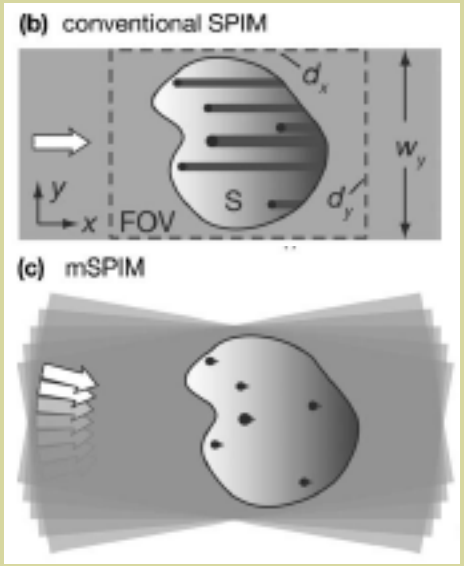


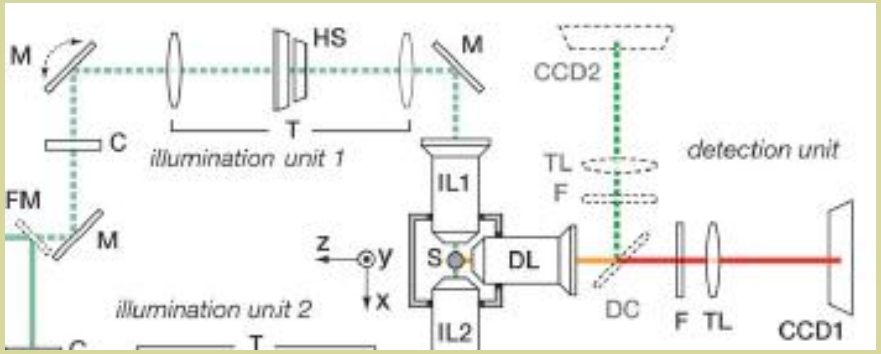
regular beam / side view

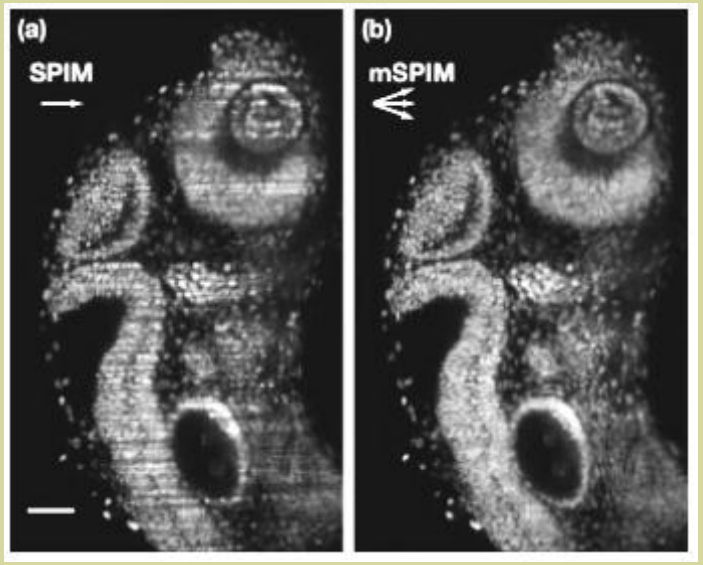
TS 0.2µm in agarose, dz = 50 nm, 10x objective

light sheet formation - mSPIM: [Huisken, Stainier, Development 136, 2009.]







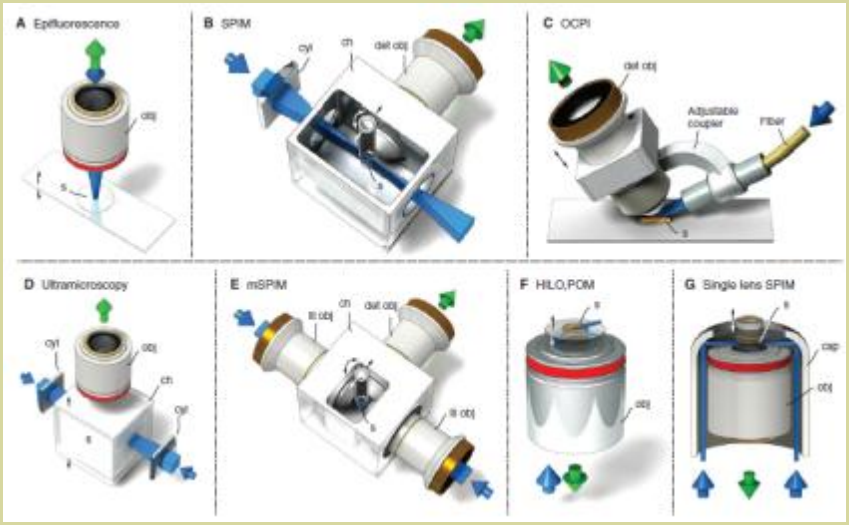


mSPIM:

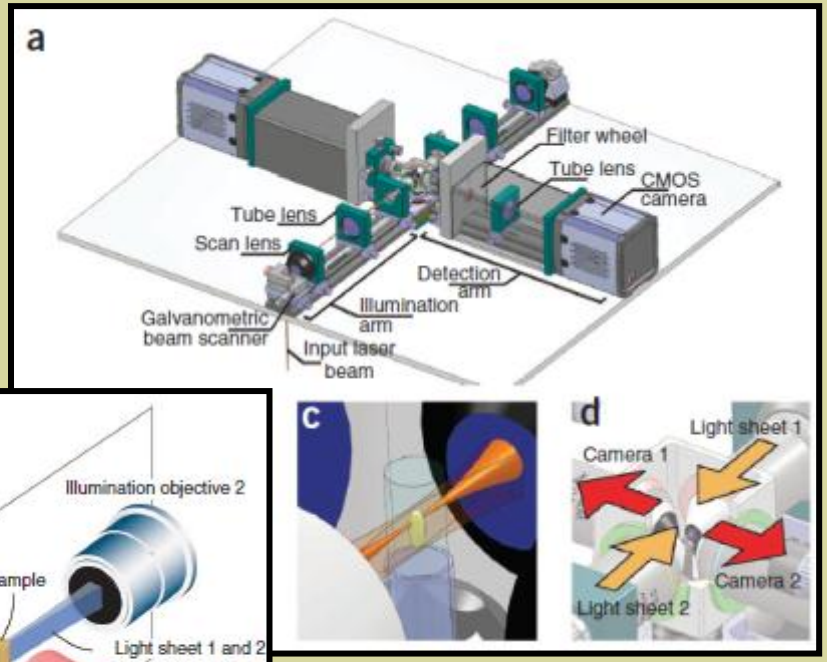
- pivot light sheet around focus
- fast scanning mirror required
- reduce shadowing effect

[Huisken, Stainier, Optics Letters 32(17), 2007.]

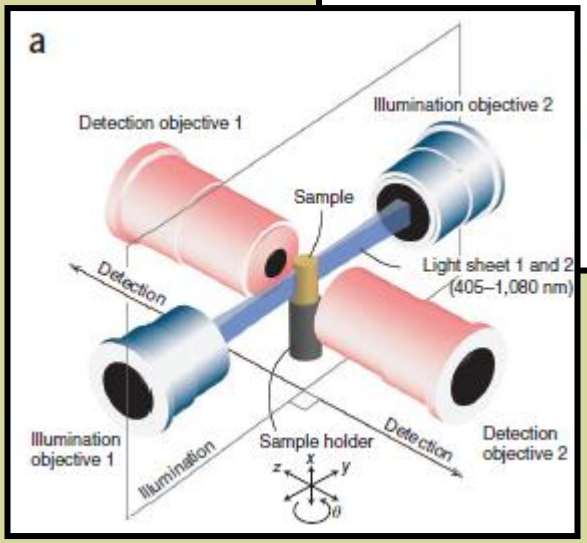
light sheet formation:



[Huisken, Stainier, Development 136, 2009.]



[Krzic et al., Nature Methods 9(7), 2012.]

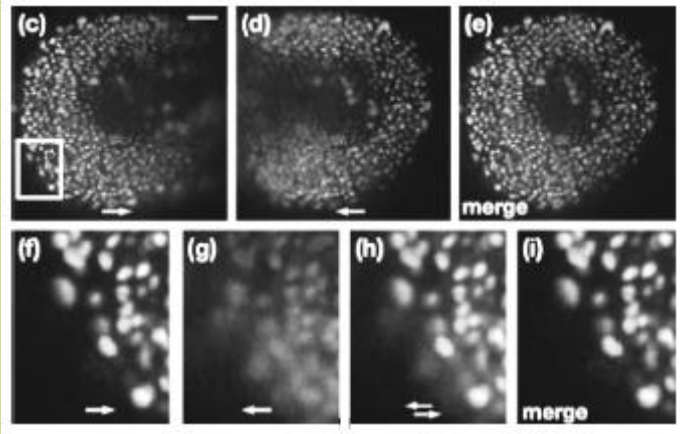


[Tomer et al., Nature Methods 9(7), 2012.]

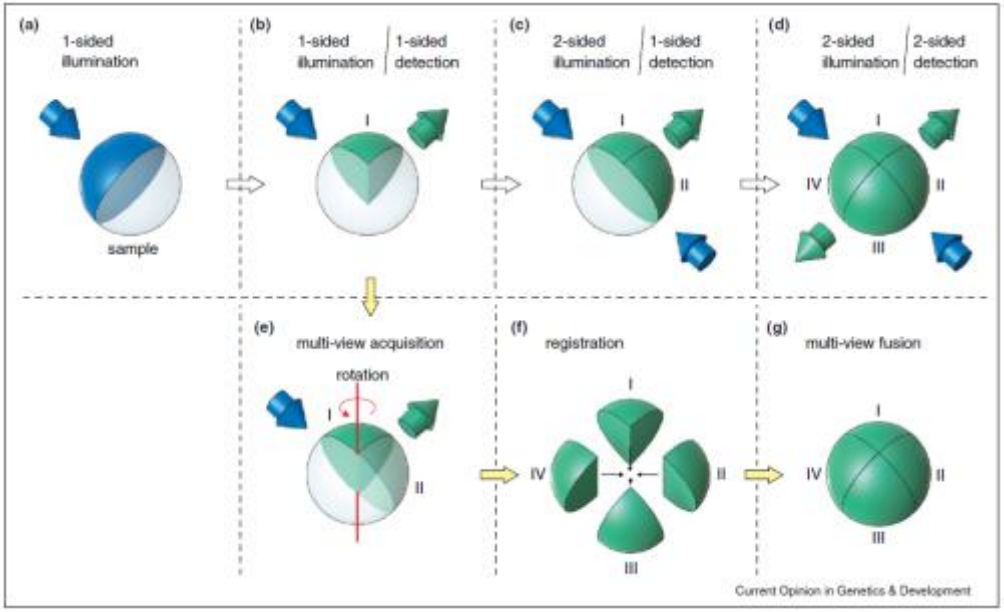
multi view detection and fusion:

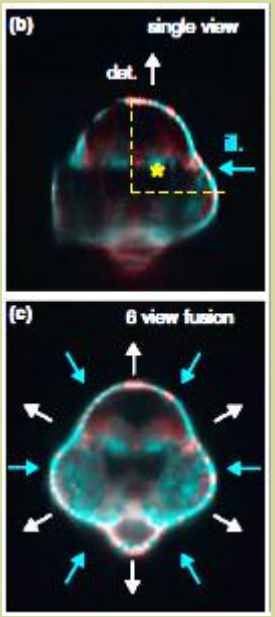
- illumination intensity loss in thick specimen
- fluorescence signal degraded for deeper layers

➔ two-sided illumination *and* detection or sample rotation



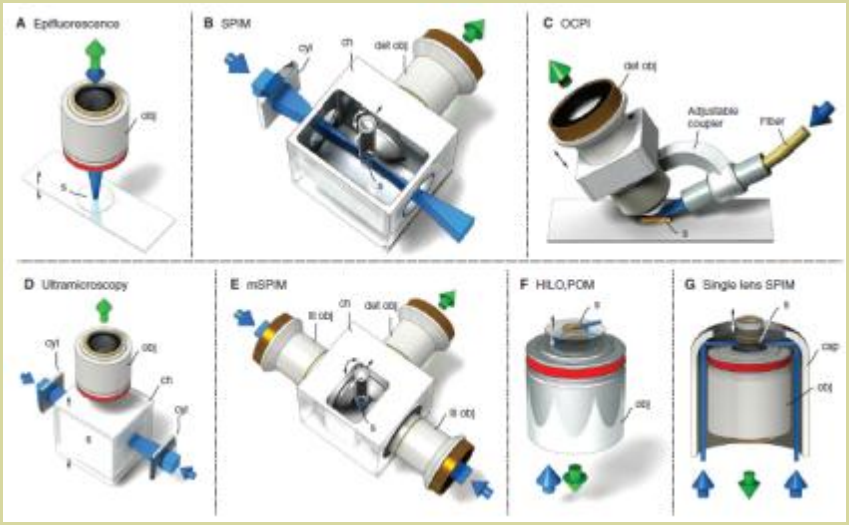
[Huisken, Stainier, Optics Letters 32(17), 2007.]



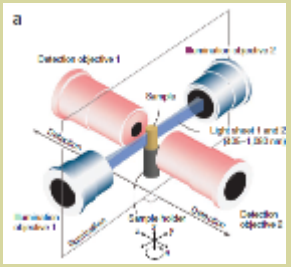


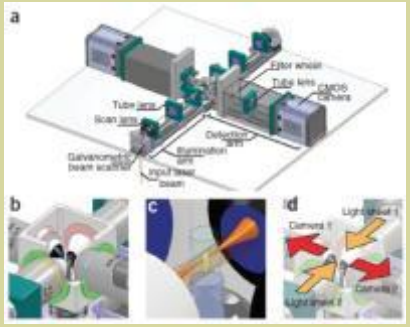
[Weber, Huisken, Curr. Op. Genetics & Development 21, 2011.]

light sheet formation:



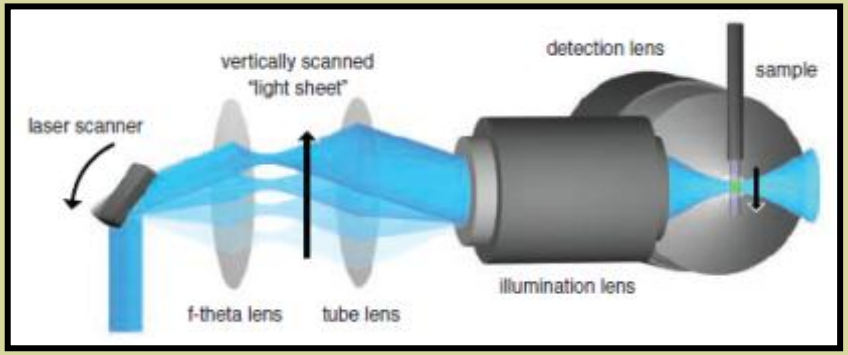
[Huisken, Stainier, Development 136, 2009.]





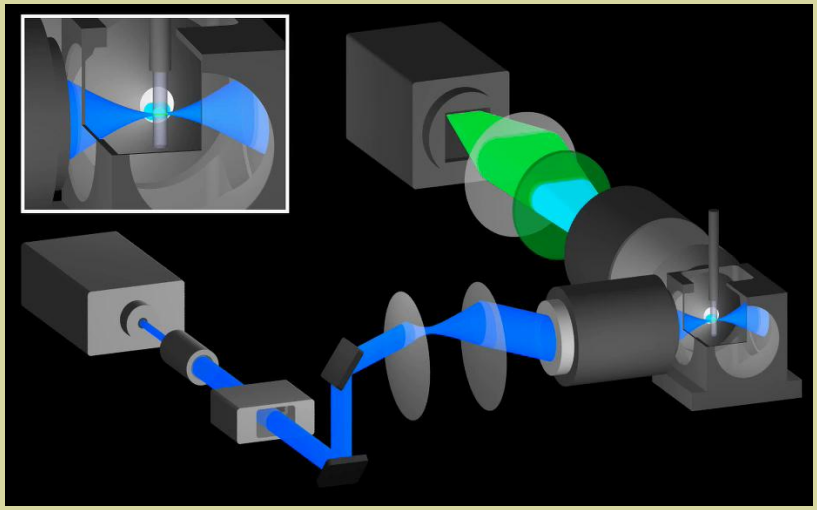
[Krzic et al., Nature Methods 9(7), 2012.]

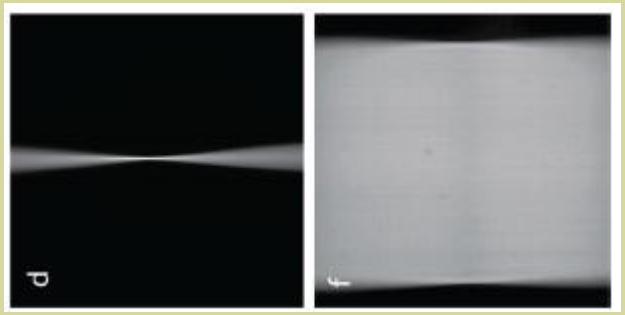
[Tomer et al., Nature Methods 9(7), 2012.]



[Keller et al., Science 322, 2008.]

[Keller et al., Science 2008.]





Digitial Scanned Light sheet Microscopy:

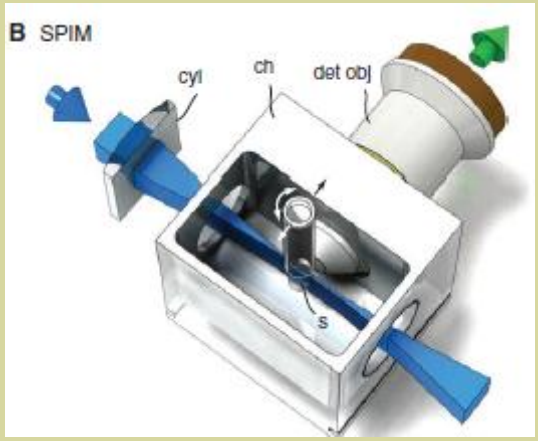
- sweep spherical beam across image plane
- homogeneous illlumination intensity along scanned axes
- reduced shadowing

light sheet formation:

- basic – cylindrical lens

xz: cylindrical lens focusing elliptical beam

xy: collimated beam passing a glass surface

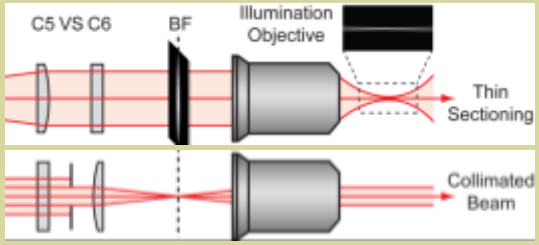


[Huisken, Stainier, Development 136, 2009.]

- advanced – illumination objective

xz: beam focused into specimen chamber

xy: cylindrical lens focusing into back focal plane

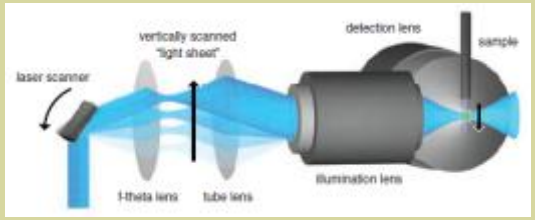


[Ritter et al., Biomedical Optics Express 2(1), 2010.]

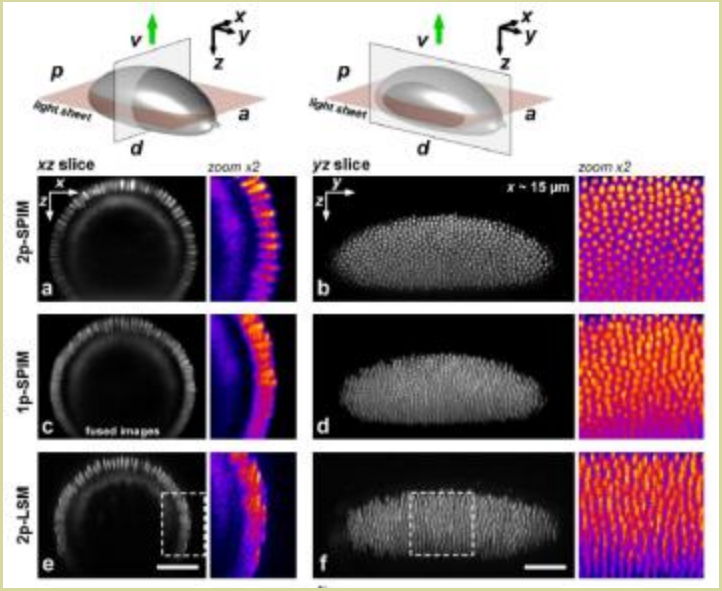
- latest – scanned light sheet

xz: spherical beam focused by objective

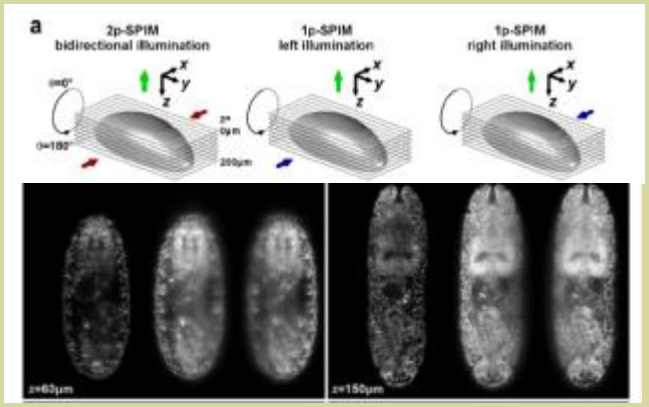
xy: beam swept across image field by scan mirror

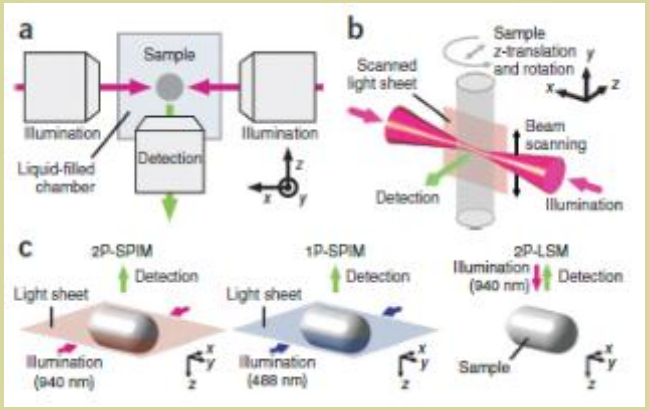


[Keller et al., Science 322, 2008.]



[Truong et al., Nat. Meth. 8(9), 2011.]



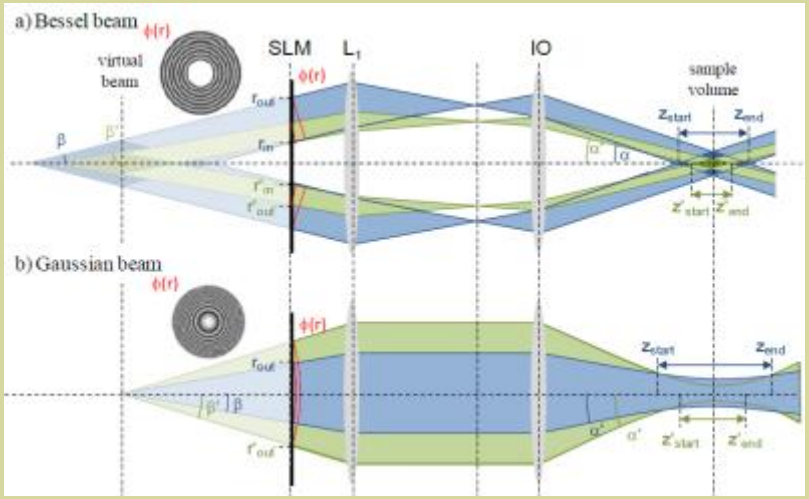


Two photon excitation:

- better confinement of fluorescence excitation
- lower impact of scattering on background
- lower autofluorescence background

-> simultaneous bidirectional illumination

-> better axial resolution than two photon point-scanning!

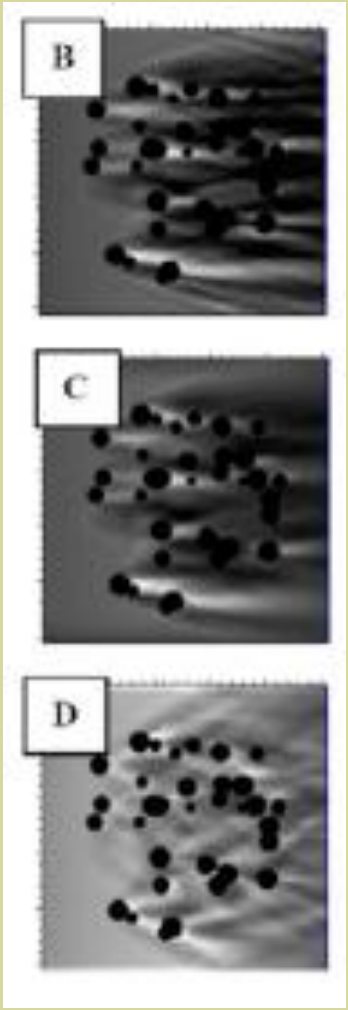


[Fahrbach, Rohrbach, Optics Express 18(23), 2010.]

Gaussian

scanned Gaussian

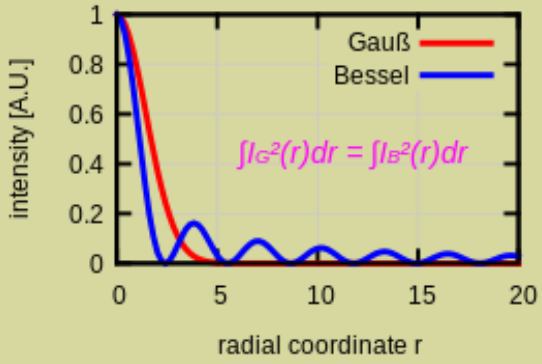
scanned Bessel



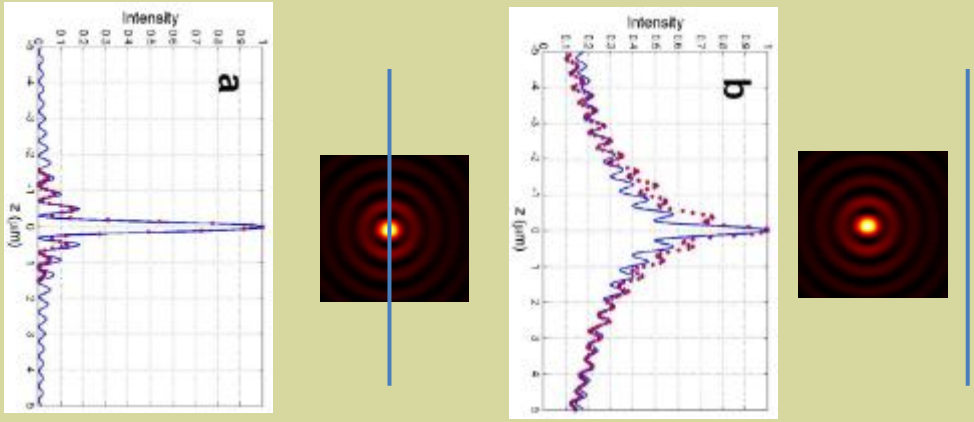
[Rohrbach, Optics Letters 34(19), 2009.]

light sheet formation with Bessel beams:

- theory: infinite Rayleigh length
- requires scanning
- scanning leads to broad axial profile
- solution: two photon excitation



[http://commons.wikimedia.org/wiki/File:Bessel_gauss.svg]

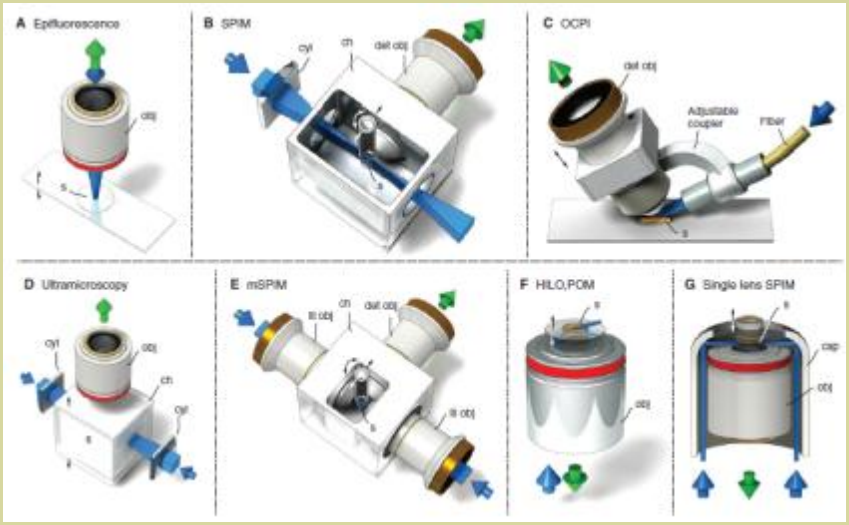


[Planchon et al., Nature Methods, 2010.]

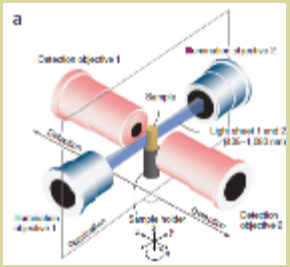
light sheet formation with Bessel beams:

- theory: infinite Rayleigh length
- requires scanning
- scanning leads to broad axial profile
- solution: two photon excitation

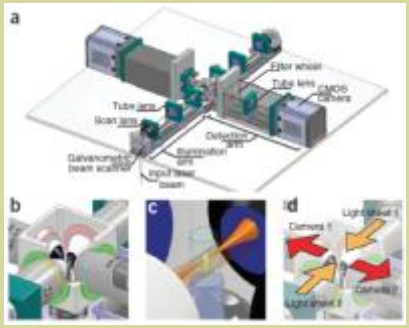
light sheet formation:



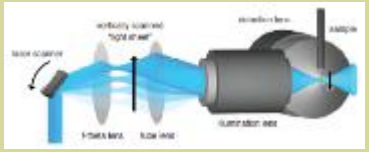
[Huisken, Stainier, Development 136, 2009.]



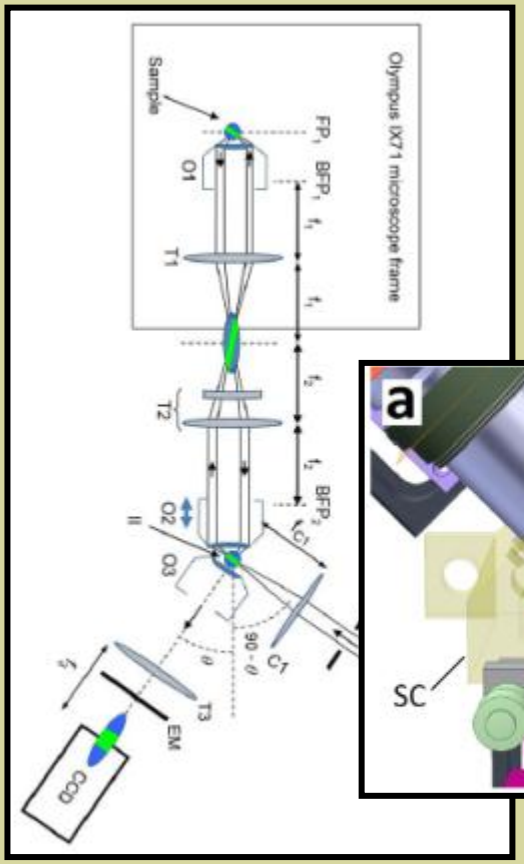
[Krzic et al., Nature Methods 9(7), 2012.]



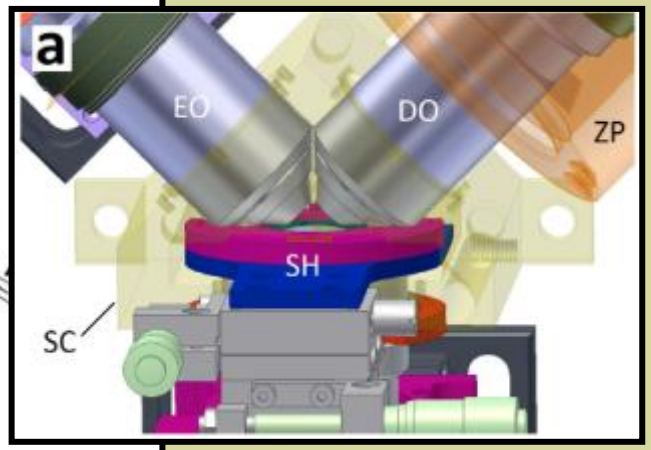
[Tomer et al., Nature Methods 9(7), 2012.]



[Keller et al., Science 322, 2008.]

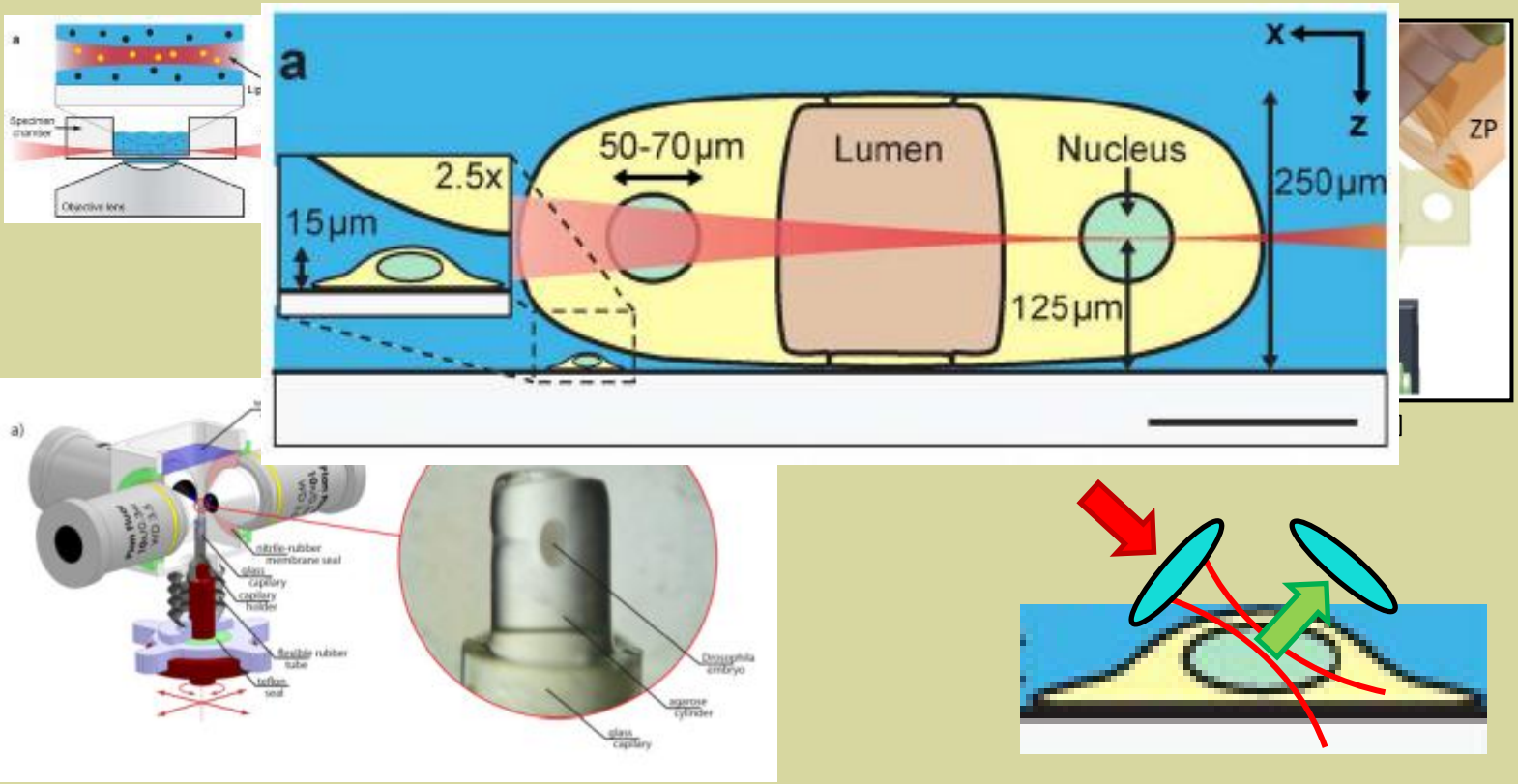


[Kumar et al., Optics Express, 2011.]



[Planchon et al., Nature Methods, 2010.]

sample height – use 45° configuration for adherent cells:



- light sheet illumination suited for large specimen
- avoid interference artifacts close to coverslip
- sample mounting in agarose

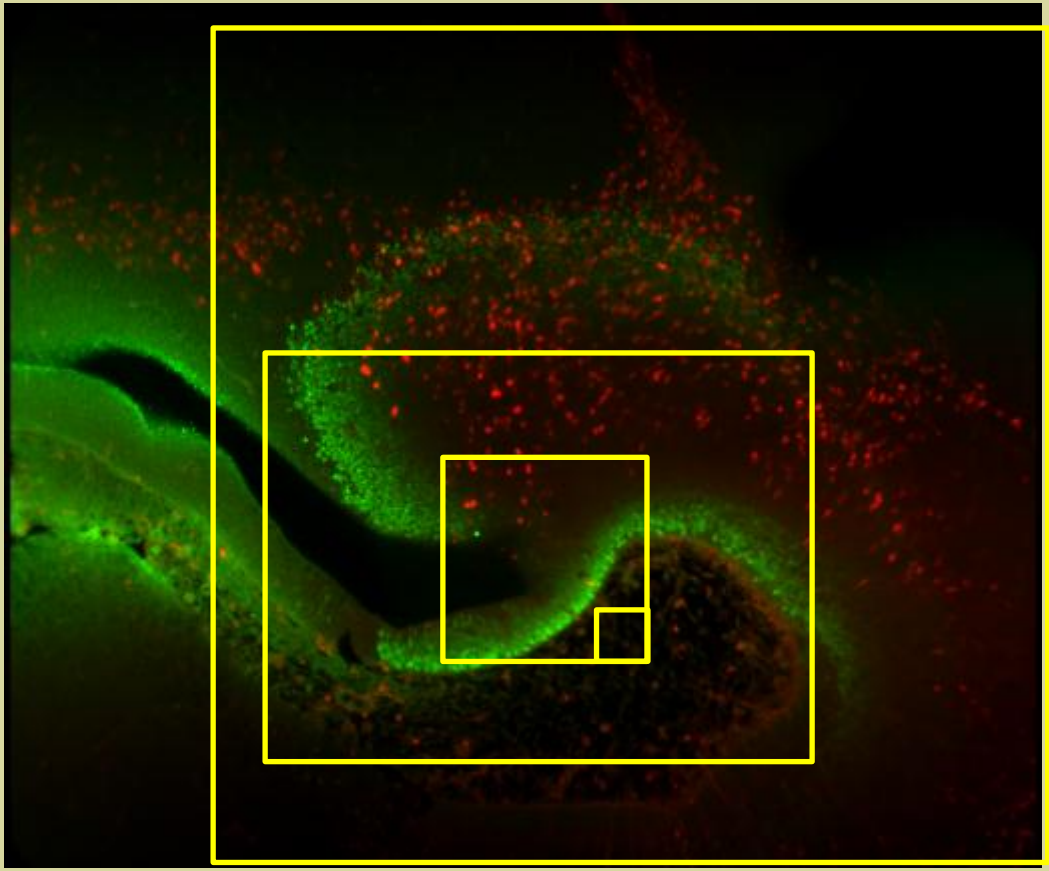
- adherent cells/small samples: use 45° configuration

fluorescence detection:

camera	Andor Ixon DU897	Andor Ixon DV860	Hamamatsu Orca Flash 4.0
type	EMCCD	EMCCD	sCMOS
chip	512 x 512	128 x 128	2048 x 2048
pixel size (physical)	16 µm	24 µm	6.5 µm
full frame rate	35 Hz	500 Hz	100 Hz
data rate (max, 16 bit)	~18 MB / s (512 kB / frame)	~16 MB / s (32 kB / frame)	~800 MB / s (8.4 MB / frame)
QE (max)[1]	92.5 % @ 575 nm eff. ~66%	92.5 % @ 575 nm eff. ~66%	72% % @ 575 nm
read noise [e-]	< 1 e- with EM gain	< 1 e- with EM gain	1.3 – 1.5 e-
prizetag	>30 k€ (40 k$)	~30 k€ (40k$)	16 k€ (21 k$)
comment	low signal level, modest image field	low signal level, high speed	(very) large image field, very high speed full speed requires dual cameralink and SSD drive or RAID system

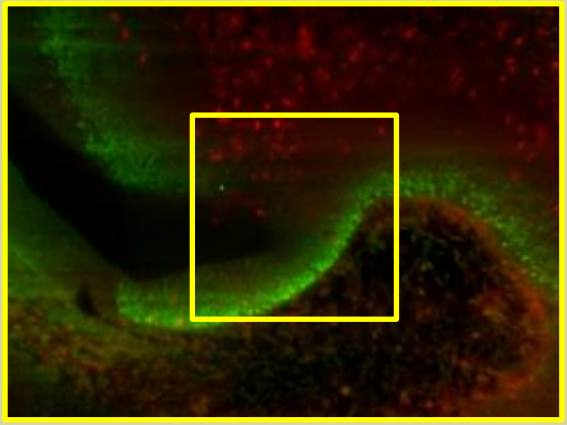
1 divide by sqrt(2) for EMCCD
for a comparison see [Long et al., Optics Express 20(16), 2012.]

fluorescence detection:

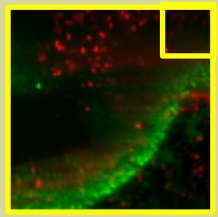


Andor Neo sCMOS (15x)
2544 x 2160 pxl, 6.5 µm/pxl
16.6 x 14.0 mm -> 1.1 x 0.9 mm

Hamamatsu Orca Flash 4.0 sCMOS (15x)
2048 x 2048 pxl, 6.5 µm/pxl
13.3 x 13.3 mm -> 0.89 x 0.89 mm



Clara Interline CCD (15x)
1392 x 1040 pxl, 6.45 µm/pxl
8.98 x 6.71 mm -> 0.61 x 0.45 mm



Ixon DU 897 EMCCD (37.5x)
512 x 512 pxl, 16 µm/pxl
8.19 x 8.19 mm -> 0.22 x 0.22 mm

Ixon DV 860 EMCCD
128 x 128 pxl, 24 µm/pxl
0.05 x 0.05 mm

data storage, processing, evaluation:

application	reference	dataset volume	data rate
single molecule tracking	Siebrasse et al., PNAS 2012	230 GB (7200 x 1000 frames x 32 kB/frame)	~1.6 MB/s 32kB @ 50 Hz Ixon DV860
super resolution imaging	Zanacchi et al., Nat. Meth. 2011	3 GB / reconstructed image (6000 frames x 512 kB / frame per reconstructed image)	~16 MB/s 512 kB @ 33 Hz Ixon DU897
full mouse brain reconstruction	Silvestri et al., Optics Express 2012	215 GB / brain ~430000 frames / brain 73 mm³ @ 0.8 x 0.8 x 1.0 µm³ voxel	5 MB/s 512 kB @ 10 Hz Ixon DU897
drosophila embryogenesis	Tomer et al., Nat. Meth. 2012	10-15 TB / embryo 10^6 images of 10 MB >2000 time points with 2Hz (17h), 4 images per slice per time point, 130 slices (dz 2µm) per time point	200 MB/s averaged; peak 350 MB/s 10MB @ 35 Hz Neo sCMOS

software:

- FIJI and plugins (e.g. S. Preibisch, "Software for bead-based registration of selective plane illumination microscopy data", Nature Methods, 7(6):418-419.)
- commercial software for segmentation, 3D rendering, tracking etc., e.g. Imaris (Bitplane), Volocity (PerkinElmer), ...
- check Supplementary Information!

commercial instruments:

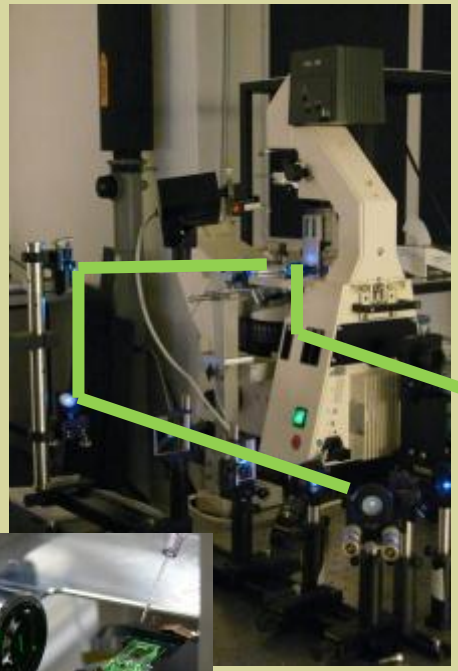
LaVision BioTec Ultramicroscope
release 11/2010

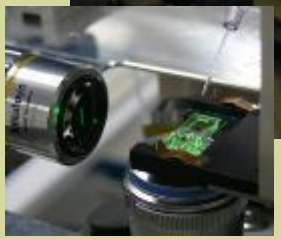
[http://lavisionbiotec-asiapacific.com/]

Zeiss Lightsheet Z.1
release 10/2012

[zeiss.com/lightsheet]

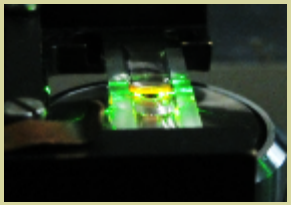
U Bonn instruments:



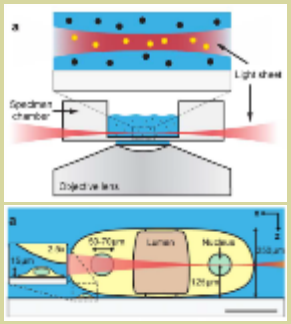


micro-injection

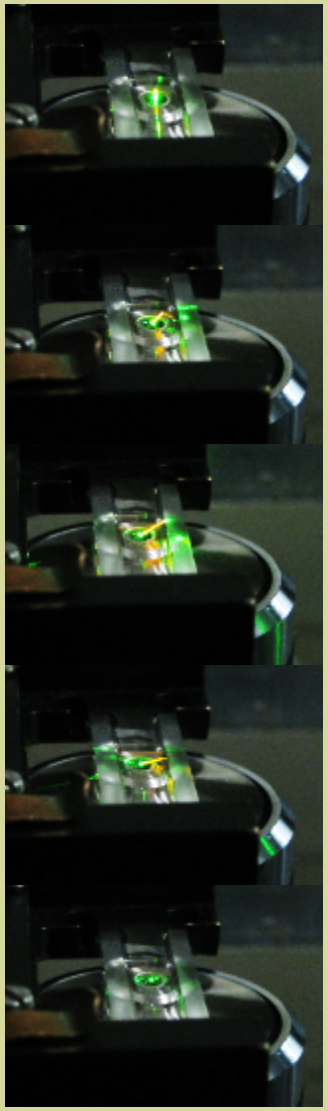
light sheet



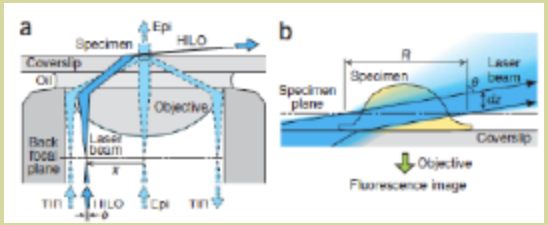
[Ritter et al., PlosOne 5(7), 2010.]



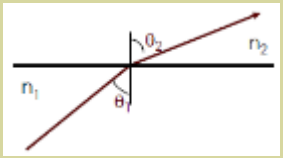
epi

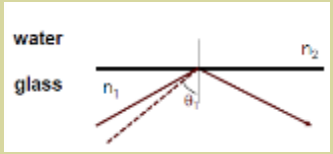


[Tokunaga et al., Nat. Meth., 2006.]



HILO





TIRF

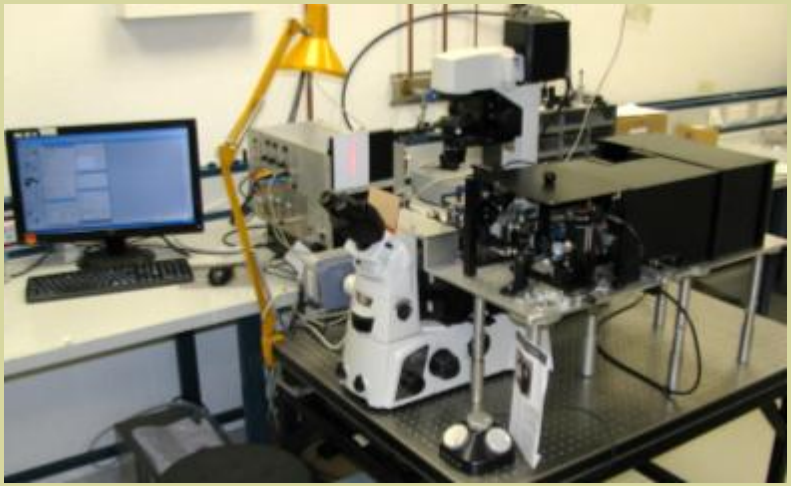
„SPIM 2.0“

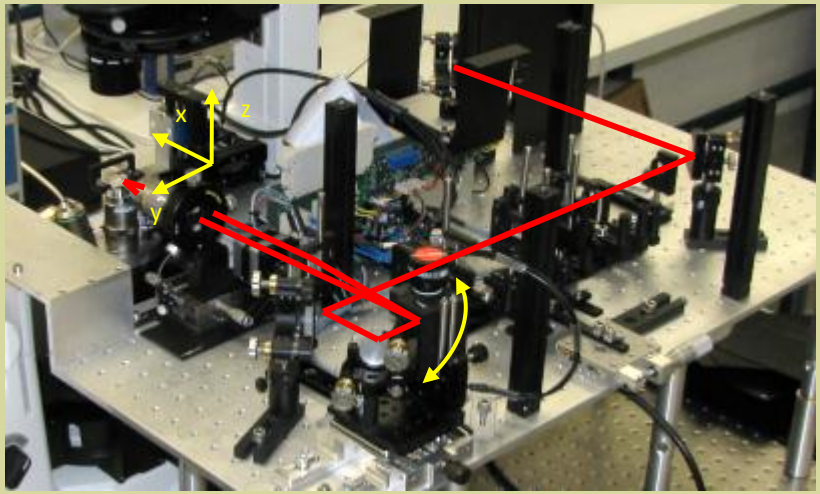
2 µm x 20 µm light sheet

epi-/Hilo-/TIRF-illumination

micro-injection

U Bonn instruments:





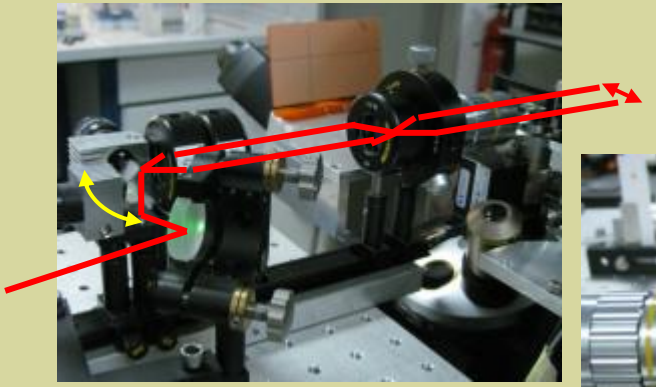
„SPIM 3.0“

motorized stage, 3D localization

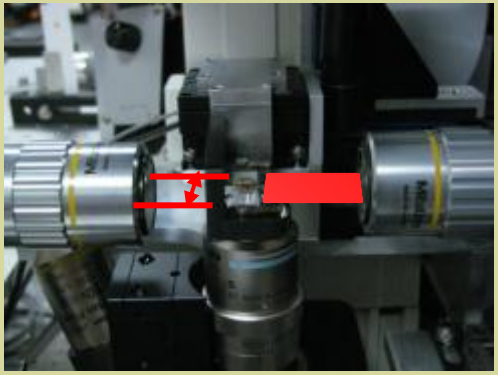
a) adjustable light sheet

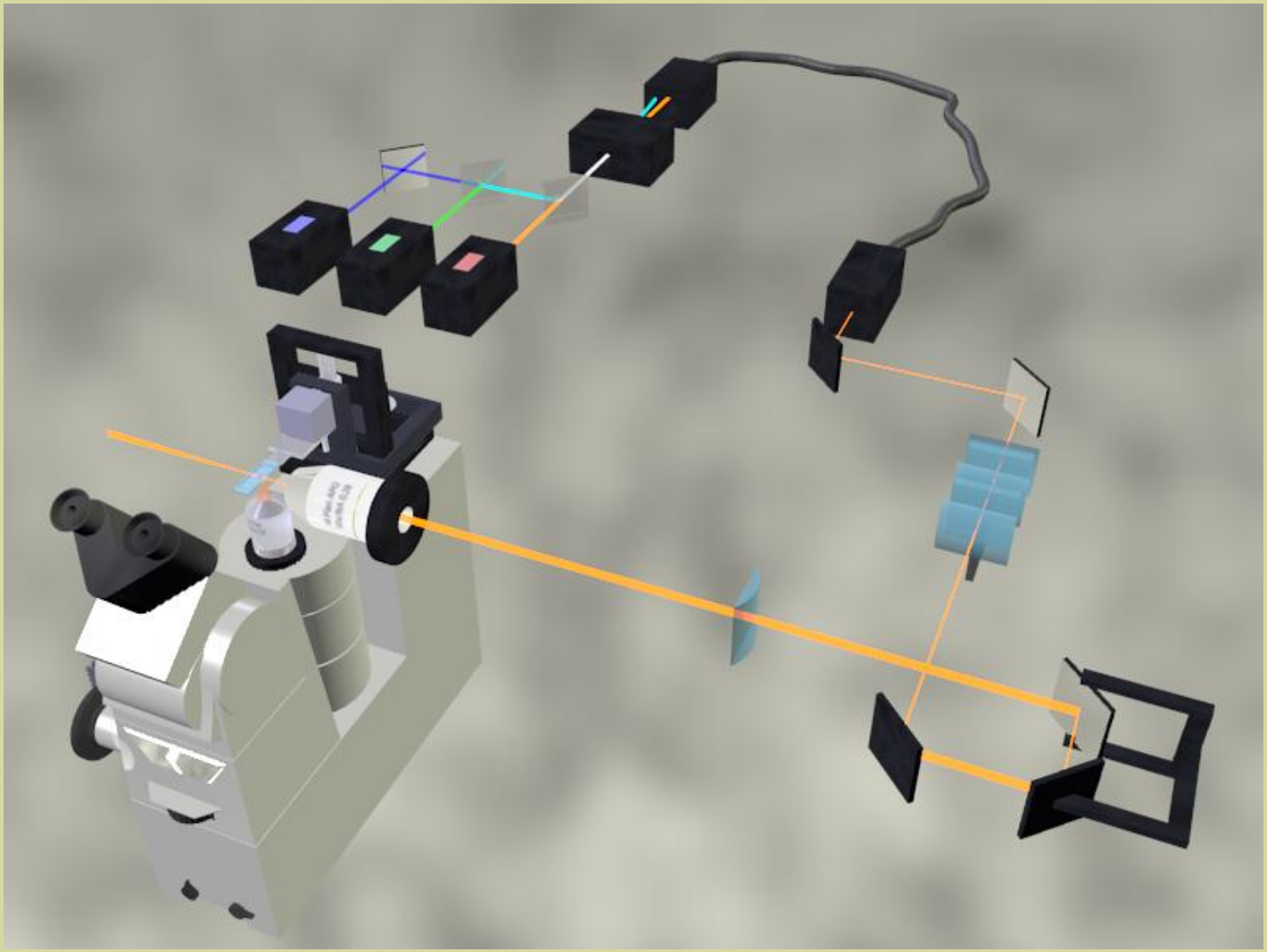
mSPIM scanner

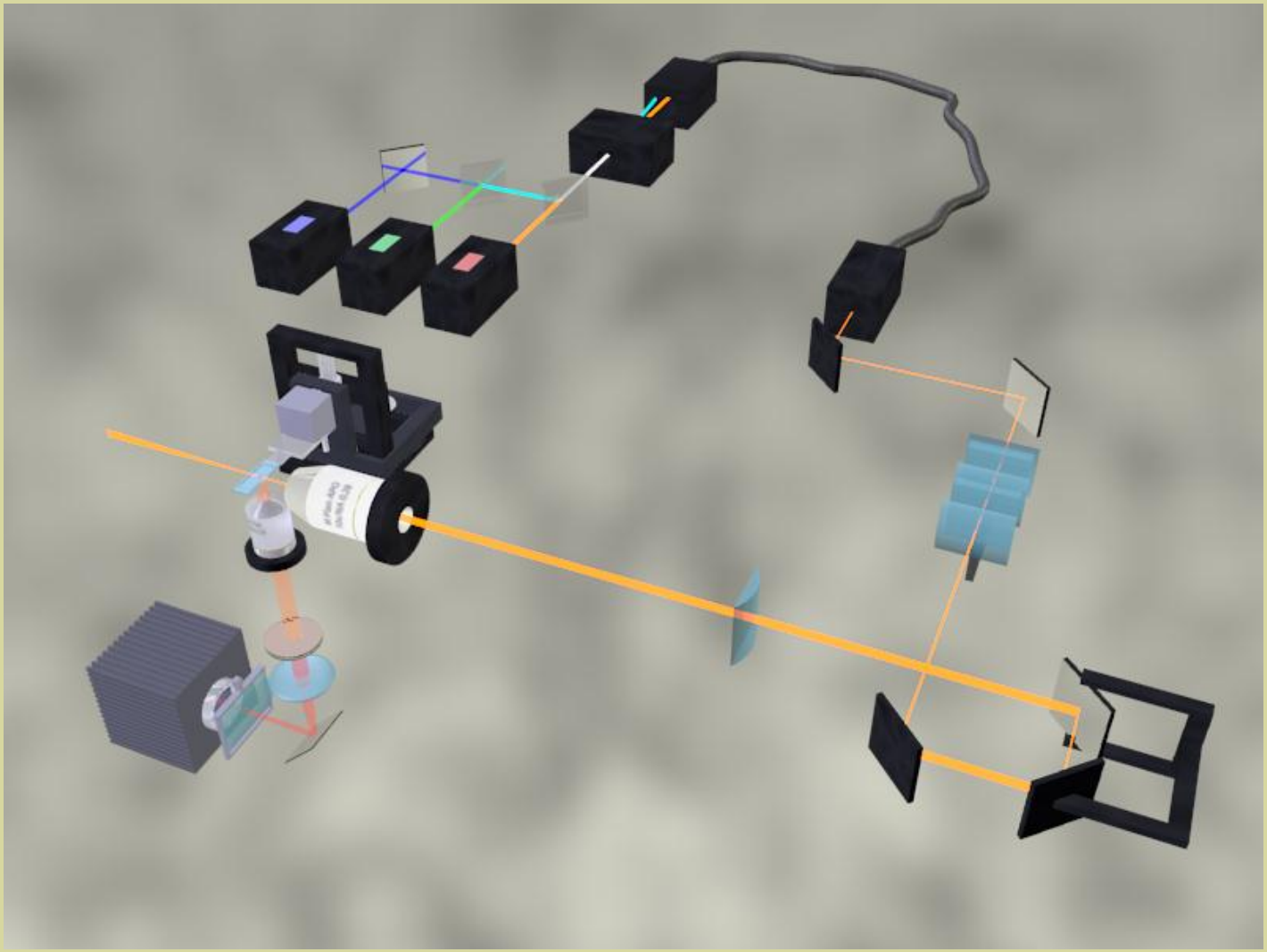
b) scanned light sheet

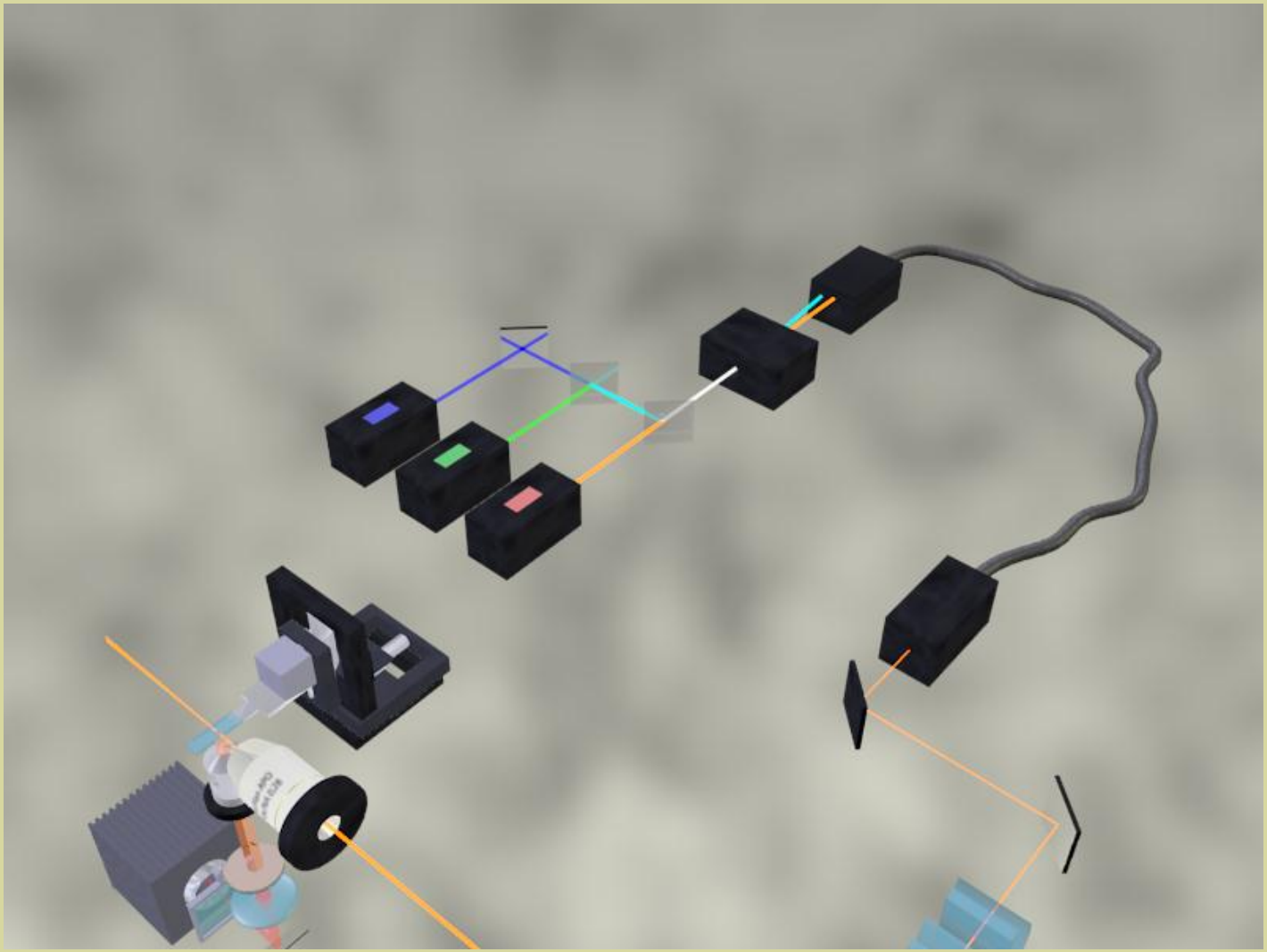


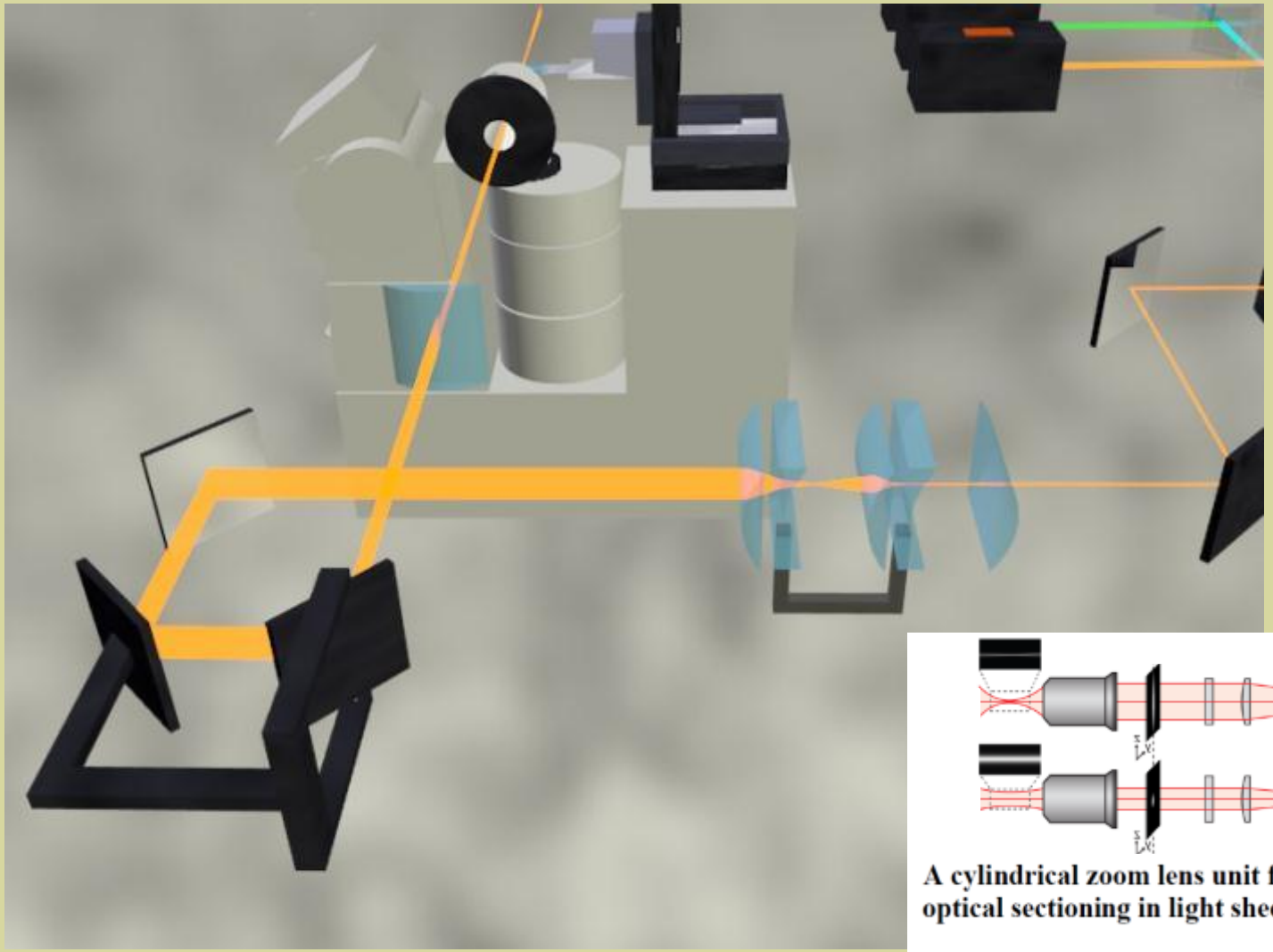
b) a)





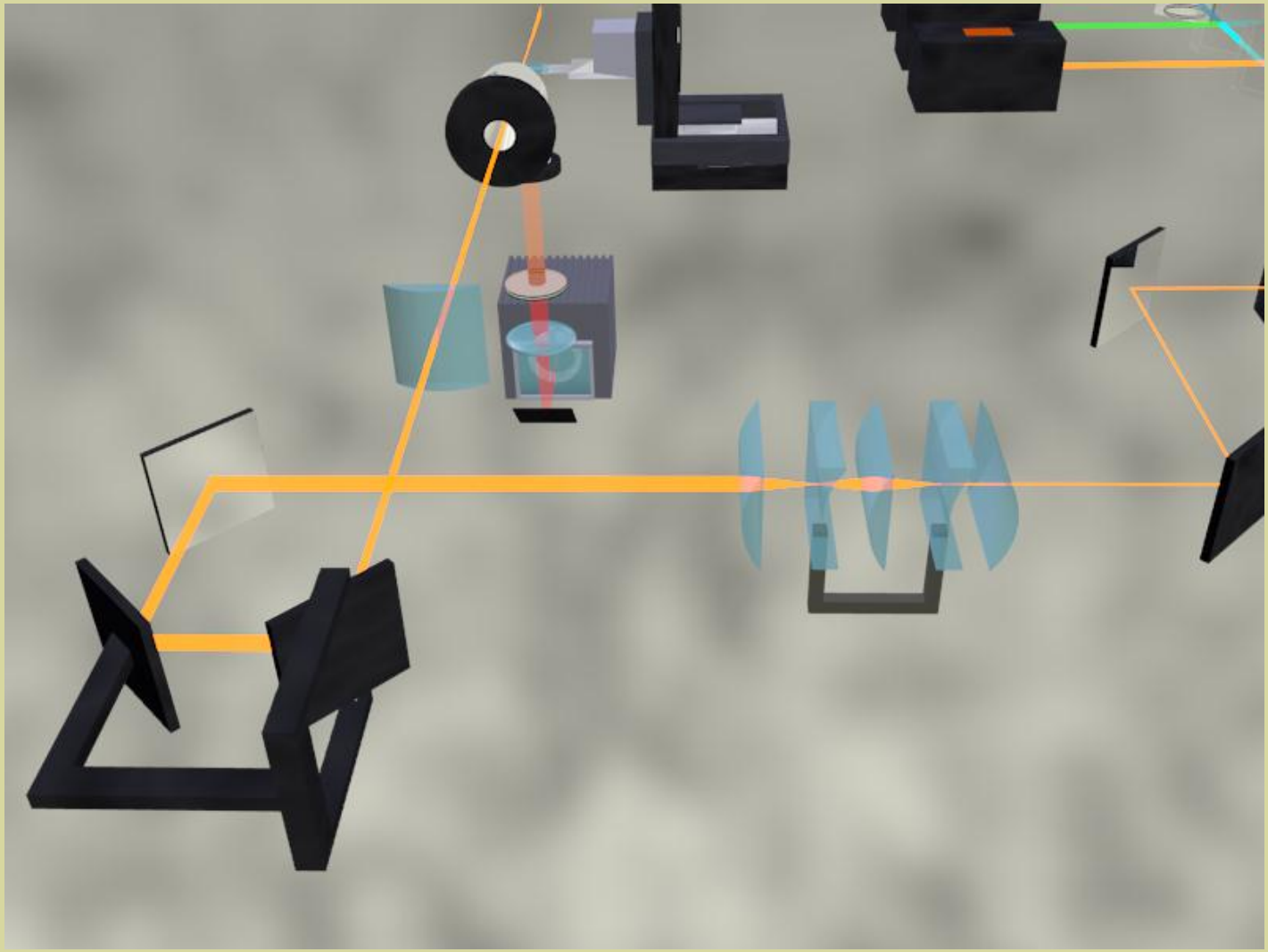


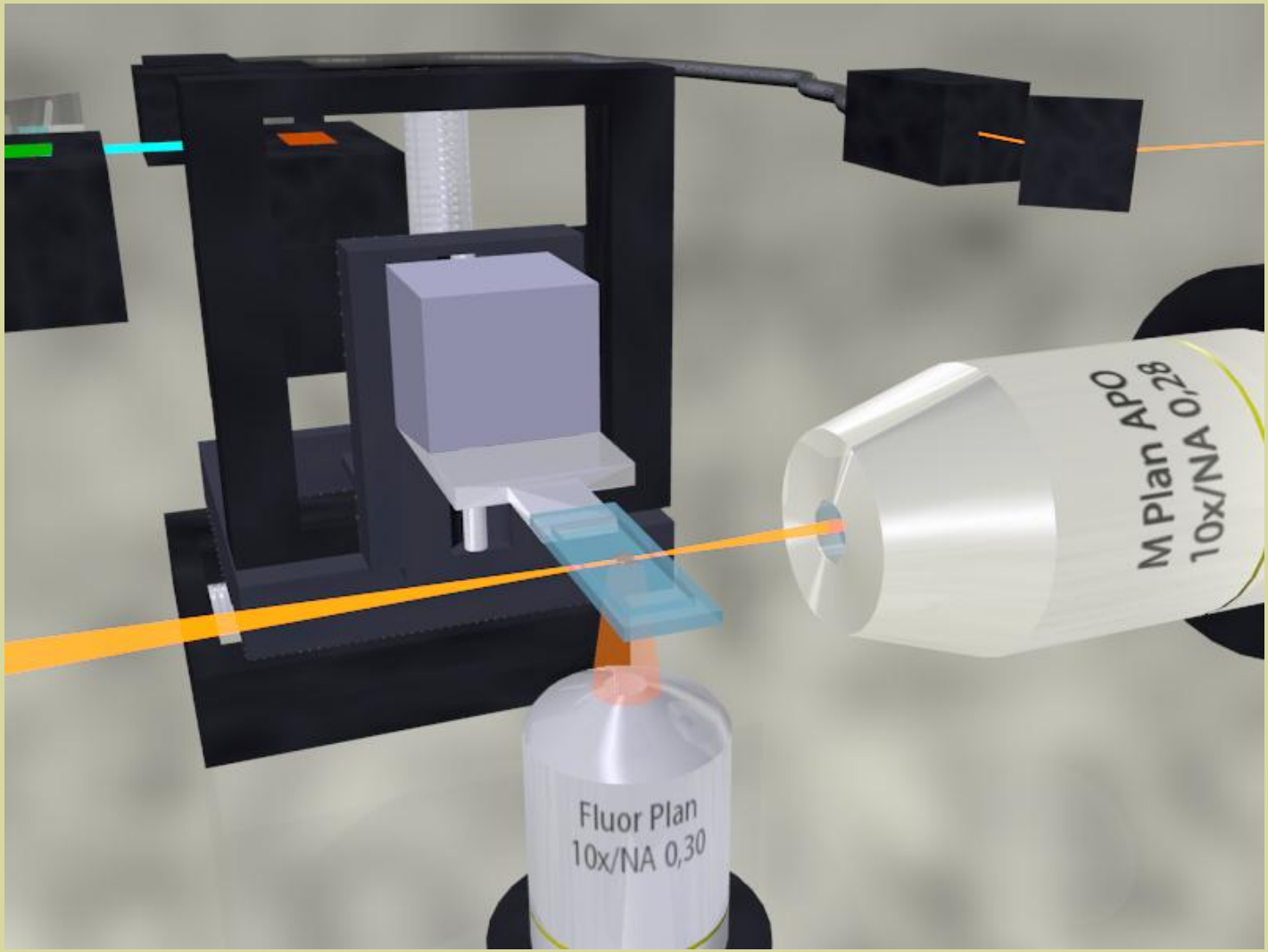




A cylindrical zoom lens unit for adjustable optical sectioning in light sheet microscopy

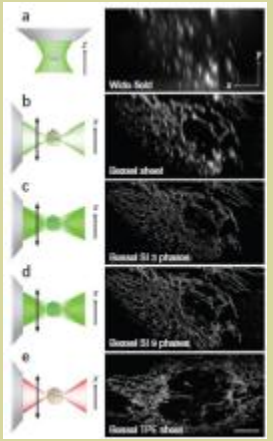
[Ritter 2010b] – thickness 1 – 6 µm




M Plan APO
10x/NA 0,28
Fluor Plan
10x/NA 0,30

Applications of light sheet microscopy

- Large specimen: Mouse, Drosophila, Zebrafish
- High speed imaging
- Superresolution and single molecule imaging



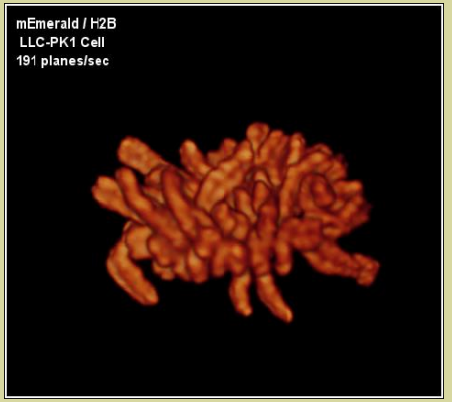
Highspeed imaging on the single cell level

- Ca2+ waves in cardiac myocytes
- High resolution imaging of adherent cells

Rapid three-dimensional isotropic imaging of living cells using Bessel beam plane illumination

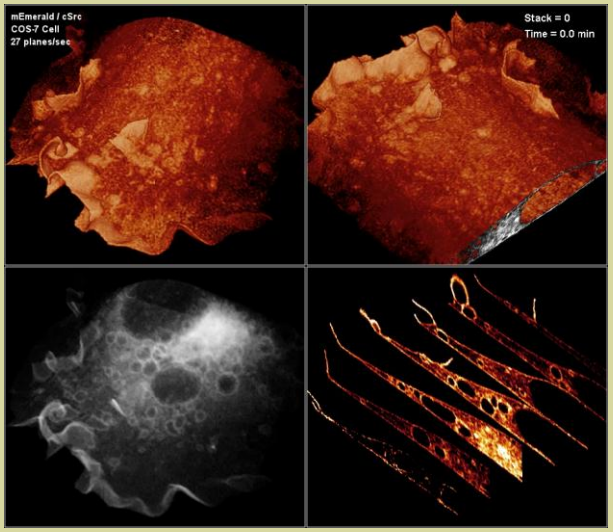
Thomas A Planchon[1,6], Liang Gao[1,6], Daniel E Milkie[2], Michael W Davidson[3], James A Galbraith[4], Catherine G Galbraith[5] & Eric Betzig[1]

[Planchon et al., Nature Methods 8, 2011.]

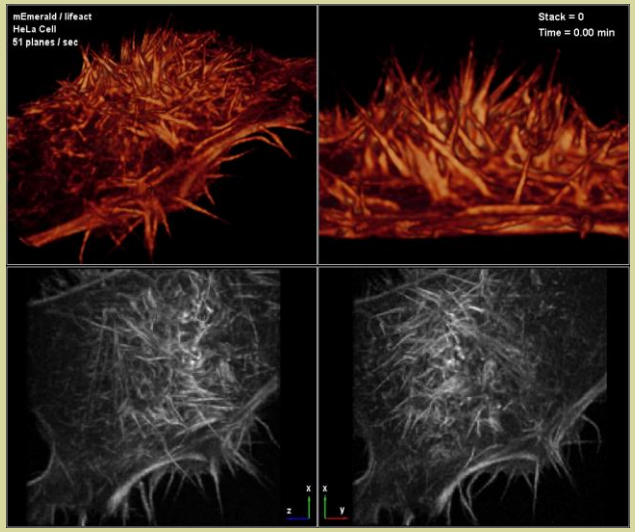


LLC-PK1 H2B staining

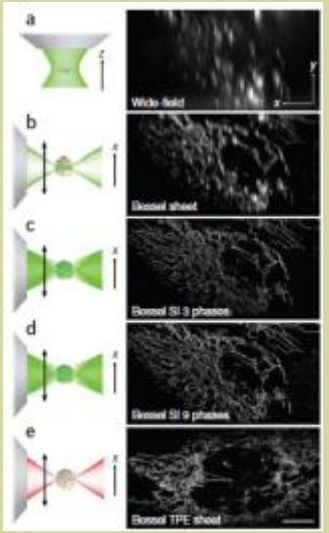
- scanned 2 photon Bessel beam
- structured illumination
- deconvolution
- imaging at up to 140 frames / s
- membrane dynamics, microtubules, cell division



COS-7 cell membrane, 1 stack / 12 sec.



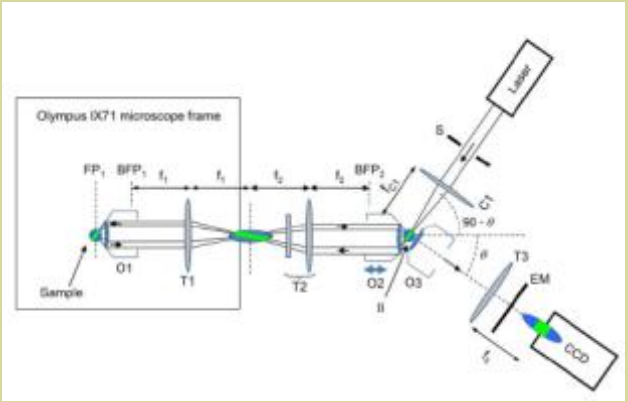
HeLa cell filopodia, 1 stack / 6 sec.



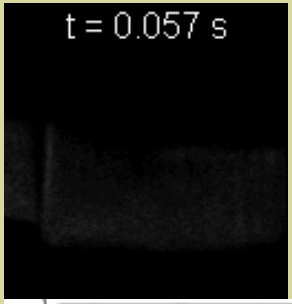
High-speed 2D and 3D fluorescence microscopy of cardiac myocytes

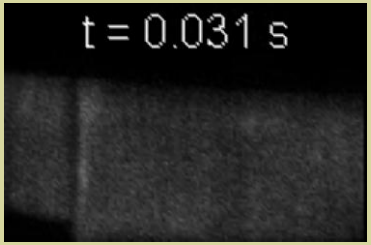
Sunil Kumar,[1] Dean Wilding,[1] Markus B. Sikkel,[2] Alexander R. Lyon,[2] Ken T. MacLeod,[2] and Chris Dunsby[1,3,*]

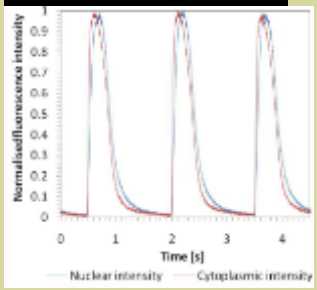
[1]Photonics Group, Department of Physics, Blackett Laboratory, South Kensington Campus, Imperial College London, London, SW7 2AZ, UK
[2]National Heart and Lung Institute, Imperial College London, London, SW3 6LY, UK
[3]Department of Medicine, Hammersmith Campus, Imperial College London, London, W12 0NN, UK
*christopher.dunsby@imperial.ac.uk

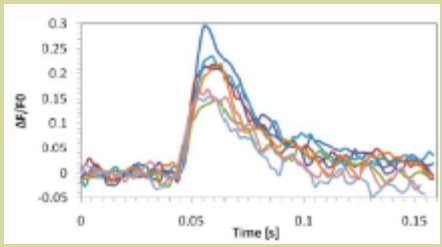


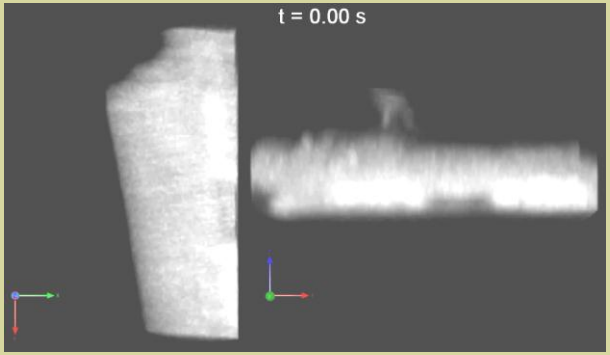
[Kumar et al., Optics Express, 2011.]



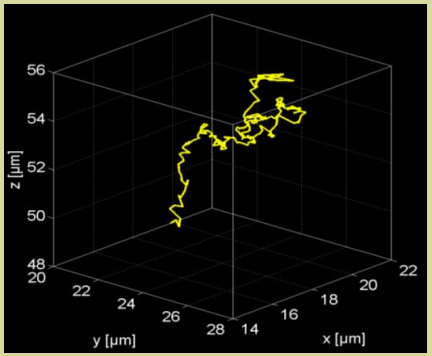








- adult rat cardiac myocytes loaded with Ca2+ indicator (Fluo-4)
- rapid scanning, 926 fps // 21 vol / s
- detection of Ca2+ waves, (a) spontaneous and (b) induced by electrical stimulation
- 3D rendering (c), induced and spontaneous sparks



Superresolution and single molecule imaging

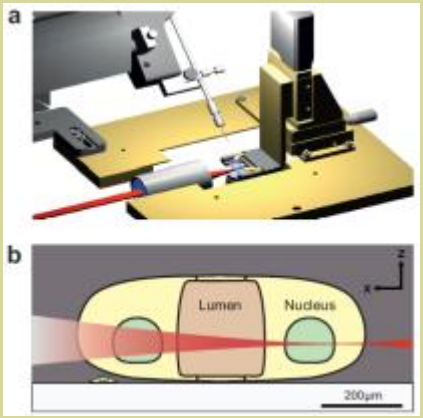
- 3D single particle tracking
- 3D superresolution imaging in thick samples

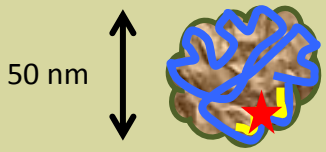
Nuclear export of single native mRNA molecules observed by light sheet fluorescence microscopy

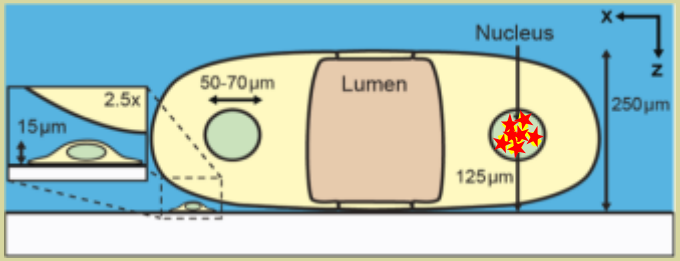
Jan Peter Siebrasse, Tim Kaminski, and Ulrich Kubitscheck[1]

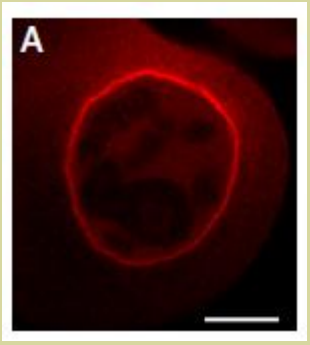
Institute of Physical and Theoretical Chemistry, Rheinische Friedrich-Wilhelms-University Bonn, 53115 Bonn, Germany

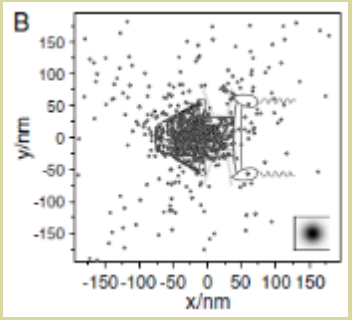
Edited by Joseph G. Gall, Carnegie Institution of Washington, Baltimore, MD, and approved April 12, 2012 (received for review February 1, 2012)

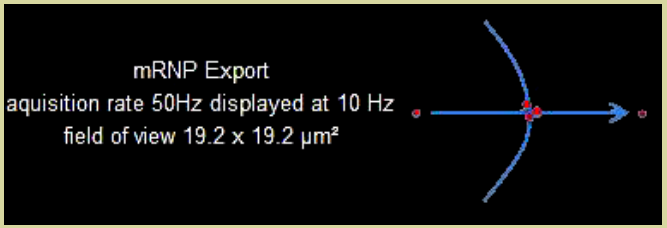


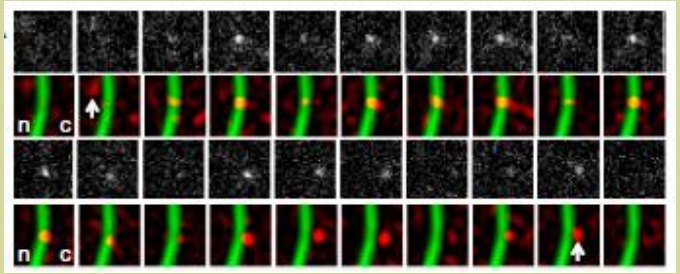












- one-sided illumination, multi-color
- micro-injection of labeled packing protein
- tracking of fluorescently labeled mRNA particles inside nucleus
- acquisition: 50 fps // displayed @ 10 fps

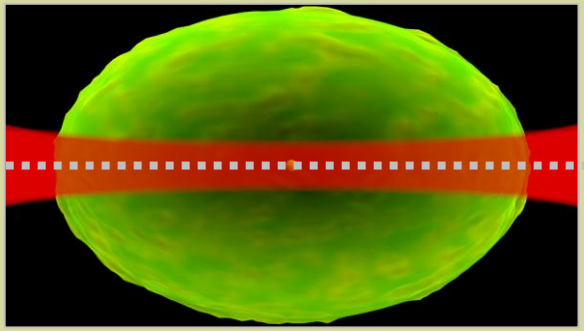
[Siebrasse et al., PNAS 109(24), 2012.]

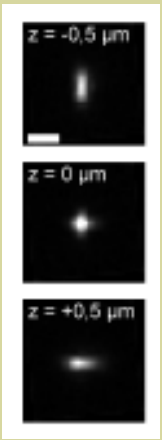
Dynamic three-dimensional tracking of single fluorescent nanoparticles deep inside living tissue

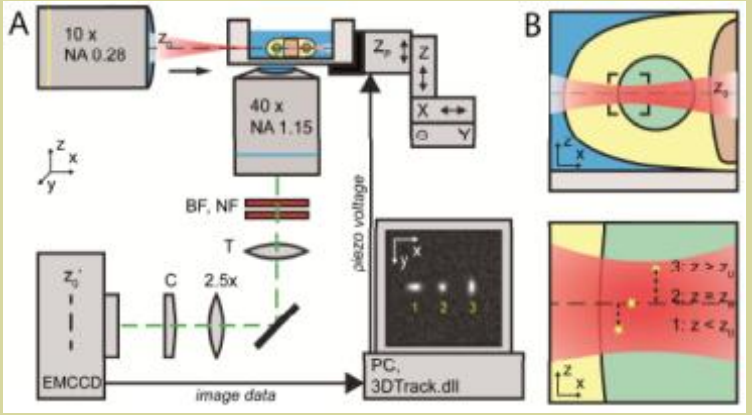
Jan-Hendrik Spille,* Tim Kaminski, Heinz-Peter Königshoven, and Ulrich Kubitscheck

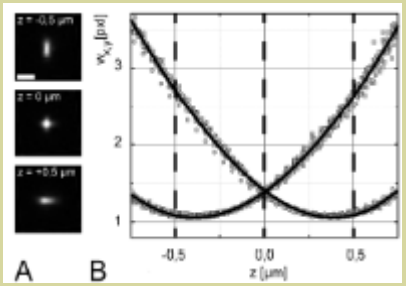
Institute of Physical and Theoretical Chemistry, Rheinische Friedrich-Wilhelms Universität Bonn, Wegelerstraße 12, D-53115 Bonn, Germany

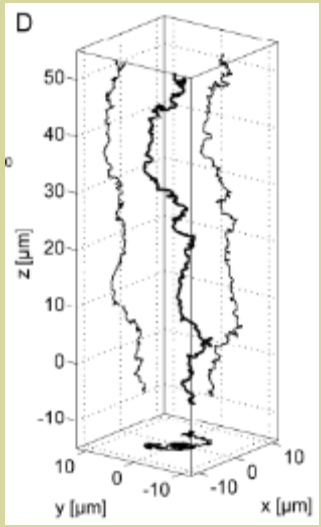
*spille@pc.uni-bonn.de

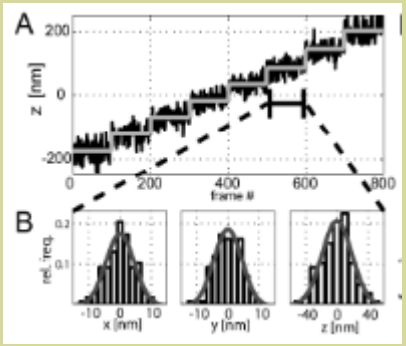






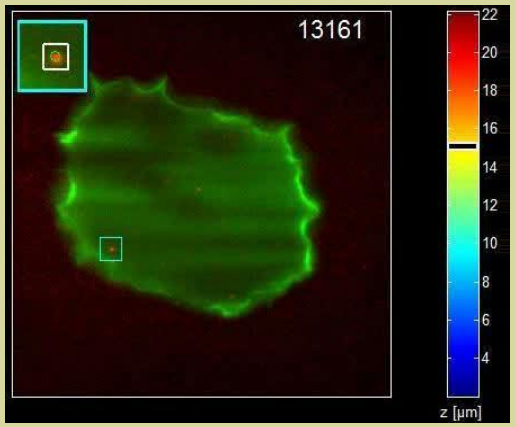


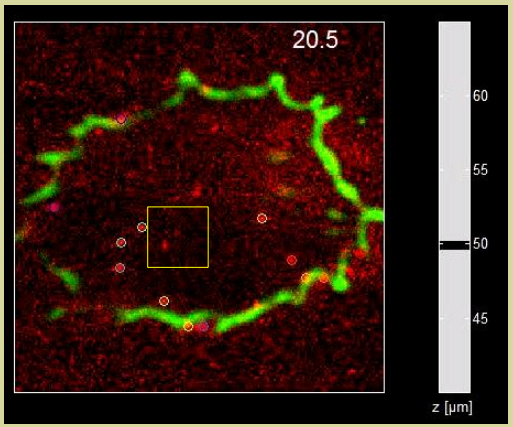


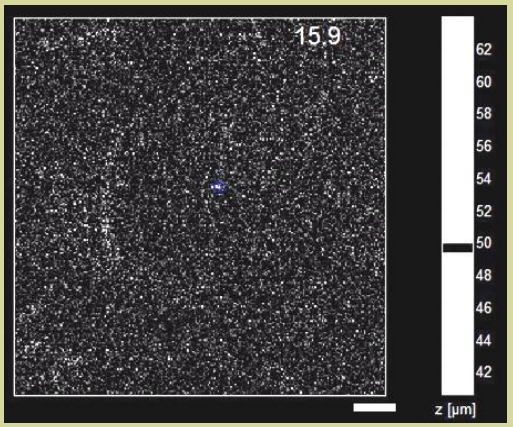


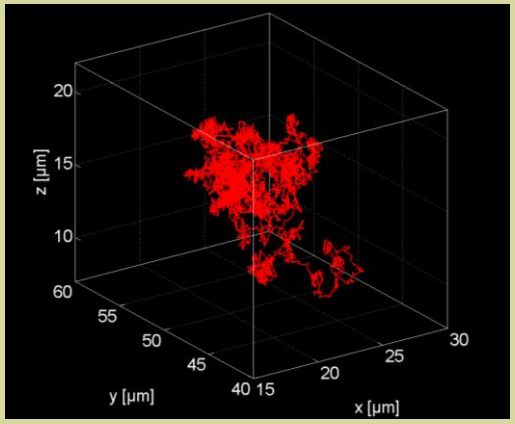
- one-sided illumination, multi-color
- astigmatic detection for 3D localization
- feedback loop to keep particle in focus

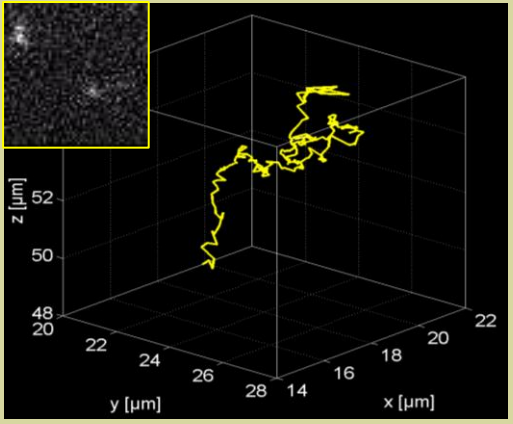
[Spille et al., Optics Express 20(18), 2012.]

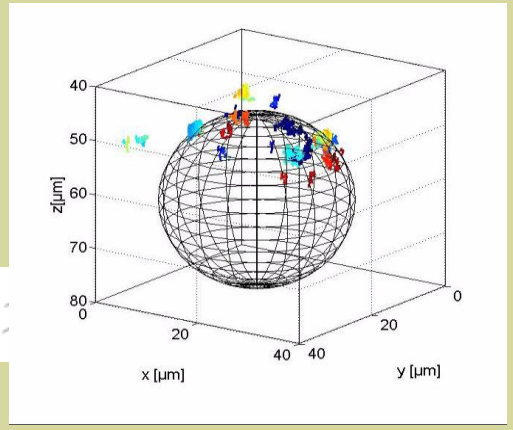












- 3D tracking of fluorescent beads in C. Tentans salivary gland cell nucleus
- 20 Hz

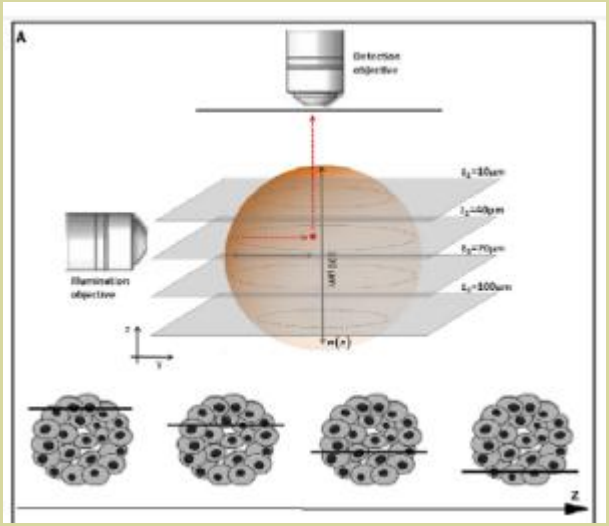
[Spille et al., Optics Express 20(18), 2012.]

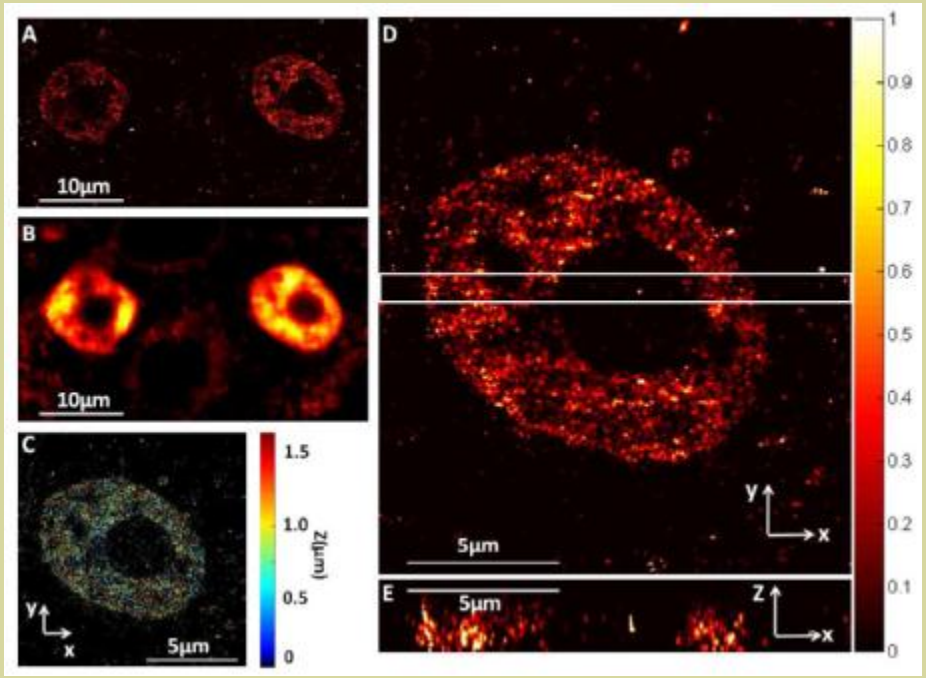
- 3D mRNA tracking
- BR2-oligonucleotide carrying 2-3 Atto647 dyes
- 50 Hz

- Lipids with single label in GUV bilayer membrane
- 63 Hz

Live-cell 3D super-resolution imaging in thick biological samples

Francesca Cella Zanacchi[1], Zeno Lavagnino[1,2], Michela Perrone Donnorso[1], Alessio Del Bue[1], Laura Furia[3], Mario Faretta[3] & Alberto Diaspro[1,2]





- live cell spheroid imaging (H2B-PAmCherry)
- photoactivation in a light sheet (λ 405 nm)
- single molecule localization (λ 561 nm) and super-resolved image reconstruction

[Zanacchi et al., Nature Methods 8 (12), 2011.]

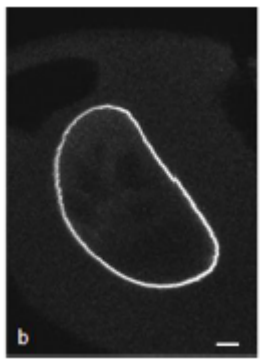
Mikroskopie mit Hochauflösung
in drei Dimensionen

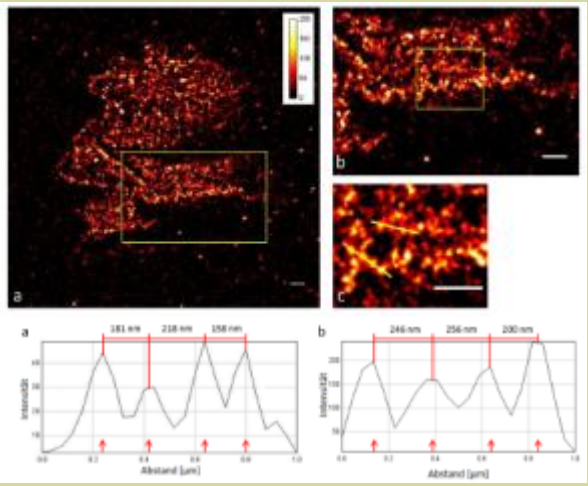
Diplomarbeit im Fach Chemie

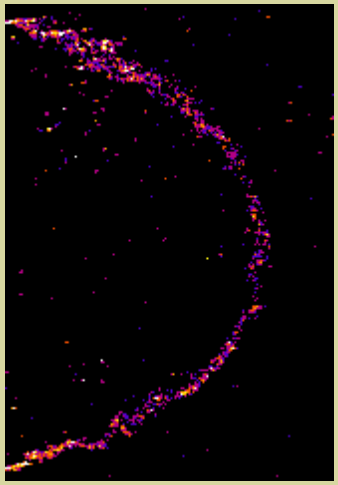
vorgelegt von

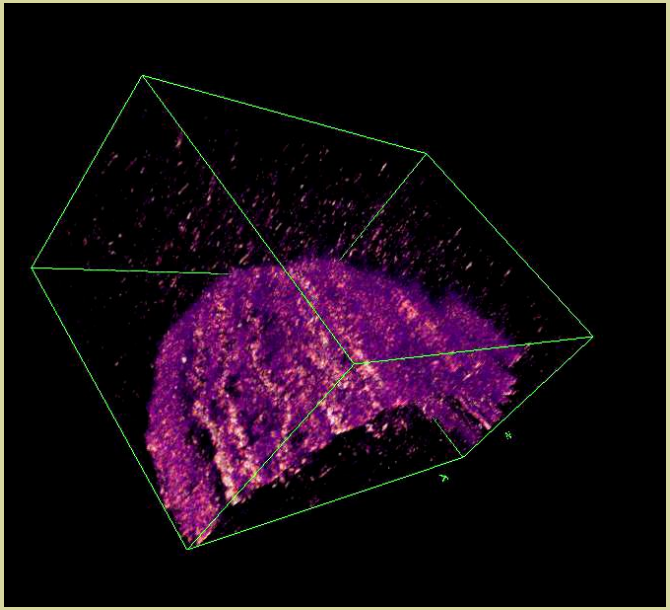
Ana Lina Meskes
im
Dezember 2011

angefertigt am
Institut für Physikalische und Theoretische Chemie
der
Rheinischen Friedrich-Wilhelms-Universität Bonn









- NPC in C. tentans salivary gland cell nucleus
- secondary antibody staining with AlexaFluor 647
- GOD/KAT – MEA STORM buffer
- 488 nm activation, 640 nm imaging
- stack volume: 20 x 25 x 15 µm³

Ultramicroscopy of cleared tissue

- Volumetric imaging of whole brains
- Tracing neurons
- Neuronal connectivity

Ultramicroscopy of cleared tissue:

Tissue is opaque, penetration < 1mm

Immerse in medium with refractive index of proteins (n ~ 1.56): Clearing solution

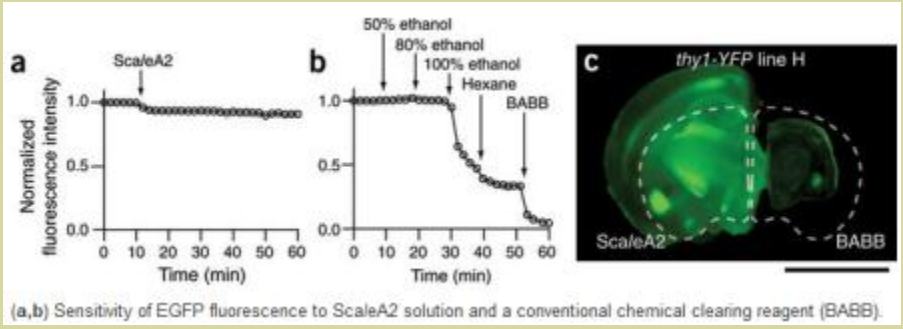
Dodt 2007 / Spalteholz 1914:

1. Fixation
2. Dehydration in graded ethanol series and hexane (1 week)
3. Immerse in benzylalcohol-benzylbenzoat (BABB, 2d)

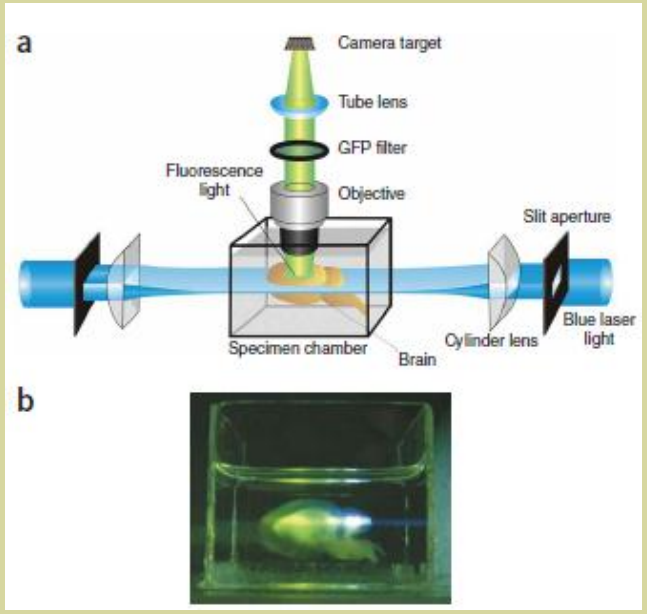
Hama 2011:

1. Fixation
2. [Urea, Triton-X, glycerol] („ScaleA2“, 5d)

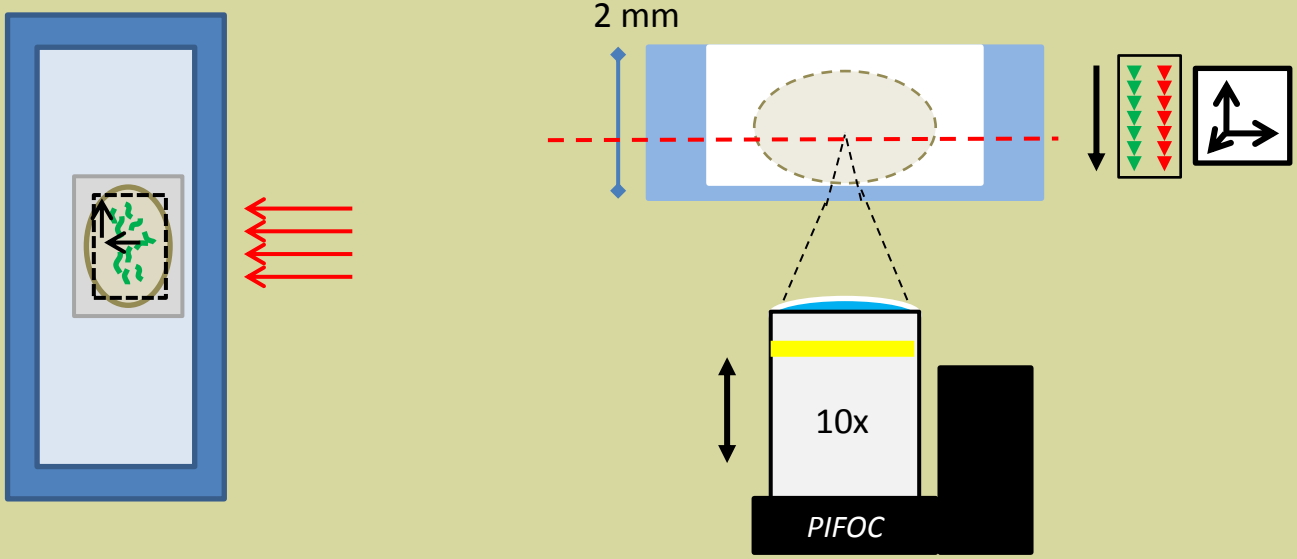
[Hama et al., Nature Neuroscience 14, 2011.]



(a,b) Sensitivity of EGFP fluorescence to ScaleA2 solution and a conventional chemical clearing reagent (BABB).

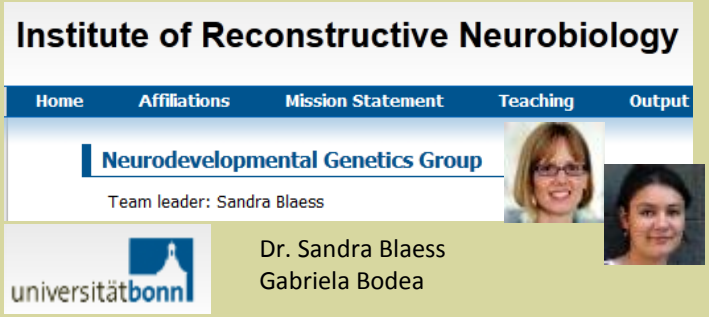


[Dodt et al., Nature Methods 4(4), 2007.]



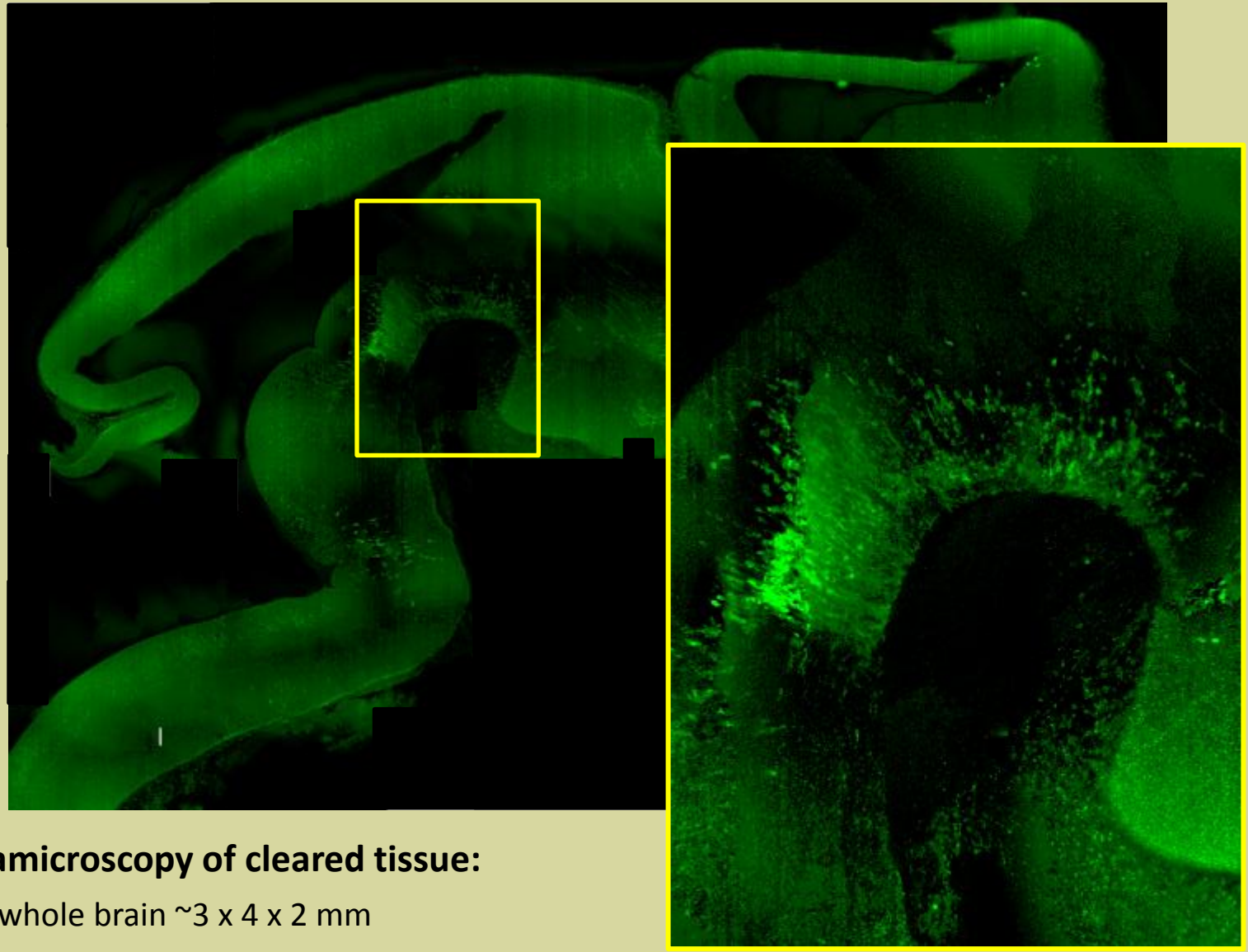
Ultramicroscopy of cleared tissue:

- progenitor cells and dopaminergic neurons
- specific labelling of both cell types
- differentiation -> migration
- identify transcription factors that regulate migration



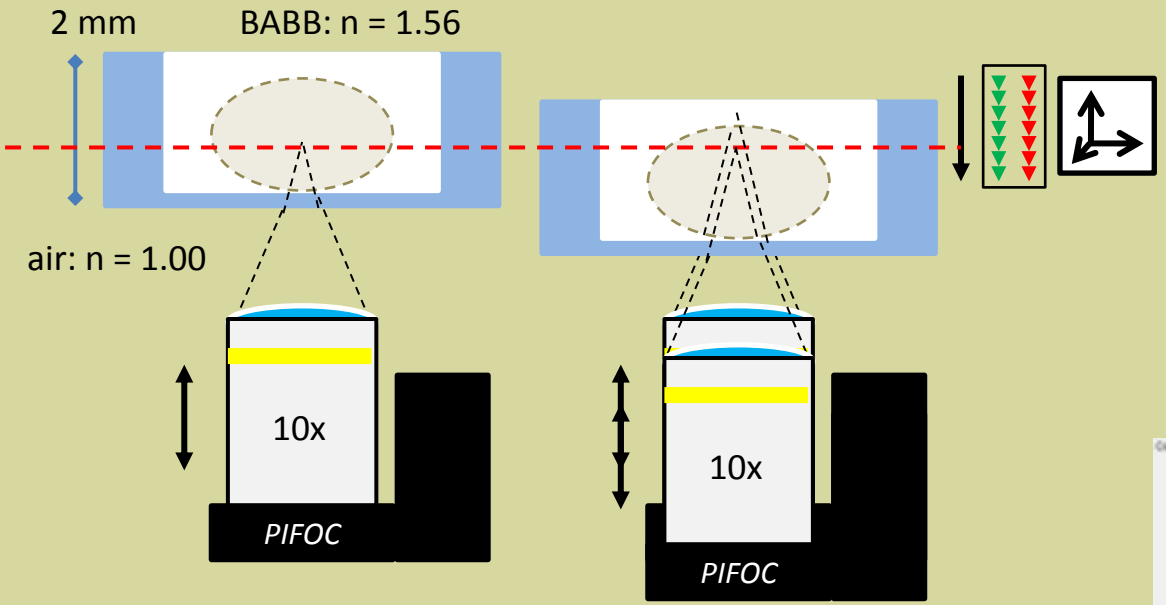
Dr. Sandra Blaess
Gabriela Bodea

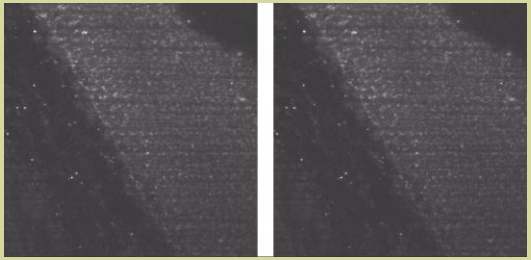
[Bodea et al., in preparation.]



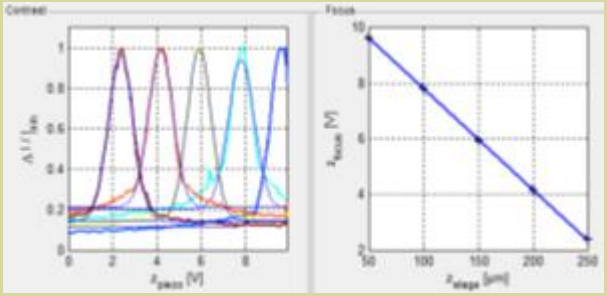
Ultramicroscopy of cleared tissue:

- whole brain ~3 x 4 x 2 mm
- midbrain ~ 700 x 700 x 500 µm





250 x 1 µm
10x



contrast vs z

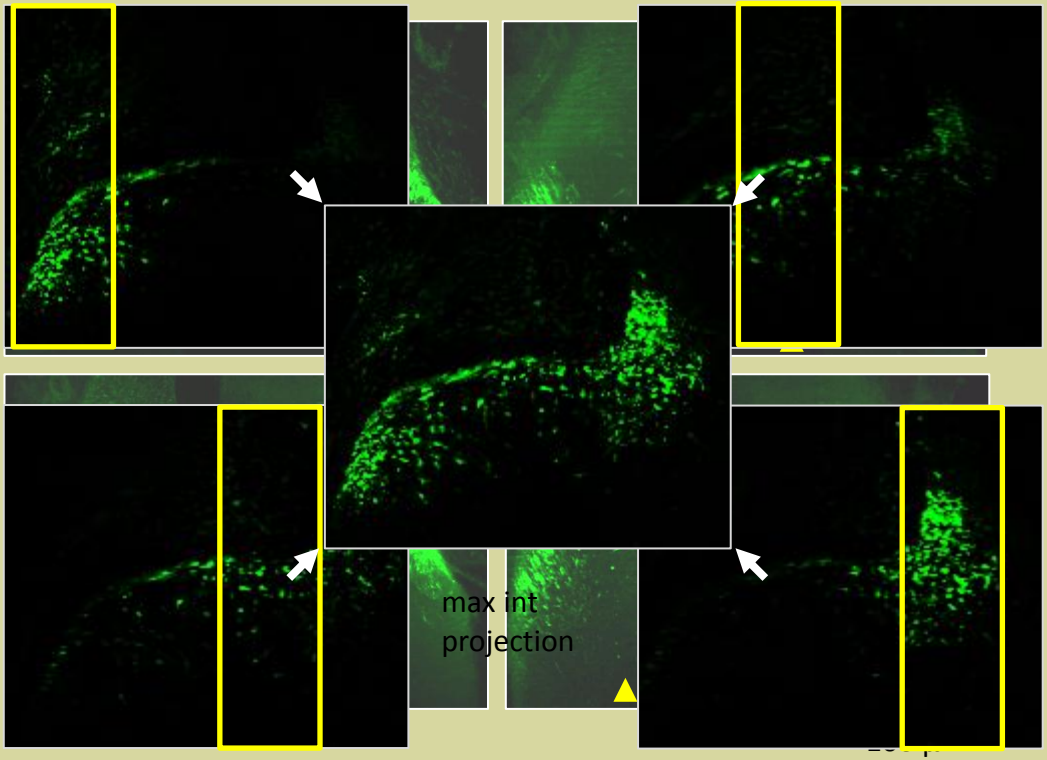
stage position
vs actual focus

$$f' - f = d' - d \approx d'\left(1 - 1/n\right) \approx 0.36\, d'$$

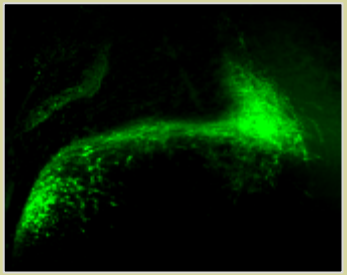
[Silvestri et al., Optics Express 20(18), 2012.]

Ultramicroscopy of cleared tissue:

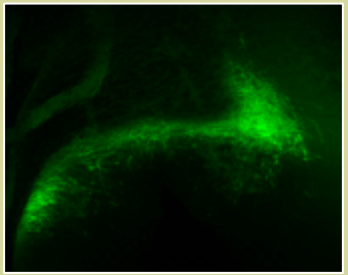
- thick sample, low magnification -> low NA objective, long working distance
- air objective -> refractive index mismatch
- use objective coupled piezo to correct automatically



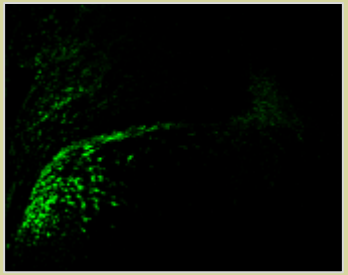
raw
slice n



background =
min(n +- 9)

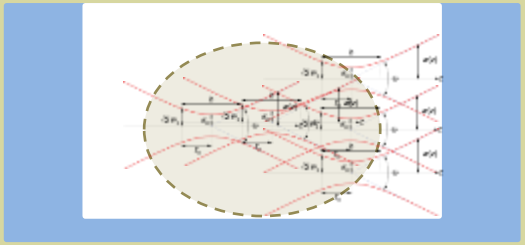


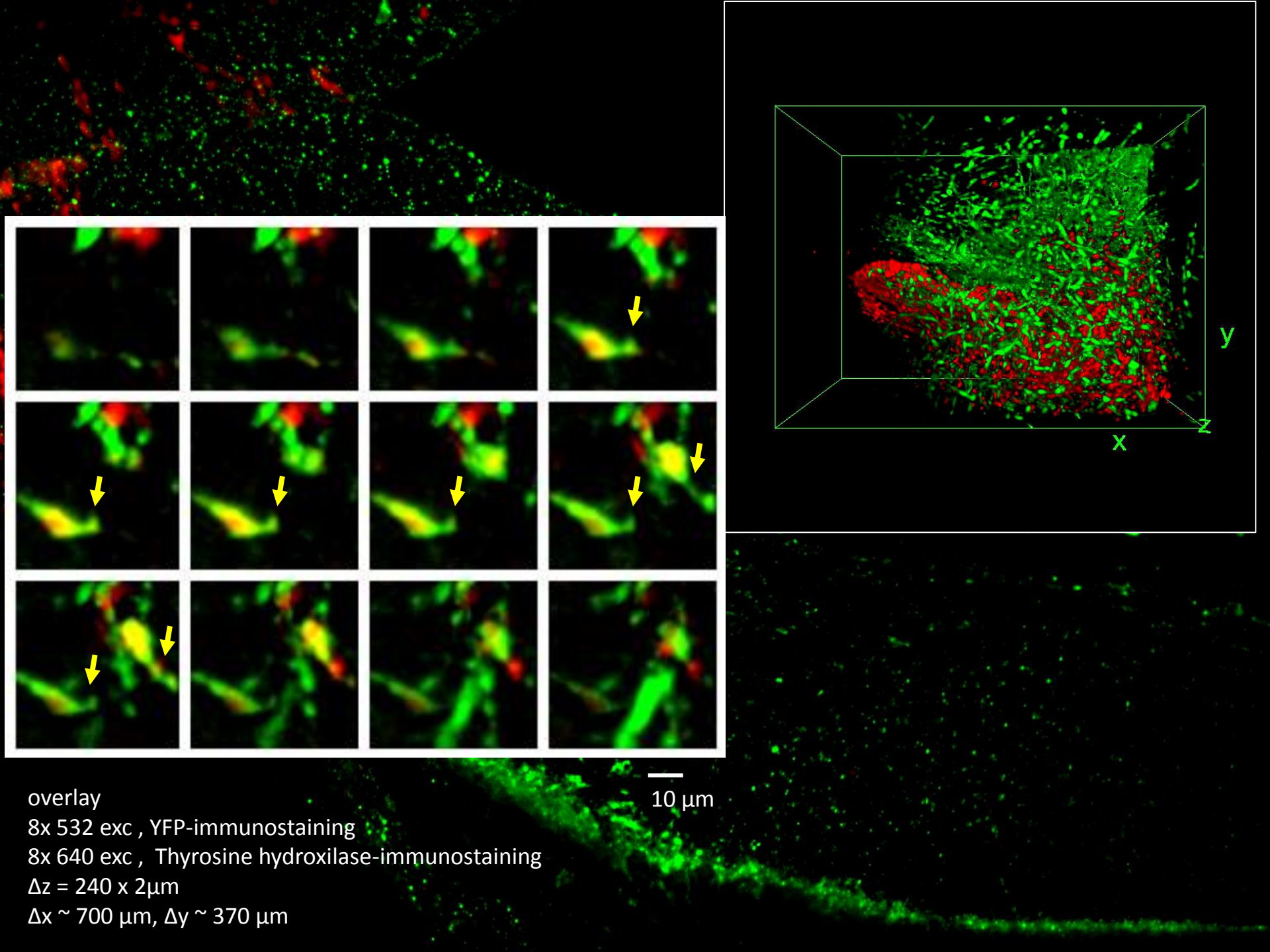
raw -
background



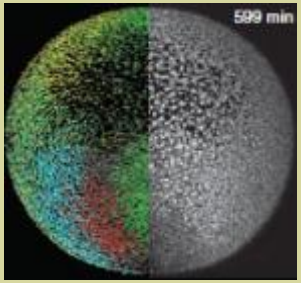
Ultramicroscopy of cleared tissue:

- subcellular resolution -> thin sectioning -> low Rayleigh length
- outside Rayleigh length: out-of-focus information
- -> background subtraction + image fusion





overlay
8x 532 exc , YFP-immunostaining
8x 640 exc , Thyrosine hydroxilase-immunostaining
Δz = 240 x 2µm
Δx ~ 700 µm, Δy ~ 370 µm



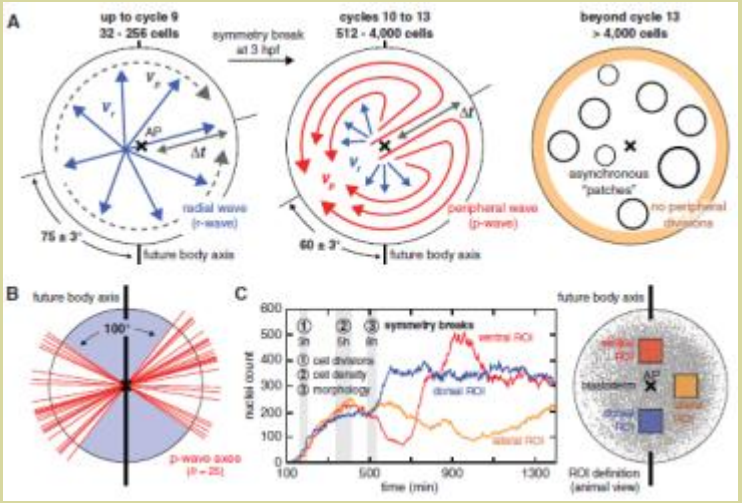
Zebrafish imaging

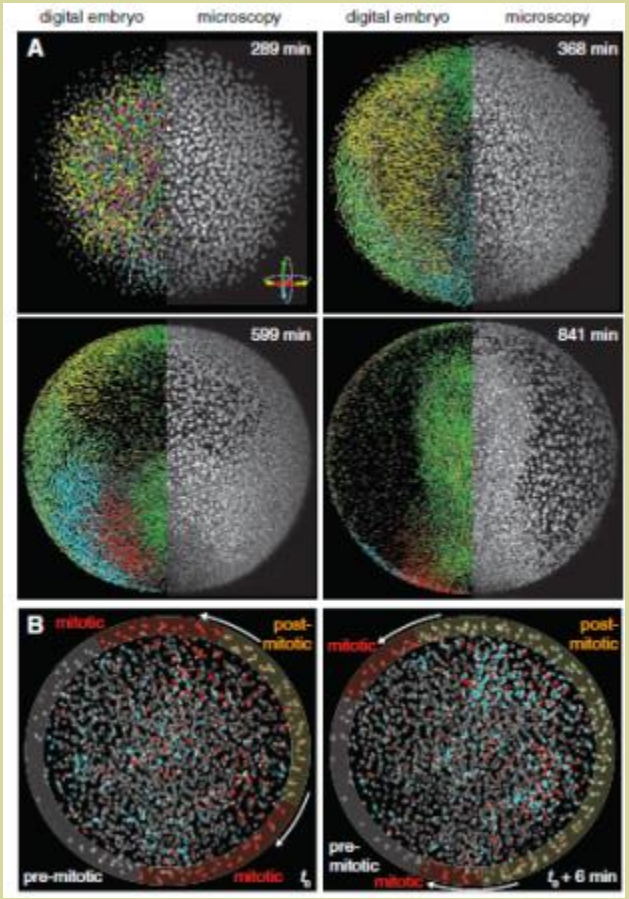
- Cell tracking
- Organ formation
- Highspeed imaging of beating heart
- Optogenetics

Reconstruction of Zebrafish Early Embryonic Development by Scanned Light Sheet Microscopy

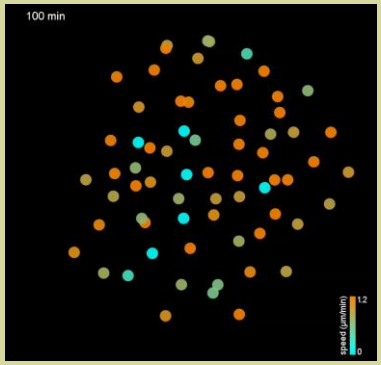
Philipp J. Keller,[1,2]* Annette D. Schmidt,[2] Joachim Wittbrodt,[1,2,3,4]* Ernst H.K. Stelzer[1]

- DSLM, one-sided illumination
- cell division patterns, H2B-eGFP staining
- organ formation in zebrafish over 24h
- „Digital Embryo“: position data for each embryo
- symmetry break in cell division pattern indicates future body axis

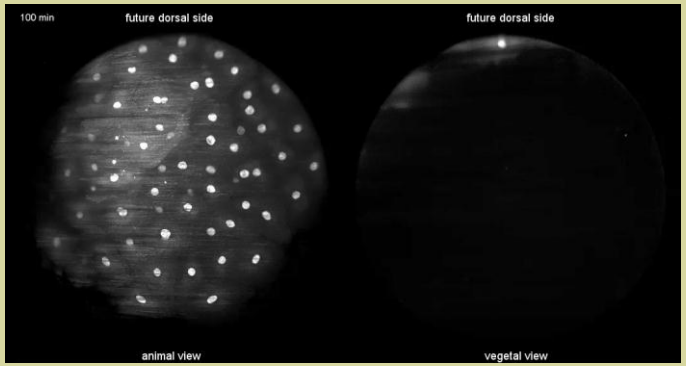




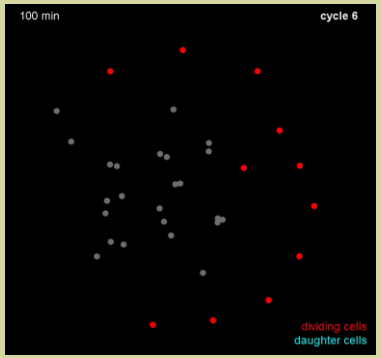
[Keller et al., Science 322, 2008.]



digital embryo

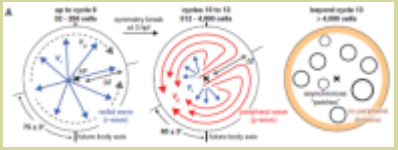


microscopy data



cell division:

radial – peripheral - patches



Reconstruction of zebrafish early embryonic development by scanned light sheet microscopy

Philipp J. Keller[1,2•], Annette D. Schmidt[2], Joachim Wittbrodt[1,2,3,4•] and Ernst H.K. Stelzer[1]

[1]Cell Biology and Biophysics Unit and [2]Developmental Biology Unit
European Molecular Biology Laboratory, Germany

[3]Institute of Zoology, Department for Developmental Physiology
University of Heidelberg, Germany

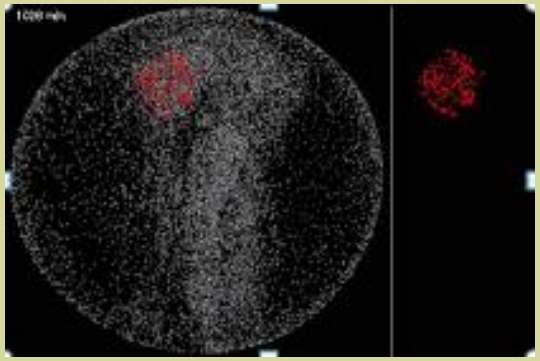
[4]Institute of Toxicology and Genetics
Karlsruhe Institute of Technology, Germany

•To whom correspondence should be addressed.
Email: keller(at)embl.de, wittbrod(at)embl.de

Science, 14 November 2008, vol. 322, no. 5904, pp. 1065-1069

Overview of the contents of this data repository

1) Digital Embryo Movies (Movies 1-16)
2) High-Quality Figures (Figures 1-6, S1-S9)
3) Digital Embryo Databases (Embryos 1-7)
4) Source Code of selected core modules of the Digital Embryo processing pipeline



(reverse) retinal progenitor tracking

[Keller et al., Science 322, 2008.]

DEVELOPMENT AND DISEASE RESEARCH ARTICLE 1179

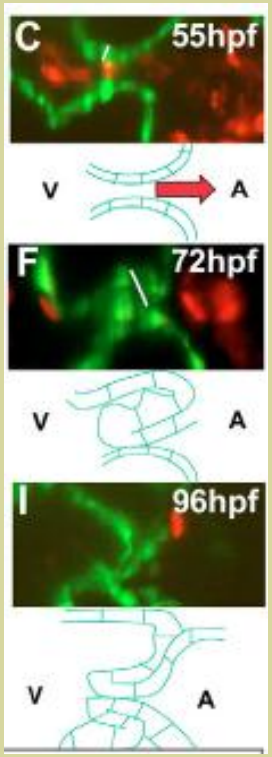
Development 135, 1179-1187 (2008) doi:10.1242/dev.010694

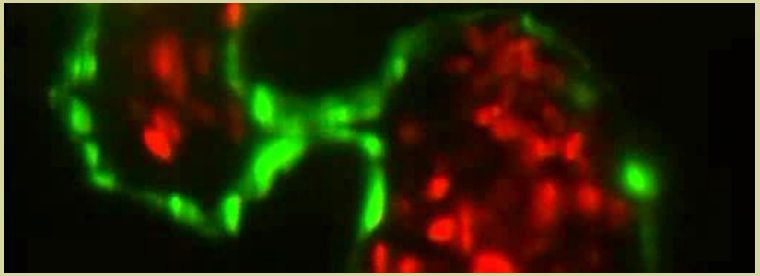
[Scherz et al., Development 135(6), 2008.]

High-speed imaging of developing heart valves reveals interplay of morphogenesis and function

Paul J. Scherz, Jan Huisken, Pankaj Sahai-Hernandez and Didier Y. R. Stainier*

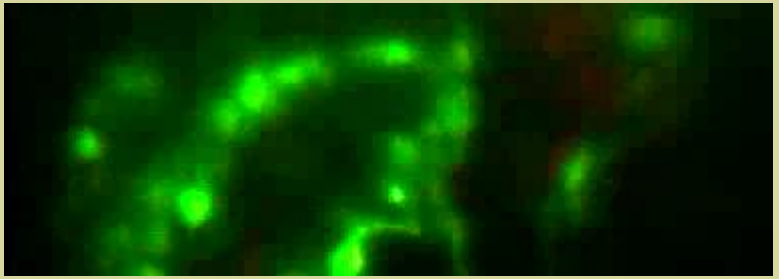
- classical SPIM, one cylindrical lens
- 2 synchronously running cameras for red/green channel
- zebrafish beating heart imaging at 70 – 160 fps
- Leaflet formation between atrium and ventricle and signaling pathways leading to morphogenesis





114 fps displayed at 30 fps

55 hpf

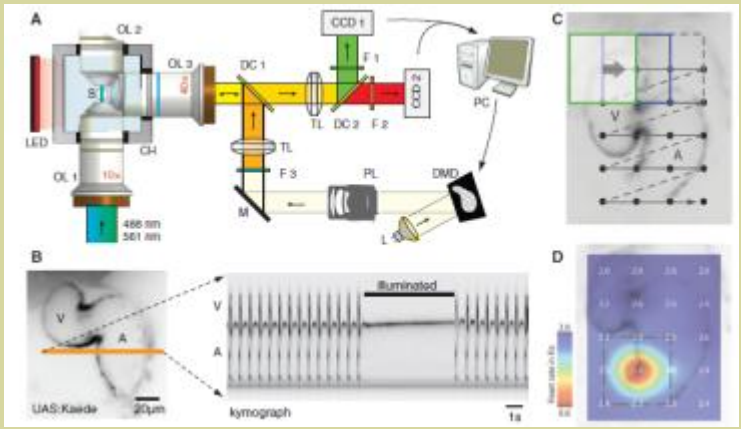


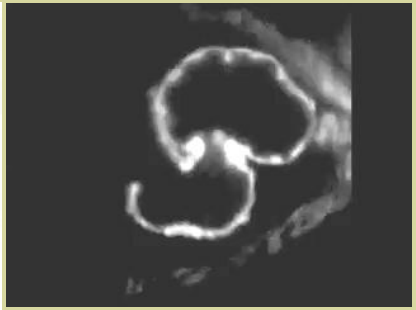
137 fps displayed at 30 fps

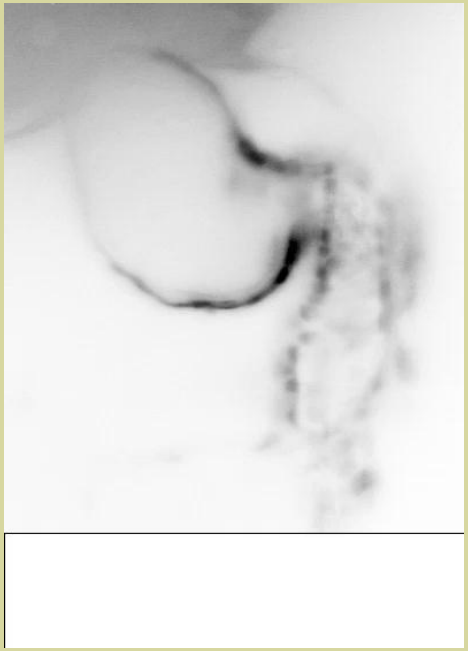
102 hpf

Optogenetic Control of Cardiac Function

Aristides B. Arrenberg,[1,3] Didier Y. R. Stainier,[2*] Herwig Baier,[1] Jan Huisken[2,4]

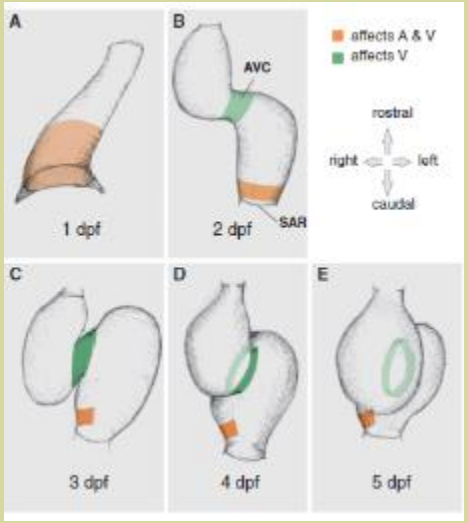


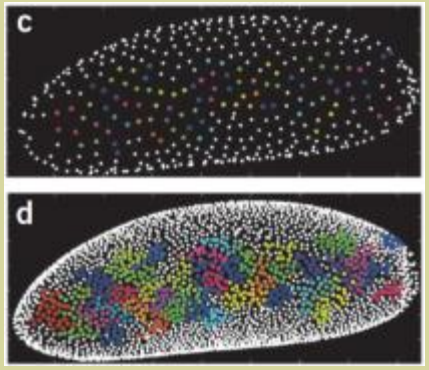




[Arrenberg et al., Science 330, 2010.]

- classical mSPIM, two-sided illumination, 40x detection
- 2 synchronously running cameras for red/green channel
- Illumination pattern for optogenetic control
- expression of light-gated ion-channels (halorhodopsin NpHR-mCherry; channelrhodopsin-2 H134R ChR2) to control cardiac pacemaker function
- illumination with orange light activates chloride pump NpHR, hyperpolarization of cell
- identify pacemakers on single cell level





Drosophila embryogenesis

- Cell tracking
- Gene expression patterns

Gene expression patterns during embryogenesis:

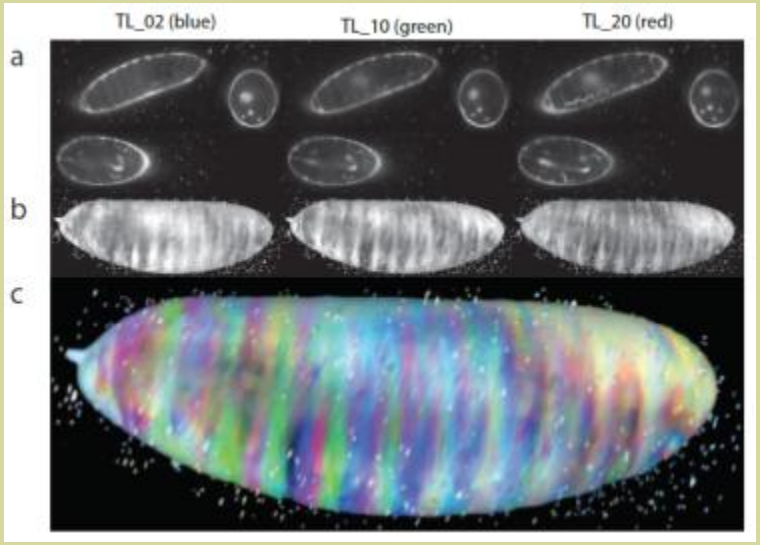
Observe spatial distribution of gene expression during embryogenesis

BRIEF COMMUNICATION

Nature Methods **6**, 435 - 437 (2009)
Published online: 24 May 2009 | doi:10.1038/nmeth.1334

A toolkit for high-throughput, cross-species gene engineering in *Drosophila*

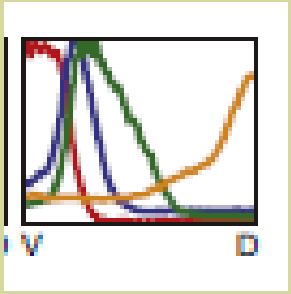
Radoslaw K Ejsmont[1], Mihail Sarov[1], Sylke Winkler[1], Kamil A Lipinski[1] & Pavel Tomancak[1]

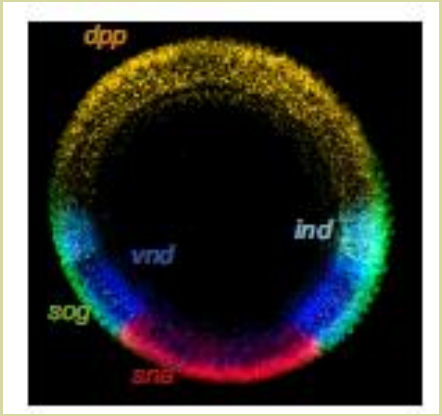


[Ejsmont et al., Nature Methods 6(6), 2009.]

Dorsal-Ventral Gene Expression in the *Drosophila* Embryo Reflects the Dynamics and Precision of the Dorsal Nuclear Gradient

Gregory T. Reeves,[1,3,4] Nathanie Trisnadi,[1,4] Thai V. Truong,[2] Marcos Nahmad,[1] Sophie Katz,[1] and Angelike Stathopoulos[1,*]
[1]Division of Biology
[2]Beckman Institute
California Institute of Technology, Pasadena, CA 91125, USA
[3]Department of Chemical and Biomolecular Engineering, North Carolina State University, Raleigh, NC 27695, USA
[4]These authors contributed equally to this work
*Correspondence: angelike@caltech.edu
DOI 10.1016/j.devcel.2011.12.007





[Reeves et al., Developmental Cell 22, 2012.]

Multiview light-sheet microscope for rapid *in toto* imaging

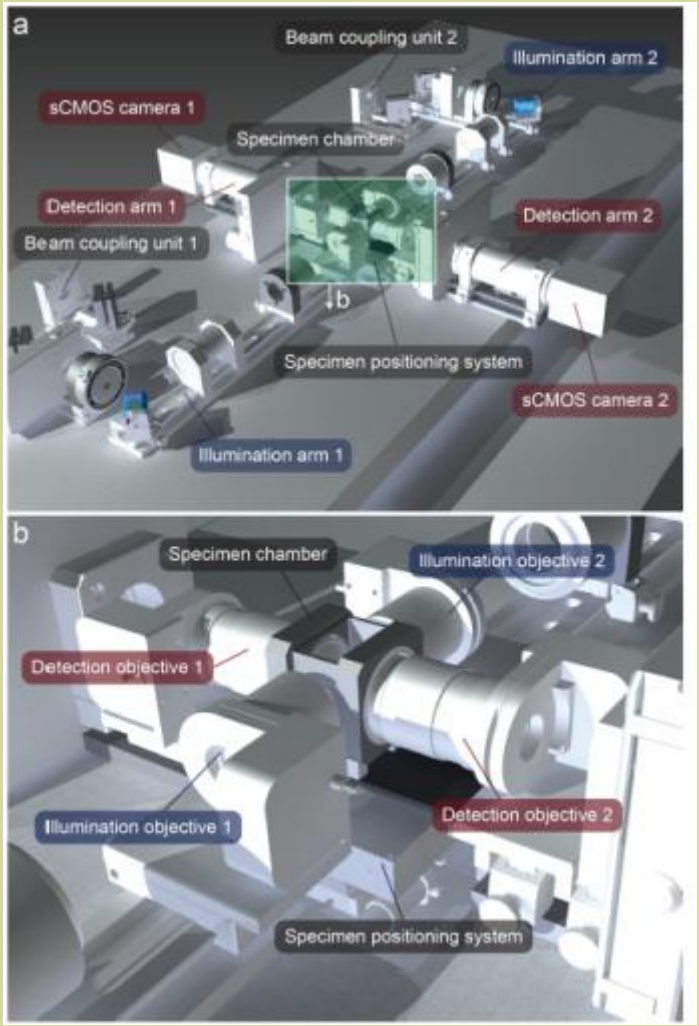
Uros Krzic[1], Stefan Gunther[1,2], Timothy E Saunders[1,2], Sebastian J Streichan[1] & Lars Hufnagel[1]

[Krzic et al., Nature Methods 9(7), 2012.]

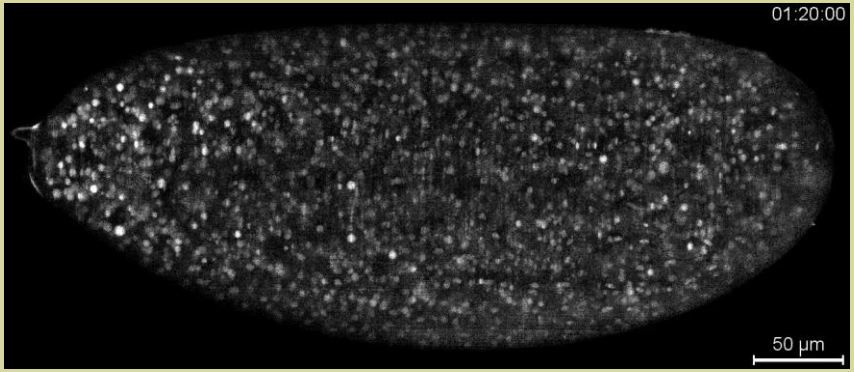
Quantitative high-speed imaging of entire developing embryos with simultaneous multiview light-sheet microscopy

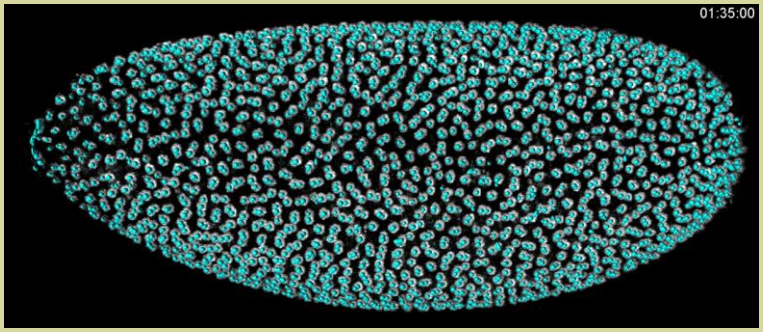
Raju Tomer, Khaled Khairy, Fernando Amat & Philipp J Keller

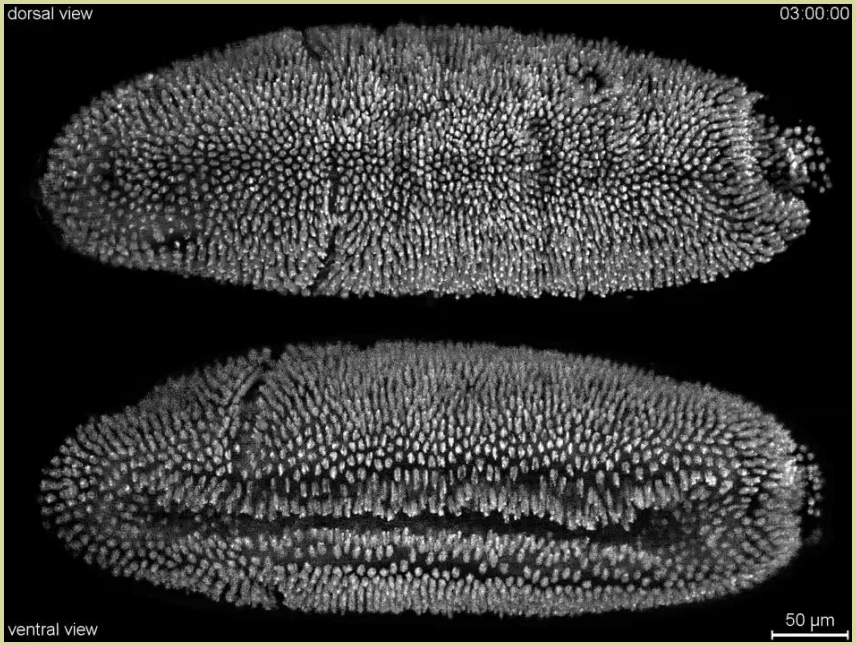
[Tomer et al., Nature Methods 9(7), 2012.]

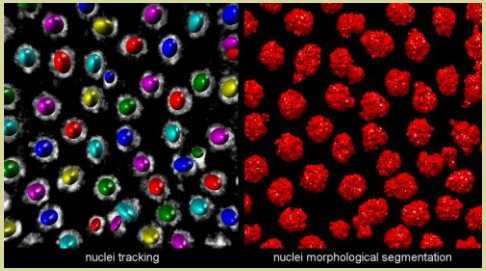


- 15s / image stack, 1 stack / 30s for 15h
- sample rotation possible (20s / 360°) but not necessary
- automated nuclei tracking
- sequential vs. simultaneous multiview
- 1-/2-photon excitation



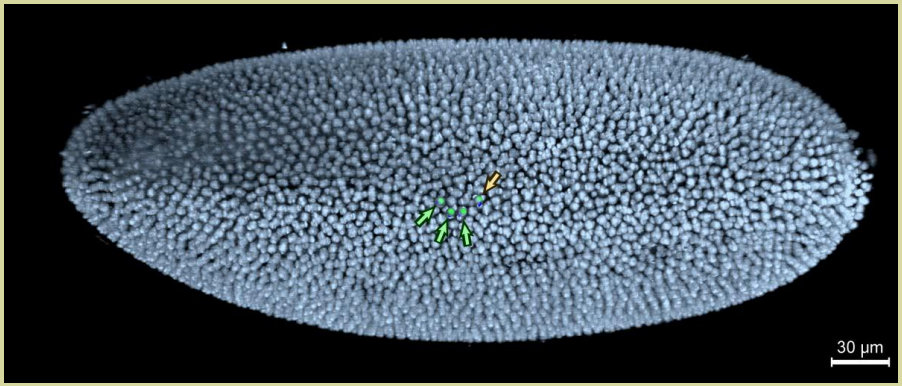


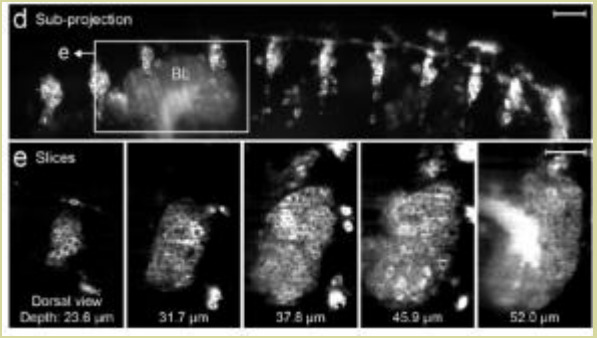


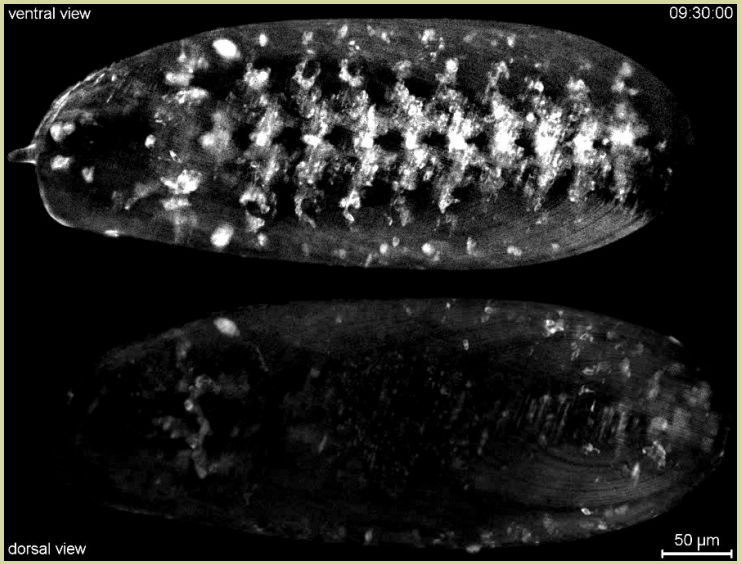


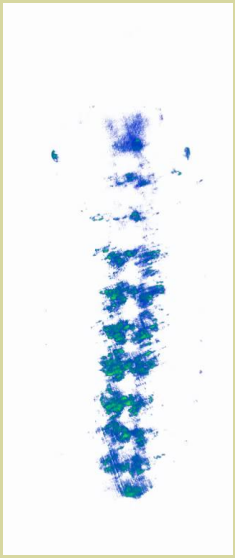
- full embryonic development until hatching
- global nuclei tracking (His2Av-GFP):
  - 1 stack / 40 seconds
  - segmentation efficiency 95%
  - tracking efficiency 99%
  - cell division detection 94%

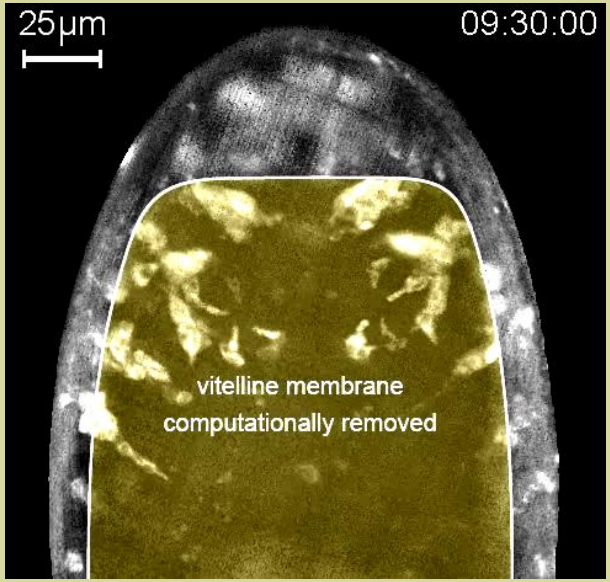
[Tomer et al., Nature Methods 9(7), 2012.]





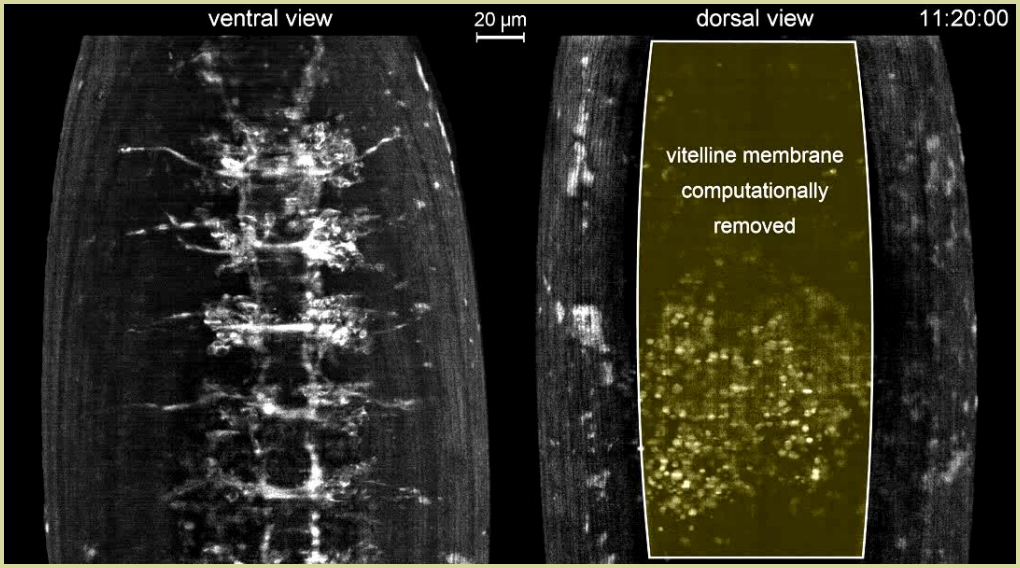


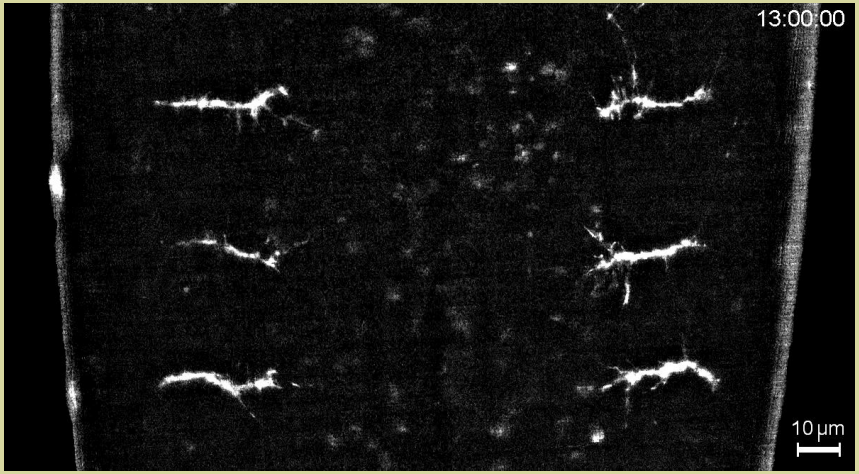


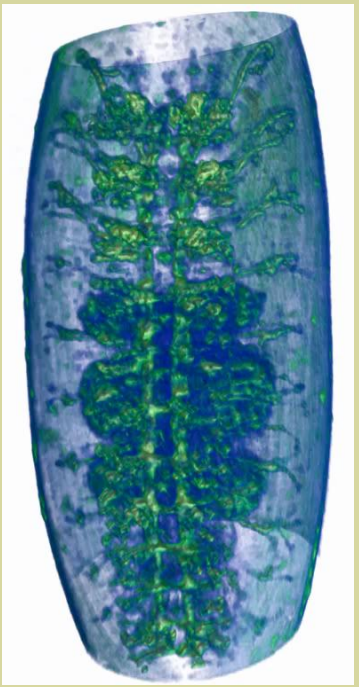


- 4 blastoderm cells differentiating into 3 neuroblasts, 1 epidermoblast
- Neural development (ventral nerve cord), axonal outgrowth with subcellular detail
- Cellular detail in brain lobe

[Tomer et al., Nature Methods 9(7), 2012.]







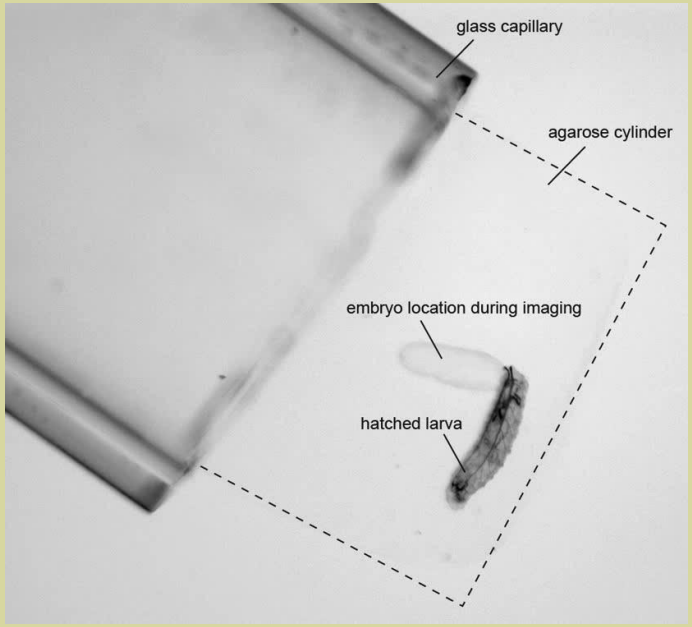
- 40x imaging
- development of central nervous system
- filopodial dynamics during axonal morphogenesis

[Tomer et al., Nature Methods 9(7), 2012.]

[Tomer et al., Nature Methods 9(7), 2012.]

The imaging technique presented here opens the door to high-throughput high-content screening, fast functional imaging and comprehensive quantitative analyses of cellular dynamics in entire developing organisms. By combining this method with advanced computational tools for automated image segmentation and cell tracking, the reconstruction of high-quality cell lineage trees, comprehensive mapping of gene expression dynamics, automated cellular phenotyping and biophysical analyses of cell shape changes and cellular forces are within reach, even for very complex biological systems.

- setup instrument
- install data acquisition and processing framework
- develop entire software environment to view data
- develop machine learning tools for segmentation and object tracking
- ...



Acknowledgements

Santiago, January 15th, 2013

Biophysical chemistry group,
University of Bonn, Germany

Prof. Dr. Ulrich Kubitscheck

Lisa Büttner c
Eugen Baumgart p *Confocal LSFM*
Julia Hockling t
Tim Kaminski b *micro-injection + C. tentans*
Florian Kotzur c *GUV preparation*
Xinliang Liu b
Claudio Nietzel t
Dr. Karl Schmitz c
Katharina Scherer c *GUV preparation*
Ulrike Schmitz-Ziffels c
Dr. Jan-Peter Siebrasse b
Andreas Veenendaal p

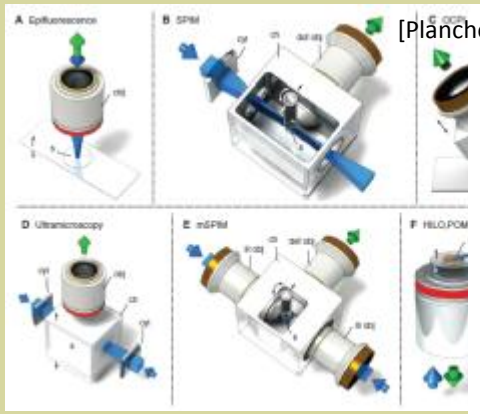
Neurodeleopmental Genetics Group,
University of Bonn, Germany
Sandra Blaess; Gabriela Bodea
mouse brain samples

Goethe Universität Frankfurt, Germany
Prof. Dr. Alexander Heckel; Jennifer Rinne
BR2 oligo

LaVision BioTec, Bielefeld, Germany
Dr. Heinrich Spiecker; Marion Zysik
Live tracking DLL interface

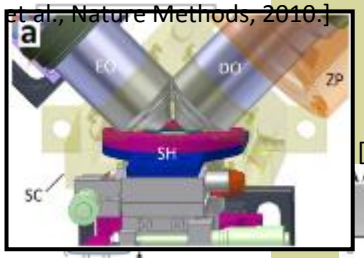
Studienstiftung des deutschen Volkes
Bundesministerium für Wirtschaft und Technologie
DFG

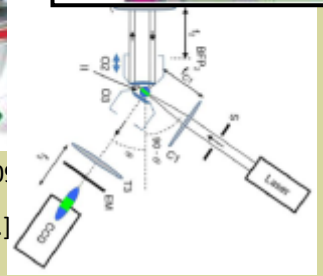
Funding by BMWi, DFG and German National Academic Foundation is gratefully acknowledged!



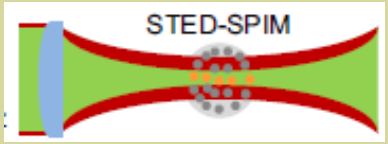
[Planchon et al., Nature Methods, 2010.]

[Huisken, Stainier, Development 136, 200

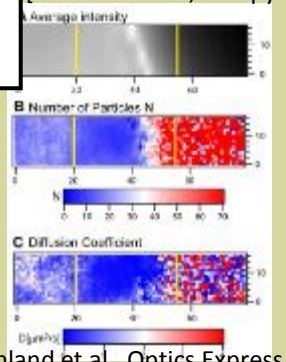




[Kumar et al., Optics Express, 2011.]



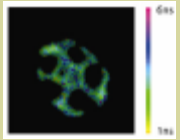
[Friedrich et al., Biohpys J 100(8), 2011.]



[Wohland et al., Optics Express 18(19), 2010.]

SPIM- FLIM

[Greger 2011]



[Karsenti 2011]

SPIM- on ship

Raman LSFM

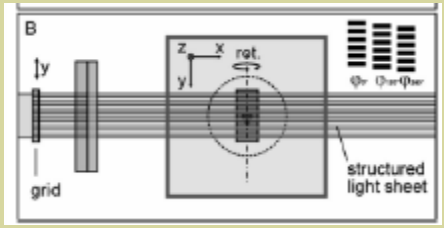
[Oshima et al., Optics Express 20(15), 2012.]

SPIM- FCS

[Truong et al., Nat Meth., 2011.]

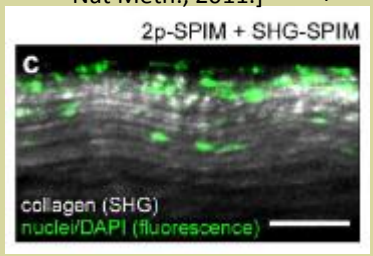
[Krzic et al., Nature Methods 9(7), 2012.]

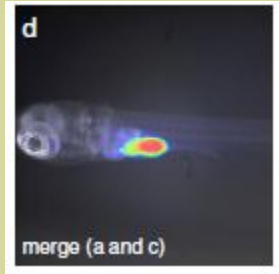
[Tomer et al., Nature Methods 9(7), 2012.]



SIM-SPIM

[Breuninger et al., Optics Letters 32(13), 2007.]



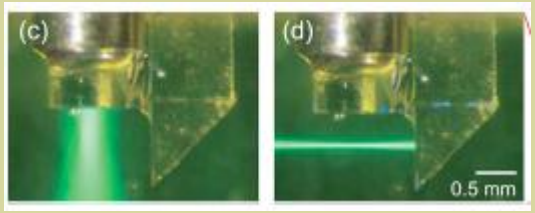


Arabidopsis root growth

[Maizel et al., The Plant Journal, 2012.]

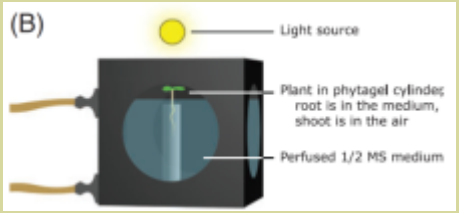
[Silvestri et al., Opt Express 20(18), 2012.]

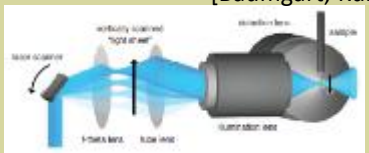
[Baumgart, Kubitscheck, Optics Express 20(19), 2012.]



miniaturized SPIM in vivo

[Engelbrecht et al., Optics Letters 35(9), 2010.]





[Keller et al., Science 322, 2008.]

Recommended literature:

Mouse brain:

- Niedworok et al., „Charting Monosynaptic Connectivity Maps by Two-Color Light-Sheet Fluorescence Microscopy", Cell Reports 2, 2012. – Reconstruction of entire mouse brains.

Drosophila:

- Tomer et al., „Quantitative high-speed imaging of entire developing embryos with simultaneous multiview light-sheet microscopy", Nat. Meth. 9(7), 2012. – Complete embryogenensis with automated nuclei segementation and tracking, CNS development.

Zebrafish:

- Keller et al., „Reconstruction of Zebrafish Embryonic Development by Digital Scanned Light Sheet Microscopy", Science 322, 2008. – First extensive use of DSLM and cell tracking in developmental biology.
- Arrenberg et al., „Optogenetic control of zebrafish heart", Science 330, 2010. – Switching zebrafish heart by light illumination, fast imaging of live specimen.

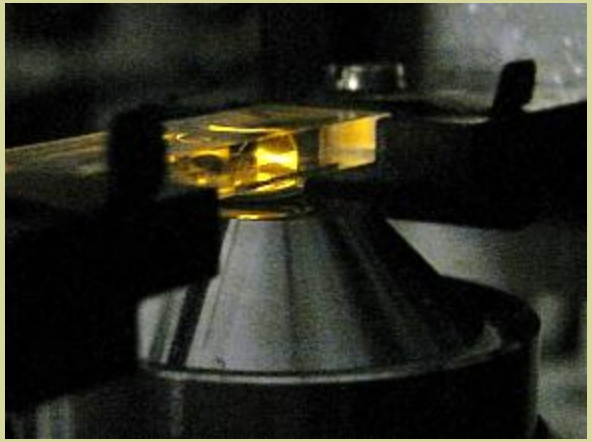
Technical development:

- Ritter et al., „Light Sheet Microscopy for Single Molecule Traching in Living Tissue", PlosOne 5, 2010. – Single molecule tracking deep inside living specimen.
- Spille et al., „Dynamic three-dimensional tracking of single fluorescent nanoparticles deep inside living tissue", Optics Express 20(18), 2012. – Extension to 3D tracking in a feedback loop.
- Silvestri et al., „Confocal light sheet microscopy: micron-scale neuroanatomy of the entire mouse brain", Optics Express 20(18), 2012 . – Confocal light sheet microscopy of entire mouse brain.
- Baumgart and Kubtischeck, „Scanned light sheet microscopy with confocal slit detection", Optics Express 20(19), 2012. – Same principle but using camera rolling shutter instead of descanning unit.
- Fahrbach and Rohrbach, „A line scanned light-sheet microscope with phase shaped self-reconstructing beams", Optics Express 18(23), 2010. – Use of Bessel beam illumination.
- Truong et al., „Deep and fast live imaging with two-photon scanned light-sheet microscopy", Nat. Meth. 8, 2012. – Characterization and use of two photon excitation for high resolution imaging with low phototoxicity.
- Planchon et al., „Rapid three-dimensional isotropic imaging of living cells using Bessel beam plane illumination", Nat. Meth., 2011.- Use of 45° illumination scheme for imaging of adherent cells with isotropically high resolution.

Reviews:

- Höckendorf et al., Quantitative Analysis of Embryogenesis: A Perspective for Light Sheet Microscopy", Developmental Cell23, 2011.
- Santi, „Light Sheet Fluorescence Microscopy: A Review", J Histochem Cytochem 59, 2011.

Thank you!



Questions?