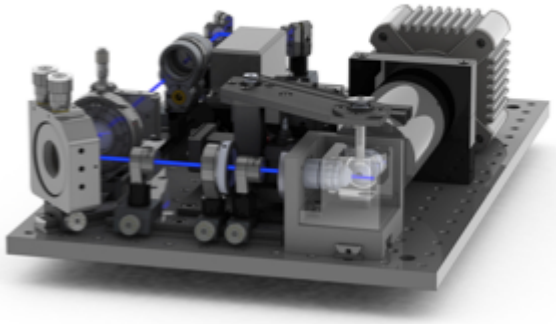
Newcastle University

Light Sheet Microscopy

Principles of imaging and construction



Bill Chaudhry MRCP PhD

Dublin November 2013

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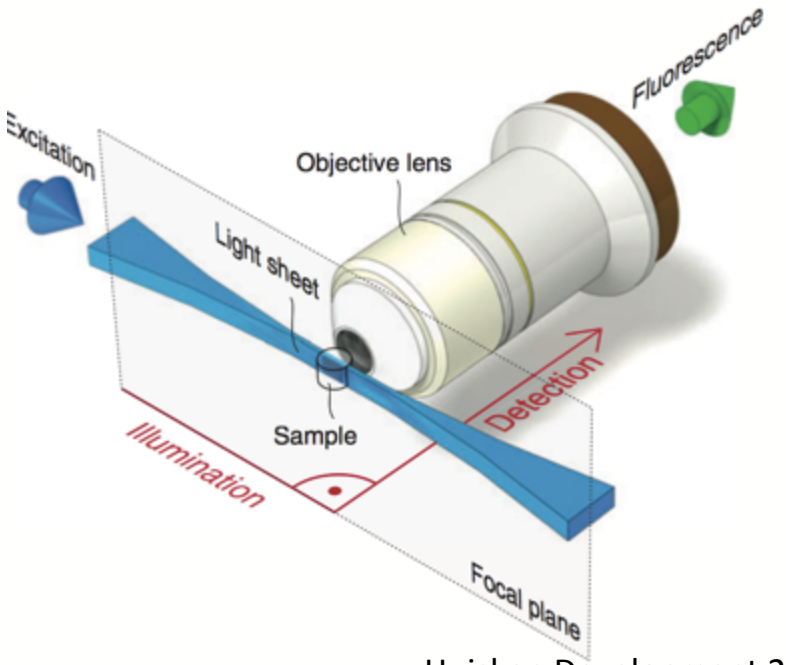

Huisken Development 2009

I. PRINCIPLES

II. SYSTEM COMPONENTS

III. EXAMPLES

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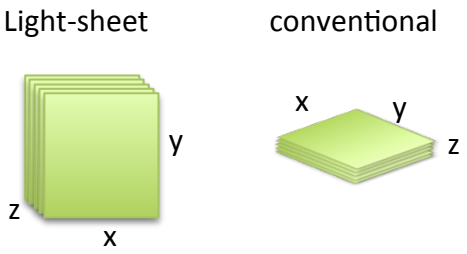
The 4WD microscope?

	xy-resolution	z- resolution	Depth	Detection Speed	Photo toxicity	User Skills
Stereo	poor	±	Mega	poor	low	Low
Epifluorescence	Excellent	Good	Good	Good	low	Fair
LS confocal	Outstanding	Outstanding	Good	Good	High	High
Multi-photon	Very good	Good	Super	Good	High	High
Sheet-light	Excellent	Excellent	Excellent	Excellent	minimal	High



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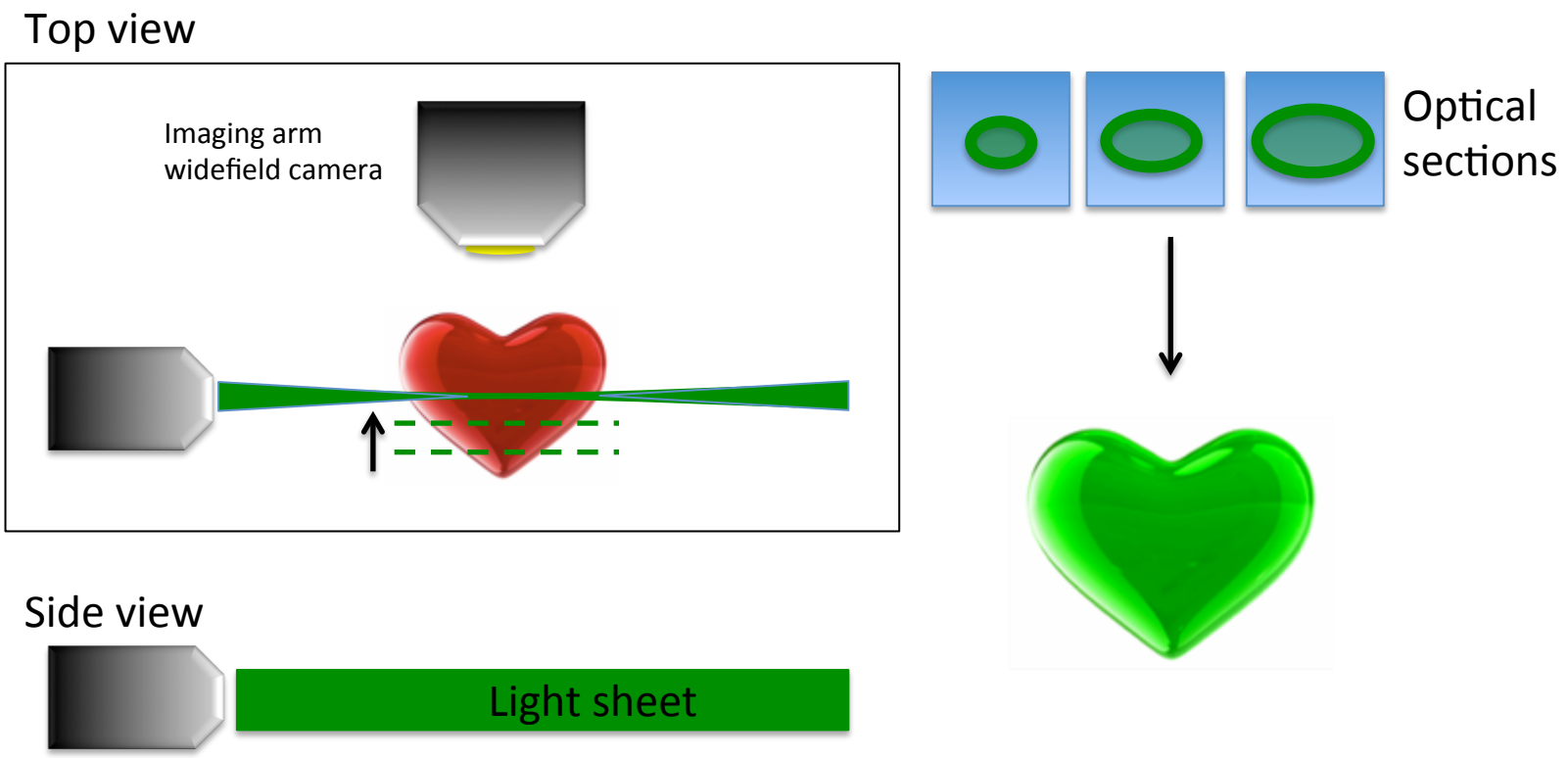
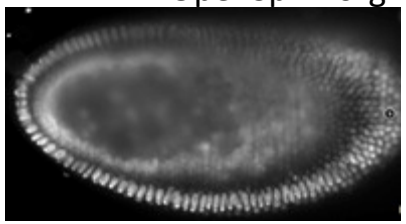
Concept of Sheet Light Imaging



Specimen Characteristics

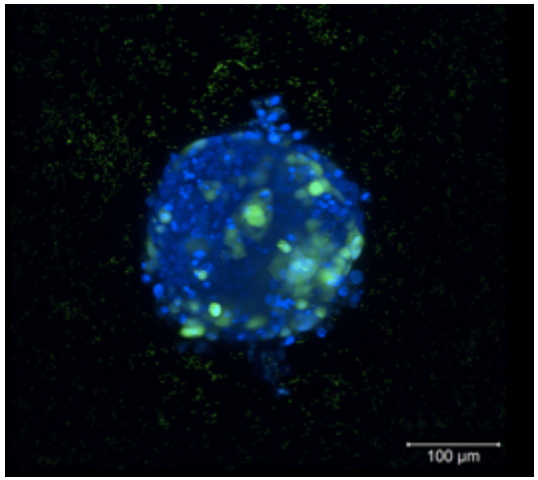
Unobstructed optical path in-and-out at 90 degrees
Optically transparent
Fluorescent label
Immobile
Smallish
Live or fixed

OpenSpim.org



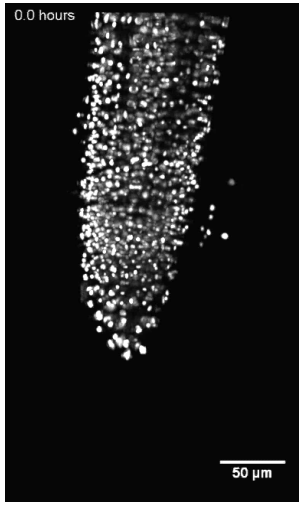
Drosophila

Zeiss



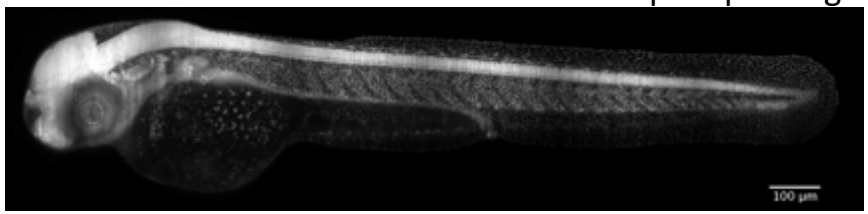
Cellular spheroids

Costa 2013



Arabidopsis thaliana

OpenSpim.org



Zebrafish embryo

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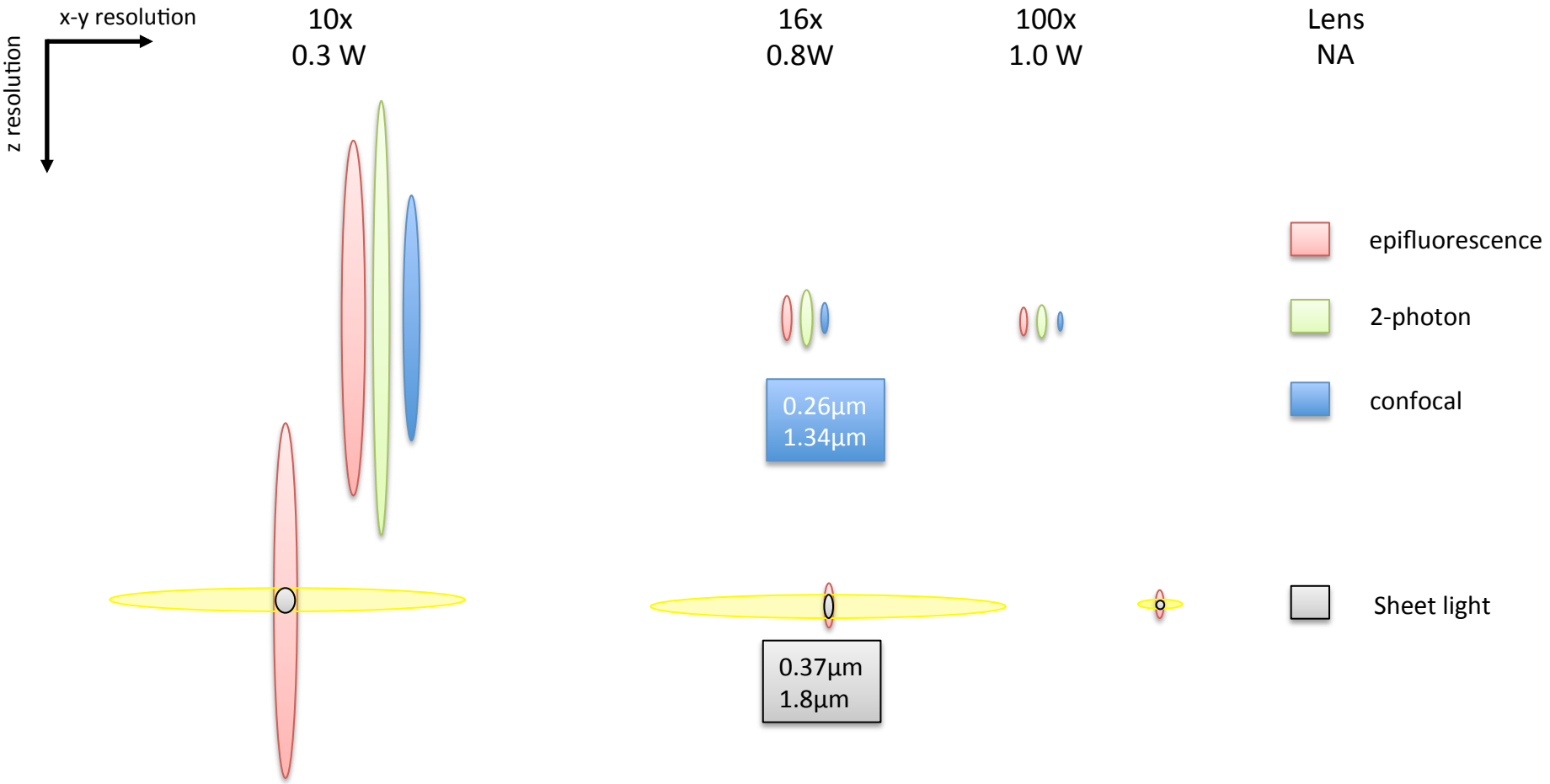
Near confocal resolution…



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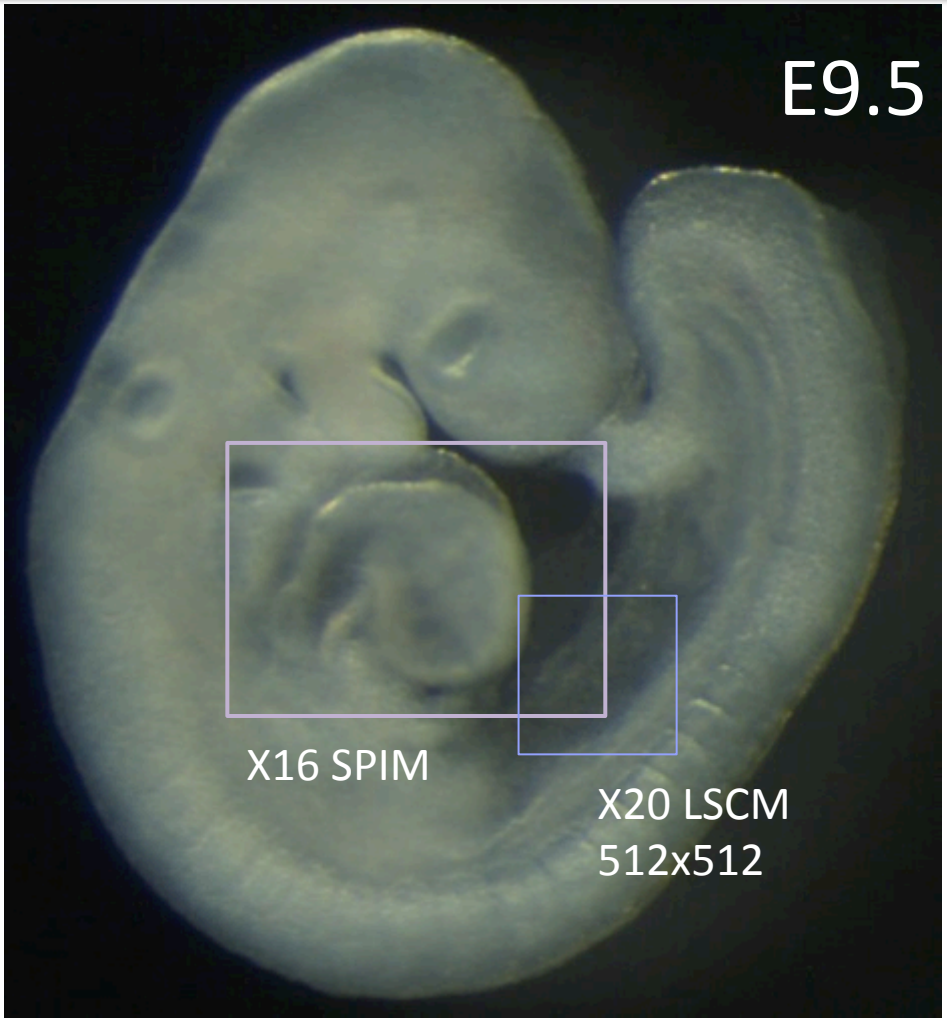
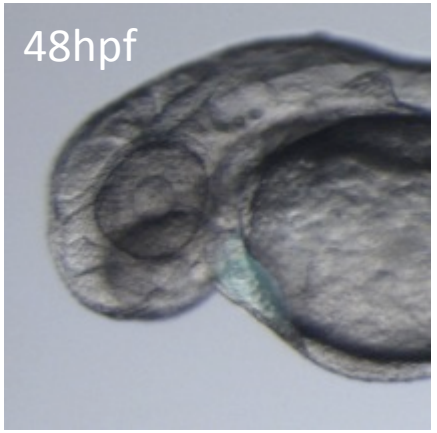
…wide field of view





light sheet: 540 X410 µm
Lens: 16x 0.8NA
Image: 1340 x1024px

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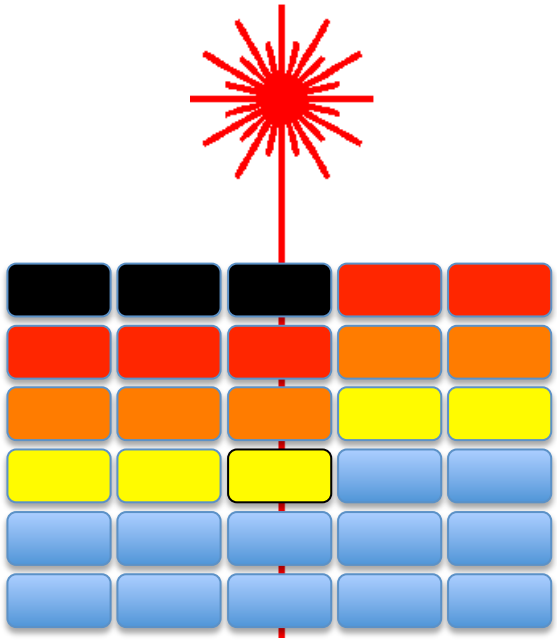
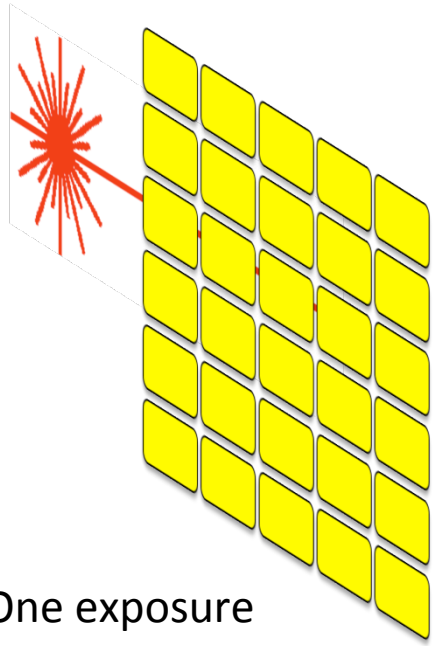
Minimal phototoxicity

- Confocal scanning light microscopy



Multiple exposures
One z image

- Sheet light



One exposure
One z image

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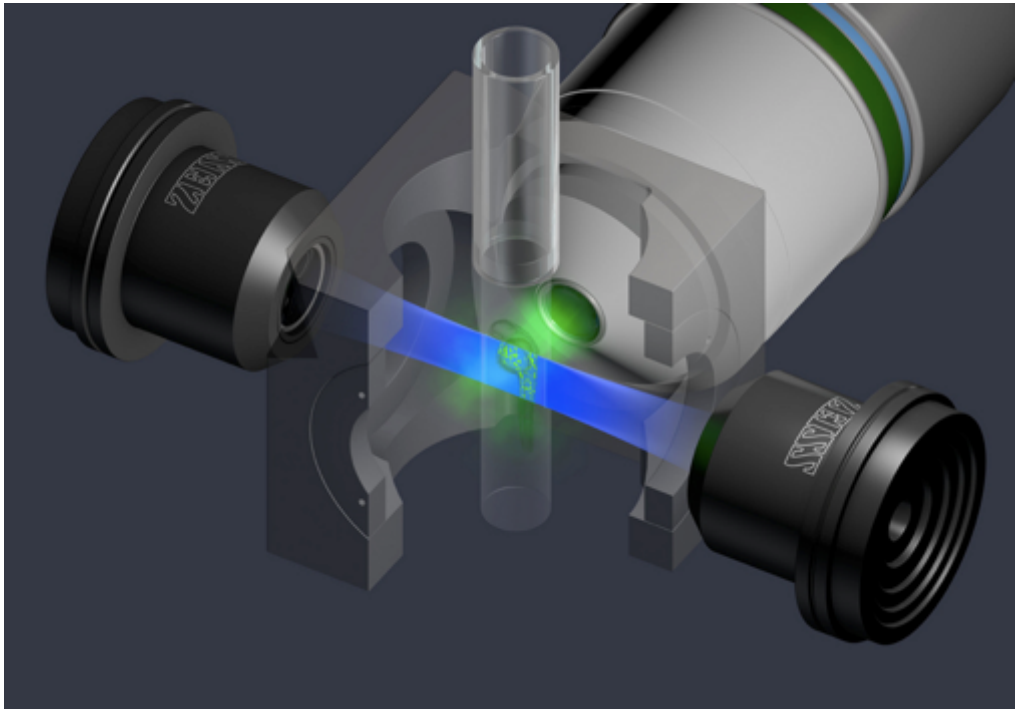
Unique specimen orientation



Zeiss Z1 – light sheet from both sides

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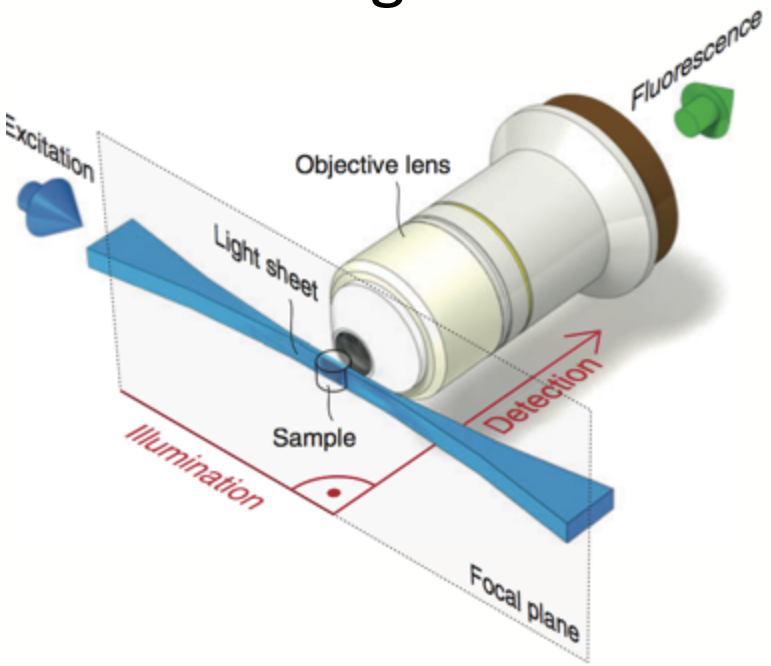
Advantages of sheet light

- Vital: No fixation artifacts
- Safe: minimise phototoxicity
- Deep: mm
- Long: days
- Fast: sCMOS camera
- Sharp: near confocal resolution
- Wide: large field of view

Orthogonal sheet



Huisken Development 2009

Huisken *Development* 2009; **136**(12); 1963
Keller *Science* 2009; **322**:1065

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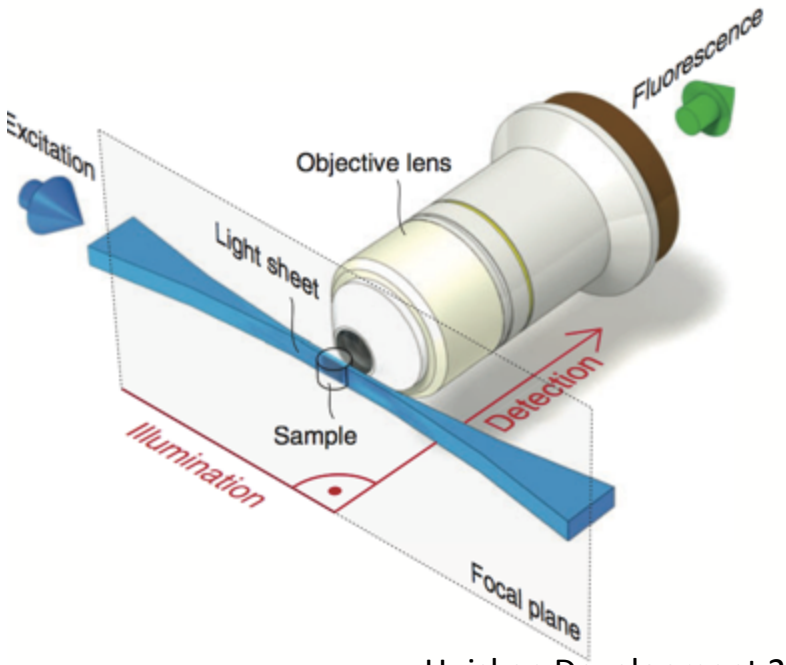

Huisken Development 2009

I. PRINCIPLES

II. SYSTEM COMPONENTS

III. EXAMPLES

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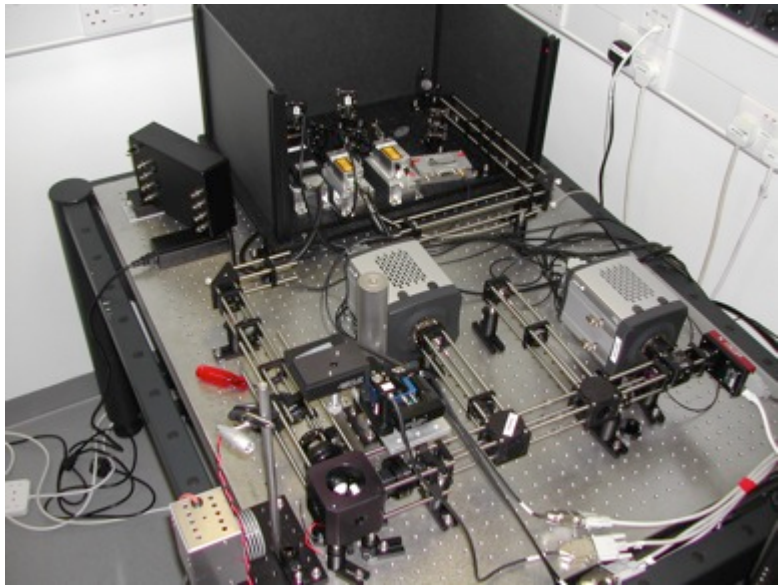
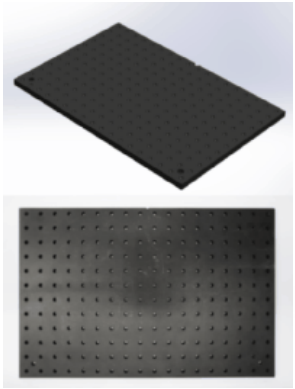
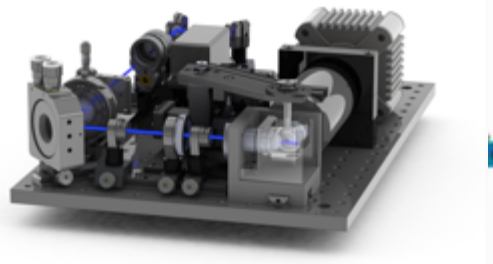
Components of LS microscope

- Sheet of light
- Camera
- Chamber
- Sample that moves
- Control system

Greger, Swoger, Stelzer.Basic building units and properties of a fluorescence single plane illumination microscope. Rev Sci Inst. 2007:78;023705







OpenSpim.org

Zeiss z1

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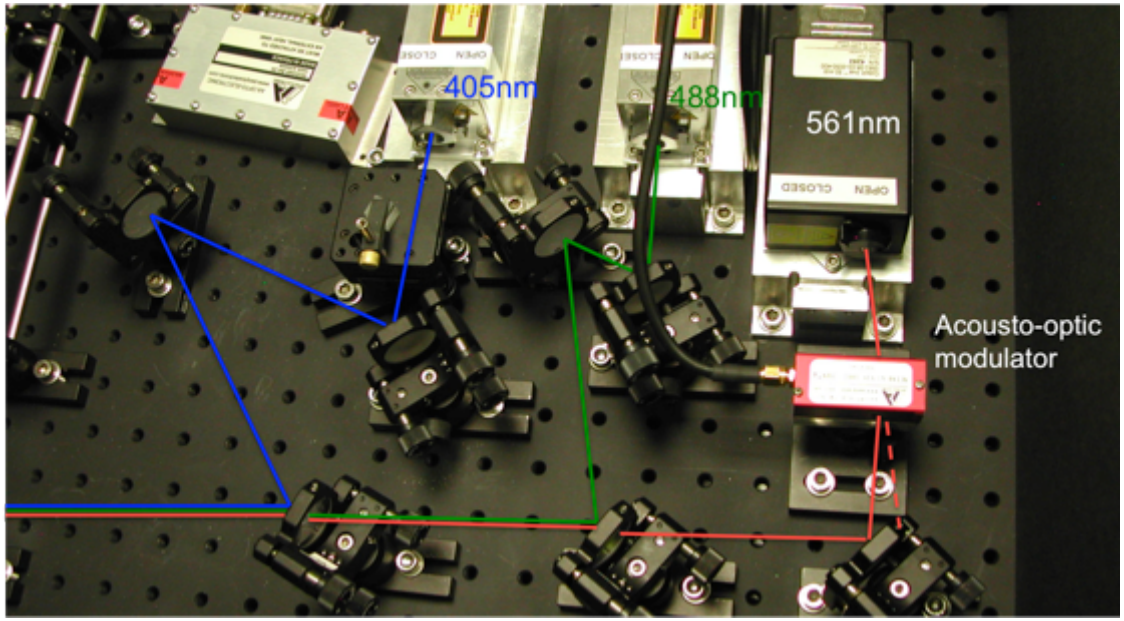
Lasers

Light Amplification by Stimulated emission of Electromagnetic Radiation



Coherent light source – focused to a tight spot, low divergence

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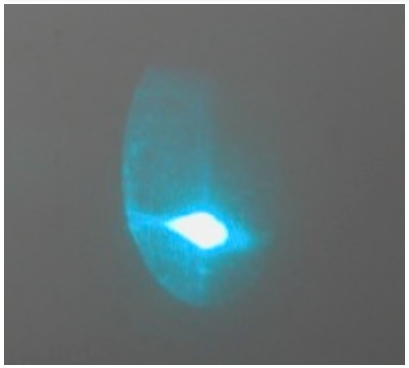
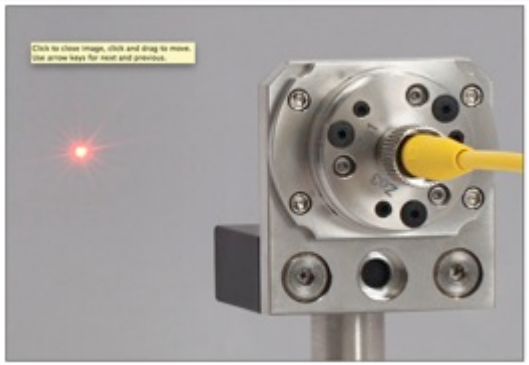
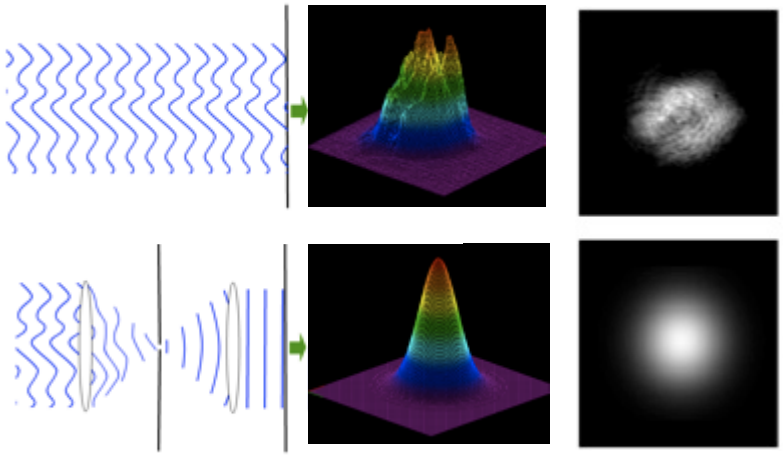
Beam conditioning



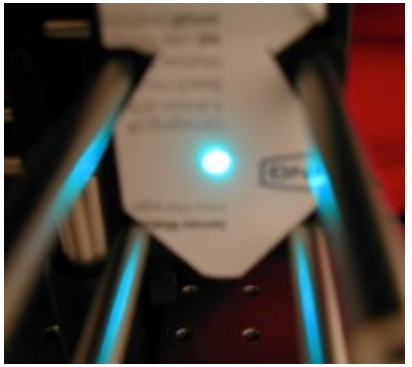
488nm diode laser



Single mode optical fiber
Light takes on properties of fiber



Spatial filter:
Kepplerian telescope with pinhole at focus "blocks" stray waves)



At least 40% power loss

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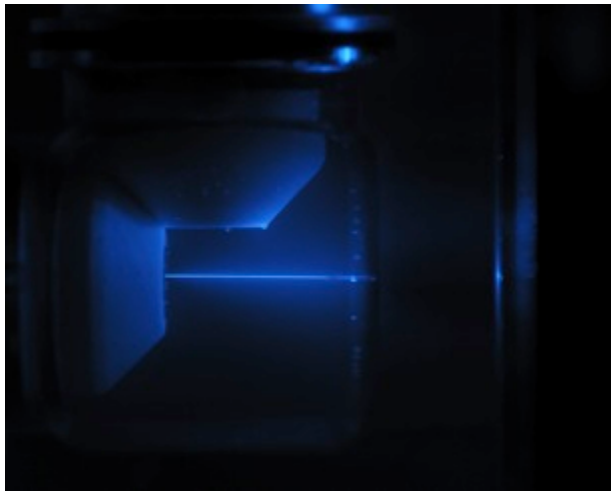
Beam shaping

Digital light sheet

Possible to produce sheet by waving the laser beam (galvo mirror)

Possible to use structured light to improve resolution

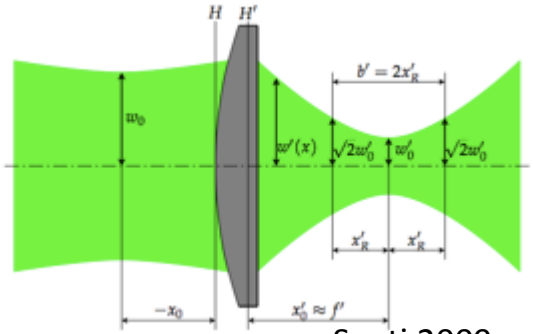
OpenSpim.org



Classical light sheet

Easiest way is a cylindrical lens in combination with the objective

Cylindrical lens overcomes the objective in one plane
Slit controls width of beam entering



Santi 2009

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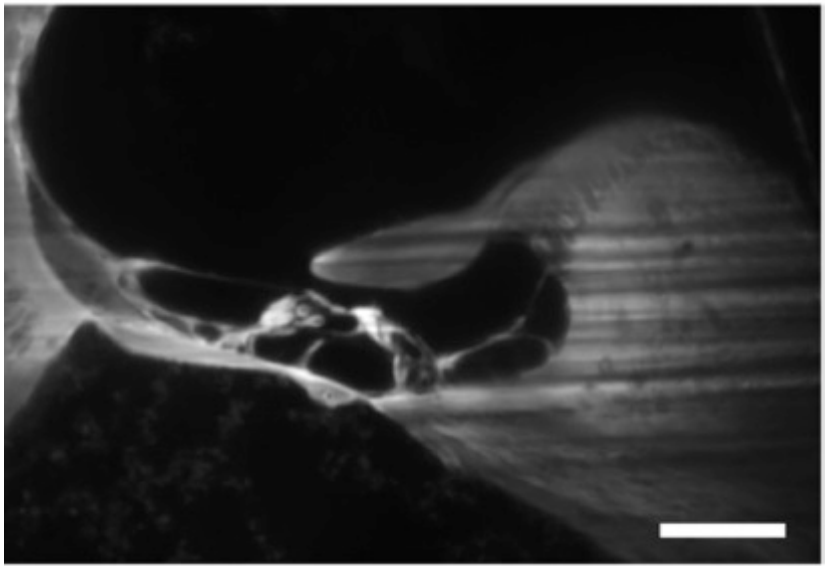
Stripes

Coherent light causes interference patterns in sample.



Santi 2009

Solution:

1. Less coherent light!
2. Wobble the sheet:

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Cameras

Pretty much any camera will work!

QI Click CCD

Cheaper, Firewire
Peltier cooling

Hamamatsu ORCA CCD

More expensive
More sensitive

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Scientific CMOS cameras

ANDOR™ TECHNOLOGY

Neo sCMOS — 5.5 Megapixel, -40°C, 1 e⁻ Noise, Rolling and Global Shutter Scientific CMOS

	Cameralink Extended Kinetic Series		Burst to 4GB Internal Memory	
Array Size	Rolling Shutter	Global Shutter	Rolling Shutter	Global Shutter
2560 x 2160 (full frame)	32	31	100	50
2064 x 2048	40	38	104	52
1392 x 1040	83	77	204	100
512 x 512	181	161	412	200
128 x 128	1098	711	1616	711

Maximum frame rates achievable from the Neo sCMOS camera.

Very sensitive
Massive chip area
Expensive
Very fast
Specialist interface
Difficult - programming, data flow, rolling/global shutter
Limited by disc –write speed

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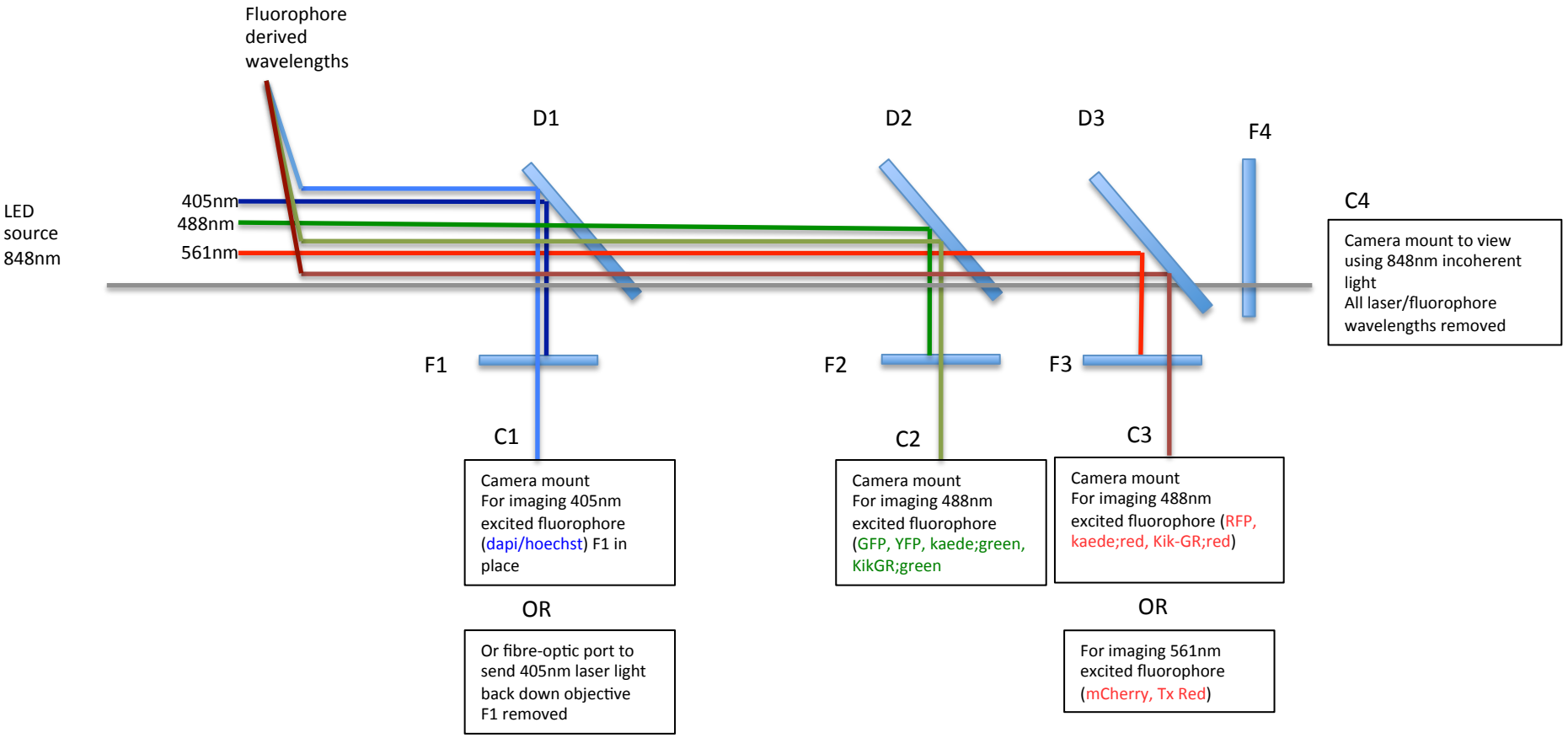
Capture every photon



Dichroics and fluorochromes

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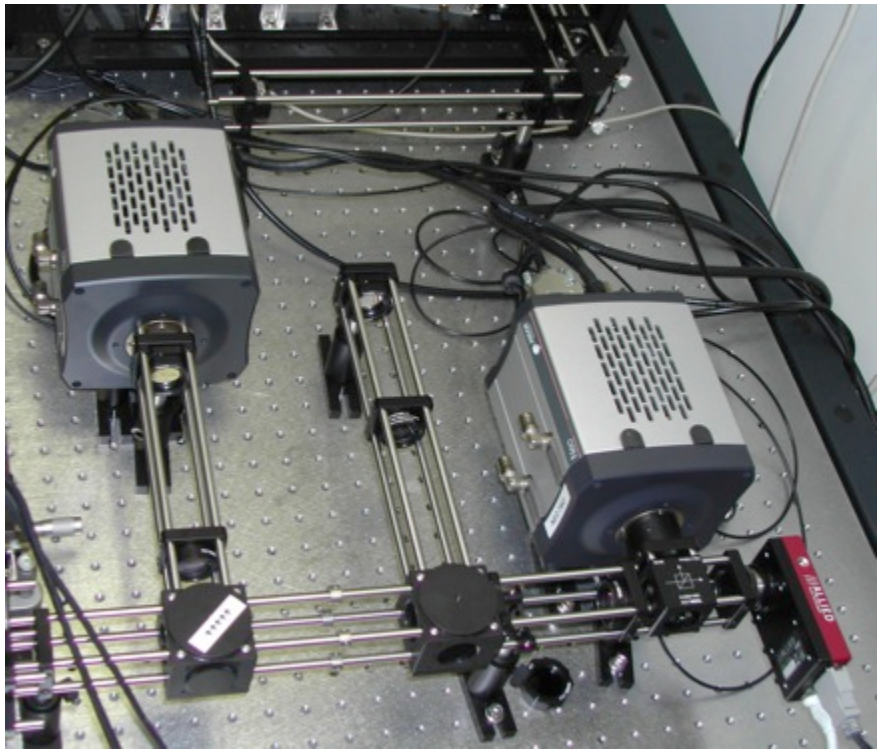
Camera arrangement



Imaging positions

IR gating
848nm 23mW LED

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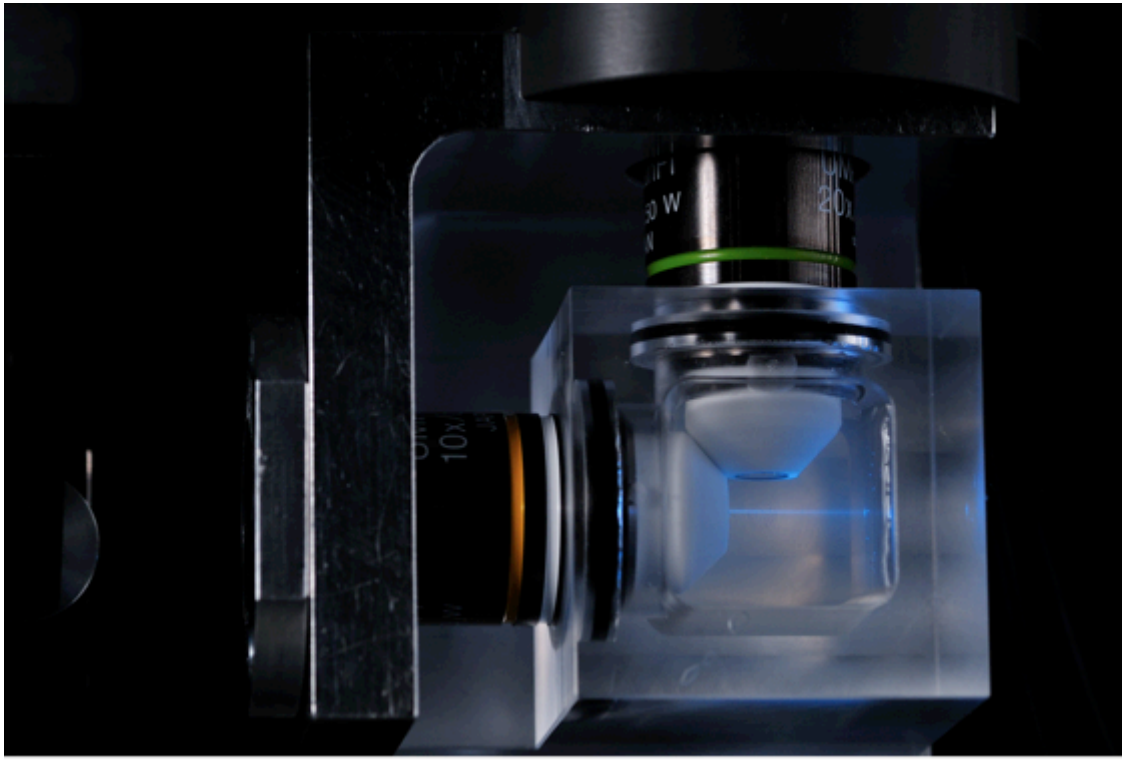
Sample chambers



OpenSpim.org design

Water dipping lenses

mechanically secured
'O' rings

Persepex chamber

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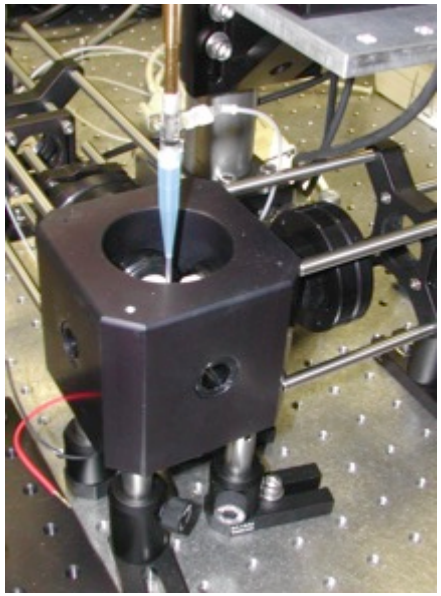
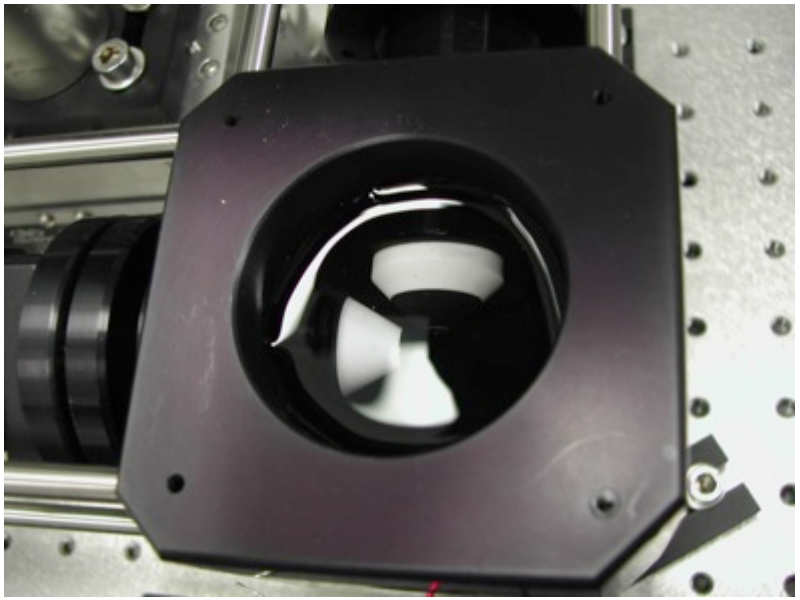
Sample chamber





Anodised aluminium chamber
Peltier cooling
25W Resistors and commercial heater controller
Fluid and gas exchange

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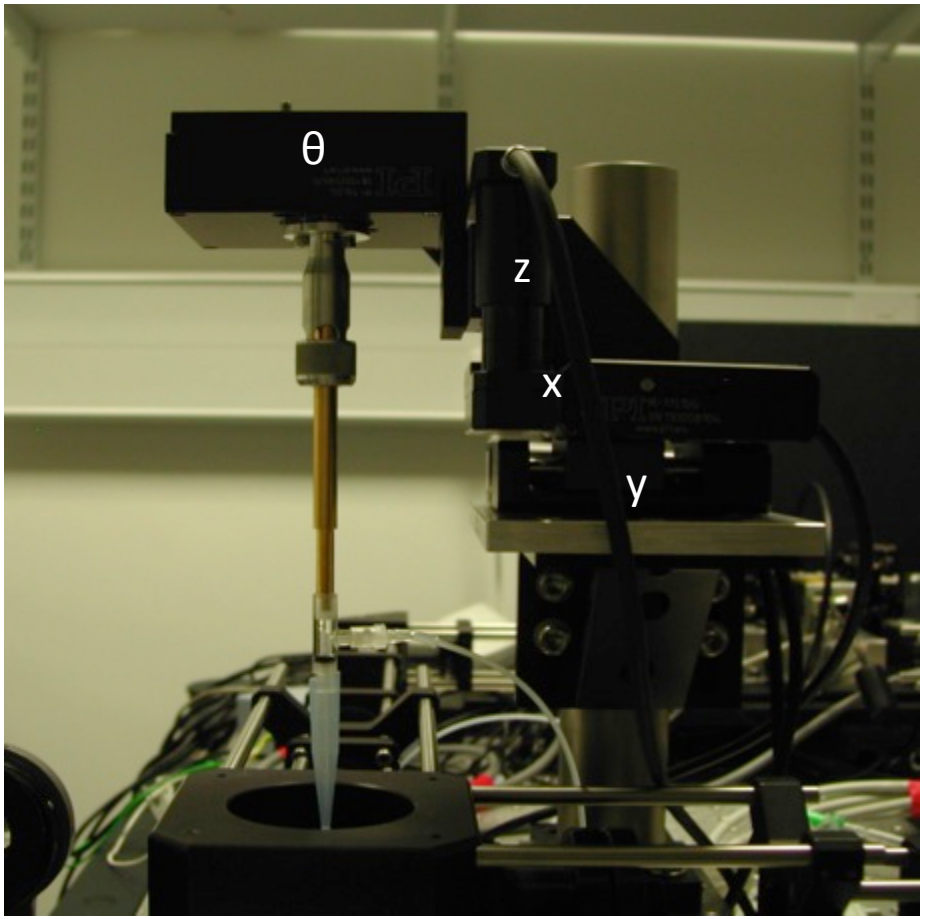
Stage stepper motors



PI micro –translation stage
50nm resolution
Repeatable (same direction)
25mm travel
Programmable
(Newport – better?)

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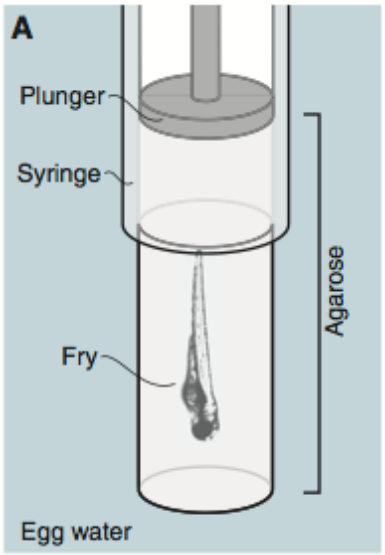
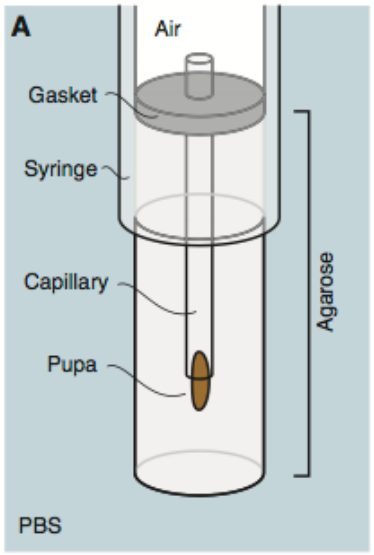
Specimen mounting





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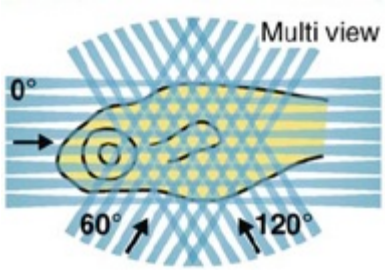
Diffusion hole

E3/Tricaine (RI = 1.34)

Flourinated ethylene propylene (FEP) tube (RI 1.338)

Plasticine plug



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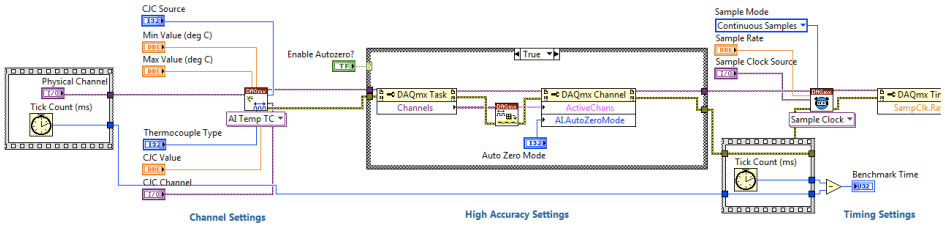
Programming

Quite hard!

Labview (National Instruments) : Instrument control



C – camera drivers
Matlab – data processing
Imaris – data visualisation
Amira – data visualisation
ImageJ/Fiji : Total package for OpenSpim.org

Computers are unreliable with timing

Not a problem with ‘slow’ applications
Labview – real time application
TTL signal communication

TTL pulse width/delay
Junction box

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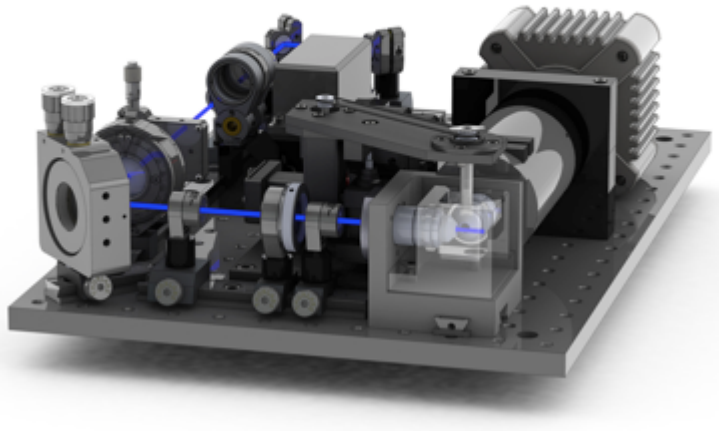
OpenSPIM: www.openspim.org

An *Open Access* platform for **Selective Plane Illumination Microscopy** (*SPIM*).



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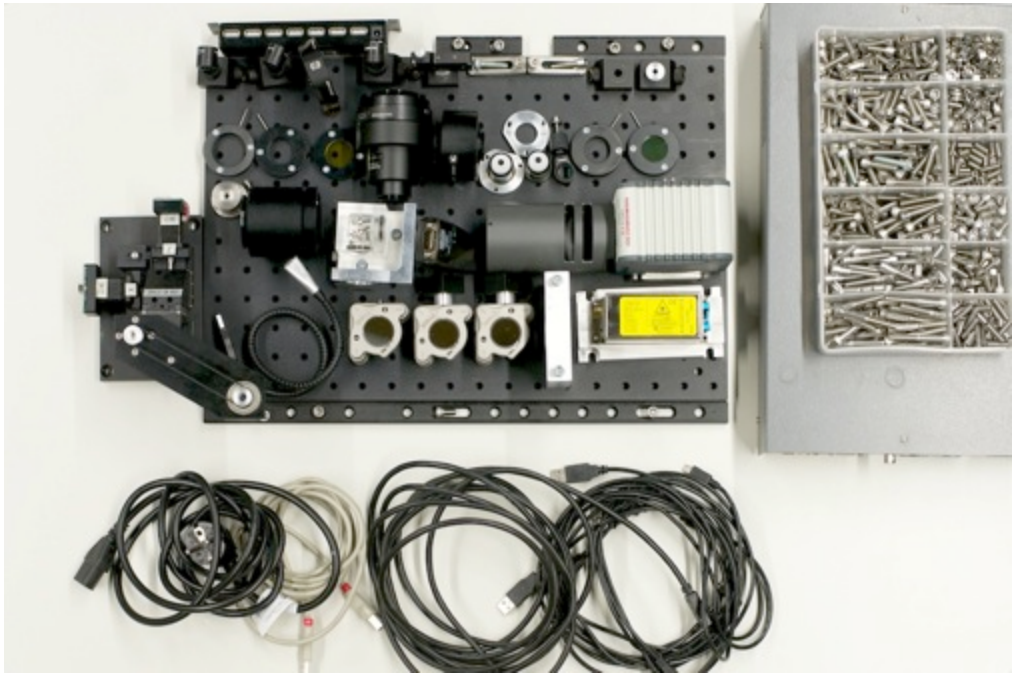
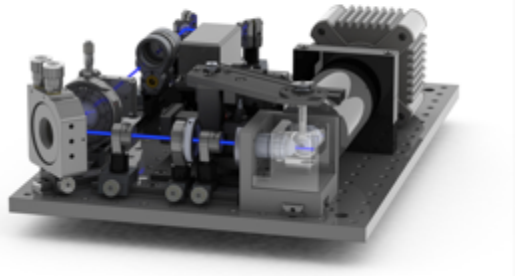
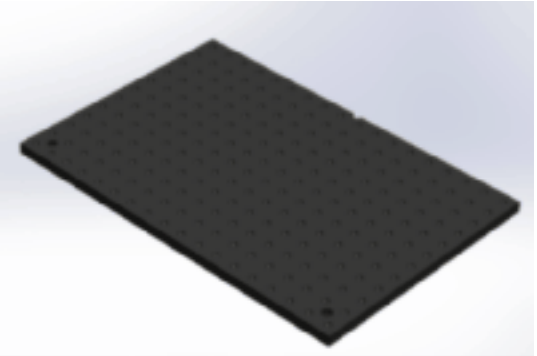
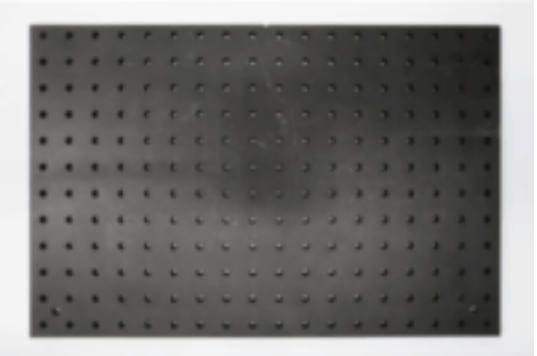
OpenSPIM:Everything you need









An *Open Access* platform for
Selective Plane Illumination Microscopy (*SPIM*).

OpenSpim.org

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Sheet light quiz

Q1 Can you make things?
a. Able to make things
b. I know someone who can
c. No

Q2 Optical physics?
a. Spatial filters, optoacoustic modulators…
b. snooker and reading glasses
c .Don't know physics

Q3 Programming?
a. Labview, Matlab, C, Java…
b. Scripting -macros
c .Prefer to click and go

Q4 Money or parts?
a. No, but can dismantle
b. Yes, but only about 20k
c .Yes, enough for a confocal

If you scored mostly:

a's. Make your own design
b's. OpenSpim.org.
c's. Zeiss Z1

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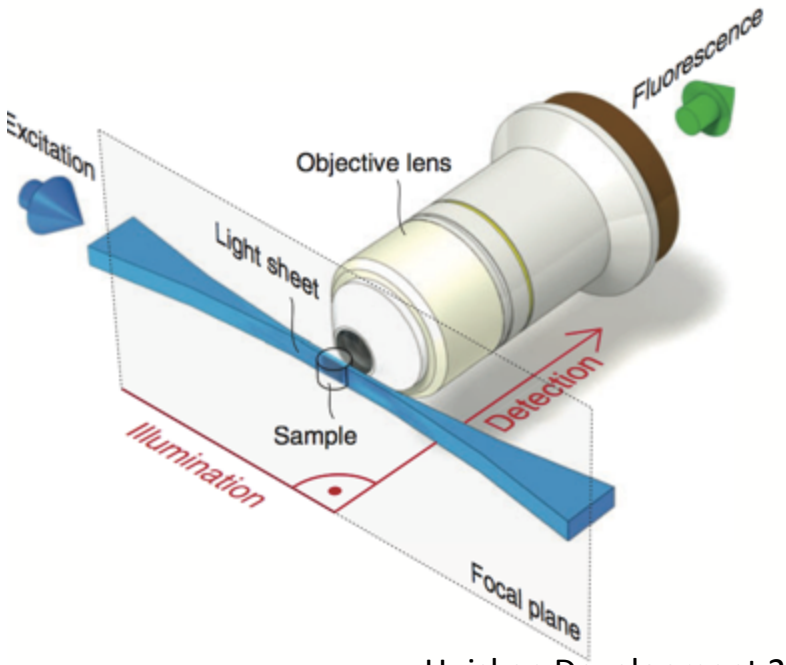

Huisken Development 2009

I. PRINCIPLES

II. SYSTEM COMPONENTS

III. EXAMPLES

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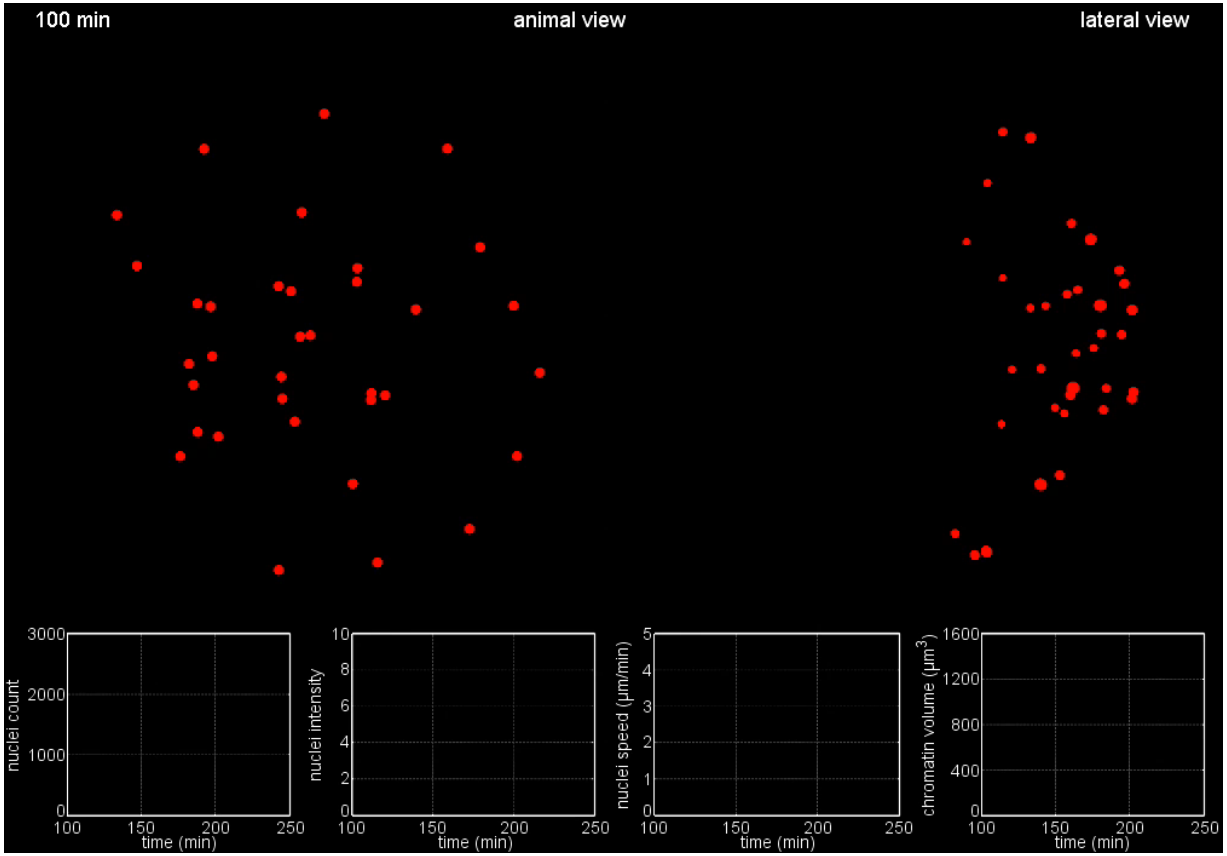
Ernst Stelzer

Science 322, 1065 (2008)



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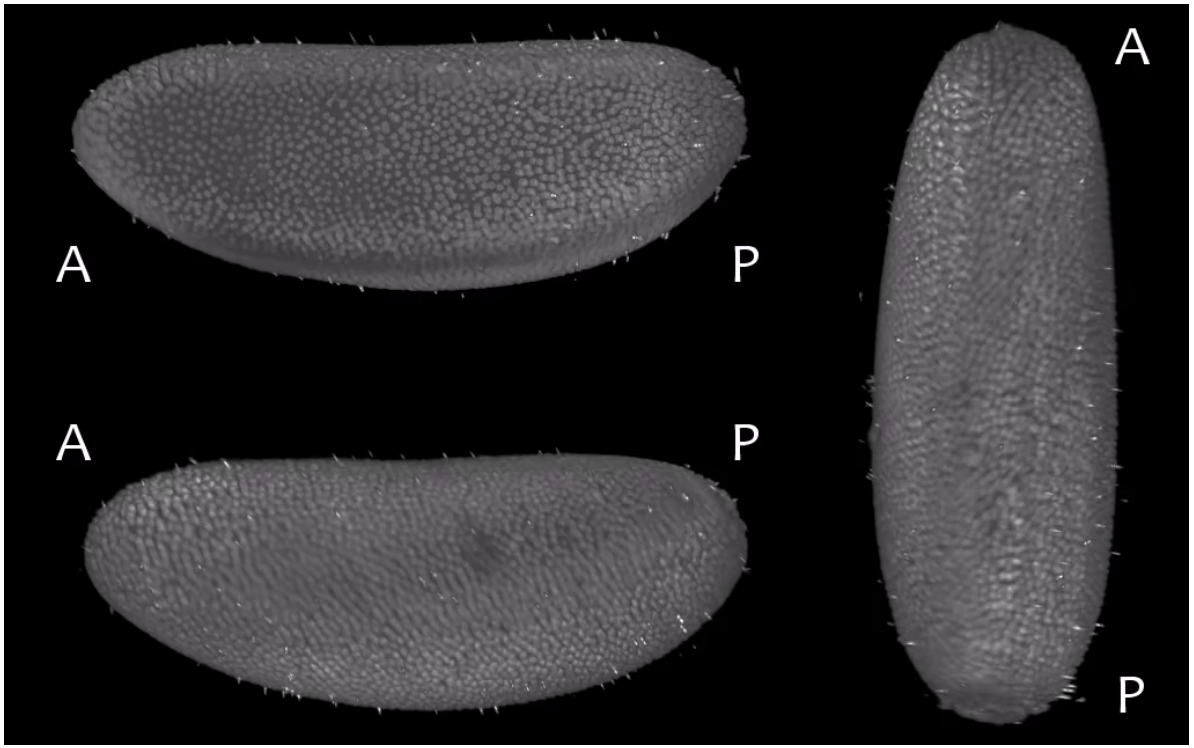
Pavel Tomancak



Nature Methods **6**, 435 - 437 (2009)

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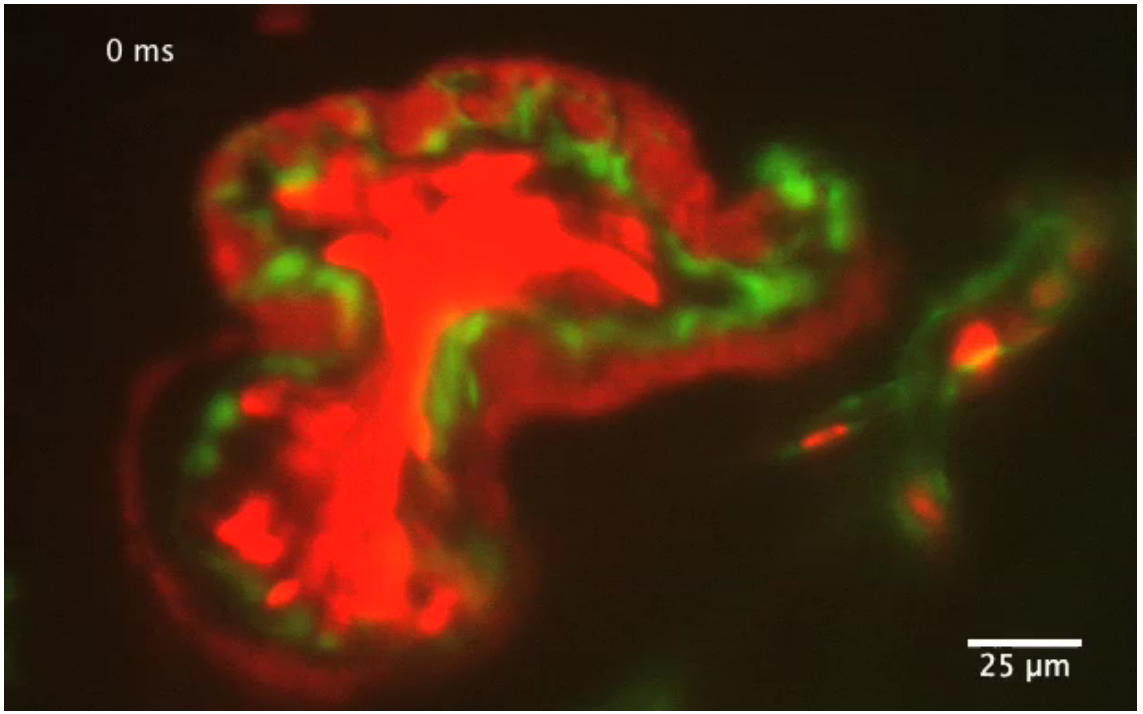
Fly – technology development

Jan Huisken



Development. 2008;135:1179-87

Heart – other developmental imaging aspects

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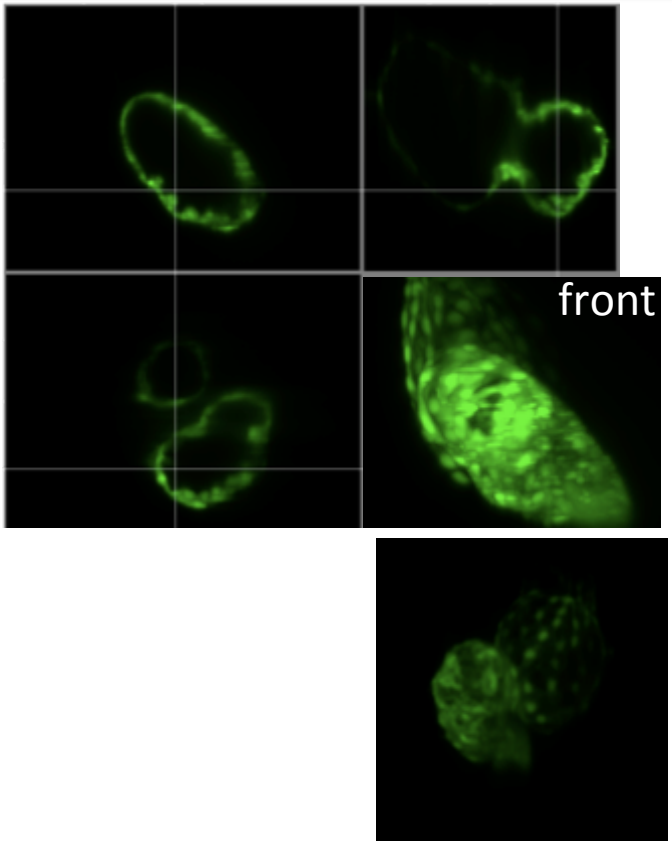
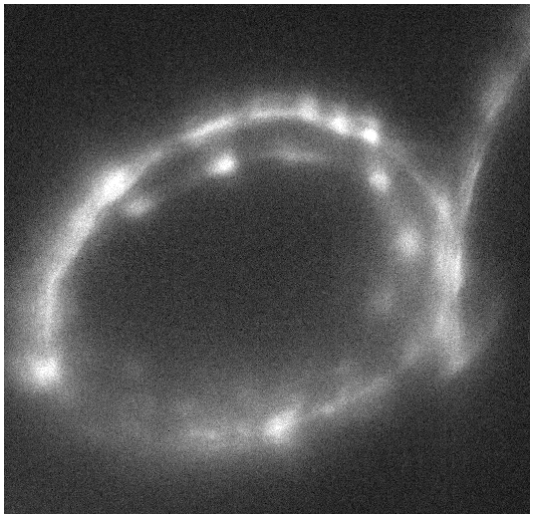
Typical Images



Raw



25um

Tg{cmcl2:gfp;fli1:gfp} 48hpf
Neo - 100fps, 4ms, 512x512
488nm, 1mW

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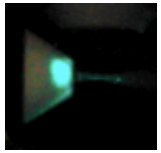
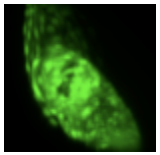
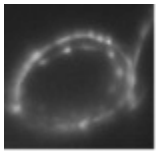
Sheet light: summary



Laser-sheet wide field imaging



Near confocal 3D optical sectioning



Non-phototoxic, time lapse, multichannel

Implementation can be straightforward

The art is of specimen mounting

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