Light-sheet and Spinning-disk microscopy

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BioImaging Day

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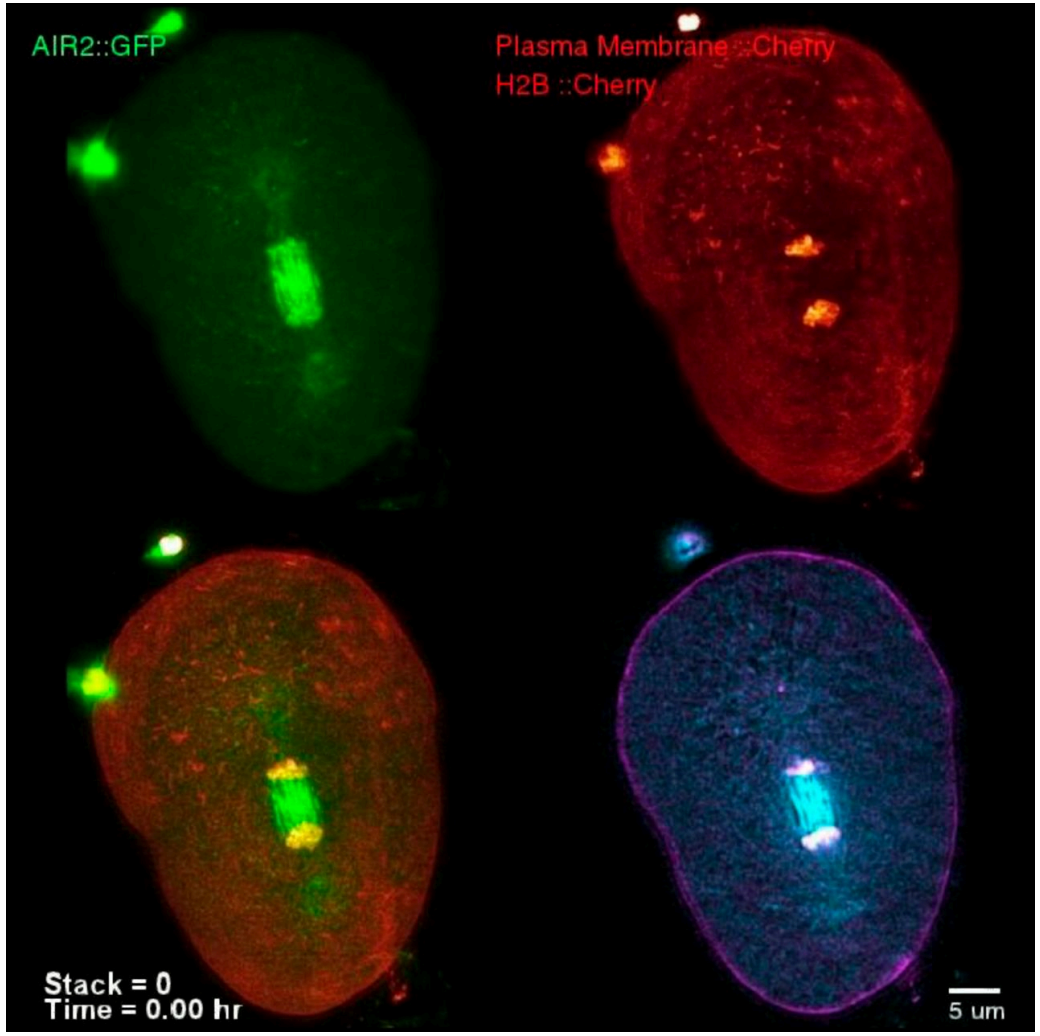
Life cell fluorescence microscopy

Movie 11 Protein localization in early embryo. Localization of the chromosomal passenger protein AIR-2 during the first few cell divisions of the early C. elegans embryo (compare with Fig. 6A and fig.

Movie_11



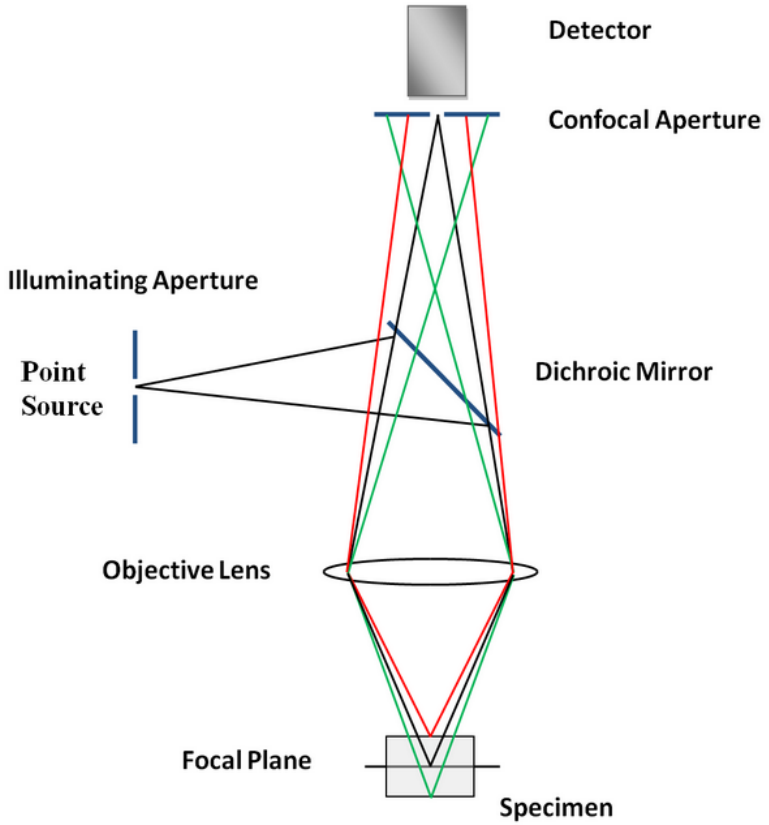
Bi-Chang Chen et al. Science 2014;346:1257998

Prerequisites for life cell fluorescence microscopy

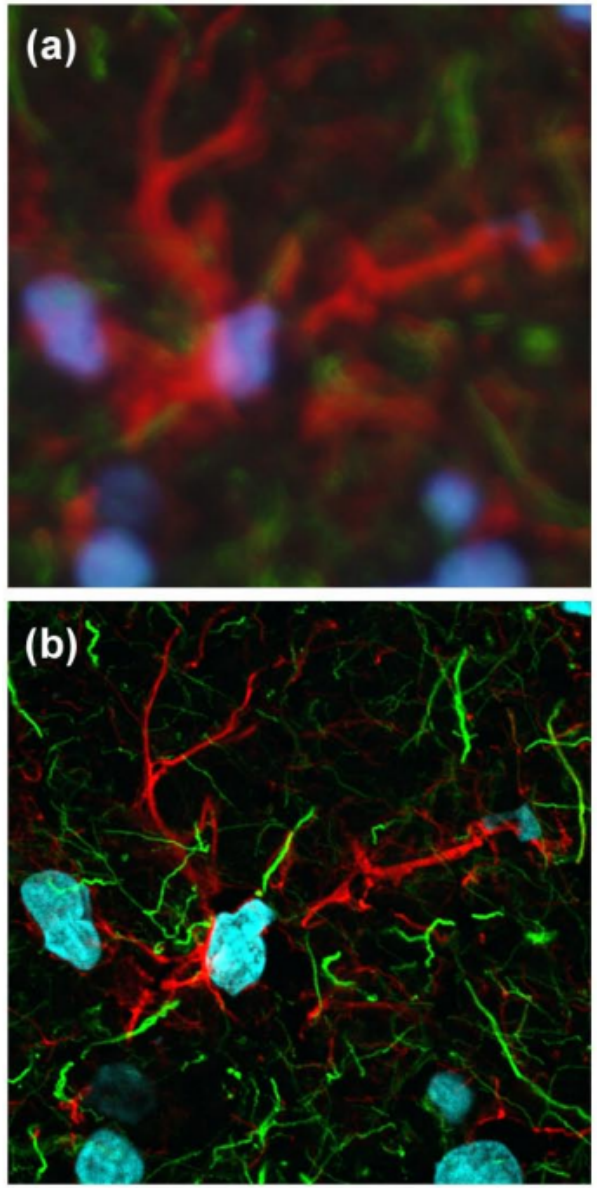
- x-, y- Resolution, sectioning
- Speed
- Signal to noise ratio
- Photo-toxicity

Sectioning

Confocal principle

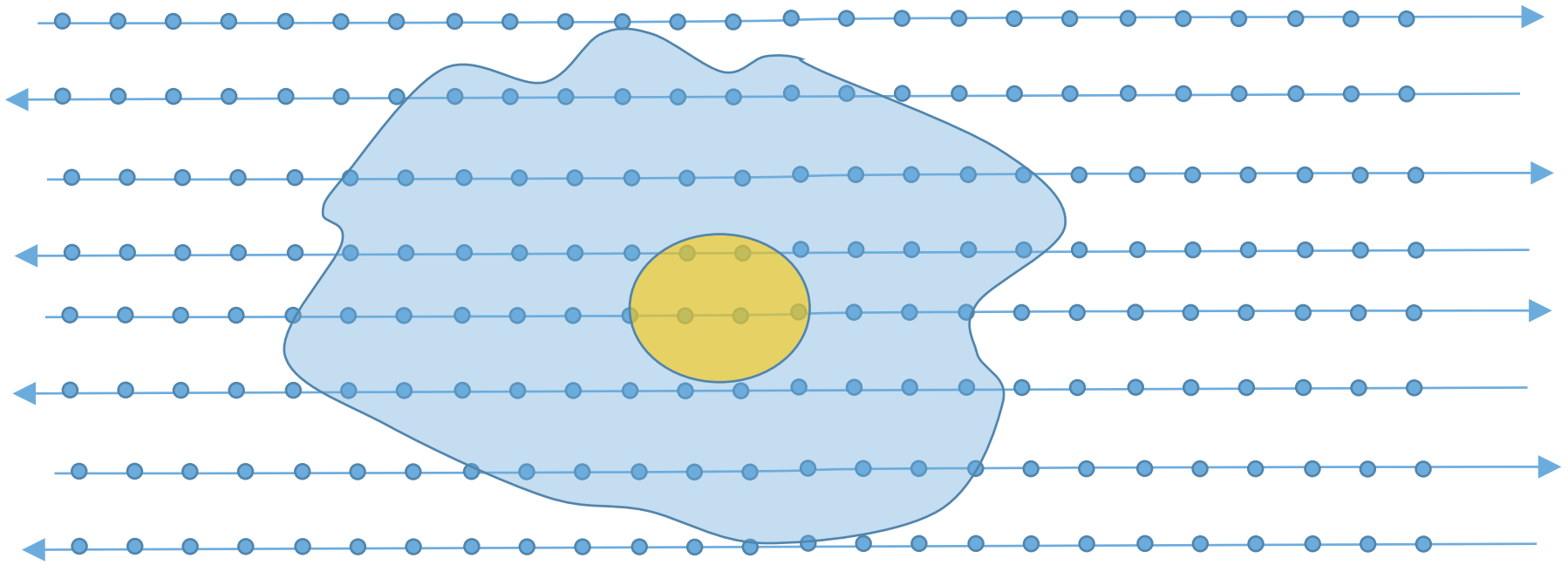


http://www.tcd.ie/Physics/photonics/research/plasmon.php



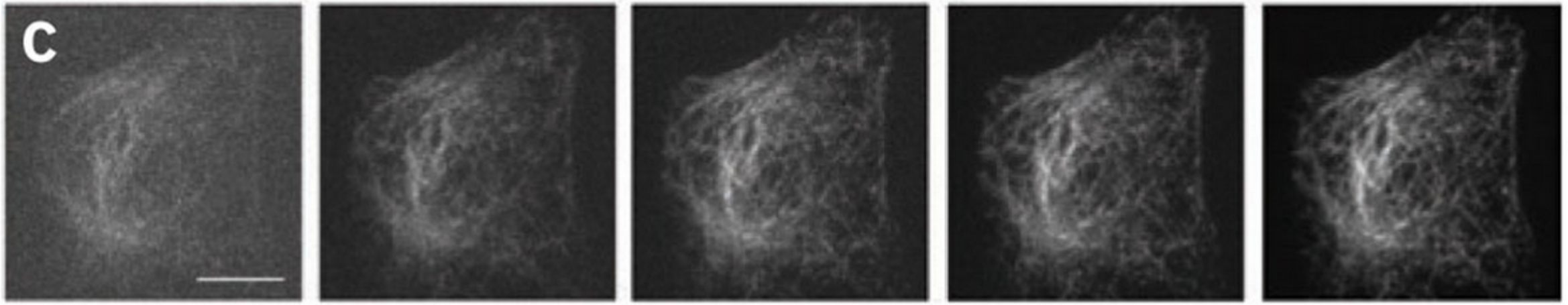
LASER SCANNING CONFOCAL MICROSCOPY Nathan S. Claxton, Thomas J. Fellers, and Michael W. Davidson

Speed



Time required for one plane: 1000 points x 1000 points x 10 µs = 10 s
Time required for a stack of 120 planes: 20 min

Signal to Noise Ratio (SNR)



418 | VOL.8 NO.5 | MAY 2011 | **nature methods**

$$\mathrm{SNR} = \frac{N}{\sqrt{N}} = \sqrt{N}.$$

N: number of photons

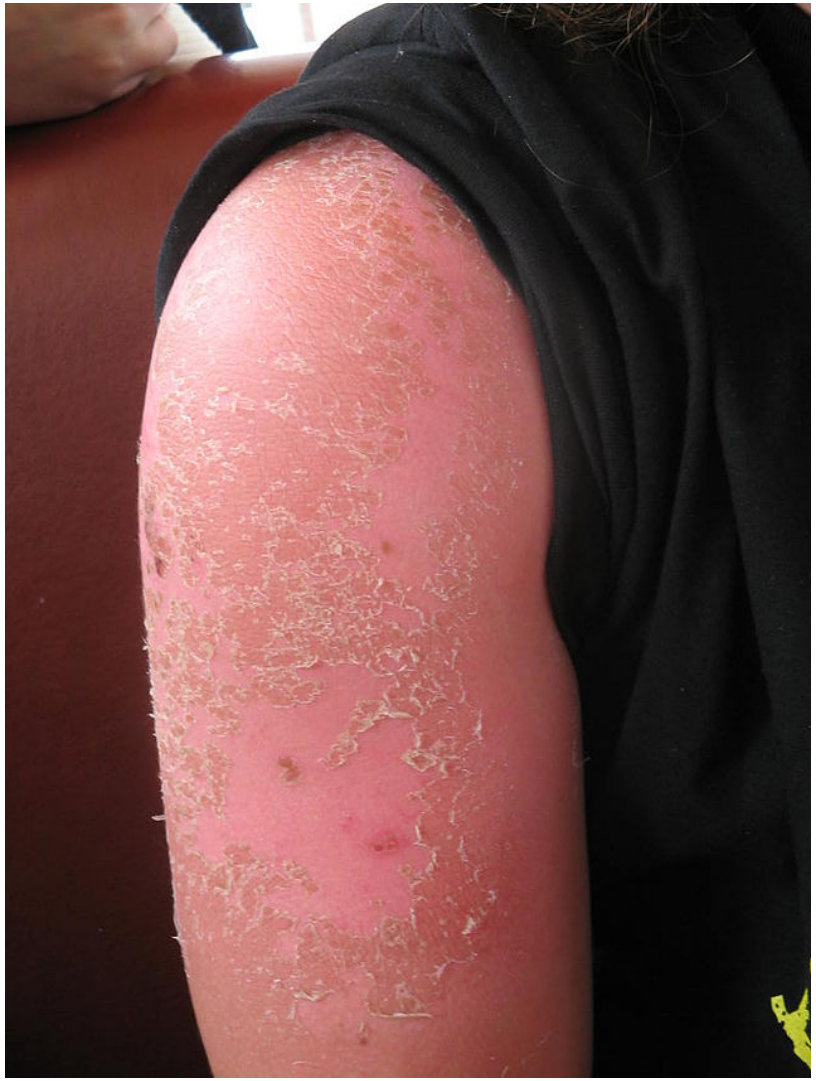
Photo-toxicity

Solar constant in central Europe
1 kW / m^2
1 nW / μm^2

In confocal microscopy you can reach µW per spot

“You do not want to live in a world with 1000 suns”

Ernst Stelzer



wikipedia

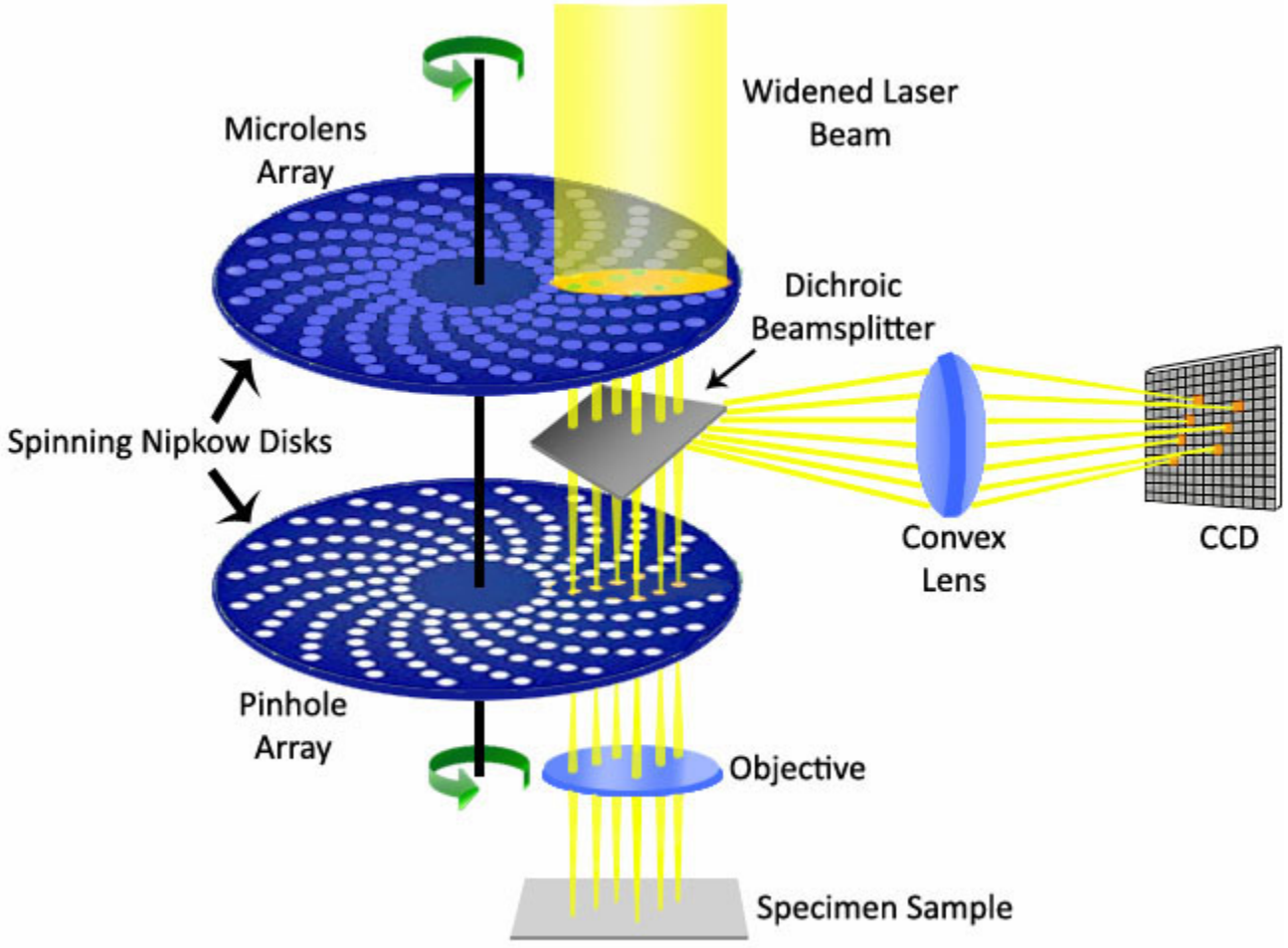
Photo-toxicity depends on the peak power

- Photo-toxicity depends on the absolute number of photons per area and time.
- The relationship between peak power and the respective phototoxic effect is non linear. Toxicity increases much faster than peak power.

Suitability for live cell fluorescence microscopy

	Confocal microscope
Sectioning	+++
Speed	- - -
Signal to noise ratio	+++
Photo-toxicity	- - -

Spinning disk confocal



A Nipkow disk typically has 20,000 To 200,000 pinholes. 1000 of them are focused on the specimen at a given time.

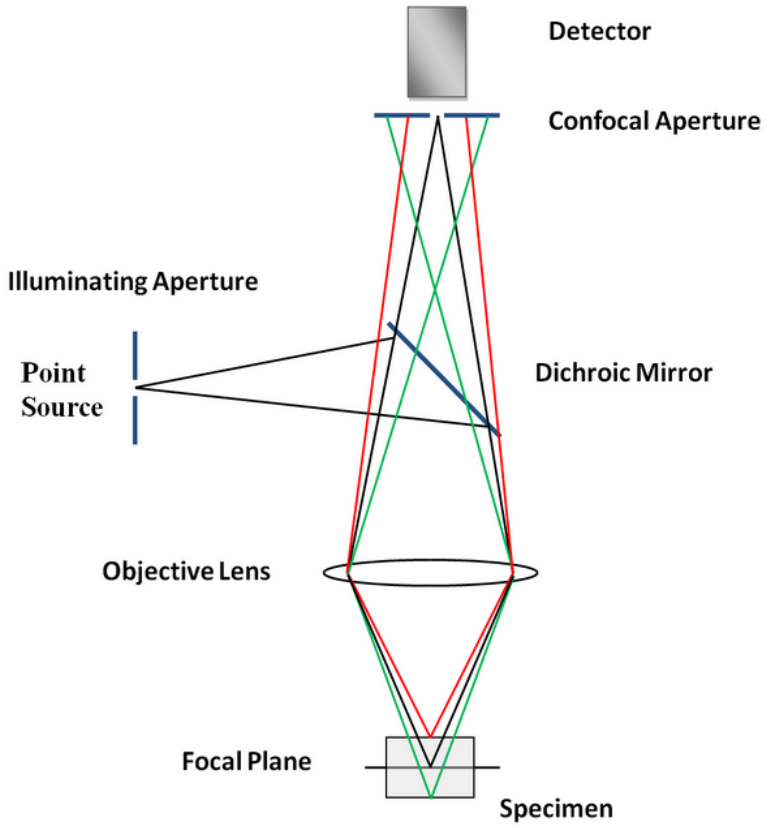
http://php.med.unsw.edu.au/

Suitability for live cell fluorescence microscopy

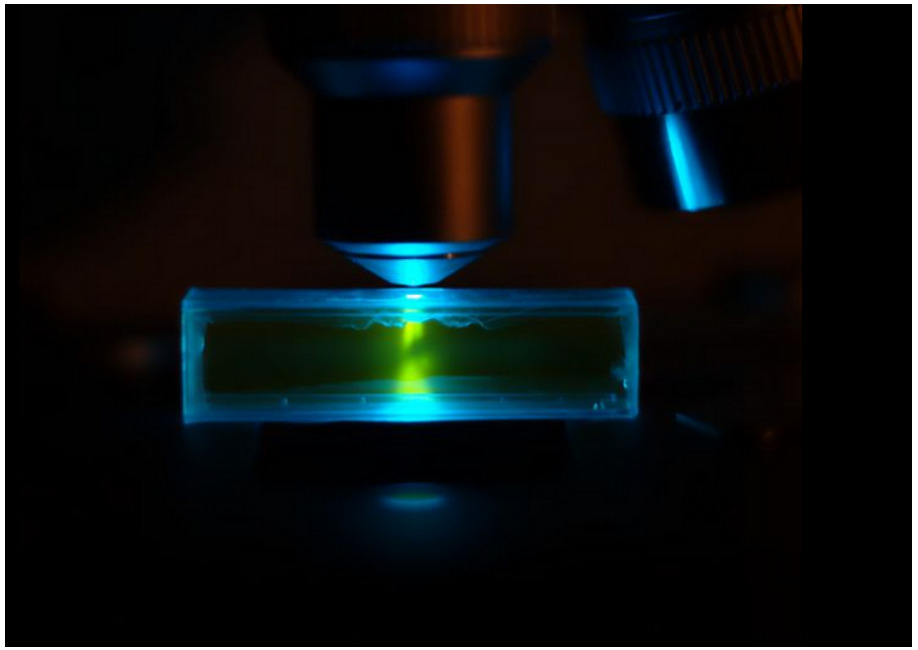
	Confocal microscope	Spinning-disk confocal
Sectioning	+++	++
Speed	- - -	+++
Signal to noise ratio	+++	++
Phototoxicity	- - -	+

Out-of-focus bleaching

Linear optical arrangement of illumination and detection axis causes out-of-focus bleaching

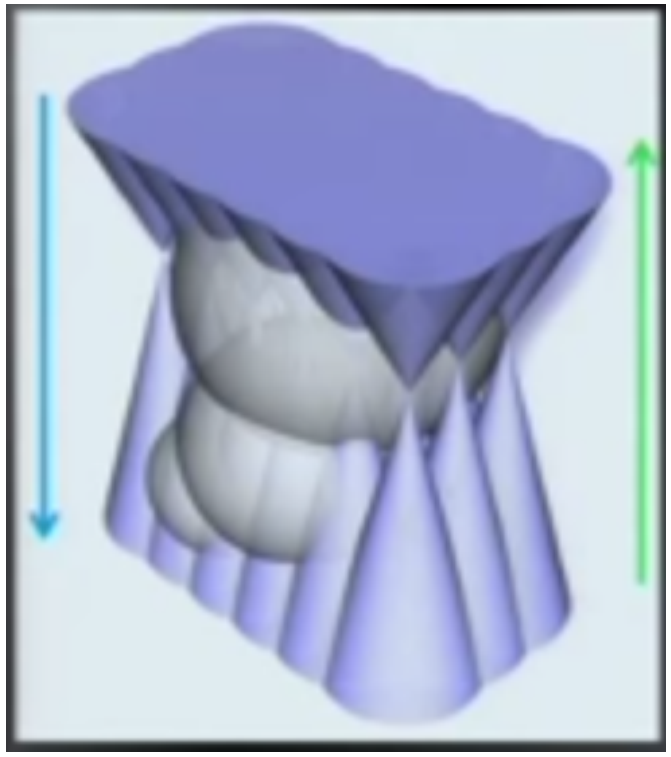


http://www.tcd.ie/Physics/photonics/research/plasmon.php



Wikipedia

Photobleaching everywhere!



iBiology.org Stelzer

A solution for this problem

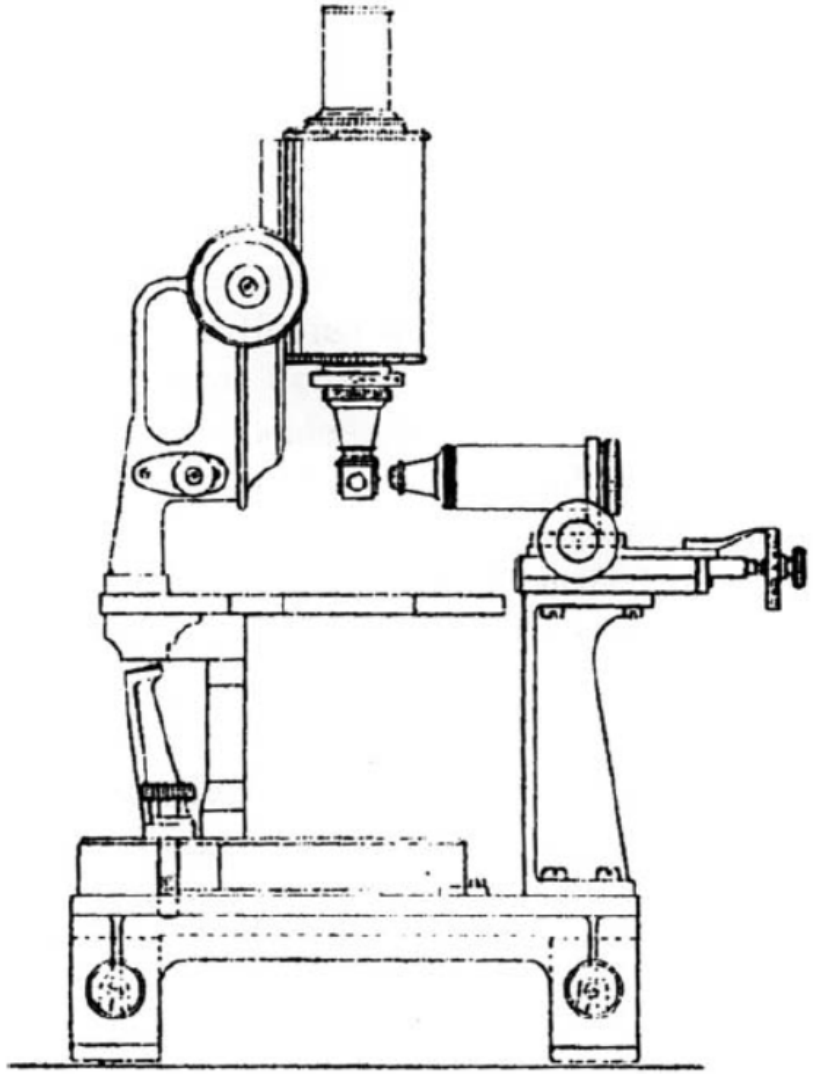
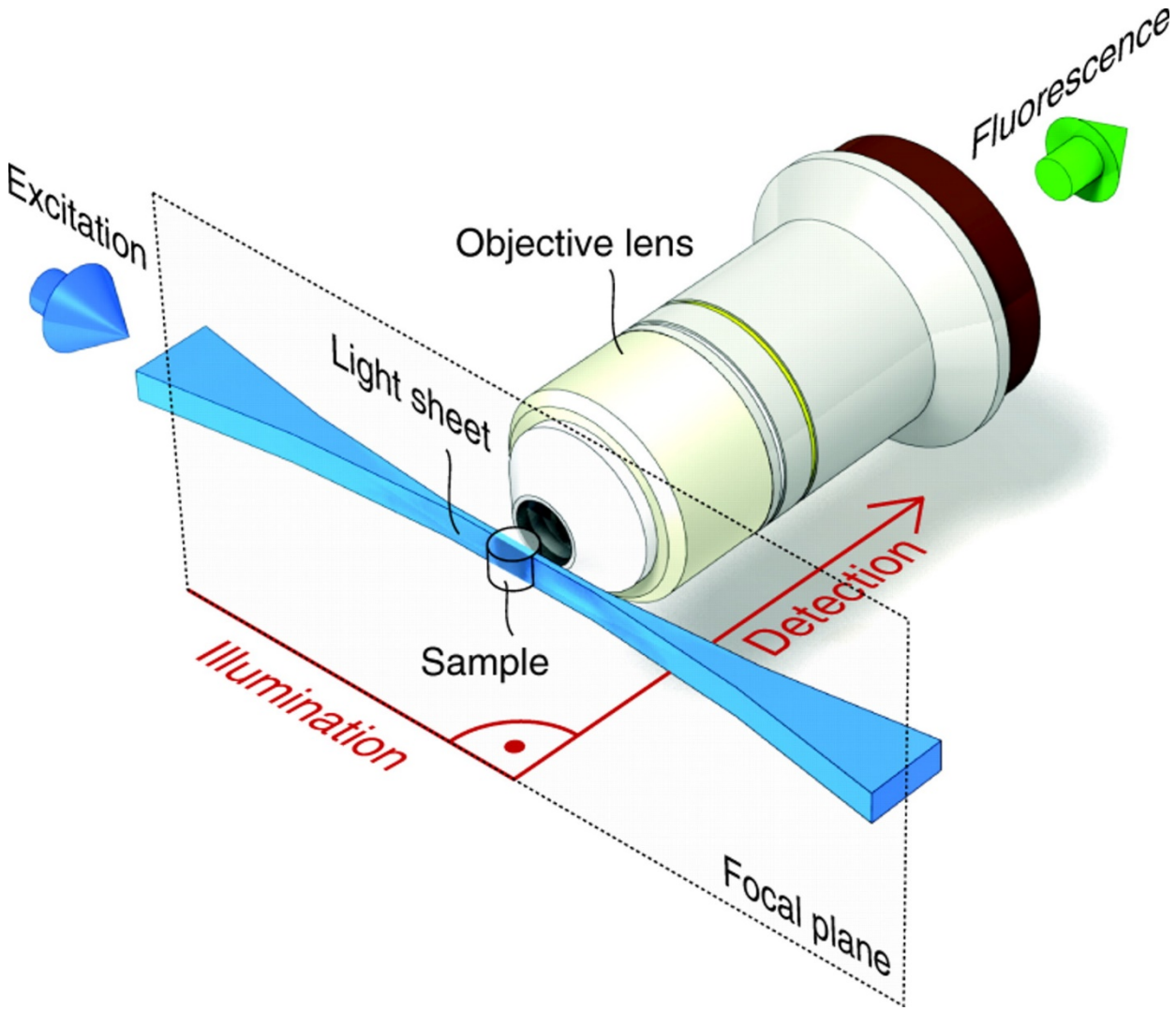


Figure 3 from Siedentopf and Zsigmondy's (1903)

Light sheet microscope

No out of focus bleaching!



Development 136, 1963-1975 (2009)

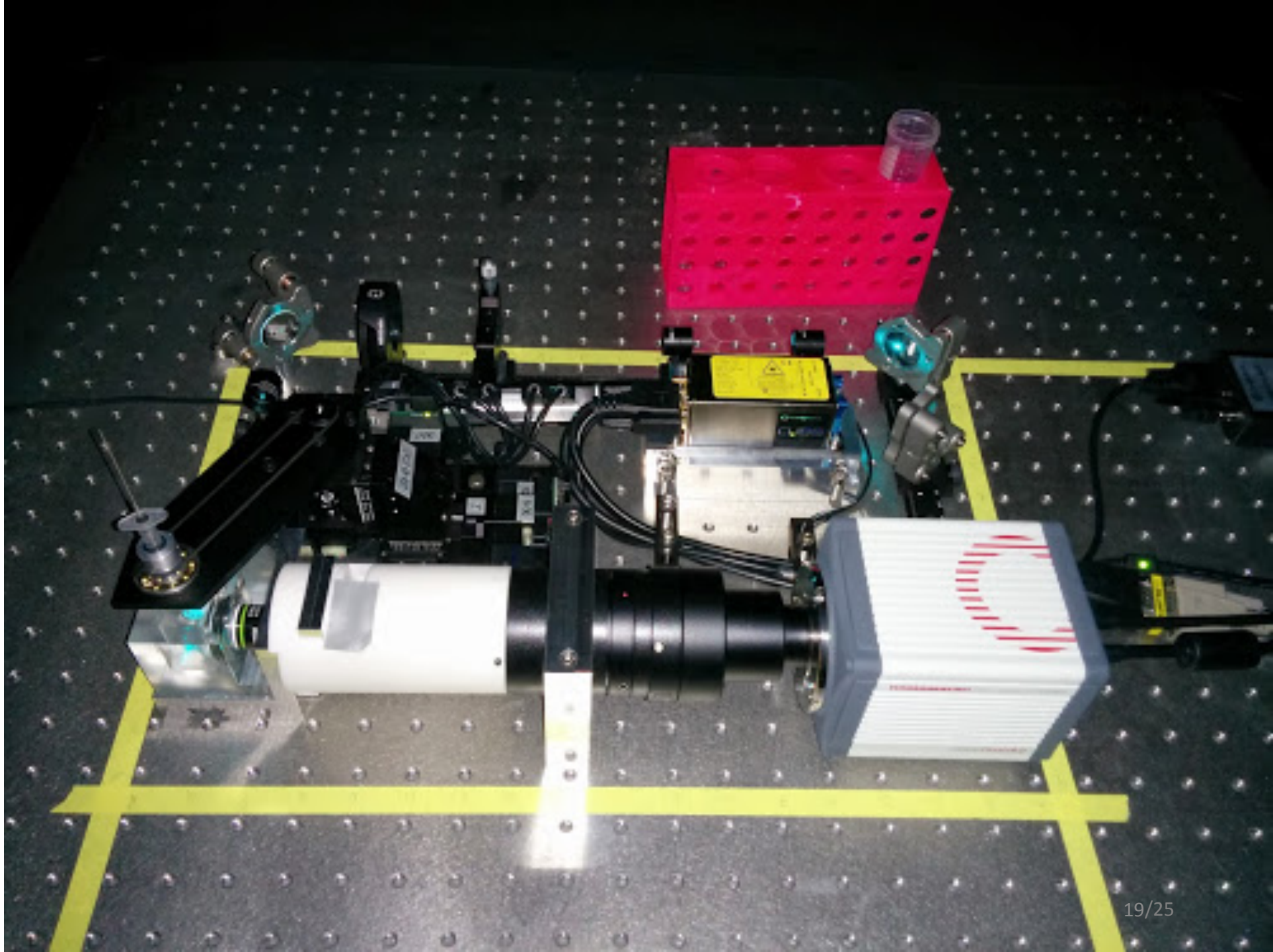
Simultaneous multiview imaging of *Drosophila* embryonic development (*His2Av-GFPS65T* transgenic stock). The embryo was recorded at 35-second intervals over a period of 19.5 hours, using an image acquisition period of 15 seconds per time point. The data set consists of 1,000,500 high-resolution images (10 terabytes).
Nature Methods **9**, 755– 763 (2012)

http://www.nature.com/nmeth/journal/v9/n7/fig_tab/nmeth.2062_SV3.html

Suitability for live cell fluorescence microscopy

	Confocal microscope	Spinning-disk confocal	Light-sheet
Sectioning	+++	++	++
Speed	- - -	+++	+++
Signal to noise ratio	+++	++	+++
Phototoxicity	- - -	+	+++

Open SPIM in the Biocenter



Located in room BU1.059

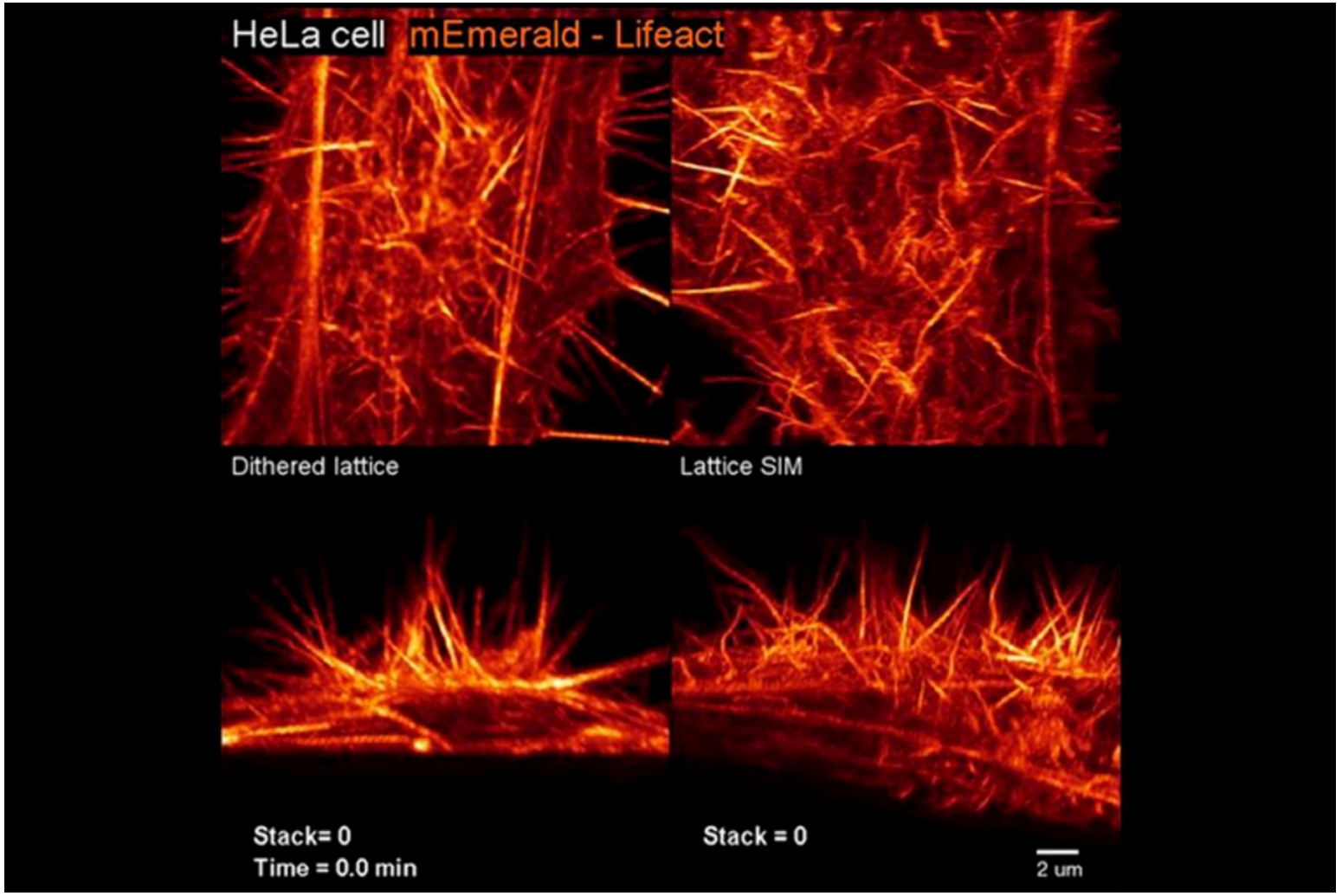
What will come next?

Conventional Gaussian light sheets are, over cellular dimensions, at least ~two to five times thicker than the depth of focus of a high-NA detection objective.

Bessel beam and lattice light sheet microscopes can overcome this limitation

Movie 1 Top and side view volume renderings of filopodia in a HeLa cell expressing mEmerald-Lifeact.The high speed of the dithered mode of lattice light-sheet microscopy (left) is compared against the high resolution of the SR-SIM mode (right).

Movie_1

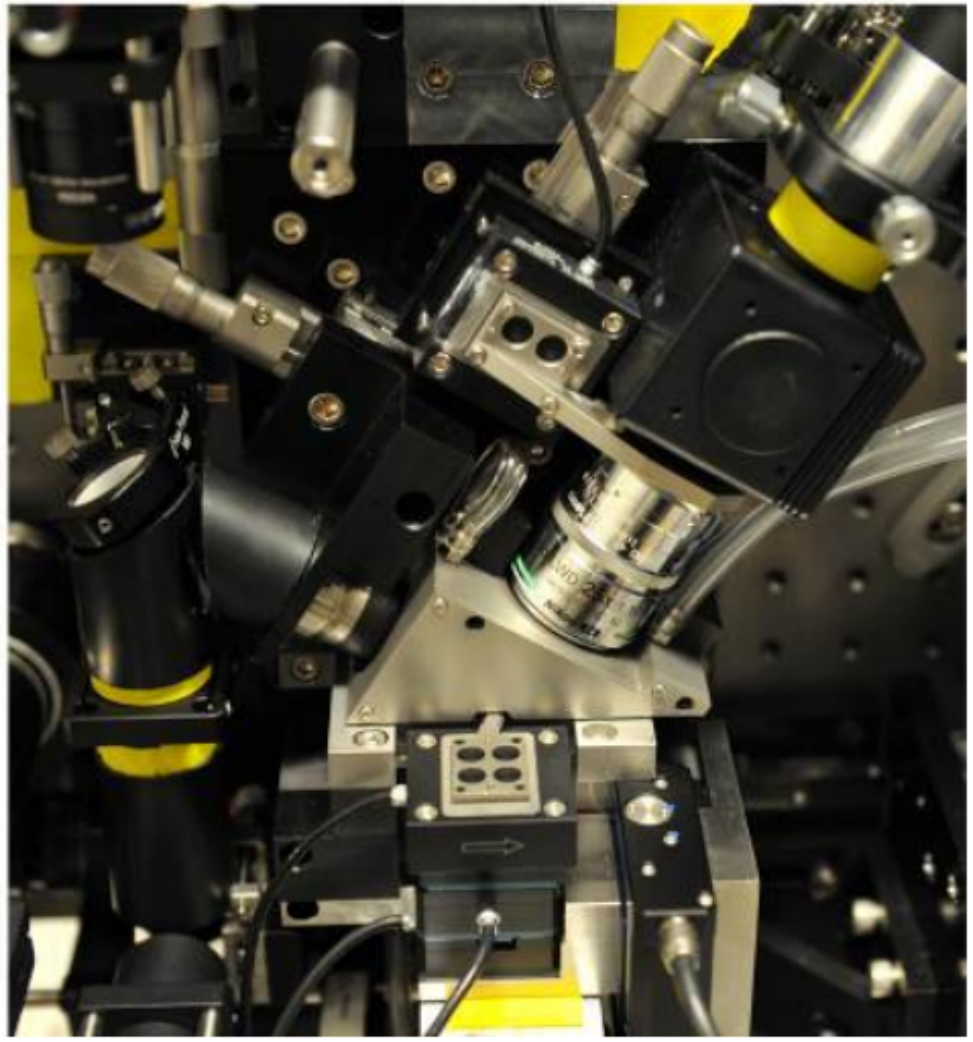


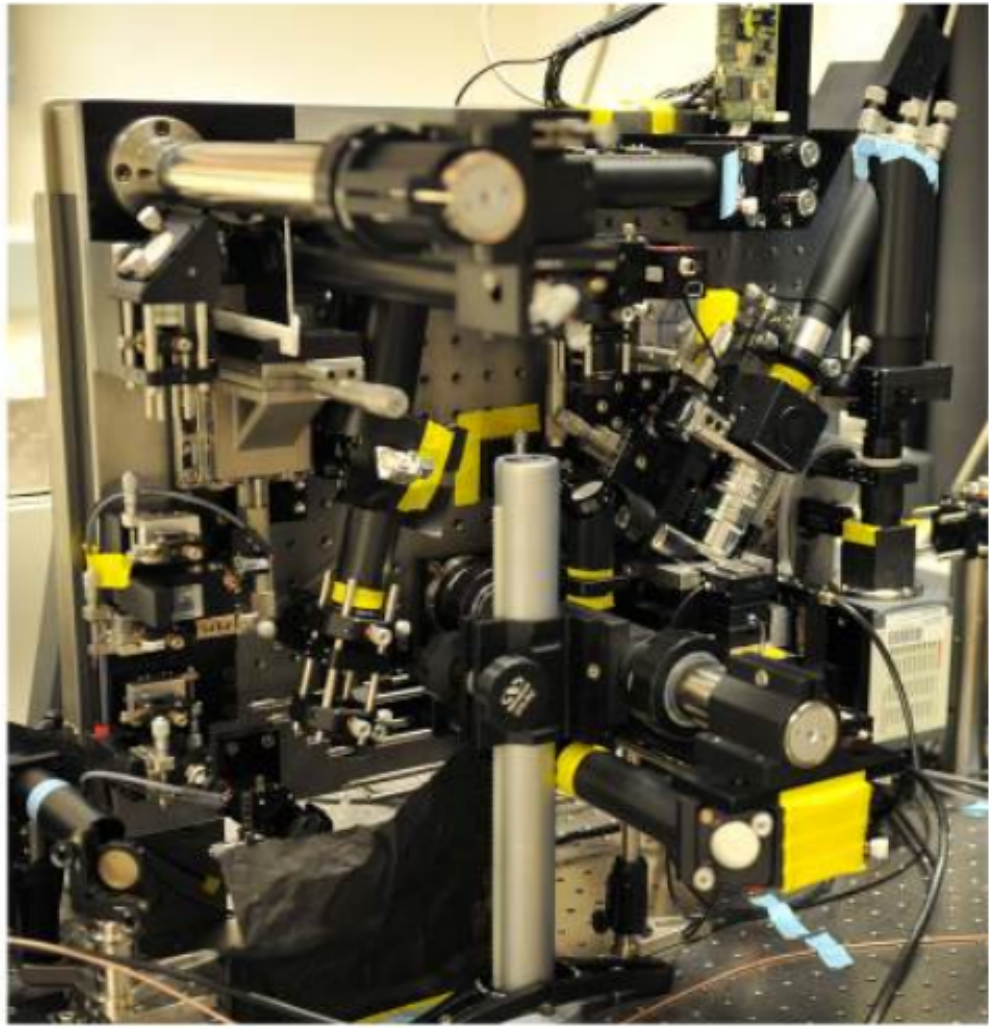
Bi-Chang Chen et al. Science 2014;346:1257998

Suitability for live cell fluorescence microscopy

	Confocal microscope	Spinning-disk confocal	Light-sheet	Lattice Light-sheet
Sectioning	+++	++	++	**++++**
Speed	- - -	+++	+++	**++++**
Signal to noise ratio	+++	++	+++	**++++**
Phototoxicity	- - -	+	+++	**++++**

Lattice Light - Sheet





Science. 2014 October 24; 346(6208): 1257998

Take-home message:

- Take care of the peak power of your light source if you want to keep your specimen alive and happy.

- Use a Spinning-disk or even a Light-sheet microscope for fluorescence live cell imaging

Thank You