

Light Sheet Microscopy

Technical Journal Club Series:

28.May.2013

Imaging and tracking cells during live imaging

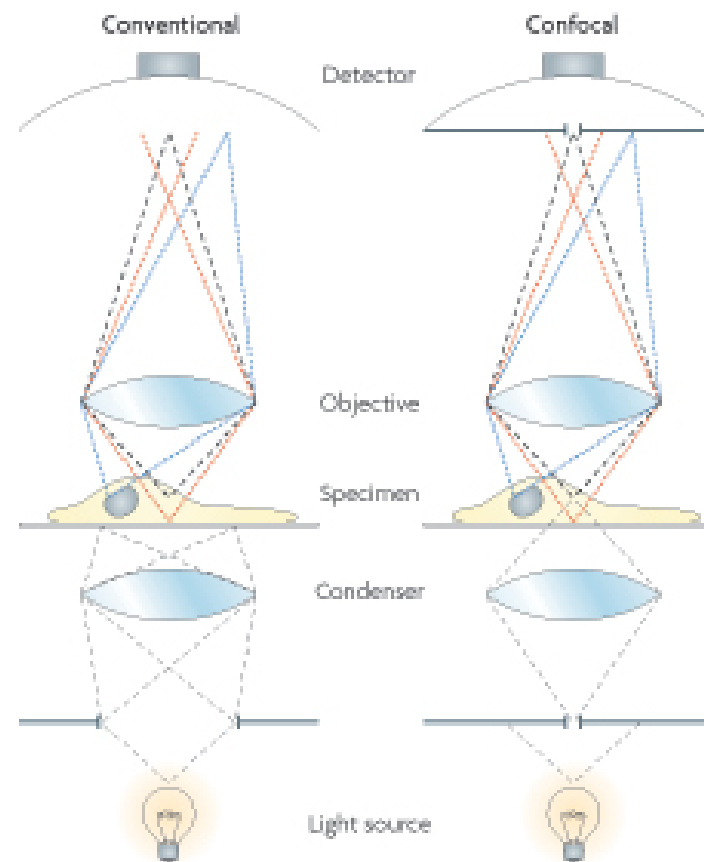
Introduction

Method: LSM

Method: RLSM

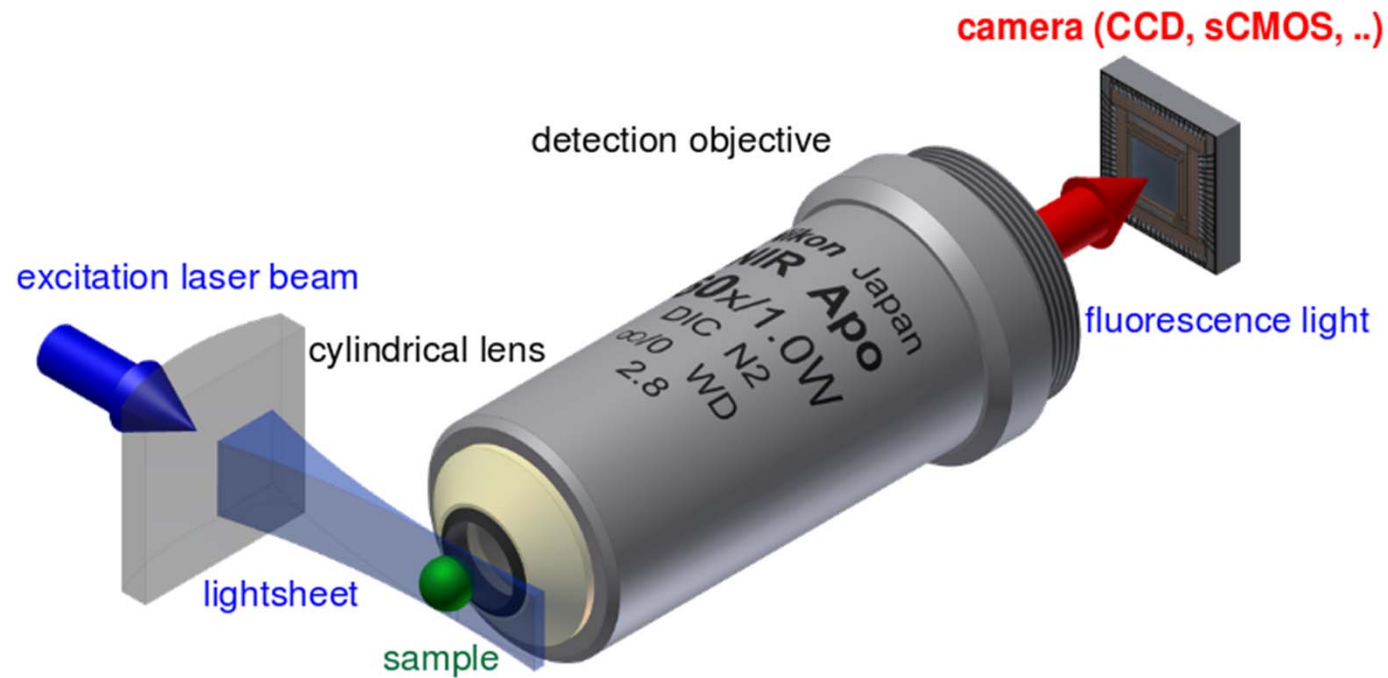
Discussion

Conventional microscopes:



Conventional microscopy illuminates the entire sample or a fraction of the sample. Detectors can be manipulated to collect only the fluorescence of interest with the plane of interest brought into focus.

Light-sheet microscopy (LSM): A method to illuminate only in the plane of focus.

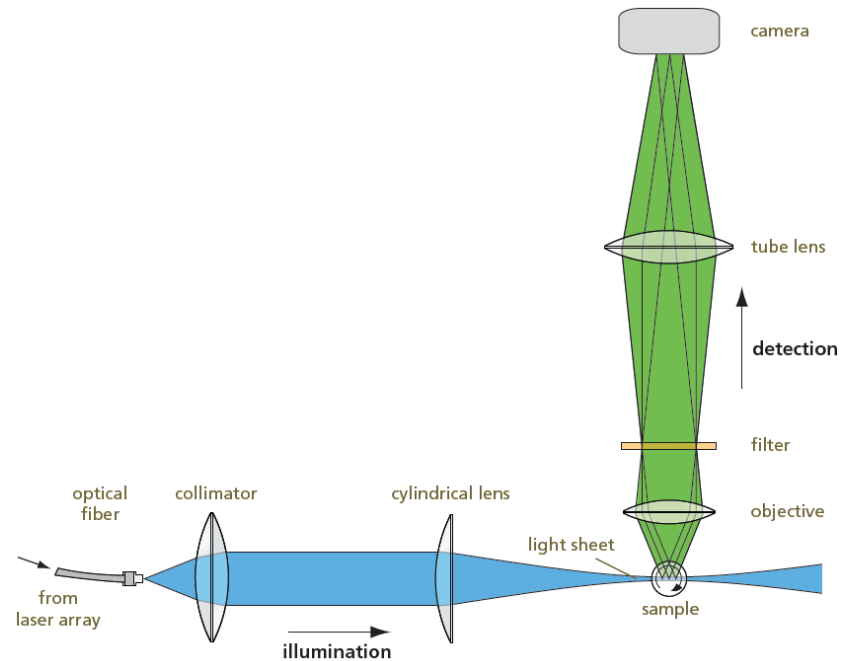
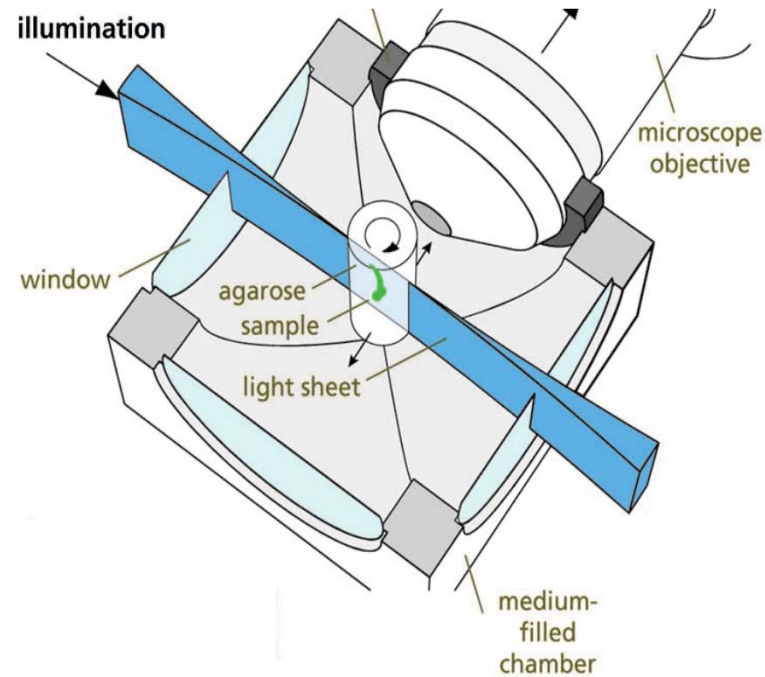


Focused sheets of laser light up to 100 μ m in thickness is used to illuminate the plane of focus perpendicularly to the detection objective.

Science 2004:

Optical Sectioning Deep Inside Live Embryos by Selective Plane Illumination Microscopy

Jan Huisken,* Jim Swoger, Filippo Del Bene, Joachim Wittbrodt, Ernst H. K. Stelzer*



Introduction

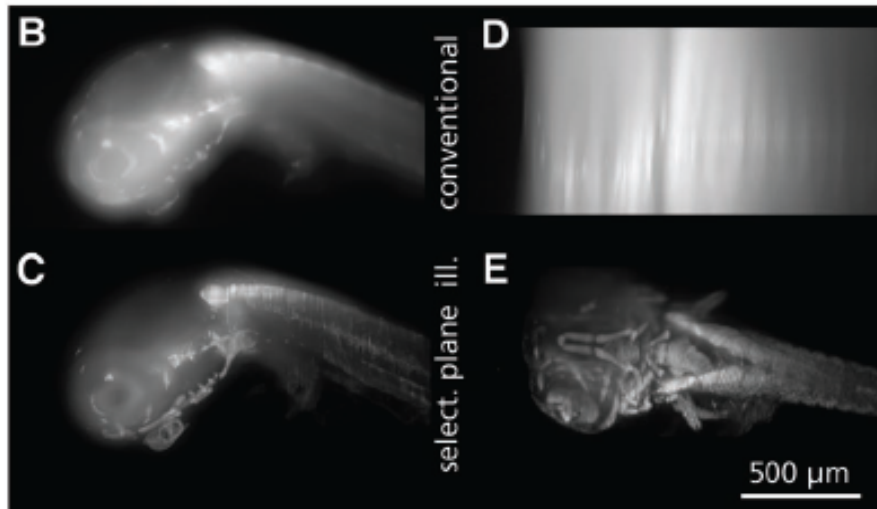
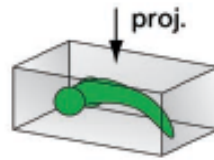
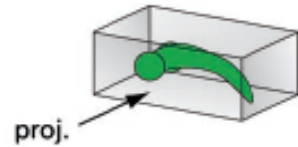
Method: LSM

Method: RLSM

Discussion

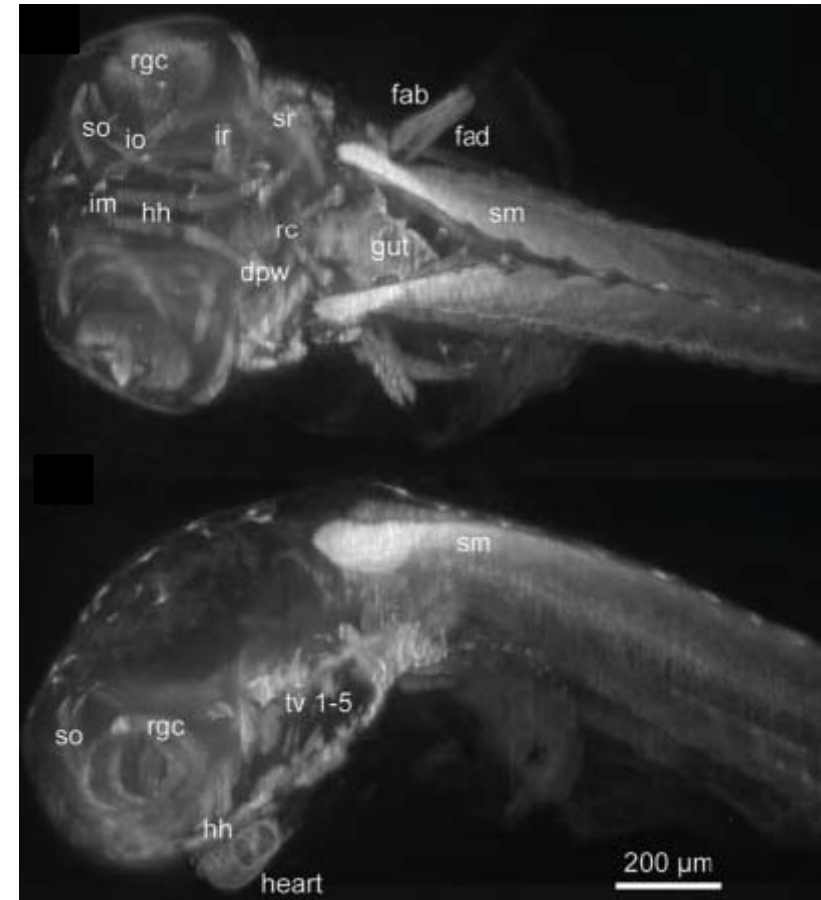
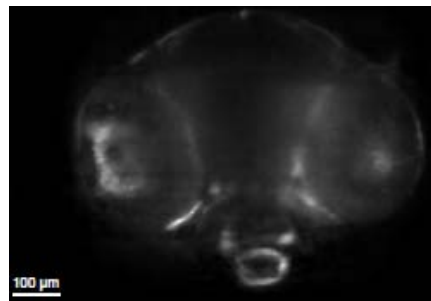
Imaging of a fixed fish embryo to a depth of 500um and with a spatial resolution of 6um

maximum intensity projections



Imaging of a fixed sample using Widefield (B & D) vs LSM (C & E)

3D reconstruction of head imaged via confocal with 10x objective



Resolution is adequate to distinguish different tissues

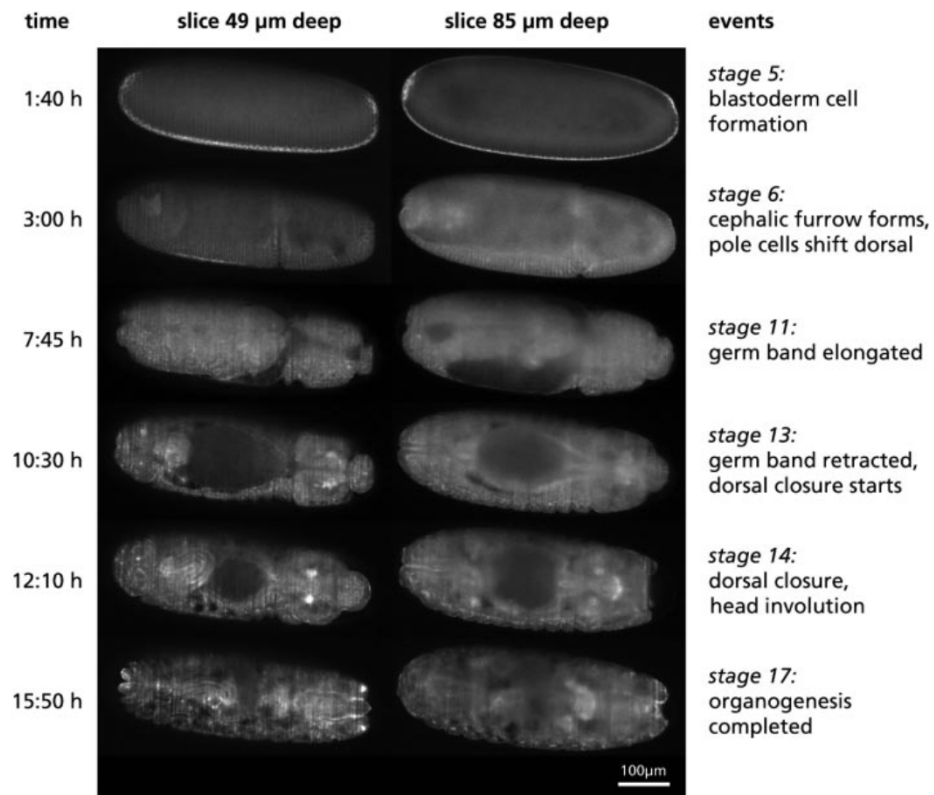
Introduction

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Discussion

The internal structures of developing fly embryos can also be distinguished by using LSM:



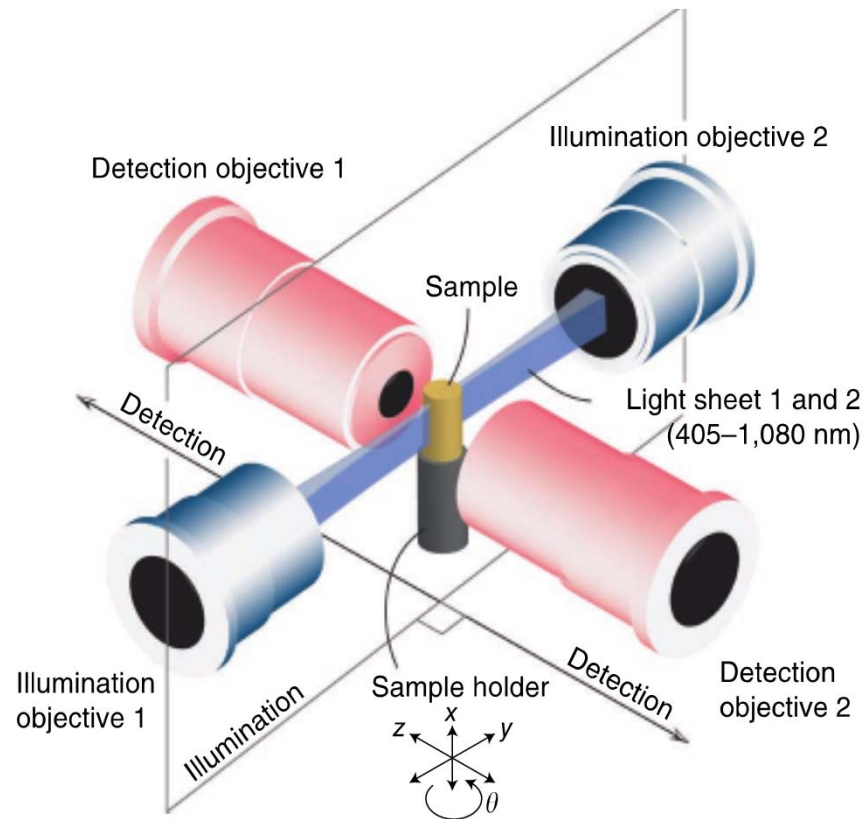
[Video](#)

However, it took 17 hours to image one embryo. The embryo developed normally after imaging.

Nature 2012:

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

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



Here they used:

1. multiple illumination objectives and detectors to image fly embryo development in multiple angles.
2. both single-photon and two-photon based illumination to obtain super-resolution or deep penetrance.

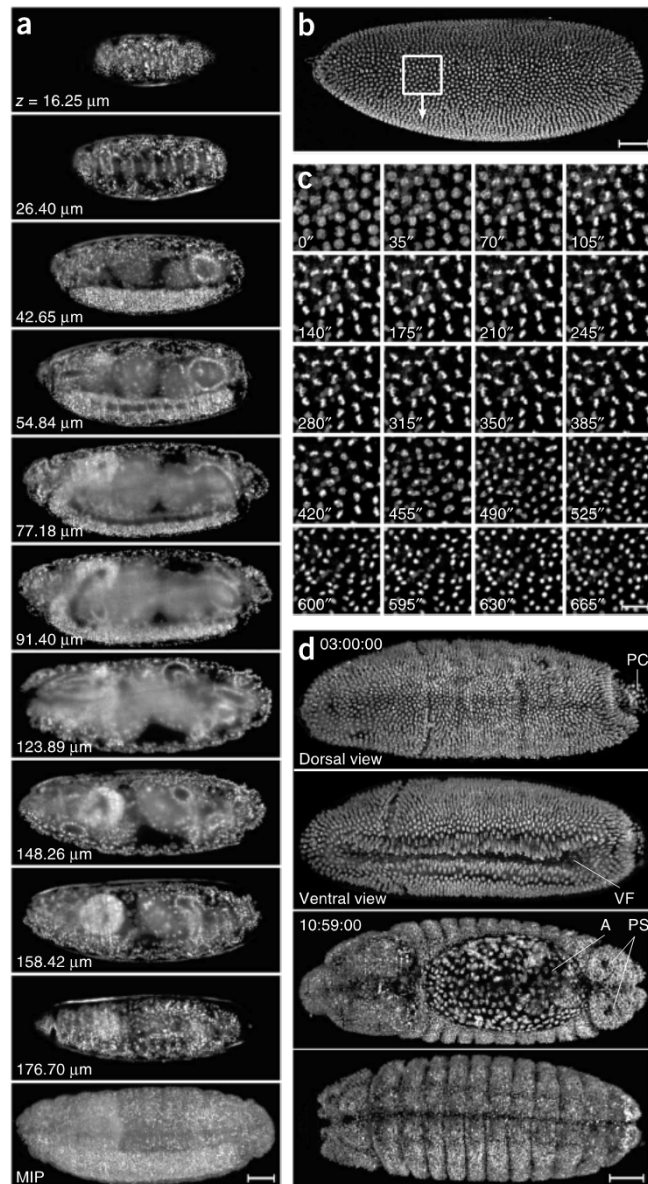
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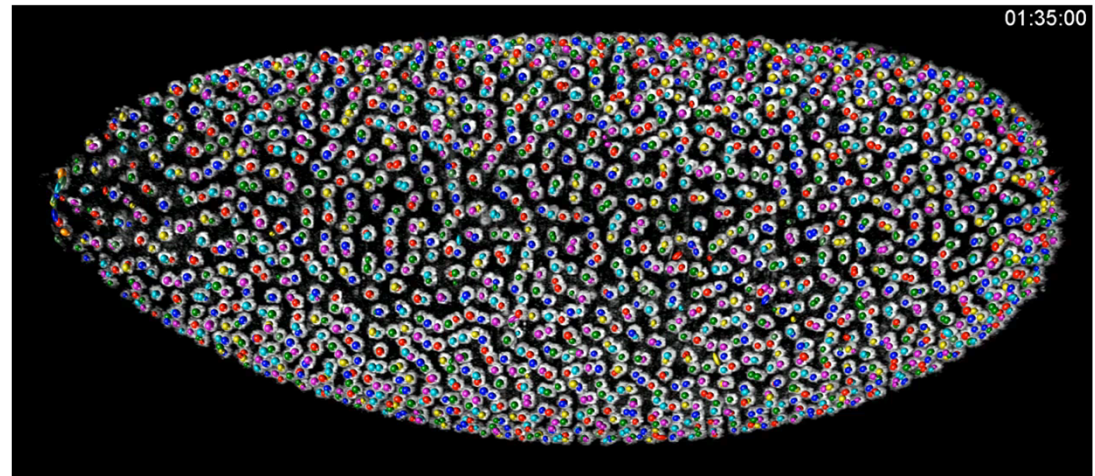
Discussion

Single-photon illumination provides single cell resolution at the surface of the embryo and pretty decent structural resolution throughout the embryo:



Introduction

Method: LSM



The dynamics of nuclei on the surface of the embryo can be followed and quantified at a single cell resolution.

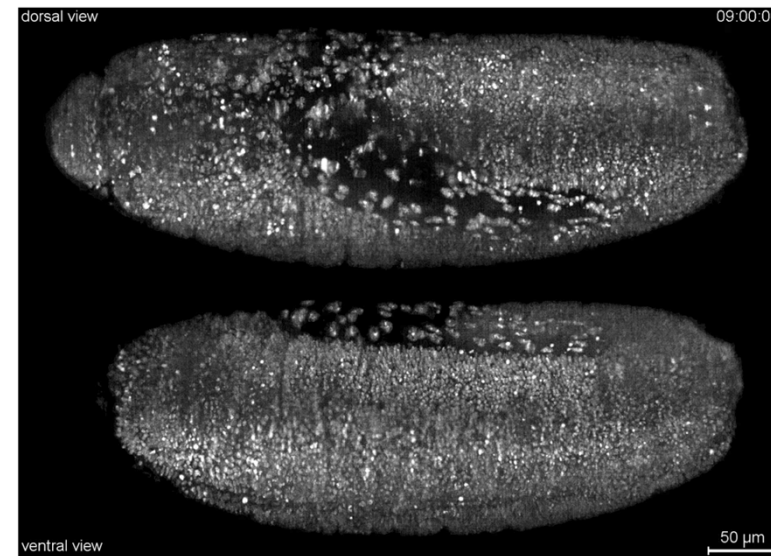
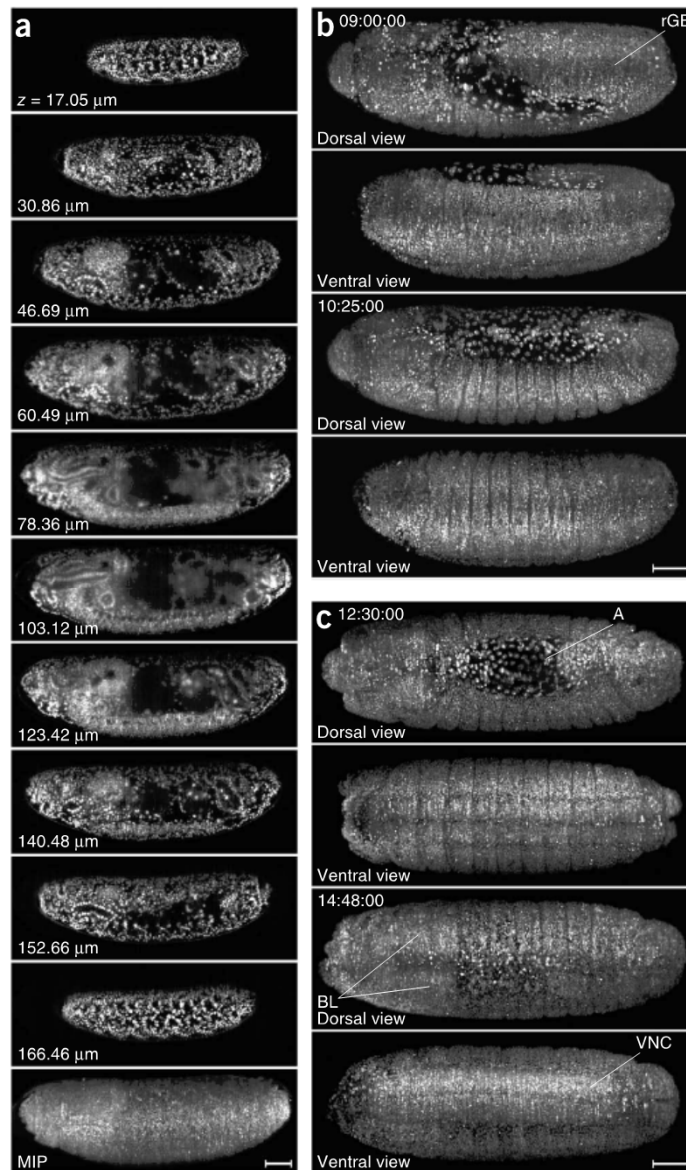
[Video](#)

The entire embryo was imaged in 15s per timepoint. Each embryo was imaged from 3h after fertilization till 18.5h.

Method: RLSM

Discussion

Two-photon illumination provides structural resolution in the embryo:



Introduction

Method: LSM

Method: RLSM

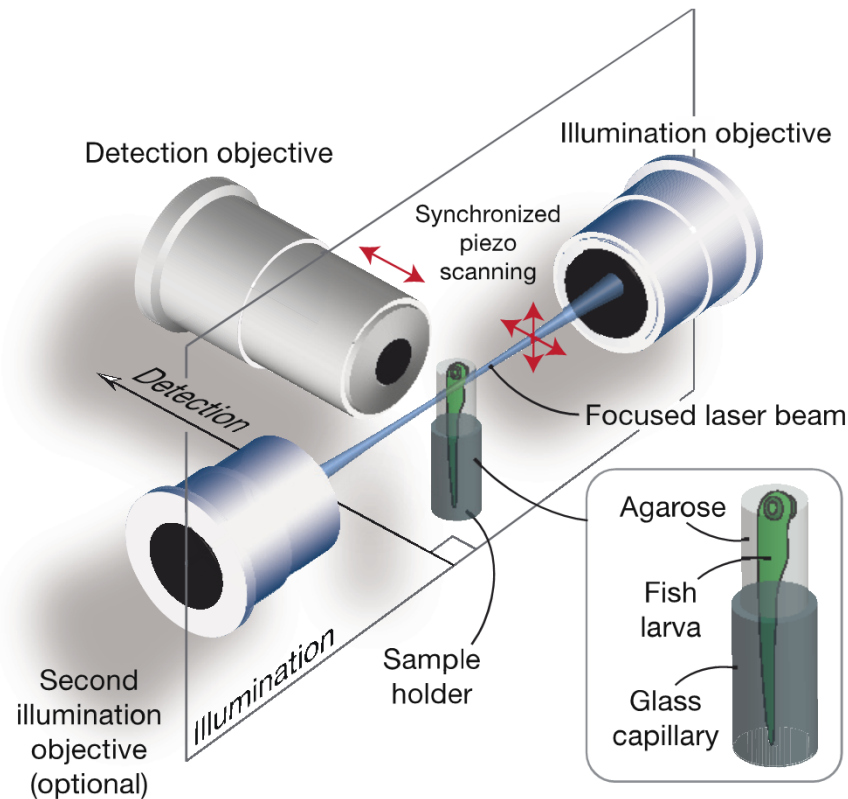
Discussion

Nature 2013:

Whole-brain functional imaging at cellular resolution using light-sheet microscopy

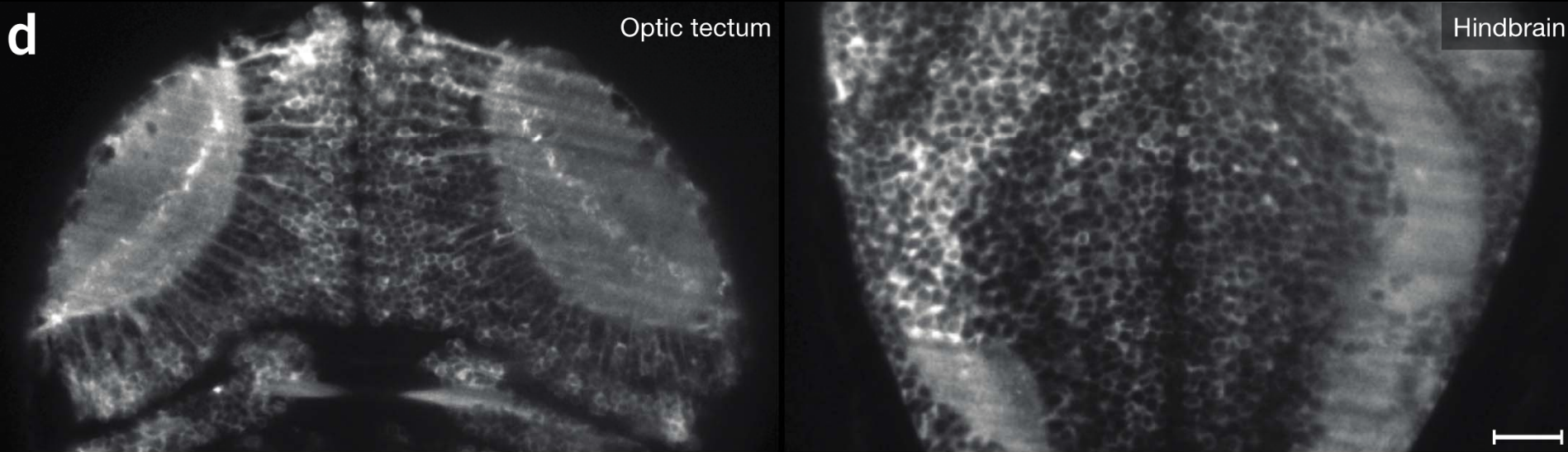
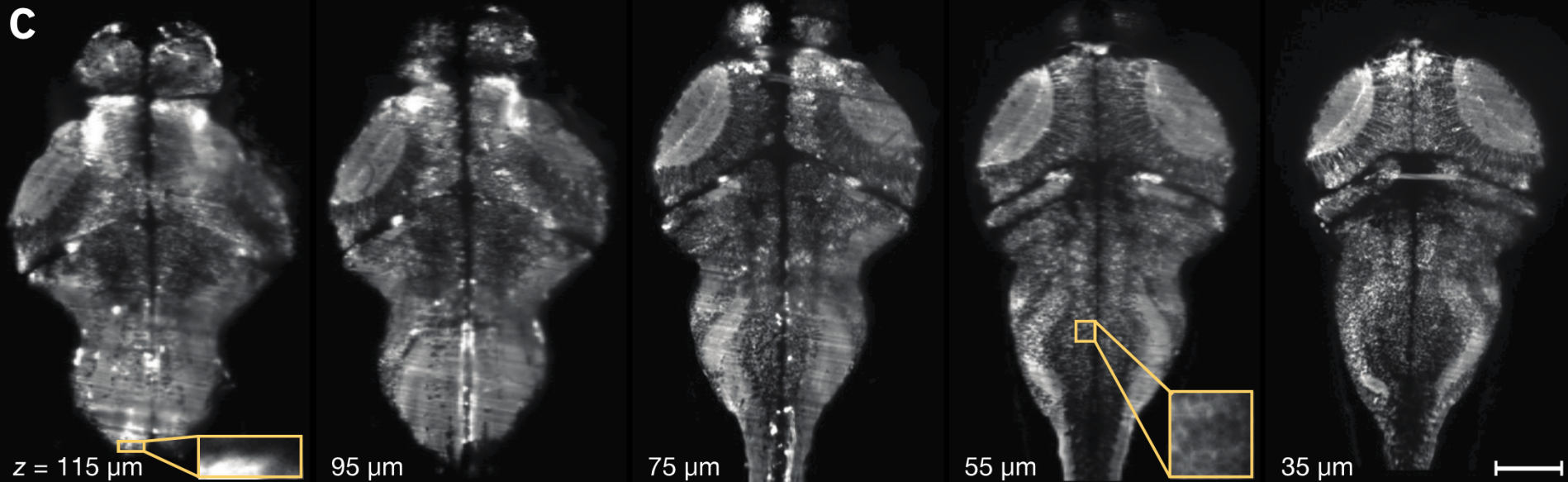
Misha B Ahrens¹, Michael B Orger², Drew N Robson³, Jennifer M Li³ & Philipp J Keller¹

Imaged entire zebrafish larval brains at a single cell resolution to record neuronal calcium activity in 1.3 seconds across the entire brain.



1. Live larvae is mounted into soft agarose.
2. Laser scanning of the larvae is performed in two directions using a fast piezo operated illumination objective.
3. Emitted light (4 μ m thick) in each illuminated plane is rapidly captured and stored using fast detectors and cameras.

The larval brain was imaged in 41 planes with a 5 μ m distance between each plane covering a total of 800 x 600 x 200 μ m³ in 1.3 seconds.



[Video](#)

Introduction

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Method: RLSM

Discussion

Identifying neuronal networks:

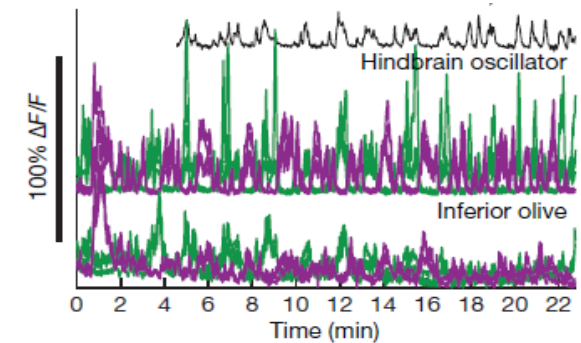
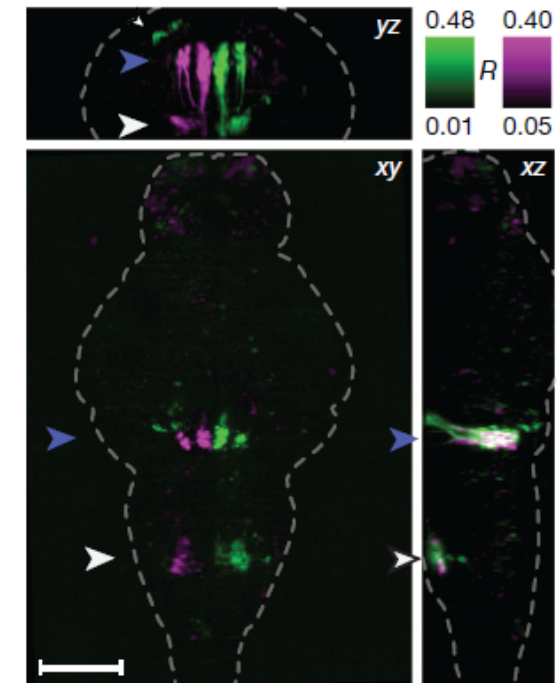
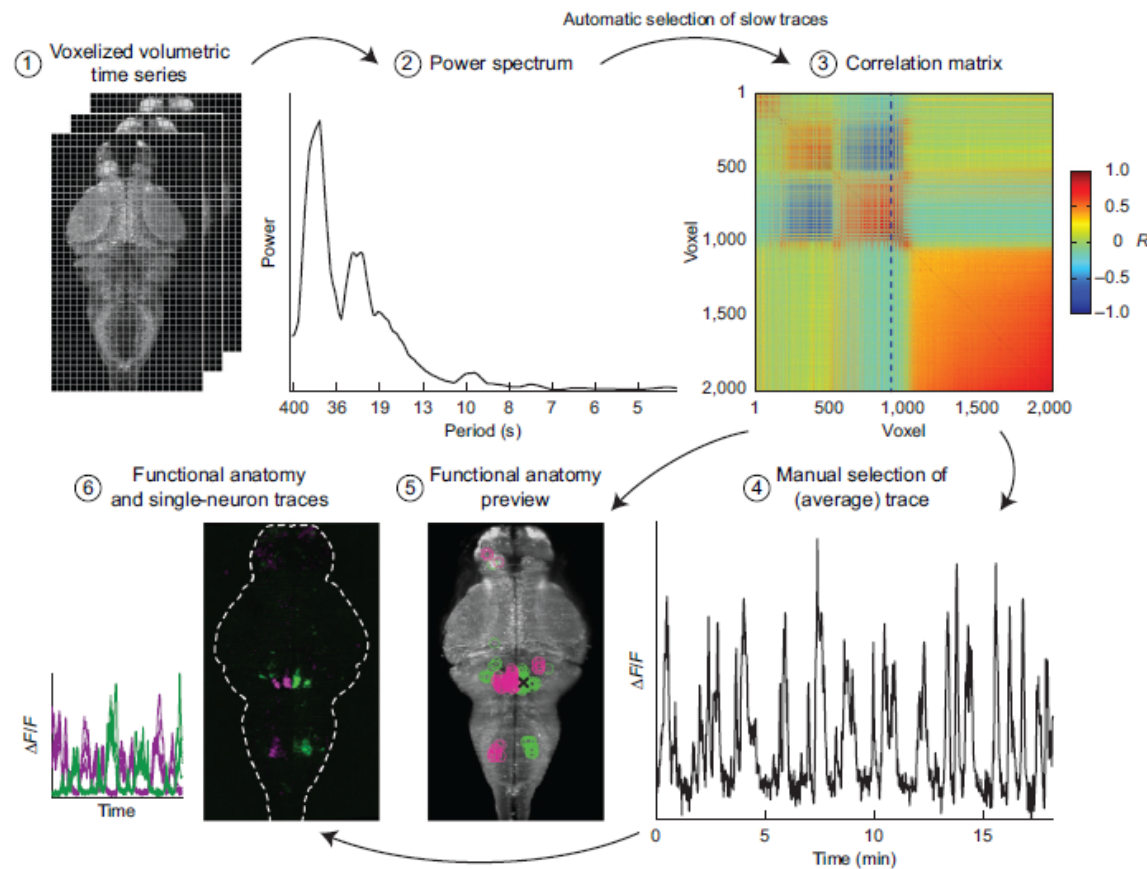
1. Zebrafish larvae of the *elavl3:GCaMP5G* line in an *albino* background were used for this study.
2. Whole brain imaging via LSM was performed on non-stimulated larvae to determine background neuronal activities in the forebrain, midbrain, and the hindbrain.
3. Whole brain was imaged during stimulation of the retina using a blue light and the neurons activated due to the stimulation were recorded.

[Video](#)

[Video](#)

4. Upon retinal stimulation they consistently observed neuronal activities in the hindbrain oscillator, inferior olive, and the spinal cord.

- More detailed observation of both the hindbrain oscillator neurons and those neurons in the inferior olive are part of the same circuit where the inferior olive neurons are driven by the hindbrain oscillator upon retinal stimulation.



Introduction

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Discussion

Summary of light-sheet microscopy:

- Light-sheet microscopy is a non-invasive form of light microscopy., e.g. there is less photodamage as compared to confocal microscopy.
 - Good for imaging tissue as thick as 200um at cellular resolution.
 - With advanced detection and image recording systems now available it is possible to use LSM for calcium imaging and quantification.
 - As the technique is non-invasive the samples can be cultured for longer periods of time for long term time lapsed imaging, e.g. during tissue development.
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- The tissue needs to be fairly opaque to minimize light scattering.
 - Live sample needs to be immobilized during imaging to provide optimal resolutions.

Live imaging of single molecules in cells

Introduction

Method: LSM

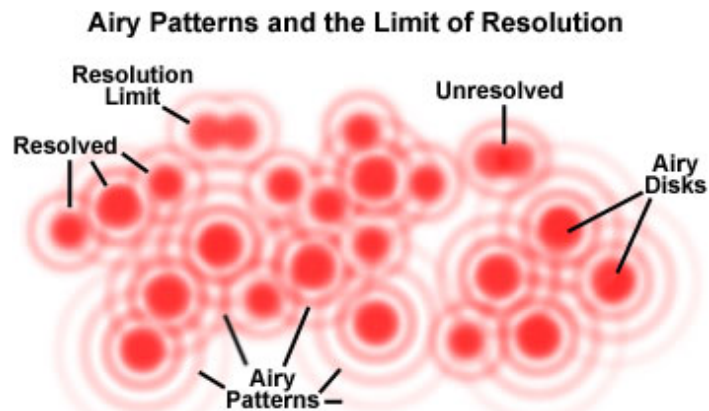
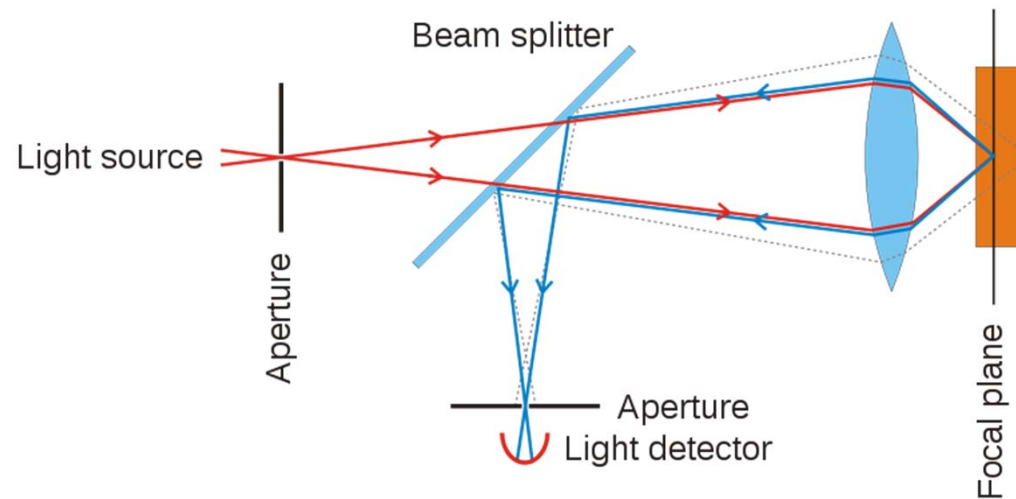
Method: RLSM

Discussion

There are two ways to try and image single molecules which have been fluorescently labeled:

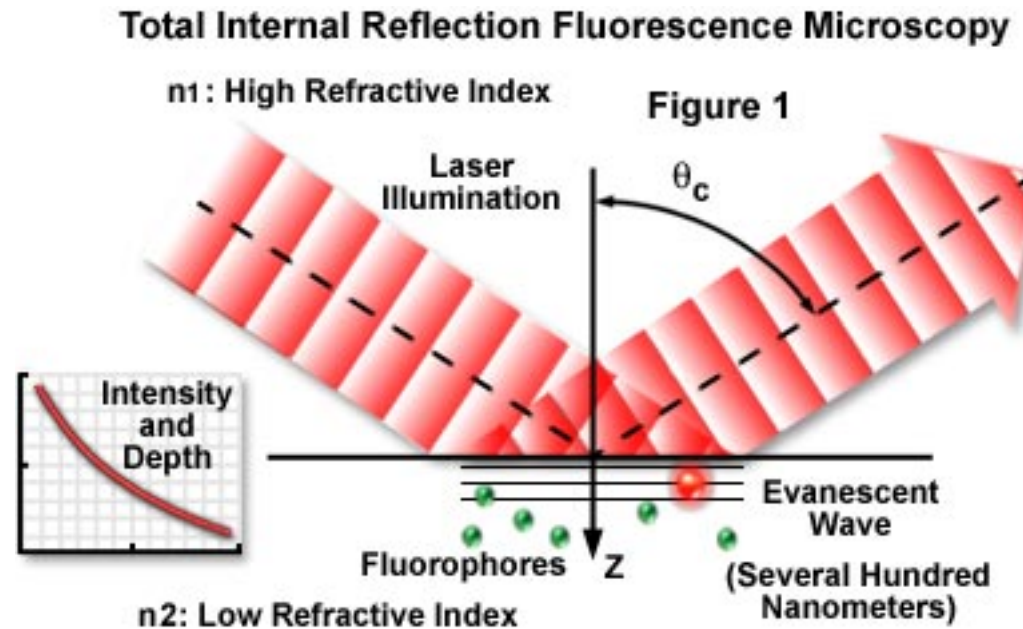
1. Reject out-of-focus background before or after imaging.
 - Laser scanning confocal microscopy
 - Multiphoton microscopy
 - Deconvolution post imaging
2. Illuminating and exciting only in the plane of focus.
 - Total internal reflective microscopy
 - Selective light sheet microscopy

Limitations of Laser Scanning Confocal Microscopy:



Difficult to distinguish two fluorophores closer than 200nm together (100x objective).

Total Internal Reflection Microscopy:



Olympus Microscopy Resource Centre

Good for:

- visualizing fluorescently labeled single molecules on the plasma membrane.
- live imaging.

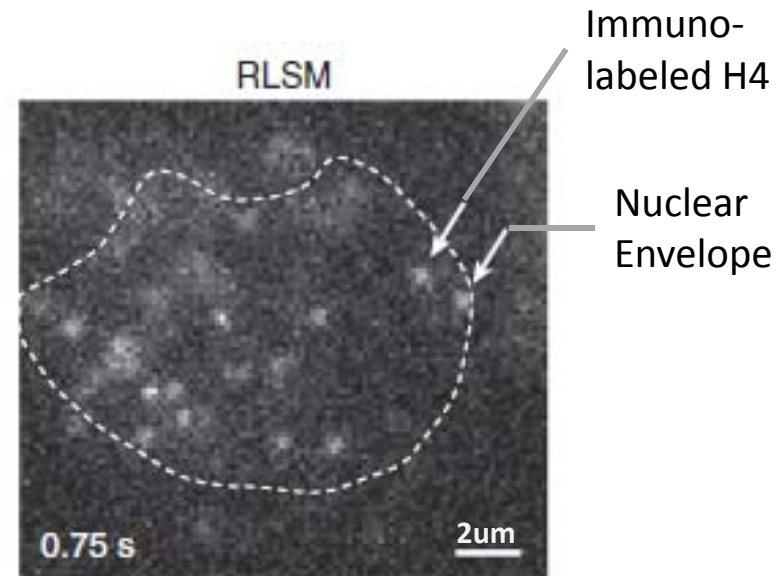
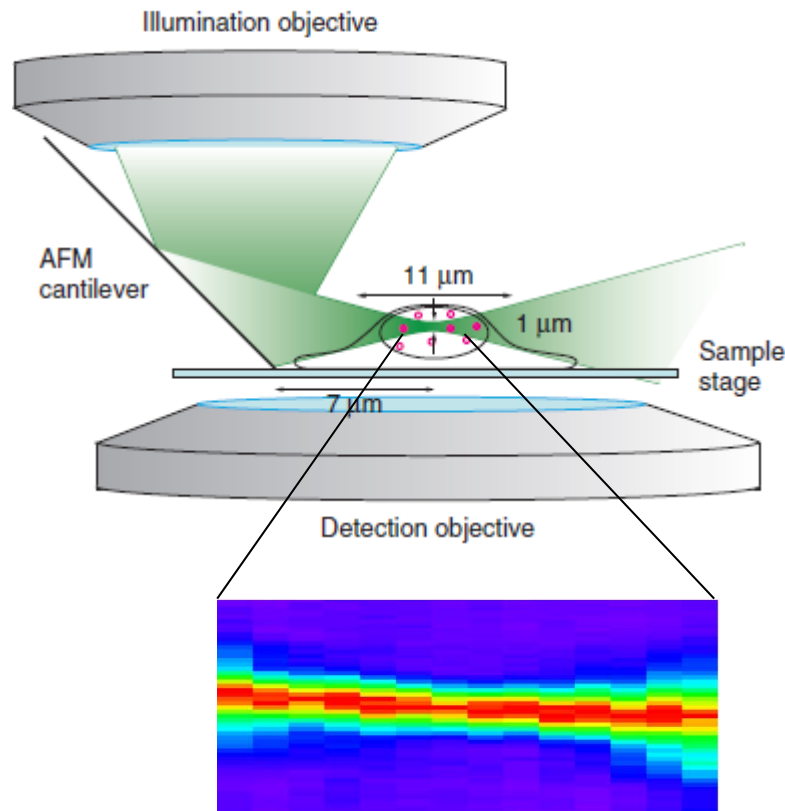
Poor for:

- visualizing fluorescently labeled single molecules further than 100nm away from glass interface, e.g. nucleus.

Nature 2013:

Single-molecule imaging of transcription factor binding to DNA in live mammalian cells

J Christof M Gebhardt^{1,5}, David M Suter^{1,5}, Rahul Roy^{1,4}, Ziqing W Zhao^{1,2}, Alec R Chapman^{1,2}, Srinjan Basu^{1,3,4}, Tom Maniatis³ & X Sunney Xie¹



Imaging of MCF-7 cell expressing mEos2-histone H4 at a resolution of $\sim 0.5 \mu\text{m}$

Introduction

Method: LSM

Method: RLSM

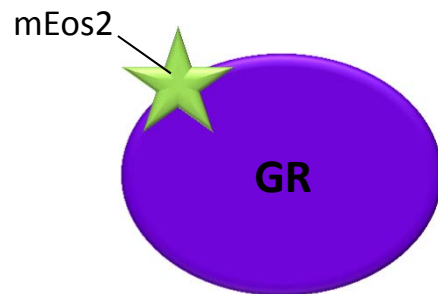
Discussion

Detecting glucocorticoid receptor (GR) binding dynamics with DNA:

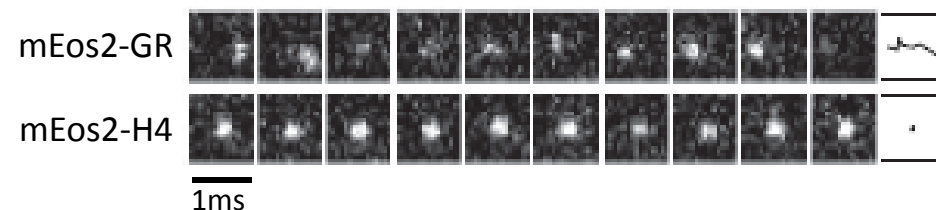
GR is a transcription factor that mainly resides in the cytoplasm in the absence of steroid glucocorticoids cortisol. Once activated it either dimerises and translocates into the nucleus to directly interact with DNA or it interacts with coactivators and binds DNA indirectly. GR regulates several hundreds of genes.

GR-DNA interactions are highly dynamic processes which are difficult to follow. Here the dynamics were recorded and quantified using RLSM.

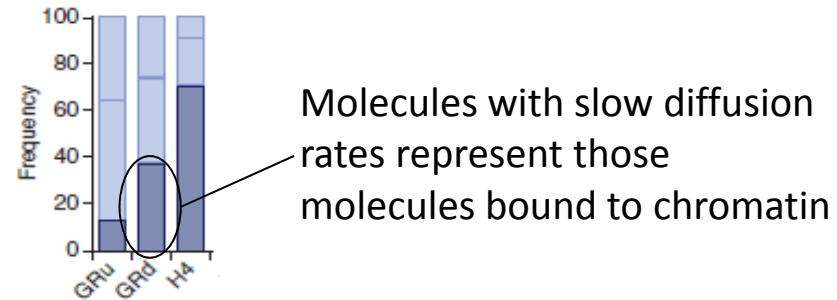
1. GR is fluorescently labeled with mEos2.



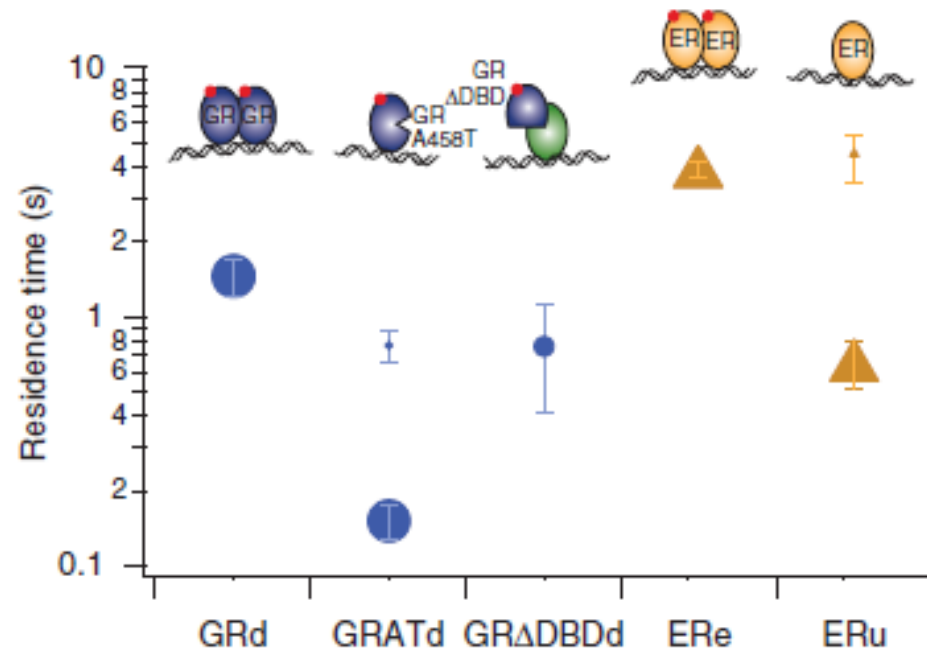
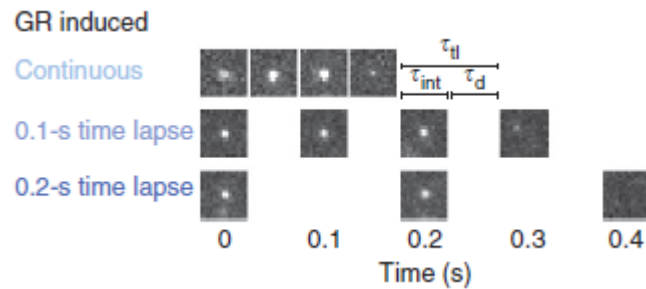
2. Few mEos2 molecules are excited and traced at a plane within the nucleus over 10ms.



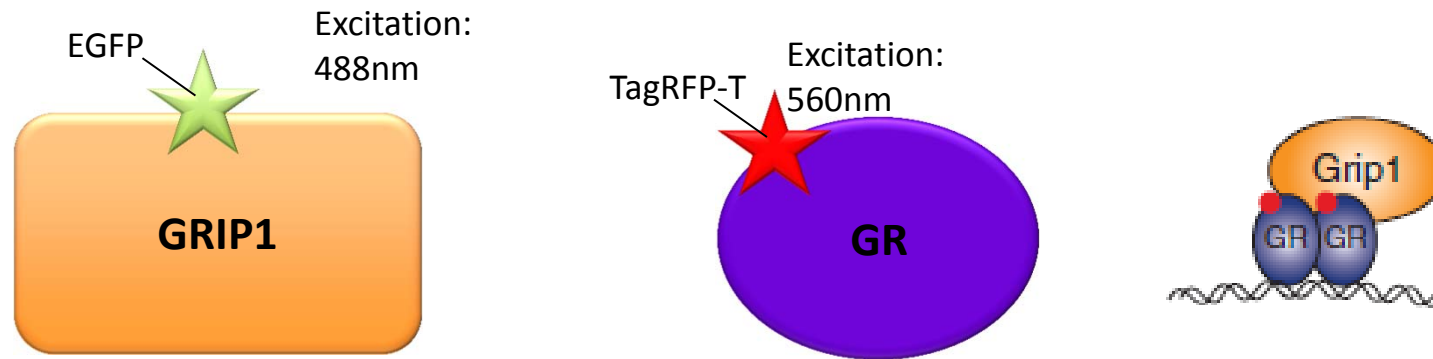
3. Add 100nM dexamethasone to the cells to stimulate GR translocation before comparing to untreated sample.



4. Residence time of DNA bound GR was measured after 100nM dexamethasone was added to the cells.

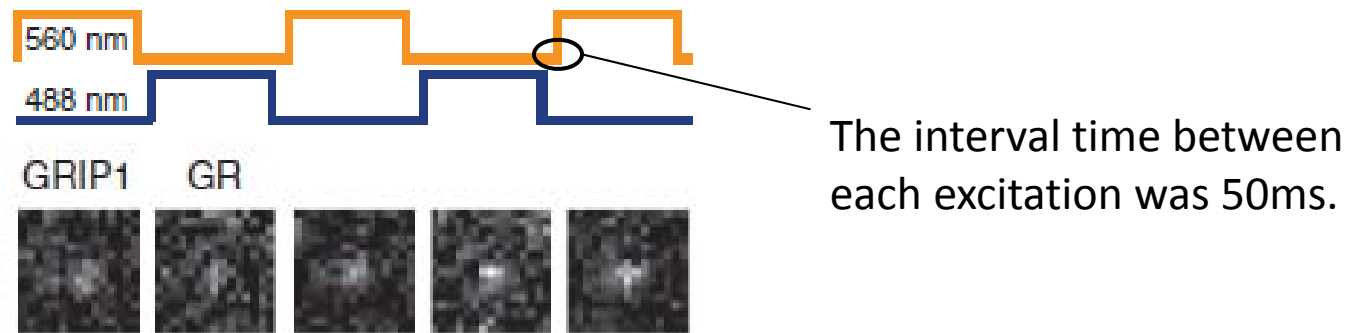


Visualization of two colocalized molecules – GRIP1 and GR:



GRIP1 is a coactivator of GR

After treatment with 100nM dexamethasone GRIP1 and GR were imaged by alternating laser excitation between 488nm and 560nm in the same light-sheet plane.



Summary of reflective light-sheet microscopy:

- Reflective light-sheet microscopy means that the laser sheet can be closer to the surface than regular light-sheet microscopy
 - The laser sheet is thinner than that used in regular LSM thus being able to improve the resolution to the molecular level
 - Due to focused lighting within the sample there is less interference of cellular activities due to photodamage.
- As the light sheet cannot get any closer than 2 μm to the glass cover slip it is not possible to detect cellular activity and the membrane level. Therefore should be paired with TIRF microscopy.

Questions?

Introduction

Method: HILO

Method: RLSM

Discussion

Thank you...