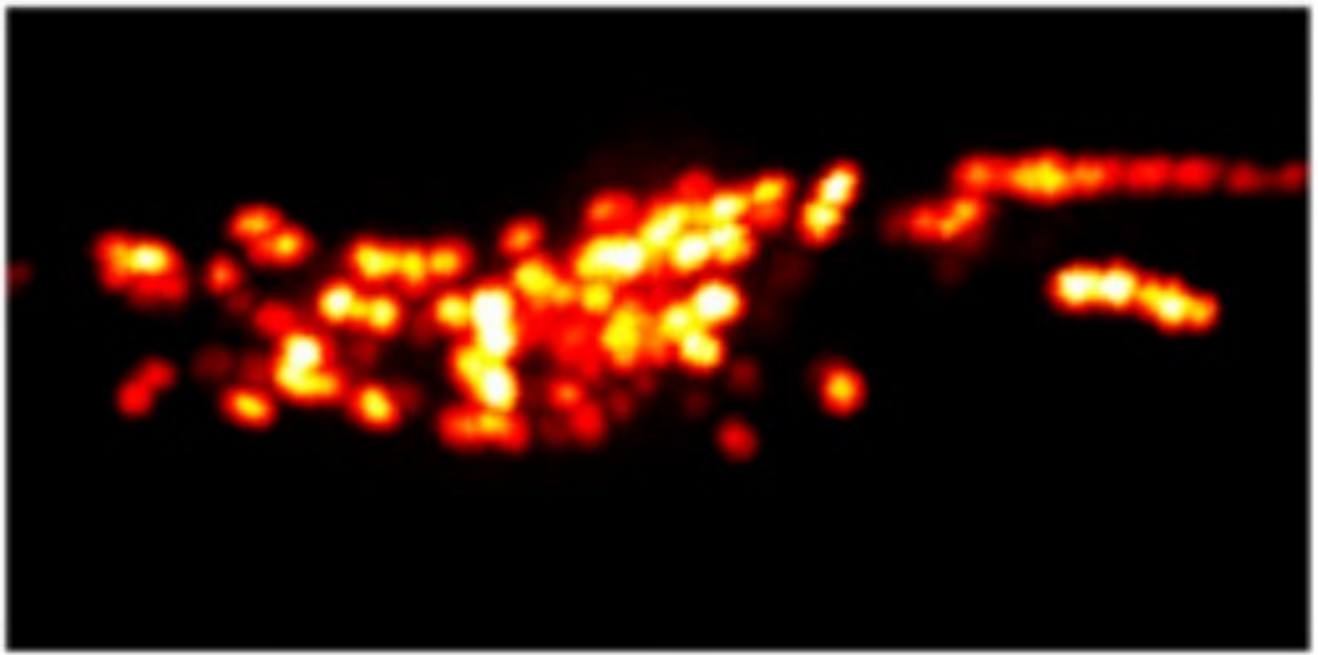
Whole-brain imaging:
Overview of microscopy techniques

Francesco Randi
Leifer Lab, Princeton University

Whole brain



Nguyen et al., PNAS 2015

Different microscopy techniques

Light field *spinning-disk confocal* *wide-field temporal focusing* *light-sheet/SCAPE*

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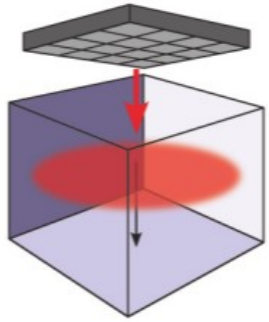

adapted from Weisenbruger, Vaziri, Annu. Rev. Neurosc. 2018

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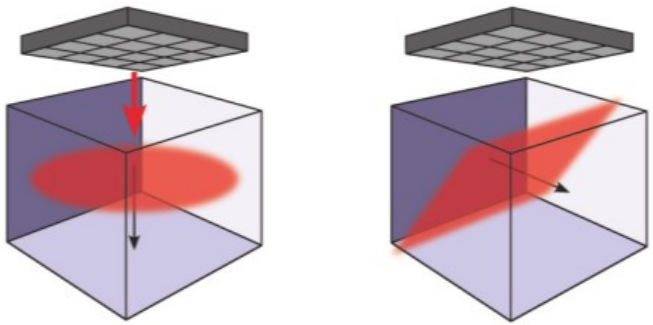

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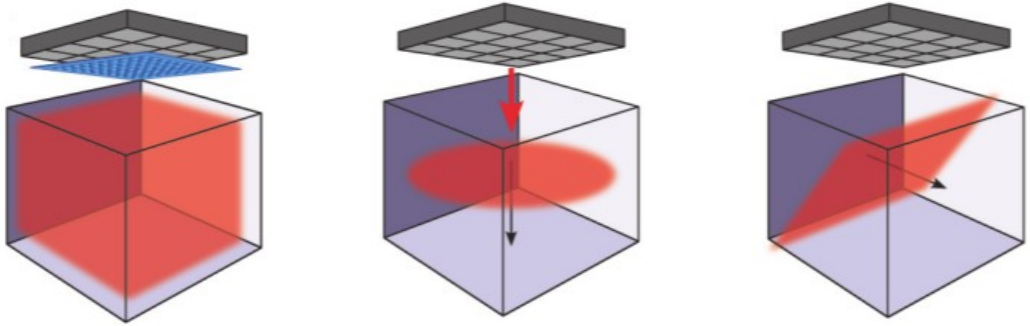

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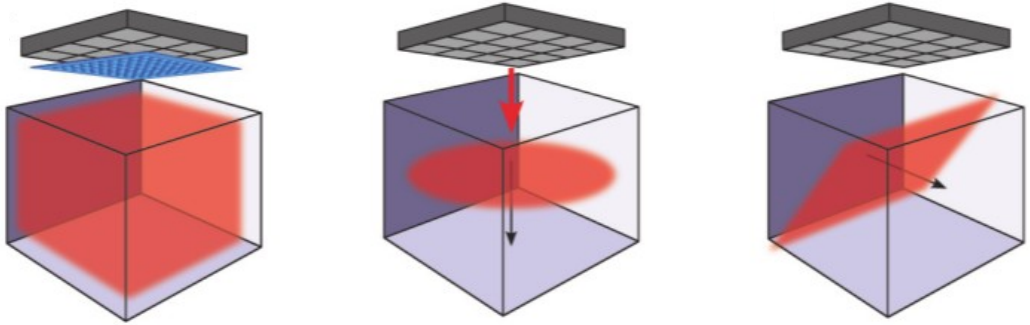
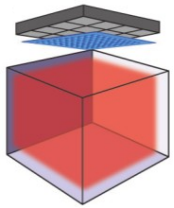


adapted from Weisenbruger, Vaziri, Annu. Rev. Neurosc. 2018

+ light sources, photo-bleaching, volume rates, post-processing...

Light field

3D volume in 1 shot



Other references
Levoy et al., Journal of Microscopy 2009

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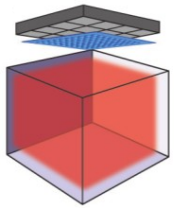

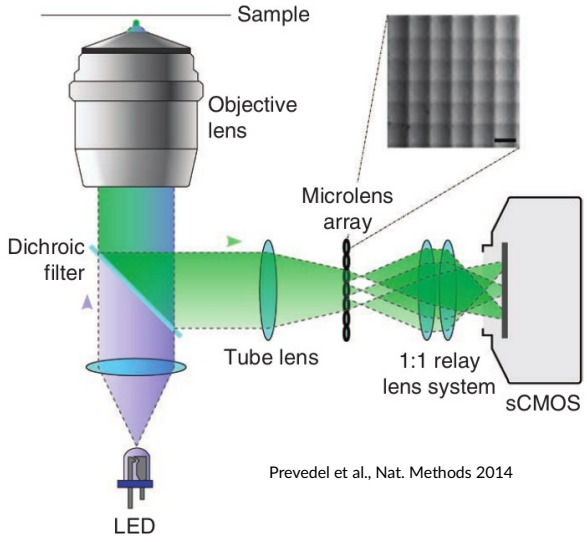

Prevedel et al., Nat. Methods 2014

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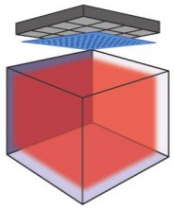

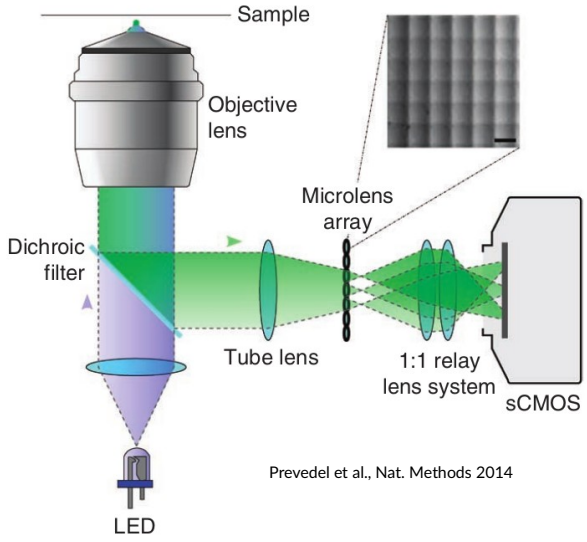

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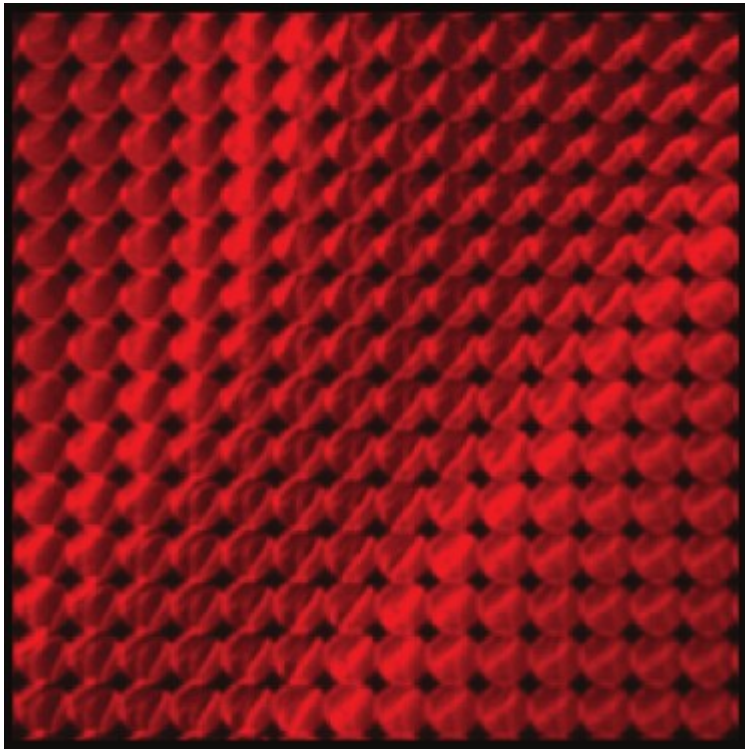

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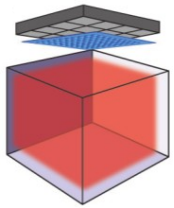
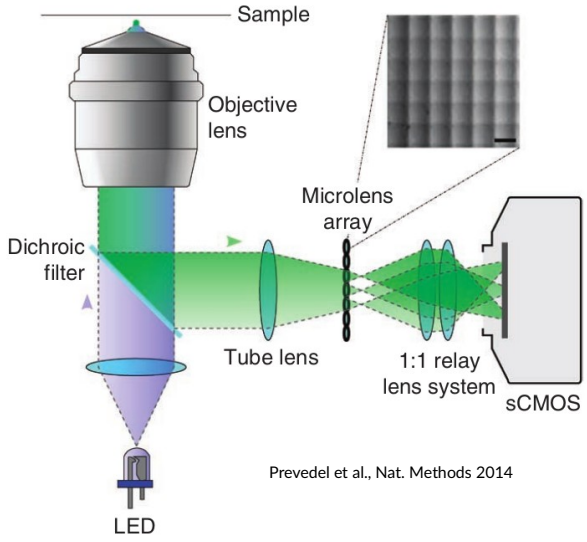
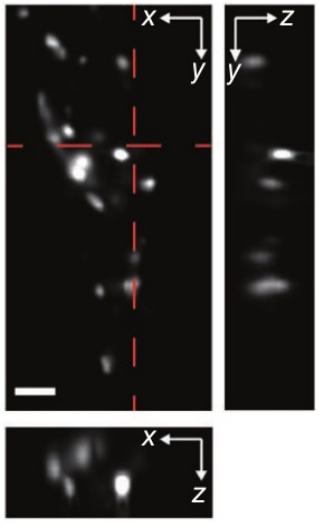




Prevedel et al., Nat. Methods 2014

Computation
(deconvolution)

2-30 min each volume



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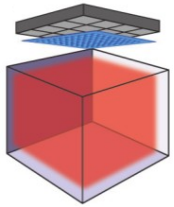

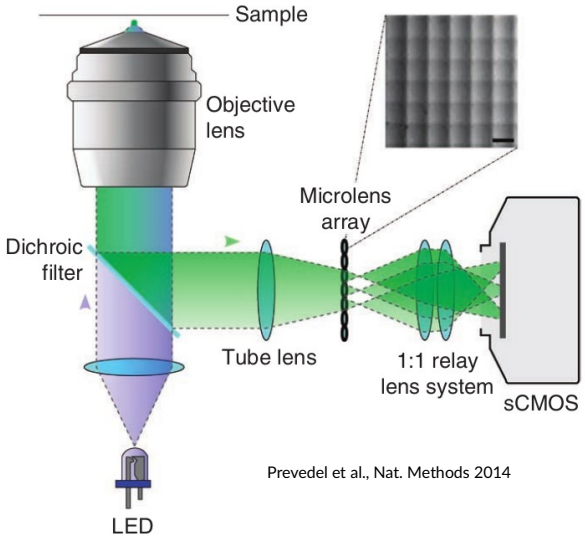

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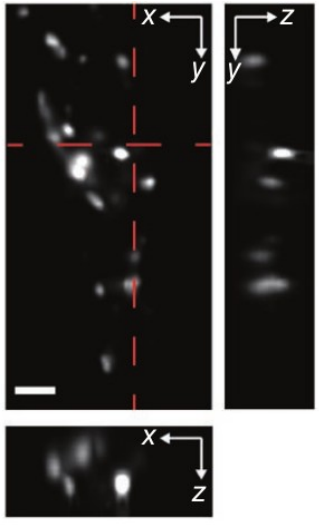

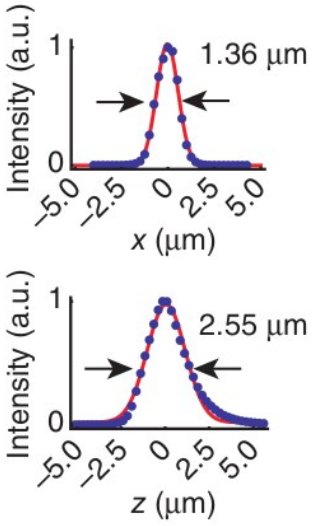

Point-spread function after reconstruction

Other references
Levoy et al., Journal of Microscopy 2009

Light field

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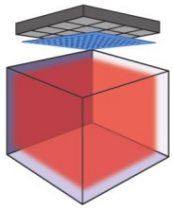

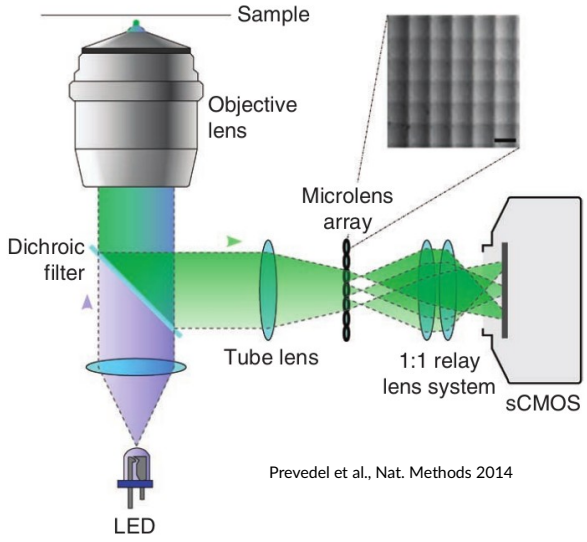

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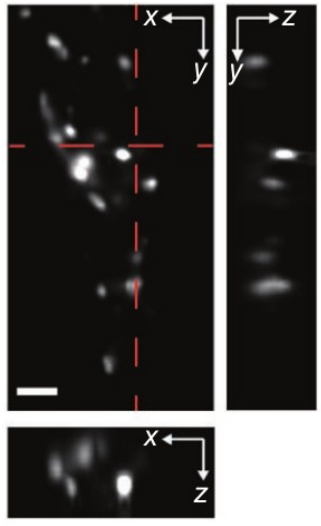
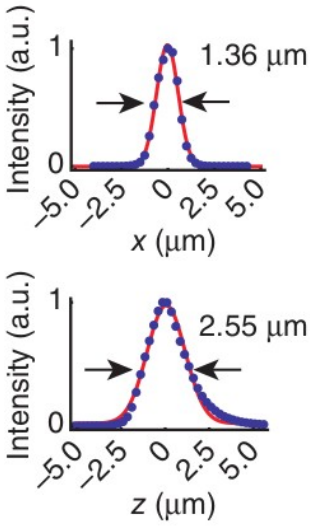




Point-spread function after reconstruction

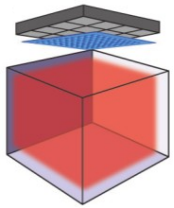
Light source LED 1-photon interaction
Field of view ~(350, 350, 30) µm with cellular resolution
Volume rate 5-50 vol/s (depends on SNR)

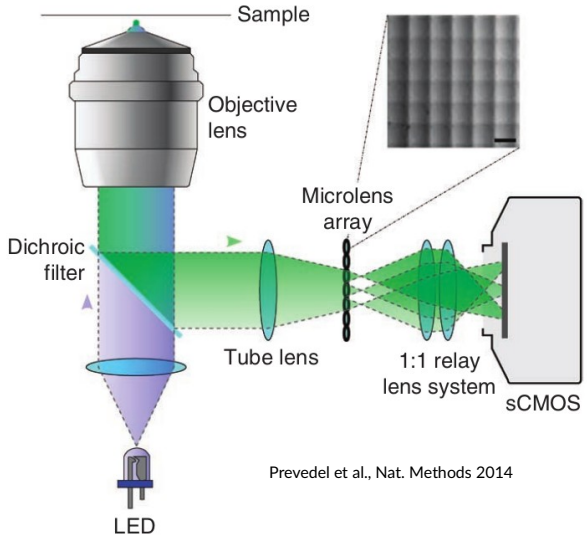
Special hardware microlens array

Other references
Levoy et al., Journal of Microscopy 2009

Light field

3D volume in 1 shot





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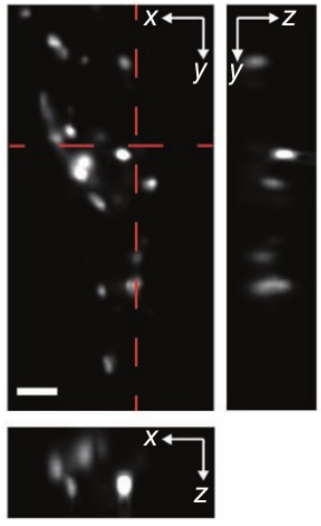
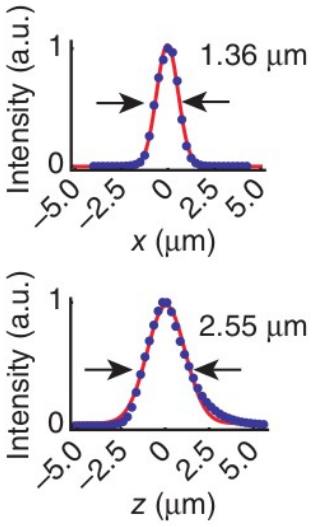




Point-spread function after reconstruction

Light source LED 1-photon interaction
Field of view ~(350, 350, 30) µm with cellular resolution
Volume rate 5-50 vol/s (depends on SNR)

Special hardware microlens array

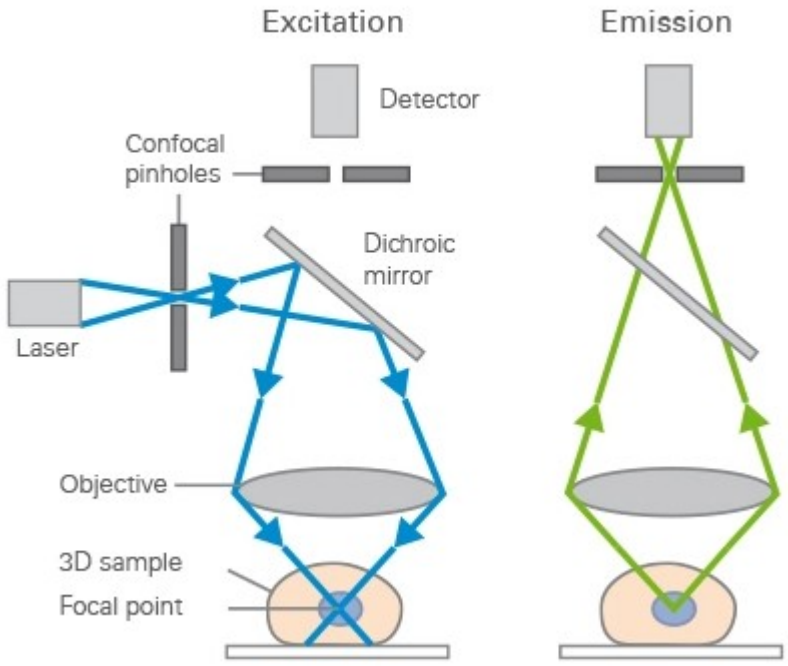
Main disadvantages

- xy resolution traded-off for z resolution
- Computation needed, no image in raw data

Other references
Levoy et al., Journal of Microscopy 2009

Spinning-disk confocal

Basic point-scanning confocal
(rejection of out-of-focus emission)



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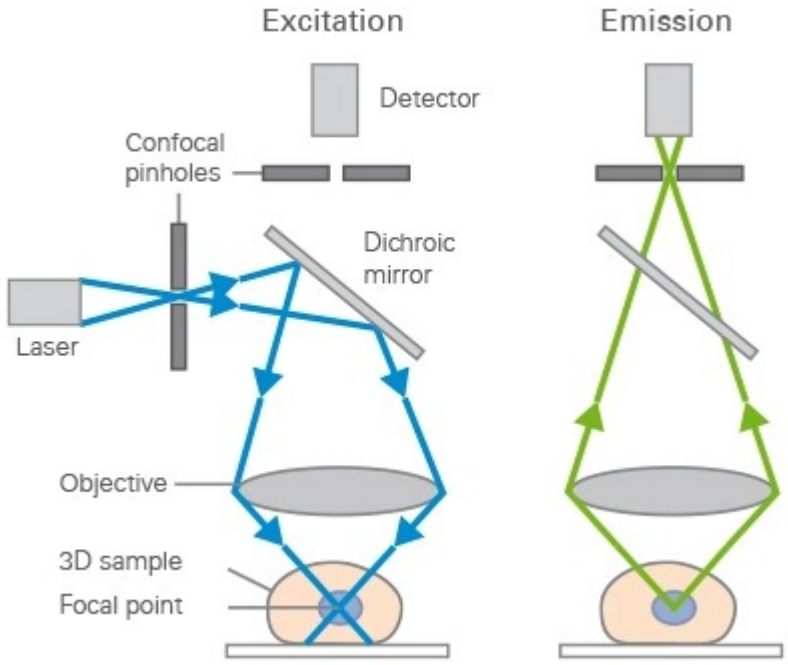
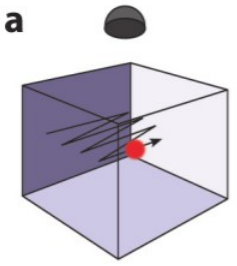
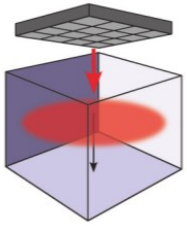


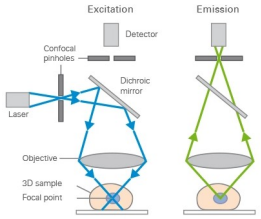
But this requires



Spinning-disk confocal

2D plane each frame
scan along z





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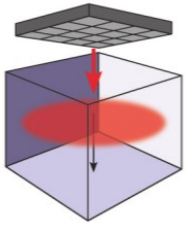
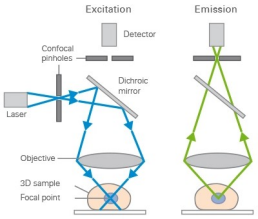
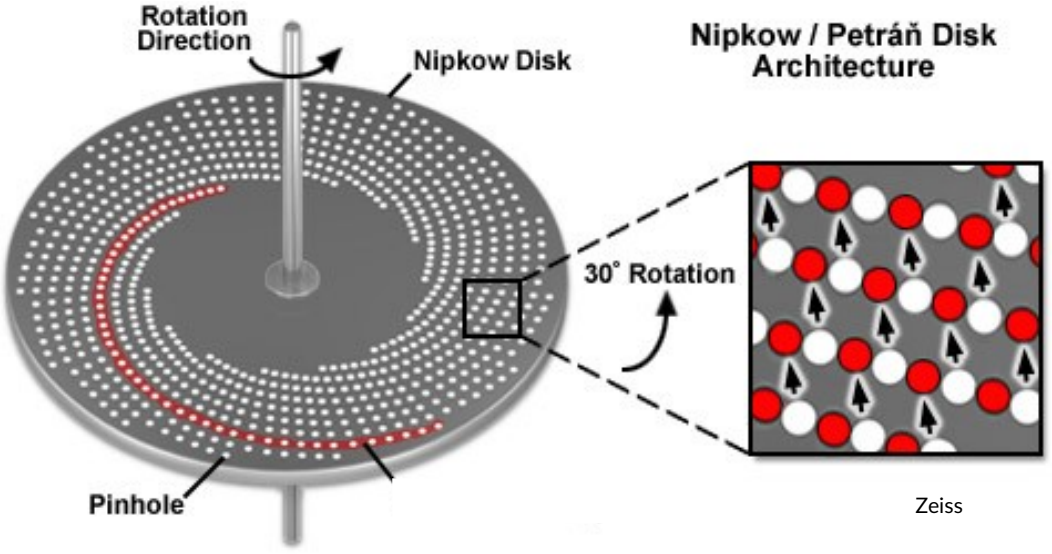




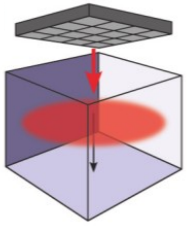
Parallel

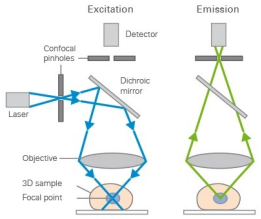


Zeiss

Spinning-disk confocal

2D plane each frame
scan along z





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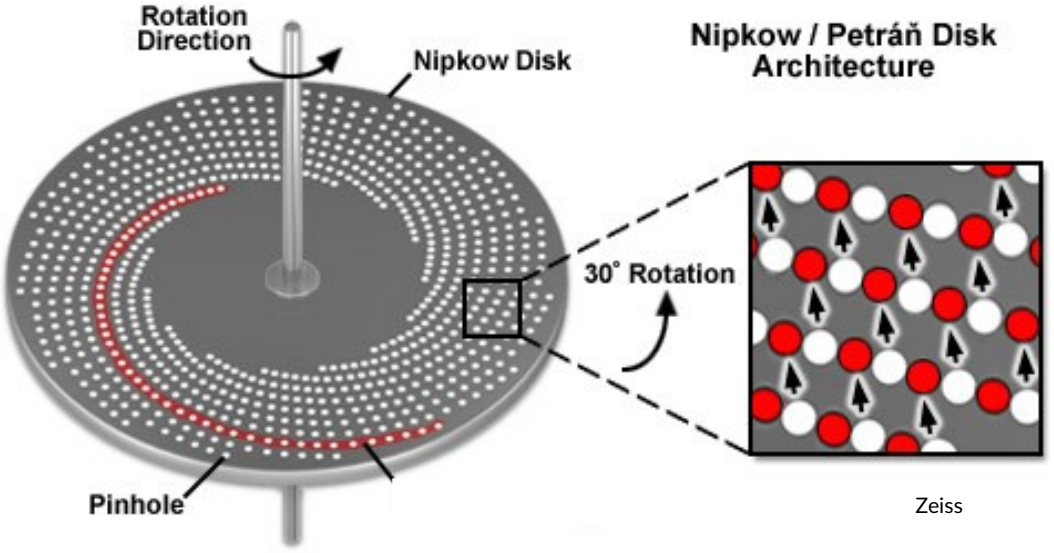


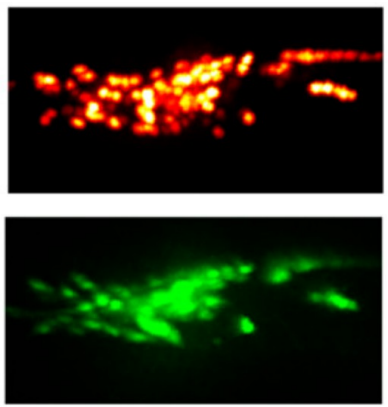
Zeiss

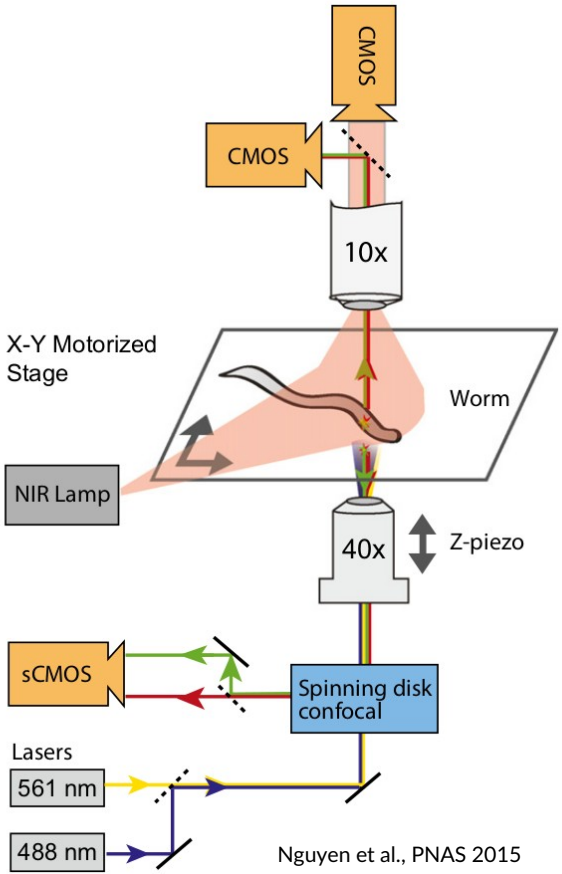
Light source continuous wave laser 1-photon interaction
Field of view max 400x400 µm at 40x (limited by spinning-disk hardware)
Volume rate ~5-10 vol/s (depends on SNR)

Special hardware spinning disk (plug-and-play)

Spinning-disk confocal

Example





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Spinning-disk confocal

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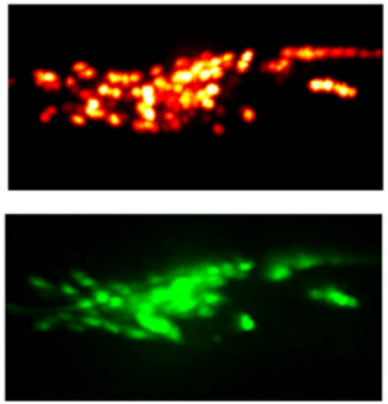

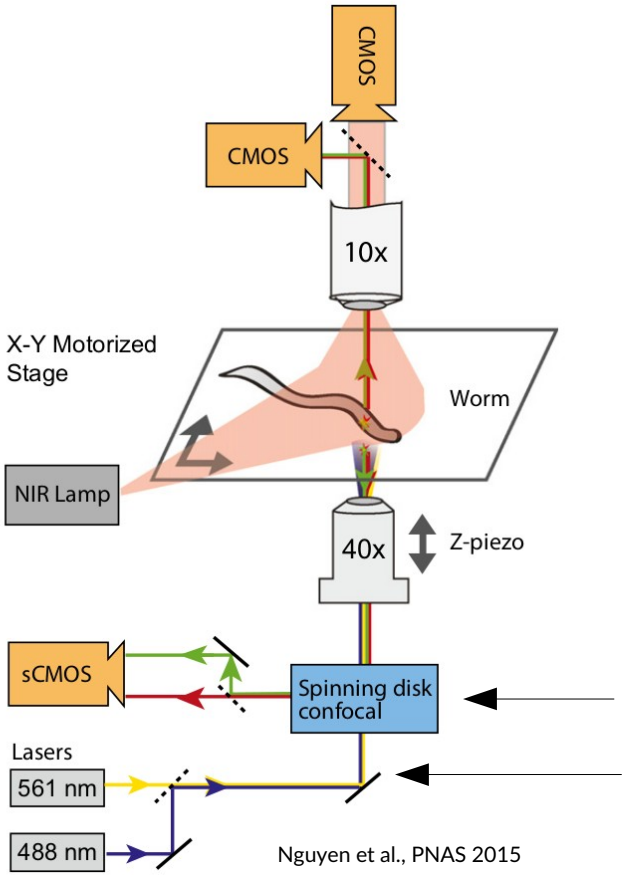

Nguyen et al., PNAS 2015

into camera port of microscope

fiber

Other references
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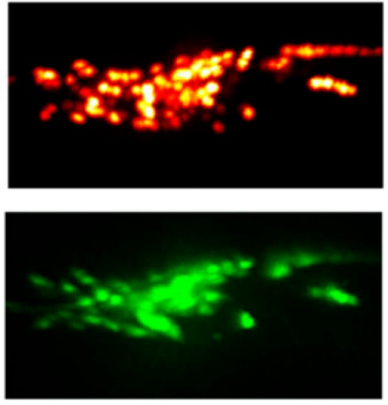
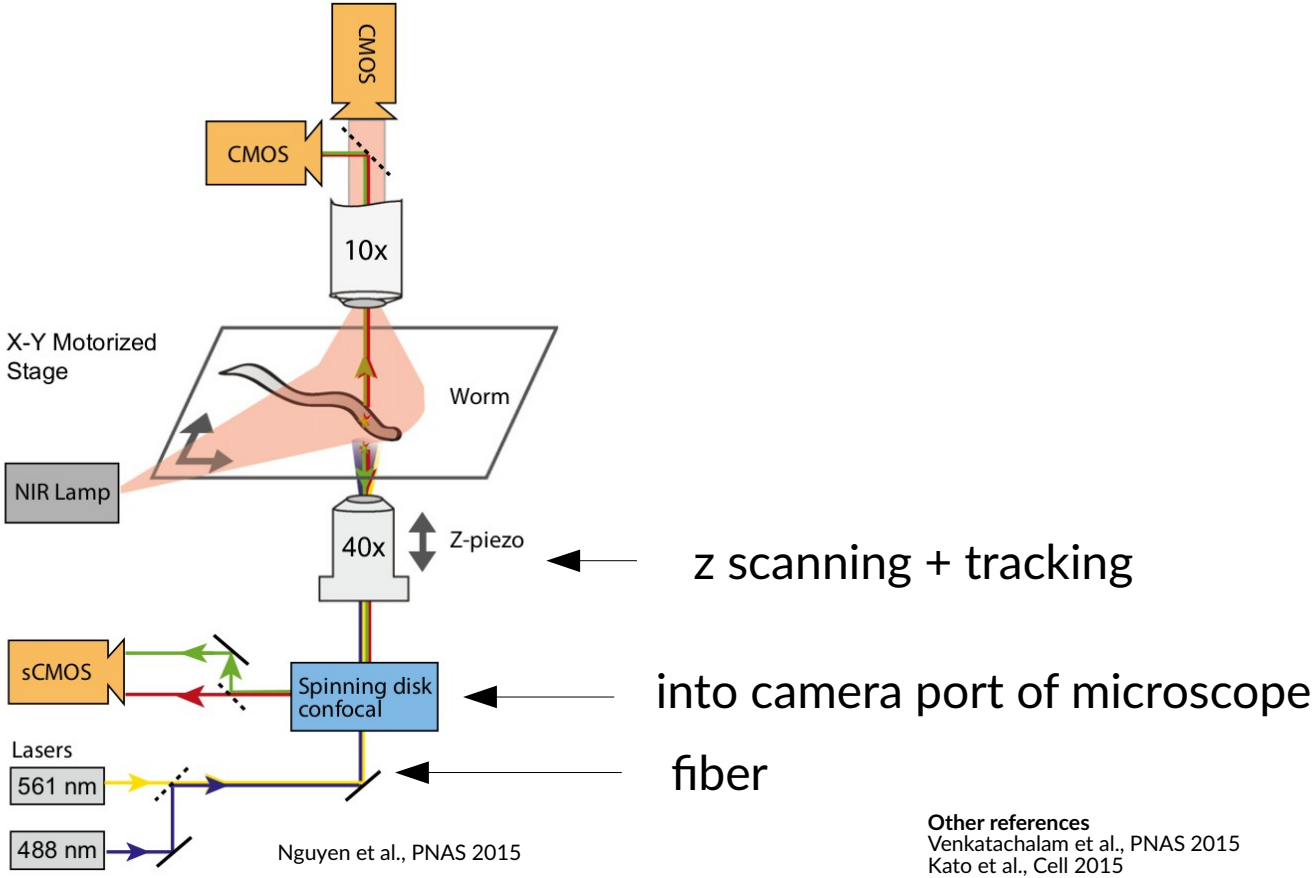
Kato et al., Cell 2015

Spinning-disk confocal

Example





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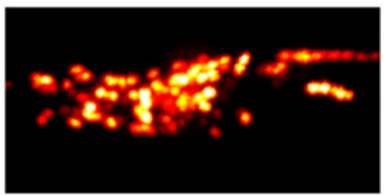
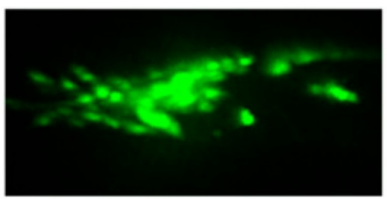
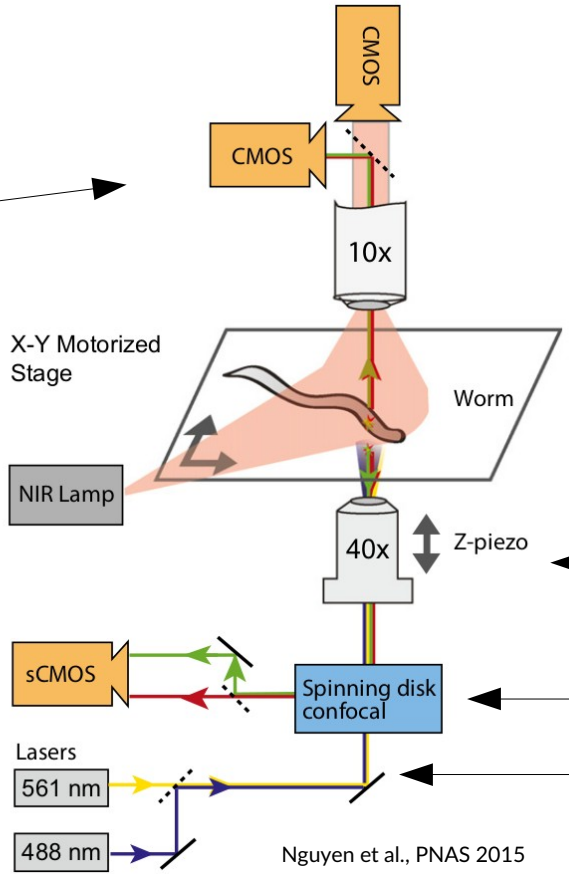




xy tracking (and behavior)
freely moving animal



z scanning + tracking

into camera port of microscope

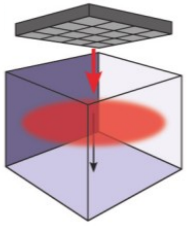
fiber

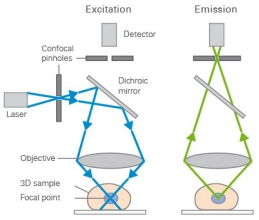
Nguyen et al., PNAS 2015

Other references
Venkatachalam et al., PNAS 2015
Kato et al., Cell 2015

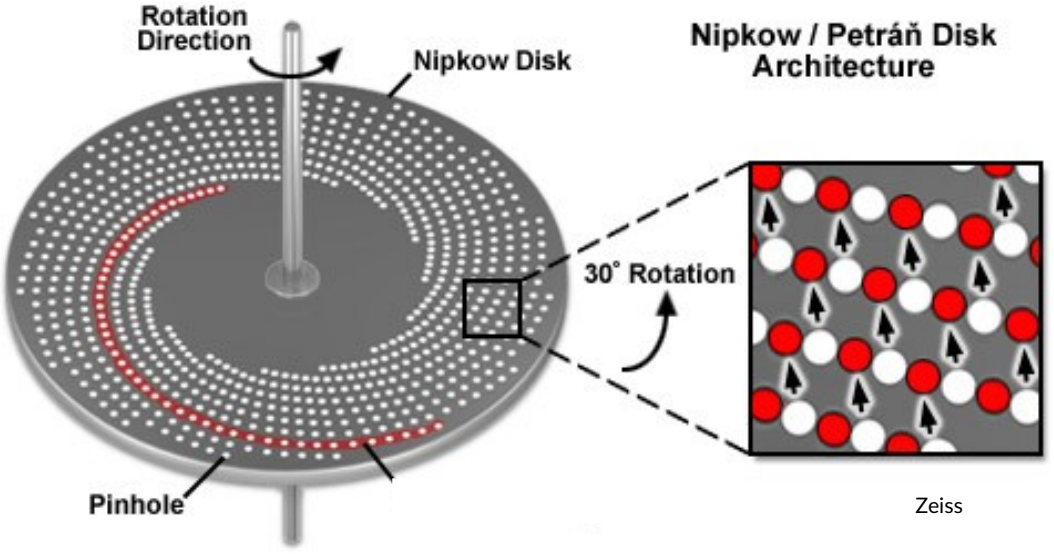
Spinning-disk confocal

2D plane each frame
scan along z





Parallel



Zeiss

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Volume rate ~5-10 vol/s (depends on SNR)

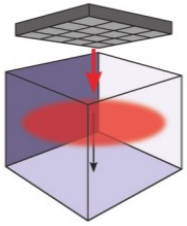
Special hardware spinning disk (plug-and-play)

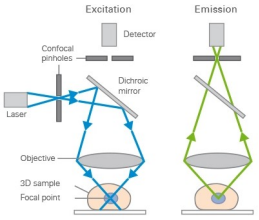
Main disadvantages

- reduced effective exposure of each point

Spinning-disk confocal

2D plane each frame
scan along z





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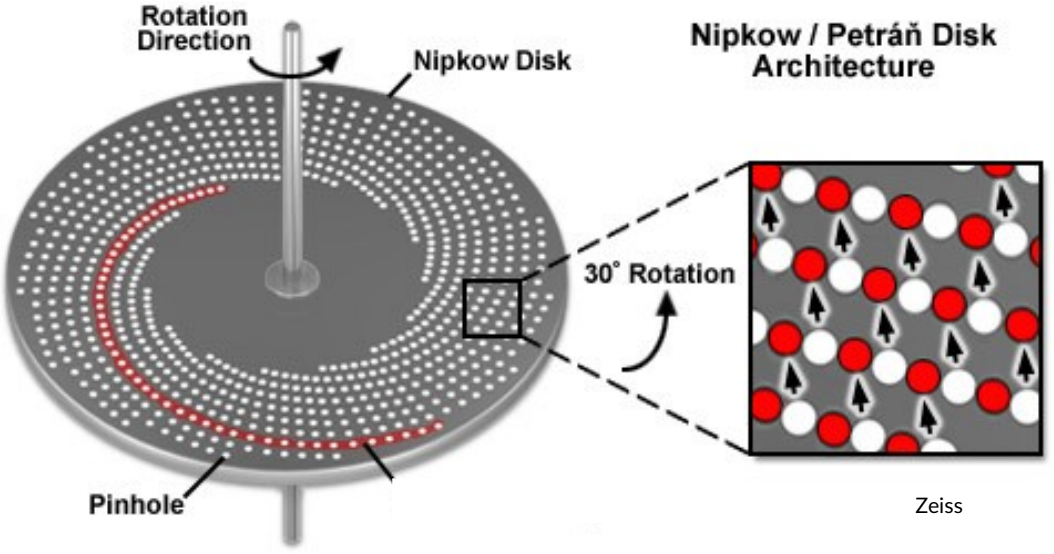

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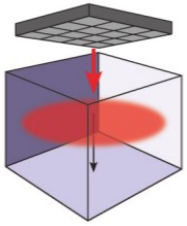
Main disadvantages

- reduced effective exposure of each point
- photo-bleaching: illumination not restricted to plane being imaged

Wide-field temporal focusing

2D plane each frame
scan along z



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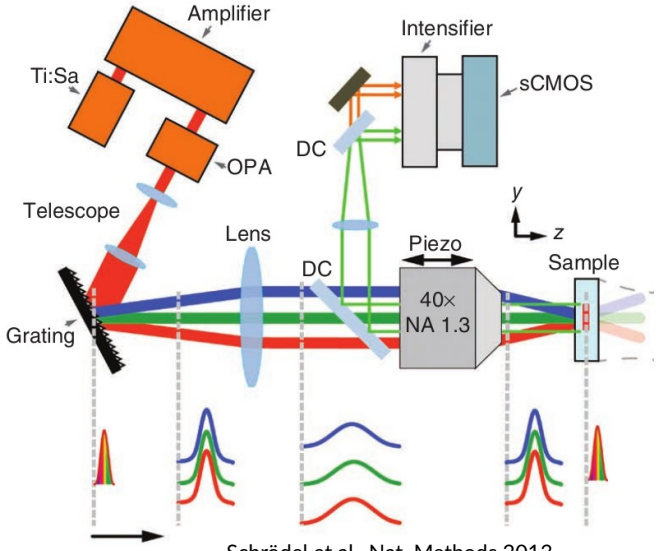


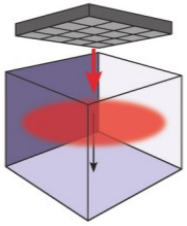
Schrödel et al., Nat. Methods 2013

No excitation outside focal plane! → less bleaching

Other references
Oron et al., Opt. Expr. 2005
Zhu et al., Opt. Expr. 2005

Wide-field temporal focusing

2D plane each frame
scan along z



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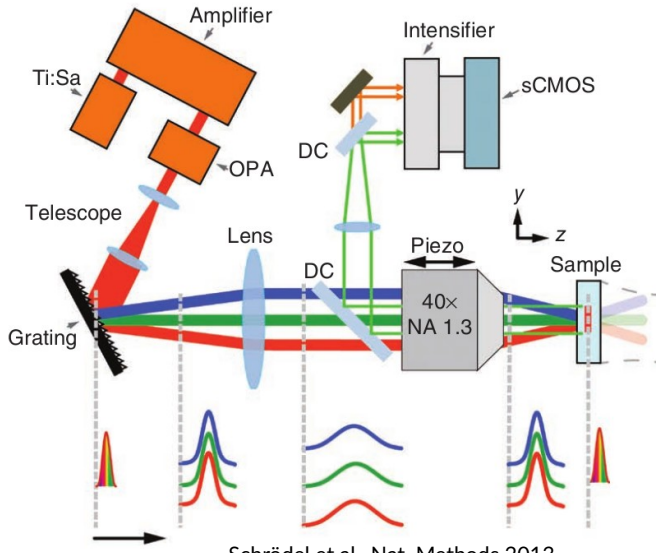
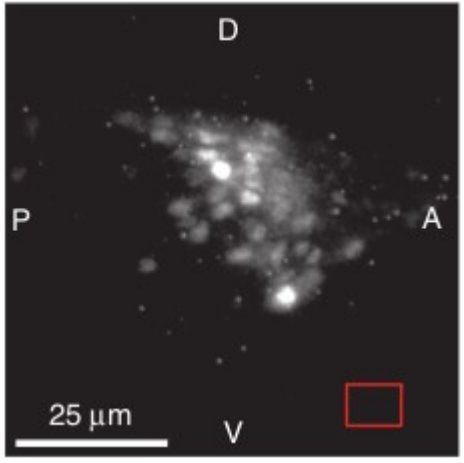


Schrödel et al., Nat. Methods 2013



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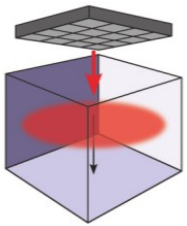

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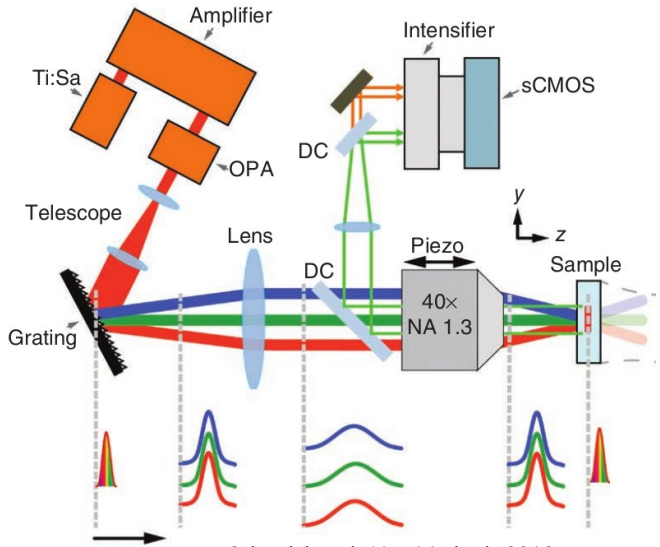
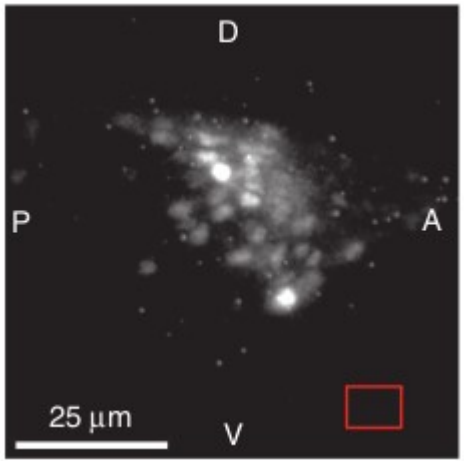


Schrödel et al., Nat. Methods 2013



Light source <u>amplified</u> pulsed laser 2-photons interaction
Field of view ~ 60 µm diameter (limited by energy/pulse)
Volume rate ~ 4-6 vol/s (depends on SNR)

Special hardware amplified pulsed laser (+ OPA)
diffraction grating
high-gain image intensifier

<u>No excitation outside focal plane! → less bleaching</u>

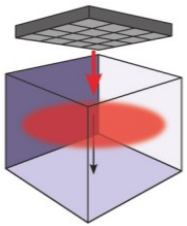
Main disadvantages

- requires custom instrument and expensive laser

Other references
Oron et al., Opt. Expr. 2005
Zhu et al., Opt. Expr. 2005

Wide-field temporal focusing

2D plane each frame
scan along z



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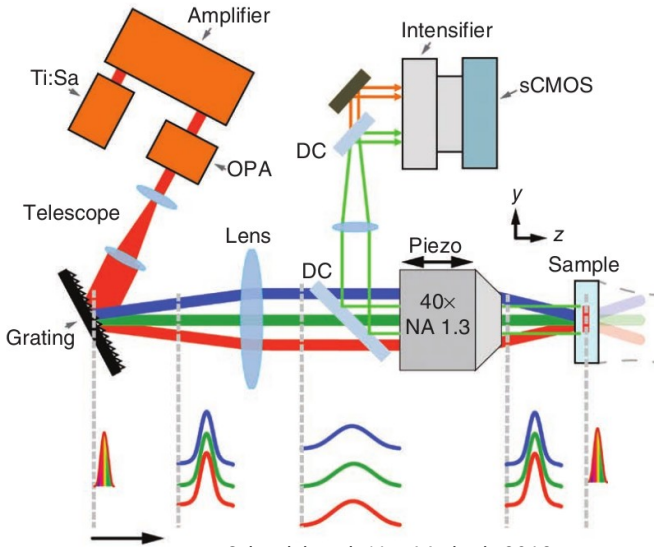
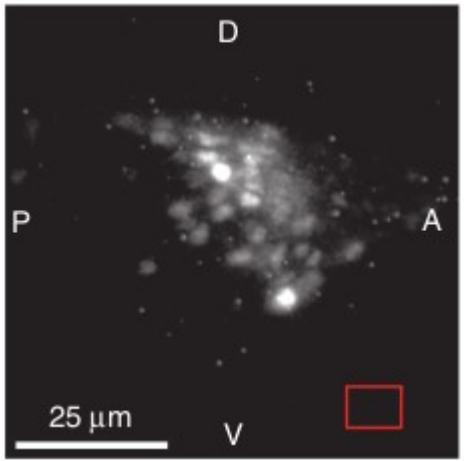


Schrödel et al., Nat. Methods 2013



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Special hardware amplified pulsed laser (+ OPA)
diffraction grating
high-gain image intensifier

<u>No excitation outside focal plane! → less bleaching</u>

Main disadvantages

- requires custom instrument and expensive laser
- few photocycles of the fluorophores (low SNR)

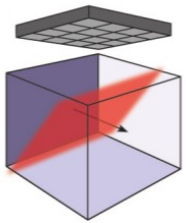
Other references
Oron et al., Opt. Expr. 2005
Zhu et al., Opt. Expr. 2005

Light sheet - SCAPE

Swept confocally-aligned planar excitation

2D plane each frame

oblique planes



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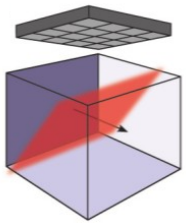
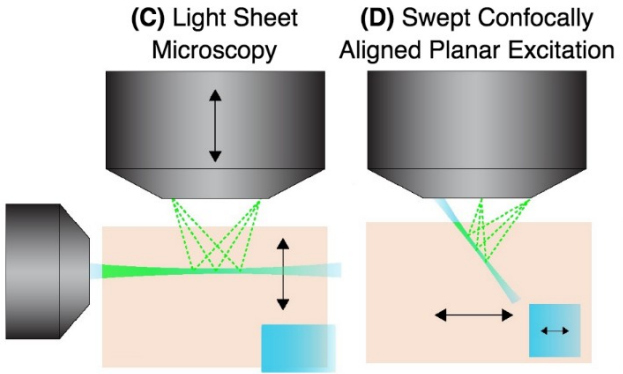
Light sheet - SCAPE

Swept confocally-aligned planar excitation

2D plane each frame

oblique planes





Hillman et al., Curr. Op. in Neurobiol. 2018

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Other references

Bouchard et al., Nat. Photonics 2015

Light sheet - SCAPE

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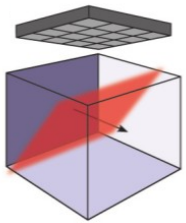
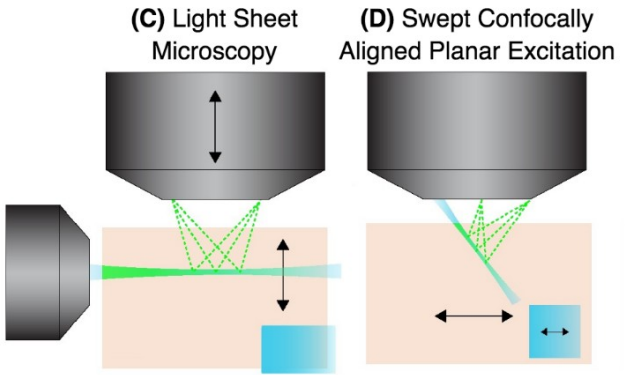
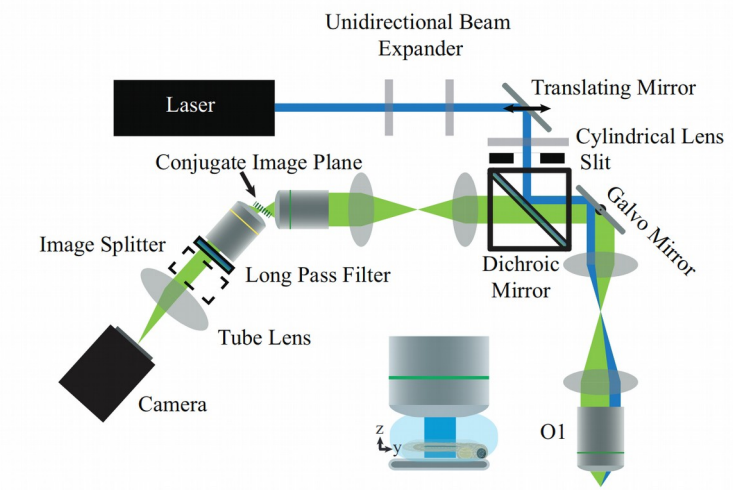




Hillman et al., Curr. Op. in Neurobiol. 2018



Voleti, Optics and the Brain 2017

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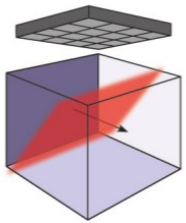
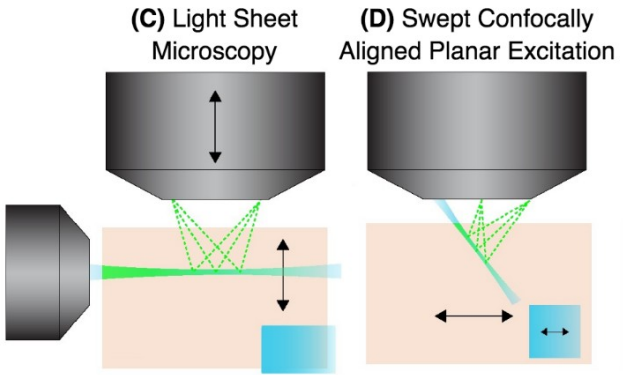
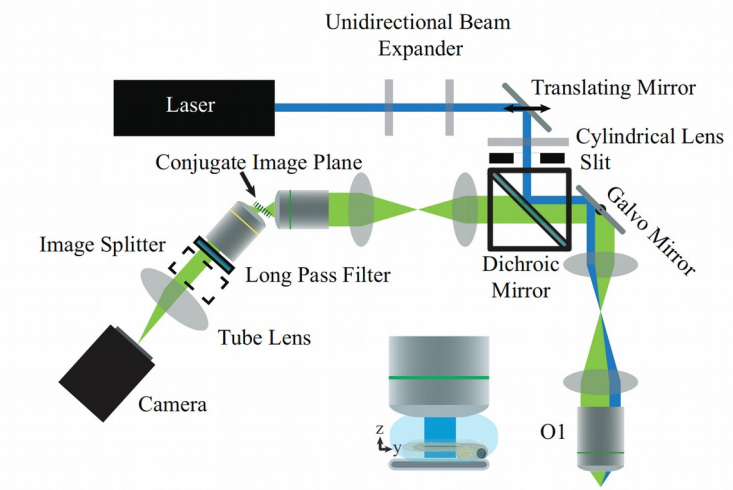




Hillman et al., Curr. Op. in Neurobiol. 2018



Voleti, Optics and the Brain 2017

Light source continuous-wave laser 1-photon interaction
Field of view
Volume rate interdependent (see Voleti, Optics and the Brain 2017)

Special hardware multiple objectives
galvo mirror

No excitation outside focal plane! → less bleaching

Main disadvantages

- Instrument not yet commercial (probably will be soon)
- Not published with worms

Other references
Bouchard et al., Nat. Photonics 2015

Comparison

	Light source	simple/ commercial hardware	volume rate	Raw data are images	Drawbacks
Light field	LED	✔	5-50 vol/s	✖	• Computation • Resolution
Spinning-disk confocal	Continuous-wave lasers	✔	~5-10 vol/s	✔	• Bleaching
Wide-field temporal-focussing	Amplified pulsed laser	✖	4-6 vol/s	✔	• Pulsed laser • Low SNR
SCAPE light-sheet	Continuous-wave laser	~	Depends on field of view	✔	• No published use on worms

PDF of presentation at: http://leiferlab.princeton.edu/publications.php under “Lecture slides”

Thanks!

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SCAPE light-sheet	Continuous-wave laser	~	Depends on field of view	✔	• No published use on worms

Freely moving worms Any works in principle. **Worm tracking + neuron tracking software needed.**

PDF of presentation at: http://leiferlab.princeton.edu/publications.php under “Lecture slides”

Thanks!