Whole-brain imaging: Overview of microscopy techniques

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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
Different microscopy techniques

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

Type of scan across the sample’s volume

adapted from Weisenbruger, Vaziri, Annu. Rev. Neurosc. 2018
Different microscopy techniques

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

Type of scan across the sample’s volume

adapted from Weisenbruger, Vaziri, Annu. Rev. Neurosc. 2018
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Different microscopy techniques

- Light field
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Type of scan across the sample’s volume

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

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

3D volume in 1 shot
Light field

3D volume in 1 shot

Prevedel et al., Nat. Methods 2014

Other references
Levoy et al., Journal of Microscopy 2009
Light field

3D volume in 1 shot

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Levoy et al., Journal of Microscopy 2009
Light field

3D volume in 1 shot

Computation (deconvolution)

2-30 min each volume

Other references
Levoy et al., Journal of Microscopy 2009
Light field

3D volume in 1 shot

Computation (deconvolution)

2-30 min each volume

Point-spread function after reconstruction

Other references
Levoy et al., Journal of Microscopy 2009
Light field

3D volume in 1 shot

- Sample
- Objective lens
- Microlens array
- Tube lens
- 1:1 relay lens system
- sCMOS

Computation (deconvolution)

2-30 min each volume

Point-spread function after reconstruction

- Intensity (a.u.)
- LED
- 1-photon interaction

- Sample
- Objective lens
- Microlens array
- Tube lens
- 1:1 relay lens system
- sCMOS

Prevedel et al., Nat. Methods 2014

**Light source**  LED  1-photon interaction

**Field of view**  \(~(350, 350, 30) \, \mu\text{m}\) 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**

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

**Special hardware**: microlens array

**Computation** (deconvolution)

- 2-30 min each volume

**Main disadvantages**

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

**Other references**

- Prevedel et al., Nat. Methods 2014
- Levoy et al., Journal of Microscopy 2009
Spinning-disk confocal

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

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

But this requires
Spinning-disk confocal

2D plane each frame
scan along z
Spinning-disk confocal

2D plane each frame
scan along z

Parallel
Spinning-disk confocal

**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

Other references
Venkatachalam et al., PNAS 2015
Kato et al., Cell 2015
Spinning-disk confocal

Example

Nguyen et al., PNAS 2015
Venkatachalam et al., PNAS 2015
Kato et al., Cell 2015

into camera port of microscope fiber

Other references
Venkatachalam et al., PNAS 2015
Kato et al., Cell 2015
Spinning-disk confocal

Example

Nguyen et al., PNAS 2015
Venkatachalam et al., PNAS 2015
Kato et al., Cell 2015

z scanning + tracking
into camera port of microscope fiber
Spinning-disk confocal

Example

xy tracking (and behavior) freely moving animal

z scanning + tracking into camera port of microscope fiber

Nguyen et al., PNAS 2015

Venkatachalam et al., PNAS 2015
Kato et al., Cell 2015
Spinning-disk confocal

- **2D plane each frame** scan along z
- **Parallel**

**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)

**Main disadvantages**
- reduced effective exposure of each point

Zeiss
Spinning-disk confocal

2D plane each frame
scan along z

Light source: continuous wave laser
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)

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

No excitation outside focal plane! → less bleaching

Schrödel et al., Nat. Methods 2013

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

No excitation outside focal plane! → less bleaching

Schrödel et al., Nat. Methods 2013

Oron et al., Opt. Expr. 2005
Zhu et al., Opt. Expr. 2005
Wide-field temporal focusing

Light source: amplified pulsed laser 2-photon 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

No excitation outside focal plane! → less bleaching

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

**Light source**  
*amplified 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*

---

No excitation outside focal plane! → less bleaching

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

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Other references
- Oron et al., Opt. Expr. 2005
- Zhu et al., Opt. Expr. 2005
Light sheet - SCAPE

2D plane each frame
oblique planes

No excitation outside focal plane! → less bleaching
Light sheet - SCAPE
Swept confocally-aligned planar excitation

2D plane each frame
oblique planes

(C) Light Sheet Microscopy
(D) Swept Confocally Aligned Planar Excitation

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

No excitation outside focal plane! → less bleaching

Other references
Bouchard et al., Nat. Photonics 2015
Light sheet - SCAPE

Swept confocally-aligned planar excitation

2D plane each frame
oblique planes

(C) Light Sheet Microscopy

(D) Swept Confocally Aligned Planar Excitation

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

No excitation outside focal plane! → less bleaching

Other references
Bouchard et al., Nat. Photonics 2015
Voleti, Optics and the Brain 2017
Light sheet - SCAPE
Swept confocally-aligned planar excitation

2D plane each frame
oblique planes

(C) Light Sheet Microscopy
(D) Swept Confocally Aligned Planar Excitation

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

Light source
continuous-wave laser
1-photon interaction

Field of view
interdependent (see Voleti, Optics and the Brain 2017)

Volume rate

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

<table>
<thead>
<tr>
<th>Light source</th>
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<th>volume rate</th>
<th>Raw data are images</th>
<th>Drawbacks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light field</td>
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<td>5-50 vol/s</td>
<td>✗</td>
<td>• Computation • Resolution</td>
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<tr>
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<td>✓</td>
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<td>Amplified pulsed laser</td>
<td>4-6 vol/s</td>
<td>✓</td>
<td>• Pulsed laser • Low SNR</td>
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<tr>
<td>SCAPE light-sheet</td>
<td>Continuous-wave laser</td>
<td>Depends on field of view</td>
<td>✓</td>
<td>• No published use on worms</td>
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PDF of presentation at: [http://leiferlab.princeton.edu/publications.php](http://leiferlab.princeton.edu/publications.php) under “Lecture slides”
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**Freely moving worms** Any works in principle. **Worm tracking + neuron tracking software needed.**