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Computational light-field microscopy enables high-throughput, scattering-mitigated, volumetric neural activity imaging

Amanda Foust, Bioengineering

Pier Luigi Dragotti, EEE

Imperial College London

13 August 2024

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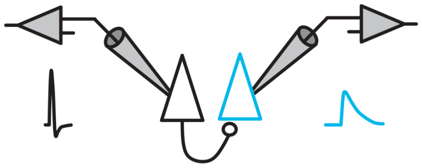
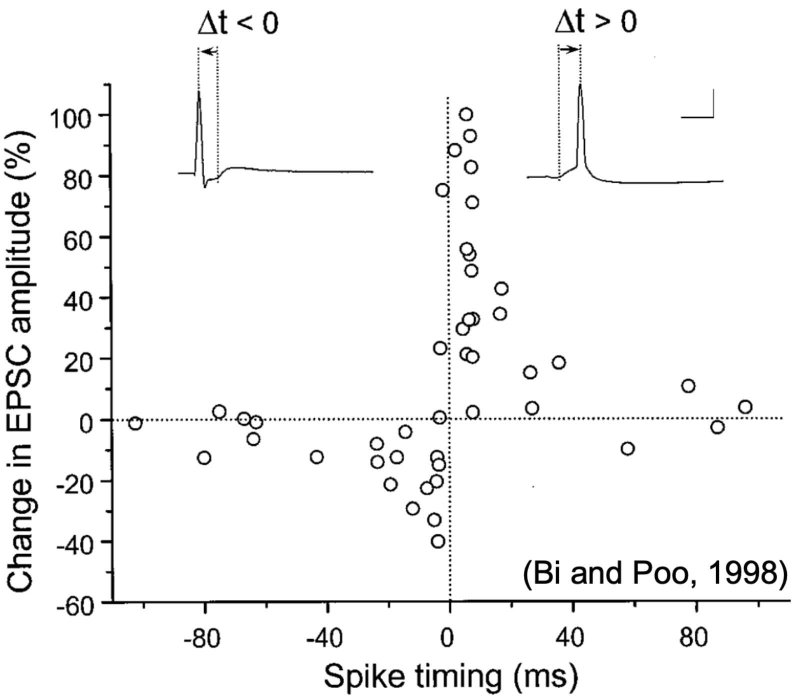
Why imaging voltage **fast** is important:

Living neuronal network update their weights on millisecond time scales





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Why **imaging** voltage fast is important:

Studying brain circuits with electrodes is “a dismaying exercise in tedium, like trying to cut the back lawn with a pair of nail scissors.”

Hubel & Wiesel, 2005

“It seems reasonable to imagine an array of 100 photodetectors that would allow simultaneous potential recordings from 100 individual cells.”

Lawrence B. Cohen, 1977

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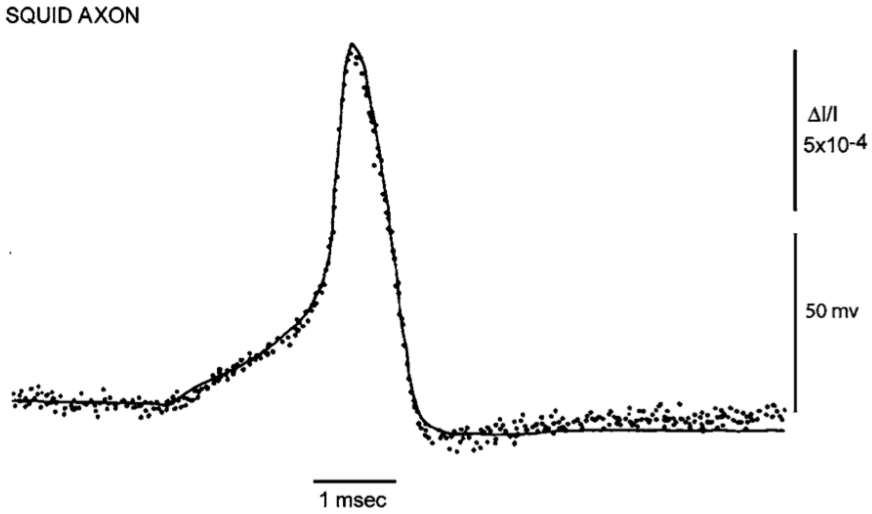
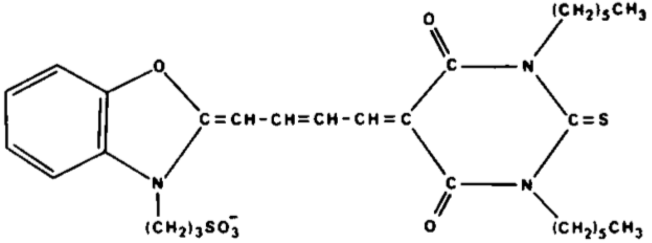
Why imaging voltage **fast** is important:

Optical voltage reporters (can) track voltage with microsecond fidelity





Ross, Salzberg, Cohen, Davila, *Biophysical Journal* 1974.

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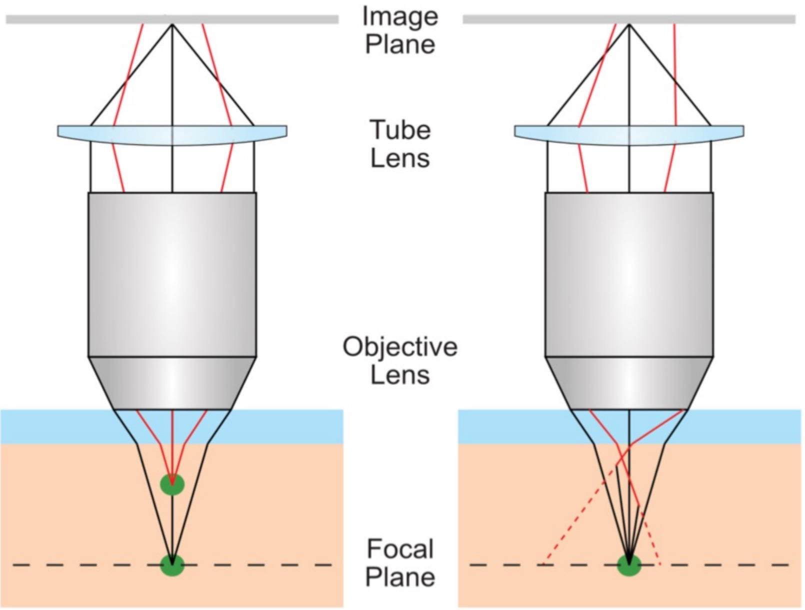
Brain Imaging Challenges

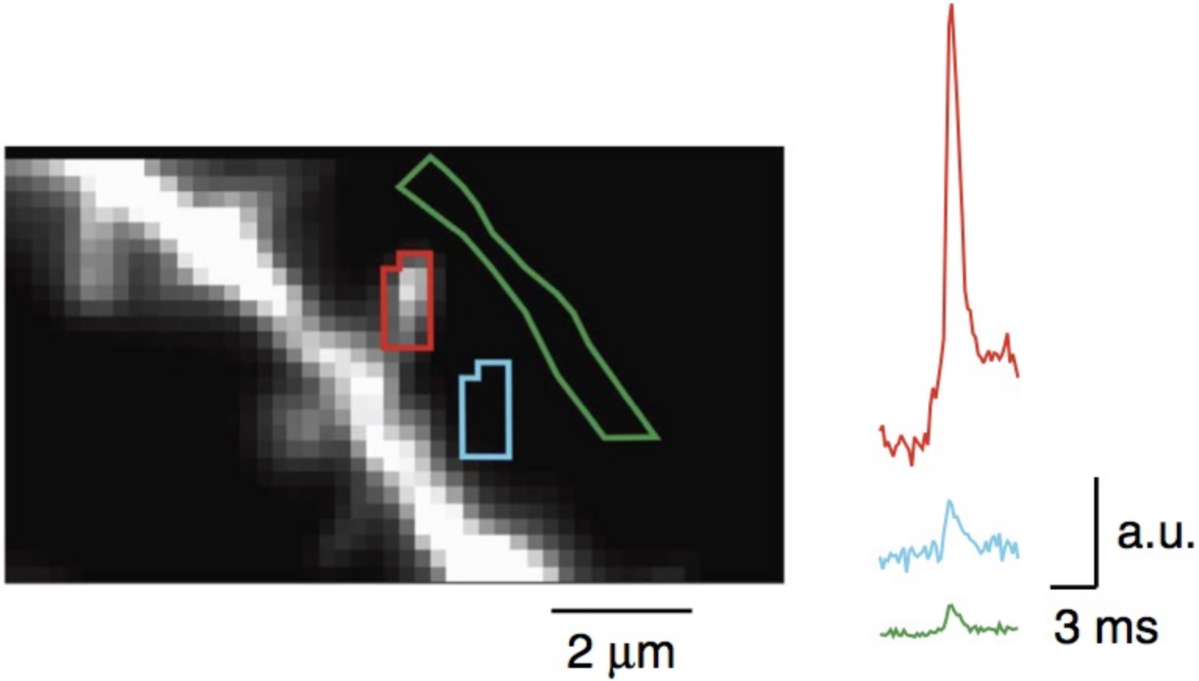
Imaging volumes

Scattering



Quicke, Howe, Foust, Balancing the fluorescence imaging budget for all-optical neurophysiology experiments
In *Neuromethods*, Humana Press 2022.

Widefield epifluorescence imaging suffers from crosstalk



Popovic, Carnevale, Rózsa, Zecevic, *Nature Communications* 2015.

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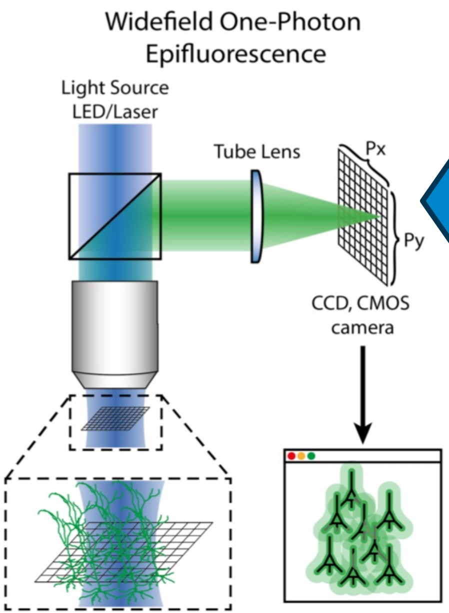
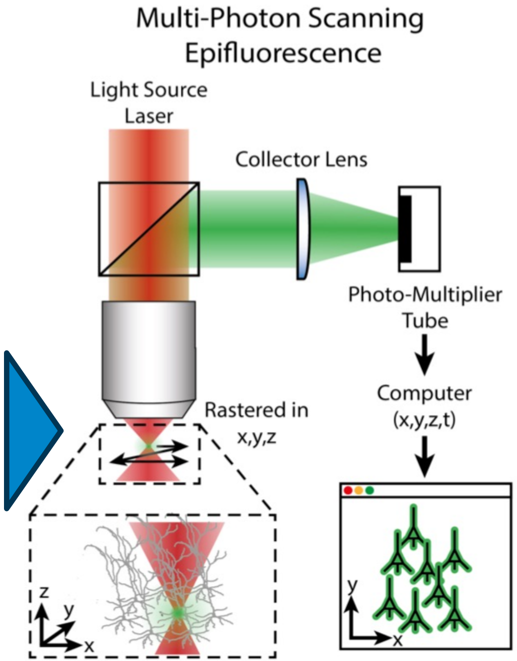
Brain Imaging Strategies



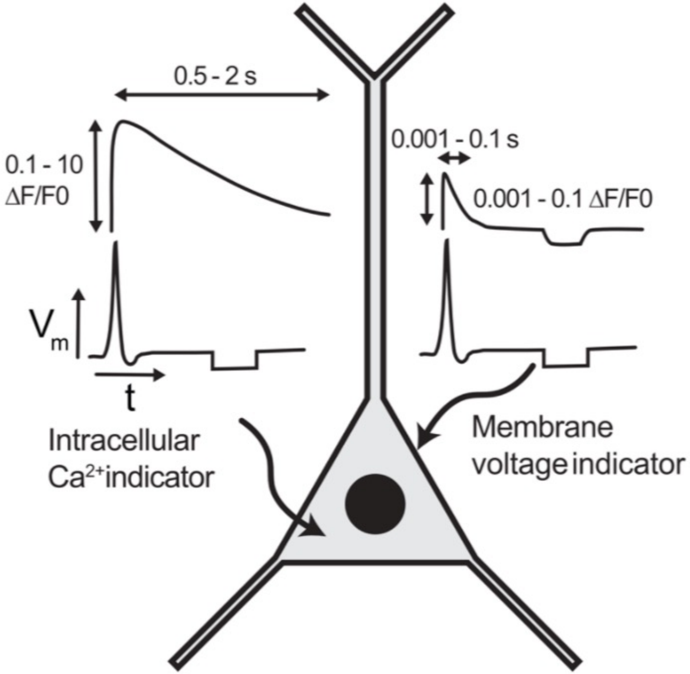
- ✓ High bandwidth
- ✗ Degraded by out-of-focus and scattered light

- ✓ Optical sectioning
- ✓ Robust to scattering
- ✗ Low-photon budget



Quicke, Howe, Foust, Balancing the fluorescence imaging budget for all-optical neurophysiology experiments
In *Neuromethods*, Humana Press 2022.

Solution 1: Image something slower (e.g., calcium)



Quicke, Howe, Foust, Balancing the fluorescence imaging budget for all-optical neurophysiology experiments
In *Neuromethods*, Humana Press 2022.

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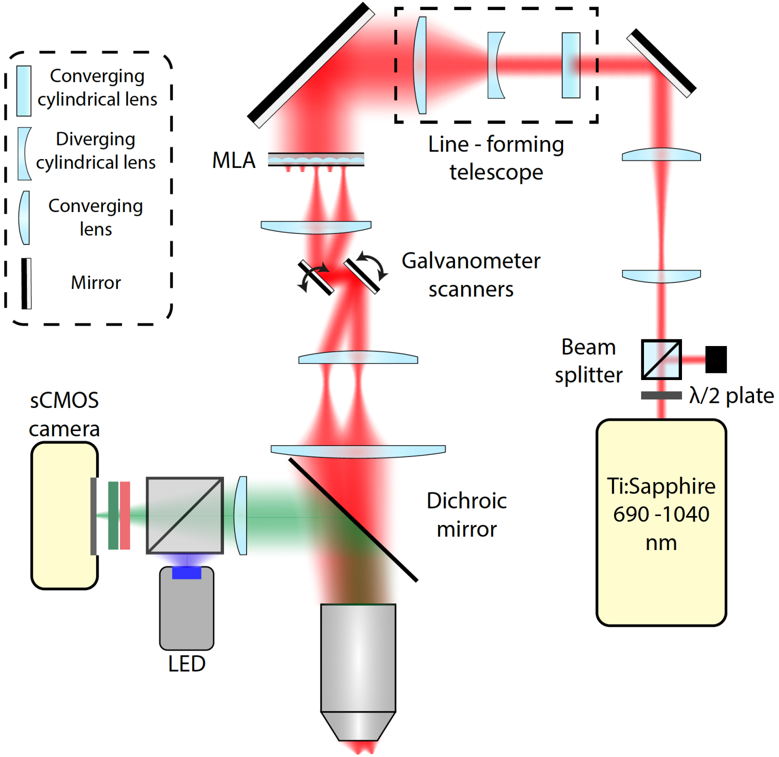
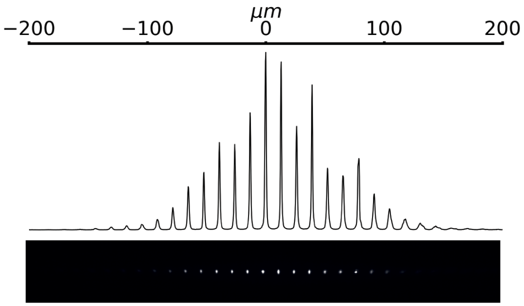
Solution 2: Scan multiple foci





Quicke, Reynolds, Neil, Knöpfel, Schultz, Foust, *Biomed Opt Exp* 2018.

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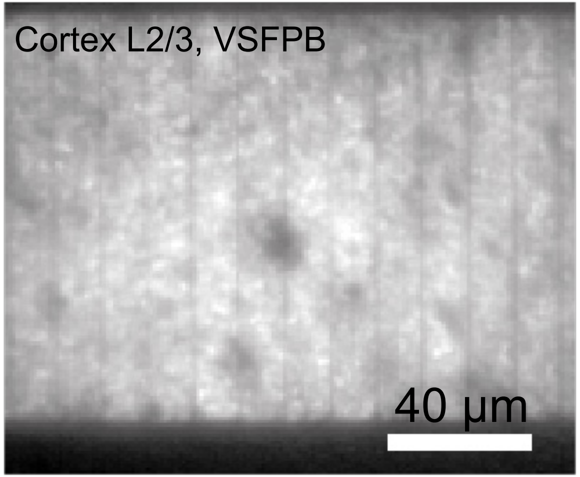
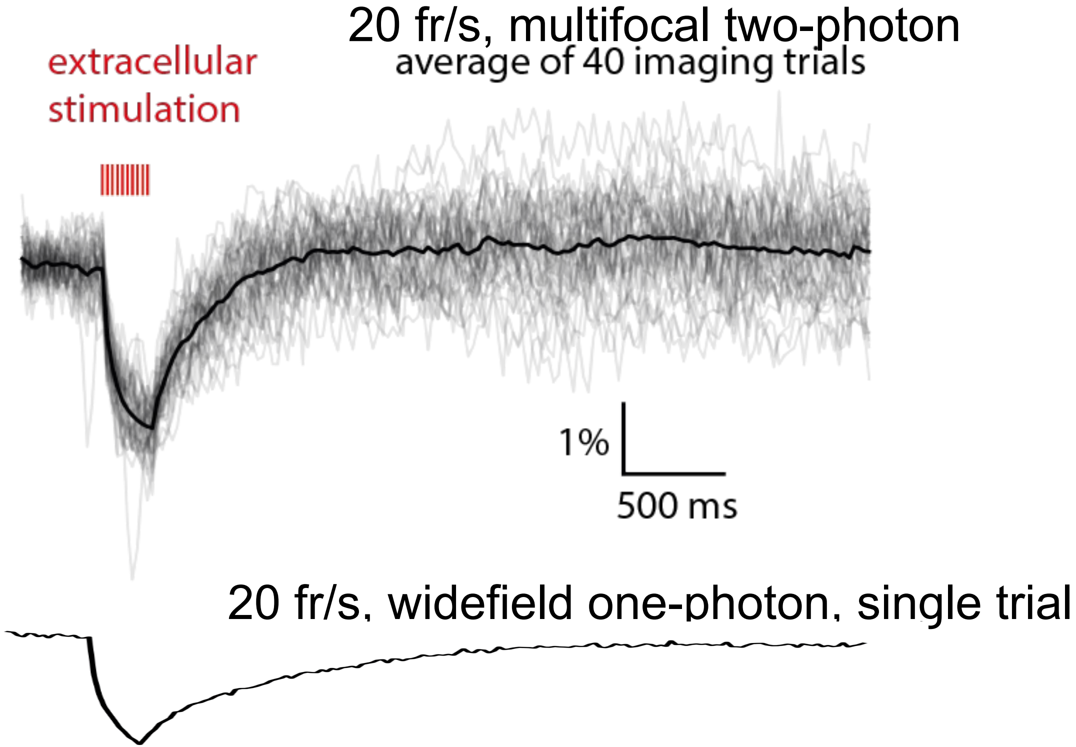
Solution 2: Scan multiple foci





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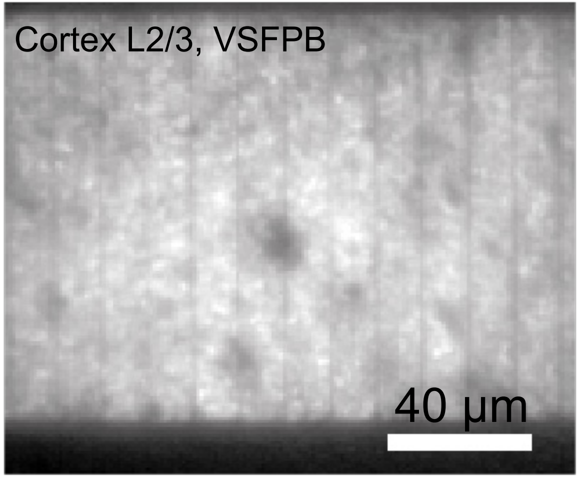
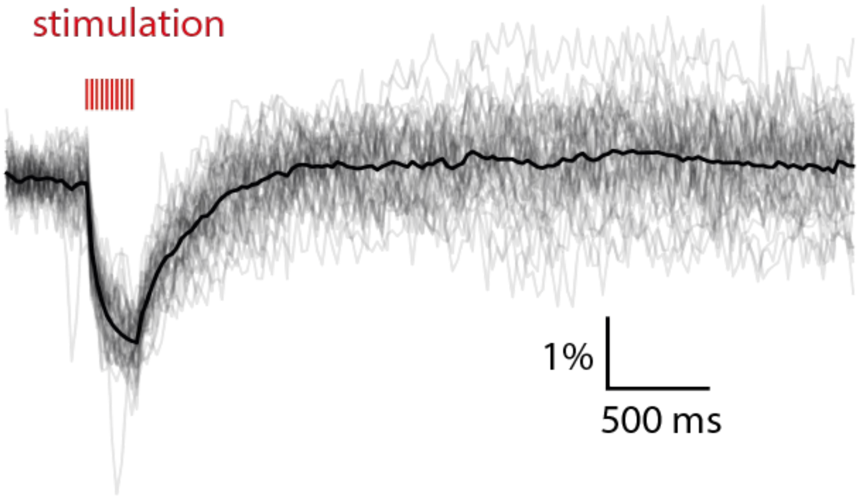


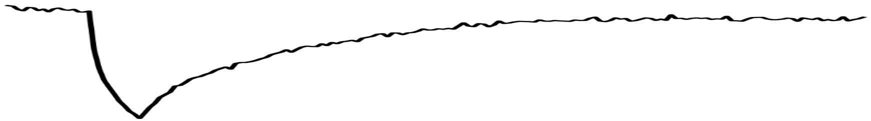
20 fr/s, multifocal two-photon

average of 40 imaging trials



$$S/N \propto \frac{\Delta F}{F}\sqrt{\phi}$$

20 fr/s, widefield one-photon, single trial



Voltage Imaging Competing Requirements

Optical Sectioning
Mitigate Scattering

vs.

Speed
Sensitivity

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Voltage Imaging Competing Requirements

Scanning

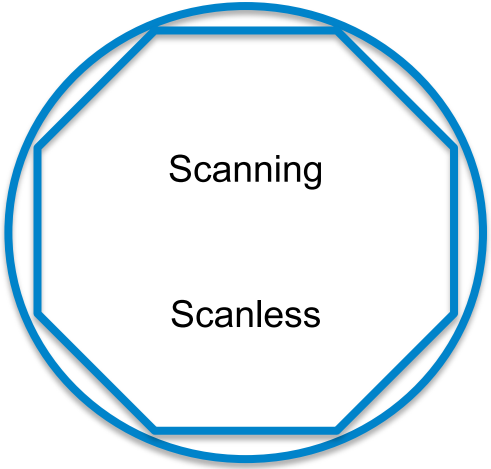
Optical Sectioning
Mitigate Scattering

vs.

Speed
Sensitivity

Scanless

Voltage Imaging Competing Requirements



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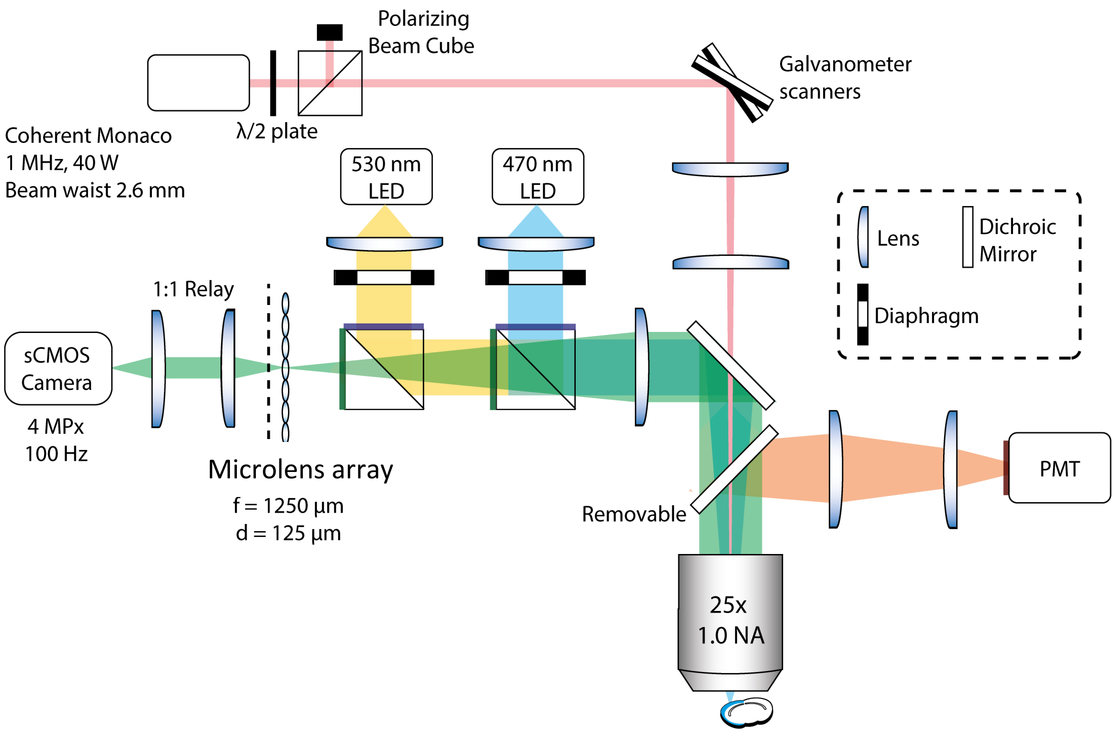
Voltage Imaging Competing Requirements

Two-photon point scanning
+
Scanless one-photon light-field imaging

Our Solution: Scattering-robust structural volumes + high-bandwidth, scanless functional volumes



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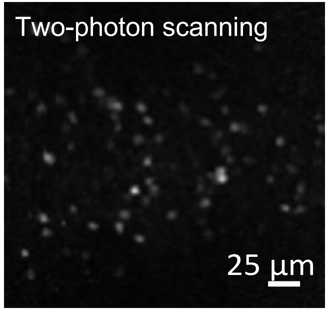
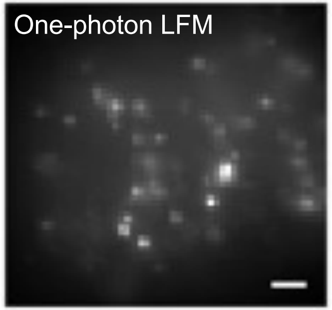
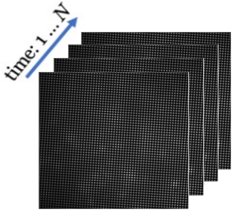
Our Solution: Scattering-robust structural volumes + high-bandwidth, scanless functional volumes

tdTomato structural marker





jGCaMP8f (world's fastest calcium indicator protein):
one-photon LFM at 100 Hz



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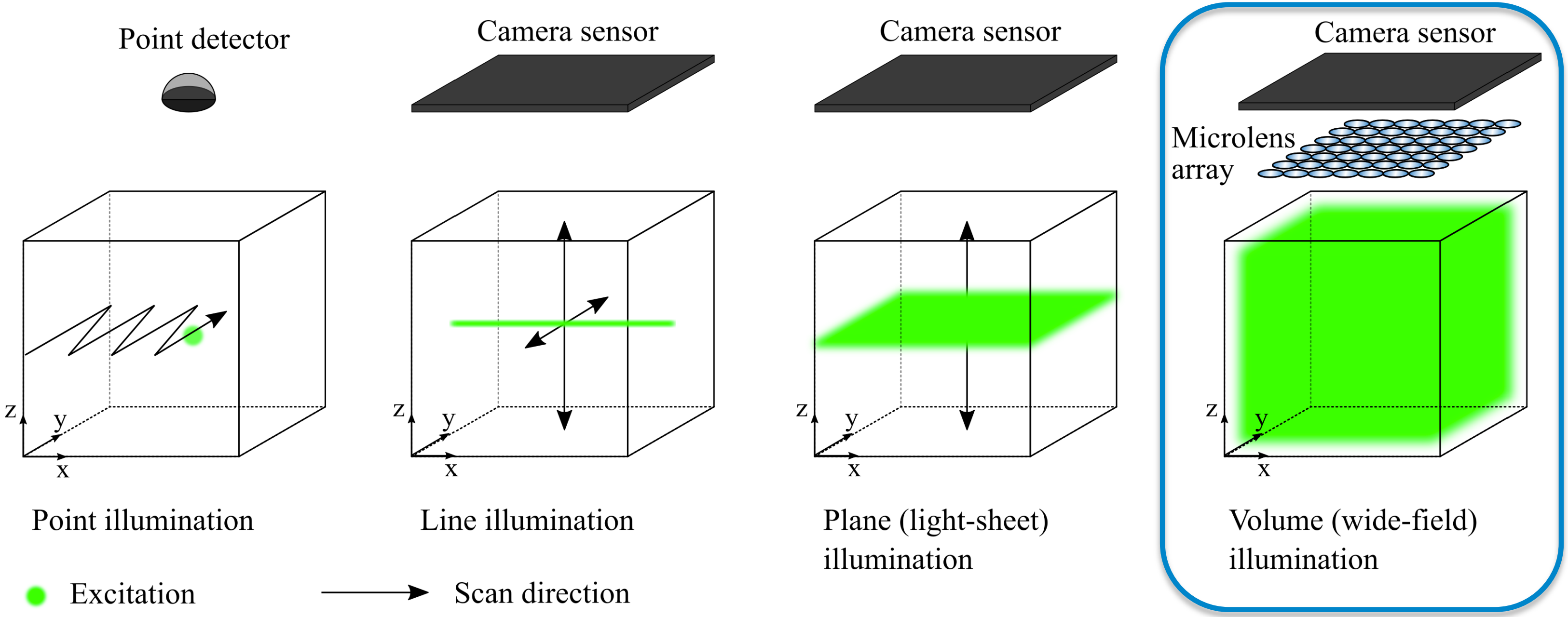
Light-field Microscopy and Illumination Strategies



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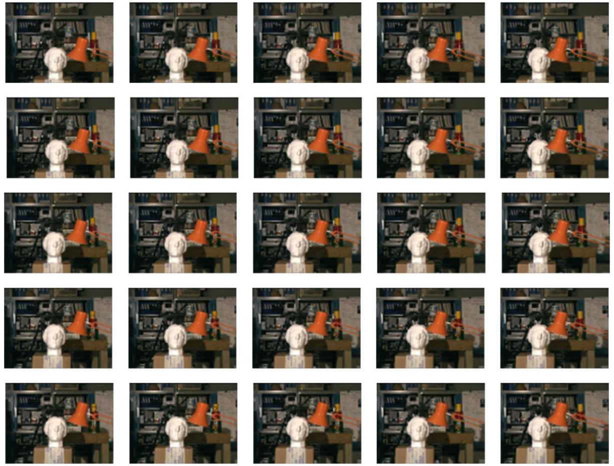
IBR Results on the Lightfield



Pearson et al. IEEE TIP 2013

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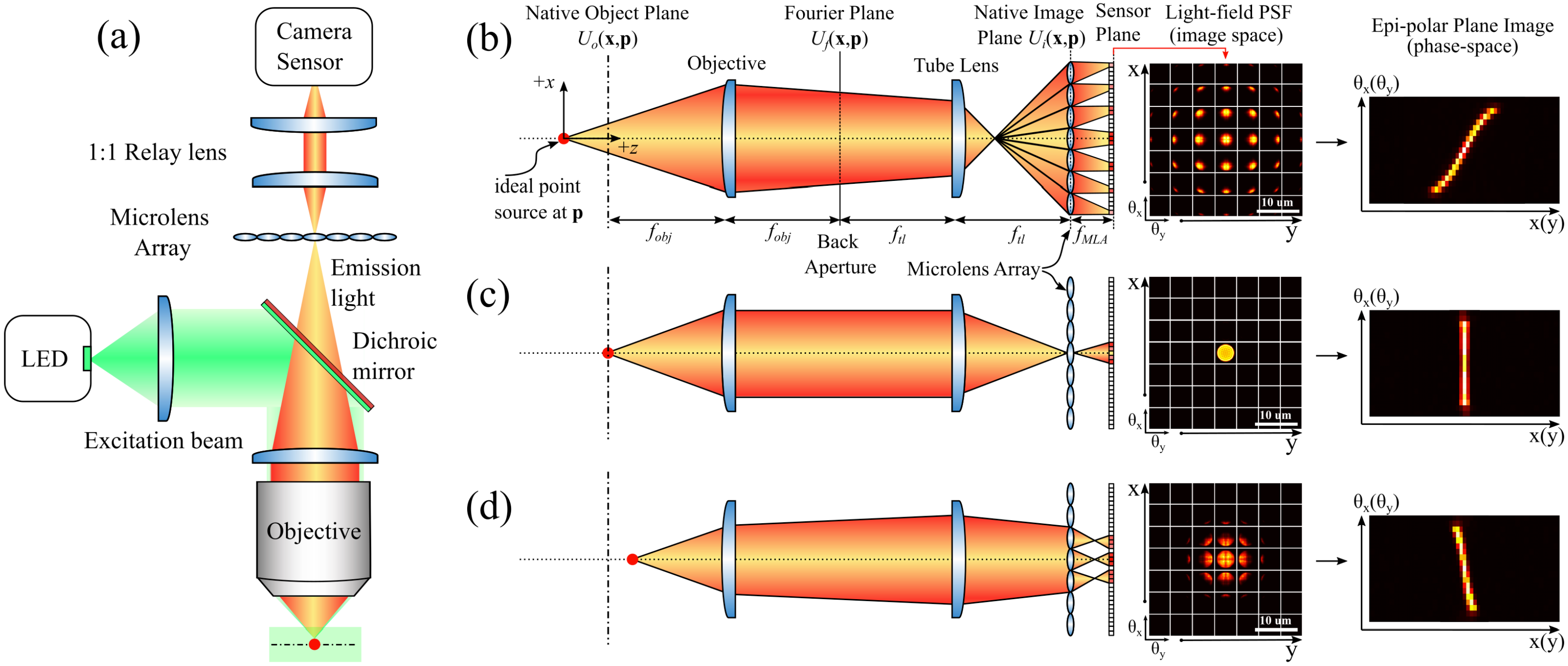
Light-field Microscopy and EPI



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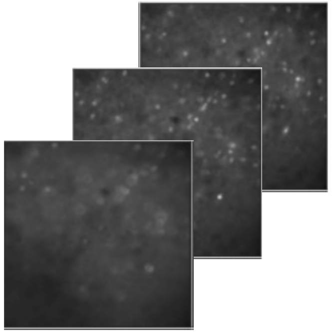
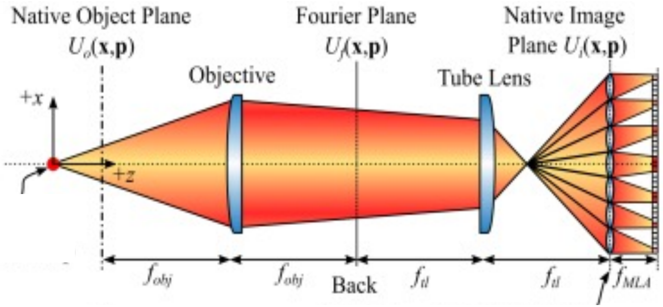
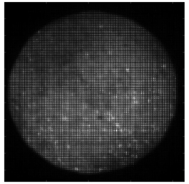
Light-field Microscopy

Challenge: given a sequence of lightfields (2-D signals), need to reconstruct a sequence of volumes (3-D+t)







2D Measured LF image

3D Input

Computational
Algorithm

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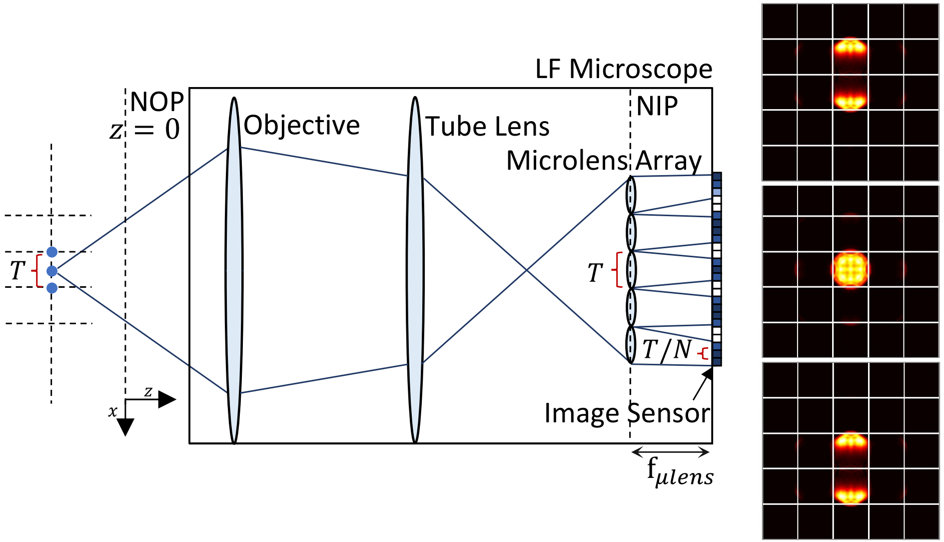
Forward Model

- Forward model is linear which means $\boldsymbol{y} = \boldsymbol{Hx}$
  - $\boldsymbol{H}$ is estimated using wave-optics
  - For each depth, $\boldsymbol{H}$ is block-circulant (periodically shift invariant) and can be modelled with a filter-bank
  - The entire forward model can be modelled using a linear convolutional network with known parameters (given by the wave-optics model)



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Neural network for volume reconstruction

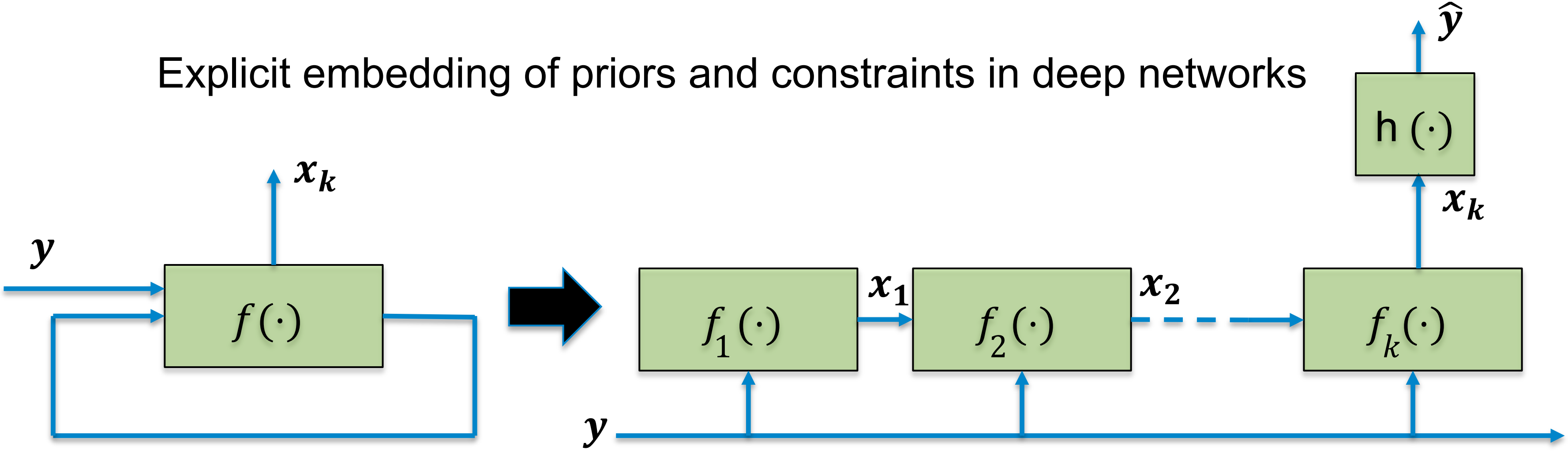
- Data is sparse (neurons fire rarely and are localized in space)
- Solve $\min_x (\|y - Hx\|^2 + \|x\|_1)$ s.t $x \geq 0$
- This leads to the following iteration:

$$x_{k+1} = ReLU(x_k - H^T H x_k + H^T y + \lambda)$$

- Approach: Convert the iteration in a deep neural network using the unfolding technique

Sparsity and Deep Unfolding Strategy

Explicit embedding of priors and constraints in deep networks



Iterative algorithm with $\boldsymbol{x}$ as input and $\boldsymbol{I}$ as output

Unfolded version of the iterative algorithm with learnable parameters

Need to re-synthesize the input, if self-supervised

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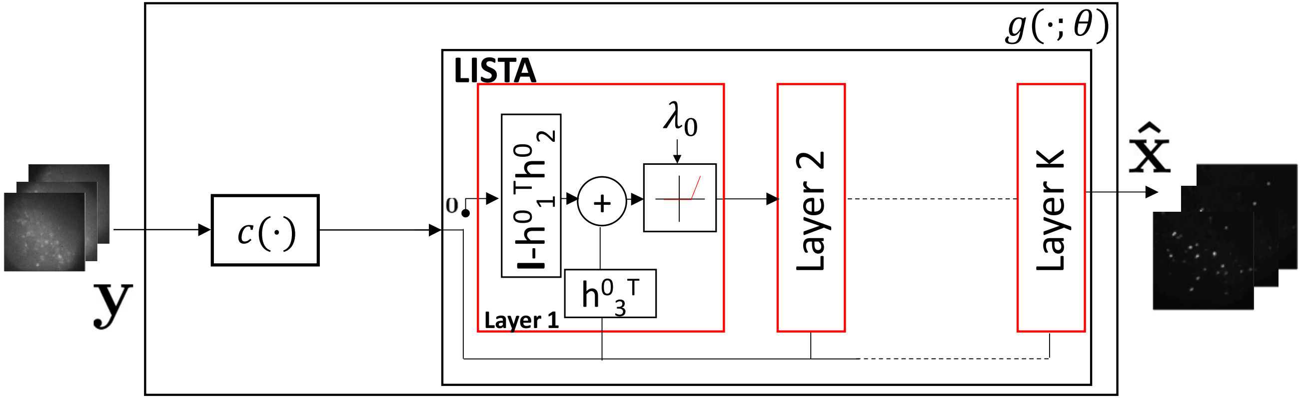
Neural network for volume reconstruction

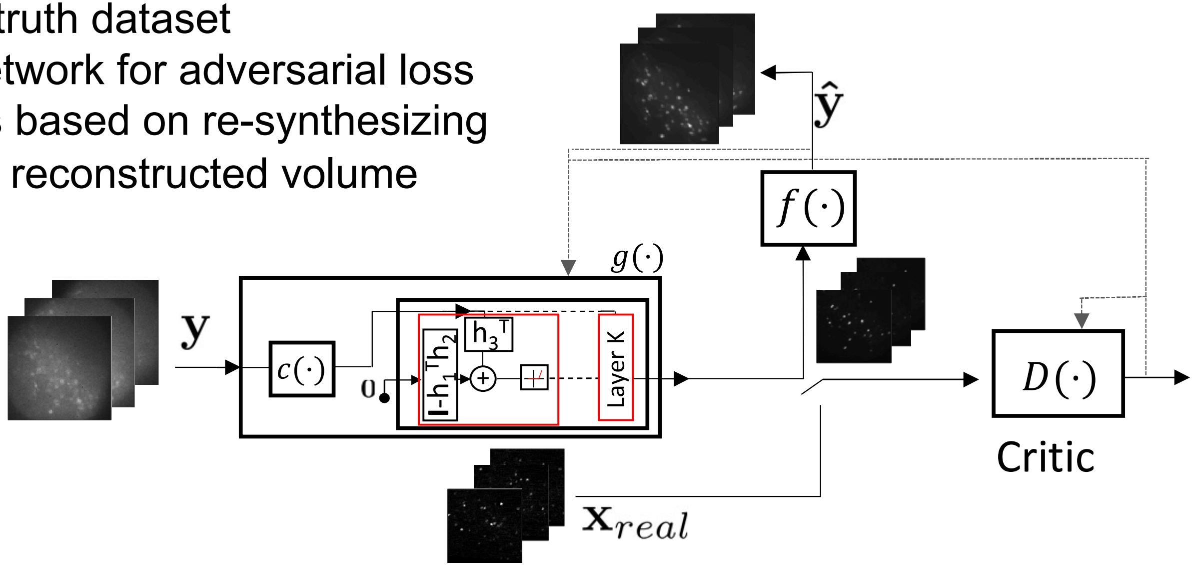
- Convert the iteration in a deep neural network using the unfolding technique

$$x^{k+1} = ReLU(x^k - H^T H x^k + H^T y + \lambda)$$



Training of the neural network

- Training, in this context, is difficult due to lack of ground-truth data
- Our approach: semi supervised learning
  - Small ground truth dataset
  - Adversarial network for adversarial loss
  - Light-field loss based on re-synthesizing light-field from reconstructed volume



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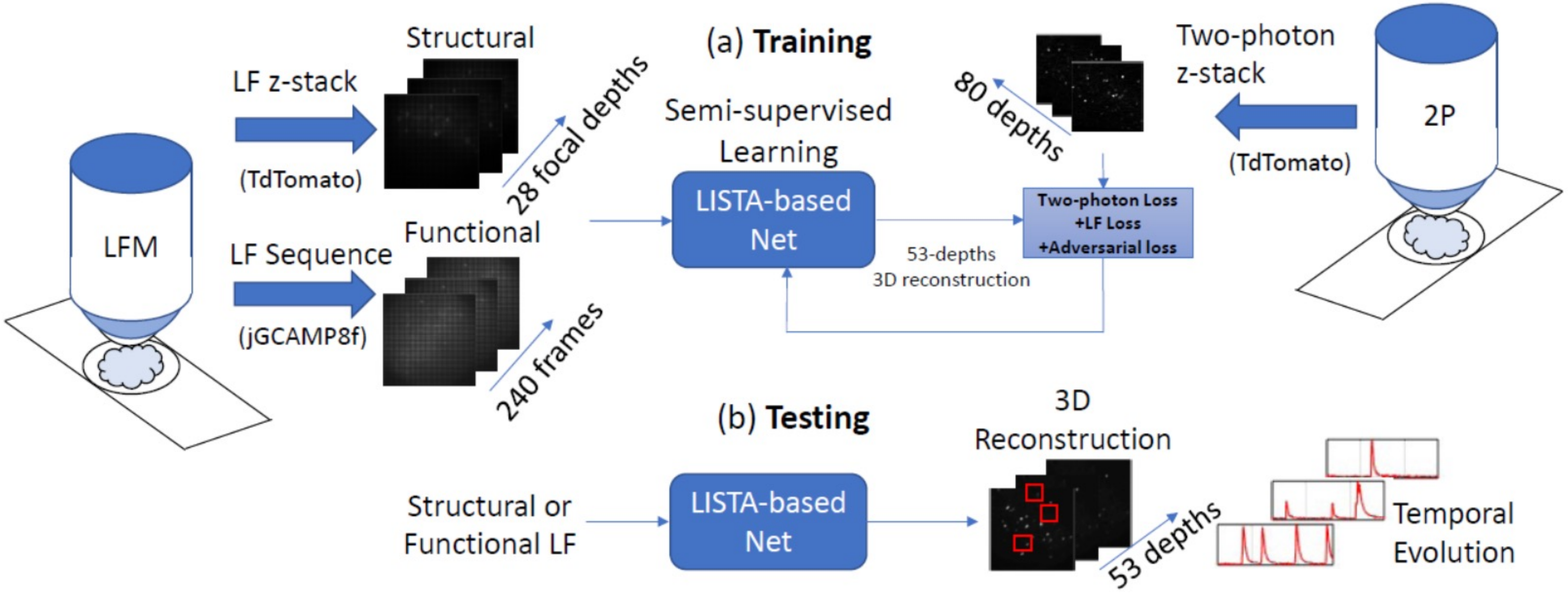
Training of the neural network



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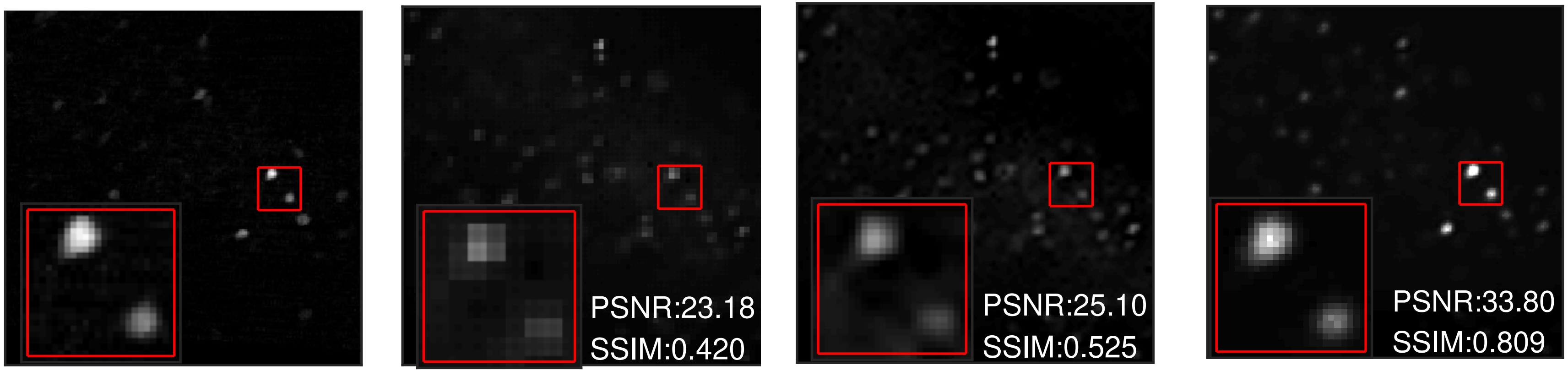
Results – Structural Data



Ground-truth | ISRA | ADMM | New method (0.3s to reconstruct one volume)

H. Verinaz et al. "Physics-based Deep Learning for Imaging Neuronal Activity via Two-photon and Light-field Microscopy", IEEE Trans. on Computational Imaging, 2023.

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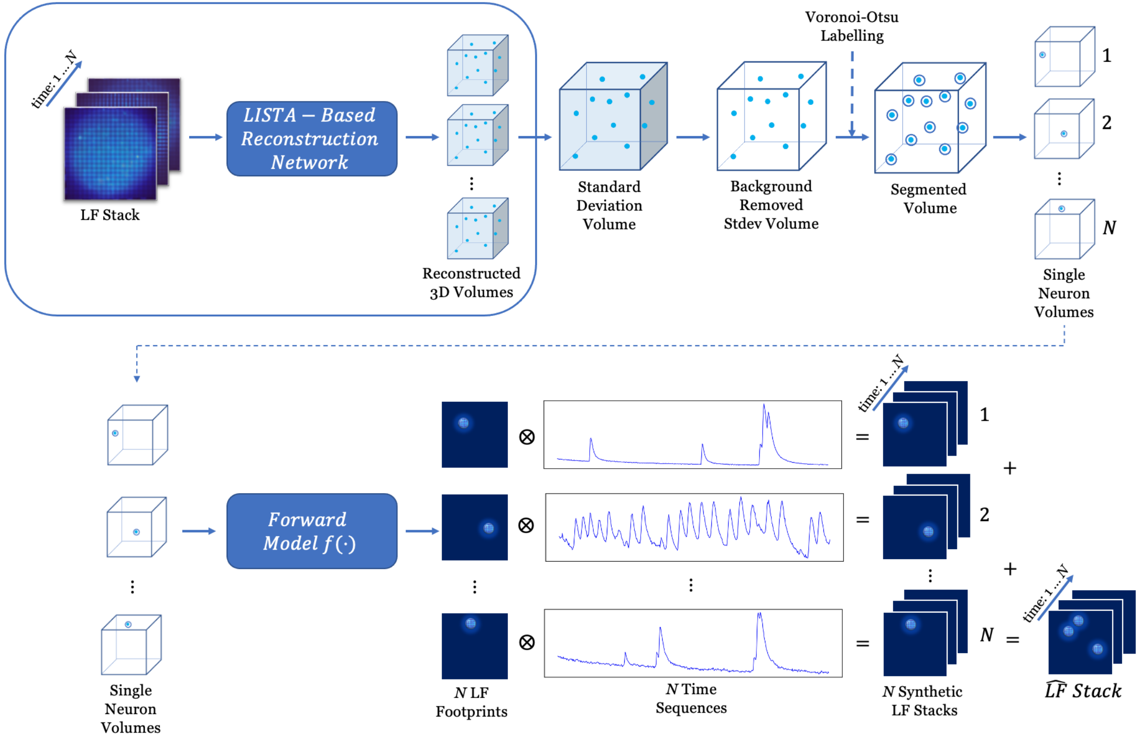
Neural Activity Extraction



$$Y = S \cdot T$$

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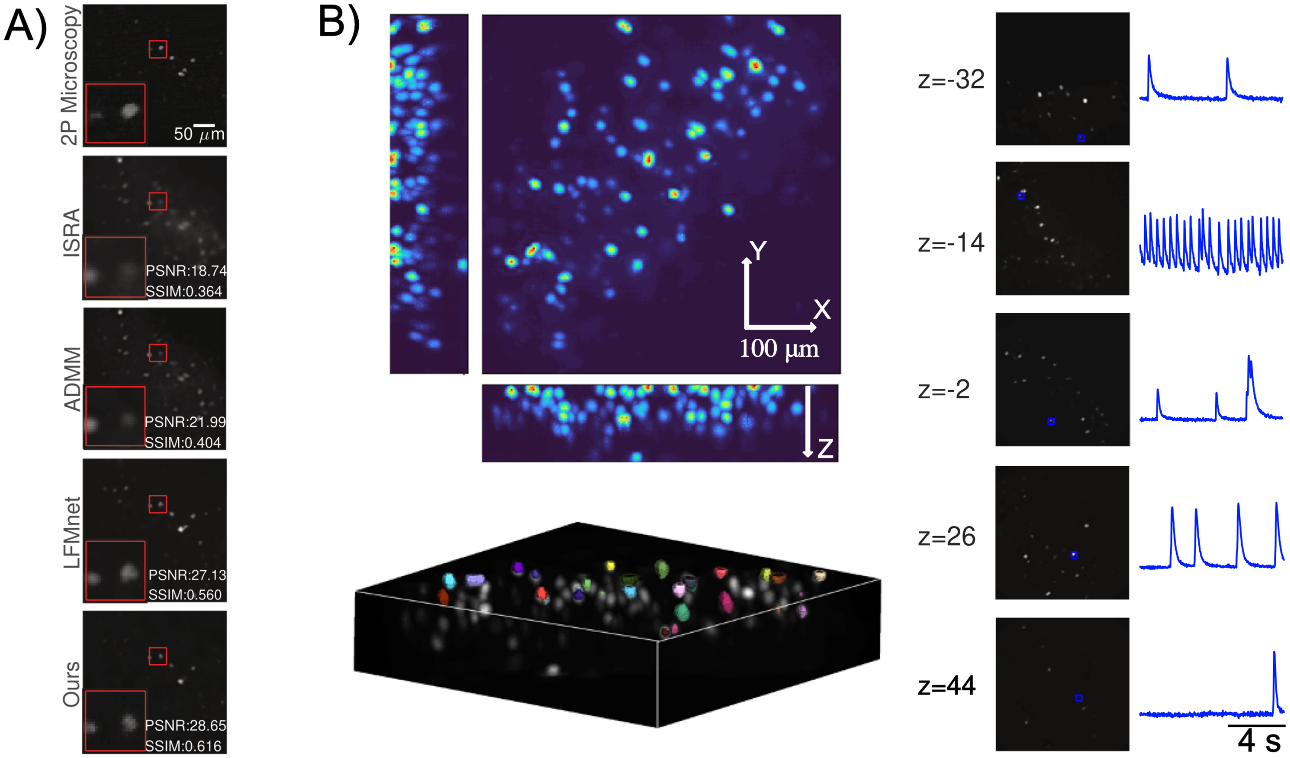
Results – Functional Data



Sample 2
(S2A3)

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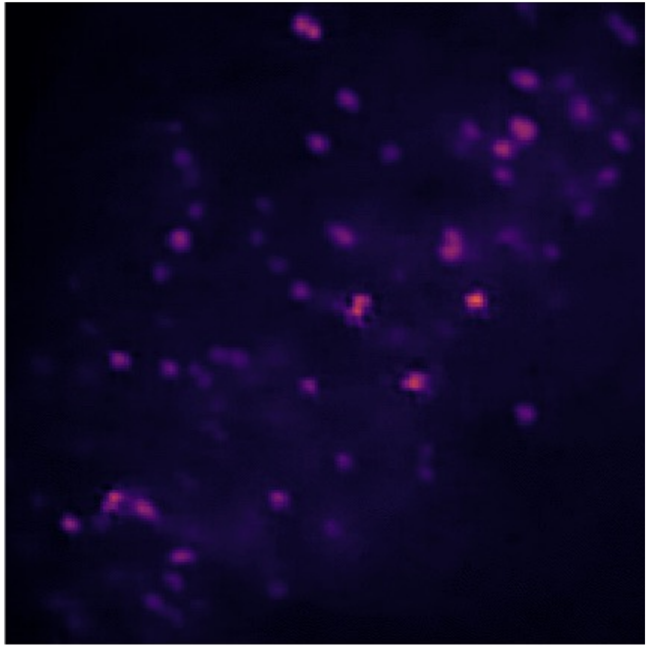
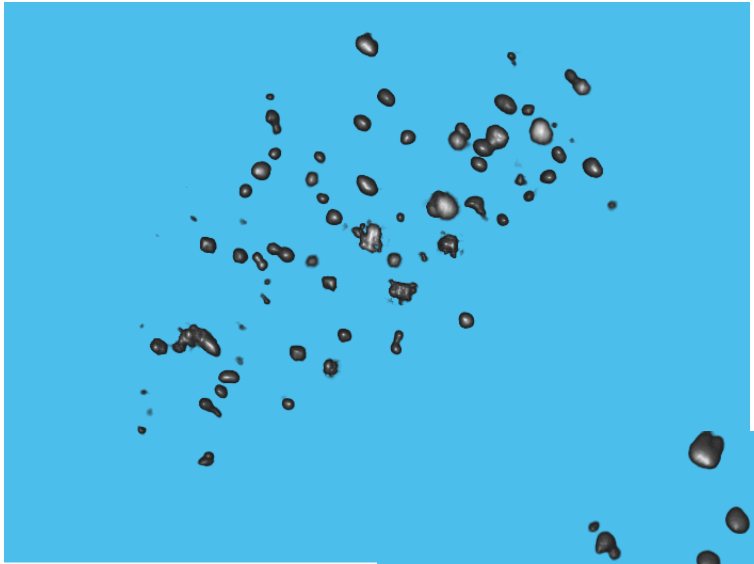
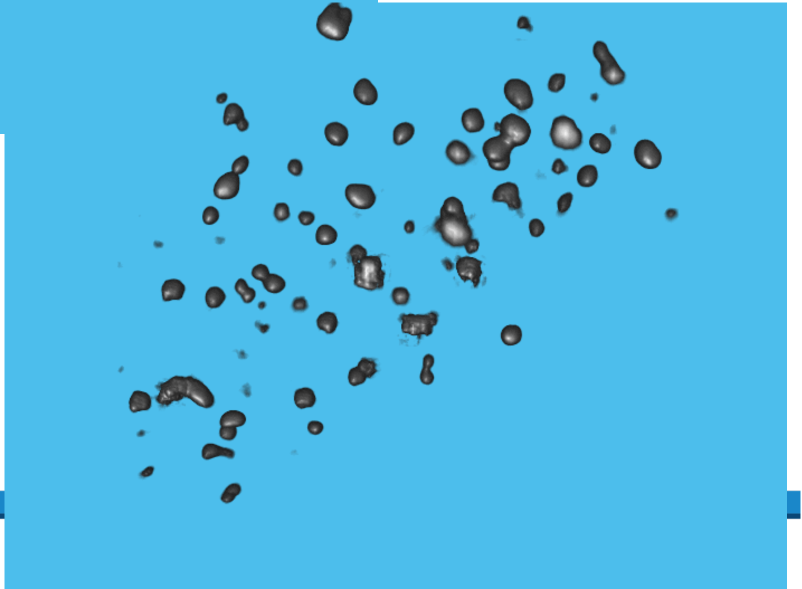


Z
Average



3D
(view 1)

3D
(view 2)



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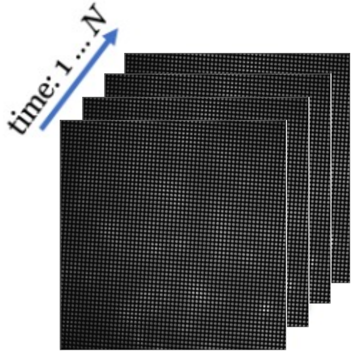
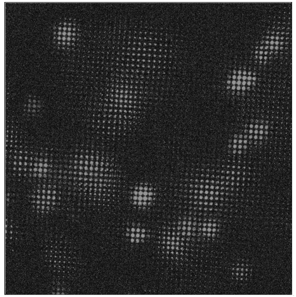
Fast volumetric jGCaMP8f time-series extraction

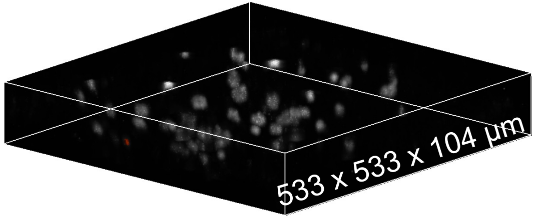


Input F(t):
LF video acquired in
brain slice
cortical layer 2/3



LF activity map:
max(ΔF)/σ(F)

LISTA-based net
Volume Estimation



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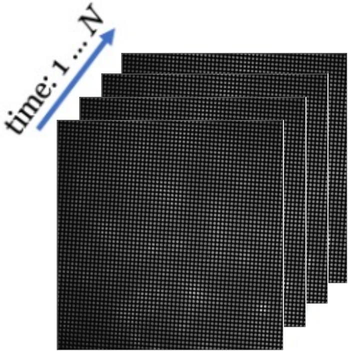

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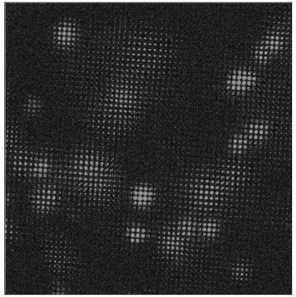
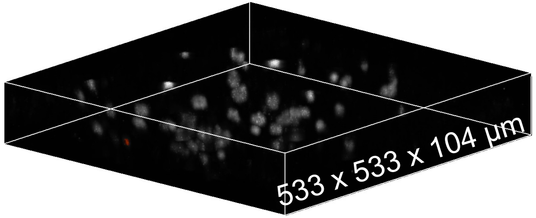
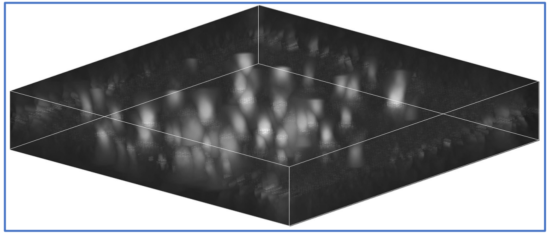


LF activity map: max(ΔF)/σ(F)

LISTA-based net Volume Estimation



8-iteration Richardson-Lucy Deconvolution



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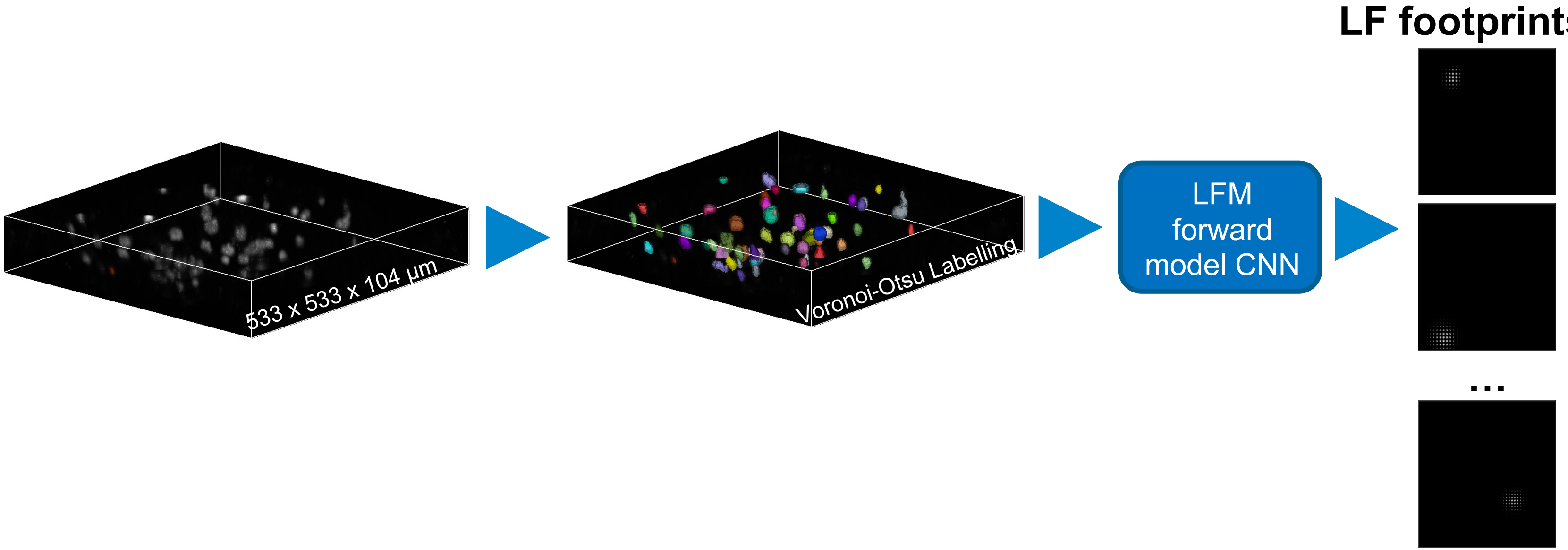
Fast volumetric jGCaMP8f time-series extraction



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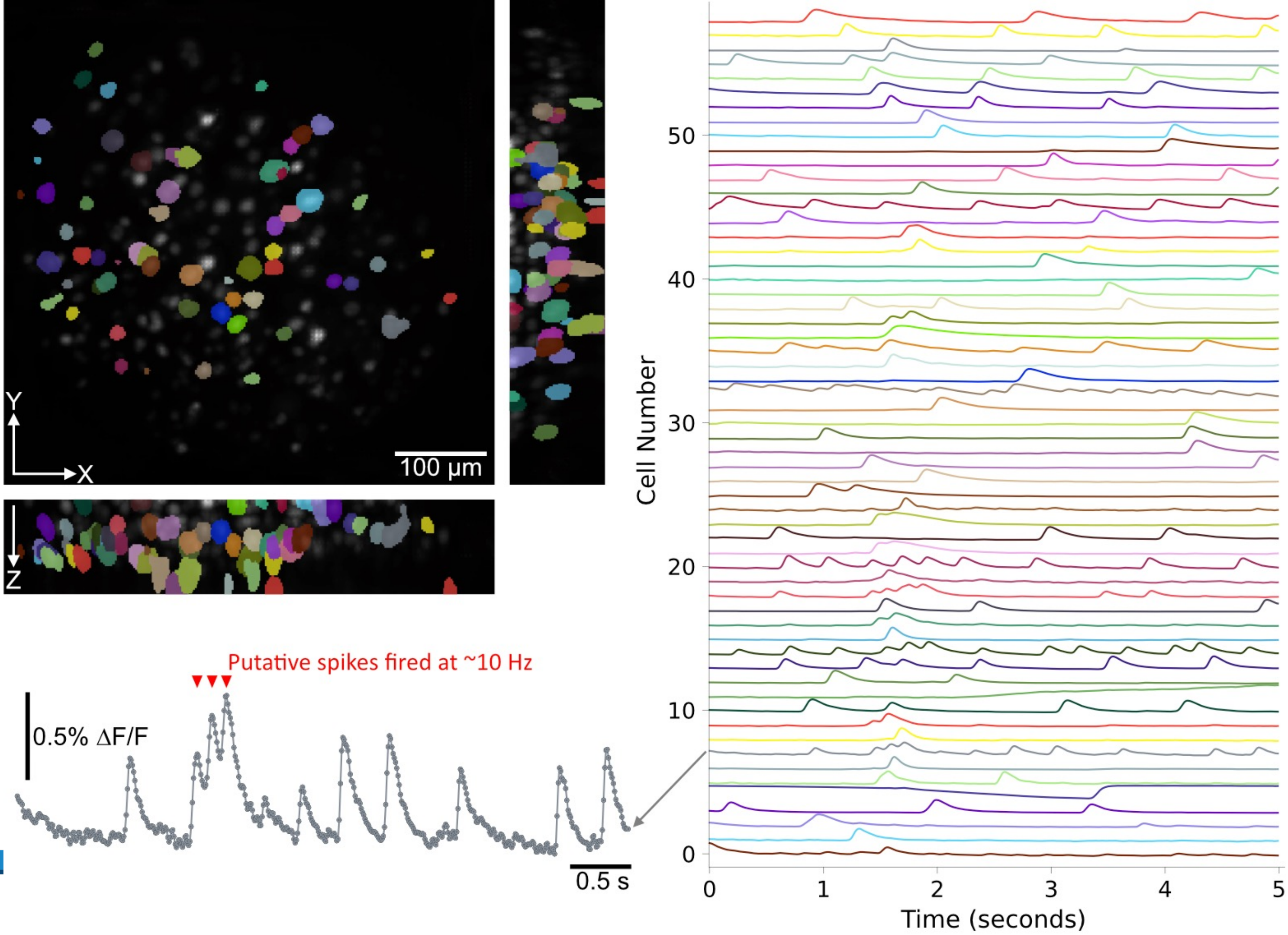

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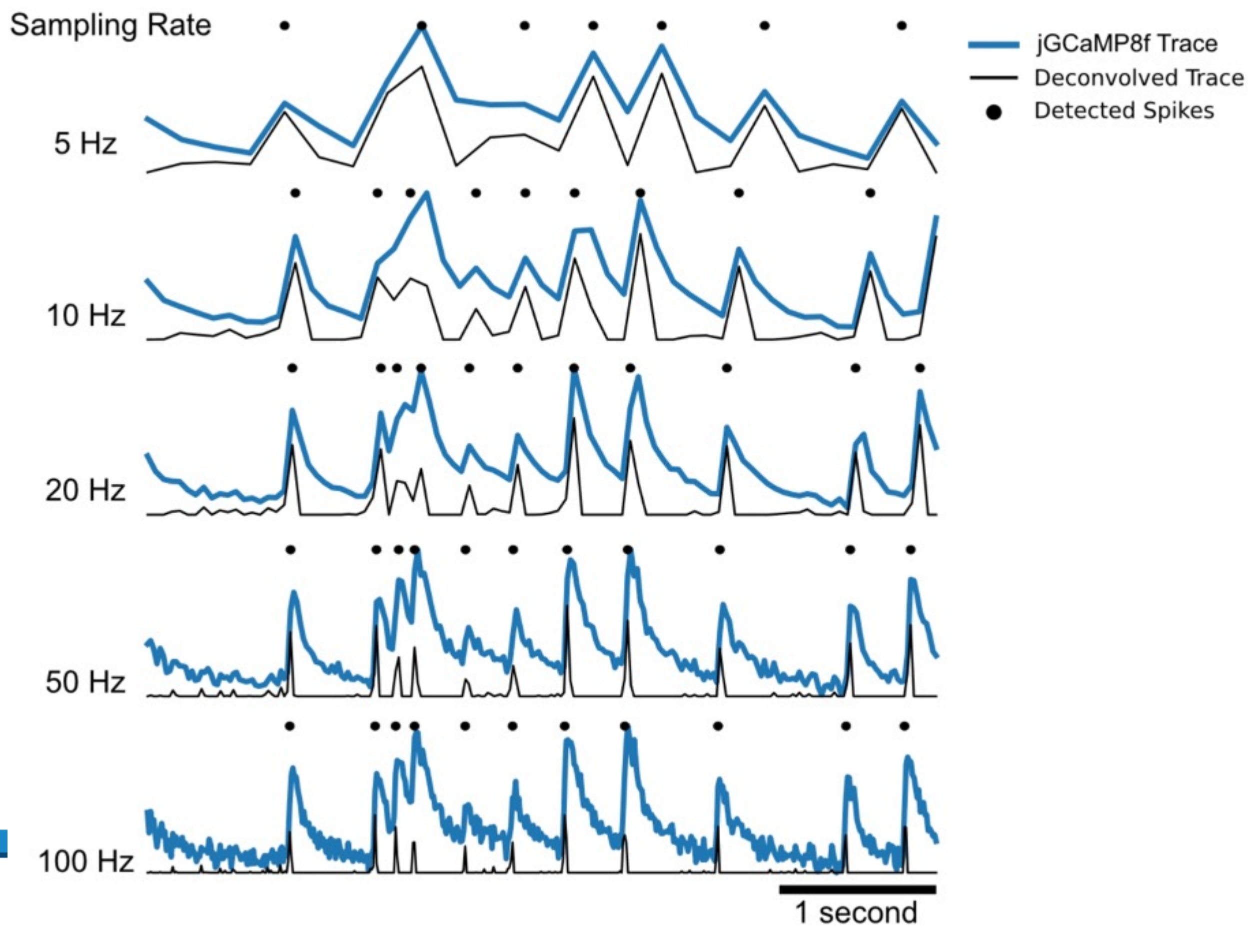
Why imaging **fast** is important:



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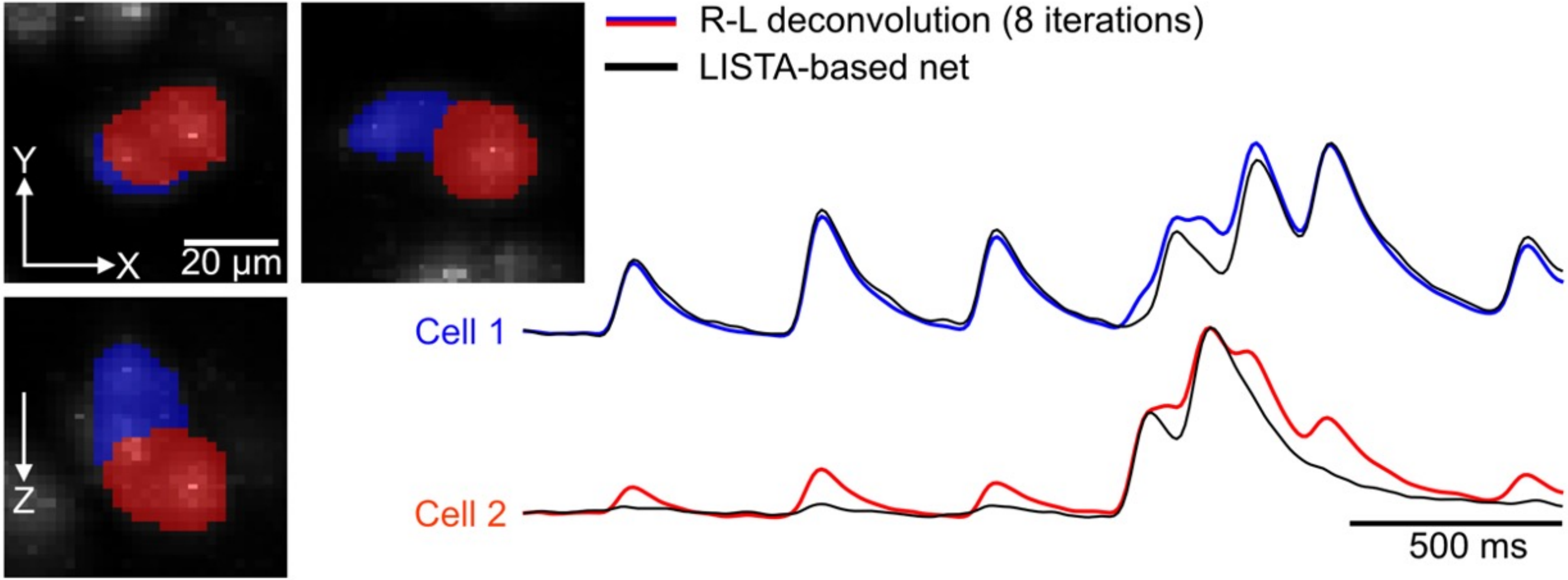
LISTA-based net decreases crosstalk between neighbouring neurons



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Conclusions

- Physics-based deep learning can powerfully exploit the advantages of light-field and two-photon microscopy.
- LISTAnet reduces calcium signal crosstalk between neighboring neurons.
- Calcium signals extracted up to 100 microns deep in neocortex. Scope to go deeper with:
  - Red-shifted indicators
  - Integrating structured illumination
- Future applications:
  - Determine living neural network learning rules in-vivo
  - Direct measurement of voltage

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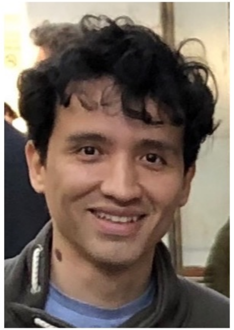
A special thank to:

Kate Zhao

Pingfan Song

Herman Verinaz



Peter Quicke

Carmel Howe

Thank you!