

IMPERIAL

Computational Light-field Microscopy for Neuroscience

Pier Luigi Dragotti
15/09/2026

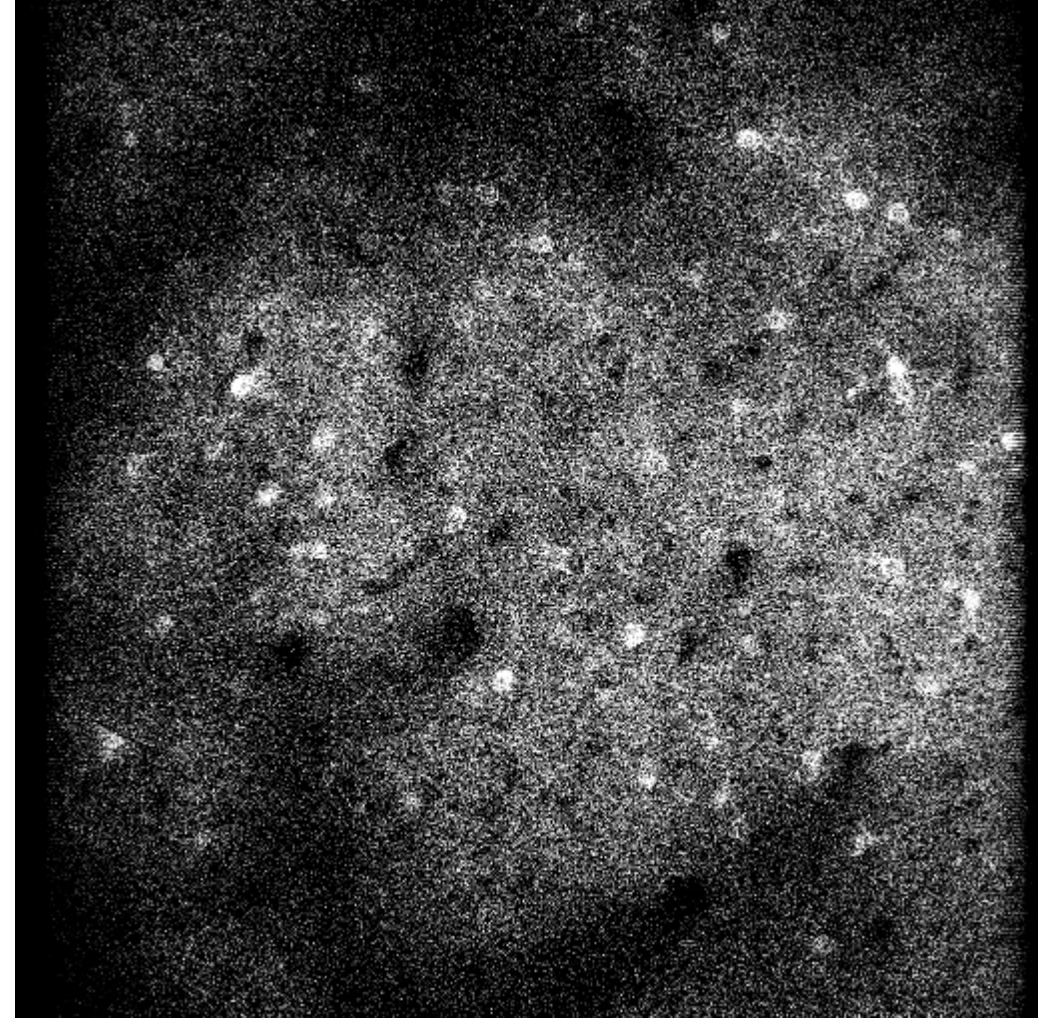
Outline

- Motivation
 - What neuroscientists want
 - Neurons and action potentials
- Neuro-Imaging Techniques
 - Scanning multi-photon microscopy
 - Alternative Scanning Techniques
 - Indicators
- Light-field Microscopy
- Computational Methods
 - Seeded demixing
 - Learning-based method
- Conclusions

Motivation

What Neuroscientists want

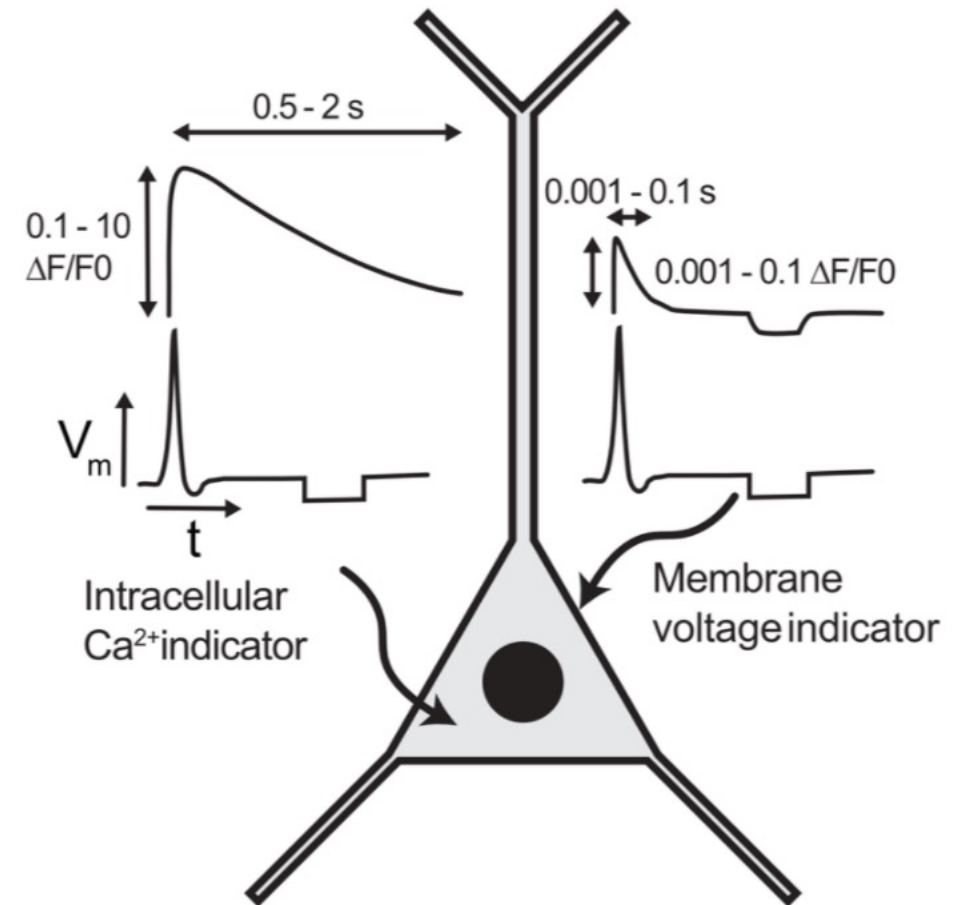
- Goal of Neuroscience: to study how information is processed in the brain
- Neurons communicate through pulses called Action Potentials (AP)
- Need to measure in-vivo the activity of large populations of neurons at cellular level resolution
- Multi-photon microscopy combined with right indicators is the most promising technology to achieve that



Motivation

Neurons and Action Potentials

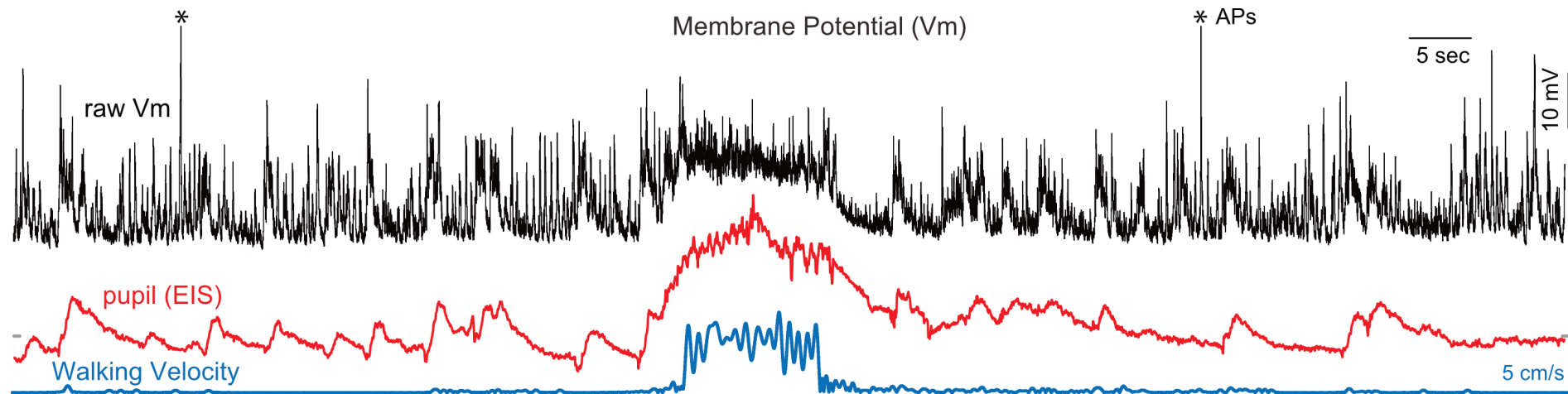
- Neurons behaves like “integrate-and-fire” systems
 - Neuron integrates the input current,
 - when the membrane potential reaches a threshold value, the neuron “fires” an action potential
 - then, the membrane potential is reset to zero.
- An action potential (AP) is a “pulse” due to rapid voltage change in the neuron’s membrane and lasts 1-10ms
- In microscopy people use “**calcium indicators**” they are indirect indicators for an AP and lasts 100-400ms
- To see voltage signals, one needs to use “**voltage indicators**” but the pulse duration is then 1-10ms



Neuro-Imaging Techniques

Challenges

- Need to image “in-vivo” (the animal, typically a mouse) should be alive
- Need to image deep in a “scattering” mammalian brain
- Need to image at cellular resolution
- Need to image fast enough in order to be able to see action potentials (and sub-threshold activities)



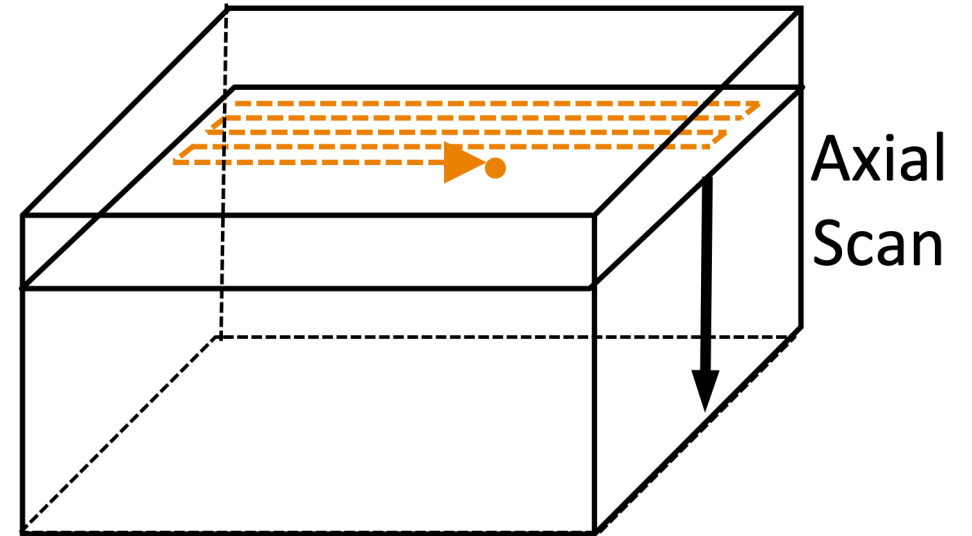
McGinley et al., Cortical Membrane Potential Signature of Optimal States for Sensory Signal Detection, *Neuron* 2015

Neuro-Imaging Techniques

Multi-photon Scanning microscopy

- Fluorescent sensors within tissues
- Highly localized laser excites fluorescence from sensors
- Photons emitted from tissue are collected
- Focal spot sequentially scanned across samples to form image
- Two-photon microscopes in raster scan modality can go deep in the tissue but are **slow**

Point scanning (2PLSM)



Neuro-Imaging Techniques

Multi-photon Scanning microscopy

- Fluorescent sensors within tissues
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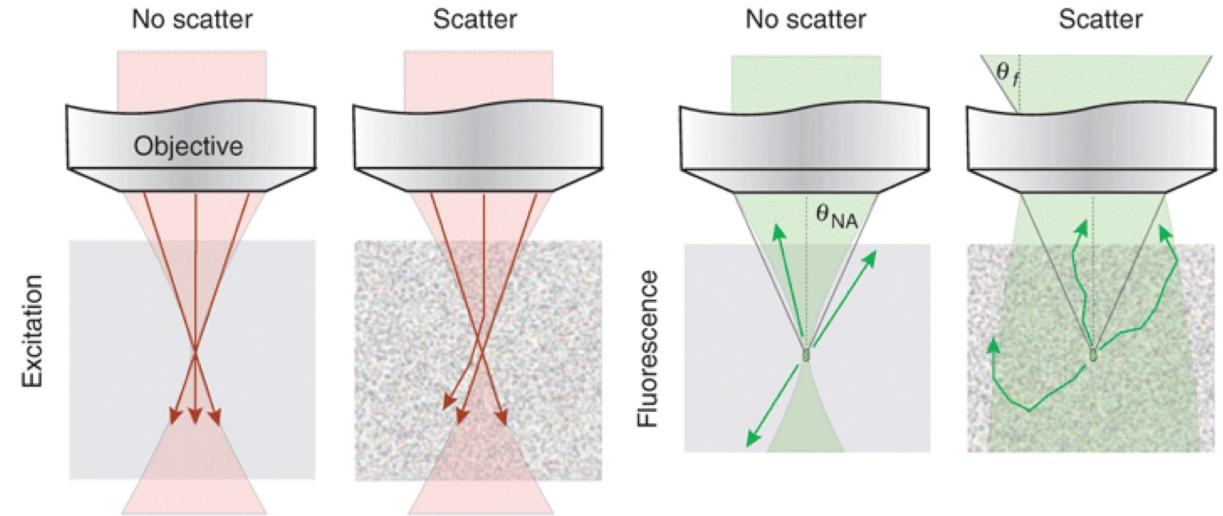
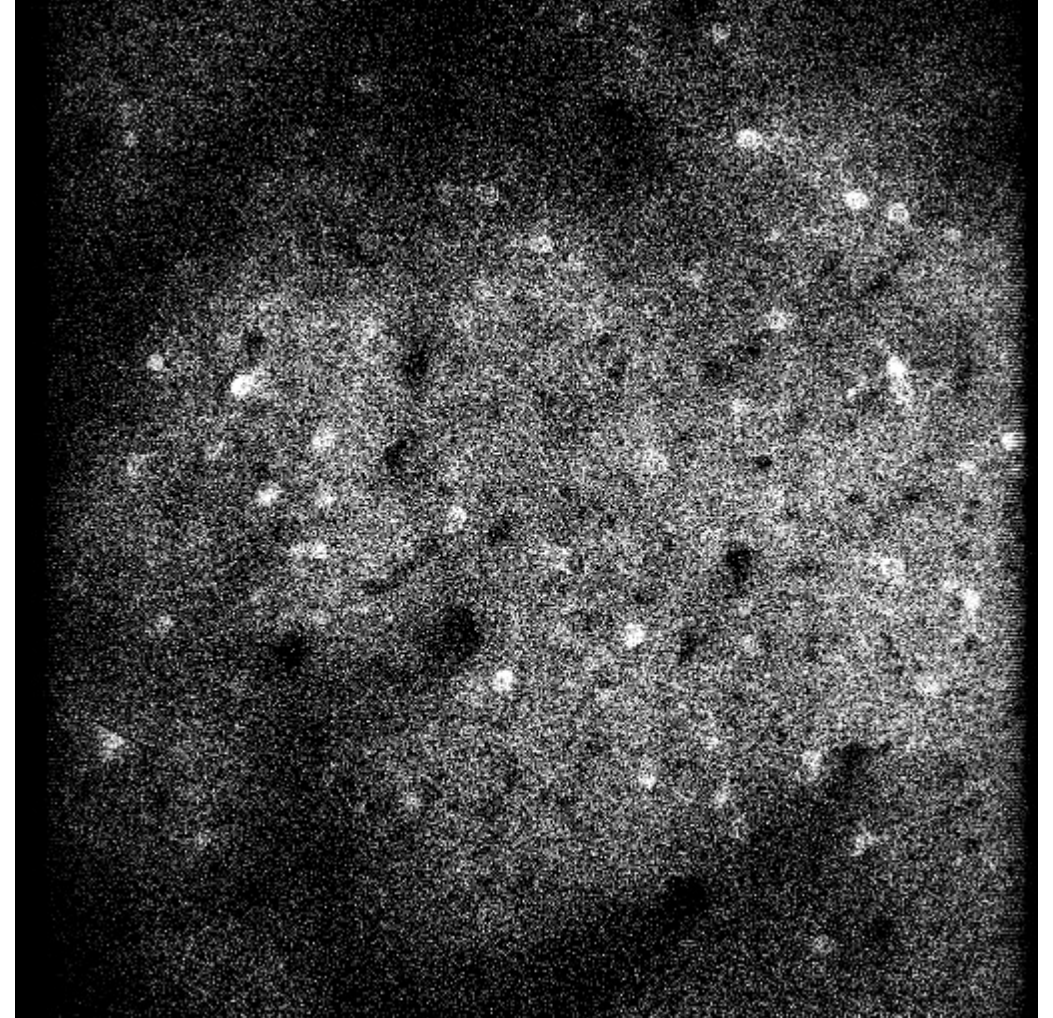


Image from: Fritjof Helmchen. Deep tissue two photon microscopy, Nature methods, 2016

Neuro-Imaging Techniques

Multi-photon Scanning microscopy

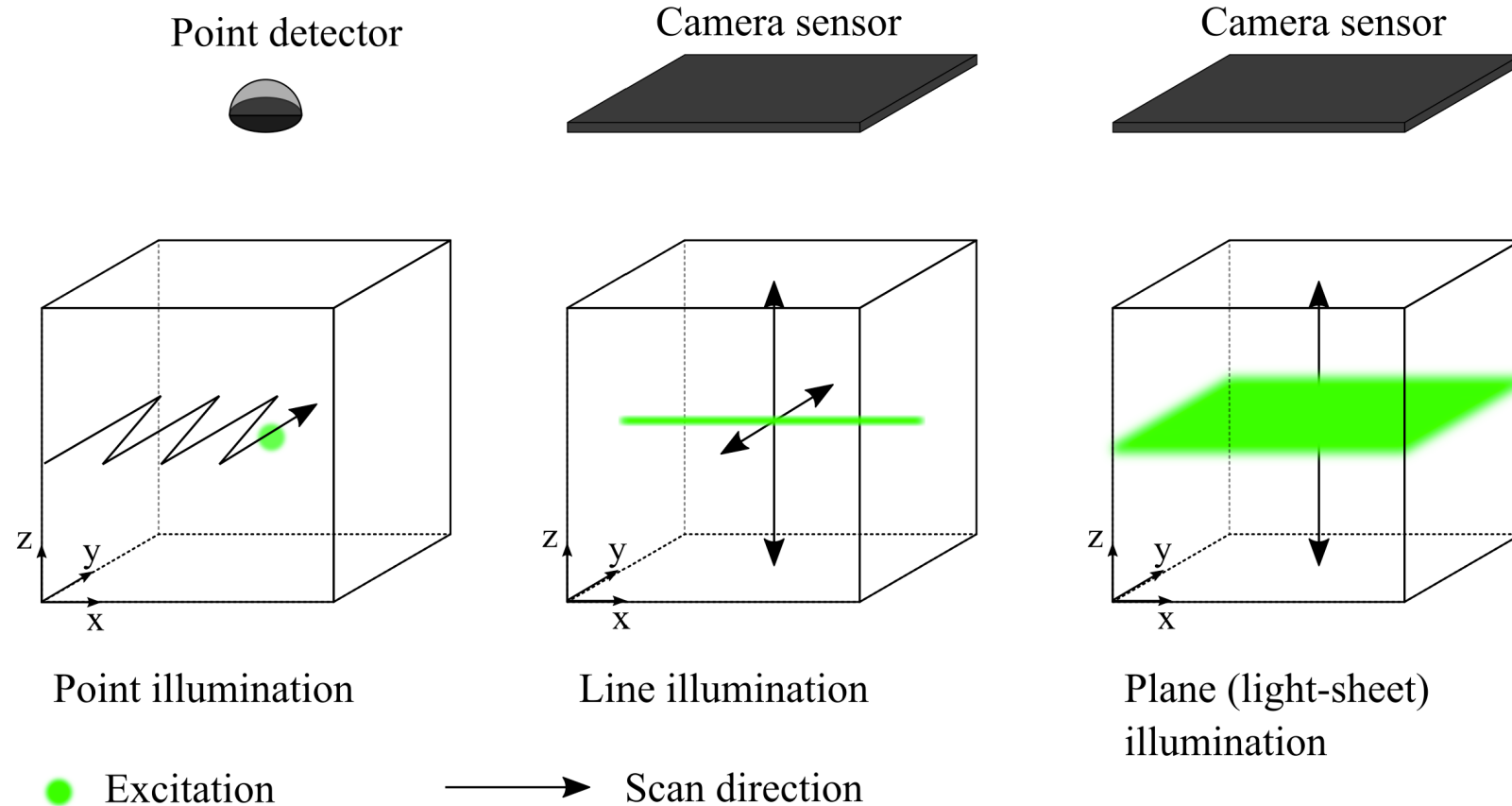
- Fluorescent sensors within tissues
- Highly localized laser excites fluorescence from sensors
- Photons emitted from tissue are collected
- Focal spot sequentially scanned across samples to form image
- Two-photon microscopes in raster scan modality can go deep in the tissue but are **slow**.
- This is why “slow” calcium indicators are used.
Frame rate: 10Hz-100Hz.



Neuro-Imaging Techniques

Alternative Scanning Techniques

- In order to speed up acquisition one can change the illumination strategy
- This mitigates the issue but does not fix it
- Issue with scattering



Neuro-Imaging Techniques

Summary and Trade-offs

Goal: fast, deep, high-spatial resolution and large volume

- **Indicators:** calcium indicators is good for slow scanning techniques, but is an “indirect” indicator of AP. Voltage indicator is the best choice but too fast
- **Scanning Techniques:** point-like, good for scattering and go deep but not fast enough. Alternatives are possible
- **Optics?**
- **Computational methods:** What is the best computational method to use?

Light-field Microscopy

How fast is fast enough?

- “**calcium indicators**” they are indirect indicators for an AP and lasts 100-400ms. So a camera operating at 10Hz-100Hz is fast enough
- To see voltage signals, one needs to use “**voltage indicators**” but the pulse duration is then 1-10ms so you need a camera operating at **1KHz**
- To image with voltage indicators we need to go scanless
- **Light-Field Microscopy (LFM)** is a high-speed imaging technique that uses a simple modification of a standard microscope to capture a 3D image of an entire volume in a single camera snapshot

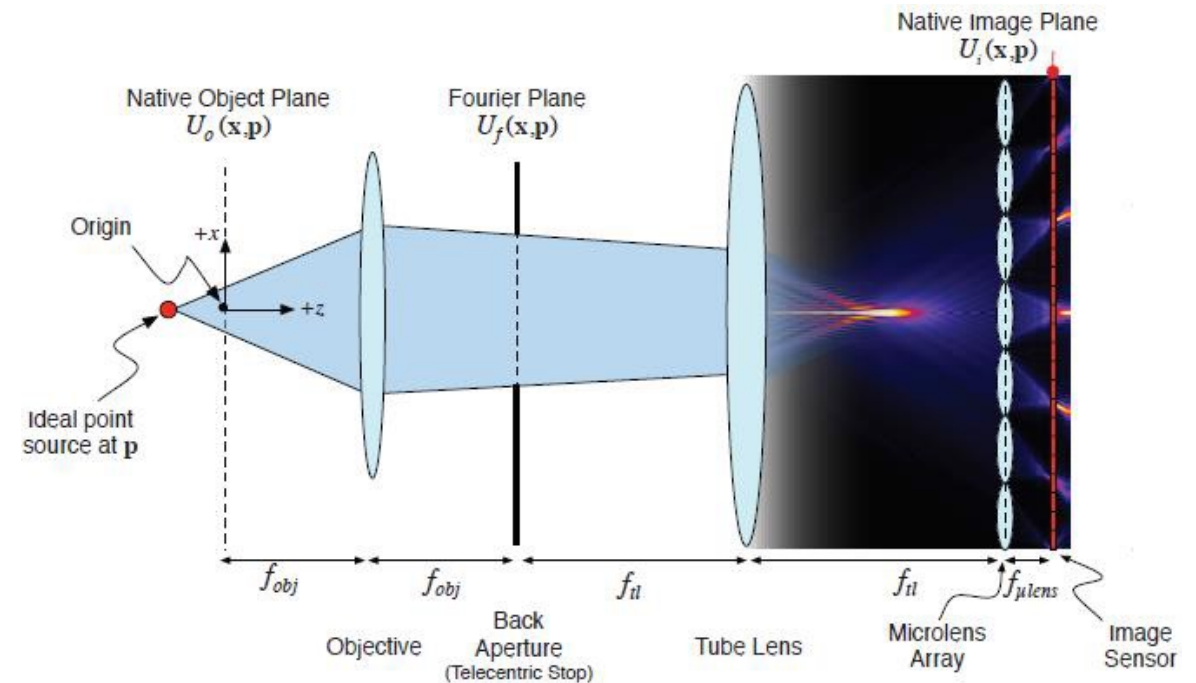
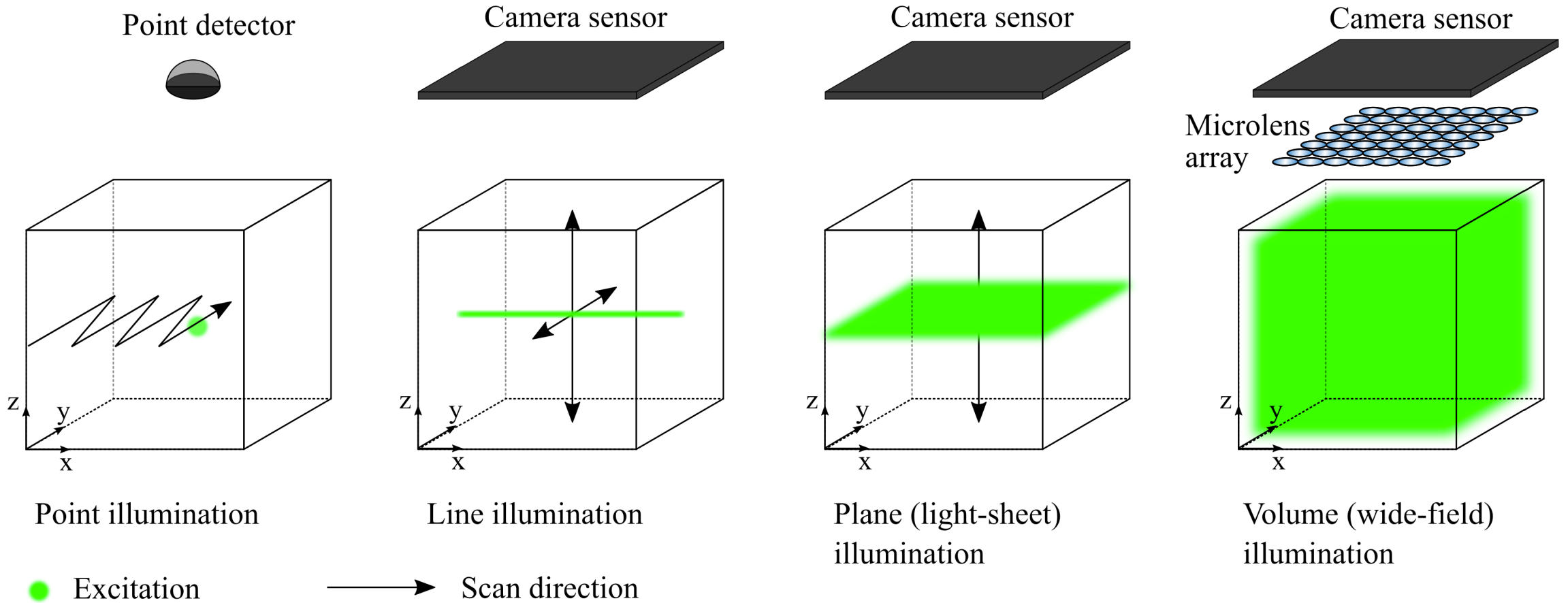


Image from: M. Broxton et al., “Wave optics theory and 3-D deconvolution for the light field microscope,” Opt. Exp., vol. 21, 2013.

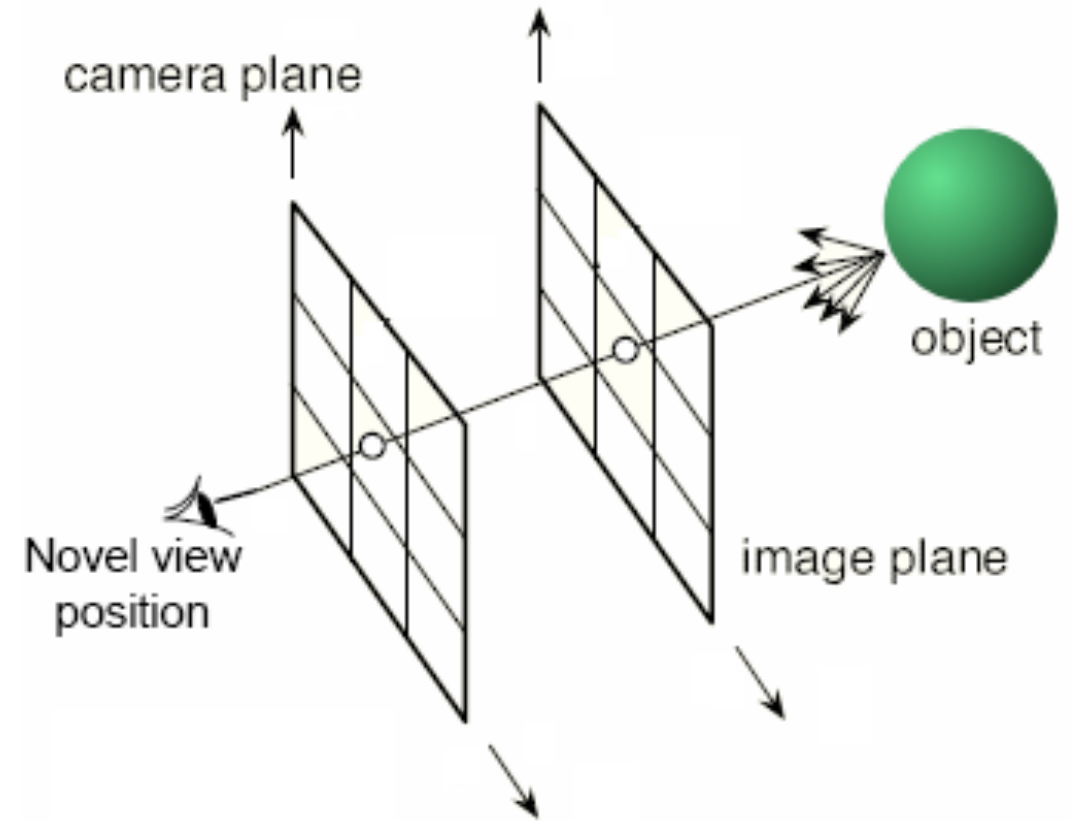
Light-Field Microscopy

Light-field optics enables scanless microscopy



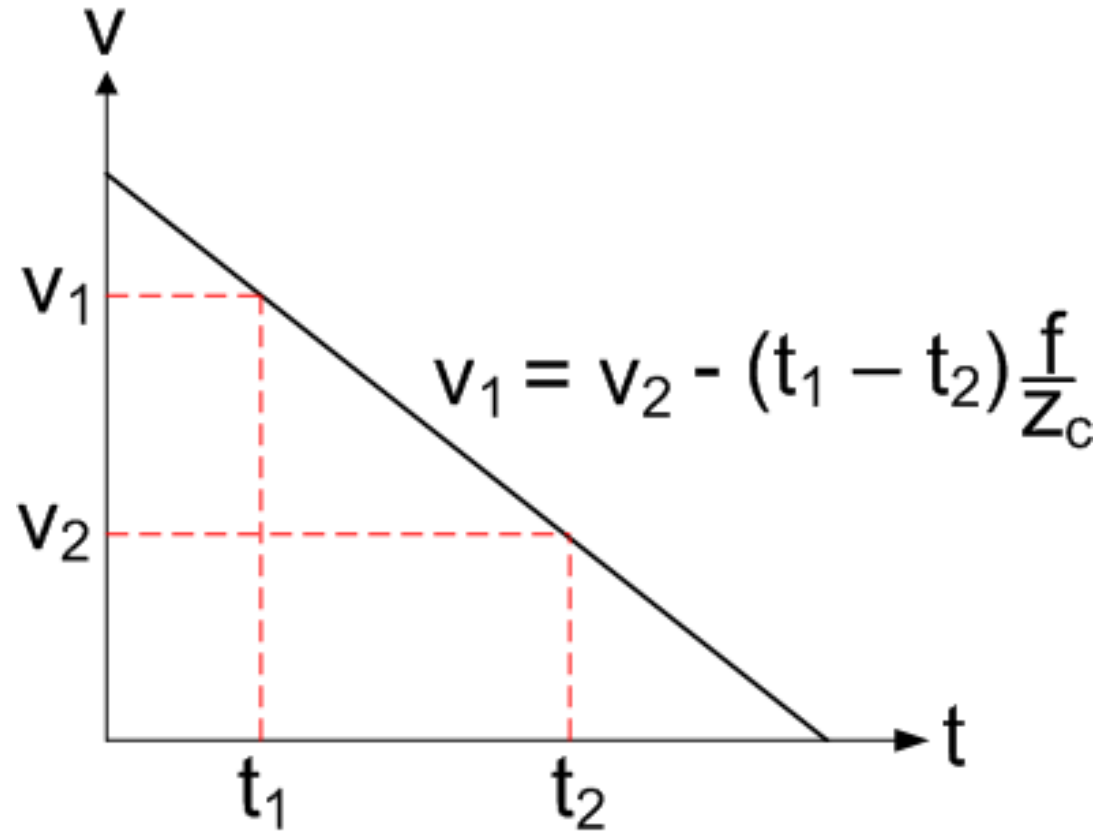
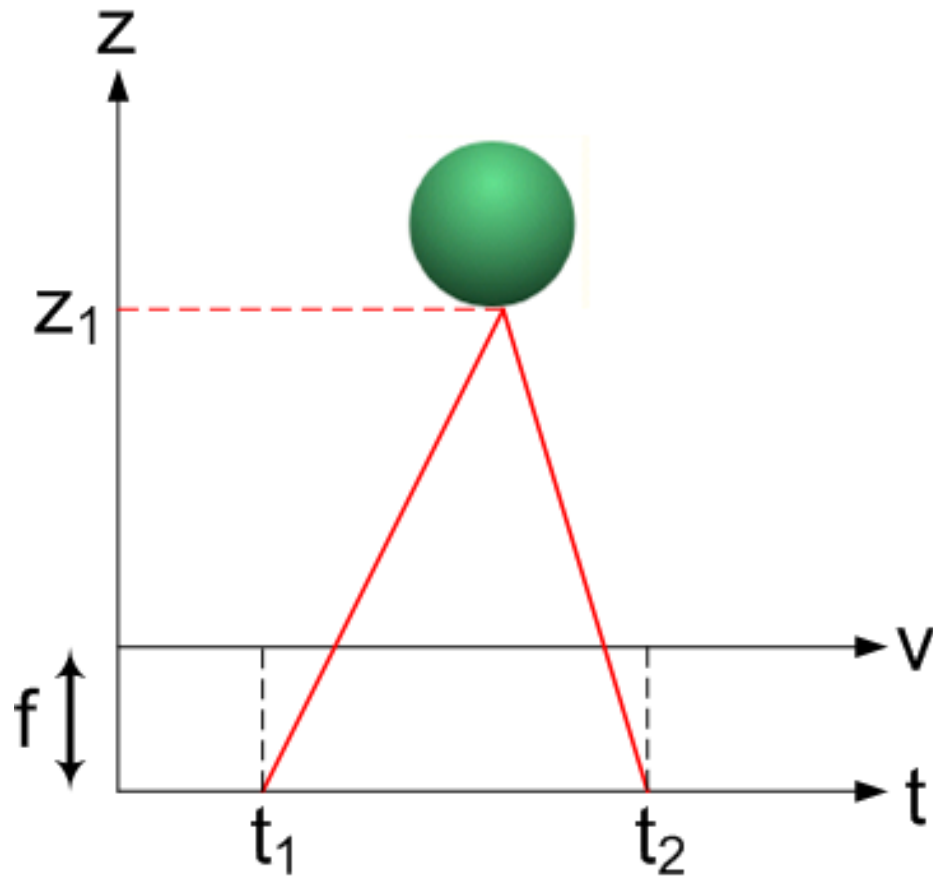
Light-Field Microscopy

Ray model (geometric optics)



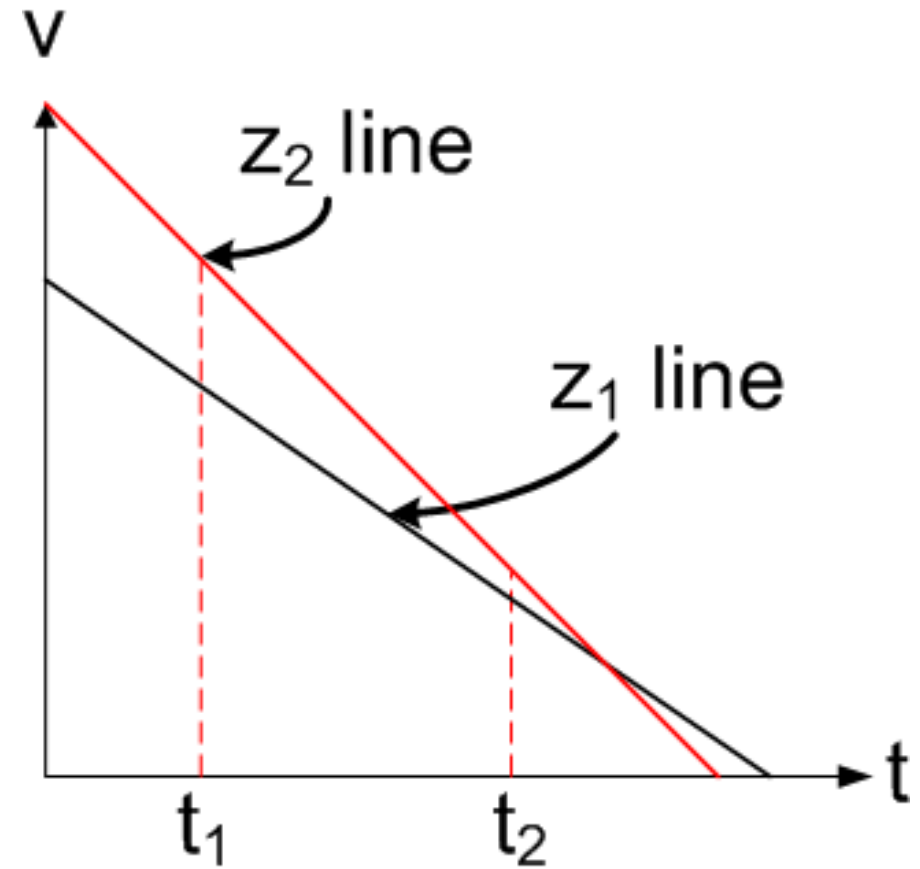
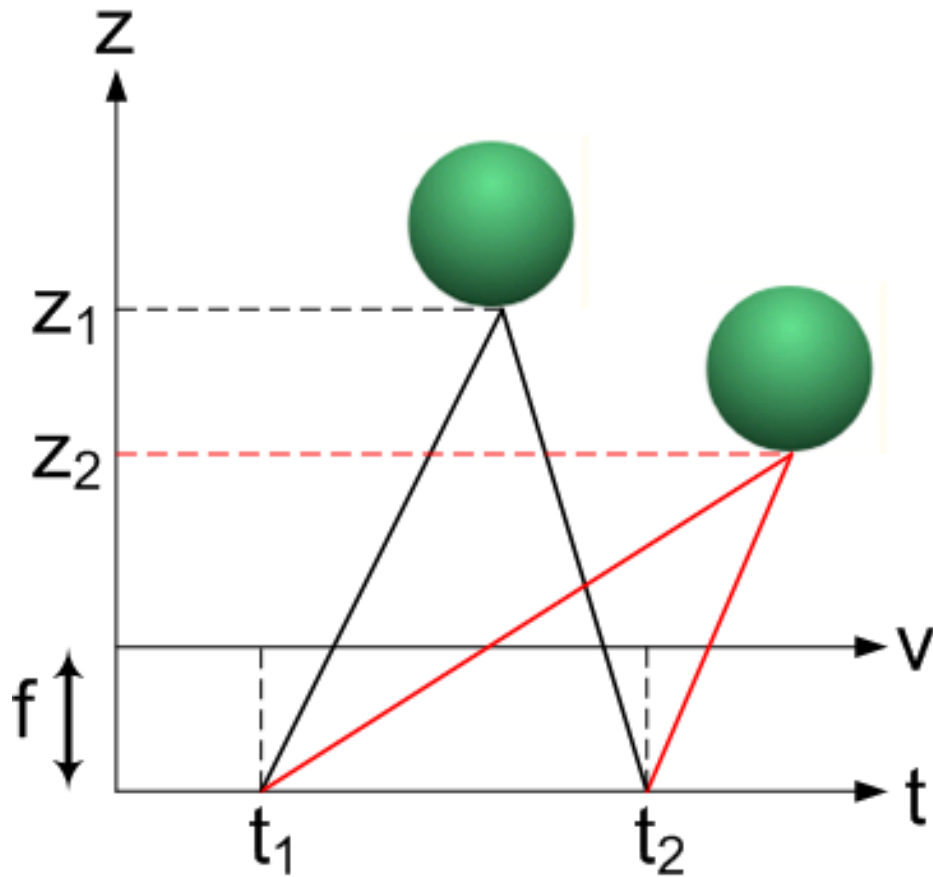
Light-Field Microscopy

Ray model (geometric optics)



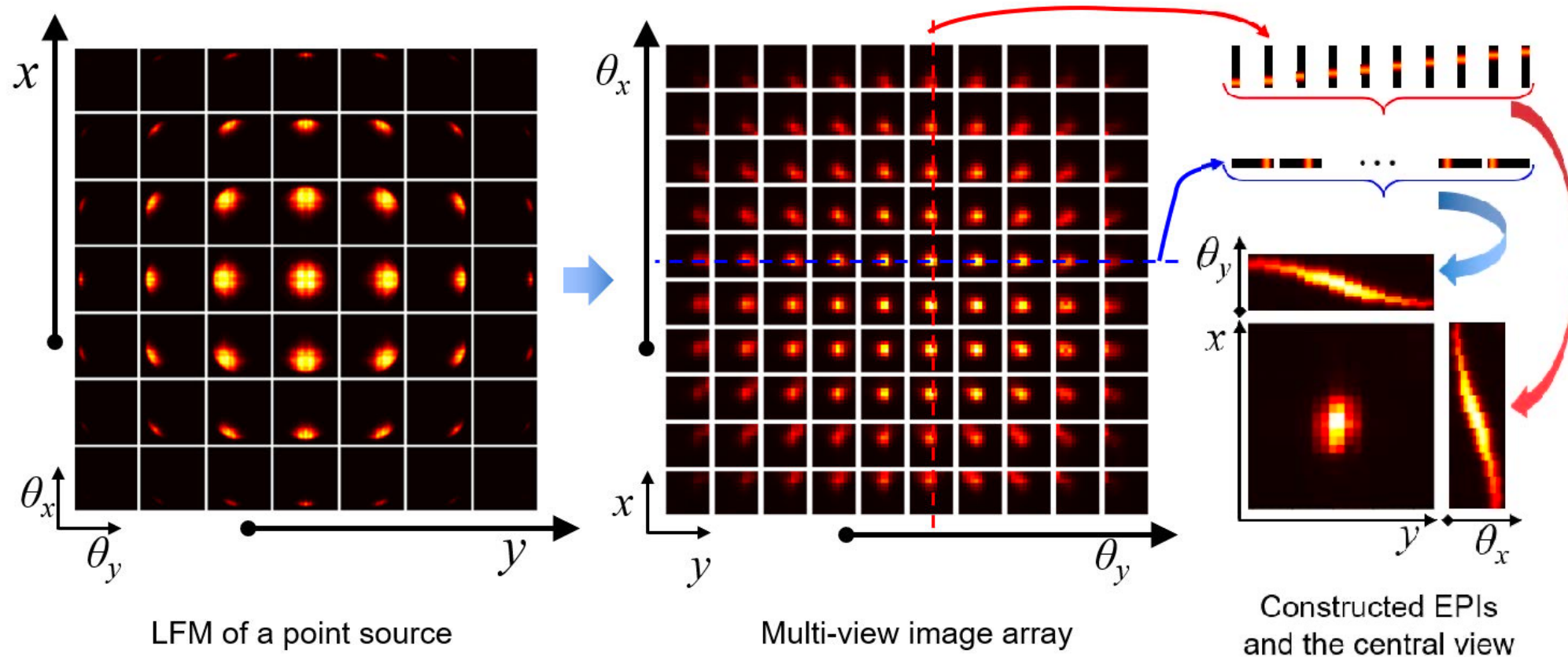
Light-Field Microscopy

Ray model (geometric optics)



Light-Field Microscopy

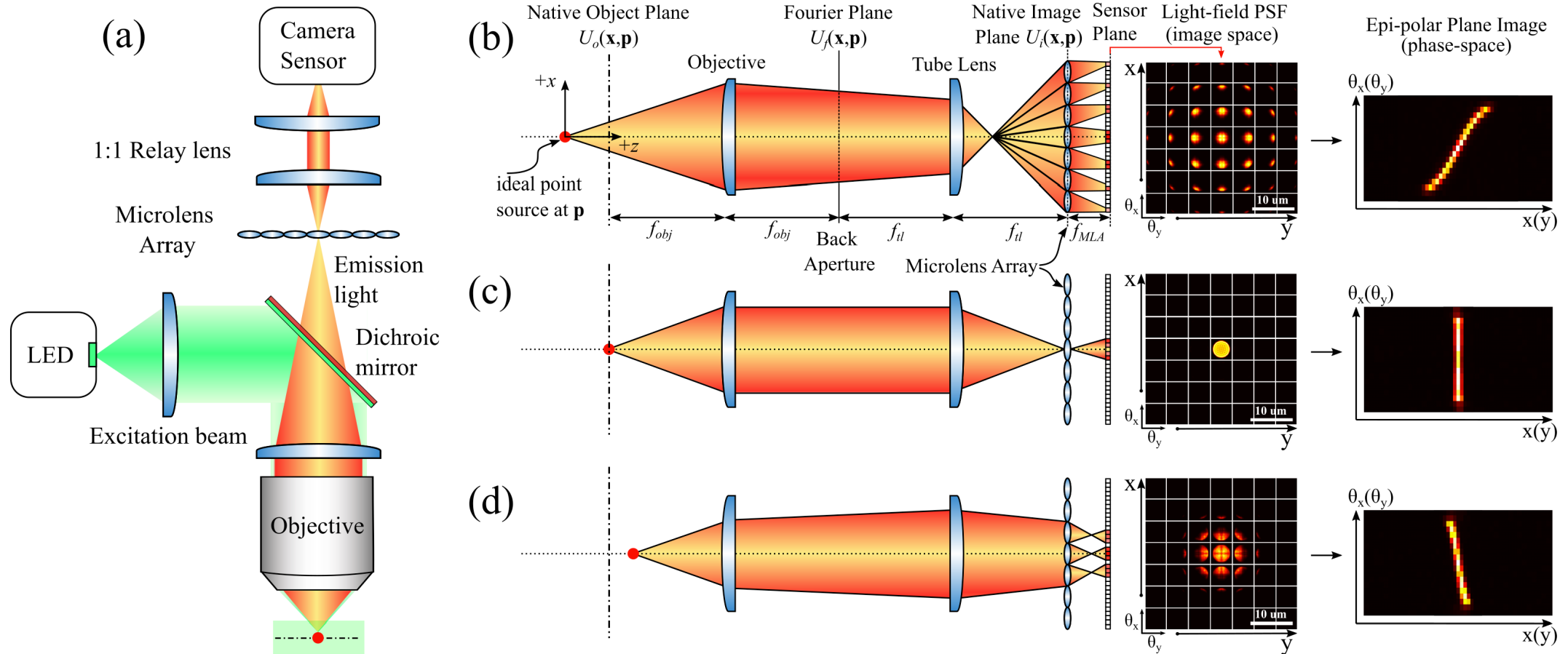
Ray model (geometric optics)



Convert an LFM image to multi-view array and EPI

Light-Field Microscopy

Wave model (wave optics)

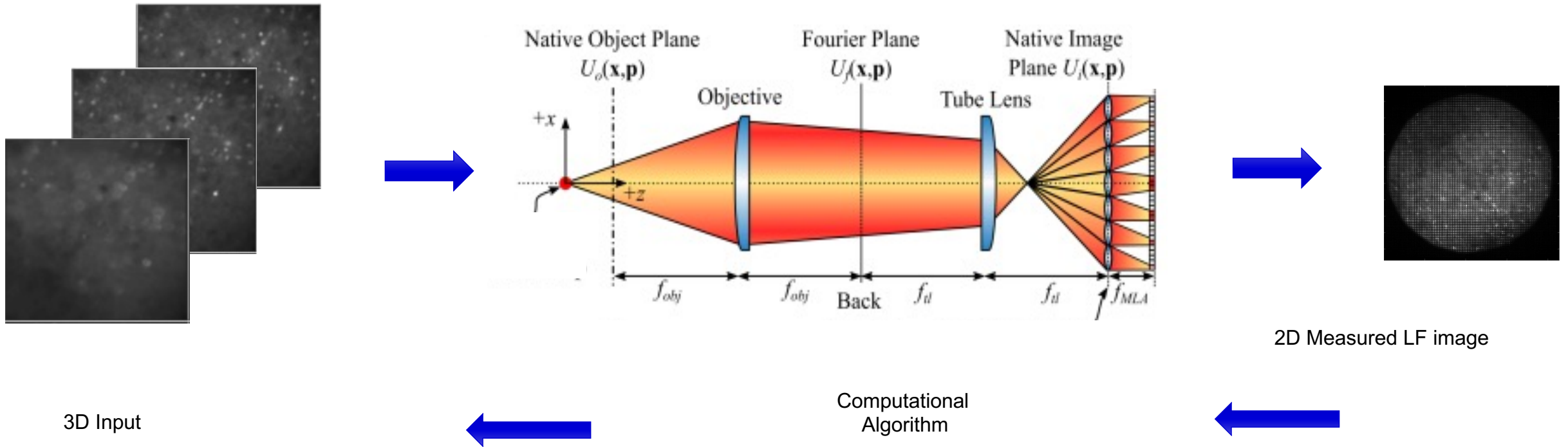


M. Broxton et al., "Wave optics theory and 3-D deconvolution for the light field microscope," Opt. Exp., vol. 21, 2013.

Light-Field Microscopy

Computational methods

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



Light-Field Microscopy

Computational methods

- **Challenges**

- Scattering induces blur, making inversion more challenging
- Lack of ground-truth data for learning

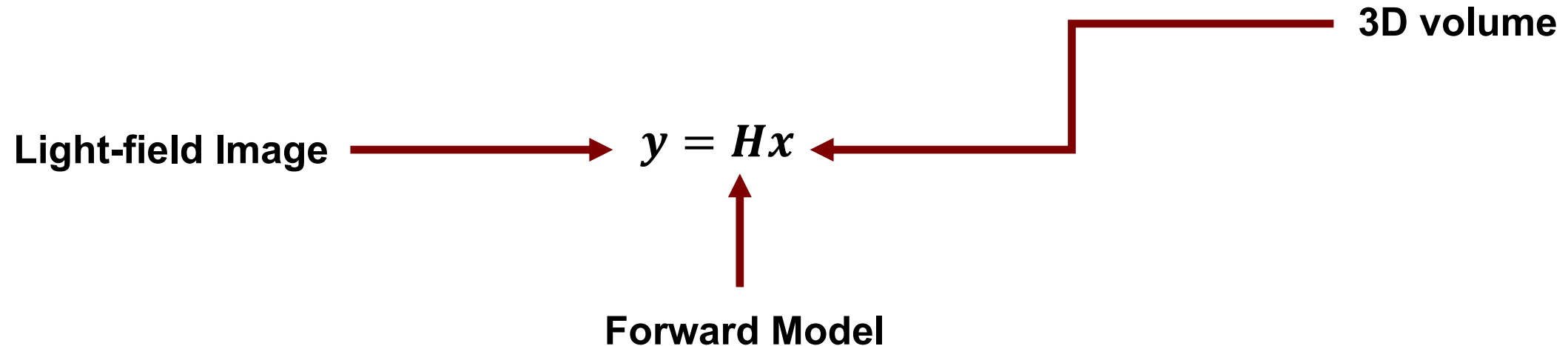
- **Opportunities**

- Forward model structured and linear
- Data is **sparse** (neurons fire rarely and are localized in space)
- Occlusion can be ignored

Light-Field Microscopy

Computational methods

- Forward model is linear which means



- Given a light field video we can express it as a matrix Y and given that the structure is fixed we can factorize it as the product in space and time: $Y=ST$

Model-Based Computational Method

Seeded Demixing (Nobauer et al. 2017)

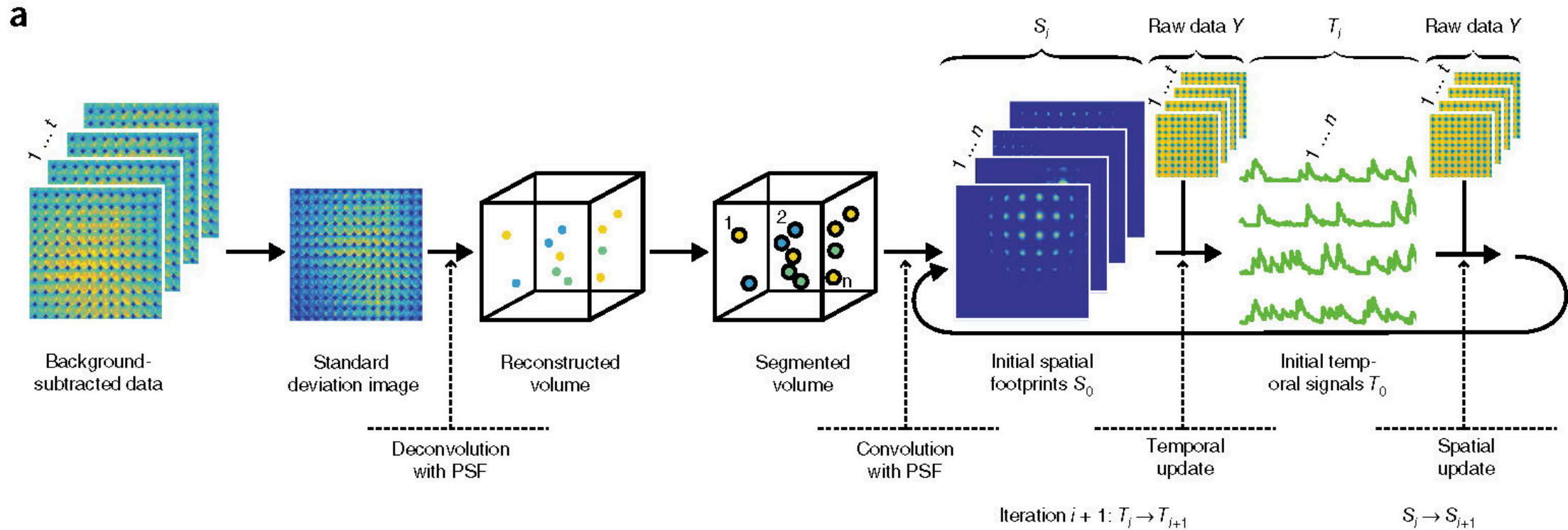


Image taken from Nobauer et al., Video rate volumetric Ca^{2+} imaging across cortex using seeded iterative demixing (SID) microscopy, Nature Methods, 2017

Model-Based Computational Method

Seeded Demixing

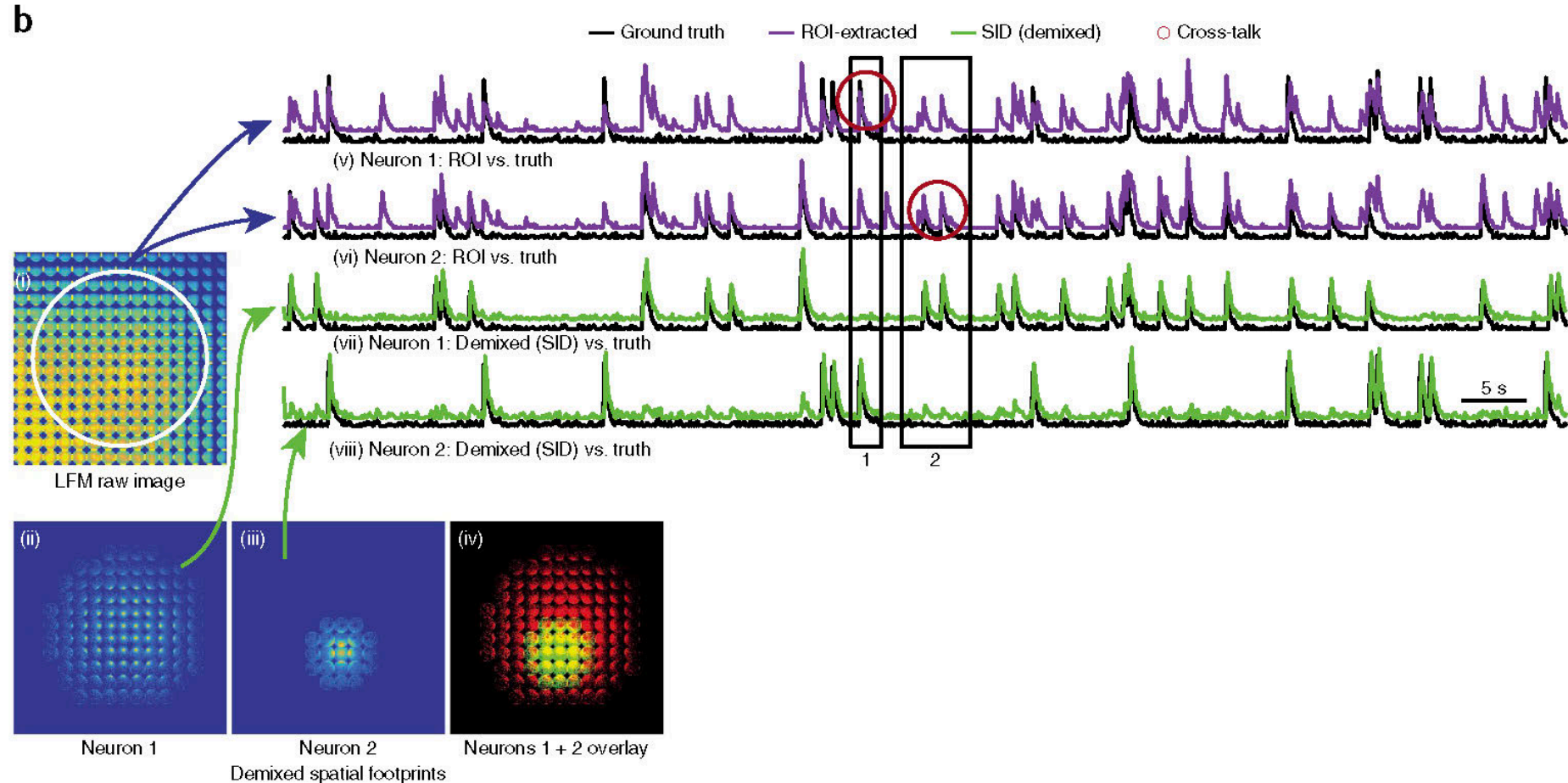
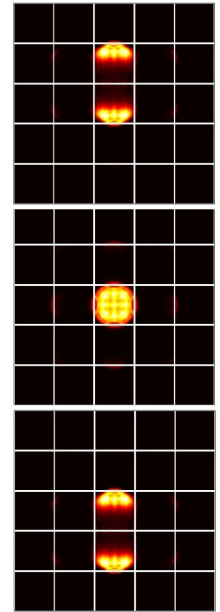
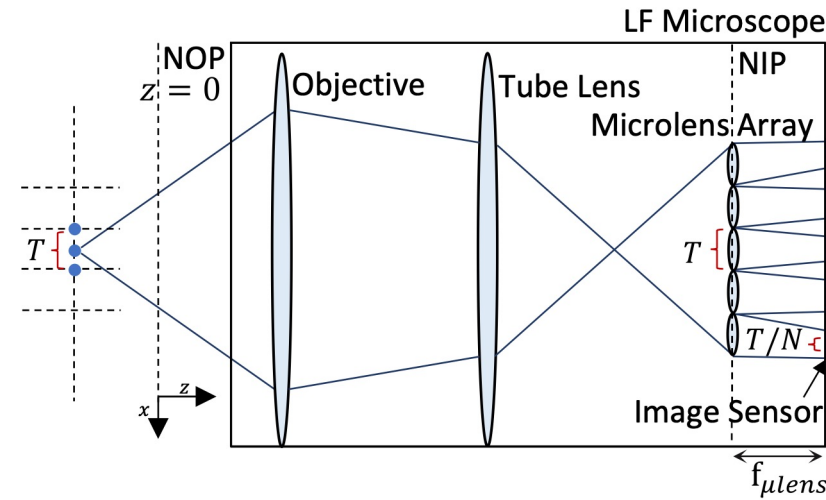


Image taken from Nobauer et al., Video rate volumetric Ca²⁺ imaging across cortex using seeded iterative demixing (SID) microscopy, Nature Methods, 2017

Learning-Based Method

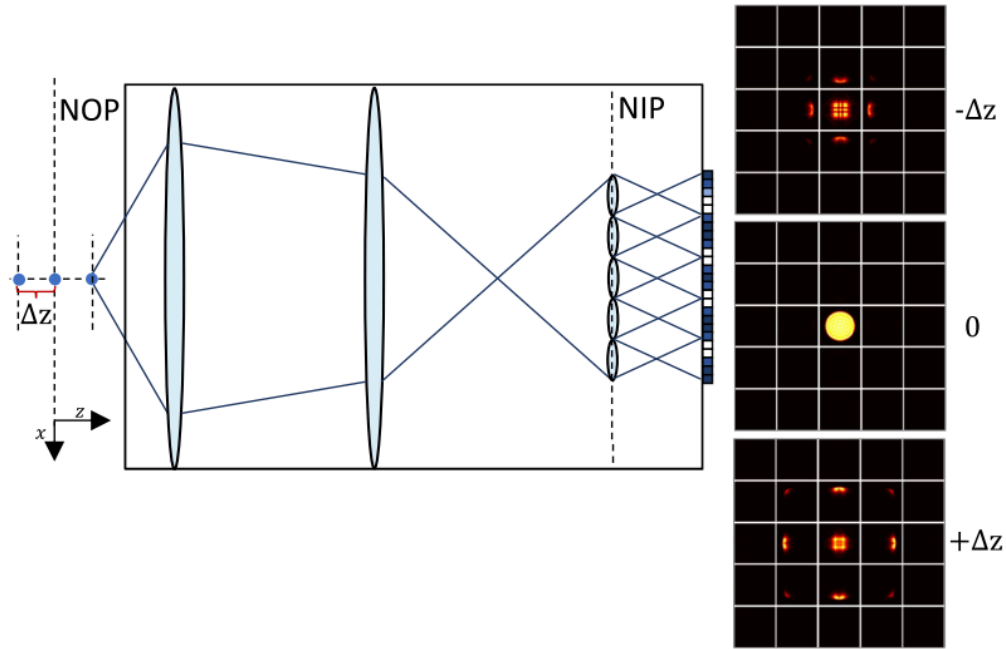
2P-informed and physics-based deep neural network (2PiLnet)

- Forward model is linear which means $\mathbf{y} = \mathbf{H}\mathbf{x}$
 - $\mathbf{H}$ is estimated using wave-optics
 - For each depth, $\mathbf{H}$ 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)



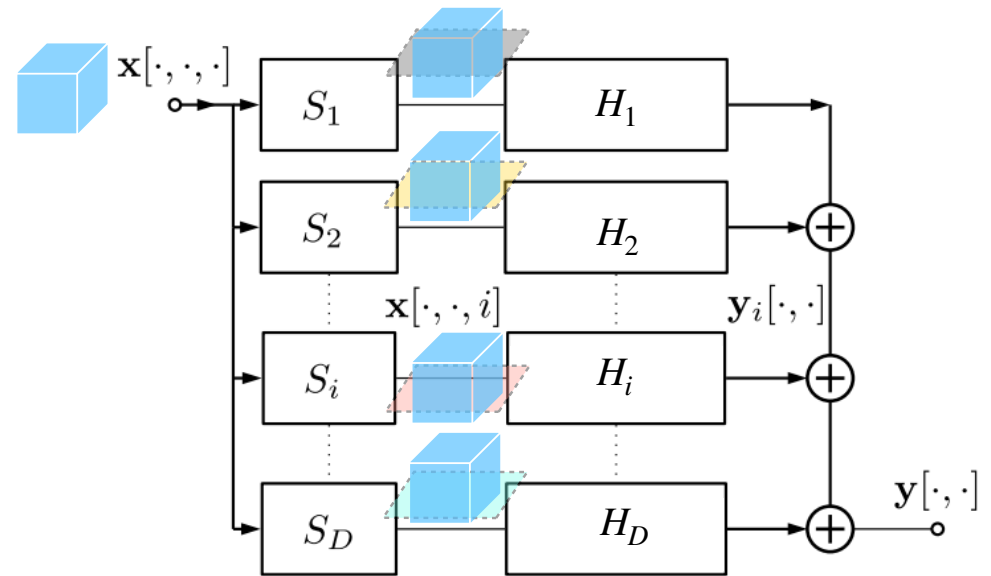
Learning-Based Method

Forward Model



- The discrete LFM system: $\mathbf{y} = \mathbf{H}\mathbf{x}$

$$\mathbf{H} = \sum_{i=1}^D \mathbf{H}_i \mathbf{S}_i$$



Learning-Based Method

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:

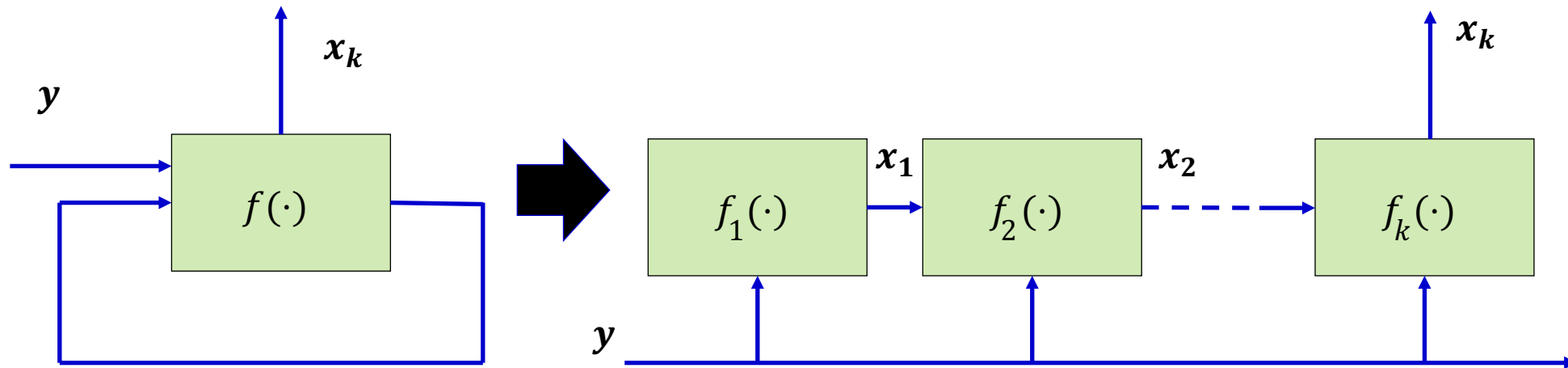
$$x_{k+1} = \text{ReLU}(x_k - H^T H x_k + H^T y + \lambda)$$

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

Learning-Based Method

Unfolding Technique

Explicit embedding of priors and constraints in deep networks



Iterative algorithm with y as input and x as output

Unfolded version of the iterative algorithm with learnable parameters

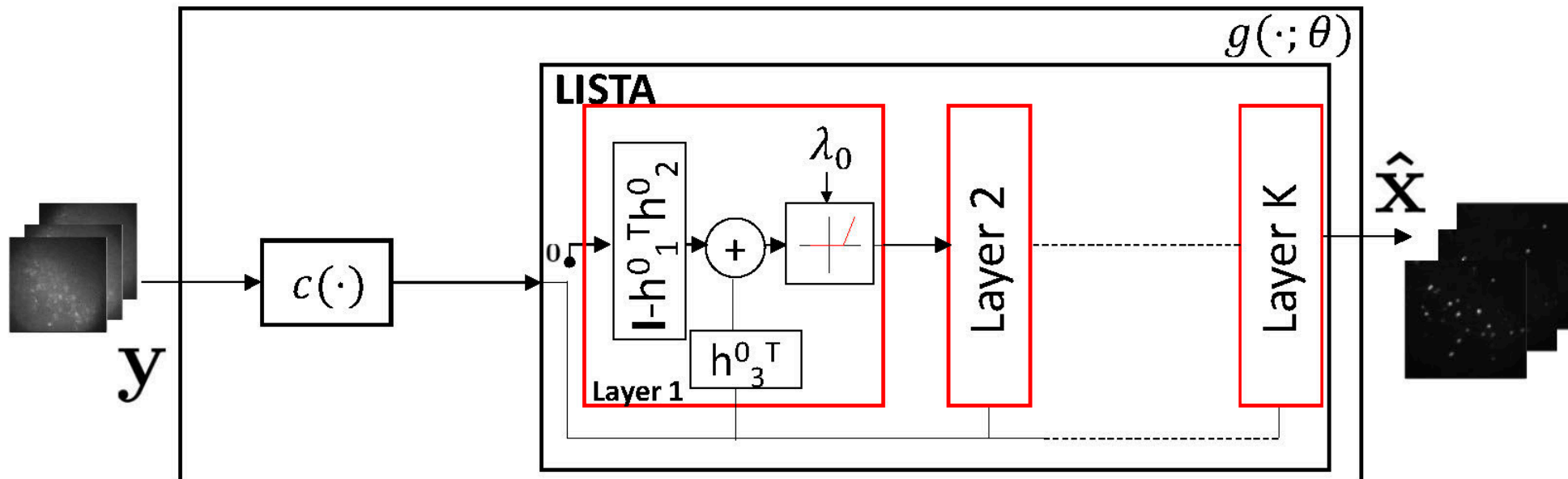
- Gregor Karol and LeCunYann, "Learning fast approximations of sparse coding ", Proceedings of the 27th International Conference on International Conference on Machine Learning, 2010
- Y. Eldar et al, "Algorithm Unrolling: Interpretable, Efficient Deep Learning for Signal and Image Processing", IEEE Signal Processing Magazine, 2021

Learning-Based Method

Network Architecture of 2PiLnet

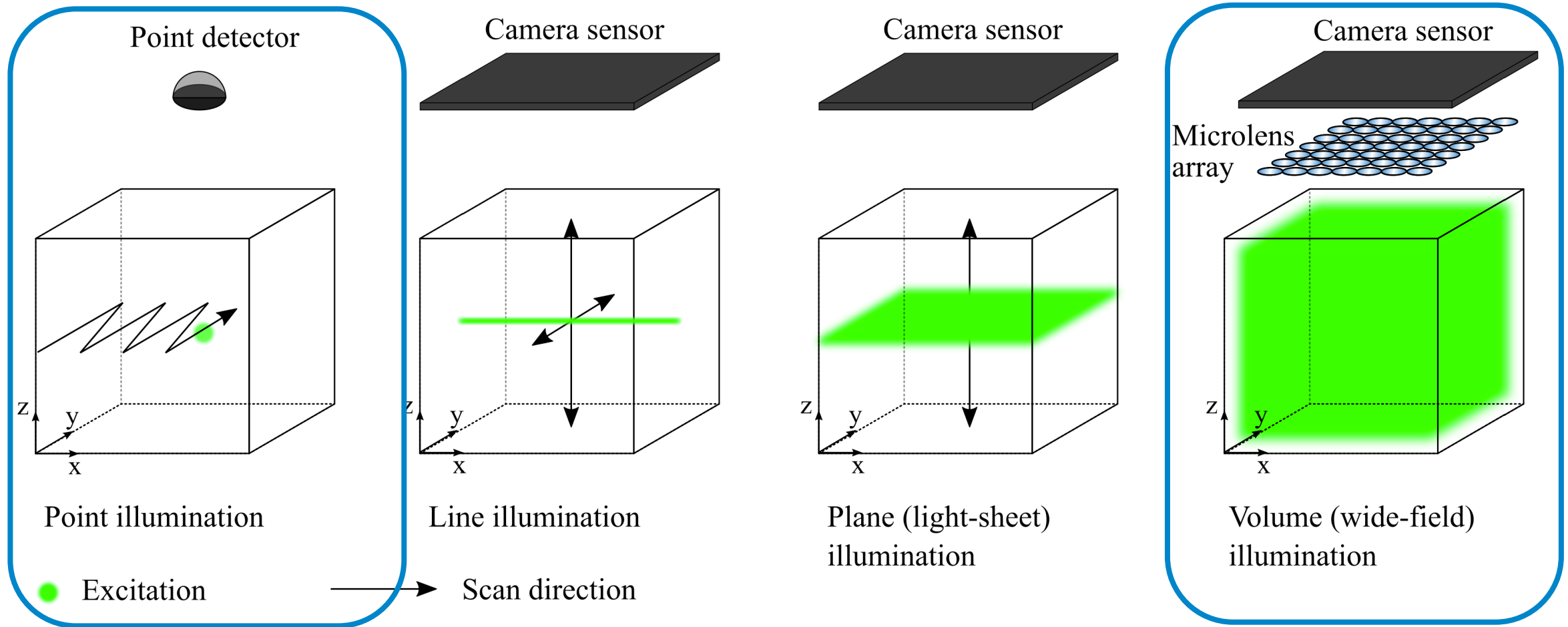
Convert the iteration in a deep neural network using the unfolding technique

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



Learning-Based Method

Data for Training

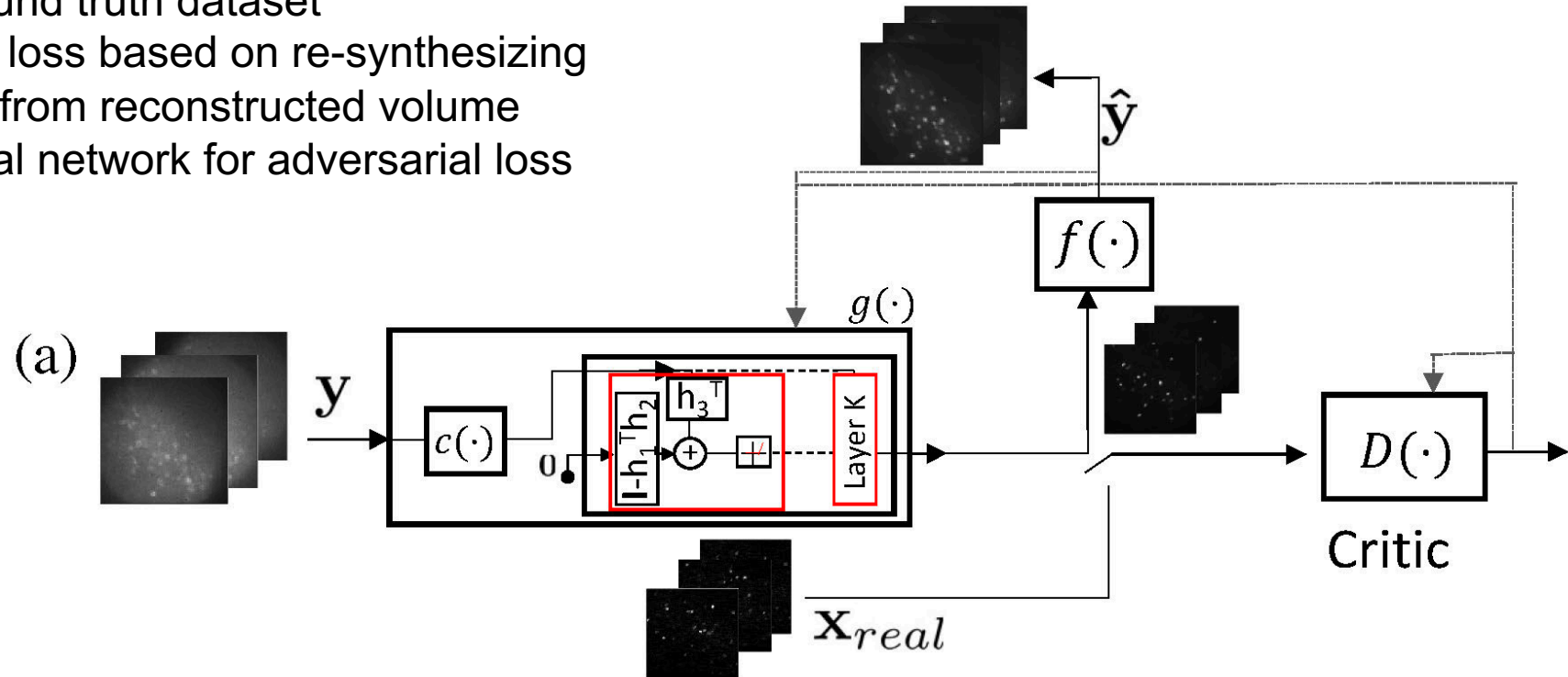


Key insight: use the 2P microscope for high-resolution structural information and the LFM for monitoring the activity of neurons.

Learning-Based Method

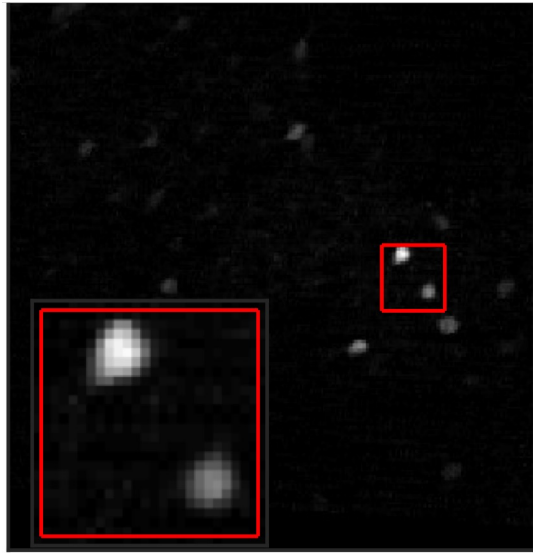
Training of the Neural Network

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

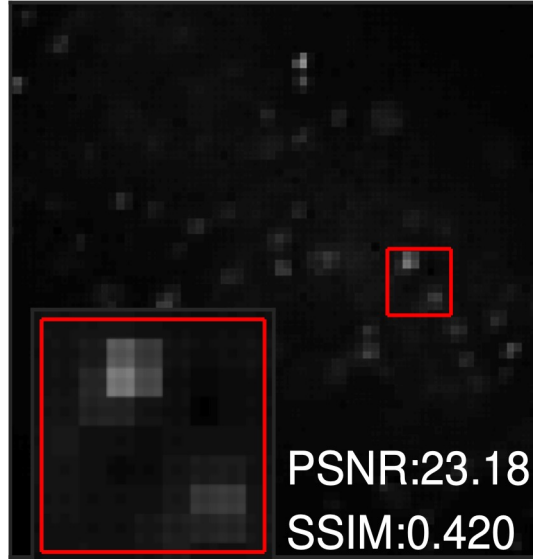


Learning-Based Method

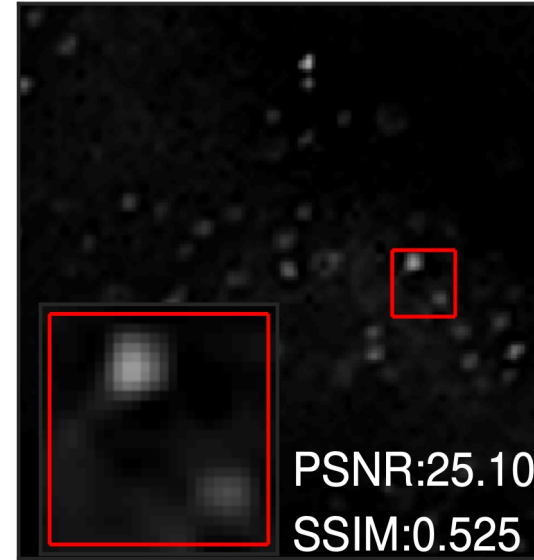
2PiLnet Results: Structural Data



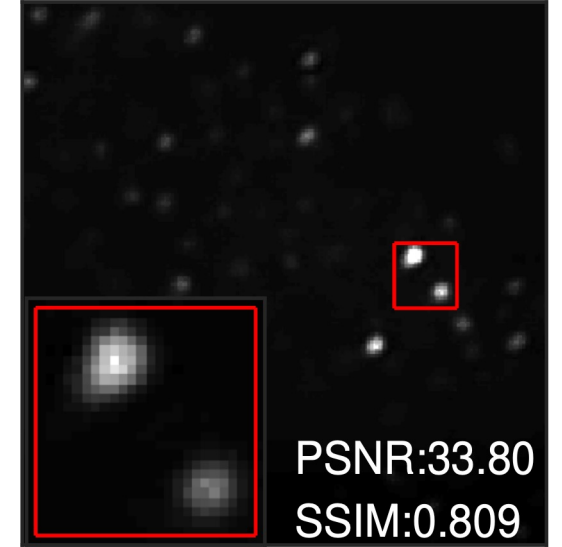
Ground-truth



ISRA



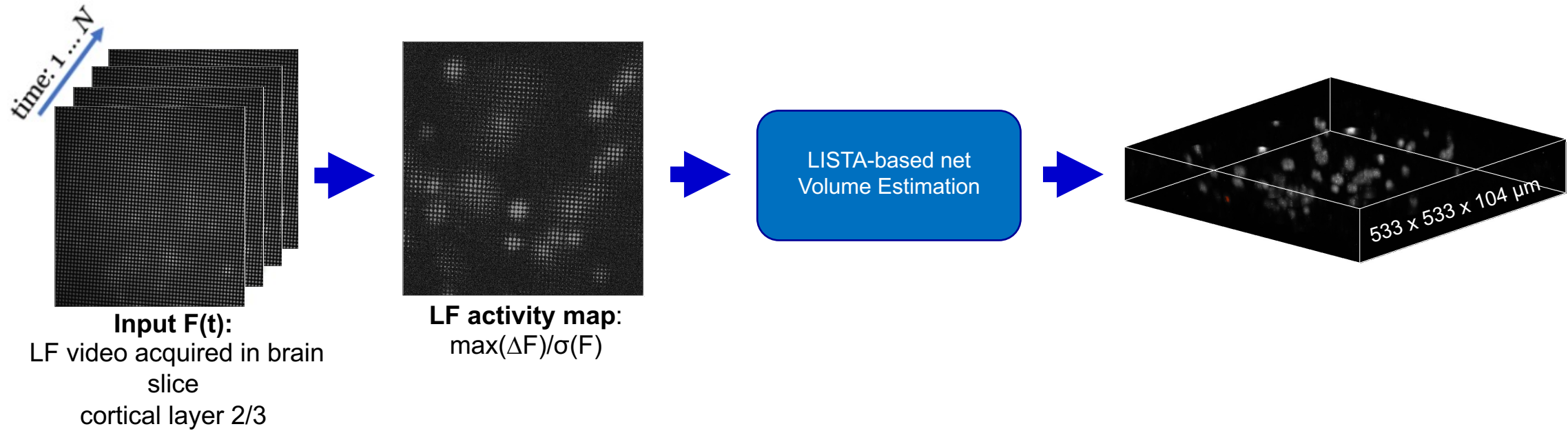
ADMM



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.

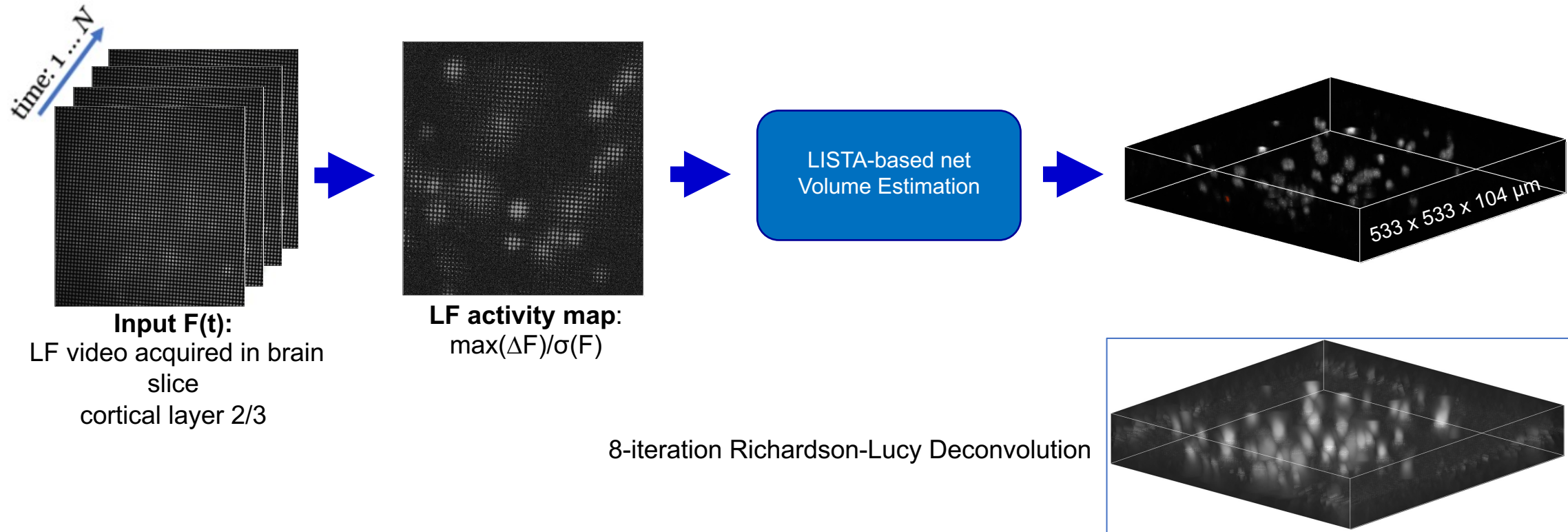
Learning-Based Method

Fast volumetric jGCaMP8f time-series extraction



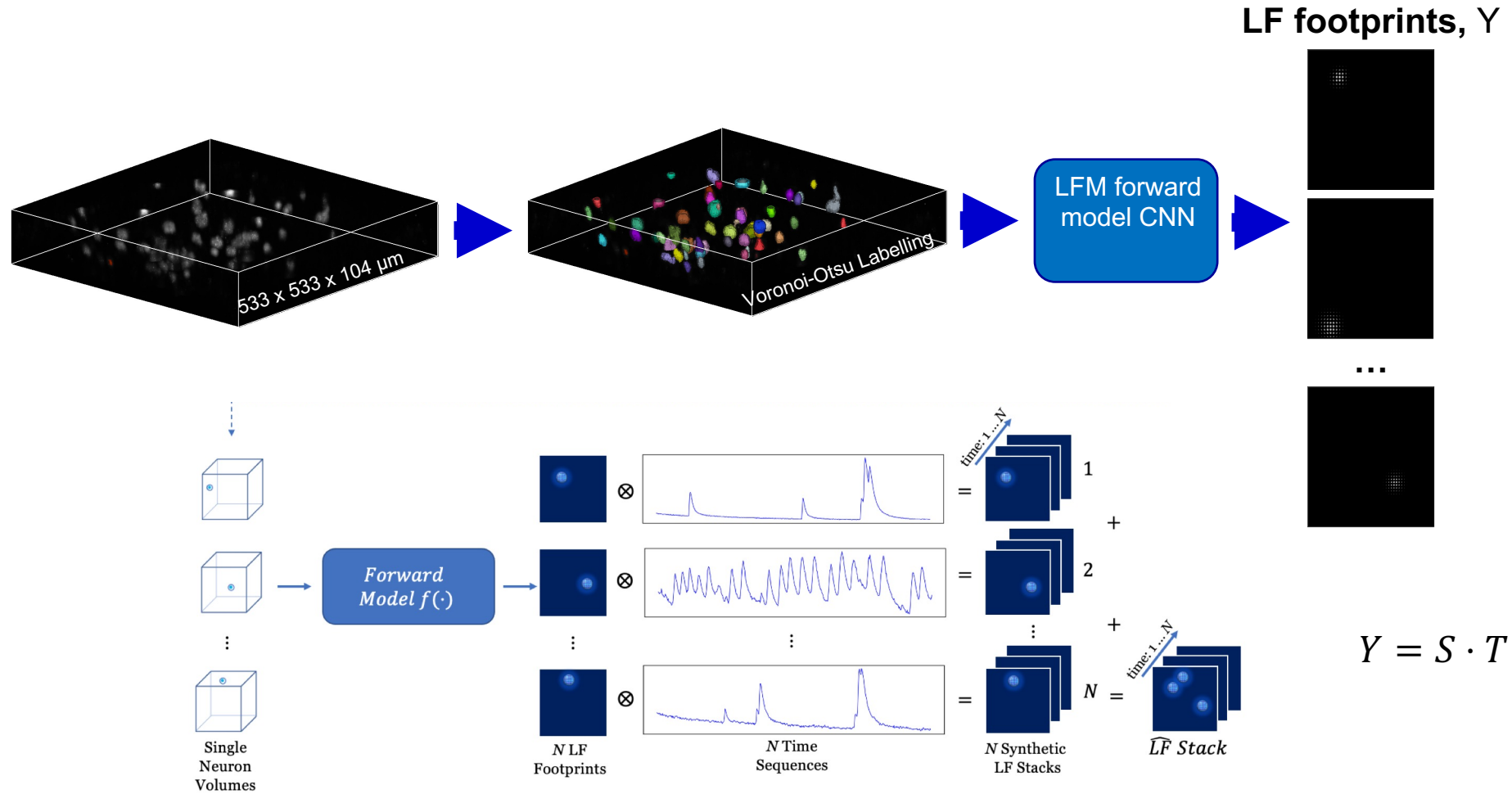
Learning-Based Method

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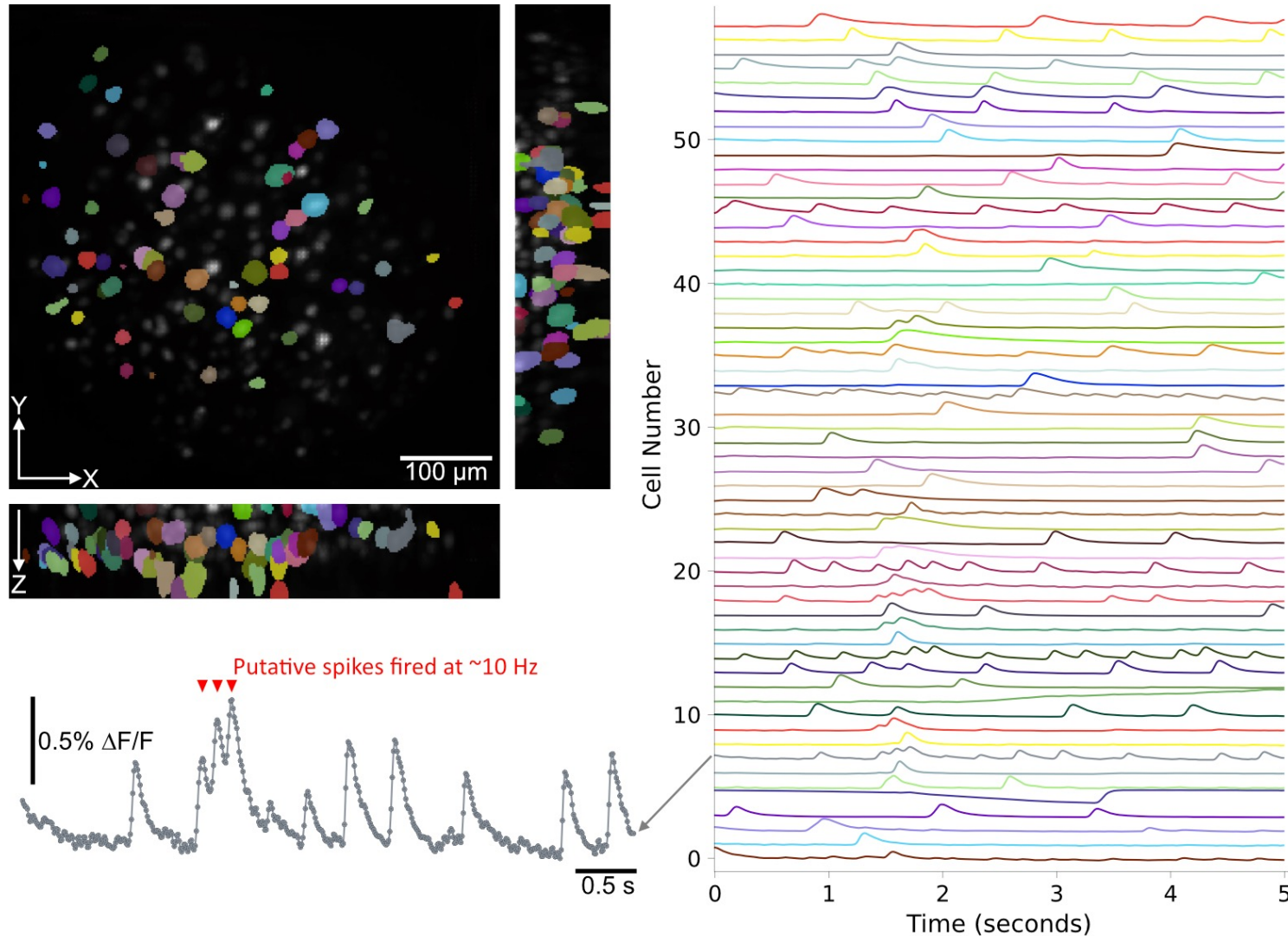
Learning-Based Method

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Learning-Based Method

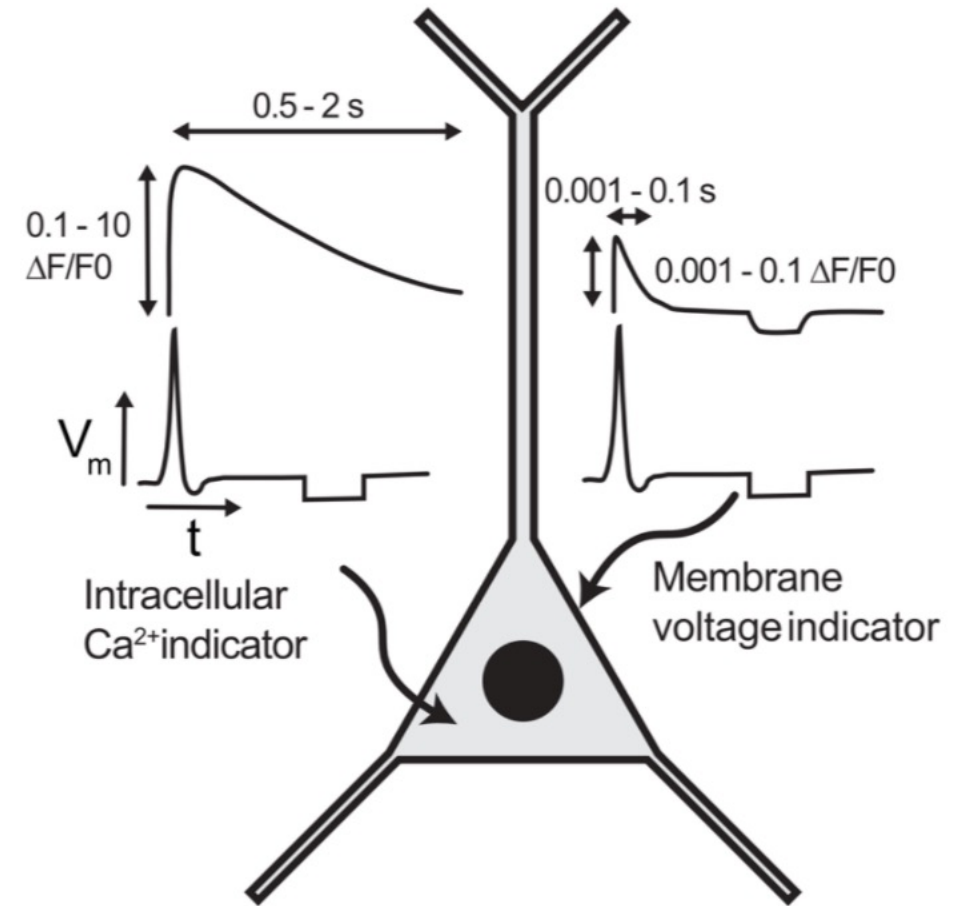
Fast volumetric jGCaMP8f time-series extraction



2PiLnet

Voltage Imaging

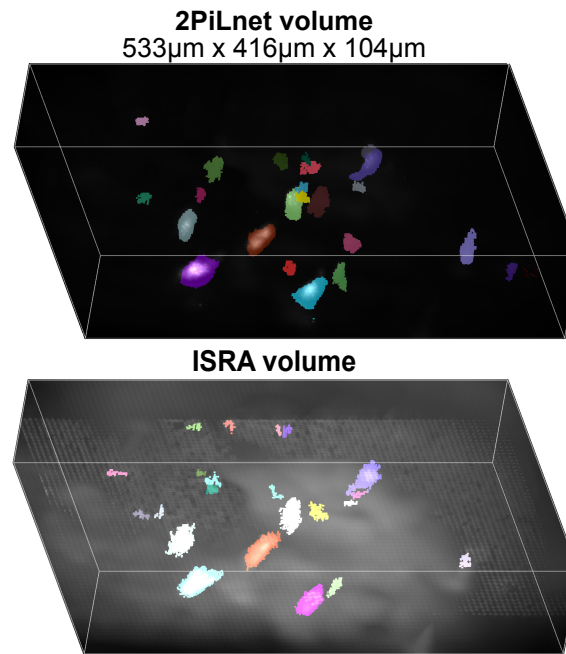
- An action potential (AP) is a “pulse” due to rapid voltage change in the neuron’s membrane and lasts 1-4ms
- In microscopy people use “**calcium indicators**” they are indirect indicators for an AP and lasts 100-400ms
- So a camera operating at 10Hz-100Hz is fast enough
- To see voltage signals, one needs to use “**voltage indicators**” but the pulse duration is then 1-4ms. So you need a camera operating at **1KHz**



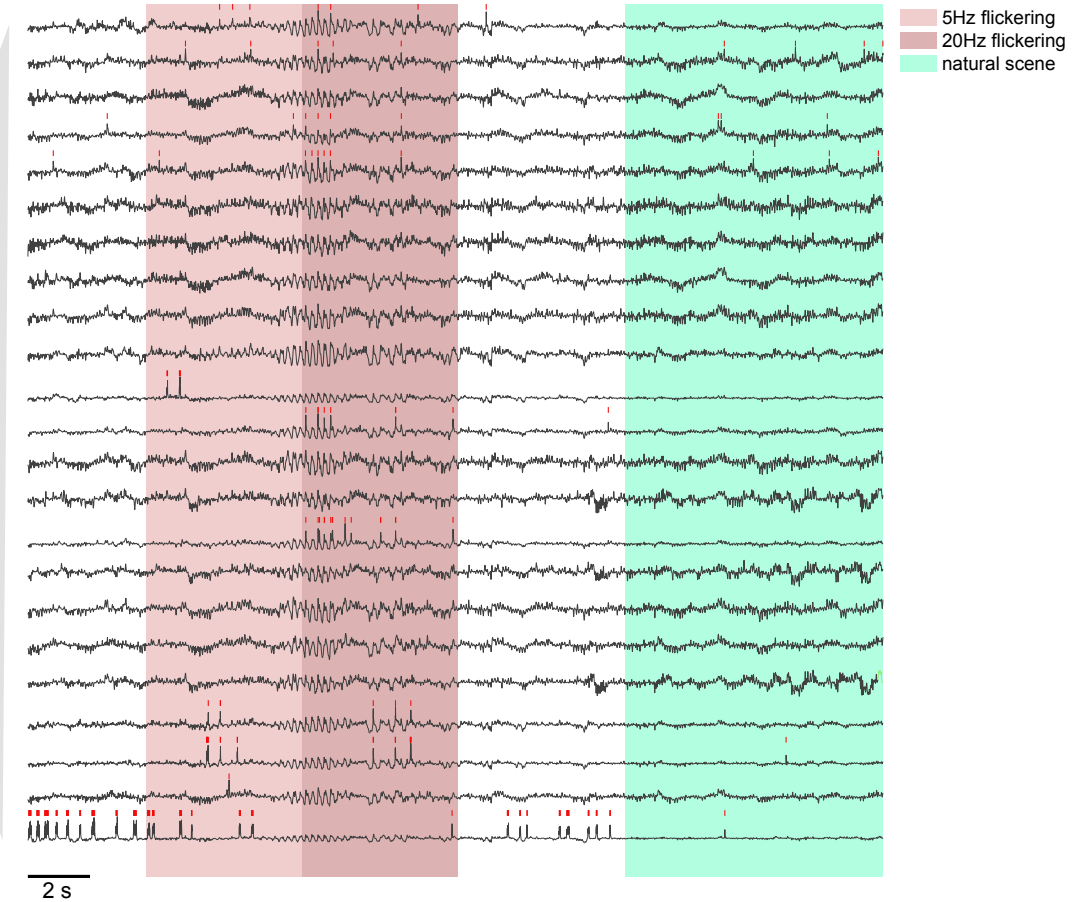
Learning-Based Method

Voltage Imaging

Lightfield field
microscopy
enables for the first
time large scale
voltage imaging at
1KHz



Neuronal voltage traces extracted from 2PiLnet volume



Conclusions

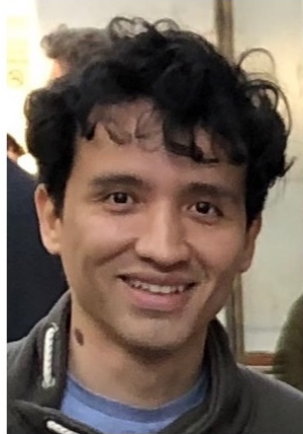
- Microscopy for neuroscience is the ideal playground for computational imaging:
 - Combine flexibility in illumination, fluorescent indicators and optics with computational methods
- In scientific imaging problems:
 - operating at the interface between maths/physics and computation is essential
 - Physics and priors regularizes neural networks
 - Structure in the problem makes computational solutions scalable
 - Lack of ground-truth data
- My message:
 - embrace computational imaging problems because they:
 - are fun 🍷🍷🍷🍷🍷
 - and inter-disciplinary

Special thanks to



Pingfan Song

Herman Verinaz



Kate Zhao



Peter Quicke



Carmel Howe

Amanda Foust



Renke Wang



And to the sponsors



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Related Publications

- C. L Howe et al. “Light-field deep learning enables high-throughput, scattering-mitigated calcium imaging”, Proceedings of the National Academy of Sciences (PNAS), 122 (48), 2025
- 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.
- P. Song et al. “Model-based explainable deep learning for light-field microscopy imaging”, IEEE Transactions on Image Processing 33, 3059-3074, 2024
- R. Wang et al., “Kilohertz In Vivo Neural Voltage Imaging with Light Field Microscopy via Physics Guided LISTA-Net Reconstruction:, Optics and the Brain, 2026
- P. Song et al. “Light-field microscopy for the optical imaging of neuronal activity: When model-based methods meet data-driven approaches”, IEEE Signal Processing Magazine 39 (2), 2022

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Thank you