Medical Imaging
Research Laboratory

Computational hyperspectral SPIM for quantitative multicolor imaging

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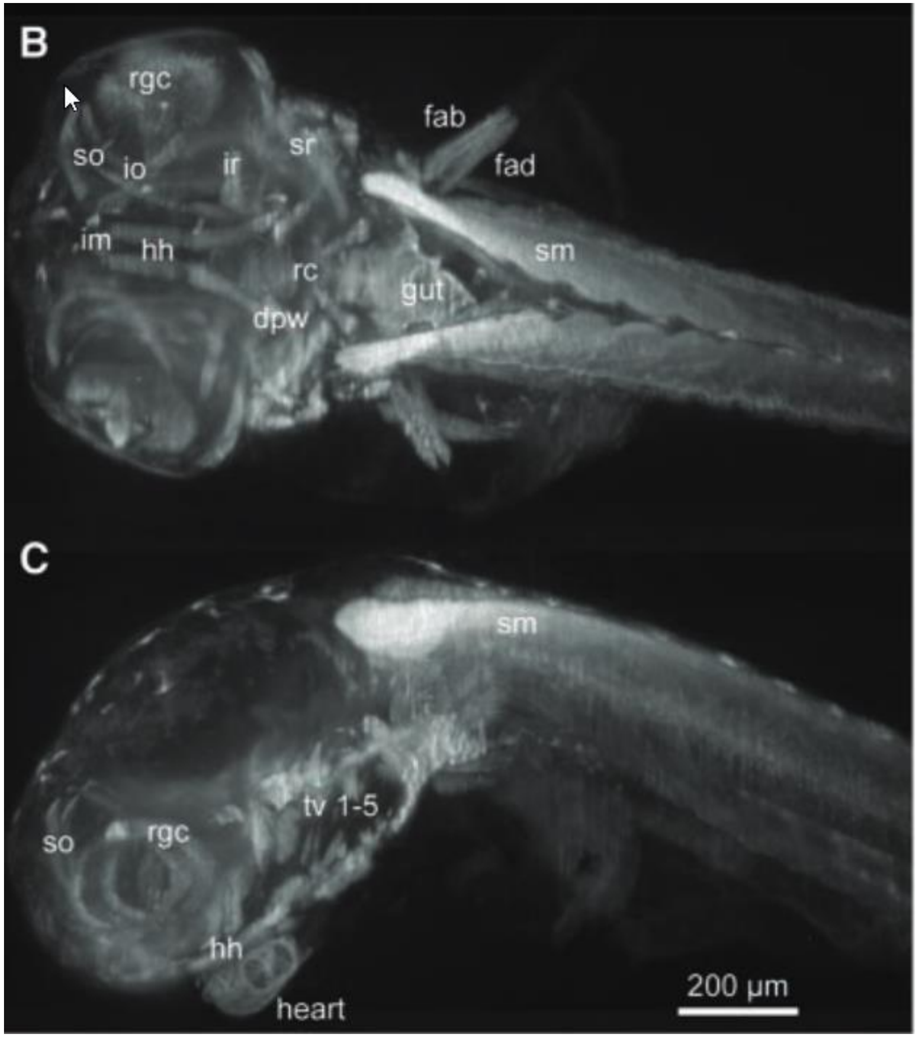
Single Plane Illumination Microscopy (SPIM)

Dorsal-ventral

lateral

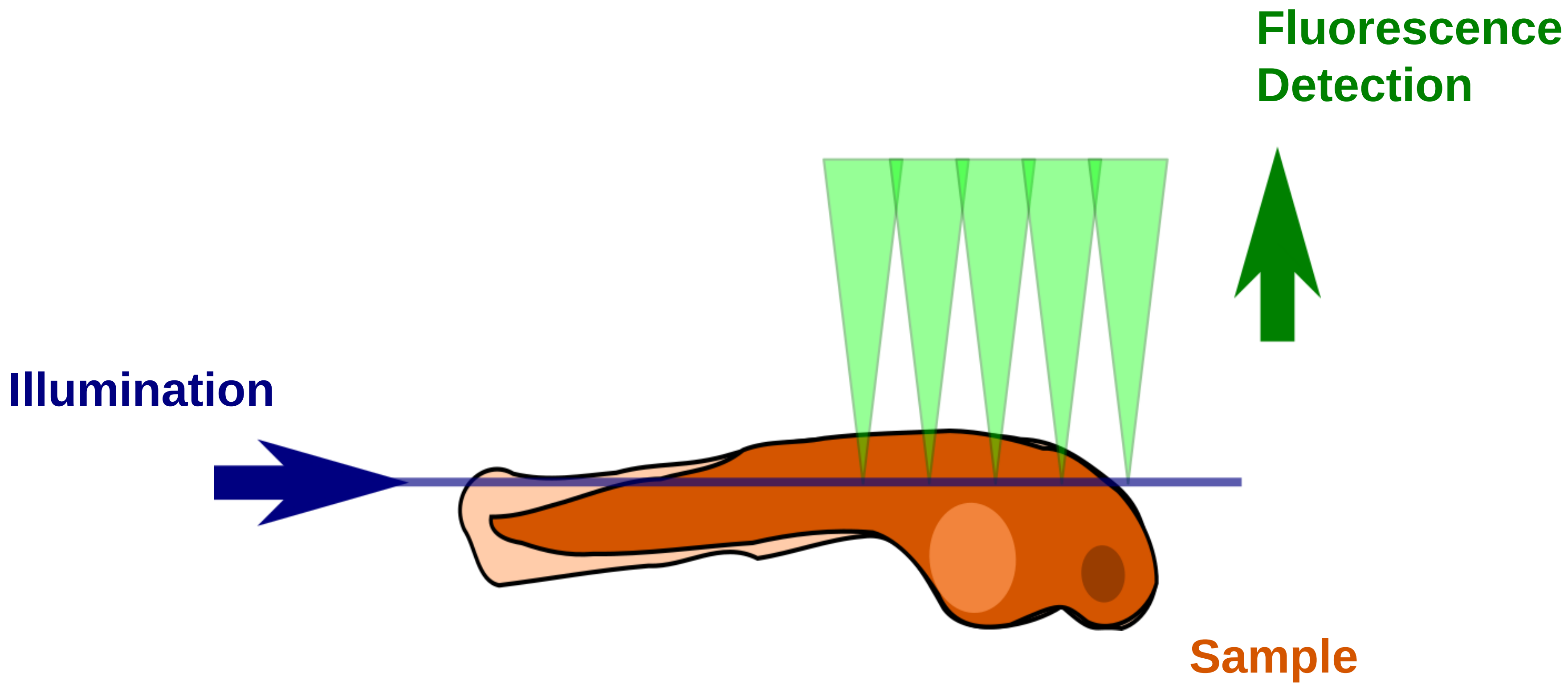


- ✓ Wide field (~mm^3),
- ✓ High res (~6 µm)
- ✓ Fast (10 fps)

- ✓ Florescence samples
- ✓ GFP-labeled transgenic embryos
- ✓ Developmental study

[J Huisken *et al*, *Science*, **305**, (2004)]

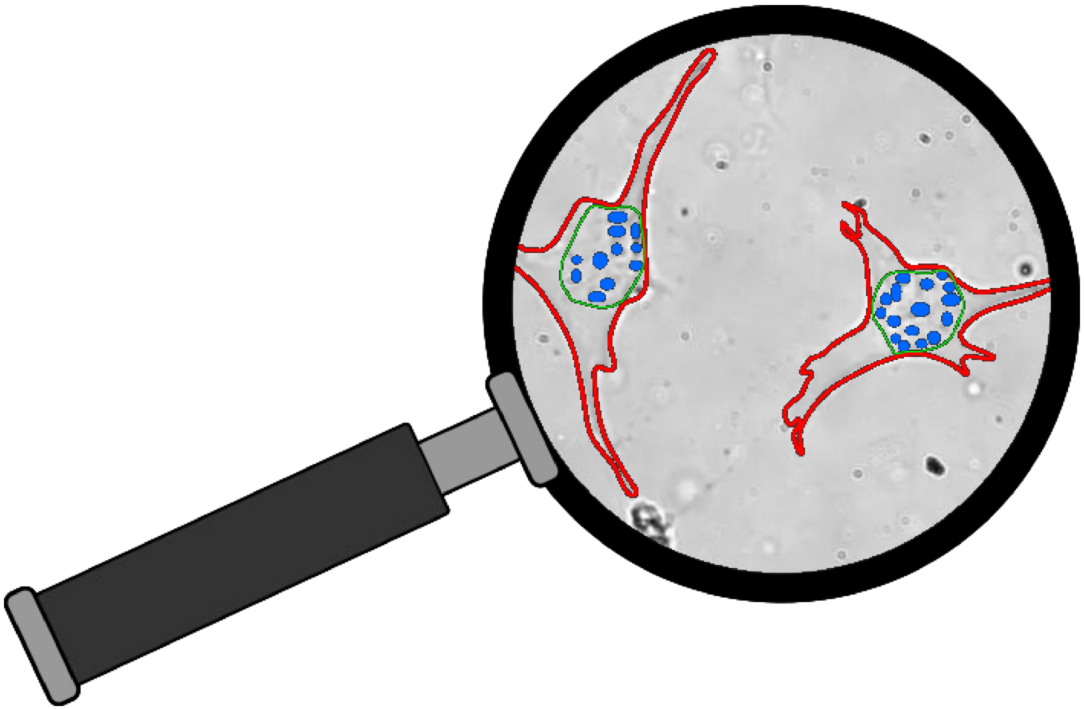
Single Plane Illumination Microscopy (SPIM)

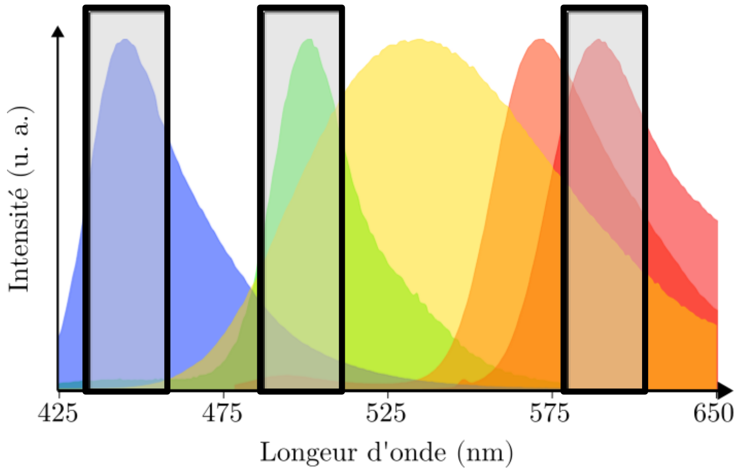


- ✓ Optical sectioning (6 µm resolution) as deep as 500 µm)
- ✓ Low photobleaching

[Image adapted from Wikimedia Commons]

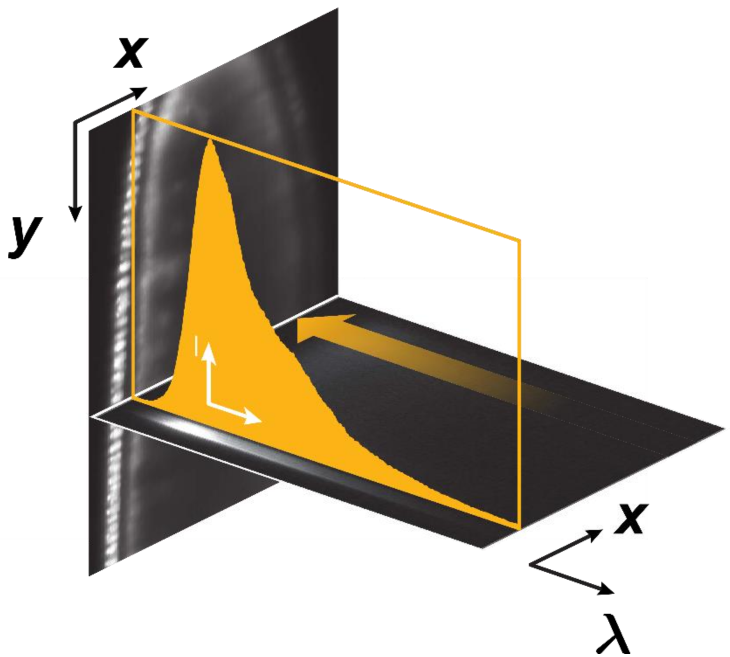
Filter-Based Fluorescence Microscopy





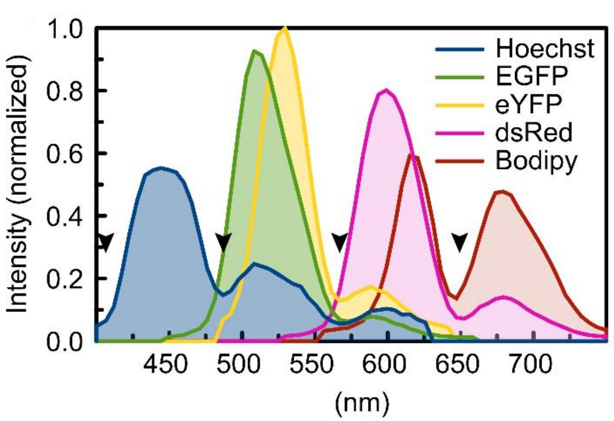
- ✓ Many photons are rejected
- ✓ Overlapping fluorophores cannot be separated
- ✓ Undesired fluorophores cannot be rejected

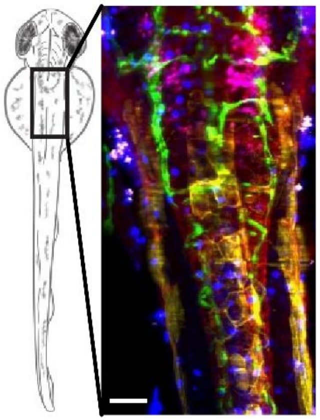
Hyperspectral SPIM



[W Jahr *et al*, *Nat. Commun.*, **6**, (2015)]

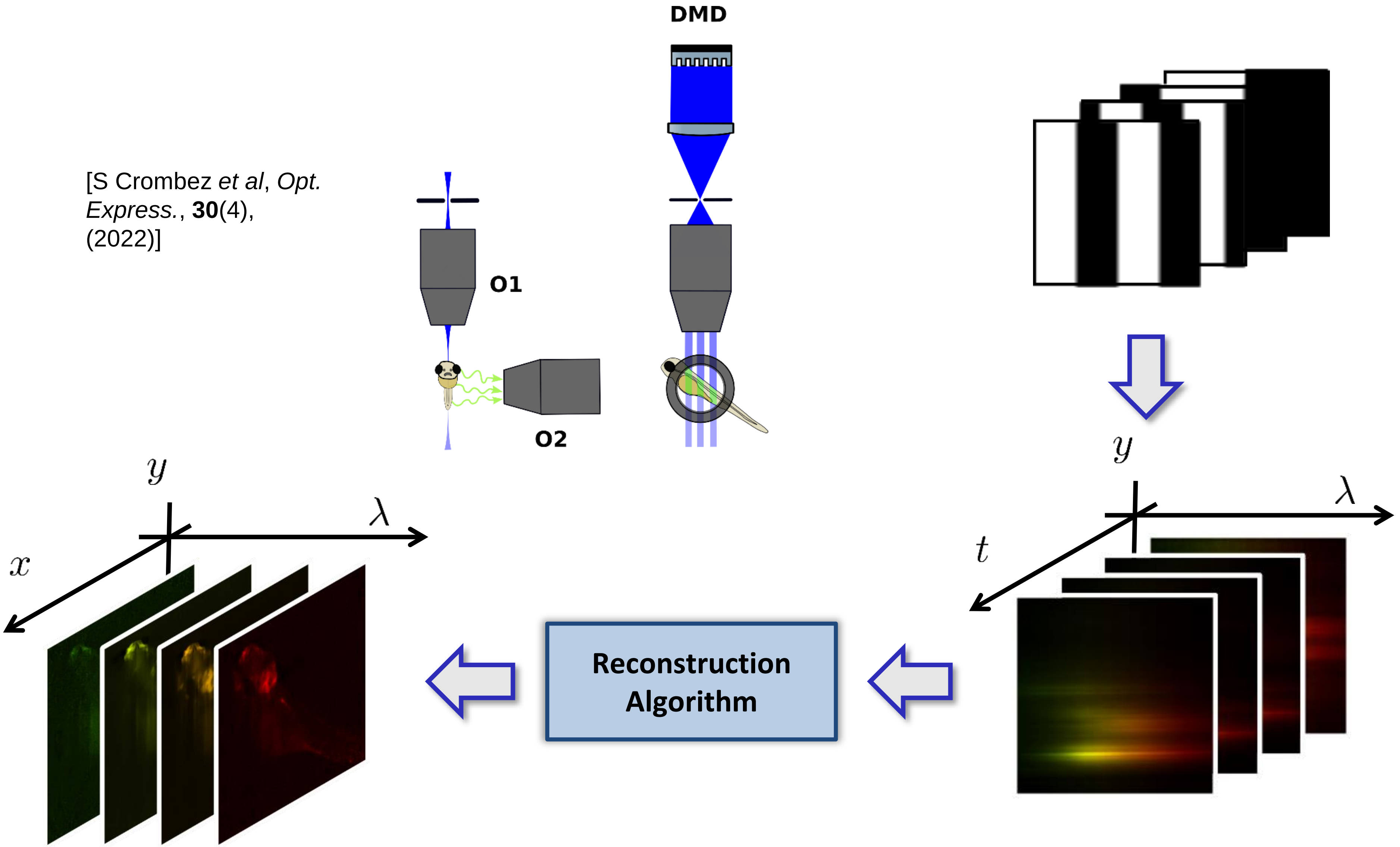
Unmixing



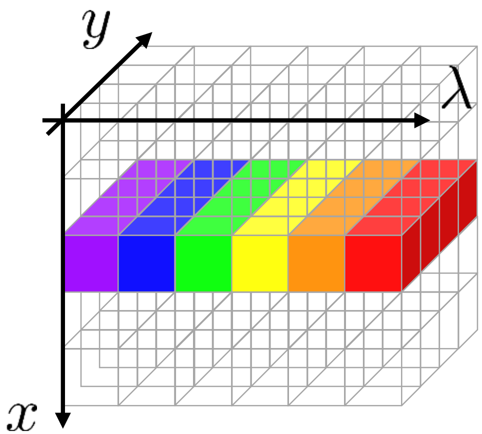


Computational Hyperspectral SPIM

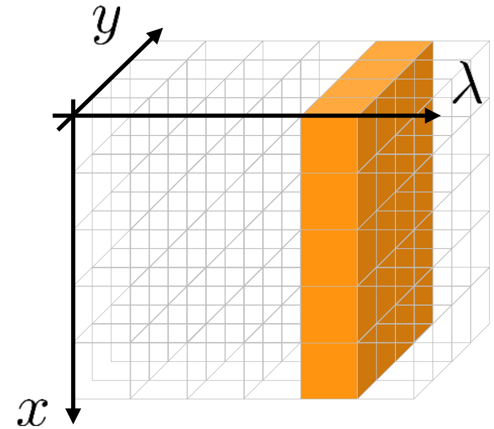
[S Crombez *et al*, *Opt. Express.*, **30**(4), (2022)]



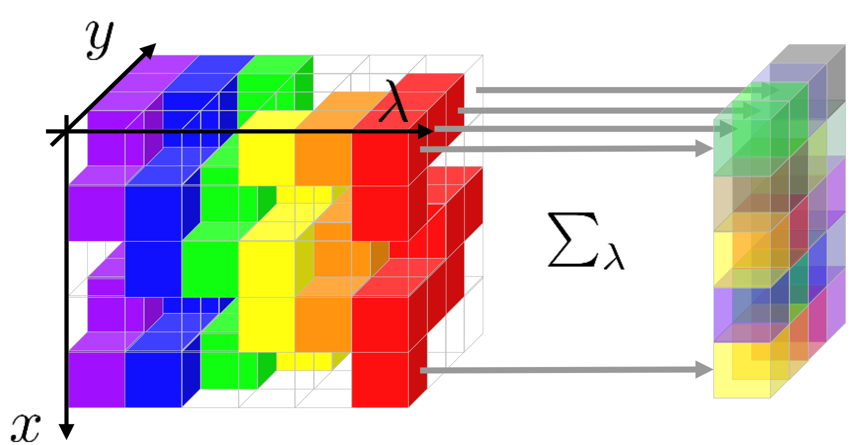
Hyperspectral Imaging



Pushbroom

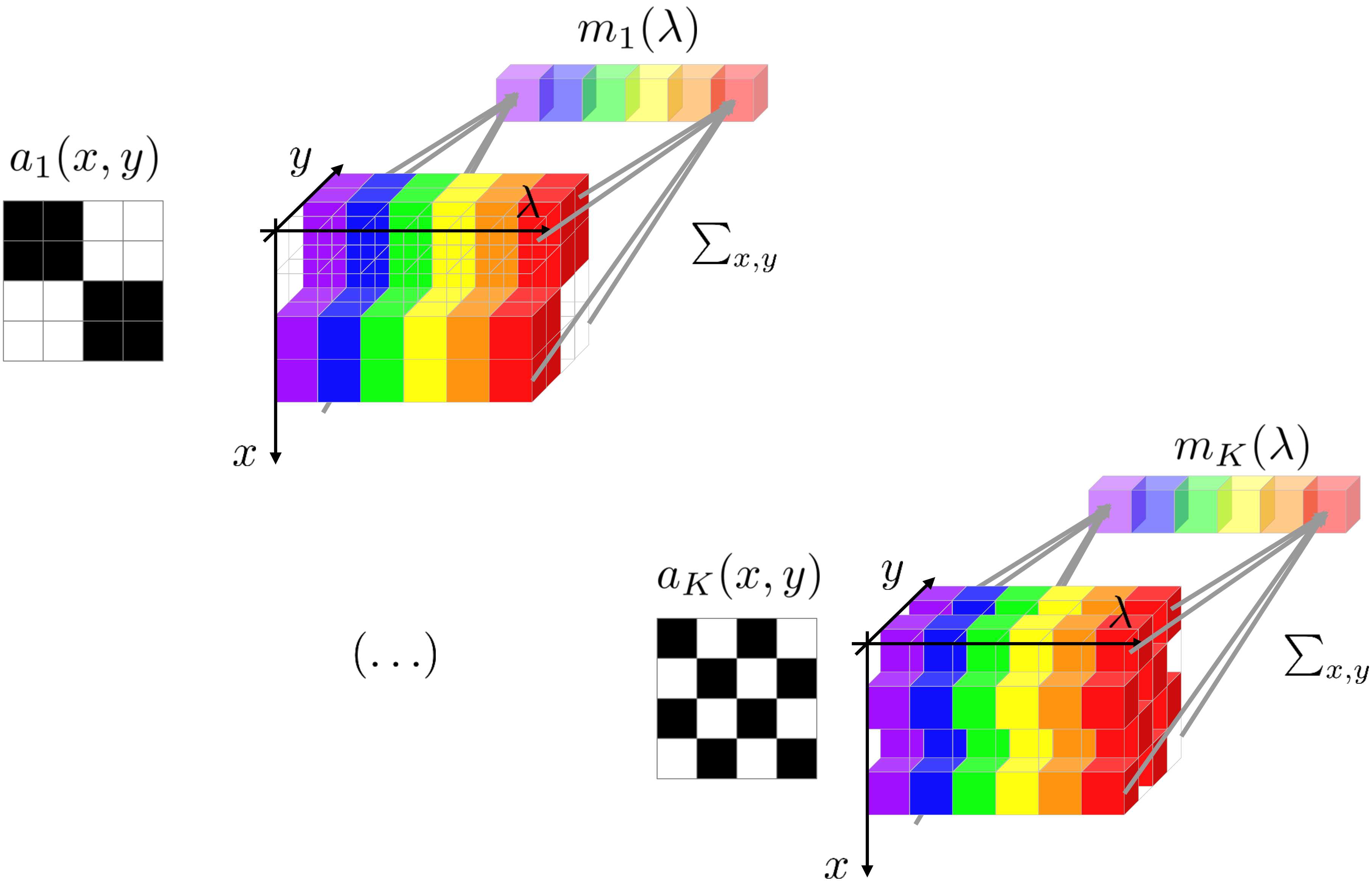


Filter-based

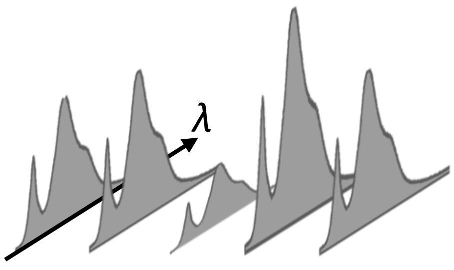


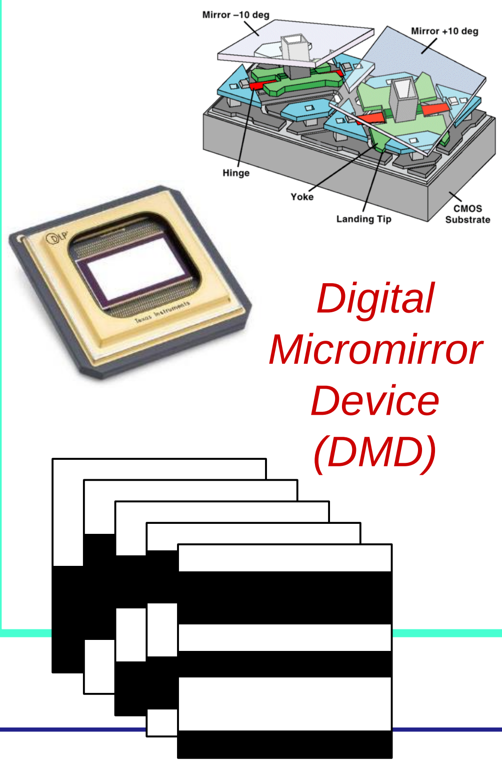
Computational

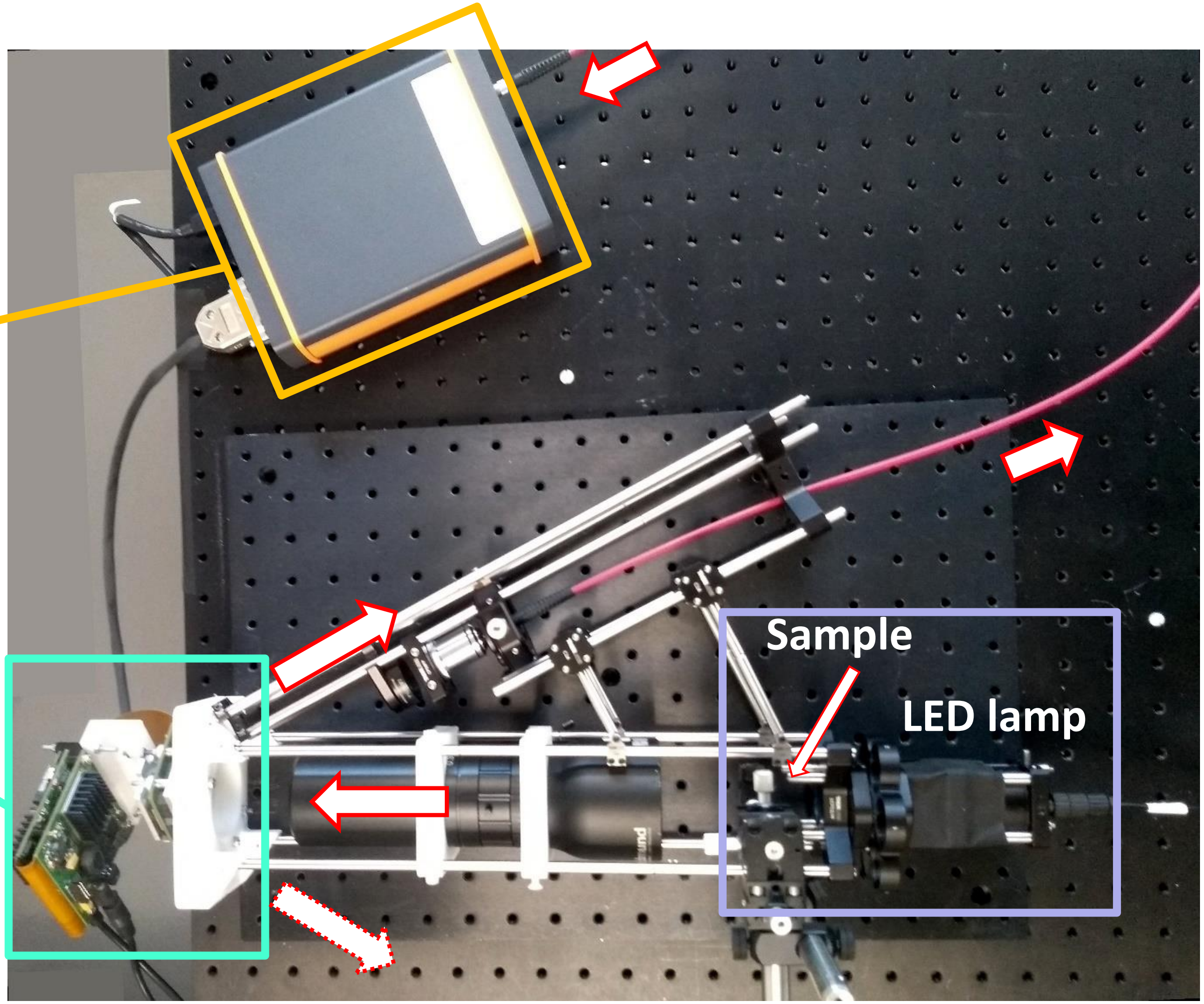
Hyperspectral Single-Pixel Imaging



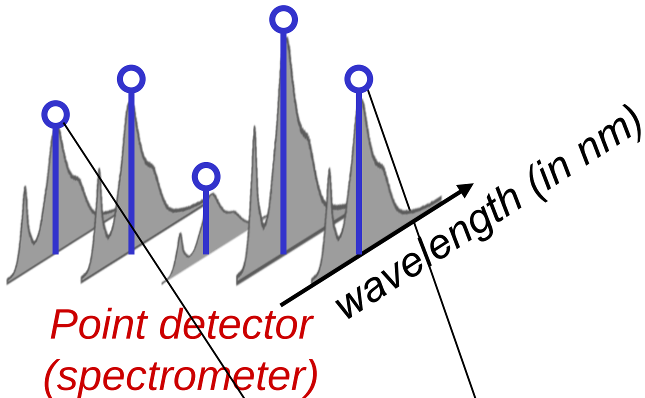
Hyperspectral Single-Pixel Imaging





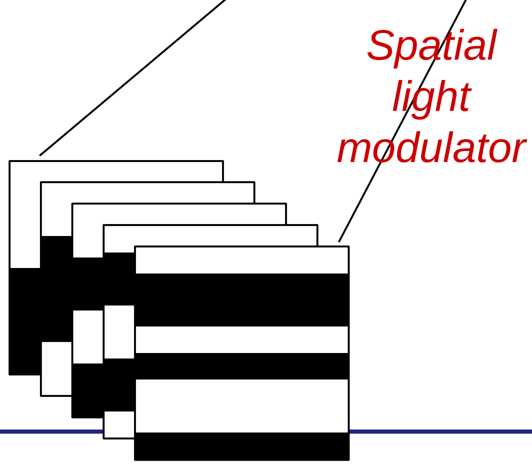


Forward model—Matrix Design—Recon



$$\boldsymbol{m}_\lambda = [m_{1,\lambda}, \dots, m_{K,\lambda}]^\top \in \mathbb{R}^K$$

$$\boldsymbol{P} = [\boldsymbol{p}_1^\top, \dots, \boldsymbol{p}_K^\top]^\top \in \mathbb{R}^{K \times N}$$



- **Linear model**

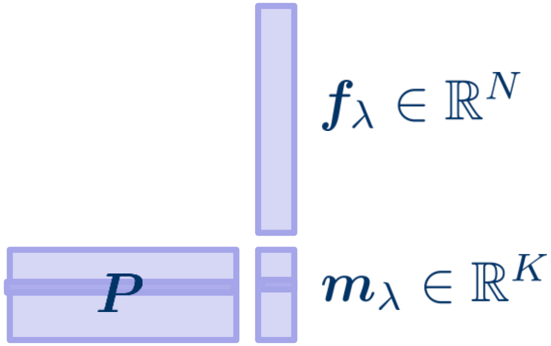
$$\boxed{\boldsymbol{m}_\lambda = \boldsymbol{P}\boldsymbol{f}_\lambda}$$

1. "Weight design": How to choose the patterns *P*?
2. Reconstruction: How to recover the image *f*?

The Compressed Sensing Answer

- **Fast acquisitions**
  - Sequential measurements lead to long acquisition times
  - → Limit to a few patterns

$K \ll N$



- Compressed sensing
  - Choose a “random” ***P***
  - Recover ***f*** from ***m*** via (constrained/L1) optimization

$$\min_{\boldsymbol{f}} \|\boldsymbol{m} - \boldsymbol{P}\boldsymbol{f}\|_2^2 + \alpha \mathcal{R}(\boldsymbol{f})$$

  - Total Variation (TV)

$$\mathcal{R}_{\mathrm{TV}}(\boldsymbol{f}) = \|\nabla \boldsymbol{f}\|_1$$

… which requires iterative algorithms

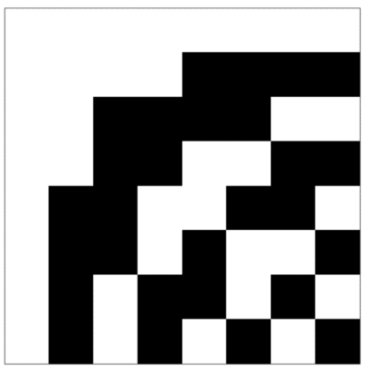
→ Fast acquisitions: long reconstructions!

256 × 256 image
M = 6,500 random measurements

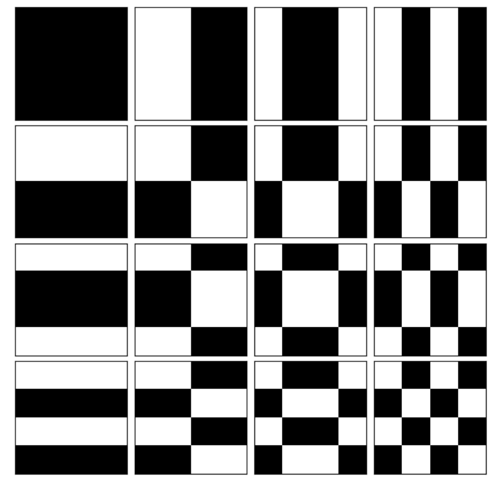
[Duarte *et. al*, IEEE SPM, 2008]

The Old Man Answer

- **In the case $K = N$, choose Hadamard patterns**



$$K = N = 8$$



$$K = N = 4^2$$

$$\boldsymbol{P}^\top \boldsymbol{P} = N\boldsymbol{I}$$

The Old Man Answer

- **Hadamard patterns are optimal for additive white Gaussian noise**

$$m_i \sim \mathcal{G}(\mu = 0, \sigma^2) \qquad 1 \le k \le N$$

- Raster scan

$$\text{var}\left(f_n^*\right) = \sigma^2$$

1	0	0	0
0	1	0	0
0	0	1	0
0	0	0	1

$$\boldsymbol{P}^\top \boldsymbol{P} = N\boldsymbol{I}$$

$$\boxed{\boldsymbol{f} = \frac{1}{N}\boldsymbol{P}^\top \boldsymbol{m}}$$

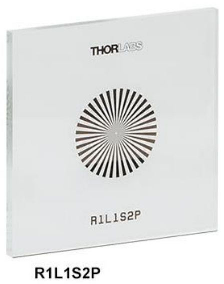
- Hadamard

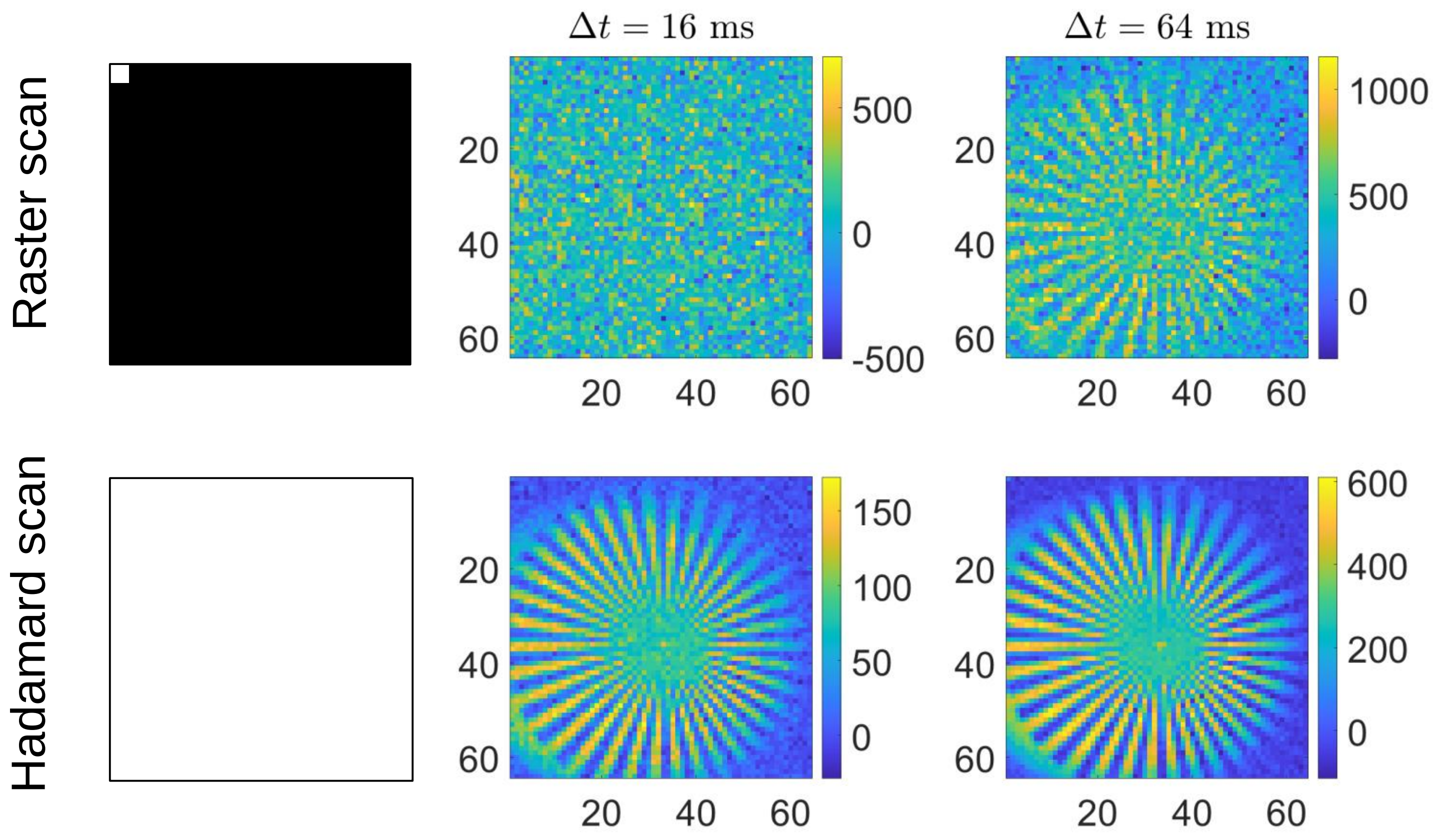
$$\text{var}\left(f_n^*\right) = \frac{1}{N}\sigma^2$$

+	+	+	+
+	+	-	-
+	-	-	+
+	-	+	-

The Felgett's Advantage

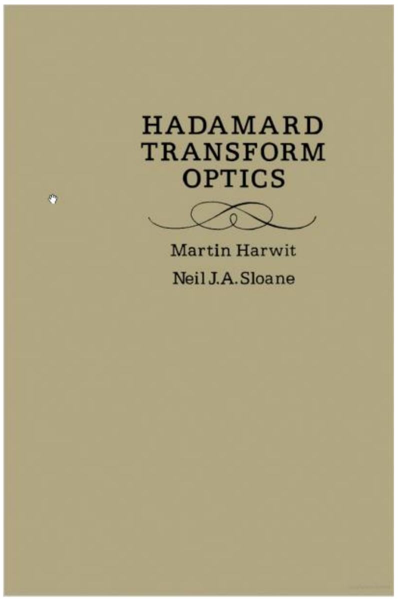
➢ Hadamard optimality, a. k. a. Felgett's advantage





[N. Ducros *et al.*, working paper, 2021]

The Felgett's Advantage for Spectroscopy



M. Harwit,
Cornell University,
Center for Radiophysics and Space Research

N. Sloane,
Bell Laboratories

(**1979**)

INLET CODING DISK

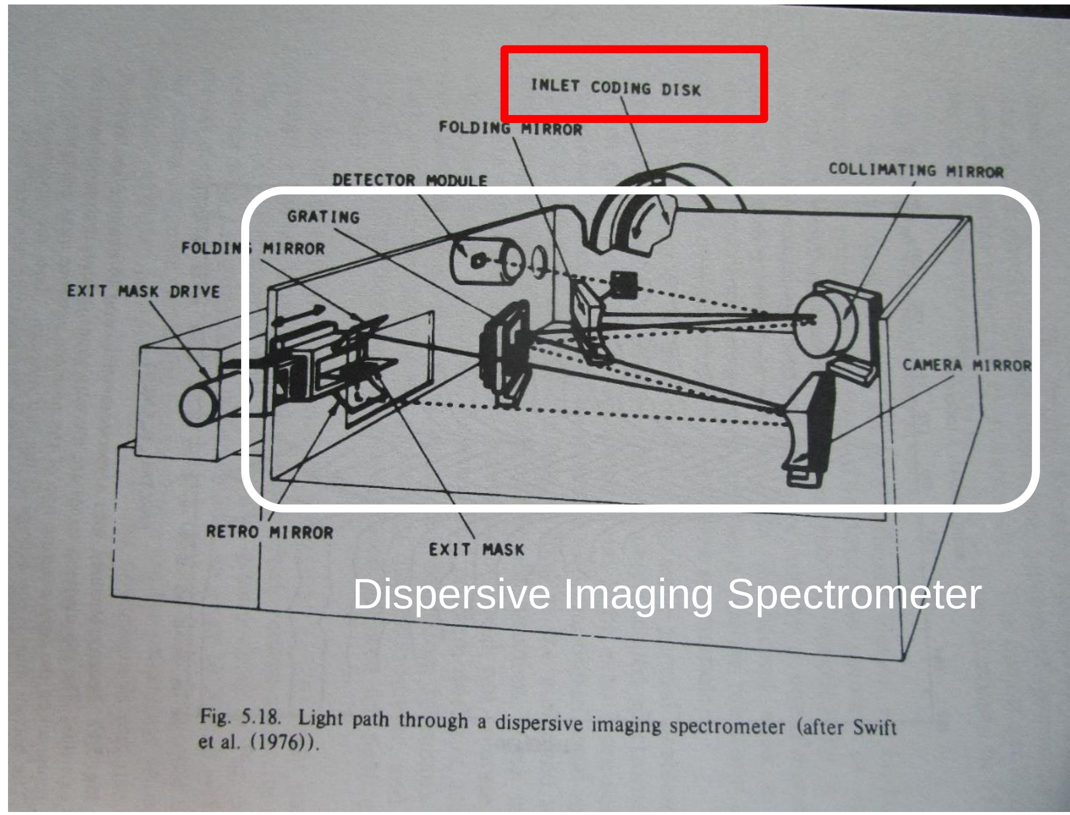
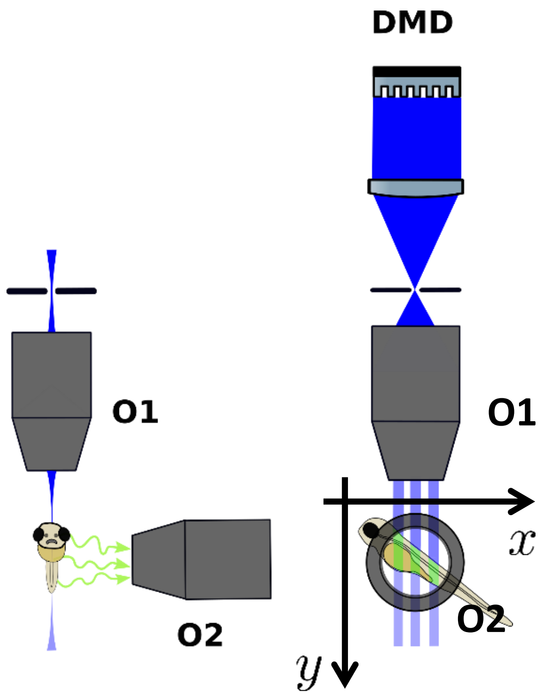
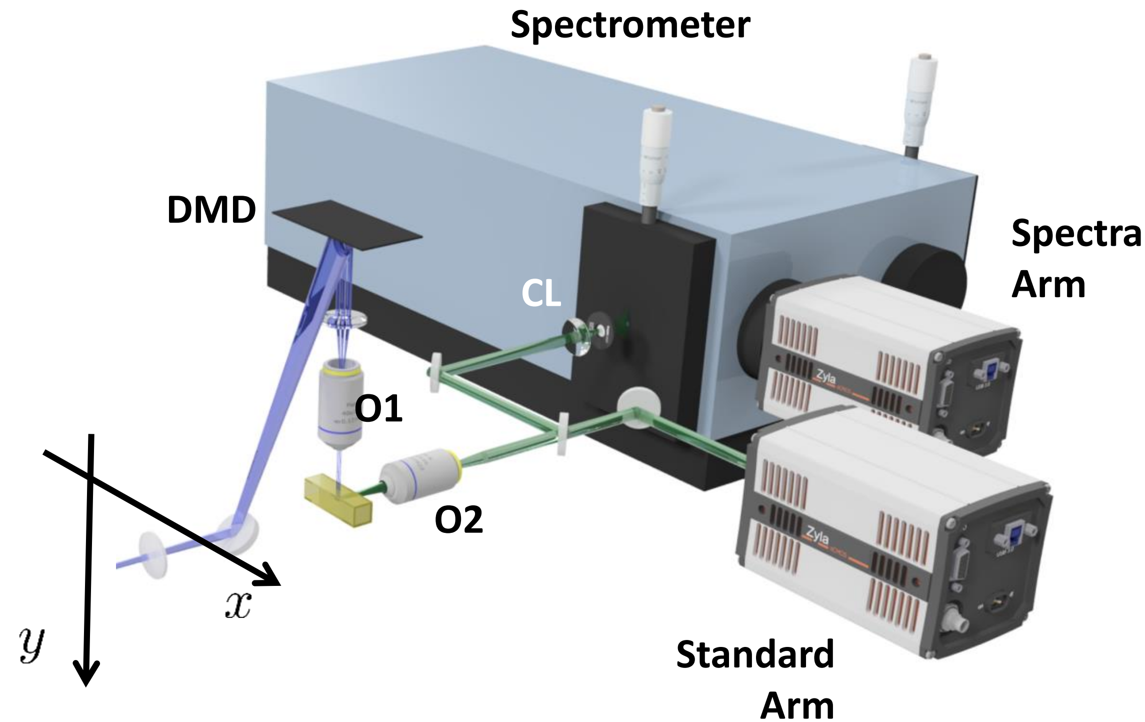


Fig. 5.18. Light path through a dispersive imaging spectrometer (after Swift et al. (1976)).

✓ Only one spatial dimension should be compressed, not two.

Computational Hyperspectral SPIM





$$m_k(y,\lambda) = \int p_k(x) f(x,y,\lambda)\,\mathrm{d}x, \quad 1 \leq k \leq K$$

- ✓ Felgett's advantage (contrary to original hspim)
- ✓ No extra compression (as in hspi)

Computational Hyperspectral SPIM

- **Image formation model**

$$M_\lambda = PF_\lambda + E, \quad 1 \le \lambda \le \Lambda,$$

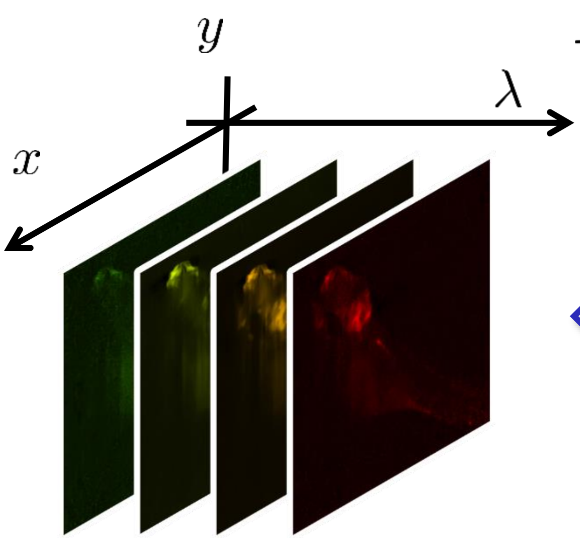
K x N_y measurement

K x K pattern matrix

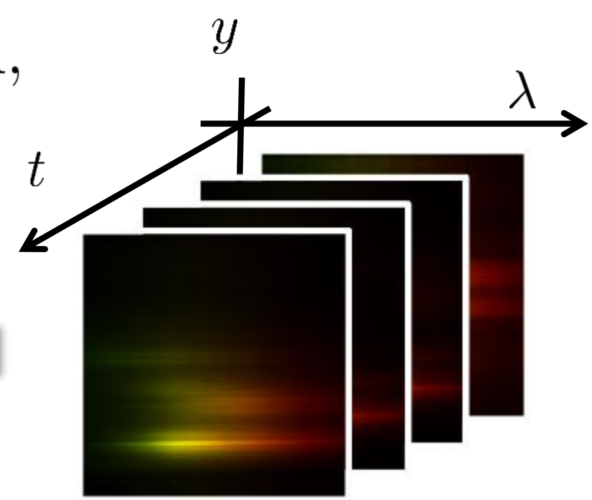
K x N_y hypercube slice

- **(Basic) Image reconstruction**

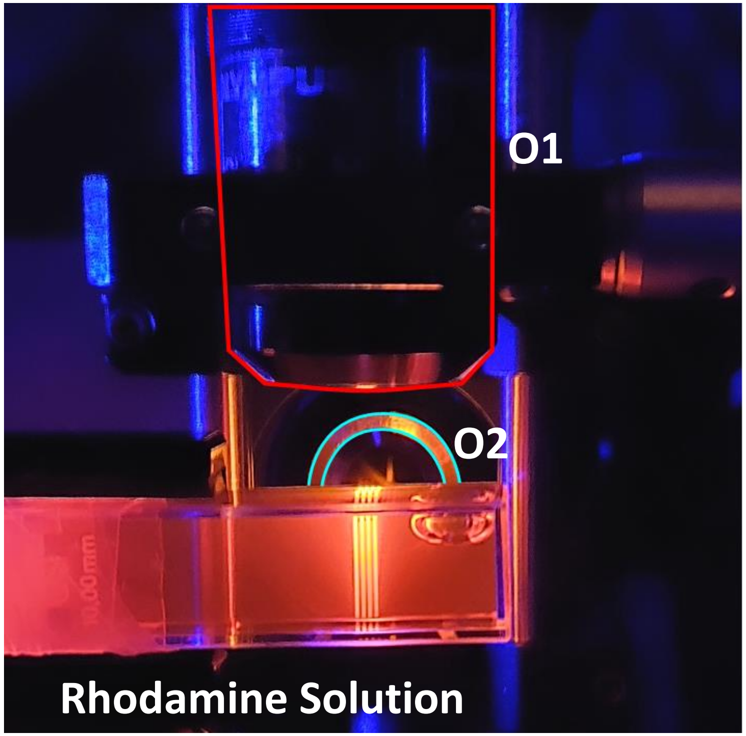
$$\hat{F}_\lambda = P^{-1} M_\lambda, \quad 1 \le \lambda \le \Lambda,$$

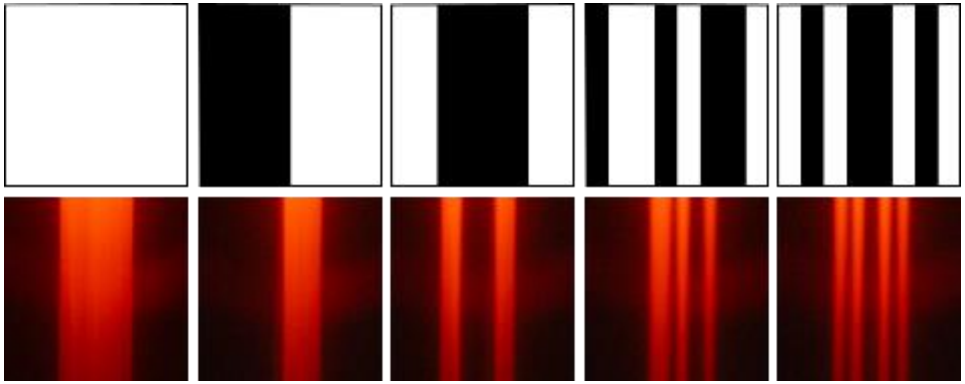


Reconstruction Algorithm



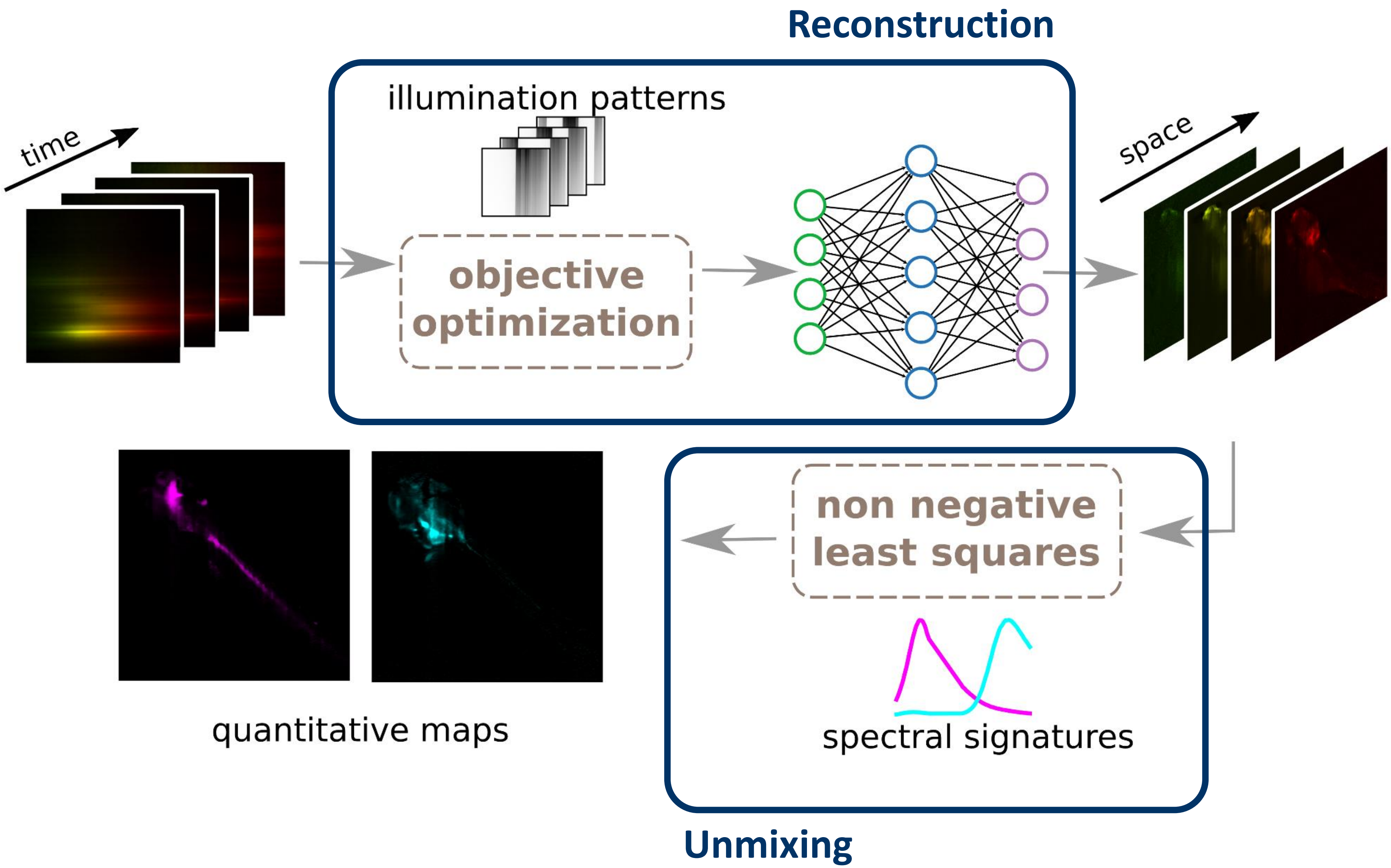
Structured Light Sheets





- ✓ Light patterns need to be calibrated
- ✓ “Good” patterns (e.g., matrix with low condition number) are highly desirable

Full Pipeline



Reconstruction

- **Learnt reconstruction**
  - Reconstruction (inference)

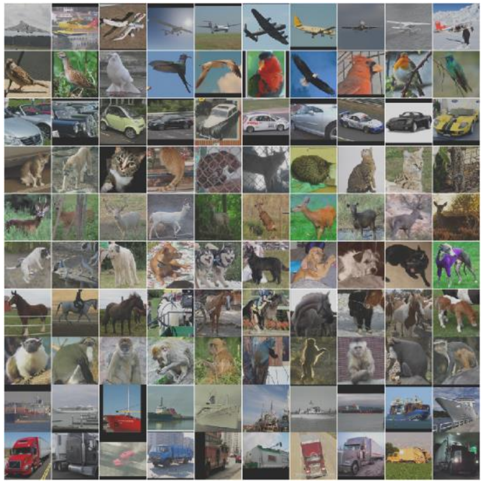
$$\boldsymbol{F}^*_\lambda = \mathcal{H}_{\boldsymbol{\theta}^*}(\boldsymbol{M}_\lambda)$$

network parameters

  - Learning

$$\boldsymbol{\theta}^* \in \arg\min_{\boldsymbol{\theta}} \frac{1}{L} \sum_{\ell} \|\mathcal{H}(\boldsymbol{\theta}; \boldsymbol{M}^\ell) - \boldsymbol{F}^\ell\|_2^2$$

STL-10 dataset

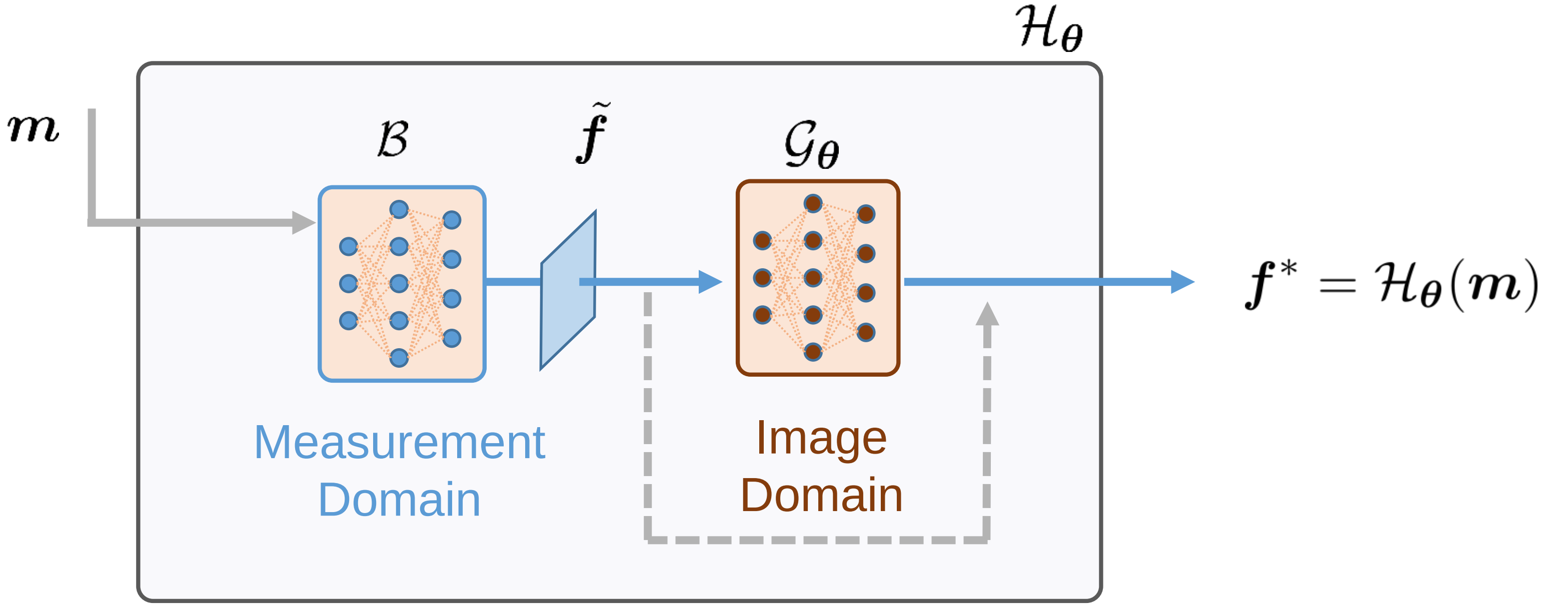


- **Computation times**
  - Training phase is slow, i.e., several hours or days
  - Inference is fast, i.e., tens or hundreds of milliseconds

Reconstruction

- **How to choose the 'model' *H*?**
  - Black box
  - Two step
  - Unrolled, plug & play

$$\boldsymbol{F}_\lambda^* = \mathcal{H}_{\boldsymbol{\theta}^*}(\boldsymbol{M}_\lambda) = (\mathcal{G}_{\boldsymbol{\theta}^*} \circ \mathcal{B})(\boldsymbol{M}_\lambda)$$



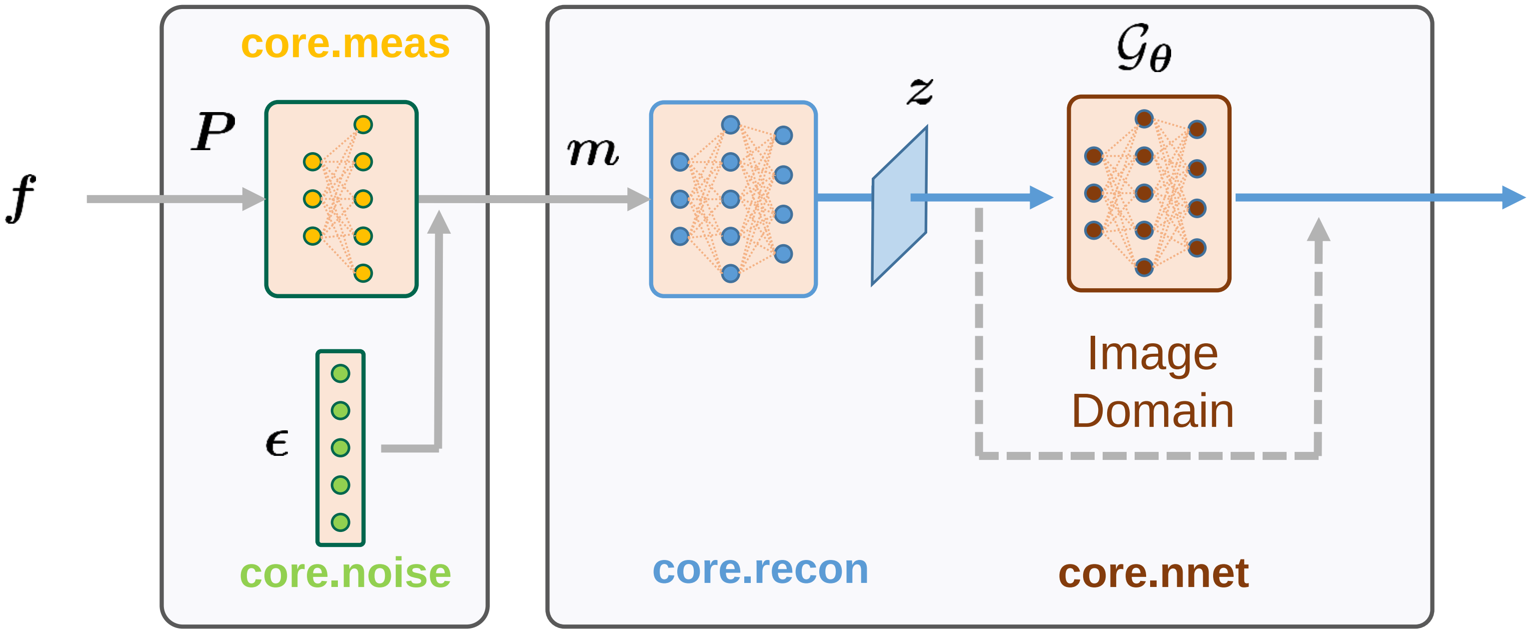
$$\mathcal{B}(\boldsymbol{M}_\lambda) = \boldsymbol{\Sigma}\boldsymbol{P}^\top \left(\boldsymbol{P}\boldsymbol{\Sigma}\boldsymbol{P}^\top + \boldsymbol{\Gamma}\right)^{-1} \boldsymbol{M}_\lambda$$

SPyRiT: Single-Pixel Reconstruction Toolkit

- **Core components** https://spyrit.readthedocs.io/en/master/

$$m = Pf + \epsilon_f$$

$$\mathcal{H}_\theta$$



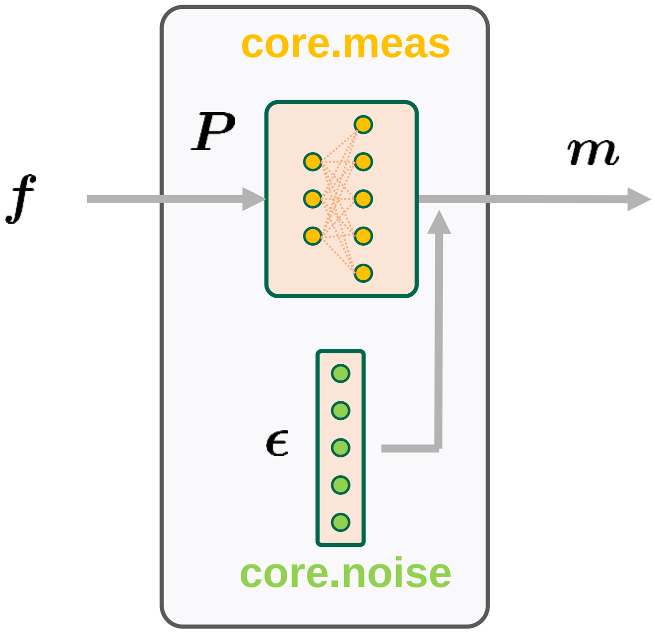
$$\theta^* \in \arg\min_{\theta} \frac{1}{L} \sum_{\ell} \|(\mathcal{G}_\theta \circ \mathcal{H})(f^\ell) - f^\ell\|_2^2$$

SPyRiT: Single-Pixel Reconstruction Toolkit

- **Core components**

https://spyrit.readthedocs.io/en/master/

$$m = Pf + \epsilon_f$$




```
# Data simulation
from spyrit.core.meas import HadamSplit
from spyrit.core.noise import Poisson

meas_op = HadamSplit(512, 128)
noise_op = Poisson(meas_op, 100)
m = noise_op(f)
```

Hyperspectral Light Sheet Microscopy

➢ Spectral unmixing

- ❖ Assuming *C* fluorophores (or combination of fluorophores, e.g., autofluorescence)

$$\min_{\boldsymbol{A}} \|\boldsymbol{F} - \boldsymbol{A}\boldsymbol{S}\|_2^2 + \alpha\mathcal{R}(\boldsymbol{A})$$

$N = K \times N_y$ pixels

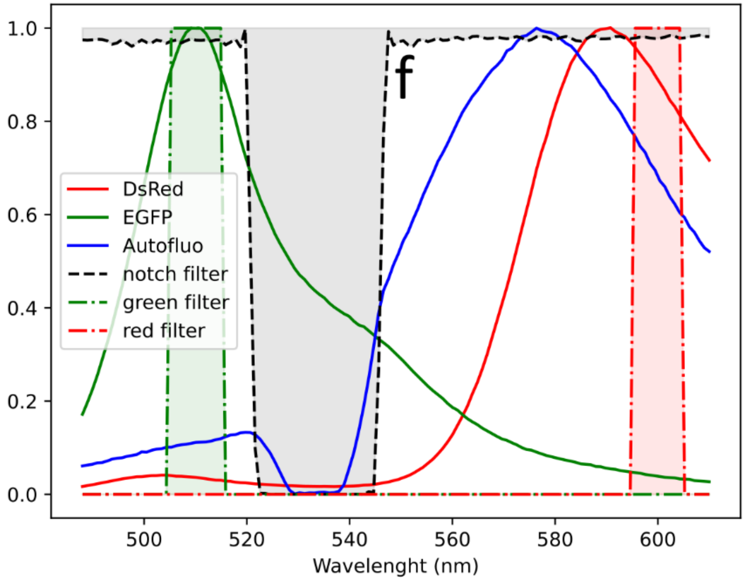
$C \times \Lambda$ spectra

$N \times \Lambda$ hypercube

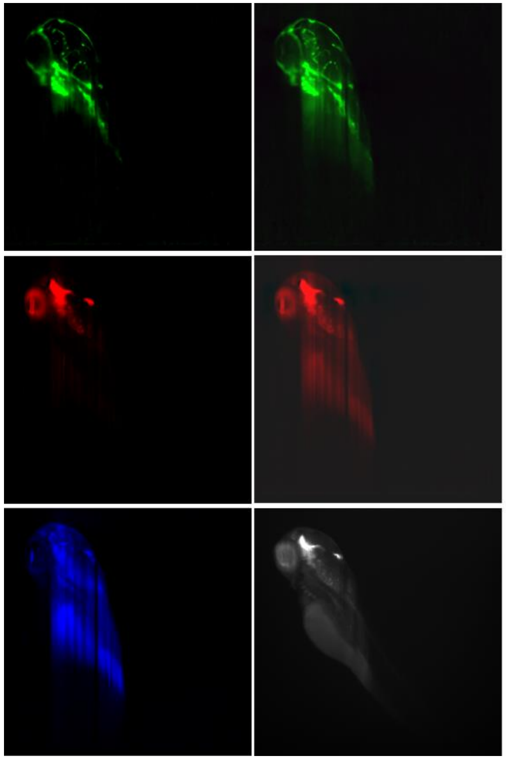
$N \times C$ abundance maps

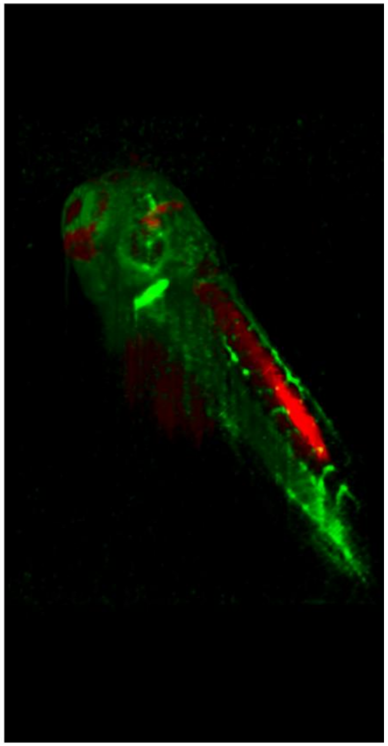
- ❖ Regularization can enforce prior knowledge about the abundance maps or be "learnt" from "examples"

Fluorescence Removal

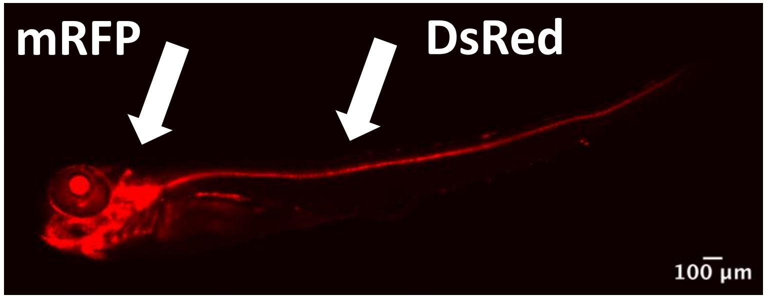


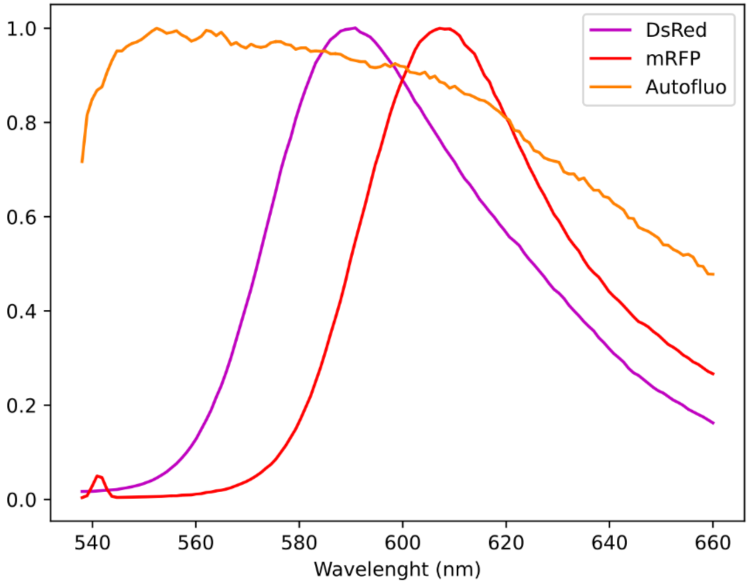
Filter **Spectral** **Autofluo free (3D)**



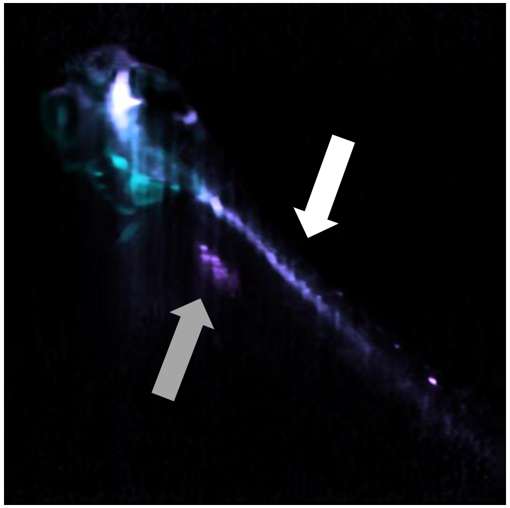


Separation of Overlapping Fluorophores

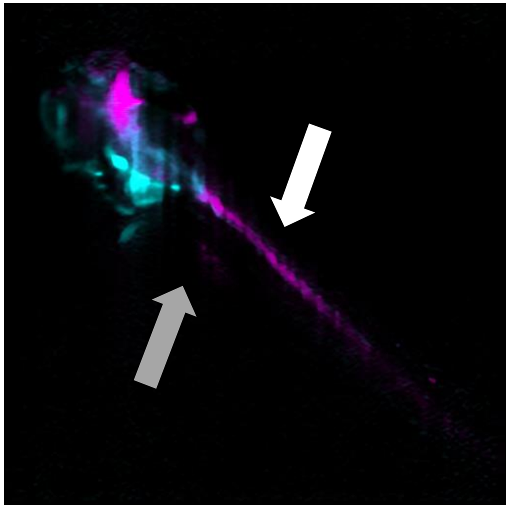




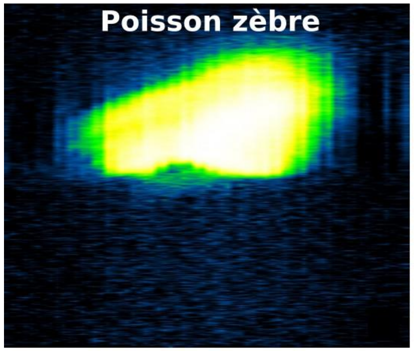
Filter



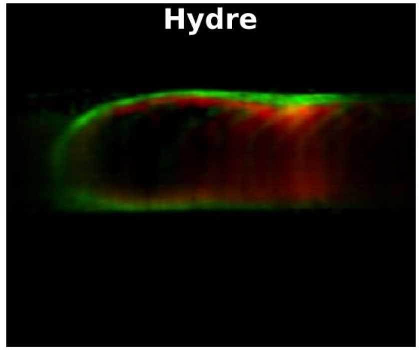
Spectral



Perspectives

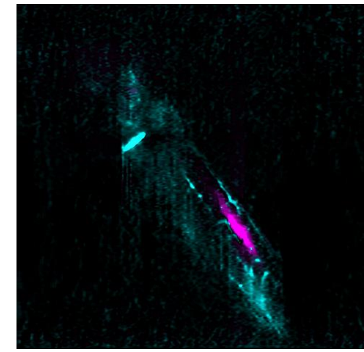


Octobre 2019



Juin 2020

Nov 2021



2023

- **Open questions :**
  - Improved reconstruction / improved unmixing
  - Joint reconstruction-unmixing
  - Choice of the patterns
  - Two-arm reconstruction (i.e., pansharpening)
  - Reconstruction hyperspectral 3D (x, y, z, λ)
  - Dynamic reconstruction (x, y, z, λ, t)