LSFM hyperspectrale structurée pour l'imagerie quantitative

S Crombez [1], C Exbrayat-Heritier [1], F Ruggiero [1], C Ray [1], Nicolas Ducros[1, 3]

[1]CREATIS, Univ Lyon, INSA-Lyon, UCB Lyon 1, CNRS, Inserm, Lyon, France

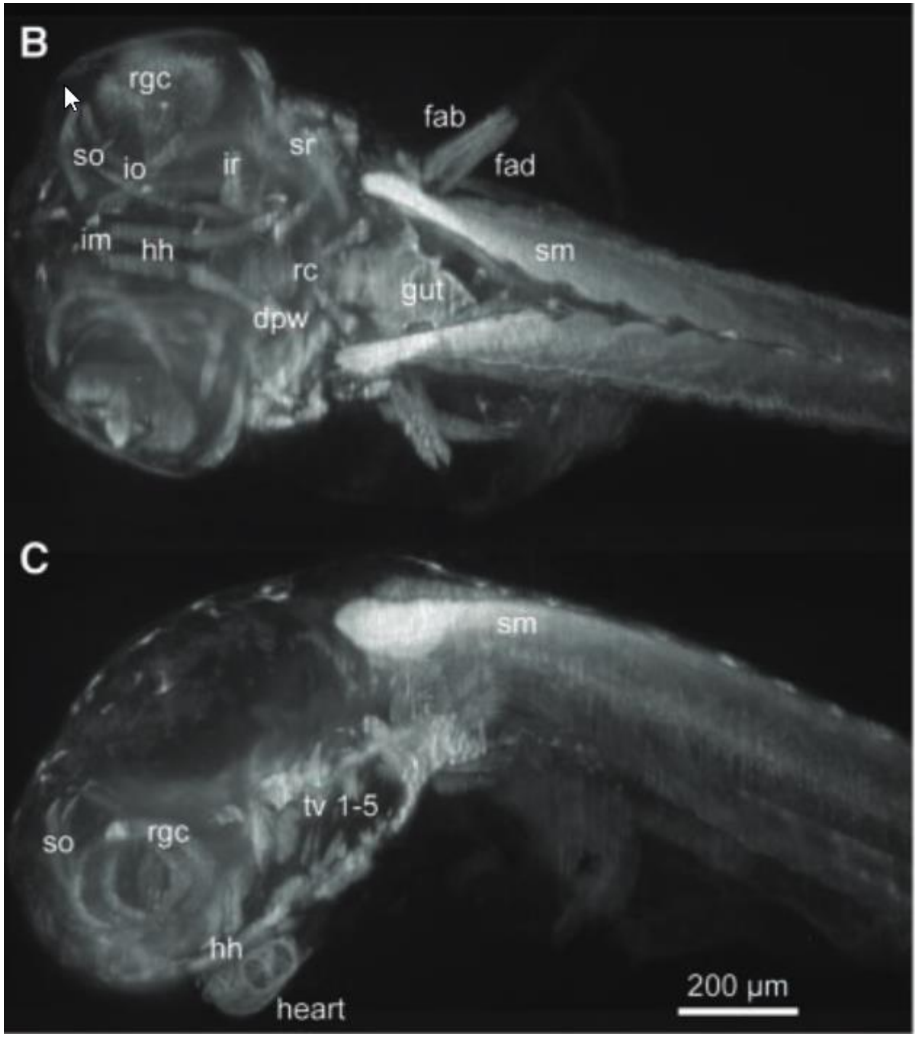
[2]IGFL, ENS Lyon, France

[3]IUF, Institut Universitaire de France

Light Sheet Fluorescence Microscopy (LSFM)

Dorsal-ventral

lateral

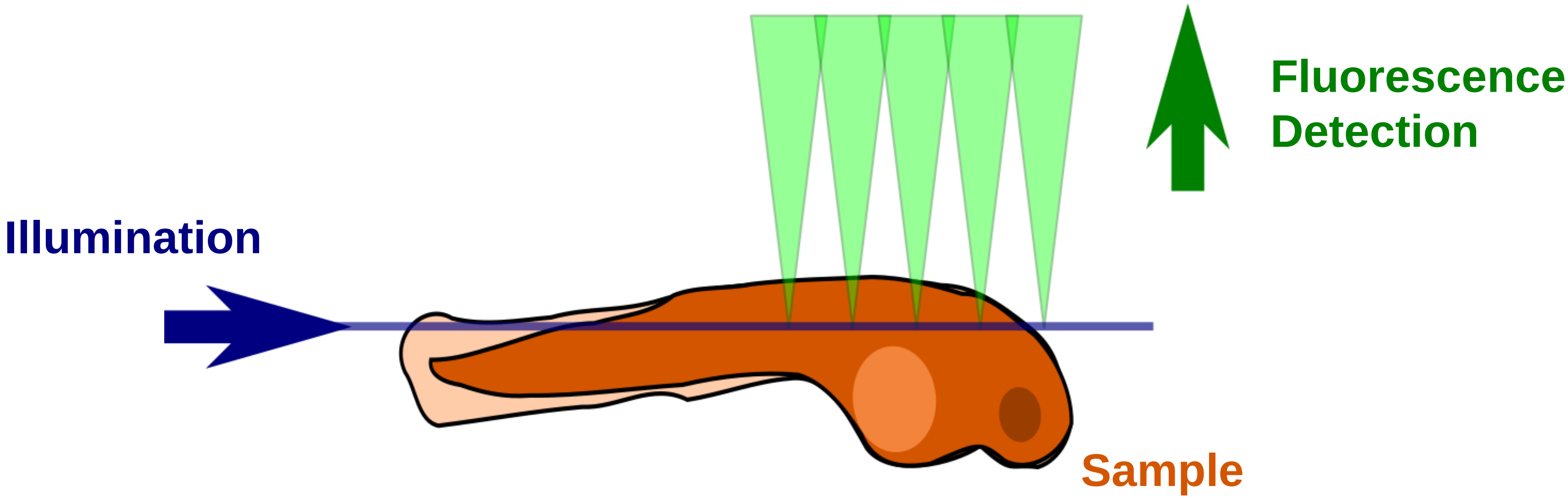


- ✓ Florescence samples
- ✓ GFP-labeled transgenic embryos
- ✓ Developmental study

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

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

Light Sheet Fluorescence Microscopy (LSFM)



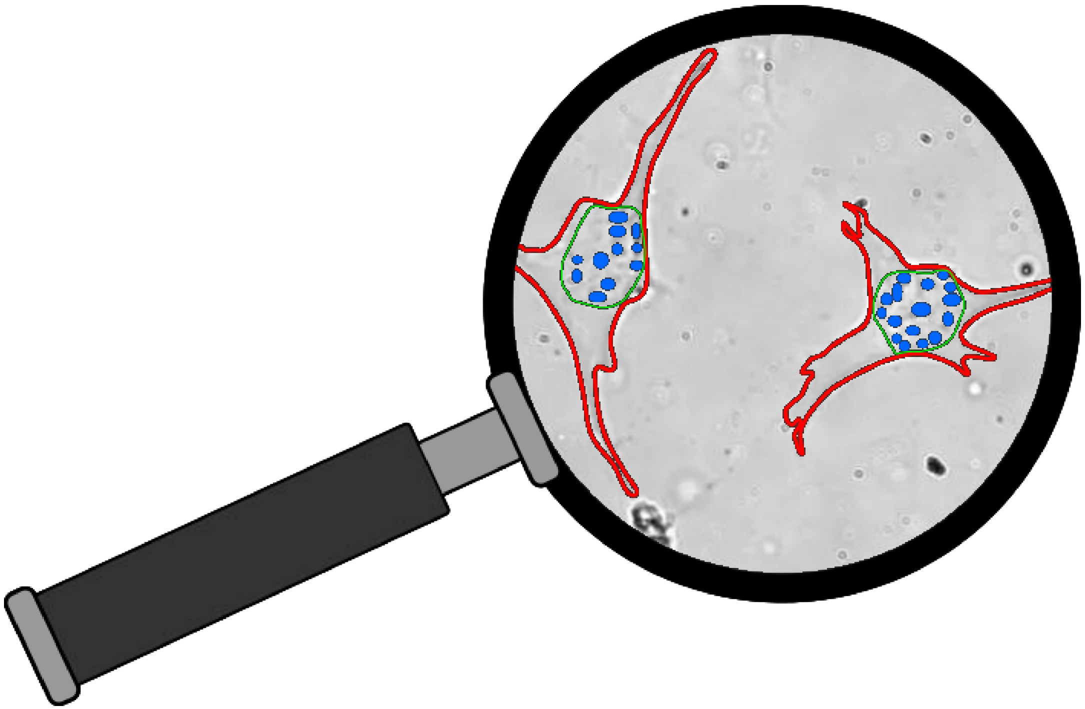
- ✓ Optical sectioning as deep as 500 µm

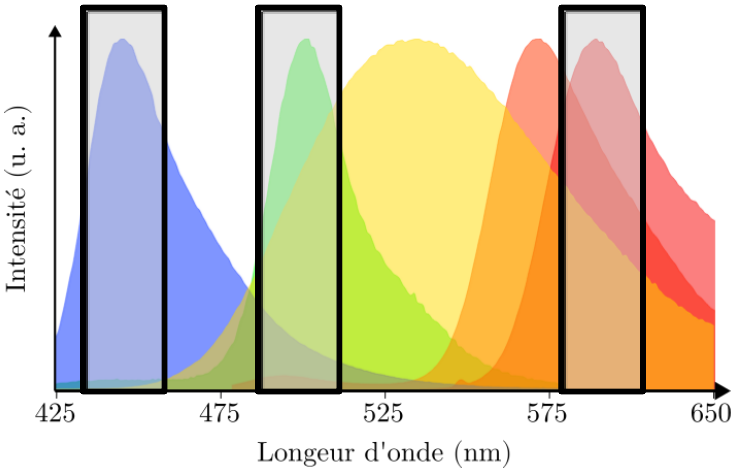
Compared to confocal

- ✓ Low photobleaching
- ✓ Fast

[Image adapted from Wikimedia Commons]

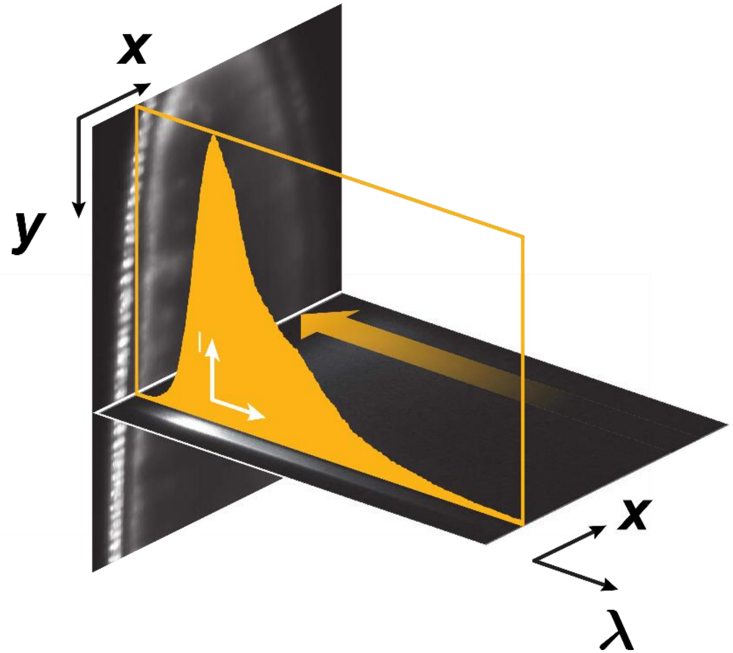
Fluorescence Microscopy usually based on Filters





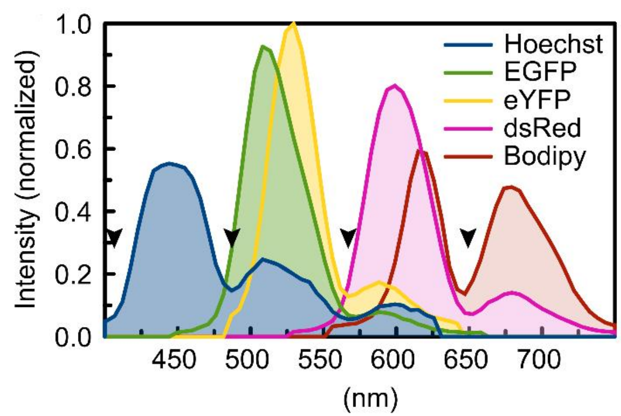
- ✓ Many photons are rejected
- ✓ Overlapping fluorophores cannot be separated
- ✓ Undesired fluorophores cannot be rejected

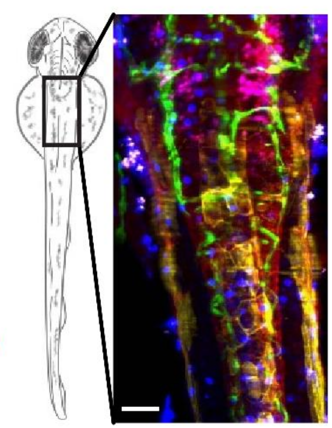
Hyperspectral LSFM



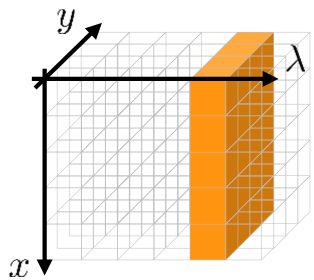
[W Jahr *et al*, *Nat. Commun.*, **6**, (2015)]

Unmixing

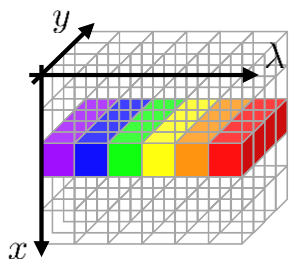




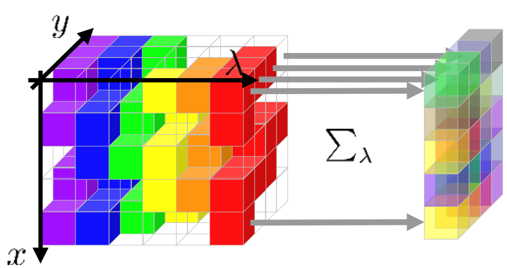
Hyperspectral imaging



Optical filters



Pushbroom



Computational

Challenge #1

Resolution

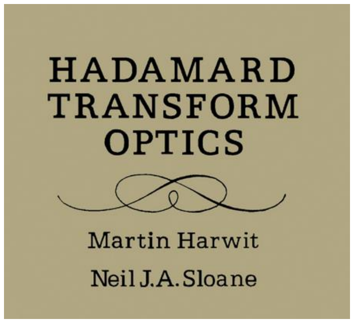
Challenge #2

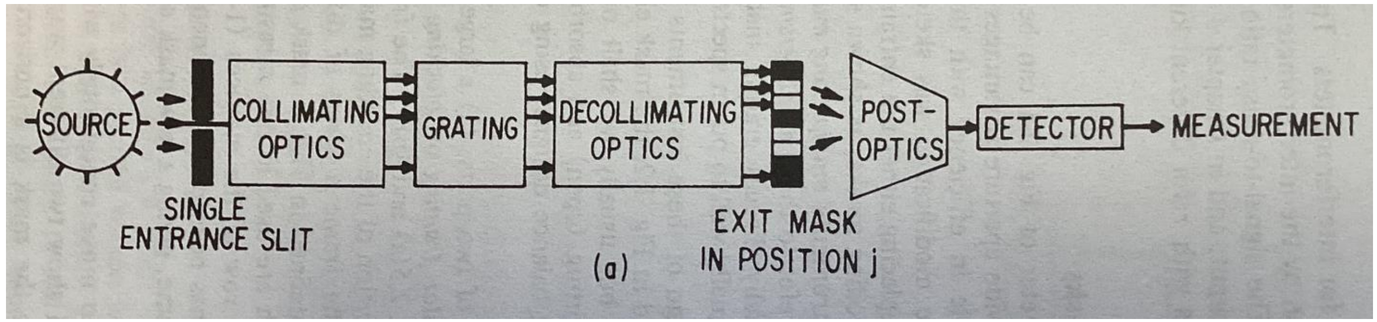
Speed

Challenge #3

Weak signals

Hadamard spectroscopy (from the 70's)



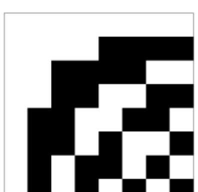


[M. Harwit and N Sloane, Academic Press, 1979]

'(…) conventional spectrometer is modified by using a <u>mask to encode the light</u> at the output'

Hadamard

$$\boldsymbol{m} = \boldsymbol{A}\boldsymbol{f}$$
$$\boldsymbol{A} \in \{-1, 1\}^{N\times N}$$



$$\boldsymbol{A}^\top \boldsymbol{A} = N\boldsymbol{I}_N$$

L2 minimization

$$\boldsymbol{f}^* = \frac{1}{N}\boldsymbol{A}\boldsymbol{m}$$

Fellgett's advantage

$$\text{Hyp}: \ \mathsf{var}\,(m_i) = \sigma^2, \forall i$$
$$\mathsf{var}\,(f_i^*) = \frac{1}{N}\sigma^2 < \sigma^2$$

Improved SNRs

Fellgett's advantage (theory, revisited)

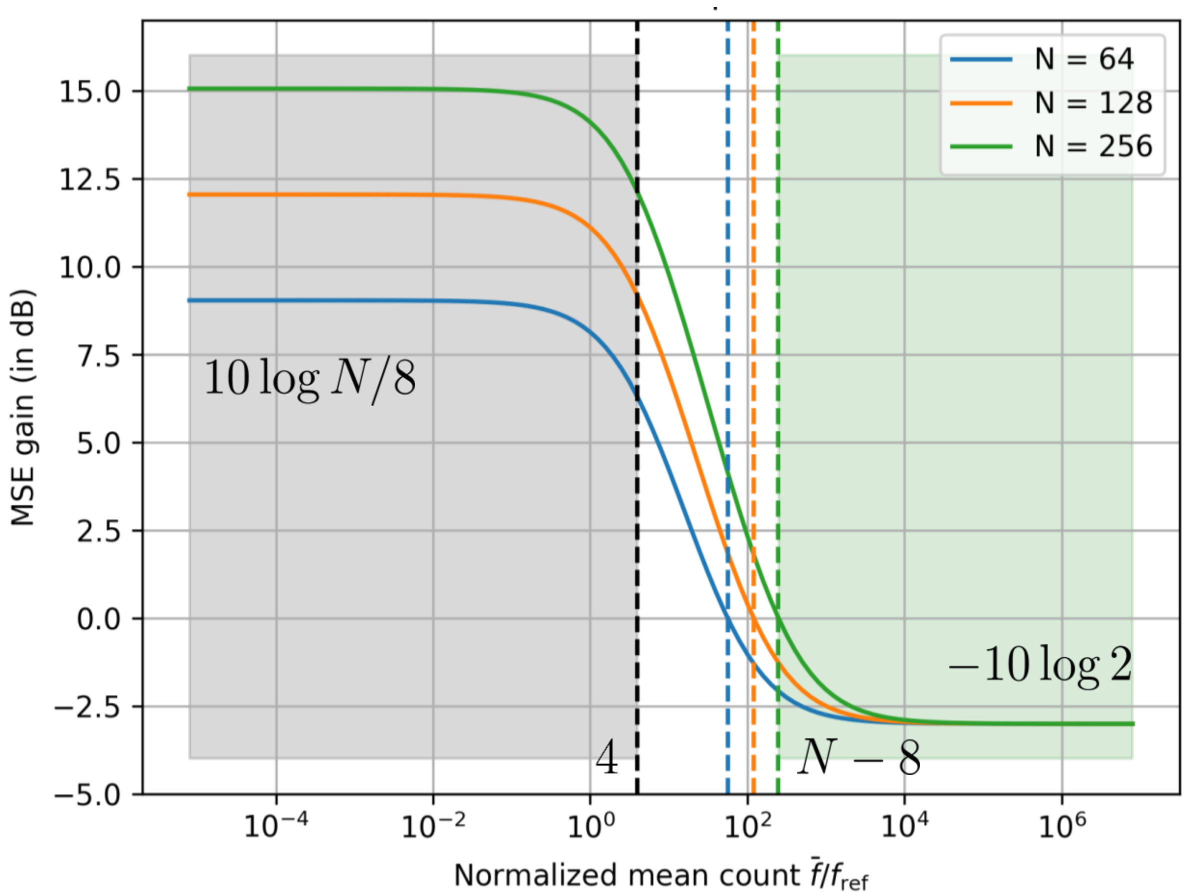
Poisson-Gaussian noise

$$\boldsymbol{m} \sim g\mathcal{P}(\boldsymbol{A}\boldsymbol{f}\Delta t) + \mathcal{N}(\mu_\mathrm{d}, \sigma_\mathrm{d}^2)$$

[EMVA standard, 2021]

Fixed time budget

$$\Delta t = \frac{T}{2N}$$

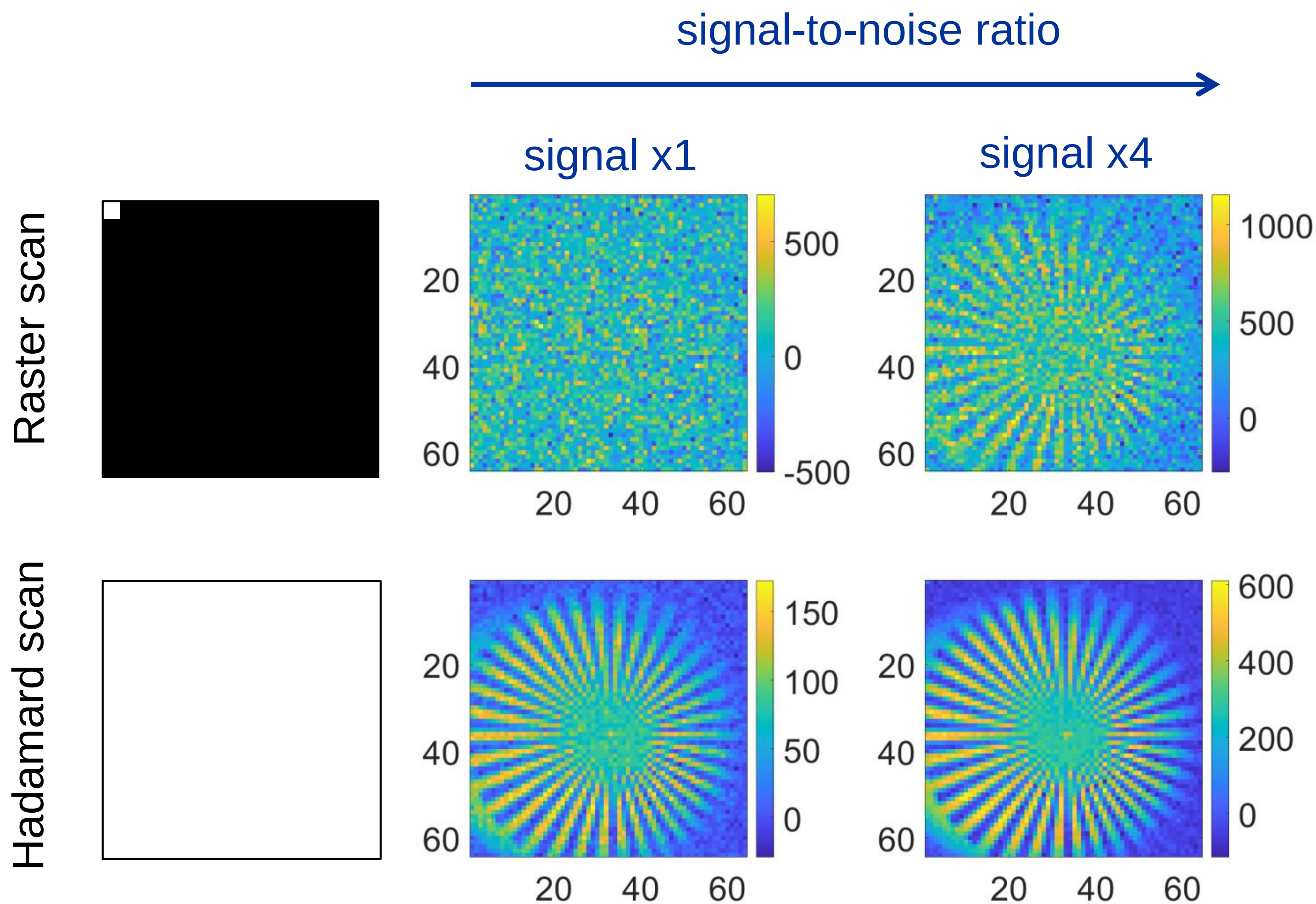


$$\bar{f} = \tfrac{1}{N}\textstyle\sum_n f_n$$

$$f_\mathrm{ref} = \frac{\sigma_\mathrm{d}^2}{g^2 T}$$

Fellgett’s advantage (experimental)

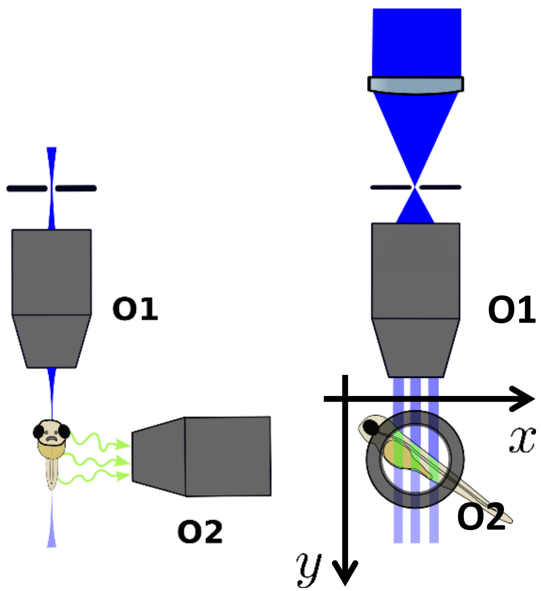
R1L1S2P

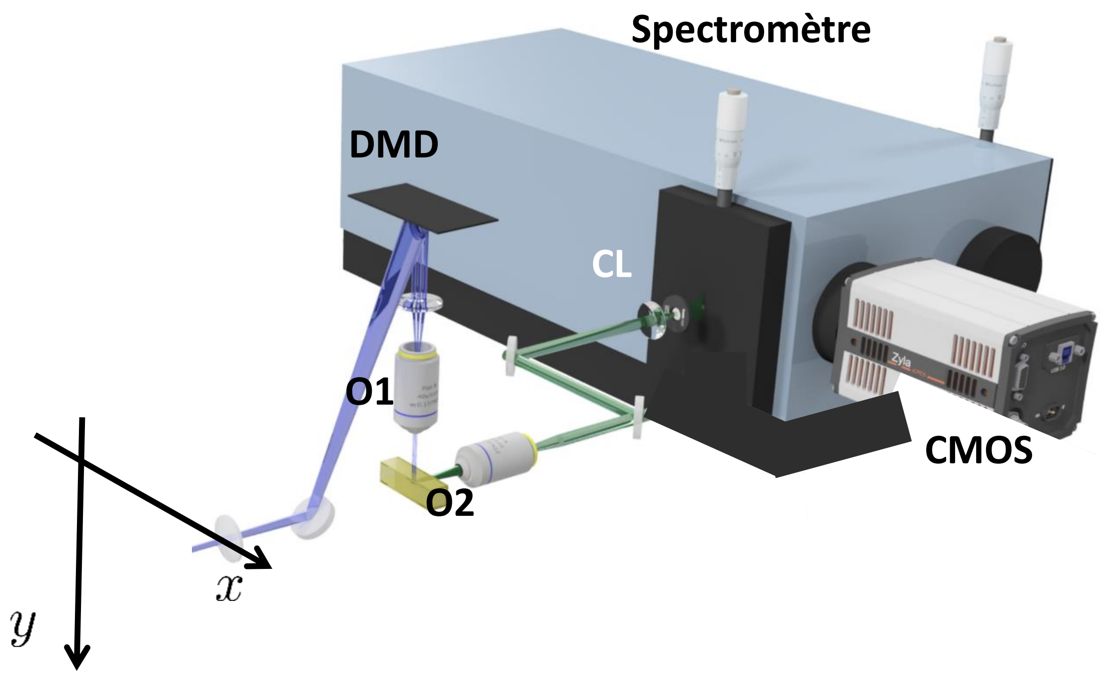


[N Ducros, 2020]

Proposed Method

Hyperspectral structured FLSM (HS-FLSM)

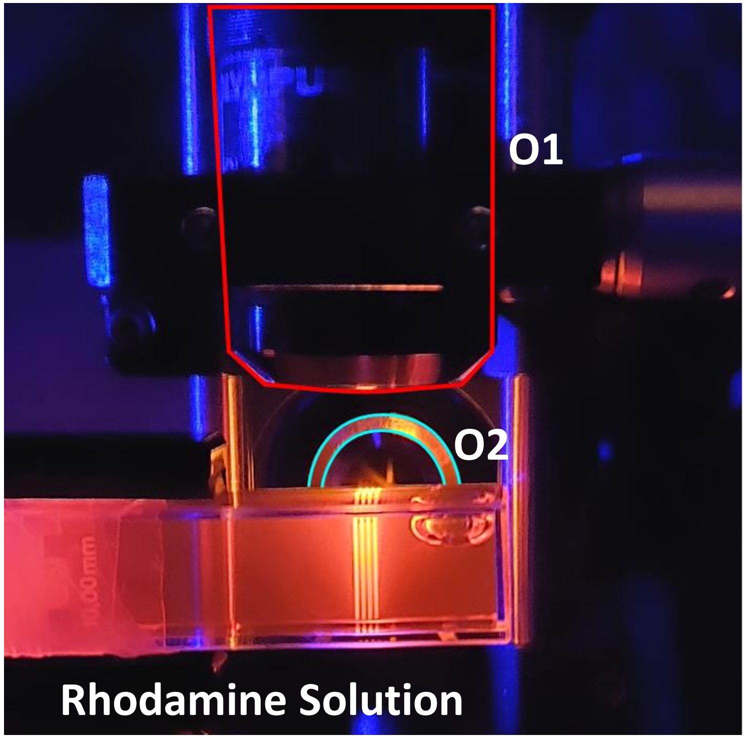




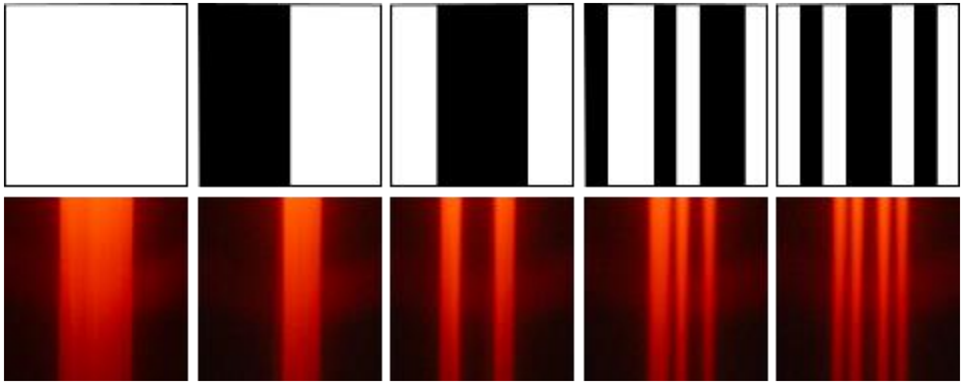
$$m_i(y,\lambda) = \int a_i(x) f(x,y,\lambda)\,\mathrm{d}x, \quad 1 \le i \le M$$

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

Structured light sheets



DMD (top) vs experimental (bottom) light patterns



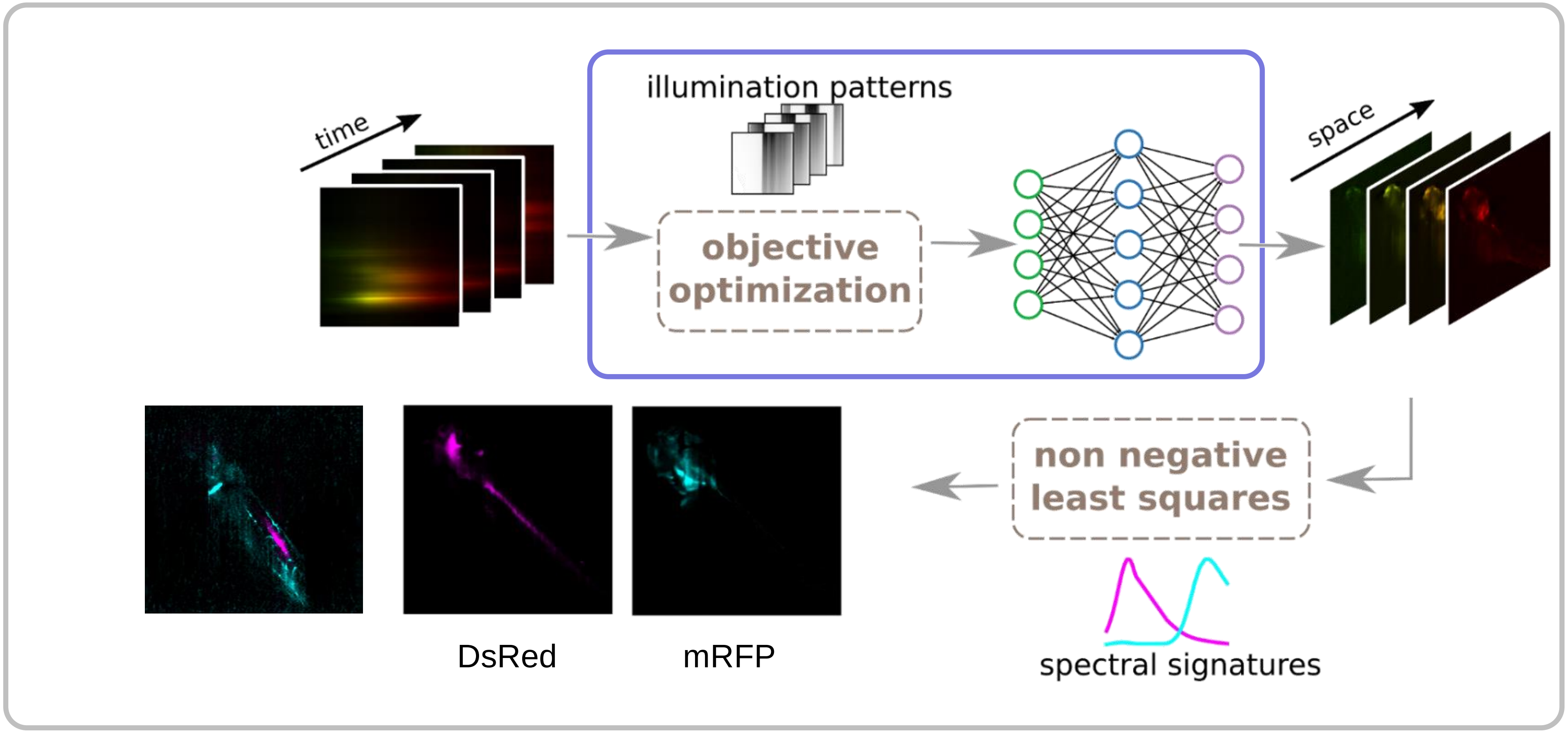
- ✓ Light patterns need to be calibrated
- ✓ “Good” patterns (e.g., system matrix with low condition number) are highly desirable

- ✓ Spatial resolution: ~60 x 4 µm^2

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

High-resolution quantitative HS-FLSM

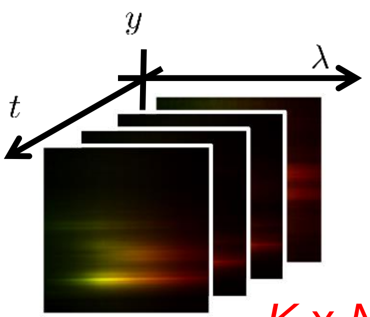
Proof of principle



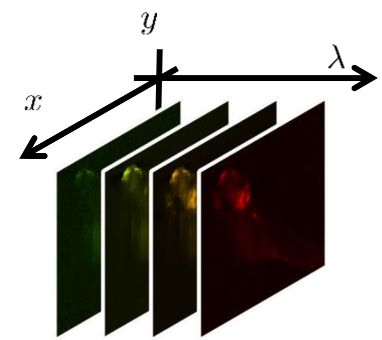
[S. Crombez *et al*., preprint, 2024]

https://hal.science/hal-04824372v1

HS-FLSM data-driven reconstruction



$$\boldsymbol{M}_\lambda = \boldsymbol{A}\boldsymbol{F}_\lambda + \boldsymbol{E}_\lambda, \quad 1 \leq \lambda \leq \Lambda,$$



K x N_y
measurements

K x N_x
system matrix

N_x x N_y
hypercube slice

Slice-by-slice reconstruction

$$\boldsymbol{F}^*_\lambda = \mathcal{R}_{\boldsymbol{\theta}^*}(\boldsymbol{M}_\lambda) \text{ for all } \lambda$$

Fast inference/small network
Training from grayscale image

End-to-end training / MMSE

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

No *regularization/prior* along the spectral axis

HS-FLSM data-driven reconstruction

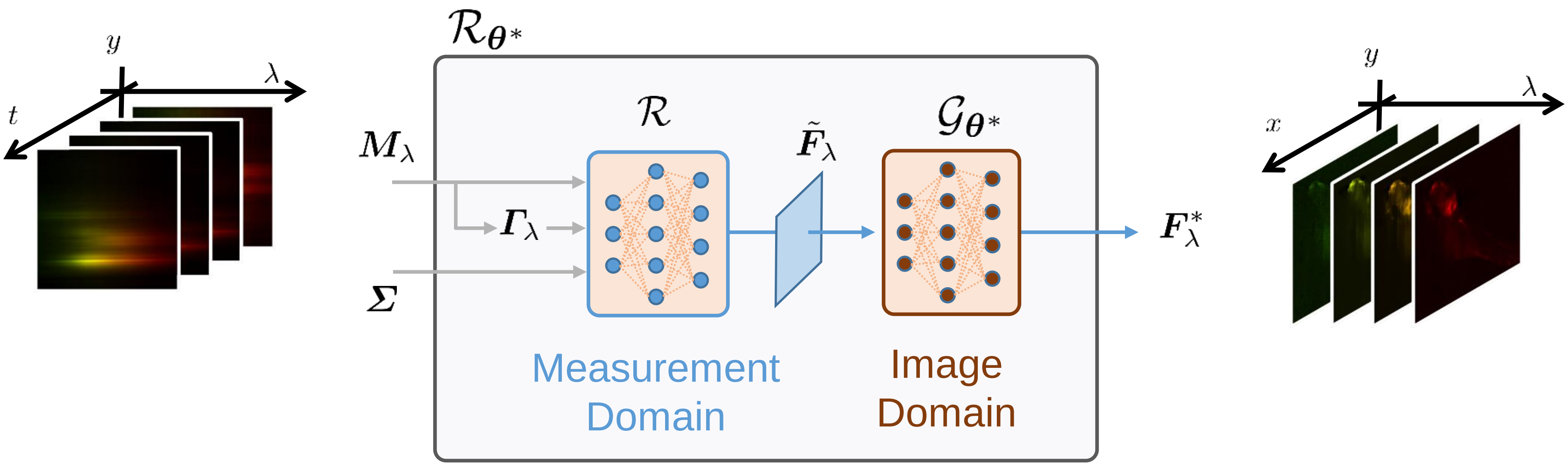
Reconstruction challenges
- ✓ Varying noise levels
- ✓ Reconstruction time
- ✓ Avoid spectral distortions

image profile covariance

measurement covariance

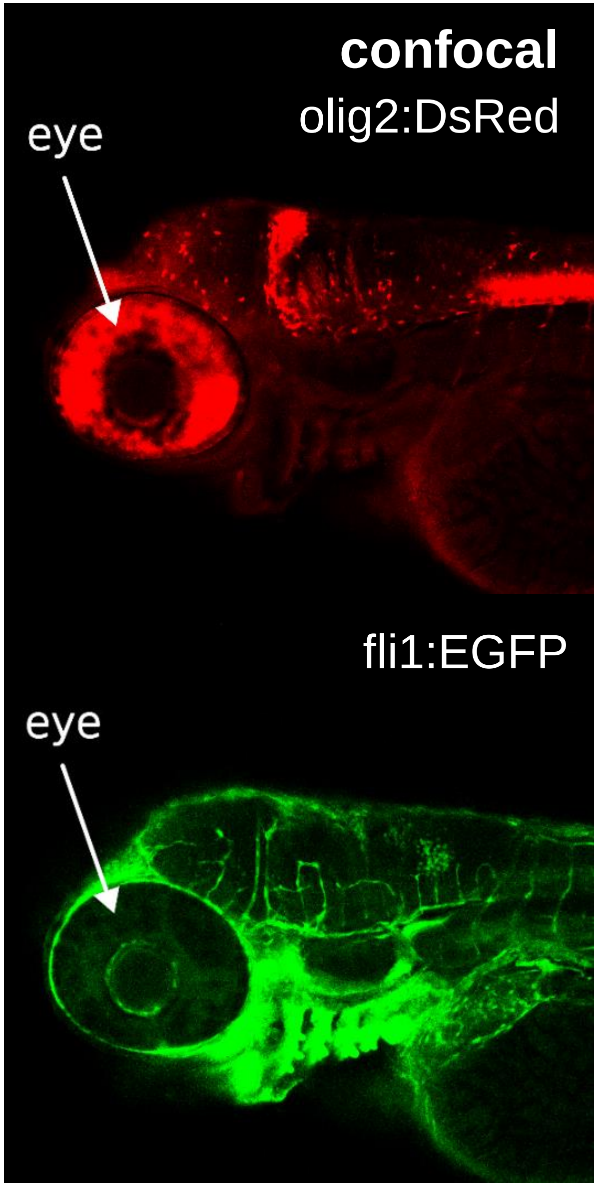
Tikhonov (*x*-axis only) : $\mathcal{R}(\boldsymbol{M}_\lambda) = \boldsymbol{\Sigma}\boldsymbol{A}^\top \left(\boldsymbol{A}\boldsymbol{\Sigma}\boldsymbol{A}^\top + \boldsymbol{\Gamma}_\lambda\right)^{-1} \boldsymbol{M}_\lambda$

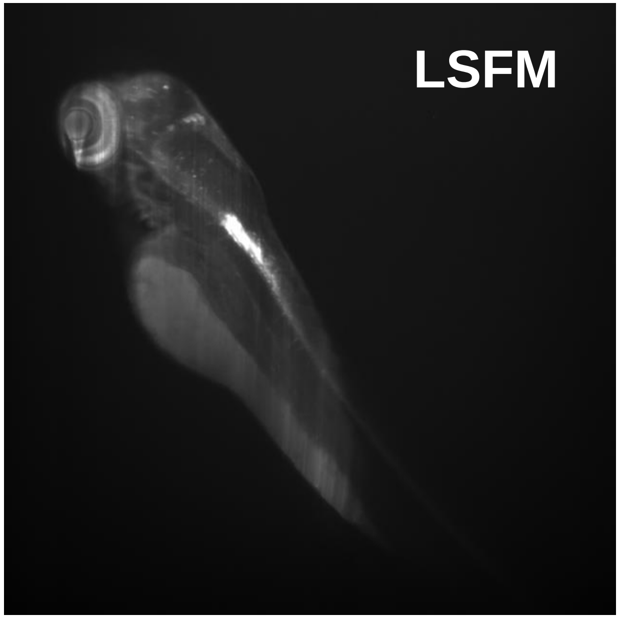
Data-driven (*x*- and *y*-axis) : $\mathcal{R}_{\boldsymbol{\theta}^*}(\boldsymbol{M}_\lambda) = (\mathcal{G}_{\boldsymbol{\theta}^*} \circ \mathcal{R})(\boldsymbol{M}_\lambda)$

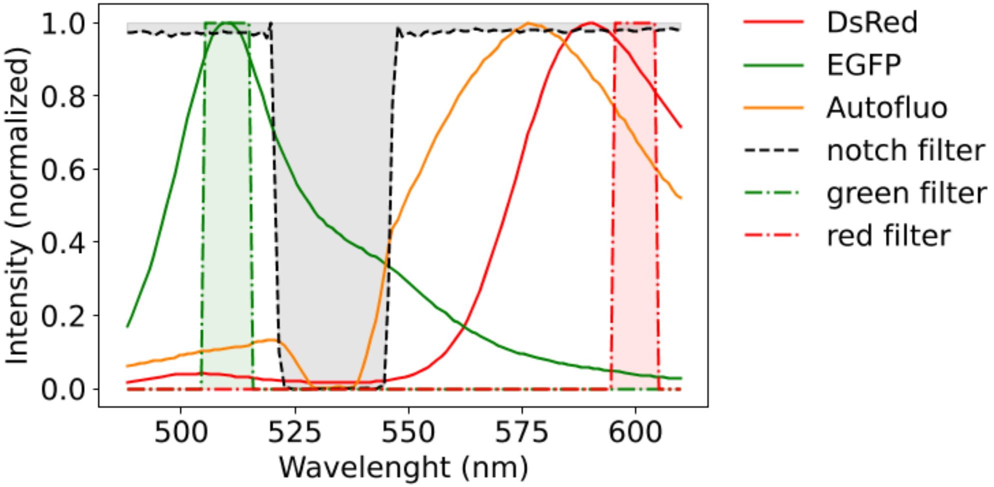


Results

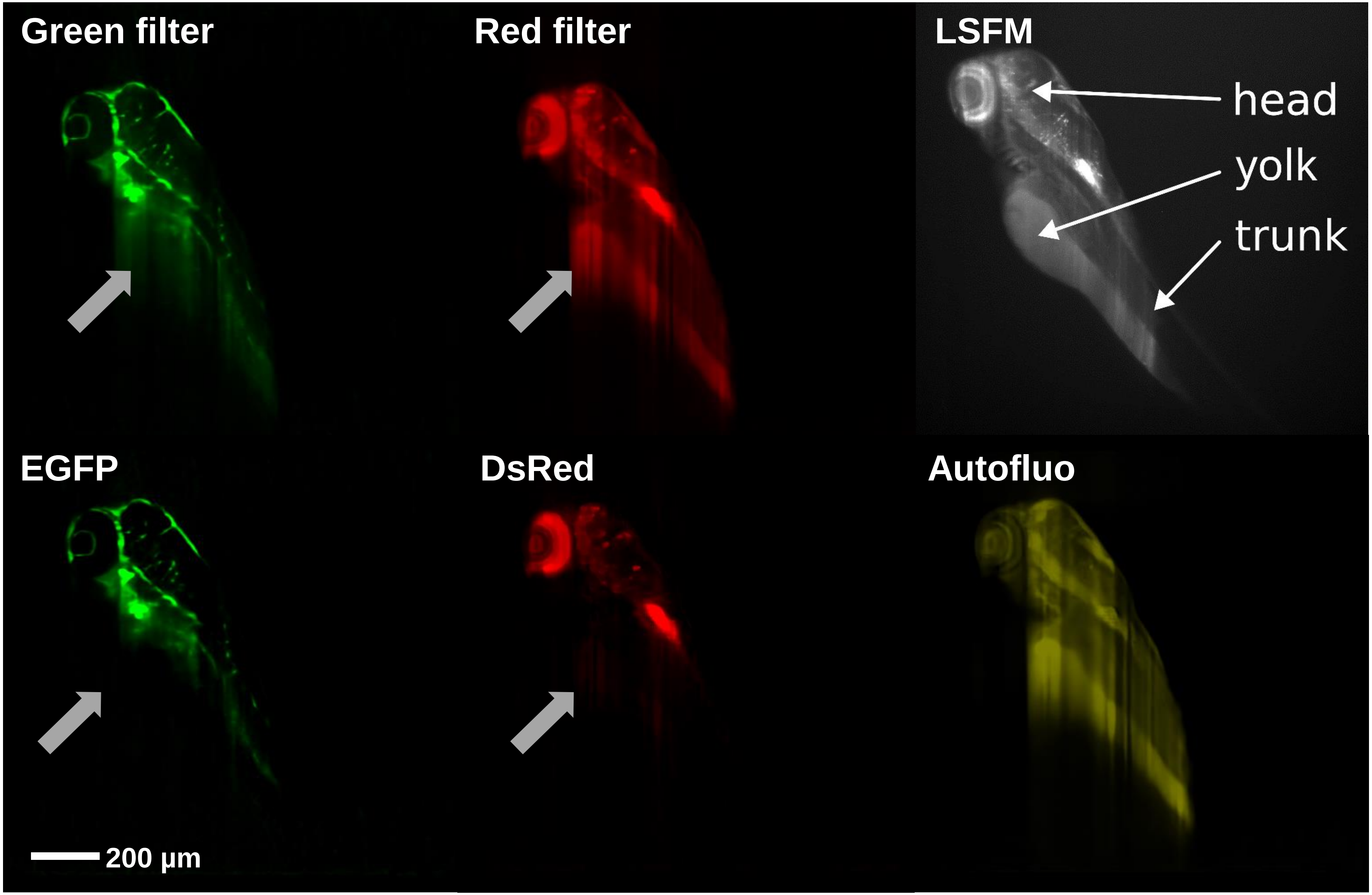
Sample #1:(fli1:EGFP;olig2:DsRed)



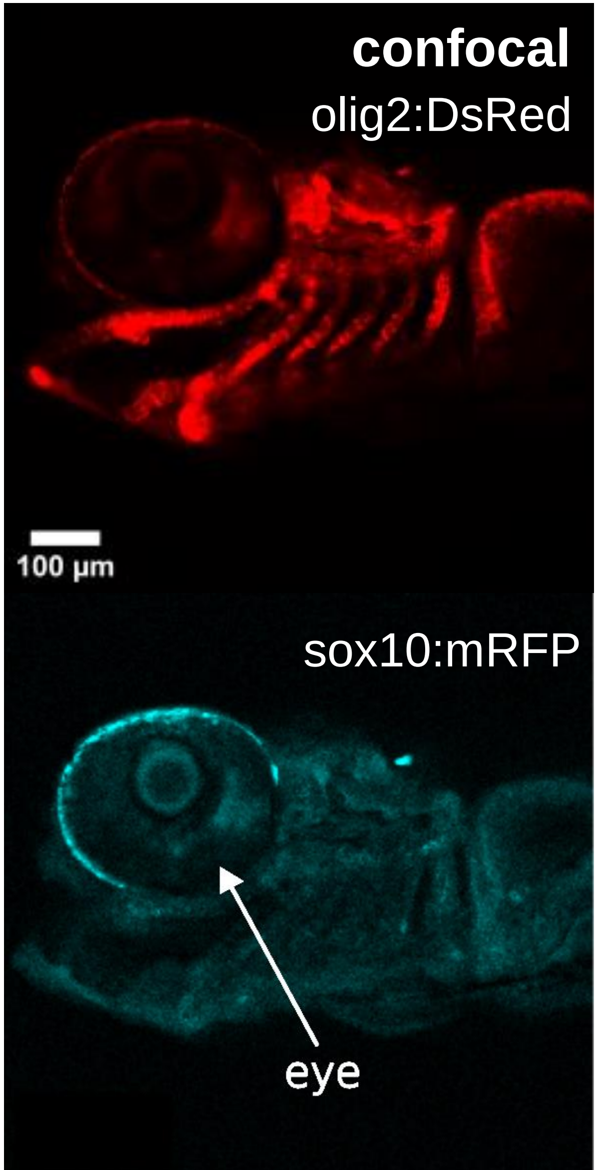


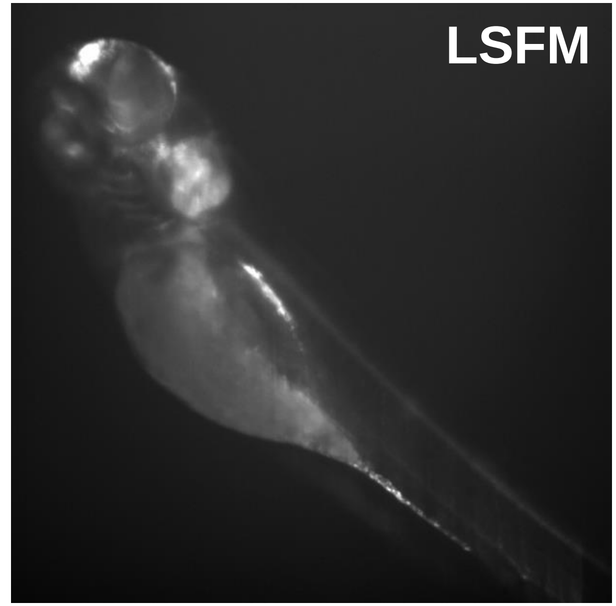


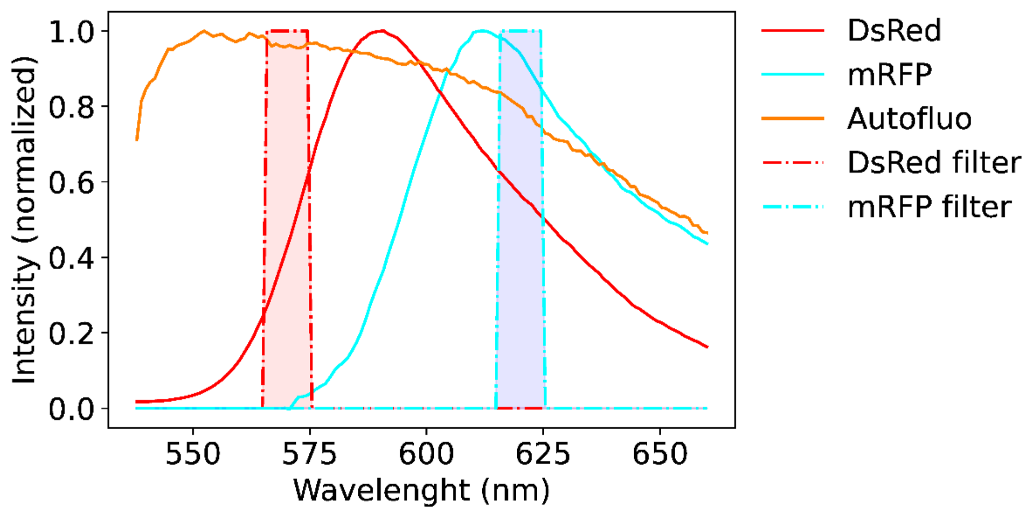
Sample #1: (fli1:EGFP;olig2:DsRed)



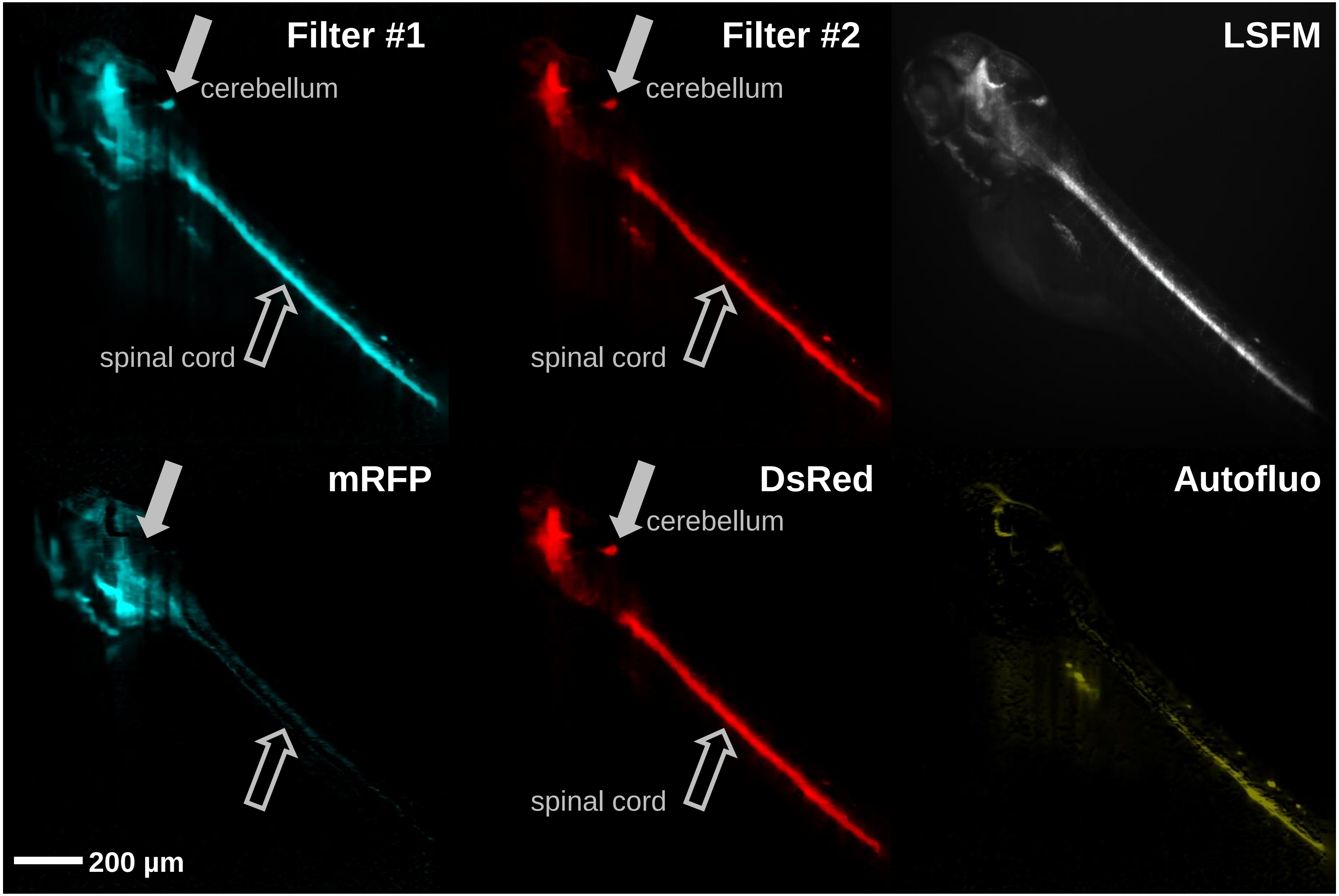
Sample #2: (sox10:mRFP;olig2:DsRed)







Sample #2: (sox10:mRFP;olig2:DsRed)



Limitations and future work

Limitations

LSFM
- Spatial resolution ~7 x 2 μm^2
- Acquisition speed ~8 min/slice

Passive imaging
- Extended FOV using experimental measurements
- Typical acquisition time is ~10 s

Future work (hardware)

LSFM
→ Improved beam shaping along *x*-axis (~500 x 500 nm^2 ?)
→ Z-axis beam shaping

Passive imaging
→ *x*-axis only modulation

Future work (software)

→ Reconstruct and unmix jointly [S. Hariga *et al.*, EUSIPCO, 2024]
→ Data-driven (e.g., unrolled, PnP) for 3D spectral reconstruction
→ 2-arm acquisition/data fusion
→ Design of the patterns
→ Etc.

Code (SPyRiT Python package): https://spyrit.readthedocs.io/
Data: https://pilot-warehouse.creatis.insa-lyon.fr/