PnP COL0RME : Fluorescence image deconvolution microscopy using a Plug and Play Denoiser

Vasiliki Stergiopoulou
(Morpheme team, I3S Laboratory, France)

In collaboration with:

Luca Calatroni & Laure Blanc-Féraud
(Morpheme team, I3S Laboratory, France)

Sébastien Schaub
(LBDV, Sorbonne Université, France)

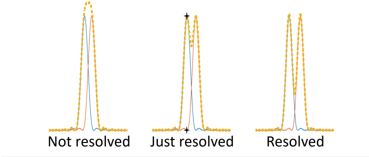
Subhadip Mukherjee & Carola-Bibiane Schönlieb
(DAMTP, University of Cambridge, UK)

Mathematical Models for Plug-and-play Image Restoration

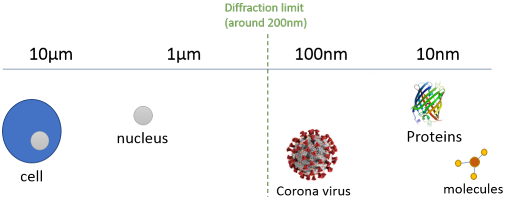
Table of Contents

Conventional Fluorescence Microscopy Limitations

- Lateral resolution is limited by light diffraction phenomena

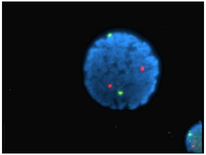


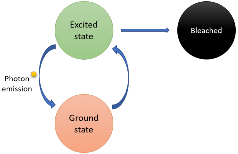
- Smallest resolvable distance (Rayleigh Criterion): $d = \frac{0.61\lambda}{NA}$ $(\approx 200nm)$
  λ: emission wavelength, NA: Numerical Aperture.



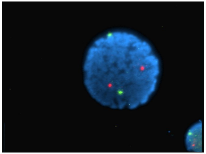
We want:

- Use of standard equipment / conventional fluorophores
- Harmless excitation levels / no sample fixation
- Improved temporal resolution



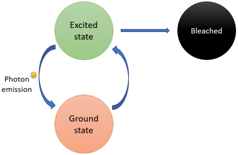


Fluorophore states

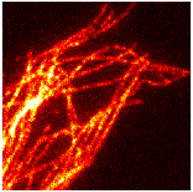


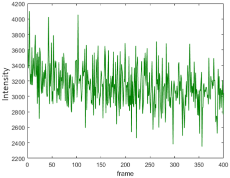
We want:

- Use of standard equipment / conventional fluorophores
- Harmless excitation levels / no sample fixation
- Improved temporal resolution



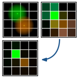
Fluorophore states





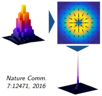
Temporal profile of a pixel

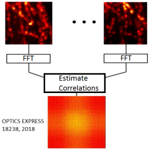
Fluctuation-based Super-Resolution Methods



SOFI (Dertinger *et al.*, '09)
Super resolution Optical Fluctuation Imaging

SRRF (Gustafsson *et al.*, '16)
Super-Resolution Radial Fluctuations



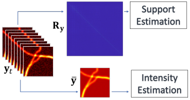


SPARCOM (Solomon *et al.*, '19)
SPARsity based super-resolution COrrelation Microscopy

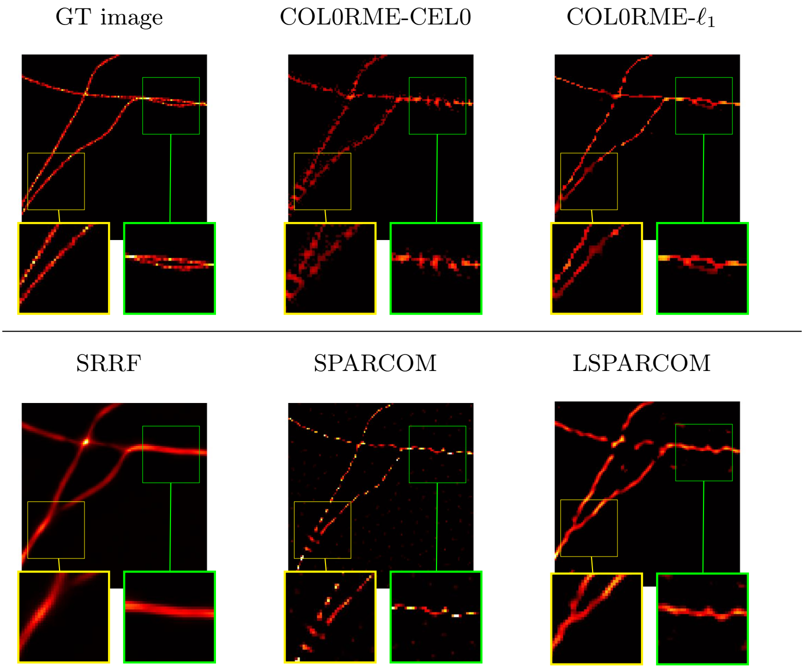
LSPARCOM (Dardikman-Yoffe *et al.*, '20)
Learned SPARCOM

COL0RME (Stergiopoulou *et al.*, '21, '22)
COvariance-based ℓ_0 super-Resolution Microscopy with intensity Estimation

- Exploits sparsity in the covariance domain
- Estimates real intensity values
- Does not have artifacts
- Extends to 3D (3D MA-TIRF COL0RME)



Simulated Data



Real TIRF Data

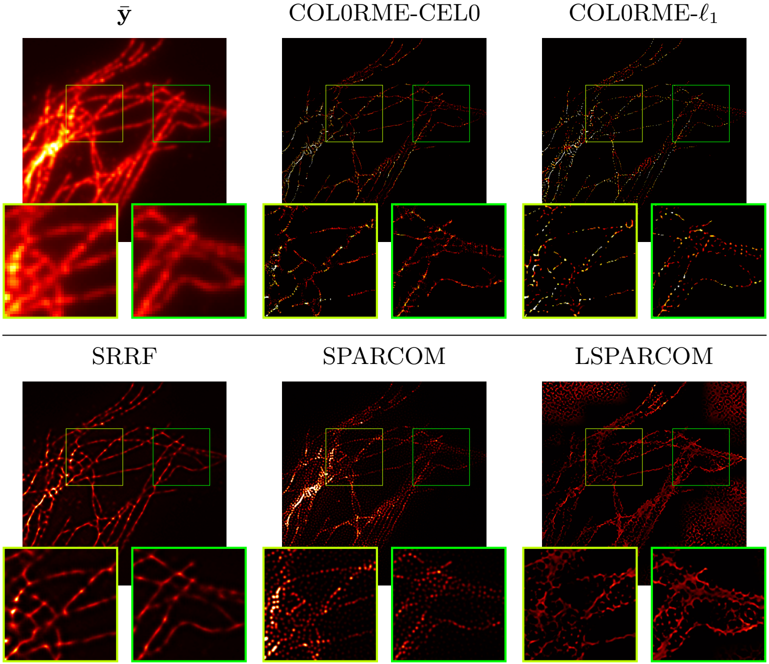
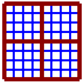
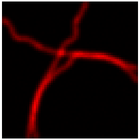


Table of Contents

Mathematical Modeling



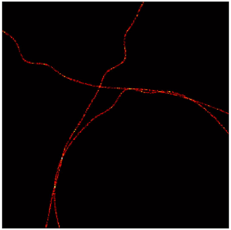
$\mathbf{Y_t} \in \mathbb{R}^{N\times N}$



$=\Bigg($ $\mathbf{H}$

$*$

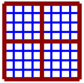
$\mathbf{X_t} \in \mathbb{R}^{L\times L}$



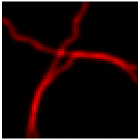
$\Bigg)_{\downarrow q} + \mathbf{N_t} + \mathbf{B}$

$$\mathbf{Y_t} = M_q(H(\mathbf{X_t})) + \mathbf{N_t} + \mathbf{B} \xrightarrow{\text{vec}(\cdot)} \mathbf{y_t} = \mathbf{\Psi}\mathbf{x_t} + \mathbf{n_t} + \mathbf{b}, \quad \forall t = 1, \ldots, T$$

Mathematical Modeling



$\mathbf{Y_t} \in \mathbb{R}^{N \times N}$

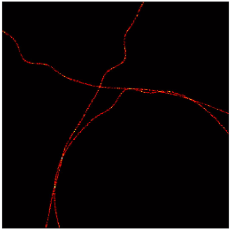


$=\Big($

$\mathbf{H}$

$*$

$\mathbf{X_t} \in \mathbb{R}^{L \times L}$



$\Big)_{\downarrow q} + \mathbf{N_t} + \mathbf{B}$

$$\mathbf{Y_t} = M_q(H(\mathbf{X_t})) + \mathbf{N_t} + \mathbf{B} \xrightarrow{\mathrm{vec}(\cdot)} \mathbf{y_t} = \mathbf{\Psi}\mathbf{x_t} + \mathbf{n_t} + \mathbf{b}, \quad \forall t = 1, \ldots, T$$

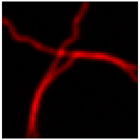
Reformulate the model in the **Covariance Domain**:

$$\mathbf{R_y} = \mathbf{\Psi}\mathbf{R_x}\mathbf{\Psi}^T + \mathbf{R_n}$$

$$\mathbf{R_y} \approx \frac{1}{T-1}\sum_{t=1}^{T}(\mathbf{y_t} - \overline{\mathbf{y}})(\mathbf{y_t} - \overline{\mathbf{y}})^T$$
$$\in \mathbb{R}_+^{N^2 \times N^2}$$

Mathematical Modeling

$\mathbf{Y_t} \in \mathbb{R}^{N\times N}$

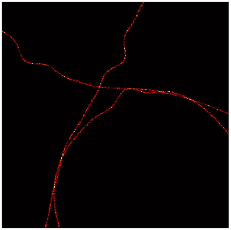


$=$ $\Big($

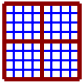
$\mathbf{H}$

$*$

$\mathbf{X_t} \in \mathbb{R}^{L\times L}$



$\Big)_{\downarrow q} + \mathbf{N_t} + \mathbf{B}$



q=4
L=qN

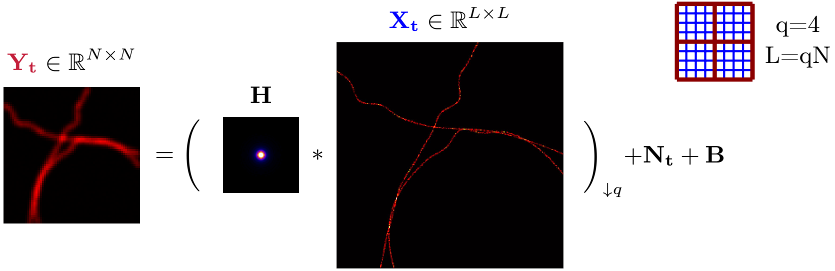
$$\mathbf{Y_t} = M_q(H(\mathbf{X_t})) + \mathbf{N_t} + \mathbf{B} \xrightarrow{\text{vec}(\cdot)} \mathbf{y_t} = \boldsymbol{\Psi}\mathbf{x_t} + \mathbf{n_t} + \mathbf{b}, \quad \forall t = 1, \dots, T$$

Reformulate the model in the **Covariance Domain**:

$$\mathbf{R_y} = \boldsymbol{\Psi}\mathbf{R_x}\boldsymbol{\Psi}^T + \mathbf{R_n}$$

- $\mathbf{R_x}$ is diagonal: $\mathbf{r_x} = \text{diag}(\mathbf{R_x}) \in \mathbb{R}_+^{L^2}$
- $\mathbf{R_n} = s\mathbf{I}$, if $\mathbf{n_t} \sim \mathcal{N}(\mathbf{0}, s\mathbf{1}_{N^2})$
- $\Omega = \{i : (\mathbf{r_x})_i \neq 0\} = \{i : \mathbf{x}_i \neq 0\}$

$$\mathbf{R_y} \approx \frac{1}{T-1}\sum_{t=1}^{T}(\mathbf{y_t} - \overline{\mathbf{y}})(\mathbf{y_t} - \overline{\mathbf{y}})^T \in \mathbb{R}_+^{N^2 \times N^2}$$



$$\mathbf{Y_t} = M_q(H(\mathbf{X_t})) + \mathbf{N_t} + \mathbf{B} \xrightarrow{\text{vec}(\cdot)} \mathbf{y_t} = \mathbf{\Psi x_t} + \mathbf{n_t} + \mathbf{b}, \quad \forall t = 1, \dots, T$$

Reformulate the model in the **Covariance Domain**:

$$\mathbf{R_y} = \mathbf{\Psi R_x \Psi}^T + \mathbf{R_n} \xrightarrow{\text{vec}(\cdot)} \boxed{\mathbf{r_y} = (\mathbf{\Psi} \odot \mathbf{\Psi})\mathbf{r_x} + s\mathbf{I_v}}$$

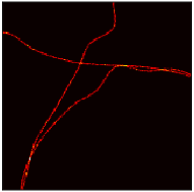
- $\mathbf{R_x}$ is diagonal: $\mathbf{r_x} = \text{diag}(\mathbf{R_x}) \in \mathbb{R}_+^{L^2}$
- $\mathbf{R_n} = s\mathbf{I}$, if $\mathbf{n_t} \sim \mathcal{N}(\mathbf{0}, s\mathbf{1}_{N^2})$
- $\Omega = \{i : (\mathbf{r_x})_i \neq 0\} = \{i : \mathbf{x}_i \neq 0\}$

$$\mathbf{R_y} \approx \frac{1}{T-1}\sum_{t=1}^{T}(\mathbf{y_t} - \overline{\mathbf{y}})(\mathbf{y_t} - \overline{\mathbf{y}})^T \in \mathbb{R}_+^{N^2 \times N^2}$$

Support Estimation

Find the pixels in the fine grid that contain **at least** one fluorescent molecule. (Covariance Domain)

Intensity Estimation

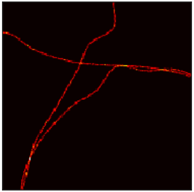


Compute intensity values for the pre-estimated support.

Support Estimation

$$\underset{\mathbf{r_x}\in\mathbb{R}_+^{L^2},\ s\in\mathbb{R}_+}{\arg\min} \frac{1}{2}\|\mathbf{r_y} - (\mathbf{\Psi}\odot\mathbf{\Psi})\mathbf{r_x} - s\mathbf{I_v}\|_2^2 + \mathcal{R}(\mathbf{r_x};\lambda)$$

Intensity Estimation

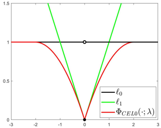


$$\underset{\mathbf{x}\in\mathbb{R}_+^{|\Omega|},\ \mathbf{b}\in\mathbb{R}_+^{N^2}}{\arg\min} \frac{1}{2}\|\overline{\mathbf{y}} - \mathbf{\Psi}_\Omega\mathbf{x} - \mathbf{b}\|_2^2 + \alpha\|\nabla_\Omega\mathbf{x}\|_2^2 + \beta\|\nabla\mathbf{b}\|_2^2$$

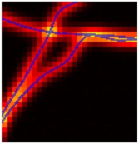
- CEL0[1]: $\mathcal{R}(\mathbf{r}_{\mathbf{x}};\lambda) = \sum_{i=1}^{L^2} \lambda - \frac{\|\mathbf{a}_i\|^2}{2}\left(|(\mathbf{r}_{\mathbf{x}})_i| - \frac{\sqrt{2\lambda}}{\|\mathbf{a}_i\|}\right)^2 \mathbb{1}_{\{|(\mathbf{r}_{\mathbf{x}})_i| \leq \frac{\sqrt{2\lambda}}{\|\mathbf{a}_i\|}\}}$

- ℓ_1-norm: $\mathcal{R}(\mathbf{r}_{\mathbf{x}};\lambda) = \lambda\|\mathbf{r}_{\mathbf{x}}\|_1$

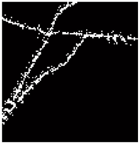
- TV: $\mathcal{R}(\mathbf{r}_{\mathbf{x}};\lambda) = \lambda\|\nabla\mathbf{r}_{\mathbf{x}}\|_{2,1}$



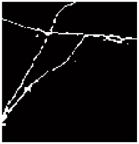
$\bar{\mathbf{y}}$ + GT



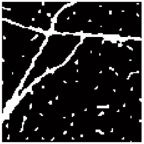
CEL0 result[2]



ℓ_1 result



TV result



[1] Continuous Exact ℓ_0 (CEL0) penalty (Soubies *et al.*, '15)

[2] An algorithmic restarting approach has been considered to improve the support reconstruction quality

Table of Contents

$$\underset{\mathbf{r_x} \geq 0, s \geq 0}{\arg\min}\ \mathcal{F}(\mathbf{r_x}, s) + \lambda \mathcal{R}(\mathbf{r_x})$$

where $\mathcal{F}(\mathbf{r_x}, s) = \frac{1}{2}\|\mathbf{r_y} - (\mathbf{\Psi} \odot \mathbf{\Psi})\mathbf{r_x} - s\mathbf{I_v}\|_2^2$

Algorithm COL0RME, Support Estimation

Require: $\mathbf{r_y} \in \mathbb{R}^{M^4}, {\mathbf{r_x}}^0 \in \mathbb{R}^{L^2}$, $\lambda > 0$

repeat

$s^{k+1} = \frac{1}{M^2}\mathbf{I_v}^\intercal(\mathbf{r_y} - (\mathbf{\Psi} \odot \mathbf{\Psi}){\mathbf{r_x}}^k)$

$\mathbf{z}^{k+1} = {\mathbf{r_x}}^k - \tau \nabla \mathcal{F}({\mathbf{r_x}}^k, s^{k+1})$

${\mathbf{r_x}}^{k+1} = \mathrm{prox}_{\tau\lambda\mathcal{R}(\cdot)}(\mathbf{z}^{k+1})$

until convergence

return $\Omega = \{i : (\mathbf{r_x})_i \neq 0\}$, s

Definition of proximal operator: $\mathrm{prox}_{\gamma\mathcal{R}(\cdot)}(\mathbf{z}) := \underset{\mathbf{x} \in \mathbb{R}^{L^2}}{\arg\min}\{\frac{1}{2}\|\mathbf{x} - \mathbf{z}\|_2^2 + \gamma\mathcal{R}(\mathbf{x})\}$.

$$\underset{\mathbf{r_x} \geq 0, s \geq 0}{\arg\min}\ \mathcal{F}(\mathbf{r_x}, s) + \lambda \mathcal{R}(\mathbf{r_x})$$

where $\mathcal{F}(\mathbf{r_x}, s) = \frac{1}{2}\|\mathbf{r_y} - (\mathbf{\Psi} \odot \mathbf{\Psi})\mathbf{r_x} - s\mathbf{I_v}\|_2^2$

Algorithm PnP - COL0RME, Support Estimation

Require: $\mathbf{r_y} \in \mathbb{R}^{M^4}, \mathbf{r_x}^0 \in \mathbb{R}^{L^2}, \cancel{\lambda}\, \sigma > 0$

repeat

$s^{k+1} = \frac{1}{M^2}\mathbf{I_v}^\intercal(\mathbf{r_y} - (\mathbf{\Psi} \odot \mathbf{\Psi})\mathbf{r_x}^k)$

$\mathbf{z}^{k+1} = \mathbf{r_x}^k - \tau \nabla \mathcal{F}(\mathbf{r_x}^k, s^{k+1})$

$\mathbf{r_x}^{k+1} \cancel{= \text{prox}_{\tau\lambda\mathcal{R}(\cdot)}(\mathbf{z}^{k+1})} = D_\sigma(\mathbf{z}^{k+1})$

until convergence

return $\Omega = \{i : (\mathbf{r_x})_i \neq 0\}$, s

Plug-and-Play (PnP) methods (Venkatakrishnan *et al.*, '13):
Replace, within a proximal algorithm, the operator $\text{prox}_{\tau\lambda\mathcal{R}}(\cdot)$ with a more general image denoiser.

Use a gradient step denoiser of the form[3]:

$$D_{\sigma}(\mathbf{z}) = \mathbf{z} - \nabla \mathcal{R}_{\sigma}(\mathbf{z}), \tag{1}$$

where $\mathcal{R}_{\sigma} : \mathbb{R}^{L^2} \to \mathbb{R}$ is a scalar function defined by:

$$\mathcal{R}_{\sigma}(\mathbf{z}) = \frac{1}{2}\|\mathbf{z} - N_{\sigma}(\mathbf{z})\|^2, \tag{2}$$

with $N_{\sigma} : \mathbb{R}^{L^2} \to \mathbb{R}^{L^2}$ being a neural network.

[3] (Cohen *et al.*, '21, Hurault *et al.*, '22a, Hurault *et al.*, '22b)

Choice of the denoiser

Use a gradient step denoiser of the form[3]:

$$D_\sigma(\mathbf{z}) = \mathbf{z} - \nabla \mathcal{R}_\sigma(\mathbf{z}), \tag{1}$$

where $\mathcal{R}_\sigma : \mathbb{R}^{L^2} \to \mathbb{R}$ is a scalar function defined by:

$$\mathcal{R}_\sigma(\mathbf{z}) = \frac{1}{2}\|\mathbf{z} - N_\sigma(\mathbf{z})\|^2, \tag{2}$$

with $N_\sigma : \mathbb{R}^{L^2} \to \mathbb{R}^{L^2}$ being a neural network.

Convergence Guarantees

In (Hurault *et al.*, '22b), they prove that $D_\sigma(\mathbf{z}) = \mathrm{prox}_{\phi_\sigma}(\mathbf{z})$, $\phi_\sigma :\in \mathbb{R}^{L^2} \to \mathbb{R}$ and derive convergence guarantees of the resulting PGD scheme.
In each iteration:

$$\mathcal{G}_\sigma({\mathbf{r}_{\mathbf{x}}}^k, s^k) := \mathcal{F}({\mathbf{r}_{\mathbf{x}}}^k, s^k) + \mathcal{R}_\sigma(\mathbf{z}^k) - \frac{1}{2}\|\mathbf{z}^k - {\mathbf{r}_{\mathbf{x}}}^k\|_2^2$$

[3](Cohen *et al.*, '21, Hurault *et al.*, '22a, Hurault *et al.*, '22b)

Introducing a scaling parameter

Introduce *denoiser scaling* parameter[4] $\mu > 0$:

$$D_{\mu,\sigma}(\mathbf{z}) := \frac{1}{\mu} D_{\sigma}(\mu \mathbf{z}), \tag{3}$$

[4] (Xu *et al.*, '20)

Introducing a scaling parameter

Introduce *denoiser scaling* parameter[4] $\mu > 0$:

$$D_{\mu,\sigma}(\mathbf{z}) := \frac{1}{\mu} D_{\sigma}(\mu \mathbf{z}), \tag{3}$$

The denoiser step will now be:

$$\mathbf{r_x}^{k+1} = D_{\mu,\sigma}(\mathbf{z}^{k+1}),$$

[4] (Xu *et al.*, '20)

Introducing a scaling parameter

Introduce *denoiser scaling* parameter[4] $\mu > 0$:

$$D_{\mu,\sigma}(\mathbf{z}) := \frac{1}{\mu} D_{\sigma}(\mu \mathbf{z}), \tag{3}$$

The denoiser step will now be:

$$\mathbf{r_x}^{k+1} = D_{\mu,\sigma}(\mathbf{z}^{k+1}),$$

In each iteration:

$$\mathcal{G}_{\mu,\sigma}(\mathbf{r_x}^k, s^k) = \mathcal{F}(\mathbf{r_x}^k, s^k) + \frac{1}{\mu^2} \mathcal{R}_{\sigma}(\mu \mathbf{z}^k) - \frac{1}{2} \|\mathbf{z}^k - \mathbf{r_x}^k\|_2^2$$

[4] (Xu *et al.*, '20)

Table of Contents

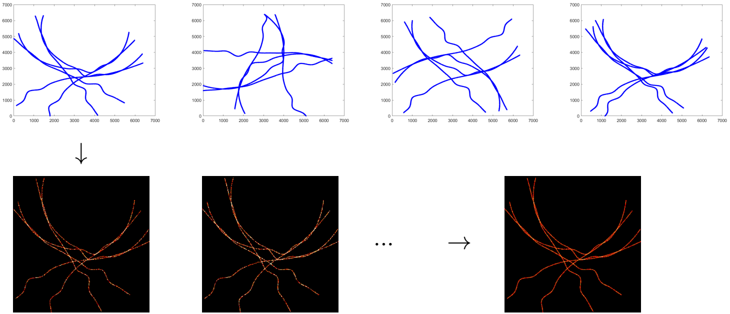
Training the denoiser

Reminder:

$$\mathbf{r_x}^{k+1} = D_{\mu,\sigma}(\mathbf{r_x}^{k} - \tau \nabla \mathcal{F}(\mathbf{r_x}^{k}, s^{k+1}))$$

The denoiser takes as an input the sample auto-covariance matrix of a fluctuating temporal sequence of images.

- **Dataset**: Create different spatial patterns → Generate fluctuating sequences → Compute the temporal variance

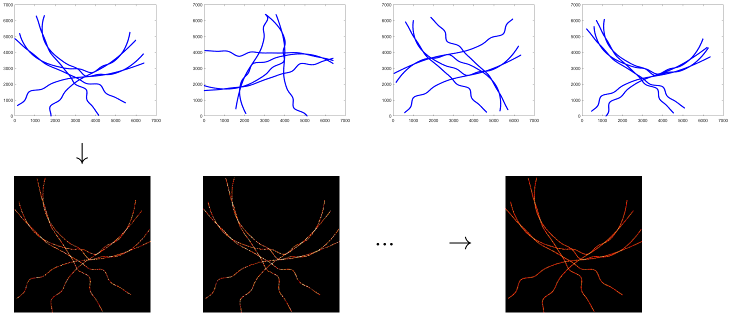


Add Gaussian noise $\boldsymbol{\eta} \sim \mathcal{N}(\mathbf{0}_{L^2}, \sigma^2 \mathbf{I}_{L^2})$, with $\sigma \sim U(0, 50/255)$.
(500 pairs of clean-noise auto-covariance images and 100 for validation)

[5] https://github.com/samuro95/Prox-PnP

Training the denoiser

- **Dataset**: Create different spatial patterns → Generate fluctuating sequences → Compute the temporal variance



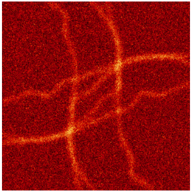
Add Gaussian noise $\boldsymbol{\eta} \sim \mathcal{N}(\mathbf{0}_{L^2}, {\color{red}\sigma}^2 \mathbf{I}_{L^2})$, with ${\color{red}\sigma} \sim U(0, 50/255)$.
(500 pairs of clean-noise auto-covariance images and 100 for validation)

- **Training**: We follow the procedure suggested in (Hurault *et al.*, '22) and use the code available online[5].

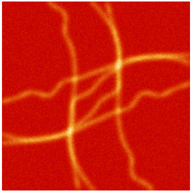
[5] https://github.com/samuro95/Prox-PnP

Preliminary Results on Simulated Data*

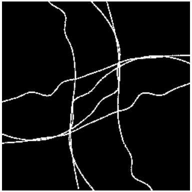
$\mathbf{y_1}$



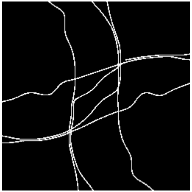
$\mathrm{diag}(\mathbf{R_y})$



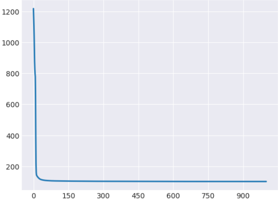
$\hat{\Omega} = \{i : (\hat{\mathbf{r}}_{\mathbf{x}})_i \neq 0\}$



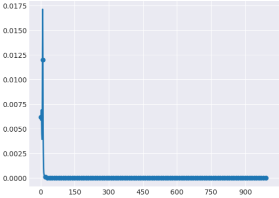
$\Omega = \{i : (\mathbf{r}_{\mathbf{x}})_i \neq 0\}$



$\mathcal{G}_{\mu,\sigma}$

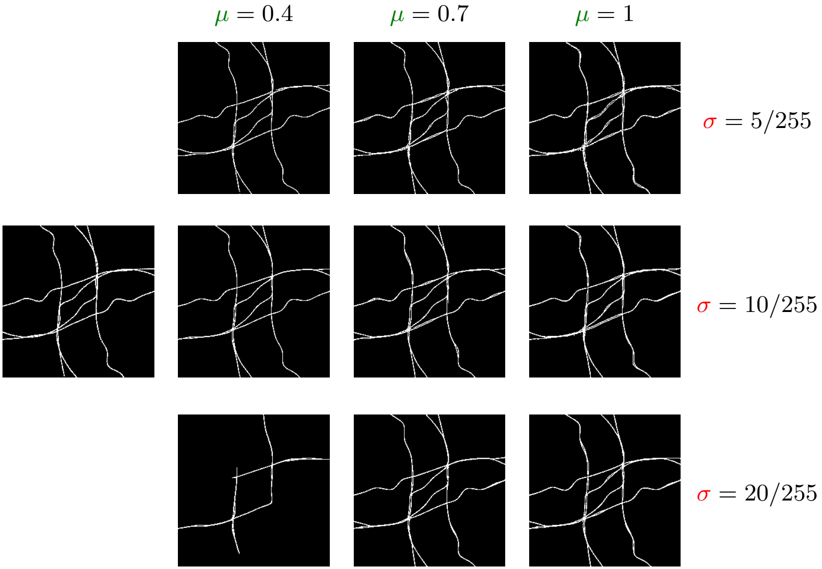


$\|\mathbf{r_x}^{k+1} - \mathbf{r_x}^{k}\|^2 / \|\mathbf{r_x}^{0}\|^2$



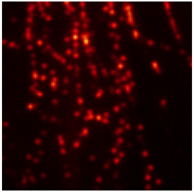
* Only for deblurring ($q = 1$ and $L = N$) and using a diagonal temporal covariance matrix $\mathbf{R_y}$.

Sensitivity to denoising and scaling parameters



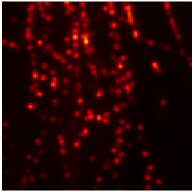
Results on Experimental HD SMLM Data

- High-density Single Molecule Localization Microscopy acquisitions
- Real dataset created for the 2013 SMLM challenge
- The dataset contains $T = 500$ images, the FWHM of the PSF is 351.8 nm.

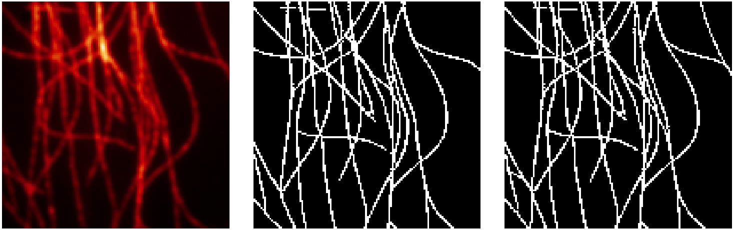


Results on Experimental HD SMLM Data

- High-density Single Molecule Localization Microscopy acquisitions
- Real dataset created for the 2013 SMLM challenge
- The dataset contains $T = 500$ images, the FWHM of the PSF is 351.8 nm.



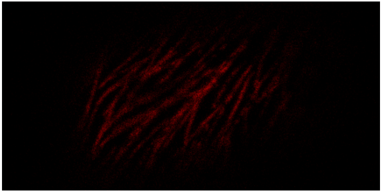
$\overline{\mathbf{y}}$ Ω Ω



$(\mu = 0.9)$ $(\mu = 1.1)$

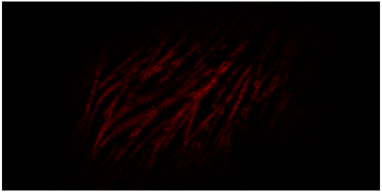
Results on Ostreopsis confocal Data

- Real biological sample of the unicellular alga *Ostreopsis*
- The dataset contains $T = 500$ images, the FWHM of the PSF is 229 nm.

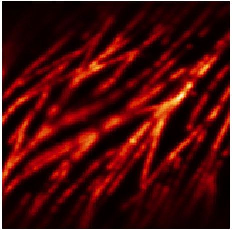


Results on Ostreopsis confocal Data

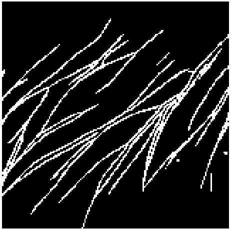
- Real biological sample of the unicellular alga *Ostreopsis*
- The dataset contains $T = 500$ images, the FWHM of the PSF is 229 nm.



$\overline{\mathbf{y}}$

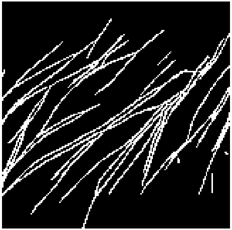


Ω



$(\mu = 0.8)$

Ω



$(\mu = 0.9)$

Conclusion and Future Work

Conclusion:

- A learned from the data proximal operator of a non-convex regularization function (or a Denoiser) can capture better the geometry of specific structures (e.g., filaments)

Future Work:

- Test the approach including also a down-sampling operator (Super-Resolution)
- Take into account the complete covariance matrix $\mathbf{R}_{\mathbf{y}}$ of our diffraction-limited images.

Thank you :)

e-mail: vasiliki.stergiopoulou@inria.fr

Do you have any questions?

References I

Regev Cohen, Yochai Blau, Daniel Freedman, and Ehud Rivlin, *It has potential: Gradient-driven denoisers for convergent solutions to inverse problems*, Advances in Neural Information Processing Systems (M. Ranzato, A. Beygelzimer, Y. Dauphin, P.S. Liang, and J. Wortman Vaughan, eds.), vol. 34, Curran Associates, Inc., 2021, pp. 18152–18164.

T. Dertinger, R. Colyer, G. Iyer, S. Weiss, and J. Enderlein, *Fast, background-free, 3D super-resolution optical fluctuation imaging (SOFI)*, Proceedings of the National Academy of Sciences, 106 (52) (2009), 22287–22292.

G. Dardikman-Yoffe and Y. C. Eldar, *Learned sparcom: unfolded deep super-resolution microscopy*, Opt. Express **28** (2020), no. 19, 27736–27763.

N. Gustafsson, S. Culley, G. Ashdown, D. M. Owen, P. M. Pereira, and R. Henriques, *Fast live-cell conventional fluorophore nanoscopy with ImageJ through super-resolution radial fluctuations*, Nature communications, 7(1) (2016), 12471–12471 (eng).

S. Hurault, A. Leclaire, and N. Papadakis, *Gradient step denoiser for convergent plug-and-play*, International Conference on Learning Representations, 2022.

References II

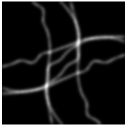
- Samuel Hurault, Arthur Leclaire, and Nicolas Papadakis, *Proximal denoiser for convergent plug-and-play optimization with nonconvex regularization*, Proceedings of the 39th International Conference on Machine Learning, Proceedings of Machine Learning Research, vol. 162, PMLR, 17–23 Jul 2022, pp. 9483–9505.
- Bastien Laville, Laure Blanc-Féraud, and Gilles Aubert, *Off-the-grid curve reconstruction through divergence regularisation: an extreme point result*, working paper or preprint, May 2022.
- E. Soubies, L. Blanc-Féraud, and G. Aubert, *A continuous exact ℓ_0 penalty (CEL0) for least squares regularized problem*, SIAM Journal on Imaging Sciences, 8 (3) (2015), 1607–1639.
- V. Stergiopoulou, L. Calatroni, J. H. de Morais Goulart, S. Schaub, and L. Blanc-Féraud, *Col0rme: Super-resolution microscopy based on sparse blinking/fluctuating fluorophore localization and intensity estimation*, Biological Imaging **2** (2022), e1.
- V. Stergiopoulou, L. Calatroni, S. Schaub, and L. Blanc-Féraud, *3d image super-resolution by fluorophore fluctuations and ma-tirf microscopy reconstruction (3d-col0rme)*, 2022 IEEE 19th International Symposium on Biomedical Imaging (ISBI), 2022, pp. 1–4.

V. Stergiopoulou, J. H. de Morais Goulart, S. Schaub, L. Calatroni, and L. Blanc-Féraud, *COL0RME: Covariance-based l0 super-resolution microscopy with intensity estimation*, 2021 IEEE 18th International Symposium on Biomedical Imaging (ISBI), 2021, pp. 349–352.

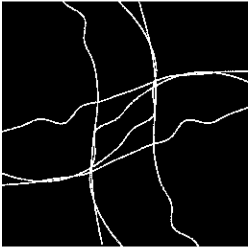
O. Solomon, Y. C. Eldar, M. Mutzafi, and M. Segev, *SPARCOM: Sparsity based super-resolution correlation microscopy*, SIAM Journal on Imaging Sciences, 12 (1) (2019), 392–419.

Xiaojian Xu, Jiaming Liu, Yu Sun, Brendt Wohlberg, and Ulugbek S Kamilov, *Boosting the performance of plug-and-play priors via denoiser scaling*, 2020 54th Asilomar Conference on Signals, Systems, and Computers, IEEE, 2020, pp. 1305–1312.

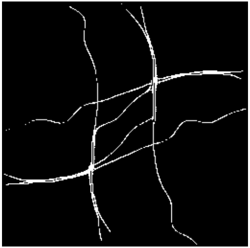
Preliminary results for PnP-COL0RME (Super-Resolution)



$\overline{\mathbf{y}}$



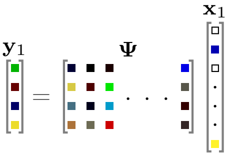
$\hat{\Omega} = \{i : (\hat{\mathbf{r}}_{\mathbf{x}})_i \neq 0\}$



$\Omega = \{i : (\mathbf{r}_{\mathbf{x}})_i \neq 0\}^*$

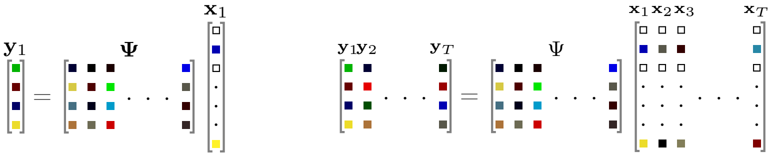
* For this reconstruction, only the temporal **auto**-covariances were used.

$$\mathbf{y_t} = \mathbf{\Psi}\mathbf{x_t}, \quad \forall t = 1, \ldots, T$$



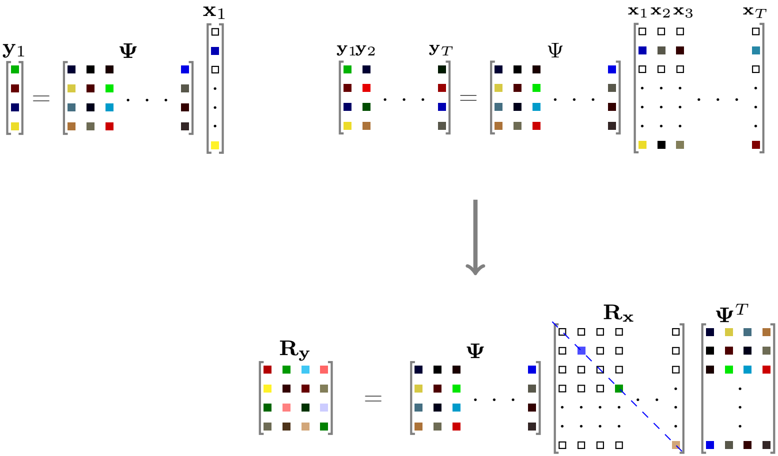
Model reformulation in the Covariance Domain

$$\mathbf{y_t} = \mathbf{\Psi} \mathbf{x_t}, \quad \forall t = 1, \ldots, T$$



Model reformulation in the Covariance Domain

$$\mathbf{y_t} = \mathbf{\Psi}\mathbf{x_t}, \quad \forall t = 1, \dots, T$$

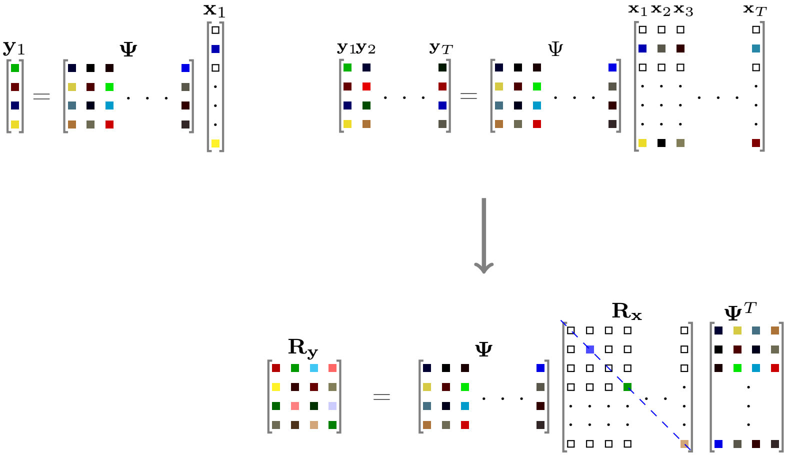


$$\boxed{\mathbf{R_y} \approx \frac{1}{T-1} \sum_{t=1}^{T} (\mathbf{y_t} - \overline{\mathbf{y}})(\mathbf{y_t} - \overline{\mathbf{y}})^T \in \mathbb{R}^{N^2 \times N^2}}$$

$$\mathbf{r_x} = \mathrm{diag}(\mathbf{R_x}) \in \mathbb{R}_+^{L^2}$$

Model reformulation in the Covariance Domain

$$\mathbf{y_t} = \mathbf{\Psi} \mathbf{x_t}, \quad \forall t = 1, \ldots, T \quad \Longrightarrow \quad \mathbf{R_y} = \mathbf{\Psi} \mathbf{R_x} \mathbf{\Psi^T}$$

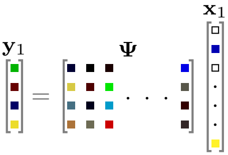


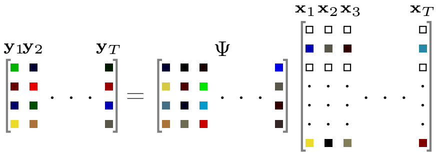
$$\boxed{\mathbf{R_y} \approx \frac{1}{T-1} \sum_{t=1}^{T} (\mathbf{y_t} - \overline{\mathbf{y}})(\mathbf{y_t} - \overline{\mathbf{y}})^T \in \mathbb{R}^{N^2 \times N^2}}$$

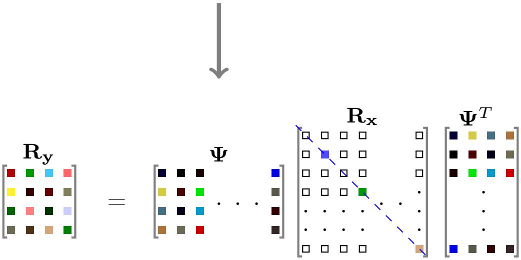
$$\mathbf{r_x} = \mathrm{diag}(\mathbf{R_x}) \in \mathbb{R}_+^{L^2}$$

Model reformulation in the Covariance Domain

$$\mathbf{y_t} = \mathbf{\Psi x_t} + \mathbf{n_t}, \quad \forall t = 1, \ldots, T \quad \Longrightarrow \quad \mathbf{R_y} = \mathbf{\Psi R_x \Psi^T} + \mathbf{R_n}$$





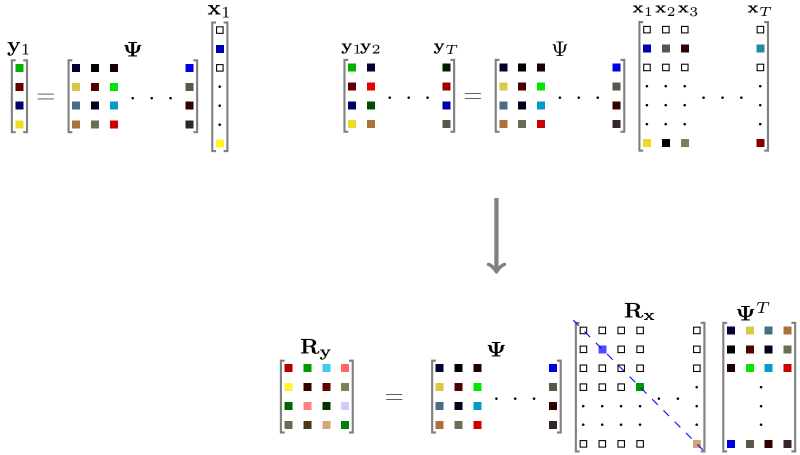


$$\boxed{\mathbf{R_y} \approx \frac{1}{T-1} \sum_{t=1}^{T} (\mathbf{y_t} - \overline{\mathbf{y}})(\mathbf{y_t} - \overline{\mathbf{y}})^T \in \mathbb{R}^{N^2 \times N^2}}$$

$$\mathbf{r_x} = \mathrm{diag}(\mathbf{R_x}) \in \mathbb{R}_+^{L^2}$$

Model reformulation in the Covariance Domain

$$\mathbf{y_t} = \boldsymbol{\Psi}\mathbf{x_t} + \mathbf{n_t} + \mathbf{b}, \quad \forall t = 1, \ldots, T \quad \Longrightarrow \quad \mathbf{R_y} = \boldsymbol{\Psi}\mathbf{R_x}\boldsymbol{\Psi}^{\mathbf{T}} + \mathbf{R_n}$$



$$\boxed{\mathbf{R_y} \approx \frac{1}{T-1}\sum_{t=1}^{T}(\mathbf{y_t} - \overline{\mathbf{y}})(\mathbf{y_t} - \overline{\mathbf{y}})^T \in \mathbb{R}^{N^2 \times N^2}}$$

$$\mathbf{r_x} = \mathrm{diag}(\mathbf{R_x}) \in \mathbb{R}_+^{L^2}$$

Support Estimation

- **Covariance Domain**:

$$\boxed{\mathbf{y_t} = \boldsymbol{\Psi}\mathbf{x_t} + \mathbf{n_t} + \mathbf{b}}$$

$$\downarrow$$

$$\boxed{\mathbf{R_y} = \boldsymbol{\Psi}\mathbf{R_x}\boldsymbol{\Psi}^{\mathbf{T}} + \mathbf{R_n}}$$

$$\mathbf{R_y} \approx \frac{1}{T-1}\sum_{t=1}^{T}(\mathbf{y_t} - \overline{\mathbf{y}})(\mathbf{y_t} - \overline{\mathbf{y}})^T \in \mathbb{R}^{N^2 \times N^2}$$

$$\mathbf{r_x} = \mathrm{diag}(\mathbf{R_x}) \in \mathbb{R}_+^{L^2} \qquad \mathbf{R_n} = s\mathbf{I}, s \in \mathbb{R}_+$$

Support Estimation

- **Covariance Domain**:

$$\boxed{\mathbf{y_t} = \mathbf{\Psi}\mathbf{x_t} + \mathbf{n_t} + \mathbf{b}}$$

$$\downarrow$$

$$\boxed{\mathbf{R_y} = \mathbf{\Psi}\mathbf{R_x}\mathbf{\Psi}^\mathbf{T} + \mathbf{R_n}}$$

$$\mathbf{R_y} \approx \frac{1}{T-1}\sum_{t=1}^{T}(\mathbf{y_t} - \overline{\mathbf{y}})(\mathbf{y_t} - \overline{\mathbf{y}})^T \in \mathbb{R}^{N^2 \times N^2}$$

$$\mathbf{r_x} = \mathrm{diag}(\mathbf{R_x}) \in \mathbb{R}_+^{L^2} \qquad \mathbf{R_n} = s\mathbf{I},\, s \in \mathbb{R}_+$$

$$\mathrm{vec}(\cdot)\downarrow$$

$$\boxed{\mathbf{r_y} = (\mathbf{\Psi} \odot \mathbf{\Psi})\mathbf{r_x} + s\mathbf{I_v}}$$

COL0RME (COvariance-based ℓ_0 super-Resolution Microscopy with intensity Estimation)

Support Estimation

- **Covariance Domain:**

$$\boxed{\mathbf{y_t} = \boldsymbol{\Psi}\mathbf{x_t} + \mathbf{n_t} + \mathbf{b}}$$

$$\downarrow$$

$$\boxed{\mathbf{R_y} = \boldsymbol{\Psi}\mathbf{R_x}\boldsymbol{\Psi}^{\mathbf{T}} + \mathbf{R_n}}$$

$$\mathbf{R_y} \approx \frac{1}{T-1}\sum_{t=1}^{T}(\mathbf{y_t} - \overline{\mathbf{y}})(\mathbf{y_t} - \overline{\mathbf{y}})^T \in \mathbb{R}^{N^2 \times N^2}$$

$$\mathbf{r_x} = \mathrm{diag}(\mathbf{R_x}) \in \mathbb{R}_+^{L^2} \qquad \mathbf{R_n} = s\mathbf{I}, s \in \mathbb{R}_+$$

$$\mathrm{vec}(\cdot) \downarrow$$

$$\boxed{\mathbf{r_y} = (\boldsymbol{\Psi} \odot \boldsymbol{\Psi})\mathbf{r_x} + s\mathbf{I_v}}$$

✓ Exploit the independent statistical behaviour of the fluorescent emitters

✓ Shrink the PSF

× We cannot compute real intensity values

Support Estimation

- **Covariance Domain**:

  $$\mathbf{r_y} = (\boldsymbol{\Psi} \odot \boldsymbol{\Psi})\mathbf{r_x} + s\mathbf{I_v}$$

- **Sparsity** on $\mathbf{r_x}$

Support Estimation

- **Covariance Domain**:

  $\mathbf{r_y} = (\mathbf{\Psi} \odot \mathbf{\Psi})\mathbf{r_x} + s\mathbf{I_v}$

- **Sparsity** on $\mathbf{r_x}$
- **Output**: $\Omega = \{i : (\mathbf{r_x})_i \neq 0\} = \{i : \mathbf{x}_i \neq 0\}\,, s$

COL0RME (COvariance-based ℓ_0 super-Resolution Microscopy with intensity Estimation)

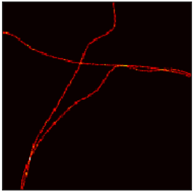
Support Estimation

- **Covariance Domain**:

  $\mathbf{r_y} = (\mathbf{\Psi} \odot \mathbf{\Psi})\mathbf{r_x} + s\mathbf{I_v}$

- **Sparsity** on $\mathbf{r_x}$
- **Output**: $\Omega = \{i : (\mathbf{r_x})_i \neq 0\} = \{i : \mathbf{x}_i \neq 0\}\,, s$

Intensity Estimation



- **Image Domain**:

  $\mathbf{y_t} = \mathbf{\Psi}\mathbf{x_t} + \mathbf{n_t} + \mathbf{b}$

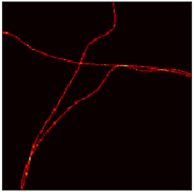
- Smoothing of **signal intensities** on the estimated support Ω and smooth **background**
- **Output**: $\mathbf{x} = \frac{1}{T}\sum_{t=1}^{T} \mathbf{x_t}, \mathbf{b}$

COL0RME: Hyper-parameter Tuning

Support Estimation

$$\operatorname*{arg\,min}_{\mathbf{r_x}\in\mathbb{R}_+^{L^2},\ s\in\mathbb{R}_+} \frac{1}{2}\|\mathbf{r_y} - (\mathbf{\Psi}\odot\mathbf{\Psi})\mathbf{r_x} - s\mathbf{I_v}\|_2^2 + \mathcal{R}(\mathbf{r_x};\lambda)$$

Intensity Estimation



$$\operatorname*{arg\,min}_{\mathbf{x}\in\mathbb{R}_+^{|\Omega|},\ \mathbf{b}\in\mathbb{R}_+^{N^2}} \frac{1}{2}\|\overline{\mathbf{y}} - \mathbf{\Psi_\Omega x} - \mathbf{b}\|_2^2 + \mu\|\nabla_\Omega \mathbf{x}\|_2^2 + \beta\|\nabla \mathbf{b}\|_2^2$$

COL0RME: Hyper-parameter Tuning

$$\underset{\mathbf{r_x}\in\mathbb{R}_+^{L^2},\ s\in\mathbb{R}_+}{\arg\min}\ \frac{1}{2}\|\mathbf{r_y}-(\mathbf{\Psi}\odot\mathbf{\Psi})\mathbf{r_x}-s\mathbf{I_v}\|_2^2+\mathcal{R}(\mathbf{r_x};\lambda)$$

- λ: $\lambda=\gamma\lambda_{max}$, $\gamma\in(0,1)$
  λ_{max}: a reference value, defined as the smallest regularization parameter for which the identically zero solution is found.

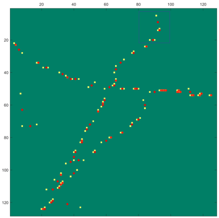
COL0RME: Hyper-parameter Tuning

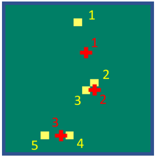
$$\underset{\mathbf{r_x}\in\mathbb{R}_+^{L^2},\ s\in\mathbb{R}_+}{\arg\min}\ \frac{1}{2}\|\mathbf{r_y}-(\mathbf{\Psi}\odot\mathbf{\Psi})\mathbf{r_x}-s\mathbf{I_v}\|_2^2+\mathcal{R}(\mathbf{r_x};\lambda)$$

- λ: $\lambda = \gamma\lambda_{max}$, $\gamma \in (0,1)$
  λ_{max}: a reference value, defined as the smallest regularization parameter for which the identically zero solution is found.

To avoid the dotted reconstruction (For CEL0 penalty) :

- We **restart** the algorithm repeatedly with a **new initialisation** each time, that depends but is different to the previous reconstruction.
- The final support image is the **superposition** of the multiple reconstructions.





■: Solution of **Run 1**
+: Initialization of **Run 2**

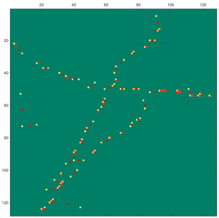
COL0RME: Hyper-parameter Tuning

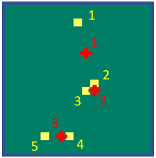
$$\underset{\mathbf{r_x}\in\mathbb{R}_+^{L^2},\ s\in\mathbb{R}_+}{\arg\min} \quad \frac{1}{2}\|\mathbf{r_y} - (\mathbf{\Psi}\odot\mathbf{\Psi})\mathbf{r_x} - s\mathbf{I_v}\|_2^2 + \mathcal{R}(\mathbf{r_x};\lambda)$$

- λ: $\lambda = \gamma\lambda_{max}$, $\gamma \in (0,1)$
  λ_{max}: a reference value, defined as the smallest regularization parameter for which the identically zero solution is found.

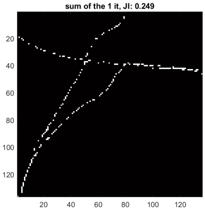
To avoid the dotted reconstruction (For CEL0 penalty) :

- We **restart** the algorithm repeatedly with a **new initialisation** each time, that depends but is different to the previous reconstruction.
- The final support image is the **superposition** of the multiple reconstructions.





■: Solution of **Run 1**
+: Initialization of **Run 2**



COL0RME: Hyper-parameter Tuning

$$\underset{\mathbf{x}\in\mathbb{R}_+^{|\Omega|},\ \mathbf{b}\in\mathbb{R}_+^{N^2}}{\arg\min} \quad \frac{1}{2}\|\overline{\mathbf{y}} - \boldsymbol{\Psi}_{\boldsymbol{\Omega}}\mathbf{x} - \mathbf{b}\|_2^2 + \mu\|\nabla_\Omega \mathbf{x}\|_2^2 + \beta\|\nabla\mathbf{b}\|_2^2$$

- μ: We can use the **discrepancy principle**, a well-known a-posteriori parameter-choice strategy to efficiently estimate the hyper-parameter.

COL0RME: Hyper-parameter Tuning

$$\underset{\mathbf{x}\in\mathbb{R}_+^{|\Omega|},\ \mathbf{b}\in\mathbb{R}_+^{N^2}}{\arg\min} \ \frac{1}{2}\|\overline{\mathbf{y}} - \mathbf{\Psi}_{\mathbf{\Omega}}\mathbf{x} - \mathbf{b}\|_2^2 + \mu\|\nabla_\Omega \mathbf{x}\|_2^2 + \beta\|\nabla \mathbf{b}\|_2^2$$

- μ: We can use the **discrepancy principle**, a well-known a-posteriori parameter-choice strategy to efficiently estimate the hyper-parameter.

Discrepancy principle strategy[6]

The regularization parameter μ will be chosen such that:

$$\|\overline{\mathbf{y}} - \mathbf{\Psi}_{\mathbf{\Omega}}\hat{\mathbf{x}}_\mu - \hat{\mathbf{b}}\|_2^2 = \nu_{DP}^2\|\overline{\mathbf{n}}\|_2^2 \tag{4}$$

$\nu_{DP} \approx 1$ is a 'safety factor', $\|\overline{\mathbf{n}}\|_2^2 = N^2\frac{s}{T}$, s: the **estimated** noise variance.

We can define the function $f(\mu) : \mathbb{R} \to \mathbb{R}$ as:

$$f(\mu) = \frac{1}{2}\|\overline{\mathbf{y}} - \mathbf{\Psi}_{\mathbf{\Omega}}\mathbf{x}_\mu - \mathbf{b}\|_2^2 - \frac{\nu_{DP}^2}{2}\|\mathbf{e}\|_2^2 \tag{5}$$

and we want $\mu^* : f(\mu^*) = 0$.
We can find iteratively μ^* using the Newton's method:

$$\mu_{n+1} = \mu_n - \frac{f(\mu_n)}{f'(\mu_n)} \tag{6}$$

[6] Per Christian Hansen,Discrete Inverse Problems: Insight and Algorithms, Society for Industrial and Applied Mathematics, USA, 2010

$$\underset{\mathbf{x}\in\mathbb{R}_+^{|\Omega|},\ \mathbf{b}\in\mathbb{R}_+^{N^2}}{\arg\min} \quad \frac{1}{2}\|\overline{\mathbf{y}} - \mathbf{\Psi_\Omega x} - \mathbf{b}\|_2^2 + \mu\|\nabla_\Omega \mathbf{x}\|_2^2 + \beta\|\nabla \mathbf{b}\|_2^2$$

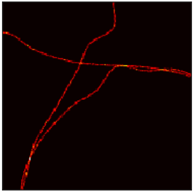
- β: Does not require fine tuning

COL0RME: Intensity and Background estimation

Support Estimation

$$\underset{\mathbf{r_x}\in\mathbb{R}_+^{L^2},\ s\in\mathbb{R}_+}{\arg\min} \ \frac{1}{2}\|\mathbf{r_y} - (\boldsymbol{\Psi}\odot\boldsymbol{\Psi})\mathbf{r_x} - s\mathbf{I_v}\|_2^2 + \mathcal{R}(\mathbf{r_x};\lambda)$$

Intensity Estimation



$$\underset{\mathbf{x}\in\mathbb{R}_+^{|\Omega|},\ \mathbf{b}\in\mathbb{R}_+^{N^2}}{\arg\min} \ \frac{1}{2}\|\overline{\mathbf{y}} - \boldsymbol{\Psi}_{\boldsymbol{\Omega}}\mathbf{x} - \mathbf{b}\|_2^2 + \mu\|\nabla_\Omega \mathbf{x}\|_2^2 + \beta\|\nabla\mathbf{b}\|_2^2$$

COL0RME: Intensity and Background estimation

- Retrieves real image intensity information
  (the only super-resolution method exploiting temporal fluctuations)

  $\rightarrow$ use the intensity information for **3D reconstruction** in e.g., MA-TIRF (Multi-Angle Total Internal Reflection Fluorescence) microscopy acquisitions.

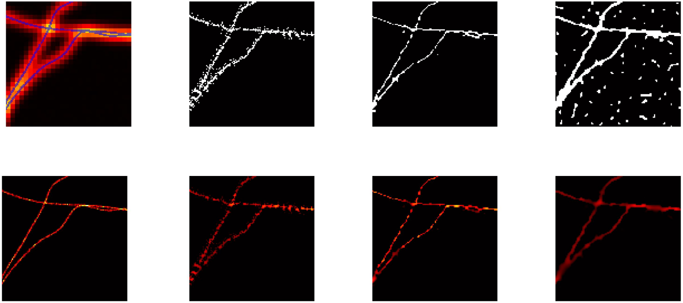
COL0RME: Intensity and Background estimation

- Retrieves real image intensity information
  (the only super-resolution method exploiting temporal fluctuations)

  → use the intensity information for **3D reconstruction** in e.g., MA-TIRF (Multi-Angle Total Internal Reflection Fluorescence) microscopy acquisitions.

- Correct artifacts:

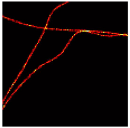
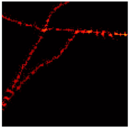
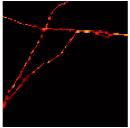
$\bar{\mathbf{y}}$ + GT | CEL0 result | ℓ_1 result | TV result



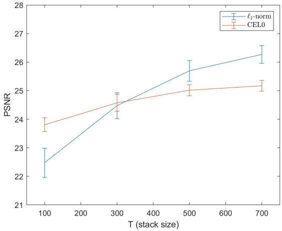
COL0RME: Intensity and Background estimation

- Results obtained using 500 frames:

GT | CEL0 result | ℓ_1 result

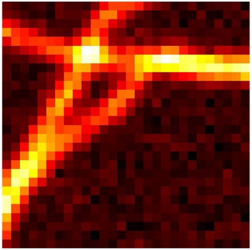
- Evaluation using the PSNR (Peak Signal-to-Noise Ratio) metric



Simulated data

Diffraction limited frames

Low Background & SNR $\approx$ 15.5dB



Size of the image: 40 x 40
Pixel size: 0.1 μm
Video rate: 100 fps
Acquisition time: 5 s

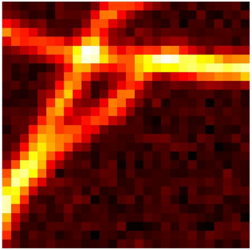
Spatial pattern: Microtubules dataset from the SMLM challenge 2016 [6]
Temporal profiles: SOFI simulation tool (Girsault *et al.*. 2016)

[6] http://bigwww.epfl.ch/smlm/datasets/index.html

Simulated data

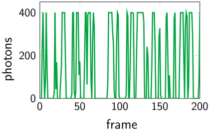
Diffraction limited frames

Low Background & SNR ≈ 15.5dB

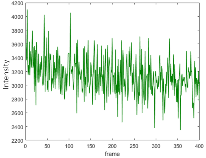


Size of the image: 40 x 40
Pixel size: 0.1 μm
Video rate: 100 fps
Acquisition time: 5 s

SOFI-tool approximation:



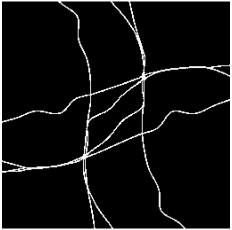
Temporal profile of a **single** molecule from SOFI-tool

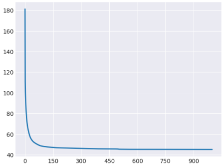


Real Temporal profile of a pixel.

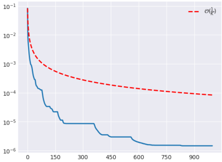
Spatial pattern: Microtubules dataset from the SMLM challenge 2016 [6]
Temporal profiles: SOFI simulation tool (Girsault *et al.*. 2016)

[6] http://bigwww.epfl.ch/smlm/datasets/index.html





180
160
140
120
100
80
60
40
0
150
300
450
600
750
900



10^{-1}
10^{-2}
10^{-3}
10^{-4}
10^{-5}
10^{-6}
0
150
300
450
600
750
900
$\mathcal{O}(\frac{1}{k})$