COL0RME: COvariance-based ℓ_0 super-Resolution Microscopy with intensity Estimation

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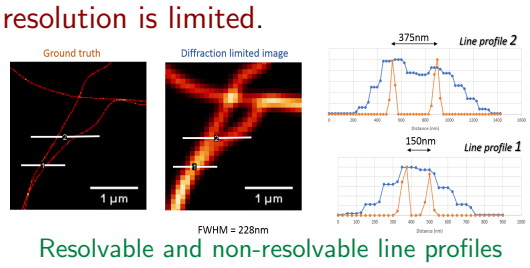
1. Conventional Fluorescent Microscopy Limitations

Fluorescence microscopy is an imaging technique that allows the investigation of living cells and their organelles.

However, due to light diffraction phenomena, resolution is limited.

Point Spread Function (PSF)



Resolvable and non-resolvable line profiles

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

2. Super-Resolution in Fluorescence Microscopy

Many super-resolution techniques have been proposed since the 1990's to overcome the "diffraction barrier" : STimulated Emission Depletion (STED), Single Molecule Localization Microscopy (SMLM), Structured Illumination Microscopy (SIM), ...

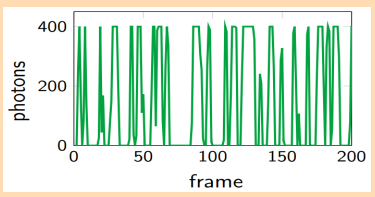
Several among them (e.g., STED and SIM) need special acquisition equipment.

SMLM requires specific photoactivable fluorophores and is very time consuming (as it relies on the acquisition of a large set of sparse images).

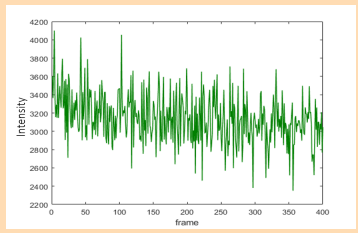
Exploiting temporal diversity

In this work, we focus on a less constraining approach: acquiring a temporal sequence of images over a short time interval and exploit the temporal diversity induced by the independent random blinking of individual fluorophores: a principle followed by e.g. the SOFI[1], SRRF[2] and SPARCOM[3] methods.

Example of temporal blinking profiles



Ideal



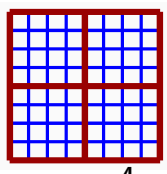
Real

3. Mathematical Modeling

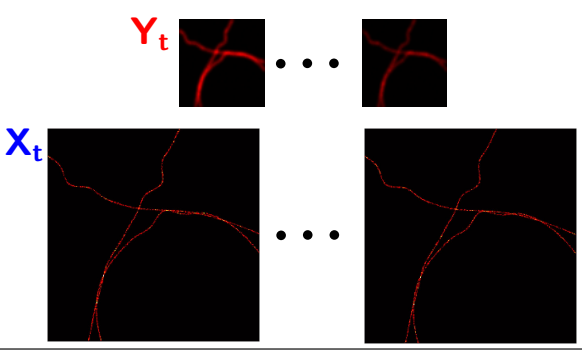
The discrete model describing the acquisition process at frame t, $t \in \{1, \dots, T\}$ is given by:

$$\mathbf{Y_t} = M_q(H(\mathbf{X_t})) + \mathbf{N_t} + \mathbf{B}, \xrightarrow{\text{Vectorise}} \mathbf{y_t} = \mathbf{\Psi x_t} + \mathbf{n_t} + \mathbf{b}, \mathbf{\Psi} \in \mathbb{R}^{N^2 \times L^2}$$

- $\mathbf{Y_t} \in \mathbb{R}^{N \times N}$: LR acquisition
- $\mathbf{X_t} \in \mathbb{R}^{L \times L}$: HR image ($L = qN$)
- $M_q \in \mathbb{R}^{N \times L}$: down-sampling operator
- $H \in \mathbb{R}^{N \times N}$: convolution operator
- $\mathbf{N_t}$: additive white Gaussian noise
- $\mathbf{B}$: spatially and temporally constant background



$q = 4$



In order to exploit the statistical behaviour of the fluorescent emitters we reformulate the model in the covariance domain:

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

- $\mathbf{Ry} := \frac{1}{T-1} \sum_{t=1}^{T} (\mathbf{y_t} - \bar{\mathbf{y}})(\mathbf{y_t} - \bar{\mathbf{y}})^T$
- $\bar{\mathbf{y}} := \frac{1}{T} \sum_{t=1}^{T} \mathbf{y}_t$
- $\mathbf{R_n} = s\mathbf{I}$, $s \in \mathbb{R}^+$

$$\mathbf{r_y} = (\mathbf{\Psi} \odot \mathbf{\Psi})\mathbf{r_x} + s\mathbf{I_v}, \ \mathbf{r_x} = \text{diag}(\mathbf{R_x})$$

4. COL0RME: Support and Intensity Estimation

Steps:

- Estimate $\mathbf{r_x}$ from which we deduce the support of $\mathbf{x}$ denoted by $\Omega_{\mathbf{x}} = \{i : \mathbf{x}_i \neq 0\} \subset \{1, \dots, L^2\}$ as $\Omega_{\mathbf{r_x}} = \Omega_{\mathbf{x}}$.

  Idea: Enforce sparsity on $\mathbf{r_x} \in \mathbb{R}^{L^2}$.

  We consider the continuous relaxation of the ℓ_2-ℓ_0 problem[4]:

  $$\arg \min_{\mathbf{r_x} \geq 0, s \geq 0} G_{\text{CEL0}} := \tfrac{1}{2}\|\mathbf{r_y} - (\mathbf{\Psi} \odot \mathbf{\Psi})\mathbf{r_x} - s\mathbf{I_v}\|_2^2 + \Phi_{\text{CEL0}}(\mathbf{r_x}; \lambda)$$

  where, defining $\mathbf{a_i} := (\mathbf{\Psi} \odot \mathbf{\Psi})_{\mathbf{i}}$ as the i-th column of the operator $\mathbf{\Psi} \odot \mathbf{\Psi}$:

  $$\Phi_{\text{CEL0}}(\mathbf{r_x}; \lambda) := \sum_{i=1}^{L^2} \phi_{\text{CEL0}}((\mathbf{r_x})_i; \|\mathbf{a_i}\|, \lambda) = \sum_{i=1}^{L^2} \lambda - \frac{\|\mathbf{a_i}\|^2}{2} \left(|(\mathbf{r_x})_i| - \frac{\sqrt{2\lambda}}{\|\mathbf{a_i}\|} \right) \mathbb{1}_{\left\{ |(\mathbf{r_x})_i| \leq \frac{\sqrt{2\lambda}}{\|\mathbf{a_i}\|} \right\}},$$

  Algorithmically: G_{CEL0} is non-convex, **but** it is continuous
  $\rightarrow$ available iterative solvers!

- We then estimate the intensity of $\mathbf{x}$ only on its support, and at the same time the spatially constant background $\mathbf{b} = b\mathbf{1}_{\mathbf{M^2}}$, $b \geq 0$, by solving:

  $$\arg \min_{\mathbf{x} \in \mathbb{R}_+^{|\Omega|}, \ b \in \mathbb{R}_+} \tfrac{1}{2}\|\bar{\mathbf{y}} - \mathbf{\Psi_\Omega x} - b\mathbf{1}_{M^2}\|_2^2 + \alpha\|\nabla \mathbf{x}\|_2^2$$

  where the i-th column of $\mathbf{\Psi_\Omega} \in \mathbb{R}^{M^2 \times |\Omega|}$ is extracted from $\mathbf{\Psi}$ for all indices $i \in \Omega$.

To the best of our knowledge, COL0RME is the only super-resolution method exploiting temporal fluctuations which is capable of retrieving real intensity and background information.

5. Results and Discussion

Simutalted Data

- Spatial distribution (8731 fluorophores) from SMLM challenge‡ 2016.
- Temporal profiles generated with SOFI simulation tool.[5]
- Emitter density is equal to 10.7 emitters/pixel/frame
- Fine-grid pixel size = 25 nm, PSF FWHM $\approx$ 229 nm.
- $T = 700$, $N = 40$, $q = 4$, acq. time $\approx$ 7 s
- Background: 2500 photons/pixel/frame, Gaussian noise of 20dB

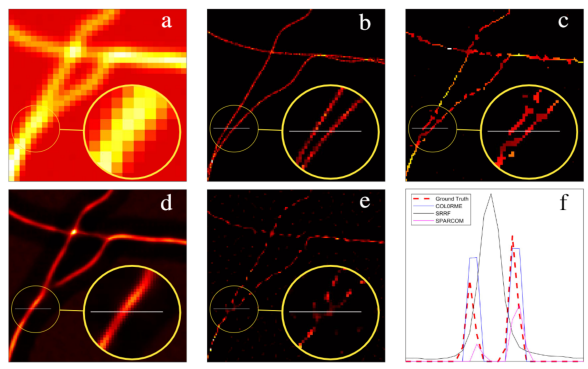


Fig. 1: Results for the simulated 'High Background' dataset: (a) temporal average of the acquired stack (4× zoom), (b) ground truth, (c) COL0RME, (d) SRRF, (e) SPARCOM, (f) intensity profiles (SRRF and SPARCOM with adapted range as they do not actually estimate real signal intensities)

COL0RME PSNR value: 28.37dB and background estimation 2454 photons/pixel/frame

Experimental Data

- A real dataset from the SMLM challenge 2013.
- We can apply these methods on high-density acquisitions obtained by SMLM techniques (the temporal behaviour of one pixel in high density videos looks like "blinking")
- Fine-grid pixel size = 25 nm, PSF FWHM $\approx$ 352 nm.
- $T = 500$, $N = 40$, $q = 4$, acq. time $\approx$ 20 s

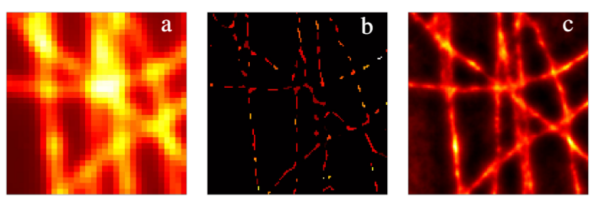


Fig. 2: Results on high-density SMLM data: (a) summation of the acquired stack (4× zoom), (b) COL0RME, (c) SRRF

Despite the high-density and short sequence, all the methods find a good reconstructed image. We observe that SRRF preserves better the broad structure of the specimen, while COL0RME better separates very close tubulins and does not create any background artifacts.

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⋆: The $\odot$ symbol stand for the column-wise Kronecker product.

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‡: http://bigwww.epfl.ch/smlm/datasets/index.html