Single-Shot Sparsity-based Sub-wavelength Fluorescence Imaging of Biological Structures Using Dictionary Learning

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Abstract: We present a novel technique to algorithmically enhance the resolution in optical microscopy. To do that, we exploit the characteristic features of biological images to construct a dictionary which enables sparsity-based reconstruction of sub-wavelength features.

OCIS codes: (100.2980) Image enhancement; (100.3010) Image reconstruction techniques; (110.0180) Microscopy; (180.2520) Fluorescence microscopy; (170.0180) Microscopy; (170.2520) Fluorescence microscopy; (170.0110) Imaging systems

An optical image can be described as a superposition of propagating and evanescent waves. The evanescent waves, which contain the high spatial frequencies, completely decay and set the resolution limit on imaging to Abbe's diffraction limit, comparable to the wavelength [1]. The information on sub-wavelength features is absent in imaging measurements. However, modern techniques are able to reconstruct sub-wavelength features; these rely on scanning (with a tip, a sub-wavelength hole or a "hot spot") or by employing fluorescence (STED, PALM [2] and STORM [3]). Specifically, PALM and STORM are based on collecting a large number of fluorescence images (a sequence), each containing just a few active isolated fluorophores (with each emitter smaller than the wavelength), followed by a second step based on super-localization methods [4] and averaging over the multiple super-localized images. A critical element here is the on-off switching ("blinking") of the florescent molecules ("labels"). The blinking, and the minimum number of photons required to distinguish between a signal and noise, pose numerous limitations, such as integration time and the number of exposures required to obtain a reliable image, etc.

In the past few years, sparsity-based techniques have shown the subwavelength information can be recovered from the Fourier transform (far-field) of a blurry image in a **single-shot** exposure [5], suggesting subwavelength resolution at ultrafast rates. The concept was first applied to images using coherent light while measuring also the phase [6], while subsequent studies demonstrated sparsity-based Coherent Diffraction Imaging with images that were sparse in a simple known basis [5]. Yet, none of these previous works managed to deal with general images or bio-images. On the other hand, related ideas taken from the field of Compressed Sensing were recently employed also for high density fluorescence microscopy [7,8], to reduce the number of exposures while maintaining the resolution. However, none of these is suitable for single-shot fluorescence microscopy.

Here, we present sparsity-based single-shot sub-wavelength fluorescence imaging of biological structures. We show that utilizing sparsity to de-convolve the epi-fluorescence images can enhance all fluorescence-based super-resolution imaging methods: it can considerably improve resolution, ***reduce the number of required exposures to a single-shot exposure***, and expedite the fluorescence imaging process. At the heart of this technique stands a learning step, where a dictionary is constructed from high-resolution data containing the characteristic features of the relevant biological objects followed by algorithmic super-resolution. Both the dictionary learning and the super-resolution process are based on sparsity: the ability to represent the data in a compact form. We demonstrate the recovery of latent high resolution images from low-resolution pixelated image obtained with fluorescence microscopy. The idea is to use the fact that a general, high resolution, image can be represented compactly, using a small number of degrees of freedom, in some basis. To use sparsity techniques, we should find a basis (a ***dictionary***) in which the information is sparse. The advantage of the dictionary is that any image with similar structure (e.g. group of proteins, etc.) can be represented as a sparse combination of the dictionary basis elements. We generate the dictionary by using a training procedure (KSVD [9]), where the learning machine is trained on high-resolution images. Subsequently, sparsity-based super-resolution recovers fine features based on the high-resolution dictionary.

We use an experimental sequence (from [10]) of the "Tubulin" protein acquired by fluorescence microscopy, and demonstrate how we considerably increase the resolution of a low-resolution image, and bring it to the level of high-resolution set by the dictionary. A typical example is shown in Fig.1. Figure 1a depicts a high-resolution image obtained using the super-localization method of fluorescence microscopy [10]. In this particular experiment, 15,000 frames were taken at a wavelength of $690nm$, NA=1.3 ($\lambda_n = 530nm$), image size 64×64, and pixel size $100nm$. The low-resolution image is a simple average over 1000 frames (Fig.1b), which physically corresponds to a single-shot experiment (or non-blinking fluorescence molecules). Figure 1c shows the high-resolution image recovered with our sparsity-based technique. Clearly, the recovered images (Fig. 1c) exhibits considerable improvement in resolution compared to Fig. 1b, almost to the level of the high-resolution image extracted from 15,000 exposures (Fig. 1a).

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The problem is formulated as follows. We denote the high resolution image $\underline{I_H}$ ($\underline{\cdot}$ represents vectors and $\underline{\underline{\cdot}}$ represents matrices) which can be approximated by a sparse linear combination of patch signals under a dictionary $\underline{\underline{D}}$ (Several patches of our dictionary are shown in Fig.1e). The dictionary is learned from experimental data of the same kind as the high resolution image. The sparsity-based dictionary learning process uses KSVD [9] on a smooth version of the high resolution images (the high resolution image is very spiky because it is made from thousands of localizations and therefore it looks unnatural). The patch $\underline{\underline{R^i}}\underline{I_H}$ is the i'th patch of the image $\underline{I_H}$ and can be represented by a sparse vector $\underline{x^i}$ under the dictionary $\underline{\underline{R^i}}\underline{I_H} = \underline{\underline{D}}\underline{x^i}$, where the high resolution image is a sum of those patches $\underline{I_H} = \sum_i \underline{\underline{R^i}}\underline{I_H} = \sum_i \underline{\underline{D}}\underline{x^i}$. We pose image recovery as an optimization problem of sparse vectors $\underline{x^i}$ with the regularization:

$$\min_{\underline{x^j}} \left\| \underline{I_L} - k \otimes \sum_i \underline{\underline{D}}\underline{x^i} \right\|_2^2 \; subject\ to\ \forall i \left\| \underline{x^i} \right\|_0 < S_0 \qquad (1)$$

where, k and $\otimes$ is the Point Spread Function (PSF) and the blurring operator of the physical system (which we approximate as Gaussian and convolution, respectively), $\|\underline{\cdot}\|_2$ is the L_2 norm and $\|\underline{\cdot}\|_0$ is the L_0 that counts the number of non-zeros, namely, $\|\underline{x^i}\|_0 < S_0$. It applies the sparsity constraint of the sparse vector $\underline{x^i}$, that is has no more than S_0 non-zeros.

To solve this optimization problem we use an iterative algorithm with three steps.

1. Modified Richardson iteration, which is usually used in image de-blurring.
2. PSF update using Tikhonov regularization.
3. Sparse projection on the dictionary using a well-known algorithm called OMP [9].

As shown in Figs.1c and 1d, the sparse reconstruction recovers sub-wavelength features, enabling us to go beyond the diffraction limit and extract high resolution images using only their low resolution version. In terms of fluorescence microscopy, we demonstrate extraction of high-resolution images from single-shot exposures that can in principle be taken with an ordinary diffraction-limited optical microscope. Also, our methods can used in fluorescence microscopy and allow super-resolution imaging from a very small number of exposures. Finally, our methods –sparsity-based dictionary learning and subsequent super-resolution, can be applied to any kind of images, provided that a database of high-resolution images of the same kind is available for the dictionary learning step.

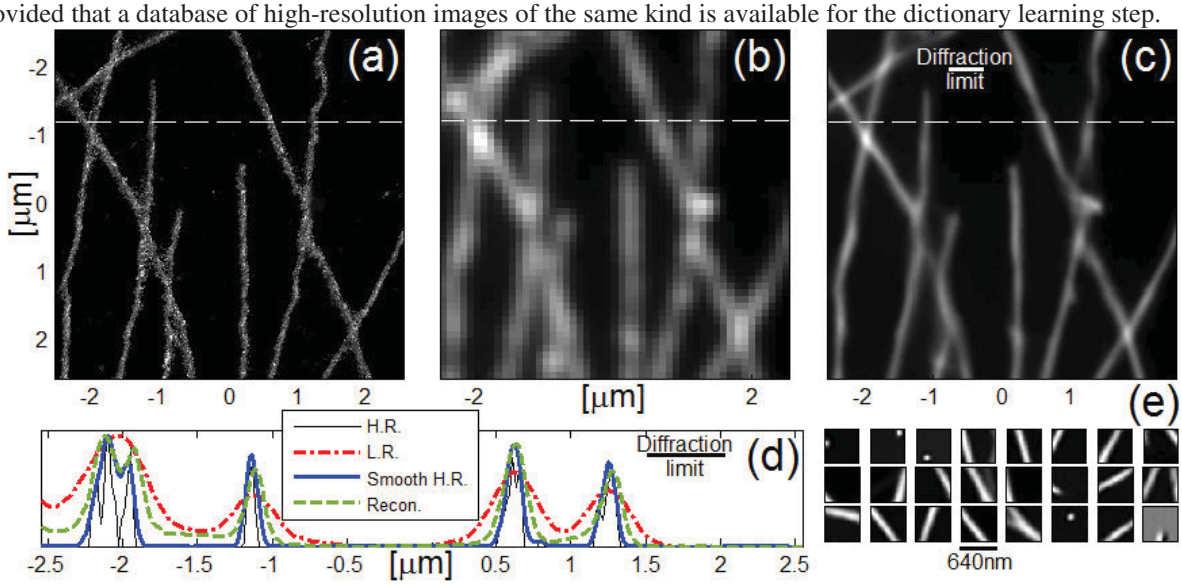

Fig. 1: (a) High resolution image of "Tubulin" obtained from a sequence of 15,000 frames using super-resolution localization fluorescence. (b) Low resolution image. (c) High-resolution recovery using our sparsity-based method. (d) Comparison between cross-sections of the three images: original high-resolution (solid black), smooth high-resolution corresponding to the true image (solid blue), low resolution (red dash-dotted) and sparsity-based reconstruction (dashed green). The reconstruction yields resolution of ~100nm. (e) Several basis elements of the dictionary $\underline{\underline{D}}$.

[1] Born, M. & Wolf, E. *Principles of Optics: Electromagnetic Theory of Propagation, Interference and Diffraction of Light*. (1980).
[2] Hess, S. T. *et al*., Ultra-high resolution imaging by fluorescence photoactivation localization microscopy. *Biophys. J.* **91,** 4258–72 (2006).
[3] Rust, M. J. *et al*., Sub-diffraction-limit imaging by stochastic optical reconstruction microscopy (STORM). *Nat. Methods* **3,** 793 (2006).
[4] Small, A. & Stahlheber, S. Fluorophore localization algorithms for super-resolution microscopy. *Nat. Methods* **11,** 267 (2014).
[5] Szameit, A. *et al.,* Sparsity-based single-shot subwavelength coherent diffractive imaging. *Nat. Mater.* **11,** 455 (2012).
[6] Gazit, S. *et al*., Super-resolution and reconstruction of sparse sub-wavelength images. *Opt. Express* **17,** 23920 (2009).
[7] Min, J. et al., FALCON: fast and unbiased reconstruction of high density super-resolution microscopy data, Sci. Rep. 4, 4577 (2014)
[8] Holden, S. J.*et al*., DAOSTORM: an algorithm for high- density super-resolution microscopy. *Nat. Methods* **8,** 279–80 (2011).
[9] Elad, M. *Sparse and Redundant Representations: From Theory to Applications in Signal and Image Processing*. 396 (Springer, 2010).
[10] Downloaded from "http://bigwww.epfl.ch/smlm/index.html".