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3D super-resolution imaging using a generalized and scalable progressive refinement method on sparse recovery (PRIS)

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ABSTRACT

Within the family of super-resolution (SR) fluorescence microscopy, single-molecule localization microscopies (PALM[1], STORM[2] and their derivatives) afford among the highest spatial resolution (approximately 5 to 10 nm), but often with moderate temporal resolution. The high spatial resolution relies on the adequate accumulation of precise localizations, which requires a relatively low density of bright fluorophores. Several methods have demonstrated localization at higher densities in both two dimensions (2D)[3, 4] and three dimensions (3D)[5-7]. Additionally, with further advancements, such as functional super-resolution[8, 9] and point spread function (PSF) engineering with[8-11] or without[12] multi-channel observations, extra information (spectra, dipole orientation) can be encoded and recovered at the single molecule level. However, such advancements are not fully extended for high-density conditions in 3D. In this work, we adopt sparse recovery using simple matrix/vector operations, and propose a systematic progressive refinement method (dubbed as PRIS) for 3D high-density condition. We also generalized the method for PSF engineering, multi-channel and multi-species observations using different forms of matrix concatenations. Specifically, we demonstrate reconstructions with both double-helix and astigmatic PSFs, for both single and biplane settings. We also demonstrate the recovery capability for a mixture of two different color species.

Keywords: PRIS, Sparse recovery, super-resolution, PSF engineering, L1-norm, multi-species imaging, multi-channel imaging, localization microscopy

1. INTRODUCTION

Super-resolution (SR) fluorescence microscopy is an indispensable tool for biological and biomedical research [13-16]. Within the family of optical SR technologies, localization based methods, such as PALM[1], STORM[2] and their derivatives, exhibit among the highest spatial resolution (approximately 5 to 10 nm), but at the disadvantage of lower temporal resolution. Higher time resolution requires a faster accumulation of localized fluorophores, which can be achieved with localizations at higher densities that require less frames of camera acquisitions. Such methods include fittings of multiple emitters that are demonstrated to work with moderate to high densities [17, 18], and compressive sensing methods such as CSSTORM[3], L1-homotopy[4] and SOFI inspired sparse recovery [19, 20].

At the same time, advances in designing three dimensional PSFs, such as double-helix PSF[5, 11], astigmatic PSF[6], saddle-point PSF[21], and tetrapod PSF[22], has led to fast growing interests and applications of localization based SR microscopy for thicker samples [23]. At the single molecule level, ZOLA-3D[24] has enabled flexible 3D localization over an adjustable axial range. At the higher density conditions: Barsic et al utilized either matching pursuit or convex optimization and a PSF dictionary with sparsity constraint to solve for the emitter localizations [5]. Junghong et al introduced sparsity reconstruction followed by refinement of localizations (FALCON^{3D}) and demonstrated its utility for an astigmatic/biplane imaging system [6]. Shuang et al developed a similar approach with open source software package that was validated on various 3D PSFs [7].

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In addition to the localizations, several further advances have been achieved at the single-molecule level with integrations of better fitting models, optics engineering and extra observation channels. For example, by fitting towards an experimental PSF model that compensates for most of the optical aberrations, minimal uncertainty of localization fitting have been achieved with video rate localizations[25]. By combining extra observation channel with modified optics, a mixture of multi-color species can be identified with SR[8, 9]. Similarly, using engineered PSFs that encodes the information of dipole orientation [26] or spectra information [12], the extra information can be encoded and recovered with SR.

However, such integrations are challenging in 3D under high-density conditions using the existing framework of open source implementation[7]. This is because the algorithm solves a sub-problem in the Fourier space that significantly reduces the computation cost, but causes the method to rely on the convolution assumption that requires the PSF to be translational invariant. We note here that when the PSF exhibits spatial variation, the observation is no longer a strict convolution between the ground truth and the PSF, therefore, the convolution assumption needs to be avoided. In order to extend the methodology advancements achieved at the single-molecule level [7-9, 12, 25, 26], a more generalized algorithm is needed for the recovery of 3D, high-density, multi-channel and multi-species conditions.

In this work, we introduce a systematic progressive refinement method for sparse recovery (PRIS) for 3D super-resolution microscopy, which is generalized and scalable for different imaging system with synchronization capability. PRIS keeps the form of simple matrix operations without relying on the convolution assumption of the observation process, allowing for convenient incorporation of aberrations, different background components, different species of signal sources and parallel measurements from different imaging channels. The sensing matrix and the reconstruction vector are defined with coarse discretization initially, and refined progressively and regionally to reach finer discretization. Such regional refinement enables higher resolution without the drastic increase in the computational cost. In principal, the discretized location coordinates in the 3D space may also be extended into a hyper-dimensional space to include extra dimensions of information, or different PSF species that co-exist and overlap in the same measurement, such as dipole orientation [26], and color [12]. We have validated PRIS and the associated generalization strategy with realistic simulations for single- or multi-channel observations (biplane) and mixture of two color species, with 3D PSFs of either Double-Helix PSF (SPINDLE[27]) or astigmatic PSF.

The rest of this paper is organized as follows: In section 2, we introduce the theory and algorithm used in our work, including both review of dependencies and emphasis on our contribution. In section 3, we validate our method and the associated generalization strategy with simulations under various conditions. We also characterized the performance of our method. In section 4, we provide a discussion highlighting the advantages of our method.

2. THEORY AND ALGORITHM

2.1 L1-norm regularized sparse recovery with progressive refinement

The image formation process of fluorescence microscopy can be described by a linear mapping process. Mathematically, we have:

$$y = Ax + \sigma, \quad (1)$$

where x is a vector accounting for all possible signal sources, y is the observation vector corresponding to the observed image, A is the sensing matrix (observation matrix) describing the linear mapping from the signal source to the observation, and σ represents an additive noise component. The effect of omitting the Poisson noise is negligible as demonstrated by the existing compressive sensing applications for SR microscopy[3, 4, 19, 20]. The following L1-norm regularized sparse recovery solves for x when A and y are known with the prior knowledge that x is sparse (possesses small portion of non-zero entries):

$$\text{minimize } \|x\|_1 \text{ subject to } y = Ax \quad (2)$$

The vector y is obtained by vectorising the observed (or simulated observation) image. An empty vector x is defined to represent a collection of discretized location coordinates, and the sensing matrix A is calculated using the knowledge of the observation process, for example, as provided by an experimentally characterized PSF or a theoretical PSF model. In cases when the noise component exhibits non-zero average, extra columns can be added to the A matrix to account for an offset, or different background components[6]. In this work, we adopted fast linearized bregman iteration[28] that constrains the inverse problem in (2) as:

$$\text{minimize } \mu \|x\|_1 + \frac{1}{2\sigma} \|x\|^2 \quad \text{subject to } y = Ax \quad (3)$$

Although (3) is different from (2), it was shown to be equivalent to (2) for large μ [29]. As shown in Algorithm 1, linearized bregman iteration involves only simple matrix/vector operations and shrinkage[28] operation consisting only Boolean operation and subtractions.

Algorithm 1. Basic linearized bregman iteration

1. Calculate sensing matrix A based on candidate signal sources and PSF model.
 2. Obtain vector y from observation image.
 3. Define μ and σ , and set iteration index $k = 0$.
 4. Initialize $x_0 = 0$, with length of total number of candidate signal sources
 5. Initialize $u_0 = 0$, with length of y
 6. While “ $\|y - A \cdot x\|$ not converge”:
 - $k = k + 1$
 - $u_k = u_{k-1} + A^T \cdot (y - A \cdot x_{k-1})$
 - $x_k = \sigma \cdot \text{shrink}(u_k, \mu)$
- End while, recovered $x = x_k$
-

Note that shrink($\cdot$) is the shrinkage operator[28]. We have incorporated fast linearized bregman iteration with “kicking” to improve the convergence speed. Interested readers are referred to reference [28] for more details.

To bring more insight, we discuss here the physical meaning of the formula of our sparse recovery task: In the basic form for 3D recovery, one element in the x vector (denote the i^{th} element as x_i) represents one candidate position for the emitters. The x vector represents a group of voxels, that collectively represent a 3D volume in the discretized manner. The value of x_i represent the signal emitted from the corresponding voxel. In addition to the basic form, extra elements can be added to the x vector (together with the corresponding columns in matrix A) to account for extra linear components of possible signal sources, such as background components and different species of fluorophores. We note here that the i^{th} column in A represents the unit profile of the observed signal created by the i^{th} possible source, as corresponding to x_i . In other words, the multiplication of the i^{th} element in x and the i^{th} column in A yields an additive component in the observation vector y , while the corresponding x element (x_i) is the coefficient of this additive component, and the i^{th} column in A is the unit observation profile created by the i^{th} signal source.

Algorithm 2. Progressive Refinement method for Sparse recovery (PRIS)

1. Obtain vector y (from observation image) and system response function (i.e. PSF).
 2. Define total PRIS iteration number N , and the list of PRIS refinement folds: $f = \{f_1, f_2, \dots, f_{N-1}\}$.
 3. Initialize sample volume V_0 , discretization size D_0 .
 4. PRIS iteration begins: For PRIS iterations $i = 0$ to $N-1$:
 - 4.1. If $i > 0$: Refine sample volume and discretization: $V_{i+1} = \text{Refine}(V_i, x_k)$, $D_{i+1} = D_i / f_i$
 - 4.2. Obtain candidate signal source collection: $C_i = \text{Sampling}(V_i, D_i)$.
 - 4.3. Calculate sensing matrix A_i based on C_i and PSF model: $A_i = \text{Sensing}(C_i, \text{PSF})$.
 - 4.4. Define μ and σ , and set iteration index $k = 0$.
 - 4.5. Initialize $x_0 = 0$, $u_0 = 0$ with length of the size of C_i .
 - 4.6. While “ $\|y - A \cdot x_k\|$ not converge”:
 - $k = k + 1$
 - $u_k = u_{k-1} + A_i^T \cdot (y - A \cdot x_{k-1})$
 - $x_k = \sigma \cdot \text{shrink}(u_k, \mu)$

End while, recovered $x = x_k$

PRIS loop ends.

 - 5. Post processing and final result rendering using C_{N-1} and recovered x .
-

In order to push for optimum performance of 3D recovery of thick sample, the inverse problem needs to account for high sampling rate of a sufficiently large 3D space. Therefore, the total number of voxels increases, leading to the increase of

RAM requirement. For example: Assuming an input image patch of 64-by-64 pixels, with a cubic volume of $6.4^3 \mu\text{m}^3$ represented by close-packed voxels of size 16^3 nm^3 , the RAM requirement for the sensing matrix $\mathbf{A}$ with single float precision is 122 Gigabytes.

The proposed progressive refinement method (PRIS) is designed as an iterative algorithm on top of the sparse recovery solver to address the computation cost. As shown in Figure 1 and Algorithm 2, the PRIS iteration is initialized by constructing the initial inverse problem accounting for candidate locations represented by close-packed coarse voxels (Figure 1(a)), and a sparse-recovery solver is used to solve for $\mathbf{x}$. The resultant $\mathbf{x}$ is inspected, where the non-zero elements in $\mathbf{x}$ are identified in accordance with the corresponding voxels to construct a new inverse problem for the next PRIS iteration (Figure 1(c)). Specifically, these selected voxels are refined into smaller voxels to yield a new sets of candidate locations (Figure 1(d)), with which a new $\mathbf{x}$ and $\mathbf{A}$ will be constructed and combined with the original $\mathbf{y}$ to form a new sparse recovery task. The refinement process is repeated progressively (Figure 1(b)): Upon completion of each sparse recovery task, zero-value voxels are discarded, and non-zero voxels are refined to construct a new sparse recovery task for the next PRIS iteration. The choice of sparse recovery solver inside PRIS iterations is not restricted. In Algorithm 2 we demonstrate the algorithm with linearized bregman iteration in its basic form. In our implementation, we incorporated “kicking” [28].

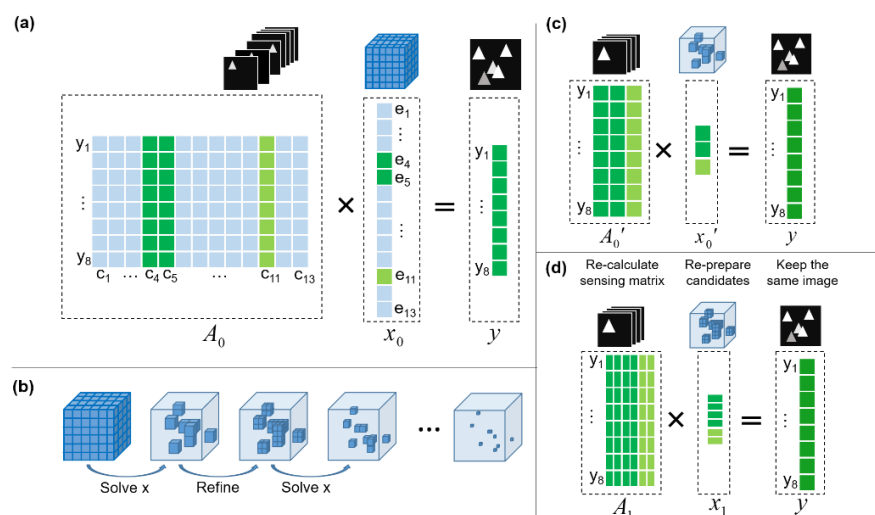


Figure 1. Sparse recovery with progressive refinement. (a) shows the formulation of the recovery problem $\mathbf{A}\mathbf{x} = \mathbf{y}$, with known $\mathbf{A}$ and $\mathbf{y}$, to recovery $\mathbf{x}$. As labeled in (a), we highlighted the one-to-one correspondence between columns in $\mathbf{A}$, elements in $\mathbf{x}$, and the linear component in $\mathbf{y}$. (b)-(d) shows the progressive refinement process of sparse recovery.

In summary, PRIS allows for sparse recovery with large pool of candidate signal sources by progressively focusing the computation power onto smaller and regional subsections of the pool, allowing for a greatly reduced RAM requirement during the recovery and therefore the ability to reach recovery with larger volume and smaller discretization.

2.2 Generalization of PRIS with matrix concatenations

With PRIS, the original framework of matrix operations for sparse recovery is retained with reduced computation cost without relying on the convolution assumption. The advances achieved at the single molecule level can be easily integrated with the spared RAM using different forms of matrix concatenations. First, vertical concatenation of the sensing matrixes allows for synchronization of multi-channel observations, such as multiple focal planes, or different phase masks. Second, horizontal concatenation of the sensing matrixes allows for incorporation of multi-species of the signal source, such as background components[6], or different PSFs(such as different colors [12] or dipole orientation [26], etc.). Hybrid of horizontal and vertical concatenations can be utilized as well. In the general sense, PRIS can be applied to sparse recovery even at a hyper dimensional space[30] and the progressive refinement can be applied at each dimension independently. We explain below the vertical and horizontal concatenations of the sensing matrix, and further generalization can be realized recursively in the same manner.

With vertical concatenation of the sensing matrixes (Figure 2), two different observation vectors are concatenated accordingly, corresponding to different observations. The $\mathbf{x}$ vector corresponds to the same pool of candidate signal sources generating multi-channel observation. The overall sparse recovery task is reduced to the same form of sparse recovery:

$$\text{Construct } A_0 \text{ and } y, \text{ with } A_0 = \begin{bmatrix} A_{c1} \\ A_{c2} \end{bmatrix}, \quad y = \begin{bmatrix} y_{c1} \\ y_{c2} \end{bmatrix}; \quad \text{and} \quad \text{minimize } \|\mathbf{x}\|_1 \text{ subject to } y = A_0 \mathbf{x}; \quad (4)$$

where A_{c1} and A_{c2} are the sensing matrixes corresponding to two different observation channels, y_{c1} and y_{c2} are the observations acquired from two different channels, and $\mathbf{x}$ is the recovery target. Such way of generalization can be applied to the recovery of different observations generated by identical signal sources (as circled by red dashed line in Figure 2 at the observation channels). Multiple observation channels could be, for example, biplane, or multiplane observations, etc. Accompanying information can be synchronized in the data analysis with direct recovery. If the sample contains different species of signal sources that respond selectively to the observation channels, the species variation is underestimated by this model, thus requires a different generalization strategy for PRIS.

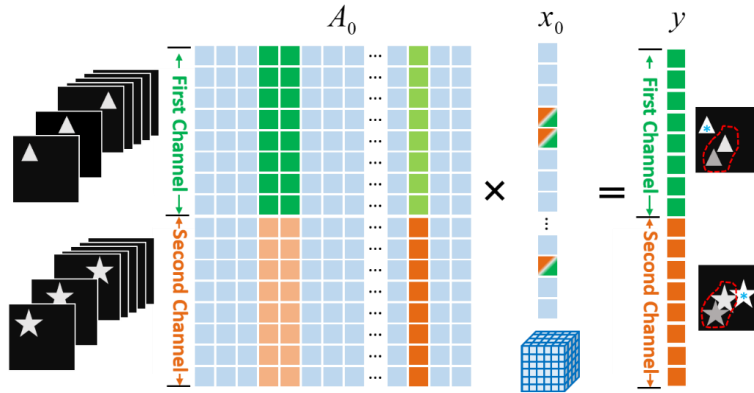


Figure 2. Generalization of PRIS with vertical matrix concatenations. Only the initial sparse recovery is illustrated and the iteration with PRIS refinement is the same as shown in Figure 1. A_0 is the overall sensing matrix, and the concatenation of two different sensing matrixes each describing the observation of two different channels. $\mathbf{x}_0$ represents the pool of candidate signal sources that uniform for both observation channels, and $\mathbf{y}$ represents the overall observation as the combination of the separate observations from different channels.

As shown in Figure 3, the sensing matrixes characterizing different species are horizontally concatenated that allows for the synchronization of different species. And the associated sparse recovery task has the conserved form as follows:

$$\text{construct } A_0 \text{ and empty } \mathbf{x} \text{ with } A_0 = [A_{c1} \ A_{c2}], \quad \mathbf{x} = [\mathbf{x}_{c1} \ \mathbf{x}_{c2}]; \quad \text{and} \quad \text{minimize } \|\mathbf{x}\|_1 \text{ subject to } y = A_0 \mathbf{x}; \quad (5)$$

In such case of dual species, the candidate locations for both species are initialized with overlapping original spatial voxels because both species exist in the same sample 3D space. However, as PRIS iteration proceeded, the recovered $\mathbf{x}_{c1}$ and $\mathbf{x}_{c2}$ vectors will possess non-zero elements corresponding to different spatial coordinates with possible partial overlapping. Therefore, the refinement was performed independently for each species. In addition, because two emitters cannot overlap, we applied a species exclusion constrain to the fast linearized bregman iteration solver by modifying the increment of μ_k shown in Algorithm 2 to favor the contribution corresponding to the species that yields a larger absolute value when the values have the same sign. To be specific, the increment of μ_k is $A_i^T \cdot (\mathbf{y} - A \cdot \mathbf{x}_{k-1})$, which has the same dimension of vector $\mathbf{x}$. Therefore, the elements in this increment vector also have a one-to-one correspondence to voxels belong to both species that has a potential to overlap in space. The mutually occupied voxels in the increment vector (corresponding to two elements in the vector of $A_i^T \cdot (\mathbf{y} - A \cdot \mathbf{x}_{k-1})$) were inspected in each iteration. If two elements have the same signs, the element with smaller amplitude was set to zero.

Following the similar strategy, hybrid concatenations can be constructed as well. We remark here that refinement has been previously used to improve resolution of sparse recovery using refinement [6] for SR microscopy. The merits of this work is the systematic progressive refinement and the generalization strategy of the refinement algorithm for sparse recovery, which allows for expansion for a variety of different fluorescence imaging systems and objects. Integration of these approaches with 3D high-density localization using compressive sensing would open up diversified possibilities for the

study of dynamics and longer-range processes in complex systems. In the more general sense, PRIS can be generalized to sparse recovery even at a hyper dimensional space with tensor formulation[30], and the progressive refinement can be applied at each dimension (each mode of the tensor) independently and/or collectively.

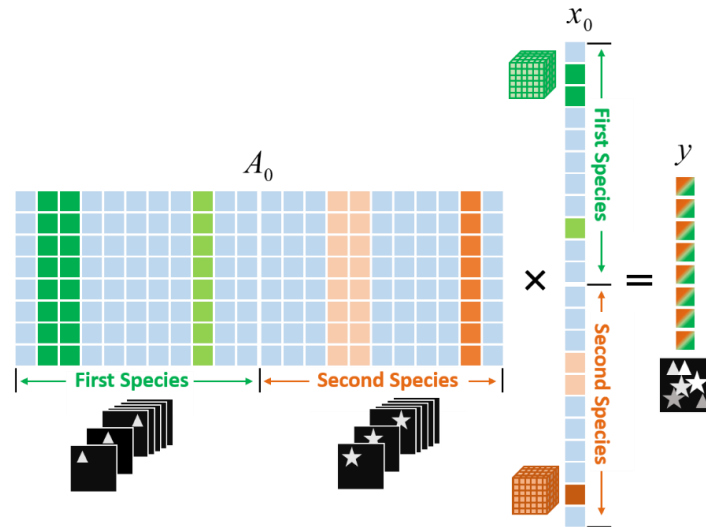


Figure 3. Generalization of PRIS with horizontal matrix concatenations. Only the initial sparse recovery task is illustrated and the iteration with PRIS refinement is the same as shown in Figure 1. A_0 is the overall sensing matrix that is the concatenation of two different sensing matrixes, each describing one portion of the observation contributed from an identical species through the same observation channel. x_0 represents the concatenated pool of candidate signal sources of two different species, and y represents the single channel observation with co-existence of two different species.

3. VALIDATION WITH SIMULATIONS

We validated and quantified the performance of PRIS on three set of simulations, and demonstrated the general applicability of the method with two different 3D PSFs: astigmatic and SPINDLE PSF (a version of double-helix PSF) [27]. As explained in details below.

3.1 Recovery with astigmatic and SPINDLE PSFs with single plane observation

In this simulation, we validated the performance of our method with both SPINDLE PSF and astigmatic PSF with single plane observations. A series of simulations were generated with randomly placed emitters in a 3D volume with various total number of emitters in the 3D volume. Figure 4 shows a representative recovery result for both PSFs, validating the performance of PRIS on sparse recovery. The PRIS result represent each candidate localization as a group of non-zero voxels. We classified the recovered 3D voxels into groups using density based classification [31], and further convert the voxel groups into a list of localization coordinates by calculating the mass center of the voxels belonging to the same group, and the corresponding brightness for each localization coordinate was obtained by taking the integral of voxel values of the same group.

Further quantification was performed with simulated samples containing 10 to 400 emitters, in a 3D volume of $6.4 \mu\text{m}$ in lateral- (XY-) dimensions, and $1 \mu\text{m}$ in the axial- (Z-) dimension. The resultant simulations include different emitter densities ranging from 0.244 to $9.766 \mu\text{m}^{-2}$ in the field of view. The photon budget for each emitter was set to be 5000, and a background level of 100 photons were added to each pixel. The simulated blur observation image was generated on initial discretization of 10 nm pixels followed by 10×10 binning to reach a final pixel size of 100 nm, with photon counting noise (shot noise) simulated with Poisson statistics. For each density condition, 20 simulations were performed with randomized 3D locations. We have quantified the performance of our method gauged by the recovered emitter density and localization precisions as shown in Figure 5. For the convenience of reading, we have plotted similar quantifications from previous works on the same graphs. We note here that the quantifications of different methods were not performed under exactly the same simulation condition (as noted in Appendix, Table 1.), and our simulation represent a challenging

condition because the signal expands over a larger area, and signal was integrated over a smaller pixel area, with non-optimized engineered PSFs and three dimensions.

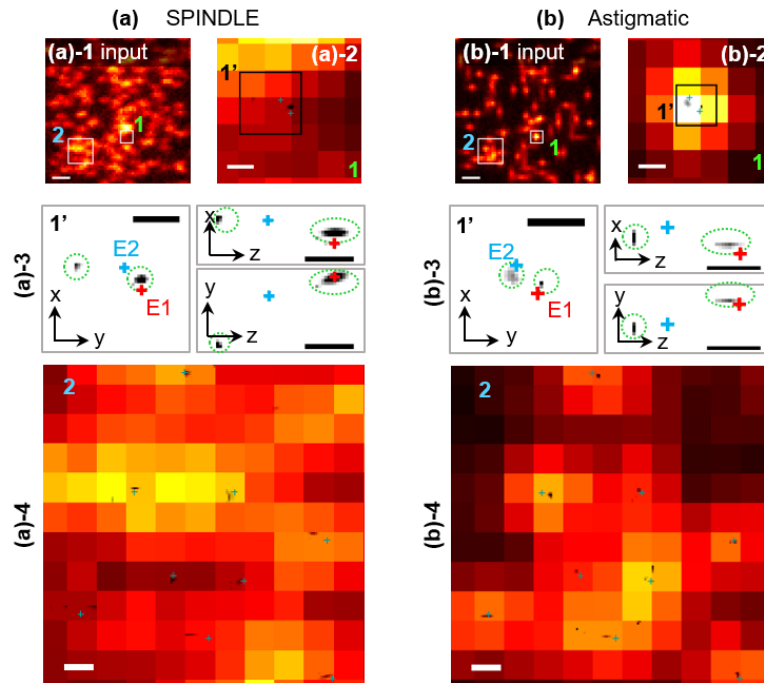


Figure 4. Representative recovery result for PRIS with (a) SPINDLE PSF and (b) Astigmatic PSF are shown here. (a/b)-1 are the input noisy blur images, where two zoom-in regions are labeled (as 1 and 2) and displayed in the rest of the panels. (a/b)-2 shows the zoom-in region 1 with two emitters, which are further amplified into region 1', as shown in (a/b)-3 for views in xy/xz/yz-planes. The reconstructed result is displayed at gray scale, appearing as groups of dark pixels (highlighted with circled with green dashed lines), and the ground truth emitter locations are labeled as red/blue crosses for E1 and E2 respectively. The red cross (E1) indicates a representative precise localization for both PSF models, with displacement of 23 nm for SPINDLE PSF, and 28 nm for Astigmatic PSF. Accordingly, the blue cross (E2) indicates a representative localization with worse precision for both PSF models, with displacement of 148 nm for double-helix PSF, and 66 nm for astigmatic PSF. The second region (Region 2, as labeled in (a/b)-1) represent a higher emitter density (equivalent to approximately $7.4 \text{ emitters } \mu\text{m}^{-2}$), the corresponding zoom-in panels are shown in (a/b)-4. Scale bars in (a/b)-1 are 800 nm, scale bars in (a/b)-2/3/4 are 100 nm.

A simulated emitter was identified as recovered if a localization was recovered within 1-pixel distance in the XY-plane. The density of recovered emitters was plotted against the actual emitter density (ground truth) in Figure 5(a). We can see that PRIS demonstrates excellent performance as compared to previous 3D recovery methods using compressive sensing [5, 7], and single- or multi-emitter localization methods. PRIS also exhibit comparable performance as compared to 2D approaches [3, 4, 19]. Additionally, the astigmatic PSF performs better than SPINDLE PSF using our method. We attribute the performance difference to the size difference between astigmatic and SPINDLE PSF. When the PSF expands a larger area (SPINDLE), we expect lower SNR, and more overlapping of the PSFs exist in the blurry observation, leading to a more challenging recovery task.

In addition, a recovered localization coordinate was identified as correct if a ground truth emitter was located within 1-pixel distance in the XY-plane. For all the correct recovered localizations, the fitting errors were defined as the coordinate displacement compared to the ground truth, and the fitting precision were calculated as the standard deviation of the fitting errors. Figure 5(b) shows the comparison of PRIS with the 2D methods [3, 4, 19] by comparing the standard deviation of fitting errors in the XY-plane (dubbed as σ_{xy}). We can see that PRIS demonstrate comparable or better performance in the lateral precision at high-density conditions ($> 2.5 \text{ } \mu\text{m}^{-2}$). Precision comparison with 3D methods [5, 7] are shown in Figure 5(c) to (e), where PRIS excels under high-density conditions ($> 1 \text{ } \mu\text{m}^{-2}$) among all the 3D methods [5, 7]. We note here that in the previous 3D approaches using compressive sensing [7], a localization result was determined as incorrect when a ground truth emitter was located within one-pixel distance (100 nm) without excluding the axial dimension, therefore, the error in Z-direction reaches a plateau slightly below 100 nm. In addition, a least square fitting approach was applied to

the localization result to finalize the localization result in the previous 3D approaches, which was not implemented in this work, but is compatible with our method and is expected to further improve the performance.

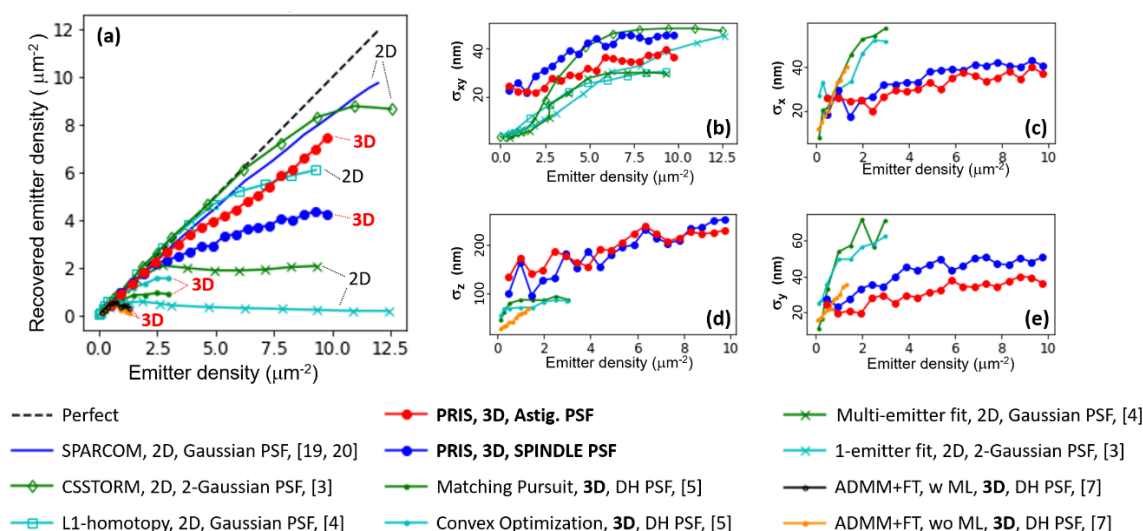


Figure 5. Performance of RIS recovery and previous works. (a) shows the recovered emitter density plotted against the ground truth emitter density. (b)-(e) shows the localization precision in the XY-, X-, Z-, Y- planes as a function of emitter density respectively. We can see that our method exhibits excellent 3D recovery capability with high fidelity at high-density conditions.

3.2 Recovery with multi-channel observation and single species (vertical concatenation)

We performed further simulations to validate the generalization scheme of our method that utilizes vertical or horizontal concatenation of sensing matrixes. Simulation of biplane observations of a single color species was used to validate the vertical concatenation strategy, and single-channel observation for a mixture of two species with two different colors was used to validate the horizontal concatenation strategy.

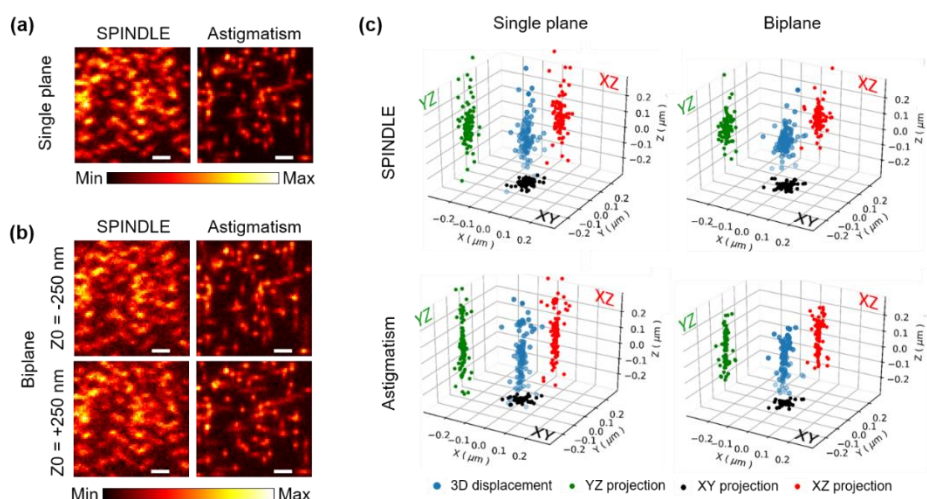


Figure 6. Validation of generalized PRIS recovery with vertical concatenation. PRIS recovery for biplane observation is demonstrated and compared to single-plane. The identical simulated sample is used for both observation configurations, using either SPINDLE PSF or Elliptical (astigmatic) PSF. (a) shows the input images for both PSF models using single plane observation and (b) shows the corresponding biplane observations, where the two focal planes are 125 nm apart from the focal plane used in single plane simulations. The recovery results are compared in (c), where each recovered localization coordinate is compared to the ground truth coordinate, and the displacement in three dimensions are plotted in the 3D scatter plot (with projections as shown in the graph). Four scattered plot demonstrate four different conditions with either single or biplane observations, using either SPINDLE or Astigmatic PSF. We can see that biplane improves localization precision for both PSF models, and Astigmatic demonstrate better precision in the lateral dimensions, while SPINDLE demonstrate better precision in the axial dimension. Scale bars: 8 μm .

Figure 6, shows the results for dual-channel observation simulations. A subset of the simulated samples was analyzed with 100 emitters randomly positioned in the sample volume of $6.4 \times 6.4 \times 1 \mu\text{m}^3$. Different observations were generated from the same simulated sample maintaining the same photon budget level of 5000 counts per emitter, to simulate biplane and single-plane observations with either SPINDLE and astigmatic PSFs. Figure 6(a) shows the single plane observation for both SPINDLE and astigmatic PSF separately, and Figure 6(b) shows the corresponding biplane observation for both PSF models separately. Due to the split of the dual planes, the total photon-budget was split between the two observation channels (accounted in the simulations). The SNR difference between the observation images for single and biplane observations is due to this split. The recovery is performed through vertical concatenation of the sensing matrixes corresponding to two different focal planes, and the corresponding recovery result is compared in Figure 6(c). We can see that although the SNR is reduced in a biplane observation, its recovery precision (as shown with our simulations) was higher due to the added constraints afforded by the biplane observation.

3.3 Recovery with single-channel observation and dual-species (horizontal concatenation)

We further tested the generalization strategy of PRIS with horizontal concatenation of the sensing matrixes (Figure 7). The simulation contains a mixture two Cy3 molecules (577 nm emission) and two Cy5 molecules (690 nm emission) both detected through the same channel. The simulated detection scenario could be (but not limited to) dual laser excitation with a multi-bands pass filter that allows for detection of both colors. For the detection path, we simulated a transparent phase mask that alters only the phase of the wave front without phase wrapping in the fabrication. We set the phase mask to be a SPINDLE phase mask that matches 577 nm emission. Emission of both colors pass through the same focusing lens and phase mask, but the phases of the wave front were altered differently by the phase mask and the defocus depth, due to different wavelengths, yielding different PSF profiles for each color. As shown in Figure 7(a), the PSF for Cy3 exhibit the expected SPINDLE PSF profile. But for Cy5 molecule with mismatched emission wavelength for the phase mask, the PSFs exhibit extra central lobe and diffraction patterns (Figure 7(a)) that mismatches with a theoretical SPINDEL PSF profile. Such differences in the PSFs enable PRIS to distinguish two species by constructing the sensing matrixes A_1 and A_2 in accordance with the two PSFs separately as shown in Figure 7(b). The corresponding experimental scenario could be independent characterizations of experimental PSFs with fluorescence beads of different colors through the same detection path.

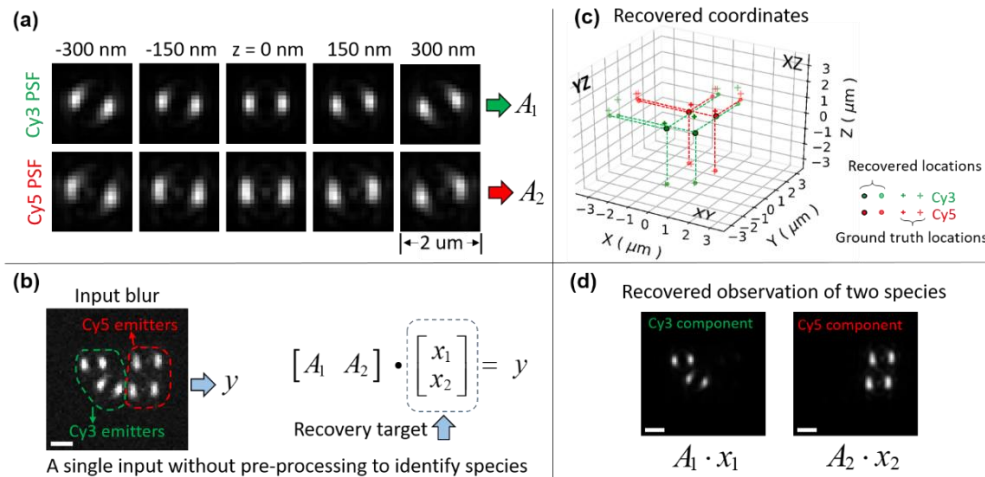


Figure 7. Validation of generalized PRIS recovery using horizontal concatenation. A single channel observation is simulated with a mixture of 4 fluorophores (two Cy3 molecules with 577 nm emission, and two Cy5 molecules with 690 nm emission). In the single observation channel, the SPINDLE phase mask is simulated to match the wavelength of Cy3. (a) shows the simulated 3D PSF slices for both fluorophores under the same detection path, for which Cy3 PSF shows the SPINDLE PSF profile, and Cy5 PSF shows extra diffraction lobes/patterns that would be unwanted in a traditional sense of double helix PSF application. (b) shows the simulated blurry noisy observation with the four emitters. The observed images of Cy3/Cy5 molecules are circled with green/red dashed lines respectively. For the sparse recovery problem, the single input image is vectorized to construct the y vector, two sensing matrixes constructed from the two species are horizontally concatenated to form the overall sensing matrix, and the sparse recovery target is the combined vector of x_1 and x_2 . PRIS refinement are applied to two species separately. The final PRIS result is the recovery coordinates shown in (c) with species tag, and compared to the ground truth coordinates. (d) shows the observation reconstructed from the recovered x_1, x_2 with PRIS iterations, which clearly indicates the separation of two color species. Scale bars: $1 \mu\text{m}$.

In the common dual-color imaging system, either two filters are applied sequentially with sequential camera exposure, or the channel is split into two with different color filters. Such hard-ware approaches to separate color channels come at the cost of detected photon budget. This example demonstrates the separation of different color species mathematically by constructing the different species into the sensing matrix, while capturing photons from both species in the same channel at the same time.

3.4 Recovery of curve feature with ultra-high labeling density

We further tested the recovery capability with a densely labeled curve in 3D with SPINDLE PSF (Figure 7(a)). The virtual sample is a curve with length of $8\text{ }\mu\text{m}$ in total, that expands approximately $5\text{ }\mu\text{m}$ in the lateral dimensions, and $0.56\text{ }\mu\text{m}$ in the axial dimension. Total number of 400 emitters are randomly placed along the line with cross-section location uncertainty of 7 nm , resulting in an average labeling density of 1 emitters per 20 nm . The emitters are all set at bright state in the simulation with randomly selected photon budgets between 4000 to 5000. The background photon count is set to be 100 per pixel with Poisson statistics. Figure 7(d) shows the simulated image measurement with the SPINDLE PSF.

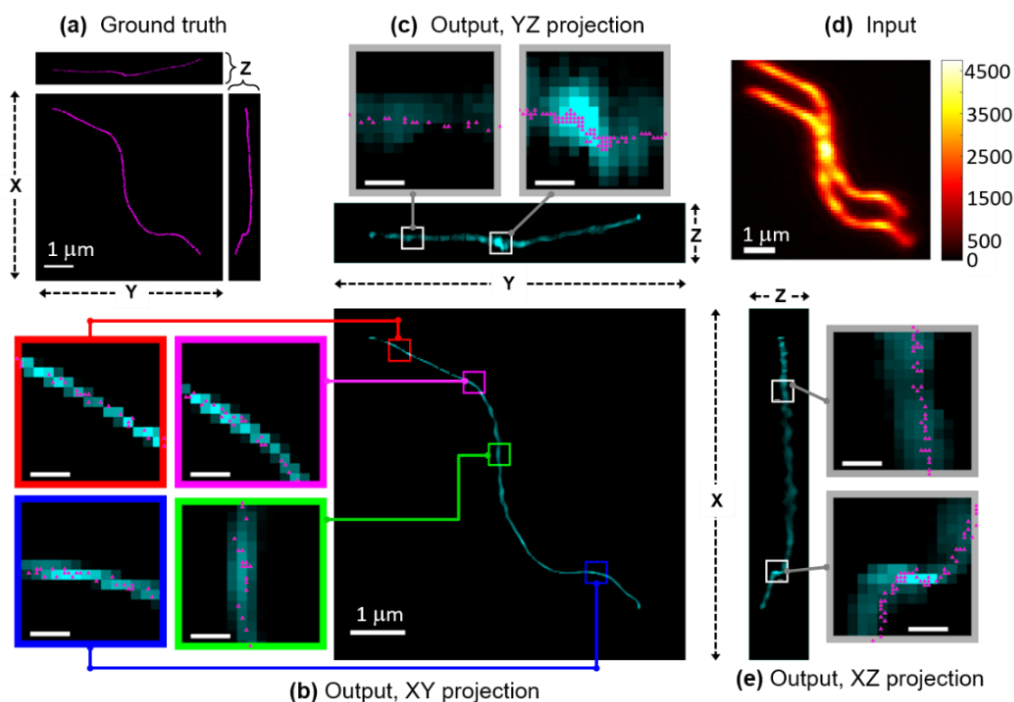


Figure 8. PRIS recovery of densely labeled curve in 3D. PRIS result of a simulate 3D densely labeled (1 emitter per 20 nm on average) curve is shown here. (a) shows the ground truth emitter locations in projection of XY-plane (center), XZ-plane (right) and YZ-plane (top). Each emitter location is labeled as a magenta dot. The displays of the ground truth appear as curves due to the high-density of labeled emitters. The simulated measurement with SPINDLE PSF is shown in (d). Note that because of the nature of the double-helix PSF (two lobes), the image of a single curve appears as two curves in the observation. The sparse recovery results are shown in (b), (c) and (e) with different projection views with zoomed panels as labeled in the major panel and connected to the zoomed panels. In the zoomed panels, the ground truth emitters are marked with magenta triangles. We can see that although the sparse recovery does not recover the location coordinates of individual emitters, but the result represents the underlying 3D curve as compared to the ground truth. Scale bars in major panels are $1\text{ }\mu\text{m}$ as labeled in the figure; scale bars in zoomed panels without labels are 100.

We note here that because SPINDLE PSF have two lobes with changing orientations to encode the defocus depth, the observation of the densely labeled curve appears as two lines. Figure 7(b)(c)(e) demonstrate the sparse recovery result of this simulated condition with different projection and zoomed panels as labeled in the figure. The sparse recovery is good in terms of recover the ground truth 3D curve, but without exhibiting individual localization coordinates. Additionally, because the intensity along the recovered line is rather smooth, the subsequent classification method couldn't identify the coordinates of individual localization results. We attribute this to the ultra-high labeling density where the inter space between adjacent emitters are limited, therefore the reconstruction lacks the ability to distinguish individual emitters at this regime of high local density of emitter, but represents the recovered feature as a curve.

4. CONCLUSIONS AND DISCUSSION

In this work, we developed a progressive refinement method for compressive sensing (PRIS) to perform 3D super-resolution recovery of fluorescence microscopy observations. The method is generalized to work with different PSF models, multiple observation channels and mixture of species of signal source. We have demonstrated high-fidelity reconstructions of simulated data sets for double-helix PSF (SPINDLE), astigmatic PSF, and for both single and biplane image acquisitions. We also demonstrated the recovery capability with densely labeled curve in 3D. Our work is useful for the algorithmic synchronization of different imaging modalities, where the synchronization is realized at the stages of data acquisition and data reconstruction. It affords a deeper level of synchronization as compared to sequential data analysis. In the more general sense, PRIS can be generalized to sparse recovery even at a hyper dimensional space with tensor formulation[30], and the systematic progressive refinement can be applied at each dimension (each mode of the tensor) independently and/or collectively. Our method can also be applied to observations with well isolated PSFs in the observation image for single molecule localization microscopy. Accordingly, proper initialization of PRIS iteration can be used with voxels covering only identified interested areas to further reduce the computation cost. Least square fittings can be applied to our algorithm to further enhance the localization precision in 3D. The highly generalized form and computation simplicity allows for scalable recovery platform with cloud computing and/or FPGA implementations.

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APPENDIX

Method	Ref.	2D or 3D	Simulated PSF	Fitting PSF	Brightness (photon budget per emitter)	Noise model	Background (counts per pixel)	Pixel size (nm)	Pixel area (unit: 100^2 nm^2)
CSSTORM	[3]	2D	Experimental	Two Gaussian	Average of 3000	Poisson	70	166	2.76
PRIS	N.A.	3D	SPINDLE	SPINDLE	5000	Poisson	100	100	1
PRIS	N.A.	3D	Astig.	Astig.	5000	Poisson	100	100	1
L1-homotopy	[4]	2D	Gaussian	Gaussian	Average of 3000	Poisson	70	167	2.79
1-emitter fitting	[3]	2D	Experimental	Two Gaussian	Average of 3000	Poisson	70	166	2.76
Multi-emitter fitting	[4]	2D	Gaussian	Gaussian	Average of 3000	Poisson	70	167	2.79
Matching Pursuit	[5]	3D	DH PSF	DH PSF	1900 to 2000	Poisson	20	160	2.56
Convex Optimization	[5]	3D	DH PSF	DH PSF	1900 to 2000	Poisson	20	160	2.56
ADMM+FT wo ML	[7]	3D	DH PSF	DH PSF	Average of 2000	Poisson	20	187.5	3.52
ADMM+FT w ML	[7]	3D	DH PSF	DH PSF	Average of 2000	Poisson	20	187.5	3.52
SPARCOM	[19]	2D	Gaussian	Gaussian	Not available	N.A.	N.A.	100	1

Table 1. Simulation conditions used for the recovery performance quantification in previous works[3-5, 7, 19] and plotted in Figure 5.