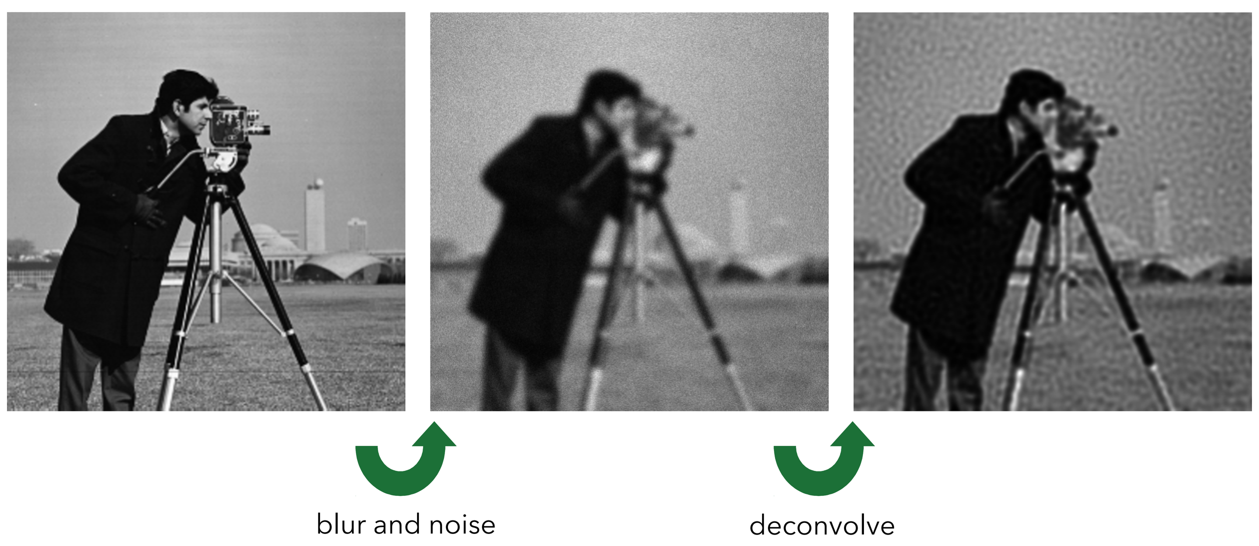
Incorporating bleaching into deconvolution

Benjamin K. Cooper and Andrew G. York

Calico Life Sciences, South San Francisco, CA

Deconvolution...



Imaging systems give blurry, noisy images of objects. Given a model of both the blurring and the noise, we can attack the inverse problem of retrieving the original image from the noisy measurement. A classic, widely-used algorithm for this deconvolution problem is **Richardson-Lucy (RL)**, an iterative maximum likelihood approach.

RL works without any assumptions about the sample, and is great for combining information from multiple images. This generality can be both strength and weakness, though, since information about the sample can greatly improve our reconstruction ability.

...and photophysics...

Superresolution techniques exploit various special situations to defeat the Abbe limit. These can be separated into two broad classes. One set of methods uses special **illuminations** to achieve superresolution. This class includes structured illumination microscopy (SIM), stimulated emission depletion (STED), and total internal reflection microscopy (TIRF). RL is already well suited for these methods because this information is straightforward to encode in the imaging model.

Another approach is localization methods like photo-activated localization microscopy (PALM) or stochastic optical reconstruction microscopy (STORM). These employ peculiarities of the **fluorophores,** such as blinking, bleaching, or photoactivation. These techniques can achieve sensational results. However, they also require special purpose analyses that can prove brittle. A more unified approach that fails gracefully would have great appeal. We think including fluorophore behavior in deconvolution is such an approach.

...together.

Can one augment deconvolution approaches with knowledge of photophysics? What gains does this yield?

As a first step toward answering these questions, we developed **spatiotemporal decon (ST)**, a deconvolution algorithm in the spirit of RL that includes a model of **photobleaching**. The algorithm is to be applied to a **time-series of fixed images**.

The choice of photobleaching was motivated by perceived simplicity more than utility. Nevertheless, the performance of ST suggests the value of this program.

Related Work

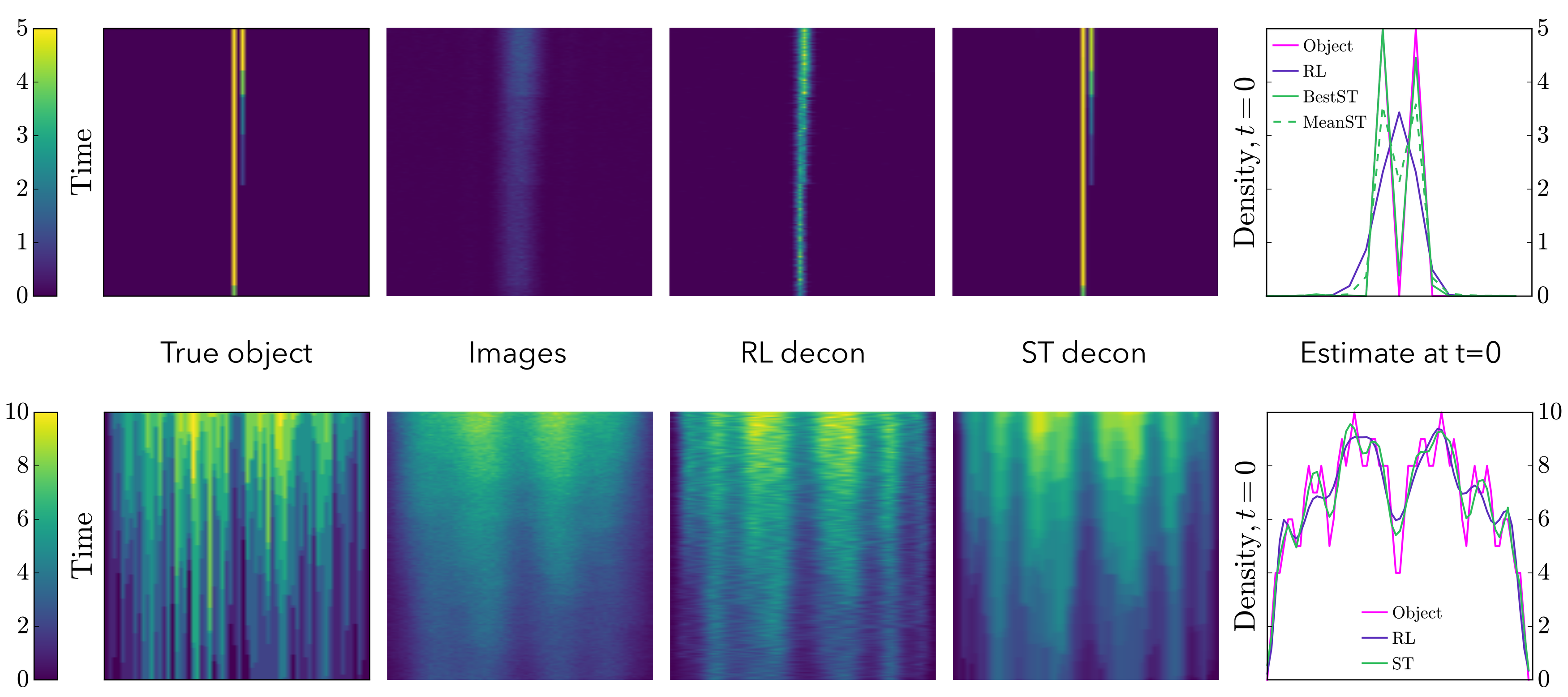
A sample of other techniques attacking similar problems:

- **BALM:** D. T. Burnette, P. Sengupta, Y. Dai, J. Lippincott-Schwartz, and B. Kachar, PNAS **108**, 21081 (2011)
- **3B:** S. Cox, E. Rosten, J. Monypenny, T. Jovanovic-Talisman, D. T. Burnette, J. Lippincott-Schwartz, G. E. Jones, and R. Heintzmann , Nat. Methods **9**, 195 (2011)
- **SOFI:** T. Dertinger, R. Colyer, G. Iyer, S. Weiss, and J. Enderlein, PNAS **106**, 22287 (2009)
- **SRRF:** N. Gustafsson, S. Culley, G. Ashdown, D. M. Owen, P. M. Pereira, and R. Henriques, Nat. Communications **7**, 12471 (2016)
- **deconSTORM:** E. A. Mukamel, H. Babcock, and X. Zhuang, Biophys. J. **102**, 2391 (2012)

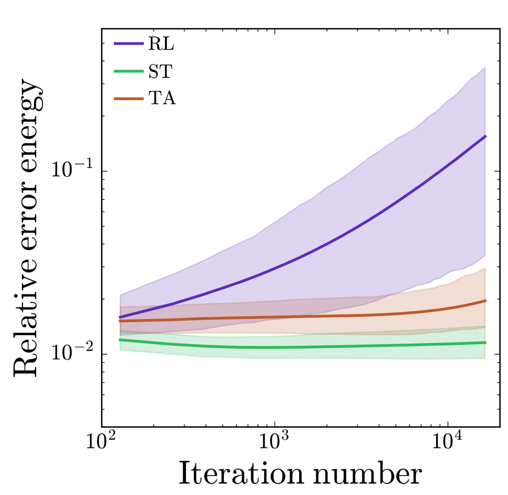
Examples

When bleached to sparse labeling, RL and ST decon can **localize** emitters at later times. ST decon can propagate that info to earlier times.

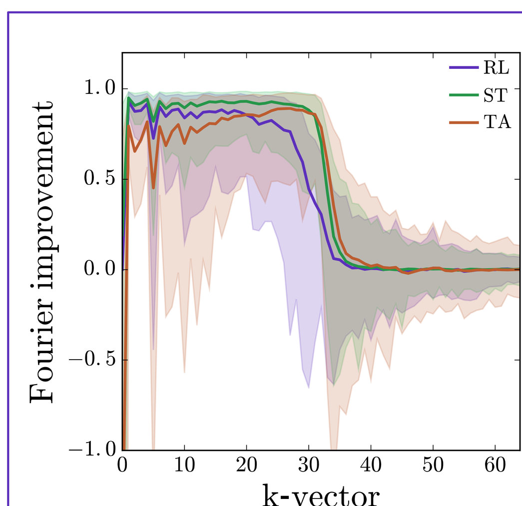
The same algorithm **outperforms RL** decon on densely labeled samples as well.



RL decon suffers from overfitting to noise if run too long. The error plot to the left shows that past a certain point more iterations gives worse results. A benefit of ST decon is an effective **regularization**. Unlike other approaches to regularization, this uses only the bleaching probability – a quantity easily measured – as a parameter. Intuitively, ST decon can aggregate more data and get an effectively lower noise floor. A naive, **time-averaged (TA)** approach shows similar regularization but less correctness.



Tests of synthetic dense 1D object over 1024 random bleaching histories and noise realizations. Filled regions represent middle 90th percentile; central line is mean.

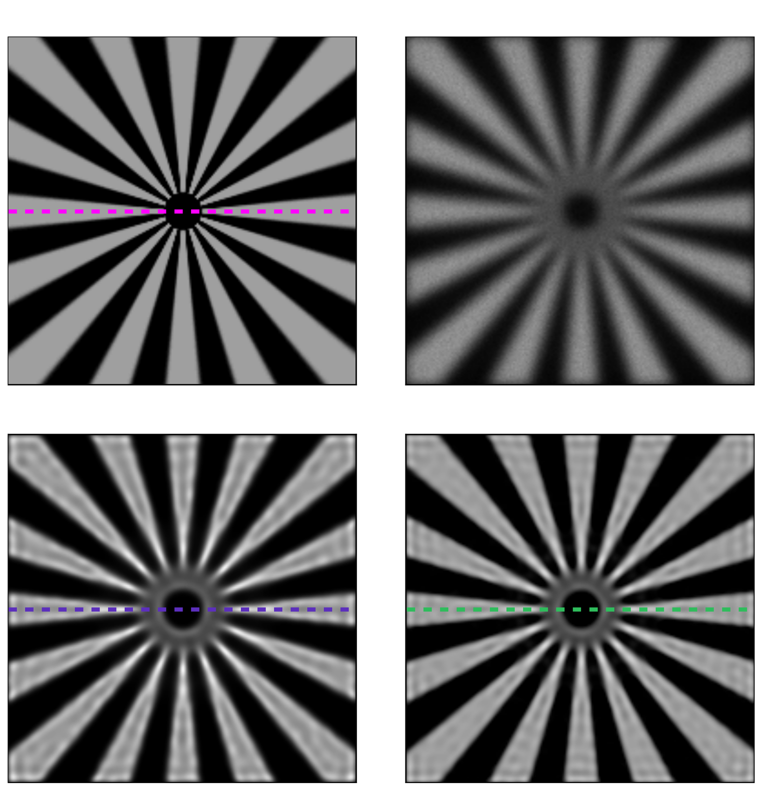


Tests of synthetic dense 1D object at first time point over 256 random bleaching histories and noise realizations. Filled regions represent middle 90th percentile; central line is median.

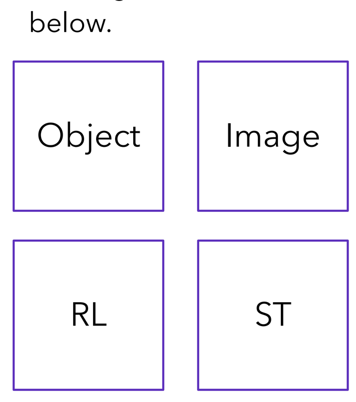
Real space shows regularization well. For correctness, It is useful to consider error in the Fourier domain. We consider a metric we call **Fourier improvement** which compares the error in the estimate to the error in the image:

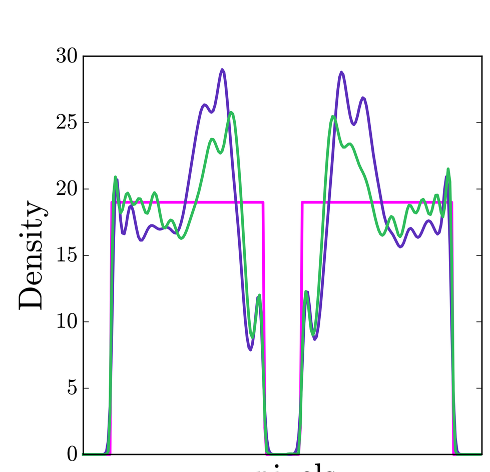
$$FI(k) \equiv 1 - \frac{\left|X(k) - \tilde{X}(k)\right|}{\left|X(k) - Y(k)\right|}$$

The plot above is of average 1D Fourier improvement at the first time point over many random bleaching histories and images. RL drops off with spatial frequency faster than ST; TA performs substantially worse at low frequencies.



Objects, images and estimates from first time point for 2D test cases. Layout of each figure shown below.

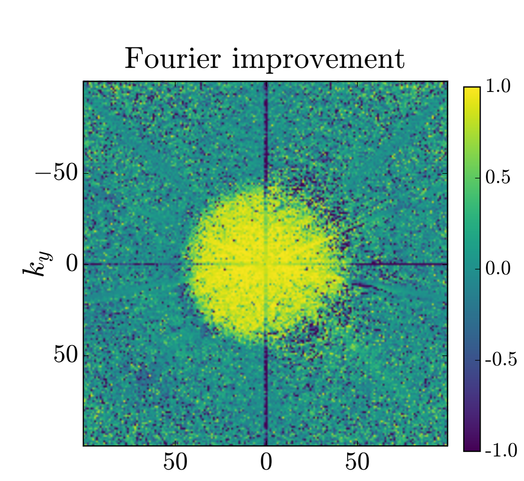




Line cuts through the spoke targets show the reduced ringing of ST relative to RL. In both the spoke and cameraman images, fewer artifacts are visible in uniform regions.

1D test cases allow visualization of space and time behavior easily, but 2D test cases are better practical examples. (The algorithm is straightforwardly extensible to 3D, but long time-series of 3D images will be impractically large.)

These 2D cases show that the regularization benefits demonstrated in 1D generalize to more realistic scenarios.



Comparing Fourier improvement in the cameraman image for ST (left) and RL (right), we see more robust performance near the edge of the band, just as in the 1D case above.

Details

Our method chooses the estimate $\vec{x}$ with **maximum probability** given data $\vec{y}$. The probability is given by:

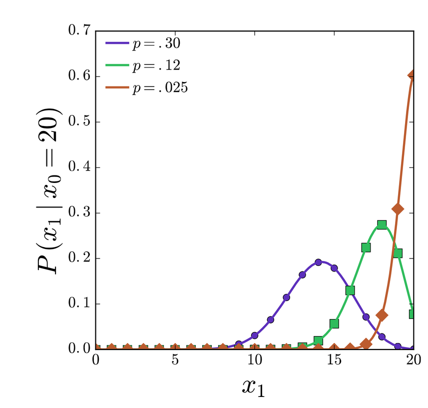
$$P(\vec{x} \mid \vec{y}) \propto \left(\prod_{i\in\vec{y}} \frac{e^{-(\mathbf{H}\vec{x})_i}\,(\mathbf{H}\vec{x})_i^{y_i}}{y_i!}\right) \times \left(\prod_{j\in\vec{x}}\prod_{t=0}^{N-2} P\big(x_j(t+1) \mid x_j(t)\big)\right)$$

The **Poisson likelihood** of the data given the estimate and an operator $\mathbf{H}$ that blurs estimates to produce an image. RL decon maximizes this likelihood.

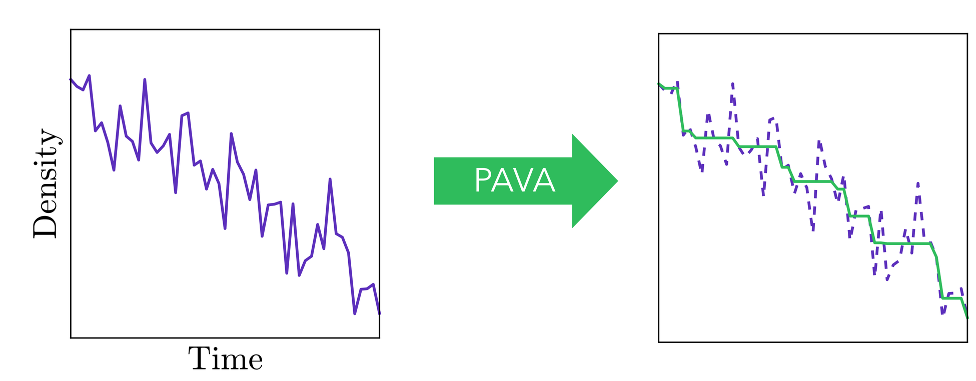
We assign a probability for the trajectory of each pixel of the estimate in time according to a model of photobleaching. We assume that a single conditional probability for a pixel at two time points is **binomially distributed**.

The binomial distribution is discrete and challenging to optimize. We use a **continuous approximation** to the binomial distribution:

$$P(x-\Delta \mid x) = \frac{x!}{\Delta!(x-\Delta)!}p^{\Delta}(1-p)^{x-\Delta}$$
$$\approx \frac{\Gamma(x+1)}{\Gamma(\Delta+1)\Gamma(x-\Delta+1)}p^{\Delta}(1-p)^{x-\Delta}$$



In the discrete case, a **nonincreasing constraint** is enforced by the probability. In the continuous approximation, this constraint doesn't always hold. We employ the pool adjacent violators algorithm (PAVA) as a **projection** back to the space of feasible estimates.



One iteration

Gradient

We take the gradient of the log probability. The gradient of the Poisson component can be taken quickly with FFT convolution. The temporal part relies on evaluation of the special digamma function.

Project

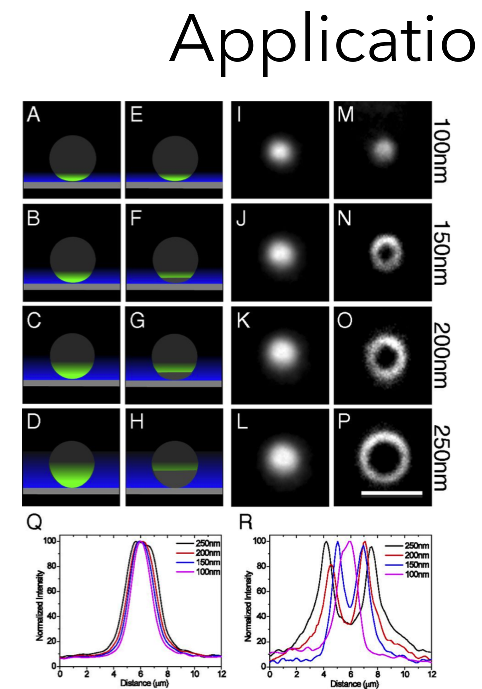
We take a step in the gradient direction and then project the result using PAVA. The constraint space is convex, so all points between the PAVA projection and the current estimate are feasible.

Line Search

We do backtracking line search between the current estimate and projection to find a next estimate that is likelihood improving.

The algorithm employed here demonstrates proof-of-principle; alternative implementations are possible. For example, a version based on just including the nonincreasing constraint can be implemented using RL with a special PSF.

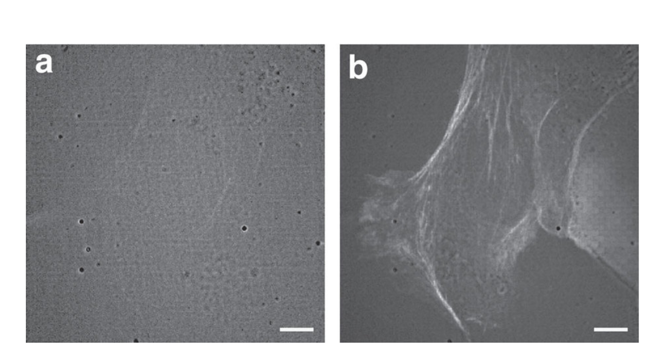
Applications and Extensions



Yan Fu, P. W. Winter, R. Rojas, V. Wang, M. McAuliffe, and G. H. Patterson, PNAS **113** 4368 (2016)

Natural application: cases where you're already taking multiple images, like **SIM** or sequential multi-angle **TIRF** with photobleaching.

Photobleaching by itself is perhaps limited, but the larger vision – a unified analysis algorithm exploiting our knowledge of photophysics – is compelling.



M. Ingaramo, A. G. York, E. J. Andrade, K. Rainey and G. H. Patterson, Nat. Communications **6**, 8184 (2015)

Including photoactivation opens the door to analyzing techniques like **PALM** or **two-step** microscopy – exploiting a special protein that photoactivates and excites at the same frequency.