Supplementary: Spectral and lifetime fluorescence unmixing via deep learning

1. *Noise generation*: In order to generate noise representative of that obtained through the HMFLI inverse solving procedure, a sequence of operations was performed:

1) Generation of a baseline random vector with a maximum of 250 photon-counts.

2) Subtraction of a random vector (comprised of values between [-50, 50]) allowing for negative values. All negative values were immediately set to zero following the subtraction.

3) Generation of a vector comprised of only ones and zeros – each element having a 40%-60% chance of being set to zero during each iteration. Multiply this vector with the noise vector generated in steps 1-2.

4) Assign Poisson noise.

5) Sum across all time-points to obtain the CW value for the particular spatial coordinate and channel.

6) Assign the "TPSF" to both a spatial coordinate and channel.

7) Iterate across all 16 channels.

The MATLAB code for the noise generation section is shown:

```
for k = 1:16
% % % #1 & #2
      cN1 = max(ones(256,1).*250 - rand(256,1).*200 + (rand(256,1).*50)-100,0);
% % % #3
      vec = rand(256,1);
      vec(vec>=(rand()*.2 + .4)) = 0;
      eC = cN1.*vec;
% % % #4
      eTC = poissrnd(round(eC'));
% % % #5
      cw(i,j,k) = sum(eTC);
% % % #6
      tpsfs(i,j,k,:) = eTC;
end
```

***2. Time Domain Reconstruction*:** The reconstruction process from single pixel data acquisitions to time domain (TD) reconstructions is accomplished through the inverse solving of: $\boldsymbol{M(t) = P\, x(t)}$ for the image $\boldsymbol{x(t)}$ of the sample plane across 256 time points, where $\boldsymbol{M(t)}$ represents the raw fluorescence decay profiles acquired per pattern $\boldsymbol{P}$. The pattern's matrix serves to calculate the corresponding weights each measured pattern has for the sample plane image. Since the pattern's matrix $\boldsymbol{P}$ and time domain measurement matrix $\boldsymbol{M(t)}$ are known, then through the use of TVAL3 [1] inverse solver, the fluorescence decay profiles per each pixel of the image sample plane, can be retrieved. To obtain continuous wave intensity images, the representations across the 256 time points can be added. In order to obtain lifetime reconstructions, each of the fluorescence decay's per pixel are bi-exponential fitted through a least-squares based minimization algorithm. The bi-exponential mean lifetime model is represented by: $(A_1/100) \times \tau_1 + (A_2/100) \times \tau_2$ where A's represent the FRETing Donor and Acceptor fraction and tau's their respective lifetime values.

References

[1] C. Li, "Compressive sensing for 3D data processing tasks: applications, models and algorithms," 2011.