Supplementary Material: Physics-enhanced machine learning for virtual fluorescence microscopy

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1 Image Formation

To supplement the brief derivation within the main text here we provide a more detailed justification for the physical model of image formation used within this work.

Considering an array of LEDs placed under a sample each LED within the array illuminates the sample with a plane wave from a unique angle corresponding to its position. Given N LEDs within the array emitting at their center wavelength λ_n, we can denote the amplitude and angle of their emitted plane waves as $\sqrt{w_n(\lambda_n)}$ and θ_n, for $n = 1, \ldots, N$. Since the LEDs are mutually incoherent with respect to each other, the image formed by an illumination composed of multiple LEDs is equivalent to the incoherent sum of images obtained by illuminating with the LEDs individually. Assuming a thin sample with complex function $o(\mathbf{r}, \lambda)$ we can express the image I' as the incoherent sum:

$$I'(r) = \sum_{n=1}^{N} \left| \left(o(r, \lambda_n) \sqrt{w_n(\lambda_n)} e^{i\mathbf{k}_n \cdot \mathbf{r}} \right) * h(r) \right|^2 \tag{1}$$

where, $\sqrt{w_n(\lambda_n)} e^{i\mathbf{k}_n \mathbf{r}}$ describes the plane wave generated by the nth LED with intensity $w_n(\lambda_n)$ at wavelength λ_n across the sample plane with coordinate $\mathbf{r}$. $\mathbf{k}_n$ denotes the plane wave transverse wavevector with respect to the optical axis, and $h(r)$ denotes the microscope's coherent point-spread function.

As the LED brightness value, $w_n(\lambda_n)$, is a scalar quantity we can factor it out of the magnitude expression in Eq. 1. Furthermore, if we denote the image of the sample formed when it is illuminated by the nth LED at a fixed brightness and wavelength as $I_n(\lambda_n) = | \left(o(\lambda_n) e^{i\mathbf{k}_n \mathbf{r}} \right) * h(\mathbf{r})|^2$, we arrive at a simple linear model for image formation. Where the detected image is equal to the weighted sum of images captured by turning on each LED individually:

$$I'(r) = \sum_{n=1}^{N} w_n(\lambda_n) I_n(\mathbf{r}, \lambda_n) \tag{2}$$

2 Neural Network Configuration Parameters

Throughout all experiments the same neural network architecture was used. We detail the key parameters of our U-Net architecture in Table 1a. Within Table 1b we report the hyperparameters used during training. With the exception of the noise level these hyperparameters were the same across tasks. For a more detailed implementation, please see the open source code associated with this paper.

Table 1: Configuration of Neural Network used in this Work

(a) Neural Network Architecture

Parameter	Value
Initial Filters	16
Filter Expansion Ratio	2
Convolutional Layers per Block	2
Convolutional Down-sampling Blocks	5
Convolutional Up-sampling Blocks	5
Convolutional Kernel Size	3×3
Activation Function	ReLU
Final Activation Function	Sigmoid
Convolutional Padding Amount	(1,1) zeros

(b) Training Hyperparameters

Hyper Parameters	HeLa Task	PAN Task
Optimizer	Adam	Adam
Initial Learning Rate	0.01	0.01
LR Reduction Factor	$\sqrt{10}$	$\sqrt{10}$
LR Reduction Patience	32	32
Training Patience	80	80
Noise Level (ψ)	0.01	0.01
L1 Penalty	0.0001	0.0001
Batch Size	8	8

3 Data and Code Sharing

The final version of the source code used to perform the experiments is attached within the supplementary material. If accepted, this code will become public through a Github repository.

The nature of our experiments creates extremely large datasets (100+GB). Due to the requirements of anonymity our data and it's size the data is uploaded in a set of multi-part tar files to *figshare* (the only platform we could find which allowed both anonymous sharing and large files). We apologize for this inconvenient data format. If accepted, we will re-upload the data in a better format to *Kaggle*, increasing accessibility.

In it's current format there are three repositories of data:

1. HeLa dataset (36.53 GB):
   https://figshare.com/s/e709e899f49b7aadb111

2. Test PAN dataset (13.2 GB):
   https://figshare.com/s/fd440616b808ca53abea

3. Train/validation PAN dataset (76.78GB):
   https://figshare.com/s/cd04f557f2f71dff5a31

Archives were created with standard *GNU* tar, and can be extracted using the command: "tar -xMf $filename".