

Introduction

Fluorescence imaging is widely used in microbiology related fields for important tasks such as pathogen detection [1] [2] [3]. Traditional fluorescence imaging techniques use physical optical filters which limits the amount fluorophores. Further, fluorophores with close emission spectra can't be used. Fourier transform imaging spectroscopy (FTIS) provides the resolution required for classifying fluorophores with close emission spectra. Typically for fluorescent imaging, FTIS is not used because of its low throughput and complexity, despite efforts being made to increase throughput [4] [5]. We combine deep learning with FTIS, and reduce sampling by 95% while identifying fluorescent signal in each pixel. We demonstrate the capabilities of our system using bovine pulmonary artery endothelial (BPAE) cells stained with fluorescent dyes.

Methods

The schematic diagram of the experimental setup used to capture the FTIS interferogram images is shown in the figure below.

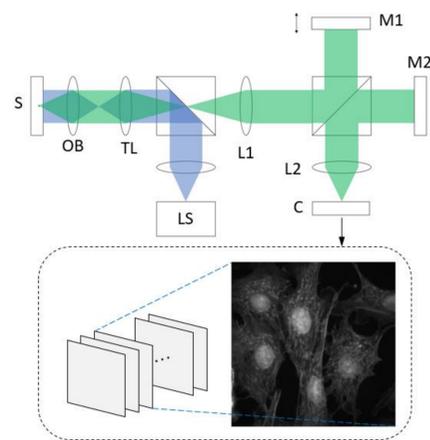


Figure 1: Schematic diagram of the optical system show the excitation beam path (blue) and emission beam path (green). LS: Light Source, S: Sample, OB: Objective Lens, TL: Tube Lens, L1 and L2: Lenses, M1: Moving Mirror, M2: Stationary Mirror, and C: Camera

Methods (Continued)

The data captured by the experimental setup is processed using the flow chart shown Figure 2. Training data is processed by computing the spectra, and finding the area under the curve of each fluorescent band. The neural network with architecture shown in Figure 3 is trained, and then used to predict and synthesize an image from an unknown sample. The training process is shown in blue, while the testing process is shown in green. Validation process followed training process except with a sample which was not trained on by the neural network.

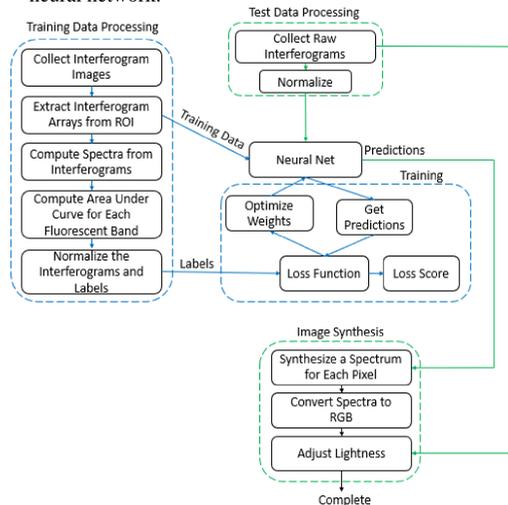


Figure 2: Data processing flowchart starting with collected interferogram images

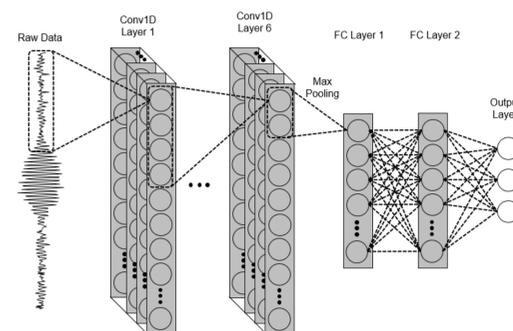


Figure 3: Diagram of our neural network architecture

Results

Using the full data set of 1000 interferogram images, with Δ OPD of 100 nm and HeNe correction the ground truth image shown in Figure 4 was computed.

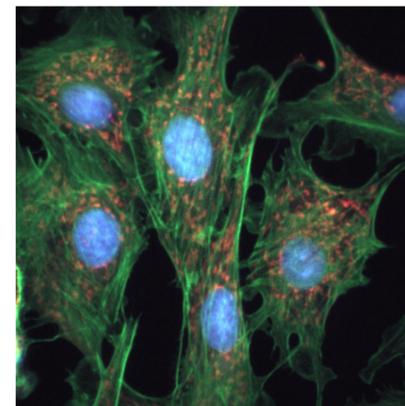


Figure 4: Ground truth test BPAE cell image

Using the trained neural network and image synthesis procedure, the image in Figure 5 was synthesized using only 1/20 of the data. The data used was 50 interferogram images with Δ OPD of 500 nm. The image synthesized by the neural network does not require HeNe correction which is typically used to remove experimental noise due to translation stage error. This leads to less optical components and less complex optical system.

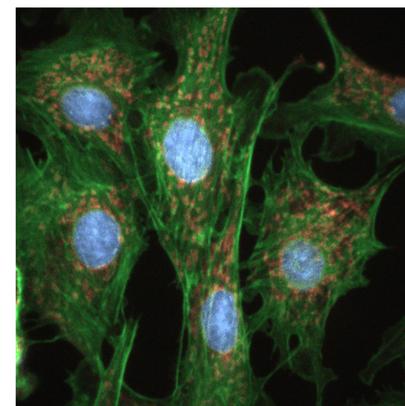


Figure 5: Image synthesized by neural network with only 1/20 of the data, without HeNe correction

Results (Continued)

For comparison, the image in Figure 6 was computed using traditional FTS with 1/20 of the data (50 interferogram images with Δ OPD of 500 nm), without HeNe correction. By observing Figure 6, we see that the fluorescent signal is not detected

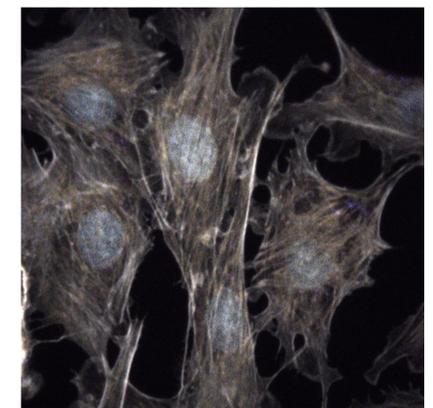


Figure 6: FTS image with 1/20 of the data, without HeNe correction

Conclusions

We have demonstrated high throughput FTIS based fluorescence imaging by combining deep learning and Fourier transform imaging spectroscopy. The fluorescent signal in each pixel of the image can accurately be determined even while reducing sampling by 95%. Using a fluorescent BPAE sample, we demonstrated the capabilities of our approach. Our developed system can be used in a wide range of applications where several fluorescent dyes with close emission spectra must be used, with much higher throughput.

Literature cited

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Contact information

Cory Juntunen
RA, Mechanical Engineering Dept., CEAS, UWM
Email: juntune3@uwm.edu

