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Low-Cost Fourier Ptychographic Microscopy for Neuroimaging Applications

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Abstract:
For a fluorescence microscopy system, a set-up often costs upwards of one hundred thousand dollars, if not multiple hundreds. This makes research inaccessible to any institutions except those with the highest level of funding. Our research addresses the challenges of high-magnification fluorescence microscopy by using low-cost off-the-shelf components to create a fluorescence imaging system. We use a cost-effective widefield system for neuroimaging using an OLED array via an Arduino microcontroller and a custom-made image processing algorithm. The OLEDs enable fluorescence microscopy with their green and blue emissions having significant overlap with the absorption spectrums for the Alexa Fluor 532 and FITC fluorescent probes. We use this system for Fourier ptychography and leverage image processing algorithms to achieve high-resolution images by combining low-resolution images within the Fourier plane. A phase retrieval algorithm and a regression model are utilized to find optimal coefficients for our transform matrices. This project is promising to democratize neuroimaging, especially in resource-constrained settings with affordable costs and portability. It is small-scale, 6'x8'x12', and compatible with any MATLAB system, thereby allowing even further reach into under-served areas.

KEYWORDS: Low-Cost Microscopy, Fourier Ptychography, Fluorescence Microscopy, Neuroimaging

Introduction:

A long-understood problem in the field of microscopy has been the idea of the "diffraction limit", the fundamental limit to a microscope's resolution[1]. For a particular field of view, the resolution is determined by the numerical aperture of the objective lens in the microscope. Objectives can be very expensive with each individual lens often costing upwards of ten thousand dollars. Imaging at high resolution over a wide area is challenging and even low-resolution systems suffer from aberations[2]. This results in difficulty for many types of imaging, but particularly neuroimaging. Neurons, which are a common target for brain function study, are unique in that they can be up to a meter long while only around 100 microns wide. This leads to the necessity for an imaging set-up that has a large enough field of view to capture the whole neuron while also having the resolution to capture the bounds of a neuron body widthwise. This is simply not possible for standard modes of microscopy because of the aforementioned tradeoff of field of view and resolution.

The costs associated with buying expensive microscopes also have resulted in unequal access to imaging that leaves groups in lower economic standing with equipment that is often outdated and ineffective[3], or there is simply no equipment for use leading to long wait times for what can be urgent procedures. In these affected low- and middle-income countries (LMICs) there is sparse access to diagnostic imaging[4,5].

Background:

Previous work in computational biology has shown the utility of a computational approach to imaging with articles starting in 2014 aiming broadly to overcome the traditional microscope tradeoff between resolution and field of view[6]. Starting off with the slower method of singular LED control before

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transitioning to patterns across an array that maximize imaging speed, these techniques gained popularity through their initial success[7]. However, a paper showing a way to computationally improve upon the diffraction limit was only published in *Nature* in 2021 which provided a fully comprehensive breakdown of the technique[8]. Between those years it has evolved from an initial demonstration to being used to its potential in research applications. Despite this now wider-spread use it remains used primarily in highly funded laboratories and has not reached its potential as a tool in biomedicine or to mitigate costs for underserved communities.

Materials and Methods:

We utilize an Arduino UNO Rev 3 to control our waveshare 1.5inch OLED Display Module with Embedded Controller Communicating for illumination. Additionally, we use an ELP 8.0 Megapixel USB CAMERA (5-50mm) for image capture. OLED control is through a manufacturer library that allows geometric lighting patterns, while camera control is through the MATLAB Image Acquisition Toolbox. The MATLAB Camera Calibration tool is also used for initial image correction. All further image processing steps are done in MATLAB.

When capturing images, we move our lighting source at a set angle through all possible locations on the OLED screen. We then vary this angle to capture additional images. From these images we use slices that are equidistant from our light to create a new set of images with uniform intensity. We then take the corresponding shift in the Fourier space and incorporate that data into our high-resolution image using the phase to iteratively move towards a higher resolution. It is important to note that currently there are images too near the light source, where blooming occurs, which we currently remove by hand, setting ranges of usable images.

Relevant code for our microscope can be found on our lab's GitHub page.

Results:

We create a dynamic illumination microscopy system where instead of panning the camera we "move" our light source by changing the location and direction of illuminated lines as they sweep across the screen. Here we see our control over shifts as each angle and intensity correlates to a unique location in the Fourier space. To maximize coverage this is compounded with a unique shift from each lighting location.

Our initial set of results is the capture and transformation of images of a sample slide set created from watercolor a "UCI" stencil. Layers of watercolor aim to emulate the thickness of biological tissue samples while also being entirely translucent to cause phase delays in light traveling through, from which we perform calculations needed for our image reconstructions.

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