Understanding Neurodynamics Through Light Field Microscopy

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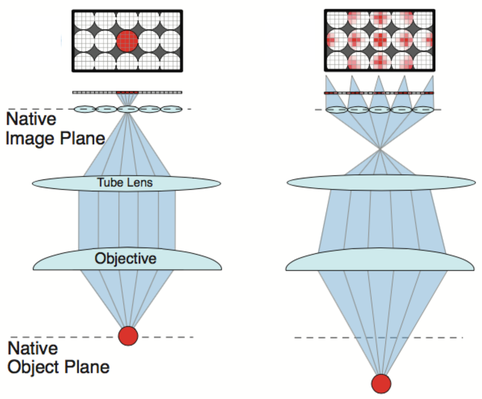
Background Information

The human brain has over 1 billion neurons, which are interconnected in approximately 10^14 ways. These connections define not only how we perceive the world around us, but also how we think about and respond to it. The number of connections in the brain make it so complex that it is almost impossible to distinguish which neurons serve which purpose. So when studying the brain where do we start? We chose to start with a model system of *caenorhabditis elegans* (hereafter called *c. elegans*). By studying the nervous system of this simple organism, we hope to gain a fundamental understanding of which neurons interact with each other so that we can more efficiently study more complex organisms such as humans. One of the primary obstacles to studying the neurology of *c. elegans* is that, in general, different neurons do not lie in the same plane. This creates difficulty to observe the interaction between neurons because any plane chosen to be the focal plane of the microscope is unlikely to contain both neurons involved in a synapse. In fact, this plane is not even likely to contain either neuron in its entirety since there is no reason that neurons must be exactly parallel to the objective lens.

Hypothesis

By building a light field microscope, we can observe objects not on the focal plane of the objective lens allowing us to observe neural interactions not on the same plane. By using a model system of *c. elegans* and the right magnification, we can see the entire nervous system of the *c. elegan* in one frame with single neuron resolution, allowing us to observe how one neuron interacts with another along the entire pathway from sensory to motor response.

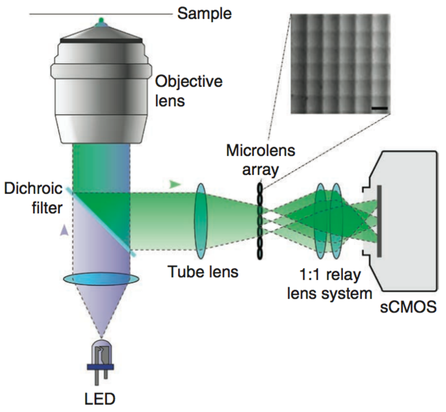
Theory



The light field microscope radically different from a conventional microscope. Left: In a conventional microscope the objective lens collimates light that originates on the focal plane, which is refocused by the tube lens onto the image plane for detection.[2]
Right: jThe objective lens in the light field microscope collects light from beyond the focal plane of the lens.[2]

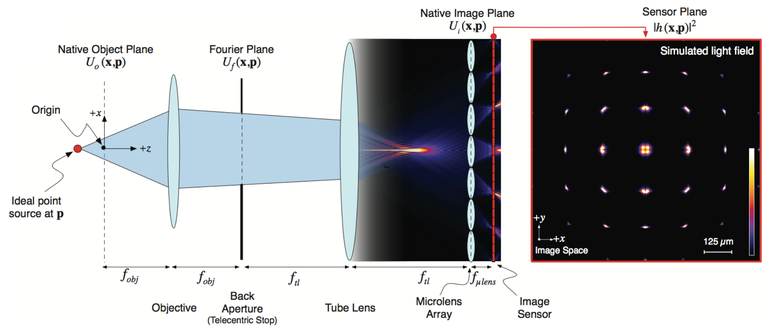
This allows the light being collected from the sample to cover the entire surface of the microlens array allowing for each lens in the array to observe the sample.[2]

Left: Diagram of the light field microscope. Light from the blue led is focused onto the stage, which stimulates the GFP in the sample. The green light from the GFP is then redirected by a dichroic mirror to the microlens array and then projected onto the camera. The lenses in the microlens array are very small so a relay system is used in order to extend the working distance of the microlens array.[3]

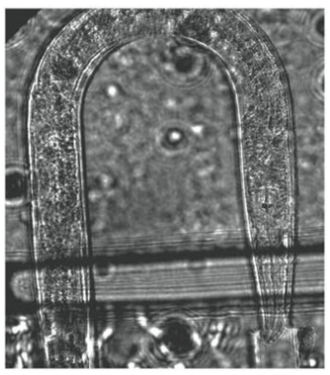


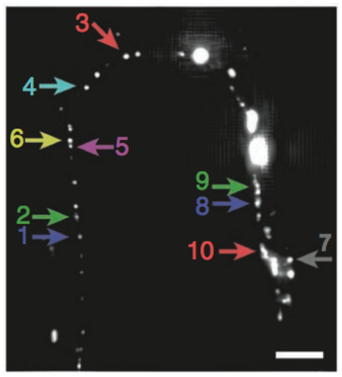
The Microlens Array

The microlens array is an array of lenses which are each only 130 um in diameter. Light coming from the specimen being observed passes through each lens in the microlens array allowing each lens to have a unique perspective of the specimen.

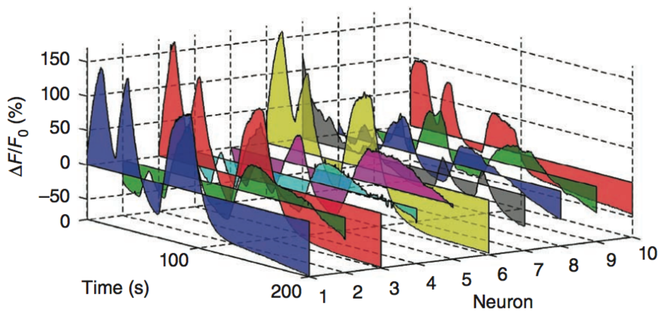


Above: The light field microscopes captures light fields instead of ordinary light. Light fields carry not only the magnitude of the intensity, but also the direction at which the light is traveling. This angular information is what allows the image to preserve the 3D nature of the sample which can be deconvoluted by a computer in order to study the physiology of the neurodynamics.[2]

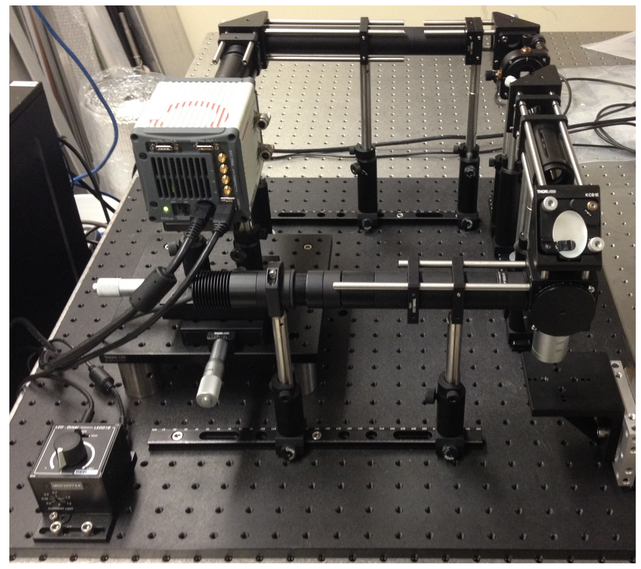




Left: Image of paralyzed worm.[3] Right: Maximum intensity projection of 14 distinct z-planes.[3]



Above: Ca^{2+} intensity traces of the neurons marked above.[3]



Conclusions

The light field microscope is a portable microscope given its small size. It is also of a relatively simple design because it is very similar to the design of a conventional microscope, with a few small modifications. The most important difference is the microlens array which allows the camera to have multiple perspectives of the sample. The major drawback we found with this microscope is the intensive computation required to render usable images from the light fields recorded by the camera. Running the software on a computer with 8 cores and 32GB of memory it takes approximately 2 hours to process one image. Using the computer cluster Hoffman2 the images still take about 40 minutes each to process. This processing time is very cumbersome when trying to take high speed video in order to have enough frames to see interactions between neurons.

Future Direction

In order to make the microscope more practical, the next step is to modify the existing software to make it more streamlined for running on Hoffman2. Currently the software operates using a graphic user interface which is not well supported by Hoffman2. Furthermore by removing the graphic user interface we can create batch jobs on the cluster which will allow it to process many frames at a time without a user having to individually process each frame.

References

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3. Prevedel R., Yoon Y., Hoffman M., Pak N., Wetzstein G., Kato S., Schrodel T., Raskar R., Zimmer M., Boyden E., Vaziri A. 2014. Simultaneous whole-animal 3D imaging of neuronal activity using light-field microscopy. Nature. 11(7): University of Vienna.

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