Confocal Synthetic Optical Holography Compatible Live-cell Imaging Module

Sreyesh Satpathy (satpath2@illinois.edu)

TA: John Capozzo

ECE 398 PSC Funding Proposal

16 December, 2016
Abstract

Synthetic optical holography is a recently described optical technique that implements holography in confocal microscopy. Demonstrating the benefits of high resolution, phase-resolved, live cell imaging is necessary to implement it for biological research. By creating and marketing a transflection mode live cell imaging module, holography in confocal microscopy can be made accessible to a wide audience of researchers who can benefit from using it. The team is composed of experienced photonics researchers at the University of Illinois, with specialized subgroups focusing on different components of the technology. Due to its ease of use, low cost compared to current technology, and large potential market, we are asking for $100,000 for 5% of the company. This will go towards finishing prototyping, producing the first batch of 24 units, and marketing. The budget for the live cell imaging component is $14900 for next semester. The initial prototyping and design was completed in December of 2016, the efficacy of the system will be tested by March 2017, and the new units will be sold to buyers by the end of May. The great improvements in imaging, low cost, potential market, and high relevance to biological research make this a valuable proposal to fund.

Project Description

Confocal Laser Scanning Microscopy (CLSM) is an optical imaging technique that utilizes a pinhole to obtain high resolution images. It is widely used in biological research and hospitals to look at histology. Holography is a class of optical techniques that uses light to encode the scattering signal from a sample by superposing an illuminated object with a reference wave. Traditional holography cannot be done through the confocal pinhole. Synthetic Optical Holography (SOH) is a recent innovation that uses a synthetic reference wave to obtain holographic images under CLSM, allowing for label-free, high resolution, phase-resolved imaging. This technique has not been demonstrated with live cell imaging (LCI), which is necessary for it to gain widespread adoption [1]. LCI compatibility would allow for SOH to offer biologically relevant information about cell movement and processes in vivo.

Our solution is the creation of a transflection mode LCI module compatible with SOH. It will make SOH easy to implement with living cells, allow for label-free phase-resolved imaging, and optically measure dry mass, cell density, and cell volume. No other high resolution phase-resolved imaging methods exist. Commercial optical coherence tomography (OCT) systems can measure scattering at high resolution and depth, but are not used for taking phase-resolved images and cost $70,000 [2]. Label-free imaging provides many benefits, most notably histopathology in living organisms.

Our objective is to create a module that adds LCI capabilities to an SOH system, allowing for high resolution, label-free phase-resolved imaging with living cells. It must keep at least 90% of cells alive over the course of 24 hours, cost less than $1000,

Team

Sreyesh Rishi Satpathy, an undergraduate at the University of Illinois Urbana-Champaign studying bioengineering, will be leading the LCI team. He has a background in photonics, light scattering, and computation optics modelling through work with Prof. Andrew Dunn and Prof. Stephen Boppart. He has four years of cell culture and histology experience.
Teams of individuals with a variety of backgrounds will focus on different components under the umbrella SOH project, such as the creation of a SOH stage insert for under $1000 or the UI of the SOH imaging software. The number of people on the LCI module team for next semester is not yet finalized. Dr. Martin Schnell, a postdoctoral researcher at the University of Illinois, invented SOH in 2014. He will be leading the overall direction of the SOH project. Prof. Paul Scott Carney, an electrical engineering professor at the University of Illinois with background in physics and optics, will be advising the teams working on the project.

**Ask**

The total SOH system costs less than $4000 to produce, with the LCI module costing well under $1000. We are asking for $100,000 for 5% of the company, which will be used to finish prototyping, produce two dozen units, and market the technology. There is a large potential market due to how ubiquitous CLSM is. Compared to the cost of a $20,000 confocal microscope, the cost of implementing SOH to obtain better images is not high. SOH provides valuable phase-resolution for a fraction of the cost of the CLSM system. The ease of implementation is a major benefit. OCT, a similar technology, commercially costs more than 15 times as much as this system and is much more difficult to set up.

**Itemized Budget**

The budget breakdown is shown in Table 1. The labor cost comes from assuming a pay of $30 per hour for 1 person to work 100 hours, then accounts for overhead with a 2.5x multiplier. All items marked with an (R) are recurring costs. All other costs are capital costs.

<table>
<thead>
<tr>
<th>Item</th>
<th>Cost:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Labor Cost</td>
<td>$7500</td>
</tr>
<tr>
<td>Okolab Uno-T System</td>
<td>$5000</td>
</tr>
<tr>
<td>Low NA Interferometry Objective</td>
<td>$1800</td>
</tr>
<tr>
<td>C2C12 Immortalized Mouse Myoblast Cells</td>
<td>$200</td>
</tr>
<tr>
<td>Standard Cell Culture Protocol Materials (R)</td>
<td>$100</td>
</tr>
<tr>
<td>Thorlabs Protected Aluminum Mirror x10 (ME05-G01) (R)</td>
<td>$103</td>
</tr>
<tr>
<td>Thermo 12/35 mm Glass Bottom Dishes x20 (150680) (R)</td>
<td>$197</td>
</tr>
<tr>
<td><strong>Total Cost</strong></td>
<td><strong>$14900</strong></td>
</tr>
</tbody>
</table>

**Timeline**
The following is the timeline specifically for the LCI team. By the start of November 2016, the transflection mode LCI module was designed and parts were ordered. By the beginning of December 2016, the LCI module prototype was completed. By the end of January 2017, experiments will be performed to determine if living cells can grow onto the treated aluminum mirror and if treating the mirror with poly-lysine will affect SOH. Compatibility with the other SOH components will be used to create a new prototype by the end of February 2017. Cell imaging with other cell types and over time periods longer than 1 hour will be carried out in March 2017. Multiple units will be assembled in April 2017 and tested by a bevy of researchers that utilize confocal microscopy for different biological applications. This will make it easy to gather clear evidence of the benefits of phase-resolved imaging. Remaining units will be sold over May 2016, as we have contacted many potential buyers excited to purchase our apparatus. Other subgroups will be working on a similar timeline to deliver their components by the time the LCI module is ready.

Long-term Impact and Conclusion

Due to the widespread applications of confocal microscopy, SOH can easily tap into a large market. The system provides label-free high resolution phase-resolved for a low cost and is specifically applicable to histology and cutting-edge biology research. Making SOH compatible with living cells and tissue gives it a wider range of applications, specifically for tracking cellular processes that occur over the course of hours. Doing so is the first step to creating a medical imaging device that utilizes this technology in a clinical setting.

Implementing SOH with an low cost, easily accessible LCI system has the potential to revolutionize the use of microscopy in hospitals and research labs. It will lead to more advanced understanding of biologically and disease relevant processes.
References
