Modular and open-source system for structured illumination microscopy

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Abstract

Introduction

Title: "Democratizing Super-Resolution Microscopy: An Adaptable Blueprint for Structured Illumination Microscopy"

Introduction:

In the world of modern microscopy, there exists a relentless pursuit of methods that enable us to explore the intricacies of life at ever finer scales. Among the many techniques at our disposal, Structured Illumination Microscopy (SIM) stands as a remarkable solution, driven by its unique capabilities and potential. This paper embarks on a journey to unveil the motivations, advantages, and challenges associated with SIM, while proposing an adaptable blueprint to democratize access to this cutting-edge technology.

The driving force behind any advancement in microscopy is the unquenchable thirst for enhanced understanding. Scientists and researchers are compelled to delve deeper into the microscopic world, to scrutinize living cells and fluorescent samples with unprecedented precision. Traditional microscopy techniques often fall short in providing the necessary resolution for such studies. It is this insatiable curiosity that motivates us to explore SIM.

Structured Illumination Microscopy presents itself as a beacon of hope in the quest for super-resolution (SR) imaging. It promises to deliver resolutions far beyond the diffraction limit while remaining fast enough to be compatible with live cells and a wide range of fluorescent samples. ~~Furthermore, SIM circumvents the stringent photophysics requirements that hinder some other super-resolution techniques.~~

Before we dive into the heart of SIM, it's essential to acknowledge the existing systems and their inherent challenges. On-chip, Spatial Light Modulators (SLM), Digital Micromirror Devices (DMD), and others have made significant contributions to microscopy, but they come with their own set of problems. These problems include high costs, limited portability, and the need for extensive optics expertise.

One significant challenge researchers face is the cost associated with implementing SIM. Not only does this encompass the setup and module itself, but it often necessitates a complete overhaul of the existing microscopy infrastructure. This financial barrier prevents access to advanced imaging techniques, especially in regions with limited resources.

Our paper seeks to provide a solution to these problems by presenting an adaptable blueprint for a SIM system that requires little optical expertise to replicate. We emphasize a customizable setup built upon open-source tools and readily available components, reducing the overall effort required for replication. Moreover, this system is designed to be compatible with multiple microscope brands, such as Nikon, Zeiss, and Olympus, making it a universal solution.

While our proposed solution aims to democratize SIM, it is important to acknowledge its limitations. This system may not be as fast as some high-end commercial setups. It introduces a new pixel scheme and mechanism that researchers must become acquainted with. Additionally, there are trade-offs between resolution and optical sectioning, and color multiplexing is limited to approximately 2-3 wavelengths.

In conclusion, our paper will delve deeper into the technical details of this adaptable blueprint for SIM, offering a bridge between cutting-edge super-resolution microscopy and researchers worldwide. By addressing the problems associated with cost, accessibility, and expertise, we hope to unlock the full potential of structured illumination microscopy for the broader scientific community.

Add something to declare the situation what we want to solve

Along with the increasement of research varieties, the required resolution of imaging is increasing steadily. The long-standing research groups have existing fluorescence microscopes, which is in good condition, but due to the fast iteration of microscope body, can’t find a proper illumination extension for it. Therefore, the expensive microscope will be shelved with the time.

Need to write something how can we cs

* Motivation
  + Why SIM?
    - SR, but fast enough to be compatible w/ live cells/most fluorescent samples
    - no photophysics requirements
  + Compare current systems (On chip, SLM, DMD, etc. w.r.t problems)
  + Resecure your old microscope
* Problems
  + too expensive = not only SIM setup/module, but usually need to renew whole microscopy setup (legacy hardware)
  + too expensive to use in “far away places” (i.e. one confocal in whole subsahara africa…)
  + not portable (?)
  + Optics expertise required
  + System integration lacking if home build system
* Here/Solution:
  + Present an adaptable blueprint to replicate a system that requires little optical expertise => protocol?
  + Customizable setup
  + based on open-source tools
  + low effort to replicate (e.g. limited numbers of externa/Thorlabs parts)
  + multiple colours
  + universal compatibility (e.g. Nikon, Zeiss\*, Olympus\* .. \*=> theoretically, not tested yet)
  + system integration using python
  + limitations:
    - not necessarily fast
    - new pixel scheme/mechanism
    - resolution vs. optical sectioning
    - color multiplexing limited to ~2-3 wavelengths

Method

* New DMD
  + Introduce the new TRP format and tell that it’s the replacement for the “old” pixel scheme
    - todo: timeline for others being deprecated?
  + Compare “old” pixel assignment and TRP
    - “edge” (TRP) versus “corner” illumination (old)
    - new design simplifies mounting, since no longer need to mount DMD rotated at 45 degree angle
    - on/off directions now along different axes
  + Theoretical description; Mathematical model; What’s the difference
    - corner illumination pixels
    - only approximate solutions to joint blaze/diffraction conditions exist, so need new math to handle this case
    - TRP makes more difficult to use on/off mirrors for different colors
  + How to use with multiple colours
    - simulation tools/scripts
    - maybe provide the diffraction solution explorer python GUI with paper
  + Maximum efficiency
  + Implications for other “coherent” microscopy methods
* Two colour SIM setup
  + sketch optical diagram
  + “calculation-driven” design; i.e. calculate angle for new wavelength and adjust 3D laser cutting files “automatically”
  + calculate angles and briefly describe selection of the components
  + Adaptation to different microscopy bodies (e.g. Tubelens + Sampling)

Results

* Validation of DMD
  + Efficiency
  + Diffraction: Theory vs. Reality
    - discuss manufacturing tolerances of angles
* Mechanical setup and selection of components
  + Control flow chart
  + DMD Evaluation board:
    - Limitations
    - How to control (e.g. using HDMI, limitations)
  + Lasers
    - Single-mode fiber-coupled lasers + control
  + Housing/Manufacturing
    - “sweet spot” between costs, stability, performance
    - 3D printing, laser cutting, Thorlabs parts
  + Final assembly
    - on Nikon Ti-e 2
    - Zeiss Axiovert ?
* Software
  + Soft- Hardware synchronization
  + GUI to collect and simultaneously reconstruct data in “realtime” => ImSwitch + Napari plugin from Peter
* Experimental results
  + Resolution calibration using Beads/Argolight
  + Multicolour results using dual-stained “Gattacells”
  + Live Imaging? Timelapse
  + Z sectioning
  + large FOV, increased resolution (e.g. 20x/NA0.75)
  + real time reconstruction?

Discussion

* Resolution improvements vs. optical sectioning
* adaptability for different microscopy manufacturers
* Software integration
* speed limitations
* possible to extend to 3D SIM?

Some thoughts:

Github repository:

* Alignment / Build Tutorial:
  + step by step guid á la <https://beniroquai.github.io/tutorials/uc2-tutorial-minibox/>
  + BOM
  + Software installation
  + Calibration points (e.g. compare camera)

TODO’s

Haoran Figures determination

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