Modular and open-source system for structured illumination microscopy

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Abstract

Introduction

Microscopy, a fundamental technique essential for delving into the micrometer and even nanometer scales, serves as a vital tool in scientific researches. Fluorescence microscopy, in particular, holds significant prominence in the realm of biological research. The resolution of microscopes is inherently confined by the diffraction limit, commonly known as the Abbe limit. Over the past two decades, numerous research groups have endeavoured to overcome this limitation through innovative approaches, including single molecule localization techniques such as Storm and PALM, as well as ground state depletion methods like STED.

Among these varied techniques, Structured Illumination Microscopy (SIM) has emerged as a noteworthy contender. Operating on the principles of widefield imaging, SIM offers distinct advantages, including low photon dosage and compact raw data size. This technique, while being a branch of fluorescence microscopy, has proven to be a valuable asset, enabling researchers to explore biological intricacies with unprecedented detail and efficiency.

Along with the increasement of research varieties, the required imaging resolution is increasing steadily. However, for super-resolution microscope, complexity and cost are always the big shortages. Due to the complicated optical path of the super-resolution illumination, it restricted the possibility of building a super-resolution microscope as nonspecialist in optics. For the long-standing biological research groups, normally they have existing fluorescence microscopes, which is in good condition but due to the fast iteration of commercial microscopes, it is hardly to find a proper illumination extension to update the imaging ability. Therefore, the expensive microscope body will be shelved when a better resolution microscope be purchased.

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

Need to write something how can we cs

In this paper, we want to demonstrate a structured illumination extension which can be applied onto epi-fluorescence microscope. With this extension, the microscope is able to get images which is 1.75x enhancement compare to widefield.

In the relentless pursuit of unraveling the mysteries hidden within the microcosm, the world of microscopy has continuously evolved, driven by unwavering motivation to push boundaries and explore new dimensions of life. Structured Illumination Microscopy (SIM) emerges as a transformative force in this scientific odyssey, offering a compelling answer to the pressing question of "Why SIM?" SIM not only ushers in super-resolution capabilities but crucially marries this high-resolution imaging with remarkable speed, rendering it compatible with the dynamic realm of live cells and most fluorescent samples. Perhaps even more enticingly, SIM liberates researchers from the intricate demands of photophysics, making it an enticing proposition in the microscopy landscape. However, as we delve deeper into the realm of SIM, it becomes evident that its potential remains partially concealed by a host of challenges, from the comparative analysis of existing systems to the prohibitive costs associated with implementation and the demanding requirements of optics expertise. This paper embarks on a journey to demystify SIM, proposing an innovative solution that envisions a future where SIM is not confined to well-funded laboratories but becomes accessible globally. Our mission is to present an adaptable blueprint that, with little optical expertise required, empowers researchers to harness the super-resolution capabilities of SIM. This blueprint, founded on open-source tools and characterized by ease of replication, heralds a new era of customizable setups, facilitating imaging in multiple colors and promising universal compatibility with a range of microscope brands. Additionally, we explore the integration of system control using Python, enhancing the accessibility and versatility of SIM. Nonetheless, as with any scientific breakthrough, there exist limitations and complexities, from the intricacies of the pixel scheme and mechanisms to the delicate balance between resolution and optical sectioning, and the constraint of color multiplexing to a limited palette. In summary, this paper embarks on an odyssey through the realm of SIM, charting a course from motivation to solution, ultimately envisioning a future where the super-resolution capabilities of SIM are within reach for researchers worldwide.

* 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

Peter

Rainer

Douglas

Benedict