Deep Learning for Structured Illumination Microscopy Image Processing

James Hughes
Supervised by Dr Edward Ward

Project Report

MPhil, Data Intensive Science

Department of Physics & Department of Chemical Engineering and Biotechnology
University of Cambridge
United Kingdom
28th June 2024

Acknowledgements

Firstly I would like to thank my supervisor, Dr Edward Ward, for all of his support over the course of this project. The project involved a lot of concepts from microscopy and image processing that were very new to me, but Dr Ward made it clear from early on in the project that this would not be a problem, and was quick to provide reading materials to help me get to grips with the subject. Having been trained in Mathematics during my time as an undergraduate, the chance to go into the laboratory and capture real microscope images that were later used in the work was incredibly exciting. Dr Ward was eager to provide this opportunity and welcomed me to the Chemical Engineering and Biotechnology (CEB) Department and his research group.

I also had the opportunity to attend some of the Laser Analytics Group (LAG) lab meetings, where I had the privilege of learning about some of the world-leading research being undertaken by the group. Later, I shared details about my own project in two presentations to the group. I would like to thank all of the members of the LAG for welcoming me, listening to my presentations and providing great feedback. In particular I wish to thank Professor Clemens Kaminski for his helpful suggestions and words of encouragement.

I would also like to thank Jeremy Wilkinson, Esther Gray, and Emilio Luz-Ricca. I had very insightful conversations about the project with all of them that helped me see the work in a new light.

Lastly, I would like to thank my parents for being a continual source of support and strength throughout my education, in particular for encouraging me to make the most of every opportunity that comes my way.

Abstract

Contents

1 Introduction

Fluorescence microscopy is an essential tool for microbiologists, enabling them to view complex biological phenomena unfolding at the sub-cellular level. This type of microscopy is particularly suited to the work of microbiology as it uses dyes to mark specific organic compounds of interest, which then release photons in response to illumination from a laser at a suitable wavelength. As a type of optical microscopy, the resolution of these systems is limited by the effects of diffraction. This limit was quantified by Abbe in 1882 as a minimal resolvable distance

$$\frac{\lambda}{2n\sin\theta}$$

Axial resolution is also an issue. For comparison, the soma (body) of a human neuron cell is approximately 10-20µm in diameter. This represents a serious obstacle to researchers attempting to view cell dynamics in greater detail.

Structured Illumination Microscopy (SIM) is a technique that combines a specialised microscope set-up, alongside computational processing of the acquired images, in order to surpass the classical Abbe diffraction limit. The theoretical foundations of the technique were first established in 2008 [1], but since then there have been a range of improvements made to the technique. While SIM does not necessarily provide the greatest improvements in resolution compared to other methods such as confocal, it has other advantages for researchers interested specifically in capturing imagery of dynamic biological processes over extended periods. This relates primarily to the issue of phototoxicity effects. Every time a fluorescence microscopy image of a cell sample is taken, the cell itself is bleached and damaged in the process. This is particularly troublesome when one wishes to view dynamic processes in live cells, because the very process of imaging has an effect on the process being captured, thereby limiting the duration of imagery that can be obtained that is faithful to the true process. SIM offers a trade-off between resolution improvements and low photo-toxicity effects.

The paper by Li. et al. [2] attempts to augment the SIM image processing pipeline with deep-learning techniques to improve this trade-off. Their research explores multiple ways in which hardware and computation can be used to improve the resolution of SIM imaging. This project investigates their 'two-step denoising method'. The core of this technique involves dramatically

lowering the illumination dose of the SIM laser, in order to mitigate phototoxicity effects. In turn, they train two networks to denoise the acquired and reconstructed images, to attempt to compensate for the low intensity illumination and reclaim lost image resolution.

The objectives for this project were

reproduce use good practice to create a robust code base

2 Methods

2.1 SIM Reconstruction process

Structured Illumination Microscopy stands in contrast to the conventional approach of using a uniform illumination to produce an image. Instead, SIM microscopes usually employ a spatial light modulator (SLM) to produce a striped illumination pattern, whose spacing is close to the Abbe diffraction limit of resolution. When the light illuminates the sample causing it to fluoresce, the excitation pattern interferes with the high spatial frequencies of the structures in the sample, causing high spatial frequency information to be exposed as lower frequency features. Figure 1 demonstrates this effect with Moiré fringes, an interference pattern with lower spatial frequency than the two patterns that generate it.

In order to correctly interpret this interference effect, and reconstruct a super-resolved image, multiple images need to be acquired from the microscope and analysed in the Fourier domain. When reconstructed properly, there will be an improvement of lateral resolution in the direction of the k-vector of the pattern. Therefore, when acquiring images for 2D SIM, 3 groups of images are almost always captured, with the patterns angled $2\pi/3$ from eachother, to obtain an (almost) isotropic improvement in *lateral* resolution, and the same is true in 3D SIM. Within these groups, the images are acquired with the illumination pattern having a different phase each time.

The reconstruction involes six key steps:

1. Parameter estimation,
2. Fourier transform,
3. Band separation,
4. Wiener filtering,

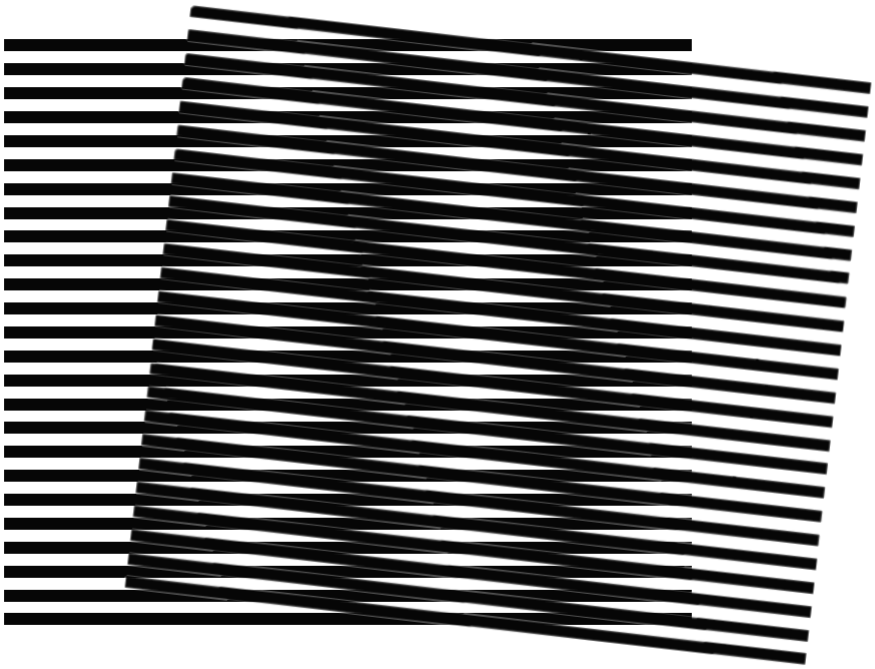

Figure 1: Moiré Fringes

5. Apodization, and,

6. Inverse Fourier transform

Parameter estimation is primarily concerned with the position of the illumination pattern, including the phase, angle, and modulation depth. This is more accurate than measuring these quantities in the physical system which would require high degree of care and precision. Then the image is converted to the frequency domain. Denoting the image intensity by $D(\vec{r})$, the pattern k-vector and phase by $\vec{p}$, ϕ_n, the modulation depth by a_m, the density of the fluorescent substance as $S(\vec{r})$ and the point-spread function by $H(\vec{r})$, we see that the effect of imaging on the 'true' ground-truth structure S is to multiply it with the excitation pattern, and then convolve with the point-spread function:

$$D_n(\vec{r}) = \sum_{m=-M}^{M} S(\vec{r}) a_m \exp(im(2\pi\vec{p}\cdot\vec{r} + \phi_n)) \otimes H(\vec{r})$$

Utilising the Convolution Theorem this becomes

$$\tilde{D}_n(\vec{r}) = \sum_{m=-M}^{M} \exp(im\phi_n) a_m \tilde{S}(\vec{k} - m\vec{p})\tilde{O}(\vec{k})$$

In turn, with sufficient acquired images at different phases, namely M, this can be used to solve a fully determined set of linear equations for

$$\tilde{S}(\vec{k} - m\vec{p})\tilde{O}(\vec{k}) \qquad m = -M, \ldots, M-1, M$$

This constitutes the band separation step, and explains why 2D SIM uses 3 sets of 3 images, while 3D SIM uses 3 sets of 5 images; the number of different phases used in imaging must correspond to the number of delta peaks that represent the illumination pattern in Fourier space.

The steps of Wiener filtering and Apodization are used to combine the separated bands of the image, whilst also adequately dealing with noise and artefacts that can occur.

fairSIM and parameters.

2.2 Data

In the research Li. et al. acquired pairs of high and low SNR images which were used to train the networks. In order to avoid to acquire these images more quickly, and to avoid the need for image registration to align the pairs of images acquired, the increased noise resulting from lowering the illumination dose was simulated in-silico.

2D real data (specifications of the Microscope, cells)

3D vh data This was generated with the help of another student's code. The code was modified to include only the most essential parts, and to generate a 3D volume of SIM acquisition stacks (15 each)

2.3 RCAN

Use elsewhere

Diagram

2.4 Pipeline

Describe building from scratch (pytorch vs tflow)
- Diagram
- Software
- Using CSD3, hardware, parallel -¿ serial

3 Results

3.1 2D Data

Parameter estimation
- Generalizability
- Tables of results, metrics
- Images

3.2 3D Data

Axial resolution
- Tables of results, metrics
- Images

4 Discussion

Results/conclusions Further work What I learned How I could have improved

5 References

[1] G. Mats G.L. *et al.*, "Three-dimensional resolution doubling in wide-field fluorescence microscopy by structured illumination," *Biophysical Journal*, vol. 94, no. 12, pp. 4957–4970, 2008. [Online]. Available: https://doi.org/10.1529/biophysj.107.120345

[2] X. Li *et al.*, "Three-dimensional structured illumination microscopy with enhanced axial resolution," *Nature Biotechnology*, vol. 41, pp. 1307–1319, 2023. [Online]. Available: https://doi.org/10.1038/s41587-022-01651-1

A Statement on the use of auto-generation tools

B High-Performance Computing Resources

This work was performed using resources provided by the Cambridge Service for Data Driven Discovery (CSD3) operated by the University of Cambridge Research Computing Service (www.csd3.cam.ac.uk), provided by Dell EMC and Intel using Tier-2 funding from the Engineering and Physical Sciences Research Council (capital grant EP/T022159/1), and DiRAC funding from the Science and Technology Facilities Council (www.dirac.ac.uk).