Phase control and measurement in digital microscopy

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Summary

The ongoing merger of the digital and optical components of the modern microscope is creating opportunities for new measurement techniques, along with new challenges for optical modelling. This thesis investigates several such opportunities and challenges which are particularly relevant to biomedical imaging. Fourier optics is used throughout the thesis as the underlying conceptual model, with a particular emphasis on three-dimensional Fourier optics.

A new challenge for optical modelling provided by digital microscopy is the relaxation of traditional symmetry constraints on optical design. An extension of optical transfer function theory to deal with arbitrary lens pupil functions is presented in this thesis. This is used to chart the 3D vectorial structure of the spatial frequency spectrum of the intensity in the focal region of a high aperture lens when illuminated by linearly polarised beam.

Wavefront coding has been used successfully in paraxial imaging systems to extend the depth of field. This is achieved by controlling the pupil phase with a cubic phase mask, and thereby balancing optical behaviour with digital processing.

In this thesis I present a high aperture vectorial model for focusing with a cubic phase mask, and compare it with results calculated using the paraxial approximation. The effect of a refractive index change is also explored. High aperture measurements of the point spread function are reported, along with experimental confirmation of high aperture extended depth of field imaging of a biological specimen.

Differential interference contrast is a popular method for imaging phase changes in otherwise transparent biological specimens. In this thesis I report on a new isotropic algorithm for retrieving the phase from differential interference contrast images of the phase gradient, using phase shifting, two directions of shear, and non-iterative Fourier phase integration incorporating a modified spiral phase transform. This method does not assume that the specimen has a constant amplitude. A simulation is presented which demonstrates good agreement between the retrieved phase and the phase of the simulated object, with excellent immunity to imaging noise.
Acknowledgements

Thanks to Carol Cogswell, my first supervisor in this project, for her ideas, energy and enthusiasm. It was Carol who got me addicted to biomedical imaging research a whole decade ago. Colin Sheppard was my second supervisor and shared with me his creativity and curiosity, his delight in discussing simple yet mind–bending optical ideas, and his ability to answer even my most primitive questions with patience and clarity.

I found Peter Török to be a most generous and supportive collaborator, who has a great passion for accurate optical modelling of important but challenging microscopy problems. He is wonderfully persistent in seeking the physical meaning behind the mathematics. Working with Peter has been very rewarding.

Kieran Larkin is one of those people who thinks in 3D Fourier space just as easily as most people think in real space. Nicholas Smith has been a great friend and colleague. I have been lucky to be able to collaborate with such a playful pair of physicists.

W. Thomas Cathey, Edward Dowski and Sara Tucker shared their pioneering expertise in the world of wavefront coding. CDM Optics provided an extended loan of a cubic phase mask and a 1 µm pinhole.

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Thanks to Andreas Schönle for gently bringing to my attention a mathematical error I made in a journal paper published as part of this project.

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- Peter Török at Oxford University;
- Satoshi Kawata, Nicholas Smith and Taisuke Ota at Osaka University;
- Joseph Braat at TU Delft; and
- Fu–Jen Kao at National Sun Yat-sen University.

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Ruby and Rudi, our canine companions, come last but not leashed. They never tire of taking simple and intuitive pleasure in mastering the physics of motion.
Declaration of originality

In this thesis I have acknowledged the contributions to my research made by my colleagues and collaborators. I have also cited the literature as appropriate. All other work presented is mine alone.

Significant contributions and collaborations included the following:

- Chapter 3: It was Colin Sheppard’s suggestion to extend the transfer function theory from his papers (Sheppard et al., 1994; Sheppard and Larkin, 1997) in order to deal with arbitrary pupil functions. Andreas Schönle gently pointed out a mathematical error in the article which chapter 3 is based on (Arnison and Sheppard, 2002), enabling me to correct the error while preparing this thesis.

- Chapter 4: Peter Török collaborated with me on the refractive index change model for cubic phase mask imaging, contributing both his theoretical expertise and his source code. My heavy use of the projected pupil integration method was inspired by a personal demonstration of the technique by Kieran Larkin.

- Chapter 5: Carol Cogswell provided the vision and leadership for high aperture wavefront coding and worked on all the experiments and processing steps for the biological imaging result presented in section 5.3. Eleanor Kable and Theresa Dibbayawan prepared the HeLa cell specimen. David Philp and Janey Lin assisted with the 1 μm fluorescent bead point spread function measurement used to restore the HeLa cell EDF image, while Edward Dowski and Claude Rosignol worked on inverse filter design and image restoration.

- Chapter 7: Colin Sheppard and Kieran Larkin both provided key ideas used in the spiral phase algorithm, as detailed in appendix A.
Publications and presentations

Chapters 3–5 and chapter 7 are based on the work presented in the following publications:


The following conference presentations highlighted work described in this thesis (* indicates presenting authors):


Acronyms, abbreviations and conventions

$\leftrightarrow$ Fourier transform relation

$\otimes$ convolution

$\ast$ correlation

1D, 2D, 3D one dimension, two dimensions, three dimensions

$\alpha$ aperture half-angle

axial parallel to the optical axis, $z$

amplitude amplitude $a$ of a complex field $ae^{i\phi}$

CCD charge-coupled device

CPM cubic phase mask

DC direct current, i.e. image background or bias

DIC differential interference contrast

EDF extended depth of field

FITC fluorescein isothiocyanate, a fluorescent dye

$f(x,y,z)$ functions in real space are usually lower case

$F(m,n,s)$ equivalent functions in Fourier space are often upper case

$g'$ projection of function $g$

$\mathcal{F}\{h\}$ Fourier transform of function $h$

$f$ vectors are set in boldface

FFT fast Fourier transform

$k_0 = 2\pi/\lambda_0$ vacuum wave number for light of wavelength $\lambda_0$

lateral orthogonal to the optical axis

$m = (m,n,s)$ vector in Fourier space, unit directional vector
<table>
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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>$n$</td>
<td>refractive index</td>
</tr>
<tr>
<td>NA</td>
<td>numerical aperture</td>
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<tr>
<td>OTF</td>
<td>optical transfer function</td>
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<tr>
<td>paraxial</td>
<td>approximate scalar field propagation for small angles to the optical axis</td>
</tr>
<tr>
<td>phase</td>
<td>phase $\phi$ of a complex field $ae^{i\phi}$; optical path length variations</td>
</tr>
<tr>
<td>PSF</td>
<td>point spread function</td>
</tr>
<tr>
<td>SNR</td>
<td>signal to noise ratio</td>
</tr>
<tr>
<td>transverse</td>
<td>orthogonal to the optical axis</td>
</tr>
<tr>
<td>vectorial</td>
<td>high aperture electromagnetic focusing theory</td>
</tr>
<tr>
<td>wave</td>
<td>unit of phase ($2\pi$ radians)</td>
</tr>
<tr>
<td>widefield</td>
<td>conventional microscope imaging, without pupil filters</td>
</tr>
<tr>
<td>$x = (x, y, z)$</td>
<td>vector in real space</td>
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