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.

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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.

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Publications and presentations

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

- Arnison, M. R. and Sheppard, C. J. R. (2002) "A 3D vectorial optical transfer function suitable for arbitrary pupil functions," *Opt. Commun.*, **211**(1-6), 53–63.
- Arnison, M. R., Cogswell, C. J., Sheppard, C. J. R., and Török, P. (2003) "Wavefront coding fluorescence microscopy using high aperture lenses," in P. Török and F.-J. Kao (Eds.), *Optical imaging and microscopy: techniques and advanced systems*, vol. 87 of the Springer series in optical sciences, chap. 6, pp. 143–165, Springer-Verlag, Berlin.
- Arnison, M. R., Larkin, K. G., Sheppard, C. J. R., Smith, N. I., and Cogswell, C. J. (2004) "Linear phase imaging using differential interference contrast microscopy," *J. Microsc.*, **214**(1), 7–12.

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

- C. J. Cogswell,* M. R. Arnison, E. R. Dowski Jr., S. C. Tucker, and W. T. Cathey, "A new generation, fast 3D fluorescence microscope using wavefront coding optics," *Microscopy Society of America*, (Oregon, USA, 1999).
- C. J. Cogswell,* M. R. Arnison,* E. R. Dowski Jr.,* S. C. Tucker,* and W. T. Cathey,* "A novel 3D fluorescence microscope using wavefront coding optics," *Optical Society of America Annual Meeting*, (Santa Clara, CA, USA, 1999).
- C. J. Cogswell,* M. R. Arnison, E. R. Dowski Jr., S. C. Tucker, and W. T. Cathey, "Extended–depth–of–focus fluorescence microscope made possible using wavefront coding," *Focus on Microscopy*, (Shirahama, Japan, 2000).
- M. R. Arnison,* P. Török, C. J. R. Sheppard, W. T. Cathey, E. R. Dowski, Jr., and C. J. Cogswell, "High resolution extended depth of field microscopy using wavefront coding," *Students International Symposium on Advanced Engineering*, (Osaka, Japan, 2000), (invited talk).
- M. R. Arnison, P. Török, C. J. R. Sheppard,* W. T. Cathey, E. R. Dowski Jr., and C. J. Cogswell, "Wavefront coding in high numerical aperture microscopy," *Australian Optical Society XIII*, (Adelaide, Australia, 2000).

- W. T. Cathey,* M. R. Arnison, C. J. Cogswell, and E. R. Dowski Jr., “Large depth of field in fluorescence imaging of live cells,” *Optical Society of America Annual Meeting*, (Rhode Island, USA, 2000).
- C. J. Cogswell, M. R. Arnison,* E. R. Dowski Jr., S. C. Tucker, and W. T. Cathey, “Wavefront coding gives rise to a fast extended–depth–of–focus fluorescence microscope,” *Optics Within the Life Sciences VI*, (Sydney, Australia, 2000).
- C. J. Cogswell,* E. R. Dowski Jr., I. A. Prischepa, G. E. Johnson, S. C. Tucker, M. R. Arnison, and W. T. Cathey, “Recent advances in extended depth of focus microscopy for live–cell imaging,” *Three-dimensional and multidimensional microscopy: image acquisition and processing VIII* at *SPIE Photonics West*, (San Jose, CA, USA, 2001).
- M. R. Arnison,* P. Török, C. J. R. Sheppard, W. T. Cathey, E. R. Dowski Jr., and C. J. Cogswell, “A model for wavefront coding in high numerical aperture microscopy,” *Optical Society of America Annual Meeting*, (Long Beach, CA, USA, 2001).
- M. R. Arnison* and C. J. R. Sheppard, “Three dimensional optical transfer functions for high aperture systems with non-symmetric pupils,” *Australian Optical Society Conference*, (Sydney, Australia, 2002).
- M. R. Arnison,* C. J. R. Sheppard, and P. Török, “High aperture wavefront coding,” *Focus on Microscopy*, (Kaohsiung, Taiwan, 2002), (invited talk).

Acronyms, abbreviations and conventions

$\Longleftrightarrow$	Fourier transform relation
$\otimes$	convolution
$\star$	correlation
1D, 2D, 3D	one dimension, two dimensions, three dimensions
α	aperture half-angle
axial	parallel to the optical axis, z
amplitude	amplitude a of a complex field $a\mathrm{e}^{\mathrm{i}\phi}$
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 λ_0
lateral	orthogonal to the optical axis
$\mathbf{m} = (m,n,s)$	vector in Fourier space, unit directional vector

n	refractive index
NA	numerical aperture
OTF	optical transfer function
paraxial	approximate scalar field propagation for small angles to the optical axis
phase	phase ϕ of a complex field $a\mathrm{e}^{\mathrm{i}\phi}$; optical path length variations
PSF	point spread function
SNR	signal to noise ratio
transverse	orthogonal to the optical axis
vectorial	high aperture electromagnetic focusing theory
wave	unit of phase (2π radians)
widefield	conventional microscope imaging, without pupil filters
$\mathbf{x} = (x, y, z)$	vector in real space

Contents