Light Microscopy

Friedrich-Schiller-Universität Jena

Prof. Dr. Rainer Heintzmann

Winter Term 2017/18

Aim

Light microscopy, although being a long-existing field of research still seems as thrilling as ever, as is seen by the recent Nobel price award to Moerner, Hell and Betzig for their contribution to super-resolution microscopy. There are multiple possible ways to understand recent optical and methodological advances. In my view, the Fourier-based approach to understanding wave-optical phenomena is particularly fruitful. This book aims at presenting optics from the wave perspective. It should teach how many phenomena in optics can be understood by considering the wave nature of light. The natural basis for a wave is a plane wave, and the Fourier transformation of a plane wave is a simple delta peak. Therefore Fourier space is immensely important for a true understanding of wave optics. By looking at phenomena from the Fourier perspective one can often gain new understanding. The switch between the real space view and the Fourier view of the same phenomenon thus serves as a guiding principle throughout this course. It may require some getting-used-to in the beginning, but mastering it holds the promise of possessing a very insightful tool for the understanding of optics and more generally physics as a whole.

Acknowledgment

I thank René Richter for the thorough improvement of the script as well as providing the exercises for the seminars. I also thank Marie Walde for providing the figures as well as Polina Feldmann and Walter Müller for their helpful input.

Contents

Nomenclature 6

1 Introduction - Understanding the Nature of a light-wave 9
1.1 Complex Numbers 9
1.2 Homogeneous Maxwell equations 11
1.3 Wave equation and Helmholtz equation 12
1.4 Superposition principle 14
1.5 Literature and References 14

2 Short Introduction to Fourier Optics 16
2.1 Properties of the Fourier-transformation 16
2.2 Convolution 20
2.3 Literature and References 24

3 Light in free space and at an interface 25
3.1 Free space 25
3.2 Free space propagation of light 26
3.3 Light in a medium 28
3.4 Light at a planar interface 28
3.5 Evanescent waves 29
3.6 Literature and References 30

4 Lenses 31
4.1 Ray optics and lenses 31
4.2 Focusing by a lens with high numerical aperture 32
4.2.1 Abbe's sine condition 33
4.2.2 The high-NA focus 33
4.3 Aplanatic factor: illumination 35
4.4 Aplanatic factor: imaging 36
4.5 Paraxial Approximation 36
4.5.1 High-NA polarisation effects 36
4.6 Defocus and other aberrations 37
4.7 Literature and References 37

5 Scattering 38
5.1 Born approximation 38
5.2 Absorption 43

5.3 Image Formation . . . 45
5.3.1 First Born approximation for incoherent illumination imaging . . . 45
5.3.2 Incoherence at large distances . . . 46
5.4 Semi-coherent illumination . . . 46

6 Sampling 48

7 Coherent Imaging 49
7.1 Dark field imaging . . . 49
7.2 Confocal scanning in Born's approximation . . . 50
7.2.1 Reflection confocal microscope . . . 50
7.3 Holography . . . 51
7.4 Optical Coherence Tomography . . . 55
7.4.1 Confocal and coherence gating . . . 55
7.4.2 Time domain OCT . . . 55
7.4.3 Frequency Domain OCT & Swept Source OCT . . . 55
7.4.4 Multiple scattering . . . 56
7.5 Oversampling Phase Retrieval . . . 57
7.5.1 Algorithms for phase retrieval . . . 57
7.6 Ptychography . . . 58
7.7 Fourier Ptychography . . . 58
7.8 Literature and References . . . 59

8 Truly incoherent linear imaging 60
8.1 Intensity transfer function OTF . . . 60
8.1.1 Geometrical shape of the support of the optical transfer function (OTF) . . . 61
8.2 Abbe's resolution limit revisited . . . 62
8.3 Comparison to spatially incoherent illumination . . . 62
8.4 Defocus in incoherent imaging . . . 63
8.5 Notes on Fluorophores . . . 63
8.6 Incoherent imaging Techniques . . . 66
8.6.1 Widefield fluorescence microscopy . . . 66
8.6.2 The confocal fluorescence microscope . . . 66

General Literature recommendations

- „*Introduction to Fourier Optics*" – Goodman (2005), Roberts and Company Publishers
- „*The strange Theory of Light and Matter*" Feynmann (2006), Princeton University Press
- „*Introduction to optical Microscopy*" Mertz (2008), Roberts & Company Publishers
- „*Handbook of Biological Confocal Microscopy*" Pawley (2006), Springer
- „*Fundamentals of Photonics*" Saleh, Teich (2007), Wiley
- „*Optics*" Hecht (2002), Addison Wesley
- „*Principles of Nano-Optics*" Novotny, Hecht (2012), Cambridge University Press
- „*Handbook of Optical Systems*, Vol.2, Physical Image Formation" Gross, Totzeck, Singer (2005), Wiley-VCH
- „*Principles of Optics*" Born, Wolf (1980), Cambridge University Press
- „*Laser – Bauformen, Strahlführung, Anwendungen*" Eichler, Eichler (2015), Springer Vieweg, > Only in German!
- „*Lasers*" Siegman (1986), University Science Books

This script can be found at: `https://sites.google.com/site/nanoimagingproject/teaching/ws-2016-17/light-microscopy`

Nomenclature

Variables

Roman variables

Short notation	*Long notation*	*Physical quantity*	*Unit*
$\mathrm{ASF}(\mathbf{r})$	$\mathcal{F}^{-1}\{\mathrm{ATF}(\mathbf{k})\}$	amplitude spread function	
$\mathrm{ATF}(\mathbf{k})$	$\mathcal{F}\{ASF(\mathbf{r})\}$	amplitude transfer function	
$\mathbf{B}$	(B_x, B_y, B_z)	magnetic field	$\frac{A}{m}$
c	$\frac{1}{\sqrt{\epsilon_0\mu_0}}$	speed of light in vacuum	$\frac{m}{s}$
$\mathbf{E}$	(E_x, E_y, E_z)	electric field	$\frac{V}{m} = \frac{N}{C}$
$h(\mathbf{r}) = \mathrm{PSF}(\mathbf{r})$	$h = (\mathrm{ASF})(\mathrm{ASF})^*$	(intensity) point spread function	
$H(\mathbf{k}) = \mathrm{OTF}(\mathbf{k})$	$\mathcal{F}\{\mathrm{PSF}(\mathbf{k})\}$	optical transfer function	
I		irradiance (sloppy: intensity)	$\frac{W}{cm^2}$
$\mathbf{k}$	(k_x, k_y, k_z)	wave vector	$(\frac{1}{m}, \frac{1}{m}, \frac{1}{m})$
k_0	$k_0 = \lvert\mathbf{k}\rvert$	wave vector	$\frac{1}{m}$
n	$\sqrt{\epsilon_r\mu_r}$	index of refraction	
$\mathbf{r}$	x, y, z	spatial coordinates	
t		time	s
T		time of oscillation	s
$\mathbf{u}$	(u_x, u_y, u_z)	vectorial amplitude	
u		scalar amplitude	
x, y, z		Cartesian space coordinates	

Greek variables

Short notation	*Long notation*	*Physical quantity*	*Unit*
ν	$\frac{1}{T}$	frequency	$\mathrm{Hz} = \frac{1}{s}$
ω	$\frac{2\pi}{T} = 2\pi\nu$	angular frequency	$\frac{1}{s}$
φ		angle	rad
α		aperture angle	rad
λ	$\frac{c}{\nu}$	vacuum wavelength	nm
ϵ_0		vacuum electrical permittivity	
ϵ_r		relative electrical permittivity	
μ_r		relative magnetic permittivity (usually 1)	
$\chi(\omega)$	$\epsilon_r(\omega) - 1$	electric susceptibility	
ρ		spatial density	s

Mathematical operators

For $\mathbf{u} = (u_1, u_2, u_3) : \mathbb{R}^3 \to \mathbb{R}^3$

f^*		Complex Conjugate	$\mathfrak{Re}(f) - \mathfrak{Im}(f) \cdot i$
$\vec{\nabla}$		Nabla	$\left(\frac{\partial}{\partial x}, \frac{\partial}{\partial y}, \frac{\partial}{\partial z}\right)$
$\vec{\nabla} u$	$\textbf{grad } u$	Gradient	$\left(\frac{\partial}{\partial x}, \frac{\partial}{\partial y}, \frac{\partial}{\partial z}\right) \mathbf{u} = \left(\frac{\partial u}{\partial x}, \frac{\partial u}{\partial y}, \frac{\partial u}{\partial z}\right)$
$\vec{\nabla} \cdot \mathbf{u}$	div $\mathbf{u}$	Divergence	$\left(\frac{\partial}{\partial x}, \frac{\partial}{\partial y}, \frac{\partial}{\partial z}\right) \cdot \mathbf{u} = \frac{\partial u_x}{\partial x} + \frac{\partial u_y}{\partial y} + \frac{\partial u_z}{\partial z}$
$\vec{\nabla} \times \mathbf{u}$	$\textbf{curl u}$	Rotation	$\left(\frac{\partial u_3}{\partial y} - \frac{\partial u_2}{\partial z}, \frac{\partial u_1}{\partial z} - \frac{\partial u_3}{\partial x}, \frac{\partial u_2}{\partial x} - \frac{\partial u_1}{\partial y}\right)$
			related to the determinant of the Jakobian
$\nabla^2 \mathbf{u}$		Laplacian	$\frac{\partial^2 u_x}{\partial x^2} + \frac{\partial^2 u_y}{\partial y^2} + \frac{\partial^2 u_z}{\partial z^2}$
$\mathcal{F}$		Fourier transformation	$\mathcal{F}\{u(\mathbf{r})\}(\mathbf{k}) = \frac{1}{(2\pi)^{\frac{n}{2}}} \int_{\mathbb{R}^n} u(\mathbf{r})\, e^{-\mathrm{i}\mathbf{k}\cdot\mathbf{r}}\, \mathrm{d}\mathbf{r}$
			n is the dimension
$\mathcal{F}^{-1}$		Inverse Fourier transformation	$\mathcal{F}^{-1}\{u(\mathbf{k})\}(\mathbf{r}) = \frac{1}{(2\pi)^{\frac{n}{2}}} \int_{\mathbb{R}^n} u(\mathbf{k})\, e^{\mathrm{i}\mathbf{k}\cdot\mathbf{r}}\, \mathrm{d}\mathbf{k}$
$f \otimes g$		Convolution	$f(\mathbf{r}) \otimes g(\mathbf{r}) = \int_{\mathbb{R}^n} f(\mathbf{r}') g(\mathbf{r} - \mathbf{r}')\, \mathrm{d}\mathbf{r}$
$f \star g$		Crosscorrelation	$f(\mathbf{r}) \otimes g(\mathbf{r}) = \int_{\mathbb{R}^n} f^*(\mathbf{r}') g(\mathbf{r} + \mathbf{r}')\, \mathrm{d}\mathbf{r}$

Calculation rules

$\vec{\nabla} \times (\vec{\nabla} \times \mathbf{u})$	$= \vec{\nabla}(\vec{\nabla} \cdot \mathbf{u}) - (\vec{\nabla} \cdot \vec{\nabla})\mathbf{u}$	Vector triple product
k_0^2	$= k_x^2 + k_y^2 + k_z^2$	Pytagoras
$\mathcal{F}\{u(\mathbf{r})\}(\mathbf{k})$	$= \frac{1}{(2\pi)^{\frac{n}{2}}} \int_{\mathbb{R}^n} u(\mathbf{r})\, e^{-\mathrm{i}\mathbf{k}\cdot\mathbf{r}}\, \mathrm{d}\mathbf{r}$	Fourier transformation

$\mathbf{k}$ is called the spatial frequency.