Light Microscopy
Recapitulation

January 21, 2019

The lecture provided you with a basic knowledge about thinking the Fourier-way and modern microscopy technology. The aim was to give you a tool for understanding new progresses in the vast field of light microscopy with ease. To substantiate this basement here is a set of questions to sum up what you have learned.

(i) Name Maxwell's equations in the presence of a source-containing material. Derive the Wave-equation out of it. What is the meaning of the Helmholtz-equation in case of a homogeneous medium?

(ii) Remember two definitions of the Fourier-transformation.

  (a) Name the space-scaling property of the Fourier-transform.

  (b) Fourier-slice theorem? Parseval-theorem? Convolution-theorem?

  (c) What is the difference between convolution, cross-correlation and auto-correlation?

(iii) Light propagation in Fourier-terms.

  (a) What is the McCutchen pupil? Why is this sufficient for describing far-field optics? What in the near-field case? How can in be used in vectorial optics?

  (b) Explain how free-space propagation of light along the axial z-direction can be described mathematically in our Fourier-formalism. How would you introduce aberrations in this formalism?

  (c) What is the Fresnel approximation with respect to the McCutchen pupil? What are benefits/disadvantages?

  (d) How does the refractive index relate to scattering of molecules?

  (e) What does the continuity of the phase of waves between to media mean to the associated k-spheres in Fourier-space?

  (f) Derive the propagation-direction of evanescent waves. Describe those waves in Real- and in Fourier-space.

(iv) Remember, that a lens is a fast Fourier-transformer.

  (a) Explain how and why the Gaussian reference-sphere is introduced for describing high-NA lens-properties in geometrical optics. Additionally: How to ensure the Hershel-Condition?

  (b) What is the aplanatic factor? Make sure to know how it was derived in Fourier-theory.

  (c) What does energy conservation on interfaces mean to polarisation? What is the influence of polarisation at high-NA?

(v) What is the Nyquist limit? Explain in terms of our Fourier-theory: Over-sampling, Under-sampling and aliasing.

(vi) Scattering

  (a) Describe how to derive the set of propagating waves in 1st-order Born approximation in case of a thin and a thick grating. Explain the Ewald-sphere and the corresponding vectors. Derive the Bragg-condition from the scattering law. Use this insight to find Abbe's law for straight and oblique illumination.

  (b) The introduction of an absorption cross section is used in various theories to describe stochastical interaction probabilities of particles with their environment. Why is there a limit to this notion in case of absorption?

(c) Draw the contributing object frequencies and scatter-vectors in case of incoherent imaging with different illumination and detection pupil functions. Now construct the resulting accessible k-range of the k-space object by convolving k_{illu} and k_{det}.

(d) Repeat the same process for an incoherent transmission imaging as well as an confocal transmission imaging. What is the benefit of such a confocal to holography in terms of object-frequency support? Explain with a sketch of a confocal transmission microscope how to derive an equation for the detected intensity at the PMT.

(vii) Explain how holography manages to capture amplitude information, even though the detector can only see intensity. What information is really contained in a holography image? How to computationally reconstruct the amplitude of the object from the holography-image?

(viii) Explain what is meant by: Time-Domain OCT (TD-OCT), Fourier-Domain OCT (FD-OCT) and Swept-Source OCT (SS-OCT)?

(ix) The incoherent side of things

(a) What is a PSF? Is a PSF a useful/possible description for coherent imaging? Find arguments for and against this. Give the link of the PSF to the OTF. How do you interpret the OTF in the coherent case?

(b) Explain the Defocus in incoherent imaging starting with a 2D in-focus OTF. What happens to the contrast?

(c) Draw the OTF-support for: a confocal-Fluorescence microscope, $4Pi$ microscope, I^5M microscope and a basic SIM-system.

(d) Explain the idea behind the Airy-Scanning (image scanning microscopy = ISM) System. What is the key-notion behind the UZI?

(e) How is the super resolution achieved in case of STED and PALM? What are limits to this techniques?