

Light Microscopy

Problem Set 5

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1 3D Optical Functions

In attached diagram the main optical functions are summarized.
Explain each of them and how they are related.

- (a) Coherent transfer function or amplitude transfer function
- (b) Coherent spread function or amplitude spread function
- (c) Optical transfer function
- (d) Point spread function

2 3D Optical Transfer Functions of Fluorescent Microscopes

(a) Confocal Fluorescence Microscopy

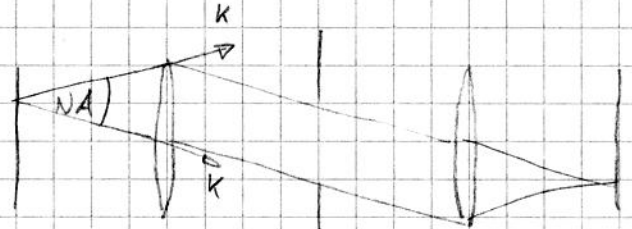
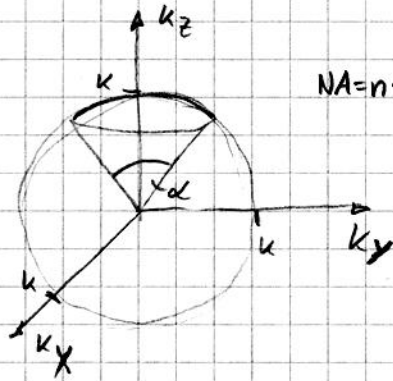
- (i) Explain why the optical transfer functions H_{Det} and H_{Em} are considered to be proportional in this imaging system. Using a graphical correlation ("paintbrush") draw the frequency support region of these functions.
- (ii) Construct, with a graphical correlation, the confocal region of support in the $k_x k_z$ -plane. Indicate the regions of higher density and explain the physical meaning of this distribution.

(b) I⁵M and 4Pi Microscopy

- (i) Illustrate a basic scheme of the optical system correspondent to these two techniques.
- (ii) Indicate the main difference between them.
- (iii) Draw the correspondent OTF support region in the $k_x k_z$ -plane and show the limiting frequencies.

Summarizing the functions of 3D imaging

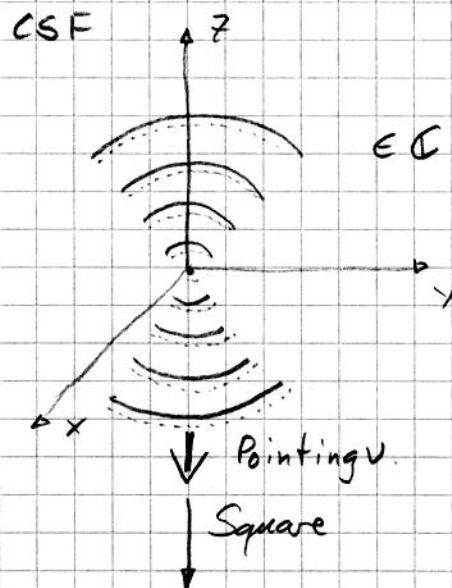
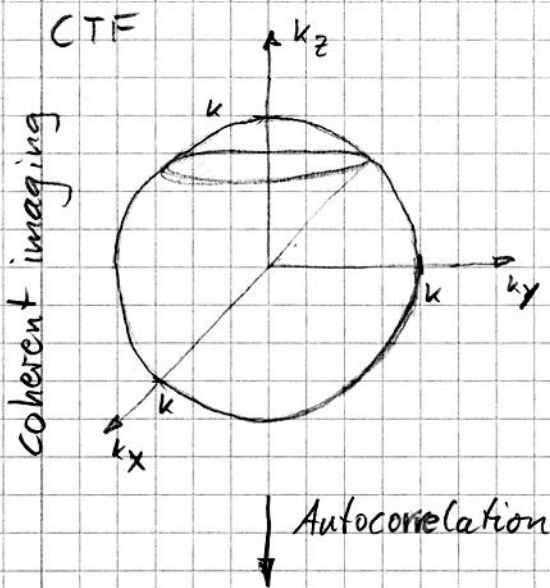
k-space / Ewald sphere



Fourierspace

Realspace

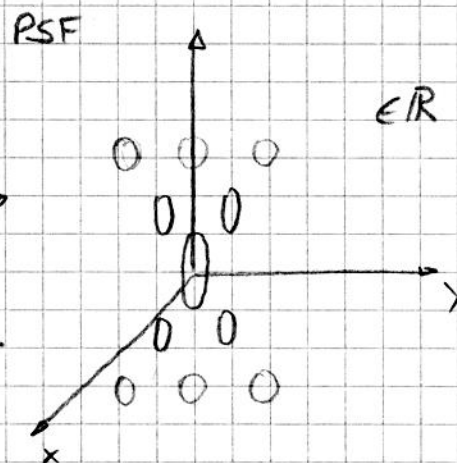
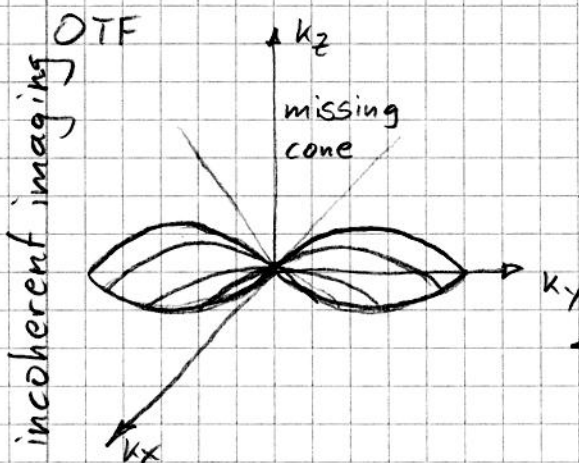
Image formation



$$E_{scatt}(\vec{r}) = E_i(\vec{r}) \cdot \delta h(\vec{r}) \otimes CSF(\vec{r})$$

$$|img(\vec{r})| = |E_i(\vec{r}) \cdot \delta h(\vec{r}) \otimes CSF(\vec{r})|^2$$

Illumin. Sample



$$|img(\vec{r})| = |h(\vec{r}) \cdot S(\vec{r}) \otimes PSF(\vec{r})|^2$$

Illumin. Sample