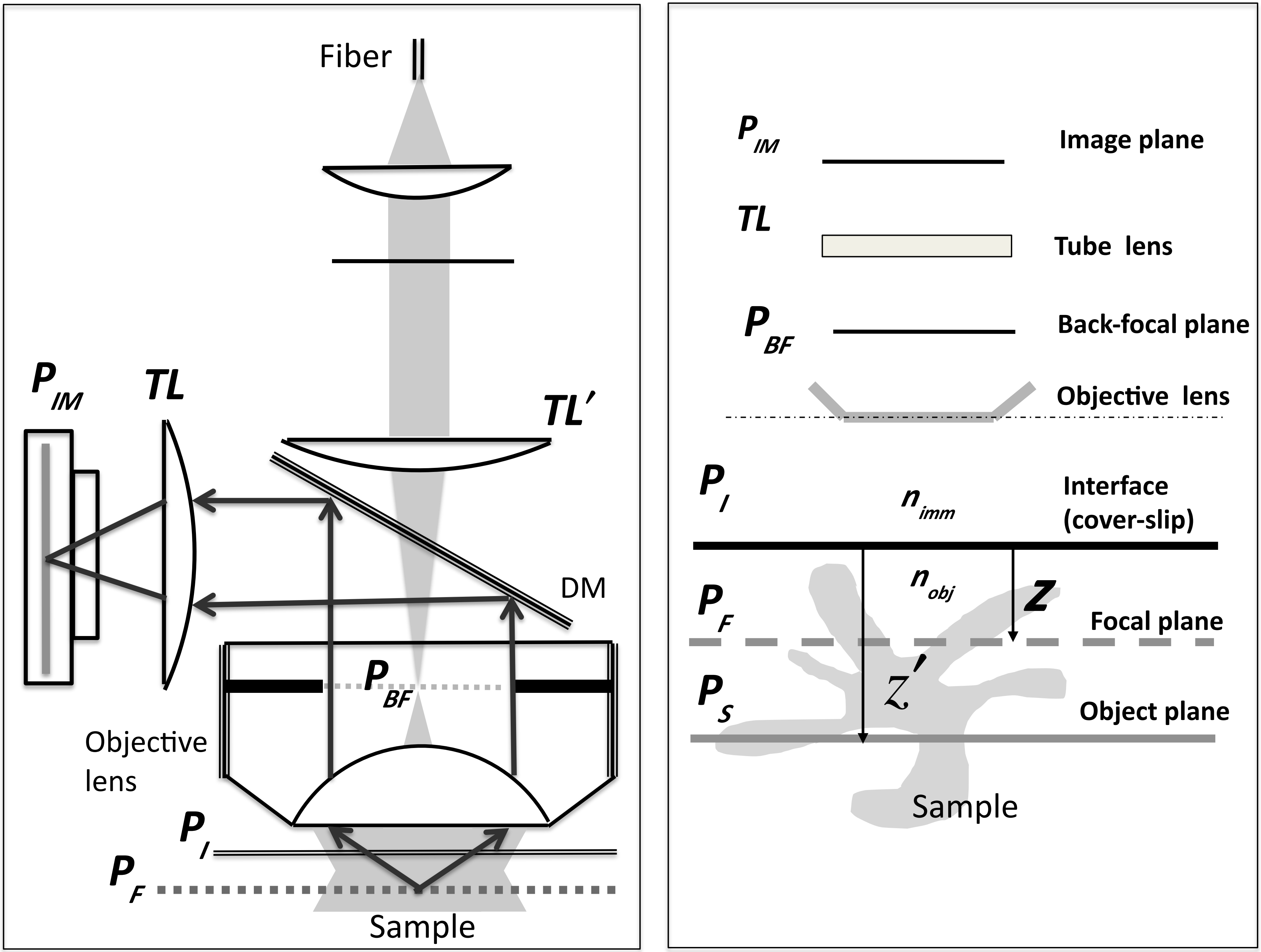
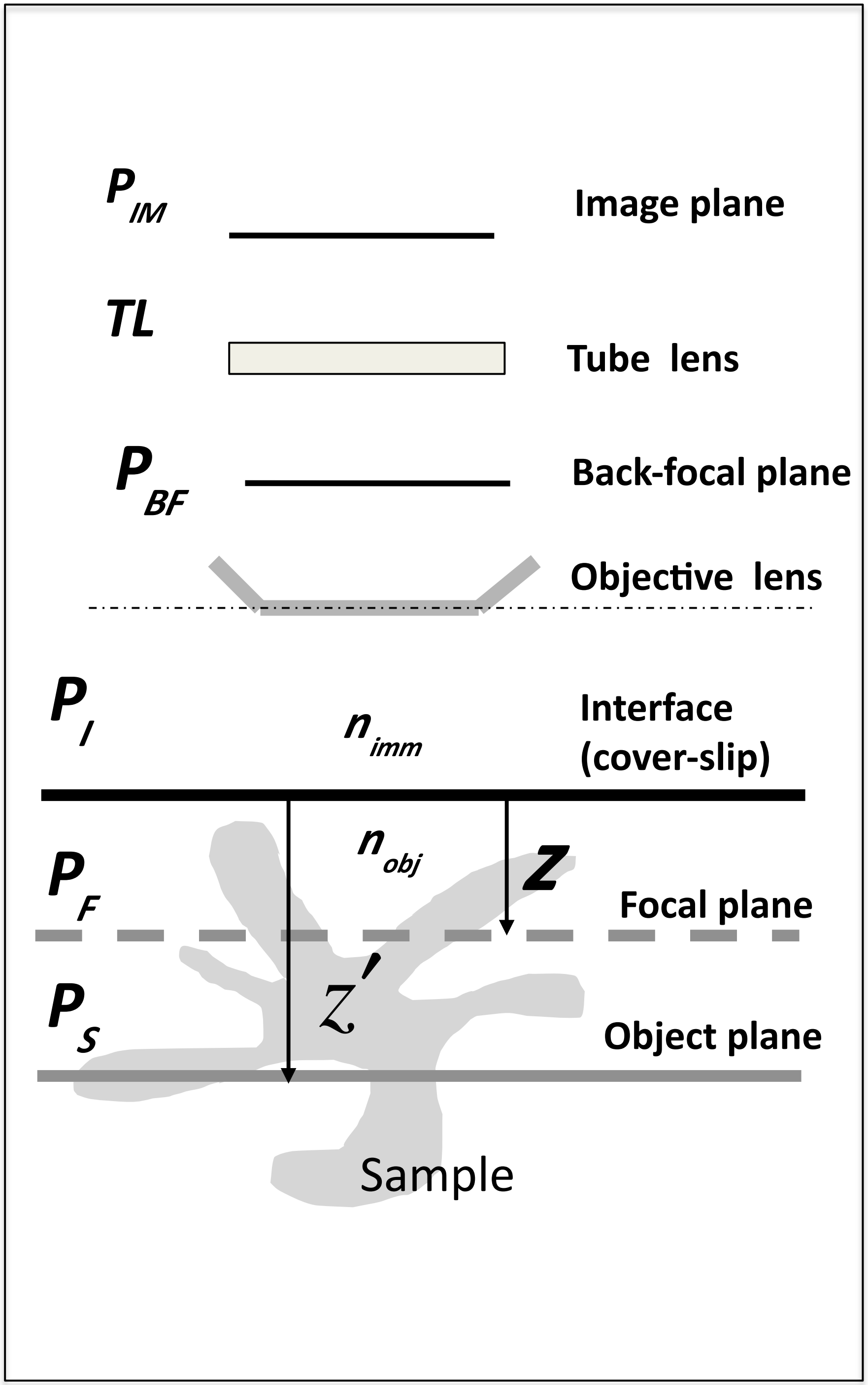
# E9 285 Biomedical imaging-Inverse problems

## Chapter 2.a
## Introduction to basic fluorescence microscopes

# The widefield microscope



Fiber
$P_{IM}$
$TL$
$TL'$
DM
$P_{BF}$
Objective
lens
$P_I$
$P_F$
Sample
$P_{IM}$
Image plane
$TL$
Tube lens
$P_{BF}$
Back-focal plane
Objective lens
$P_I$
$n_{imm}$
Interface
(cover-slip)
$n_{obj}$
$P_F$
$Z$
Focal plane
$z'$
$P_S$
Object plane
Sample




$S_a(x,y,z')$ : complex signal amplitude

$S(x,y,z') = |S_a(x,y,z')|^2$

(image to be measured)

$z$ : user depth variable

$z'$ : object depth variable

$W_{BF}(X,Y,z)$ : image at $P_{BF}$

$S_a(X,Y,z')$ : $xy$ Fourier transform of the signal.

$T(X,Y,z,z')$ : Transfer function

$$W_{BF}(X,Y,z) = \int_{z'} \begin{bmatrix} S_a(X,Y,z') \times \\ T(X,Y,z,z') \end{bmatrix} dz'$$

$$W_{BF}(X,Y,z)=\int_{z'}\left[S_a(X,Y,z')T(X,Y,z,z')\right]dz'$$

The measured image:

$$R(x,y,z)=\left|F_{xy}^{-1}\left[W_{BF}(X,Y,z)\right]\right|^2$$

$$=\int_{z'} g(x,y,z,z')\oplus_{xy} S(x,y,z')dz'$$

$$g(x,y,z,z')=\left|F_{xy}^{-1}\left[T(X,Y,z,z')\right]\right|^2$$

Assumption: $g(x,y,z,z')=g(x,y,z-z',0)$

Then

$$R(x,y,z)=\int_{z'} g(x,y,z-z',0)\oplus_{xy} S(x,y,z')dz'$$

$$= g_0(x,y,z) * S(x,y,z),$$

where $g_0(x,y,z)=g(x,y,z-z',0)$

$$g_0(x,y,z) = F_{xy}^{-1}\left[T_0(X,Y,z)\right]$$

$$T(X,Y,z) = A(X,Y)\exp(j2\pi Q(X,Y)) \times \exp\left[j\frac{2\pi nz}{\lambda}\sqrt{1-(\lambda X/n)^2-(\lambda Y/n)^2}\right]$$

$A(X,Y):$ aperture (normally circular)

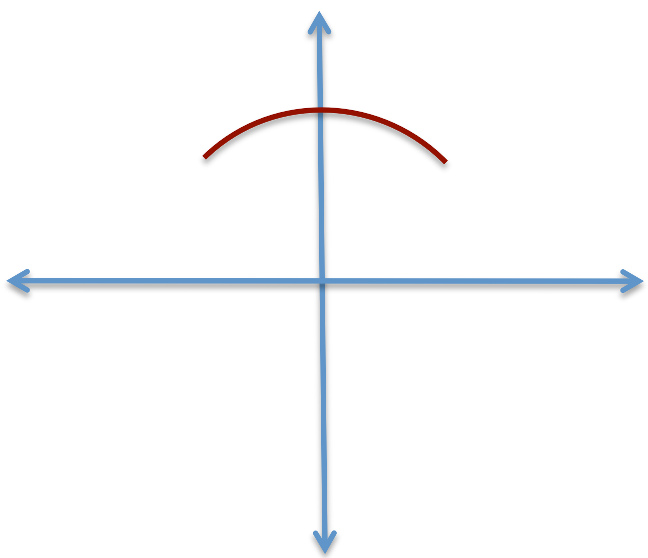
$Q(X,Y):$ smooth phase function

$\lambda:$ wavelength

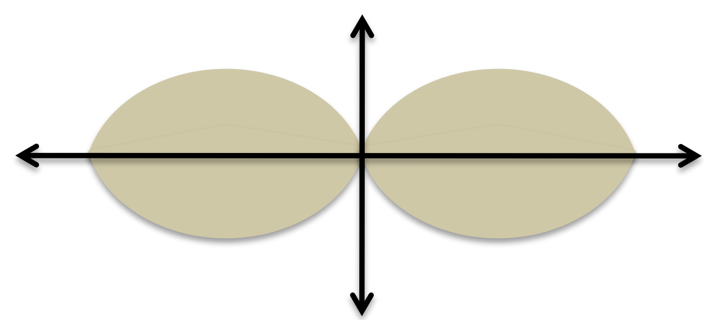
$n:$ refractive index of the medium below the object lens

$$T(X,Y,z) = A(X,Y)\exp(j2\pi Q(X,Y)) \times \exp\left[ j\frac{2\pi nz}{\lambda}\sqrt{1-(\lambda X/n)^2-(\lambda Y/n)^2} \right]$$

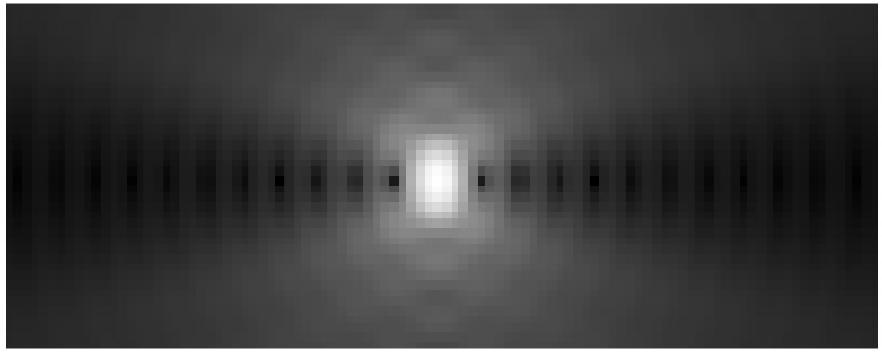
$T(X,Y,Z) = ?$



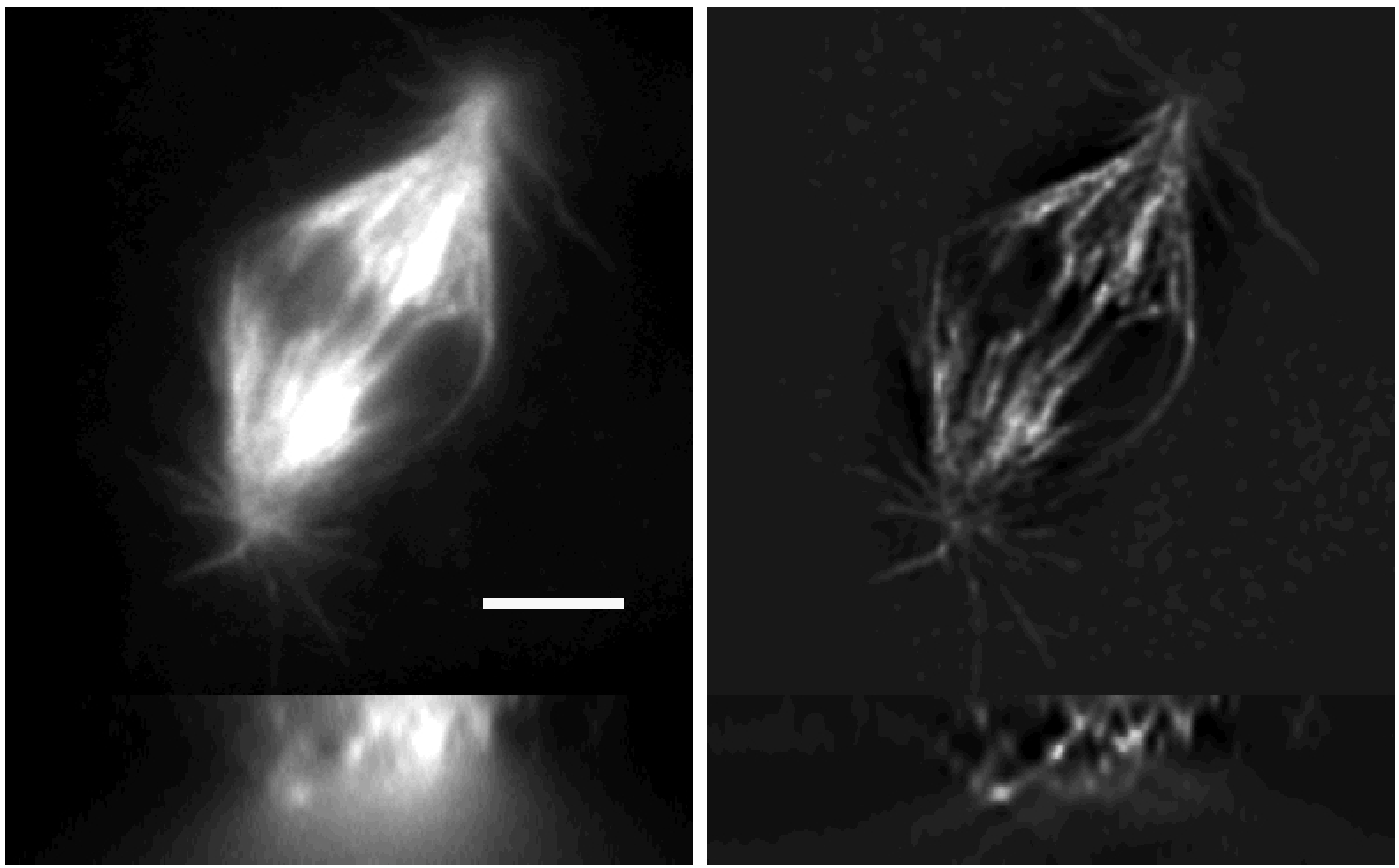
$g_0(X,Y,Z) = ?$



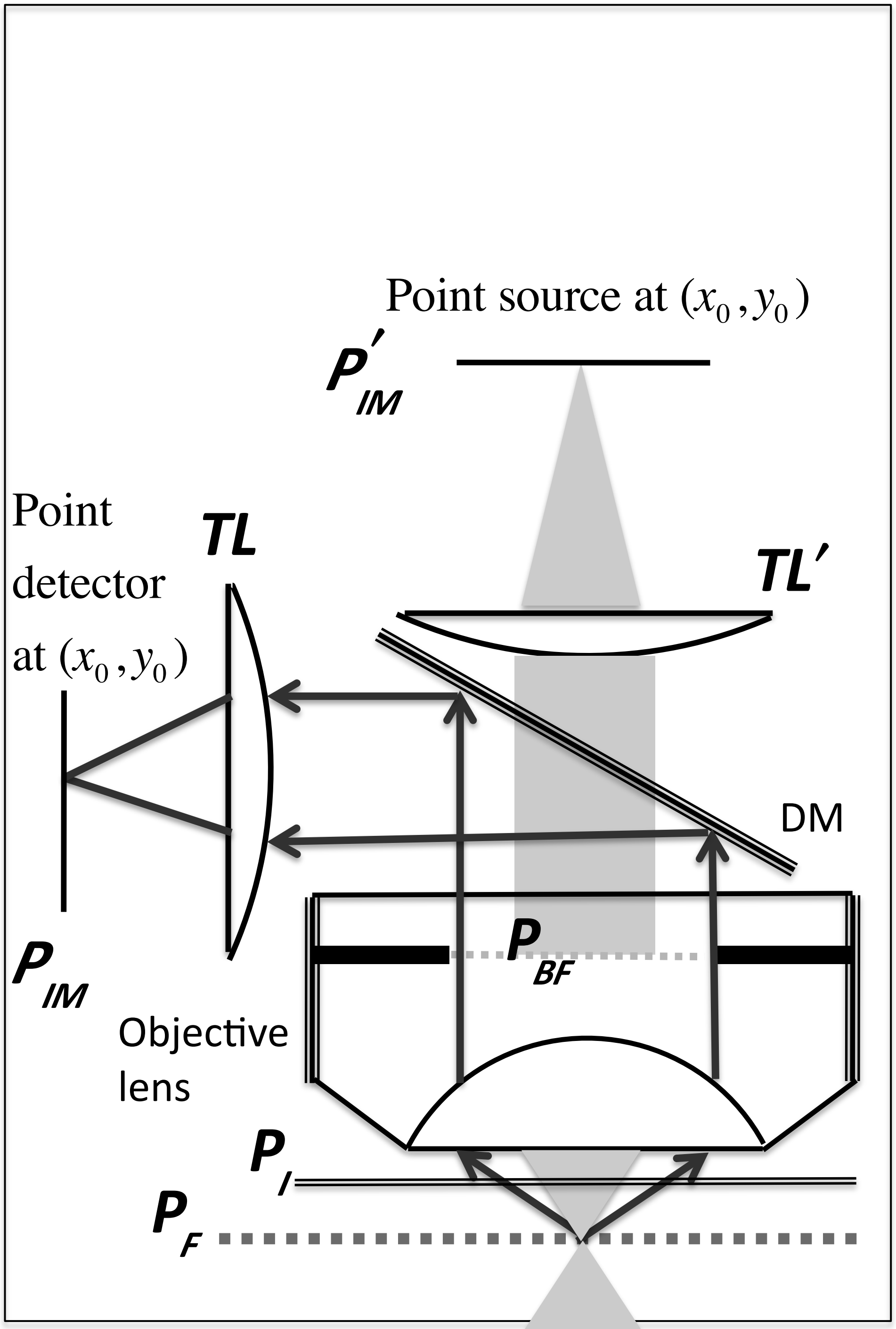
$g_0(x,y,z)$



An image example:



# The confocal microscope




$PSF$ from $P'_{IM}$ to sample space: $g'(x, y, z)$

$PSF$ from $P_{IM}$ to sample space: $g(x, y, z)$

Let the slide position be $z_0$

Fluorescence intensity at sample space:

$$F_I(x,y,z) = S(x,y,z)g'(x-x_0, y-y_0, z-z_0)$$

Intensity at detector point:

$$D(x_0, y_0, z_0) = \sum_{x,y,z} \begin{bmatrix} F_I(x,y,z) \\ \times g(x-x_0, y-y_0, z-z_0) \end{bmatrix}$$

$$= \sum_{x,y,z} \begin{bmatrix} S(x,y,z) \\ \times g'(x-x_0, y-y_0, z-z_0) \\ \times g(x-x_0, y-y_0, z-z_0) \end{bmatrix}$$

Hence

$$D(x,y,z) = S(x,y,z) * \left[ g(x,y,z)g'(x,y,z) \right]$$

# E9 285 Biomedical imaging-Inverse problems

## Chapter 2.a
## Representative reconstruction methods

# Tikhonov inverse filtering

## Recap: P-MLE for Gaussian

$$\mathbf{x}_{opt} = \underset{\mathbf{x}}{\arg\min} \left[ \sum_{\mathbf{r}\in[1:N]^D} (\mathbf{v}_{\mathbf{r}}^T \mathbf{x} - d_{\mathbf{r}})^2 + \lambda J_R(x) \right]$$

In terms of convolution:

$$x_{opt}(\mathbf{r}) = \underset{x(\mathbf{r})}{\arg\min} \left[ \underbrace{\sum_{\mathbf{r}'\in[1:N]^D} ((h * x)(\mathbf{r}') - D(\mathbf{r}'))^2}_{J_e(x)} + \lambda J_R(x) \right]$$

$d_{\mathbf{r}'} = D(\mathbf{r}'), \quad \mathbf{v}_{\mathbf{r}'}$ : scanned vector from $h(\mathbf{r} - \mathbf{r}')$

$J$ is quadratic in Tikhonov filtering:

$$J_R(x) = \sum_{\mathbf{r}'} \left( \sum_{j=1}^{N_F} \left( (L_j * x)(\mathbf{r}') \right)^2 \right)$$

First consider the data term:

$$J_e(x) = \sum_{\mathbf{r}} \left((h*x)(\mathbf{r}')\right)^2 + \sum_{\mathbf{r}} D^2(\mathbf{r}') - 2\sum_{\mathbf{r}} (h*x)(\mathbf{r}')D(\mathbf{r}')$$

Consider the individual terms:

$$\sum_{\mathbf{r}} \left((h*x)(\mathbf{r}')\right)^2 = \langle h*x, h*x\rangle = \left\langle x, h^T * h * x\right\rangle, \quad \text{where} \quad h^T(\mathbf{r}) = h(-\mathbf{r})$$

$$\sum_{\mathbf{r}} (h*x)(\mathbf{r}')D(\mathbf{r}') = \langle h*x, D\rangle = \left\langle x, h^T * D\right\rangle$$

Hence we get

$$J_e(x) = \left\langle x, h^T * h * x\right\rangle - 2\left\langle x, h^T * D\right\rangle + \langle D, D\rangle$$

$$J_R(x) = \left\langle x, h^T * h * x\right\rangle$$

Total cost: $J_e(x) + \lambda J_R(x)$

With respect to the terminology of quadratic forms:

$\mathbf{Q}$ : matrix equivalent of $h^T * h * x + \lambda \sum_{j=1}^{N_F} L_j^T * L_j * x$

$\mathbf{b}$ : scanned vector of the image $\left(h^T * D\right)(\mathbf{r})$

$c$ : $\langle D, D \rangle$

To get the gradient of $J_e(x)$:

Evaluate $\displaystyle \lim_{\alpha \to 0} \frac{J_e(x+\alpha y) - J_e(x)}{\alpha} = \langle y, u \rangle.$

$u$ will be the gradient.

$$\nabla J_e(\mathbf{r}) = \left(h^T * h * x\right)(\mathbf{r}) - \left(h^T * D\right)(\mathbf{r})$$

$$\nabla J_R(\mathbf{r}) = \sum_{j=1}^{N_F} \left(L_j^T * L_j * x\right)(\mathbf{r})$$

The required solution is given by

$\nabla J_e(\mathbf{r}) + \lambda \nabla J_R(\mathbf{r}) = 0$

$$\left(h^T * h * x\right)(\mathbf{r}) + \lambda \sum_{j=1}^{N_F} \left(L_j^T * L_j * x\right)(\mathbf{r}) = \left(h^T * D\right)(\mathbf{r})$$

# Assignment 2:Tikhonov filtering on simulated data

1) Choose a biomedical image
2) Write a program for Gaussian blurring with a possibility of adding different levels of Gaussian and Poisson noise after blurring
3) Write a program for Tikhonov filtering with $\lambda$ as an input
4) Write a program to compute

$$C(\eta) = \frac{\left\langle \widehat{x}_o, \widehat{x}_d \right\rangle_{\|\omega\|=\eta}}{\sqrt{\left\langle \widehat{x}_o, \widehat{x}_o \right\rangle}_{\|\omega\|=\eta} \sqrt{\left\langle \widehat{x}_d, \widehat{x}_d \right\rangle}_{\|\omega\|=\eta}}$$

where $\widehat{x}_o$ and $\widehat{x}_d$ are the Fourier transforms of the original and deconvolved images

5) Plot $(\eta, C(\eta))$ for different values of $\lambda$, for a given Gaussian noise level
6) Repeat 5 for Poisson noise
7) Plot $(\eta, C(\eta))$ for different Gaussian noise levels with correct value of $\lambda$
8) Repeat 7 for Poissons noise.

# Iterative constrained Tikhonov Miller method (ICTM)

[1] H.T.M. van der Voort and K.C. Strasters, "Restoration of confocal images for quantitative image analysis," *J. Microsc., vol. 178, no. 2, pp. 165–181, 1995.*

[2] G.M.P. van der Kempen, L.J. van Vliet, P.J. Verveer, and H.T.M. van der Voort, "A quantitative comparison of image restoration methods for confocal microscopy," *J. Microsc., vol. 185, no. 3, pp. 354–365, 1997.*

# ICTM

1) $\{L_1, ..., L_{N_F}\}$ : first derivative filters

2) Implements steepest descent and conjugate gradient algorithm where the gradient is clipped for negative values at each step.

# Assignment 3

Write program to compute $\nabla J_e(\mathbf{r}) + \lambda \nabla J_R(\mathbf{r})$ for Tikhonov filtering problem.
Implement regularization filters of real space