BE-OPTICAL
Advanced Biomedical Optical Imaging and Data Analysis

Image Reconstruction of the Image Scanning Microscopy (ISM)

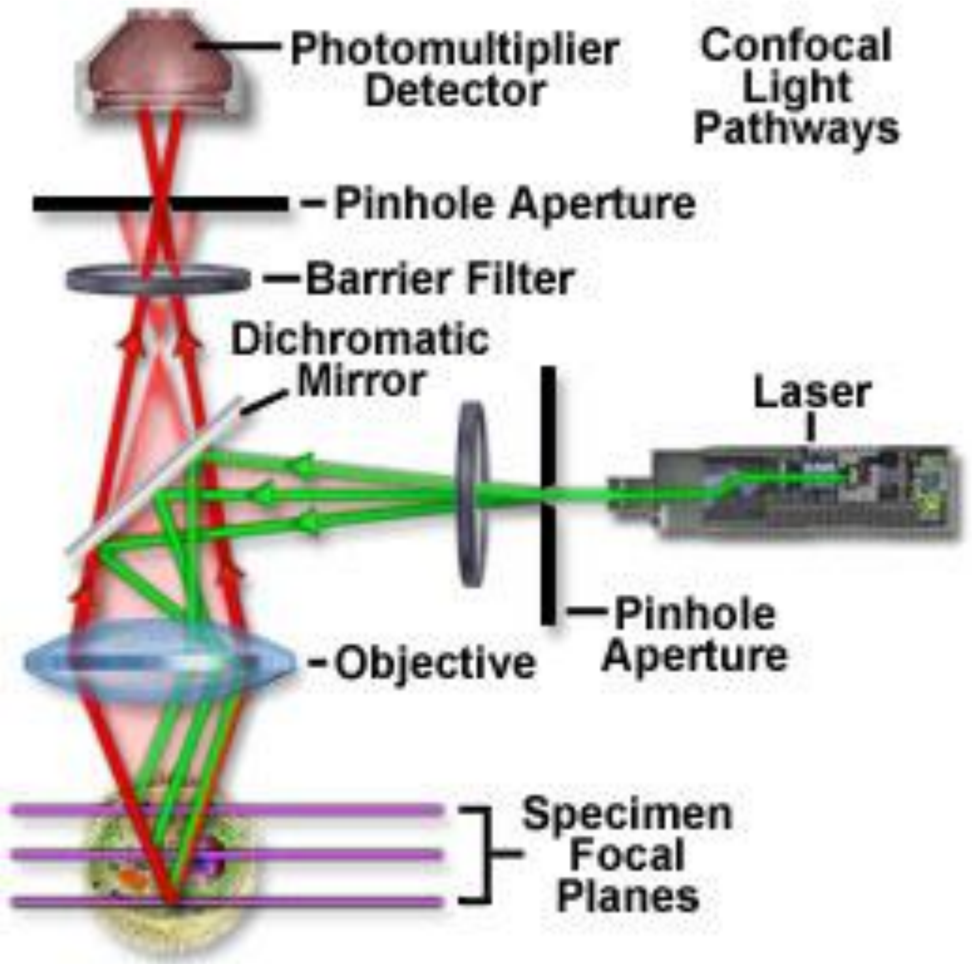
Shun Qin

III. Institute of Physics
Georg-August-University Göttingen

Review of Confocal Microscope

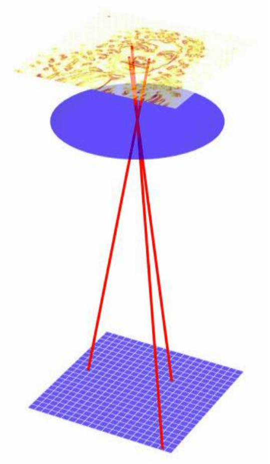
Confocal Scanning Syster

I. z-sectioning
II. low resolution



http://fluoview.magnet.fsu.edu/theory/index.html

Principle of ISM Microscopy



I. Scanning system based: general and easy to implement

II. Image each focal spot rather than single intensity at each scanned position: more information

III. Image reconstruction: simple, robust

Image Reconstruction-Theoretical

I. Let $U(s, r')$ PSF of optical imaging system, $P(r')$ the illumination funcition of laser scanning beam, $S(r')$ the sample function, where r the scaning positon, r' the space coodinate of sample, s the CCD coodinate; take the symetric of laser illumination function $P(r'-r) = P(r-r')$ into account, we have

$$I(r,s) = \int U(s+r-r')P(r-r')S(r')dr'$$

II. The optimal choice is let $r = r - \frac{s}{2}$, integrate $I(r-\frac{s}{2}, s)$ over s, then we have

$$I(r) = \int U\left(r+\frac{s}{2}-r'\right)P\left(r-\frac{s}{2}-r'\right)S(r')dr'$$

which indicates that the PSF function of ISM have the form of

$$U_{ISM}(r) = \int U\left(r+\frac{s}{2}\right)P\left(r-\frac{s}{2}\right)ds$$

IV. In order to have a deep insight to the new PSF, let us perform Fourier transform to $U_{ISM}(r)$ and then we can get the OTF function

$$U(q, k_z) = 2\pi \int U\left(\frac{q}{2}, k_z - k_z'\right)P\left(\frac{q}{2}, k_z'\right)dk_z$$

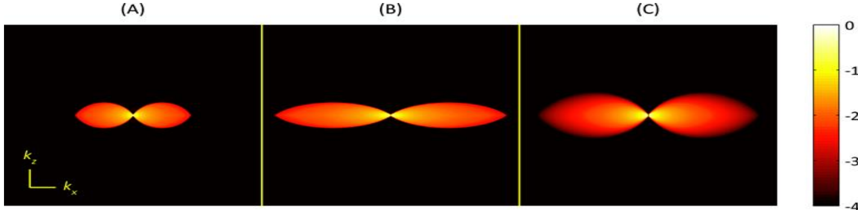
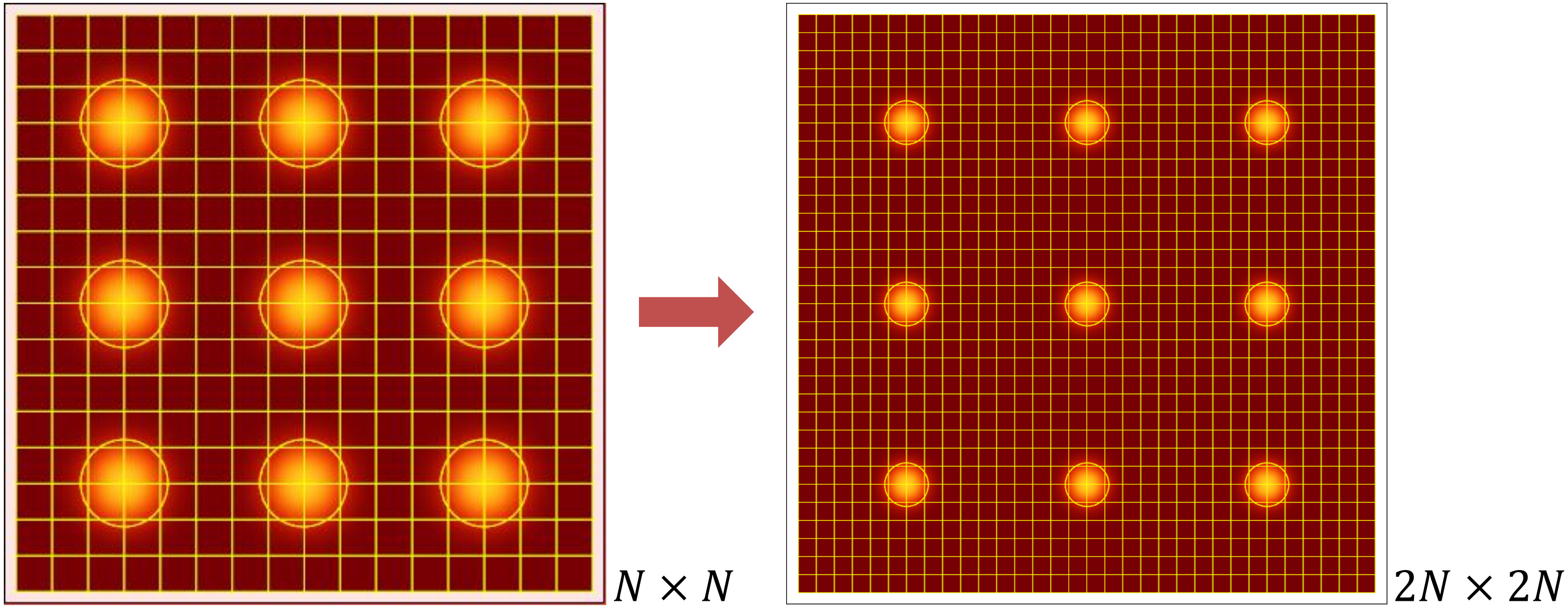


Image Reconstruction-Technological



The image reconstruction algorithm can be described as :

$$I_{ism}\left(2y-\frac{w}{2}:2y+\frac{w}{2},2x-\frac{w}{2}:2x+\frac{w}{2}\right) += I_{raw}\left(y-\frac{w}{2}:y+\frac{w}{2},x-\frac{w}{2}:x+\frac{w}{2}\right)$$

where (x, y) is the localized positition from the reference raw image and w the width of light spot.

Retrieve the True OTF by Fourier Reweighting

I. Notice the proximity between PSF and illumination function, reconsider the OTF of ISM image

SDCM

$$\widetilde{U}_{ISM}(\boldsymbol{q}) = \widetilde{U}\left(\frac{\boldsymbol{q}}{2}\right)\widetilde{P}\left(\frac{\boldsymbol{q}}{2}\right) \approx \widetilde{D}^2(\frac{\boldsymbol{q}}{2})$$

II. However, the true PSF for a super-resolution image should be $\widetilde{D}\left(\frac{\boldsymbol{q}}{2}\right)$, so reconstructing the OTF of ISM image is necessary.

III. We can define a weight function at Fourier domain

ISM

$$W(\boldsymbol{q}) = \frac{1}{\widetilde{D}(\frac{\boldsymbol{q}}{2}) + \varepsilon}$$

the final super-resoluition image can then be calculated by

$$I_{sup} = F^{-1}\left[F\left(I_{ism}\right) \cdot W(\boldsymbol{q})\right]$$

Data Analysis Procedure

I. Precisely localize each scanning position with raw image of reference sample
II. Copy each imaged light spot and paste them to a up-sampled pixel array
III. Sum up each reconstructed frames up
IV. Do Fourier Reweighting to the ISM image

Maximum Likelihood based localization

The localization can be realized by fitting the measured data, say z, to the 2D Gaussian function model. The 2D Gaussian function can be written as

$$\lambda(\theta) = \ Ae^{-\frac{(x-a)^2+(y-b)^2}{2\sigma^2}} + C,$$

where $\theta = (A, a, b, \sigma, C)$ is the parameters vector of Gaussian function. According to the Poisson noise model, there exists

$$P(z|\lambda) = \ \frac{{\lambda_i}^{z_i}}{z_i} e^{-\lambda_i}, \ \ i = 1,2, \dots, n$$

The Maximum-Likelihook function can be written as

$$L(\lambda|z_1, z_2, \dots, z_n) = log \prod_i \frac{{\lambda_i}^{z_i}}{z_i!} e^{-\lambda_i} = \sum_i (z \ln \lambda - \lambda) - \sum_i \ln(z_i!)$$

Ignoring the last term, the object function can be written as

$$\theta = \underset{\theta}{\operatorname{argmin}}[-L(\lambda)] = \sum_i (\lambda_i - z_i \ln \lambda_i)$$

The derivative of object function can be written as

$$f'(\theta) = \sum \lambda' (1 - \frac{z}{\lambda})$$

To solve aboved function, the very powerful nonlinear optimization method: BFGS algorithm with a effiecient line-searching method, is used for its robustness and efficientcy.

Secondments

Finished：PicoQuant, 14.08.2017-15.09.2017

Next one: The University of St Andrew

Topic: Deconvolution algorithm for light-sheet microscopy

Thank you for your attention!

Göttingen Graduate School for Neurosciences, Biophysics, and Molecular Biosciences(GGNB)

BE-OPTICAL