CENTER for BIOPHOTONICS SCIENCE and TECHNOLOGY

Nonlinear Structured Illumination Microscopy

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Fluorescence Microscopy

Image observed under the microscope be described by:

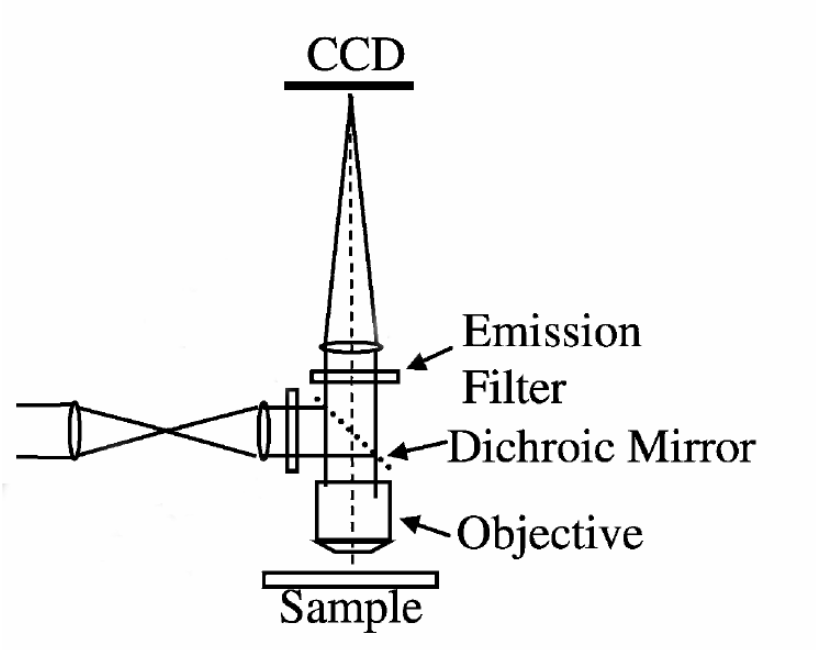
$$E(r) = H(r) \otimes \{I(r) \bullet D(r)\}$$

Image on CCD

Point Spread Function

Excitation Intensity

Fluorophore Density

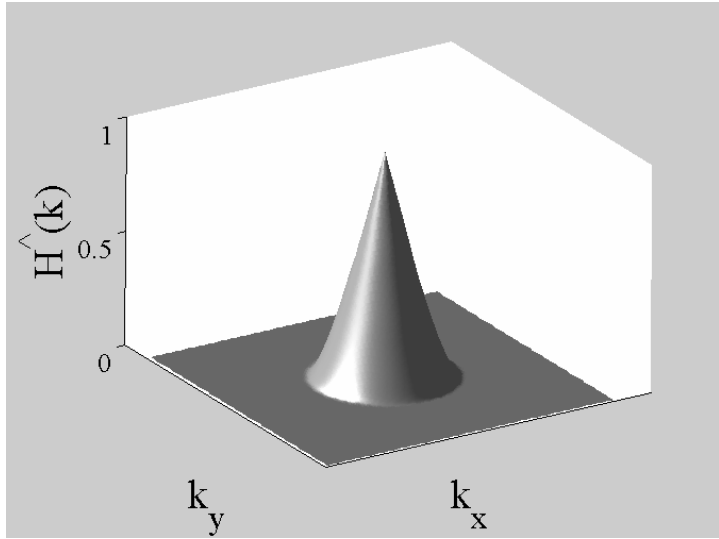


In reciprocal space the observed image is:

$$\hat{E}(k) = \hat{H}(k) \bullet \{\hat{I}(k) \otimes \hat{D}(k)\}$$

($\hat{E}(k)$ denotes F.T. of $E(r)$)

Resolution limit is given by the support of $\hat{H}(k)$ inside $k_{obs} = \frac{2NA}{\lambda}$



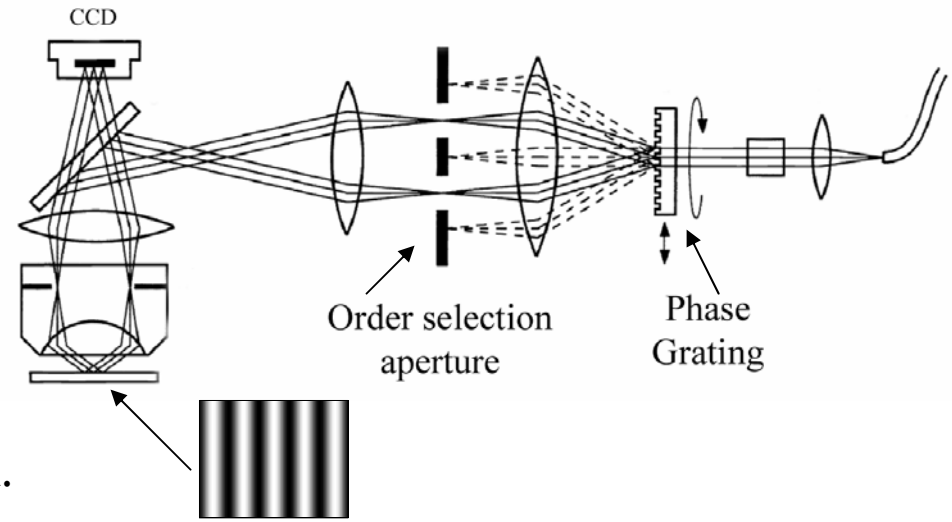
How can we overcome the resolution limit?

We can use spatially varying (structured) illumination I(r)*!

Use sinusoidal pattern in the lateral direction:

$$I(r) = 1 + \cos\left(k_0 r + \varphi\right)$$

where k_0 is wave vector of illumination pattern.



* M.G.L Gustafsson, D.A. Agard, J.W. Sedat Proceedings SPIE v.3919(2000)

In Fourier Space: $$\hat{I}(k) = \delta(k) + \frac{1}{2}\left[\delta(k+k_0)e^{\iota\phi} + \delta(k-k_0)e^{-\iota\varphi}\right]$$

Using $$\hat{E}(k) = \hat{H}(k) \bullet \left\{\hat{I}(k) \otimes \hat{D}(k)\right\}$$

We now observe:

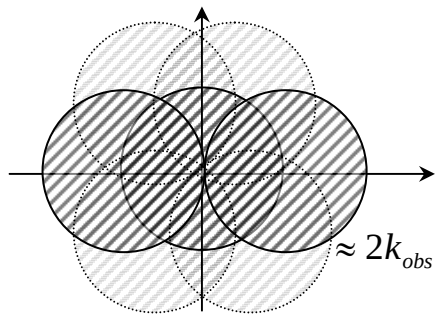
$$\hat{E}(k) = \hat{H}(k) \bullet \{\hat{D}(k) + \frac{1}{2}\hat{D}(k-k_0)e^{-i\varphi} + \frac{1}{2}\hat{D}(k+k_0)e^{i\varphi}\}$$

Image reconstruction:

1. Separate $\hat{D}(k), \hat{D}(k-k_0), \hat{D}(k+k_0)$ from three images with different φ by solving a linear equation.

2. Put $\hat{D}$ in correct place in the k-space.

3. Inverse Fourier transform to obtain D(r).



Effective observable region in reciprocal space

We can almost double the resolution with structured illumination.

Can we obtain even higher resolution?

Suppose fluorescent emission depends non-linearly on the excitation:

$$E(r) = H(r) \otimes \{F[I(r)] \cdot D(r)\}$$

Expand $F[I(r)] = \sum_n a_n (I\ \ (r))^n$

If I(r) is sinusoidal then in reciprocal space: $\hat{F}[I(k)] = \sum_n c_n \delta(k + n \cdot k_0) e^{in\varphi}$

Then we obtain

$$\hat{E}(k) = \hat{H}(k)\{\hat{F}[I[k]] \otimes \hat{D}(k)\} = \hat{H}(k)\{\sum_n c_n \hat{D}(k + n \cdot k_0) e^{in\varphi}\}$$

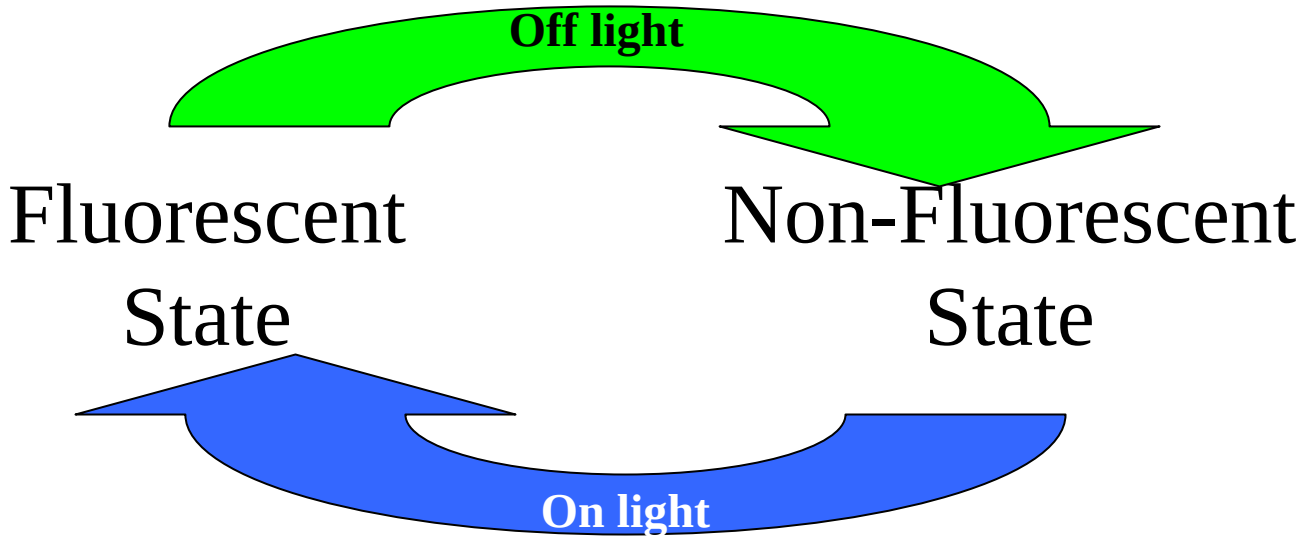
No hard limit on the obtainable resolution.

In reality, $c_n \to 0$ for large n. In the presence of noise this limits the resolution.

Possible non-linear processes:

- Two-photon fluorescence
- STED
- Saturation
- Switchable fluorescent molecules

Switchable protein markers

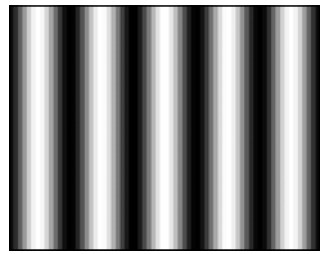


Residual fluorescence: $e^{-bTI_{off}(r)}D(r)$

where b = extinction coefficient, T = exposure time.

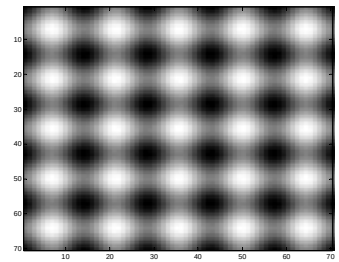
Possible illumination patterns

1D pattern $I(x) = 1 + \cos(kx + \varphi)$



Has to be rotated and shifted to obtain full resolution.

2D pattern $I(x, y) = 2 + \cos(kx + \varphi_x) + \cos(ky + \varphi_y)$

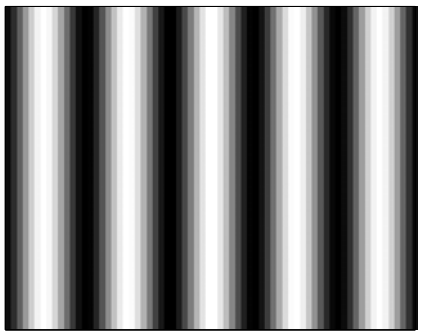


Only need to translate in x and y directions for full resolution.

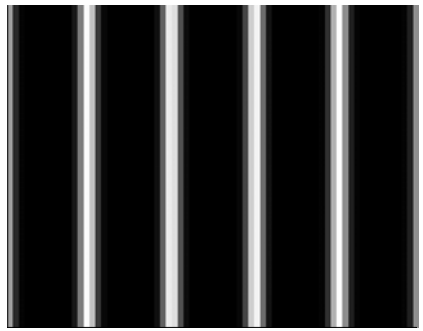
Which pattern is better?

1D Pattern

Off light pattern $I_{off}(r)$

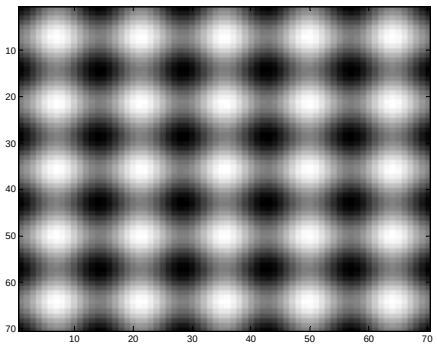


Residual fluorescence $e^{-bTI_{off}(r)}$



2D pattern

Off light pattern $I_{off}(r)$



Residual fluorescence $e^{-bTI_{off}(r)}$

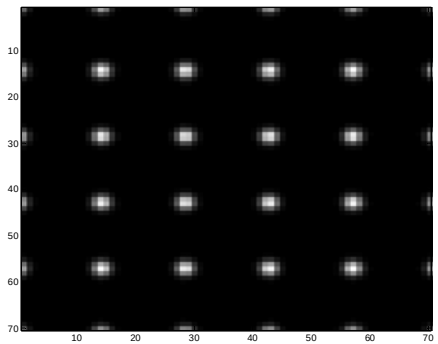


Image formation

We must consider Sources of “noise”:

- Detector noise
- Fluorophore degradation – permanently on fraction

We model image formation by:

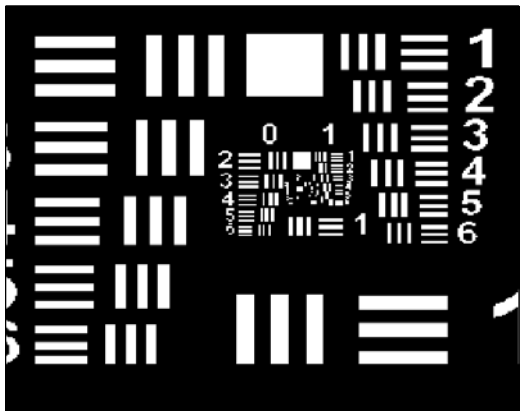
$$E(r) = (1-\alpha)H(r) \otimes \{e^{-bTI_{off}(r)} \cdot D(r)\} + \alpha H(r) \otimes D(r) + \eta(r)$$

α - permanently on fraction of fluorophore

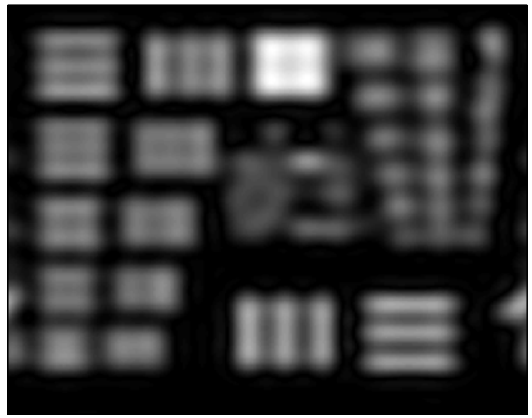
η - Poisson noise

Simulation of the Non-linear Structured Illumination

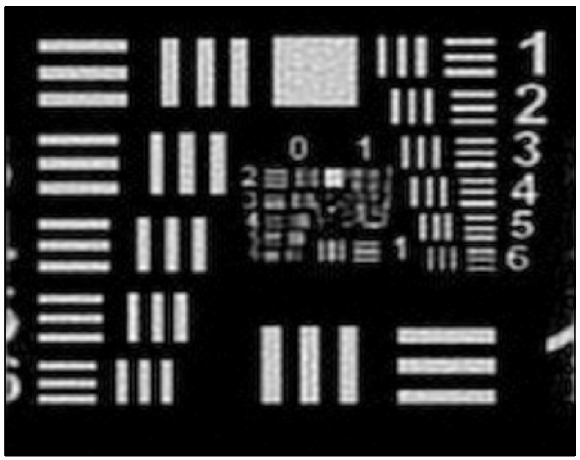
Original object



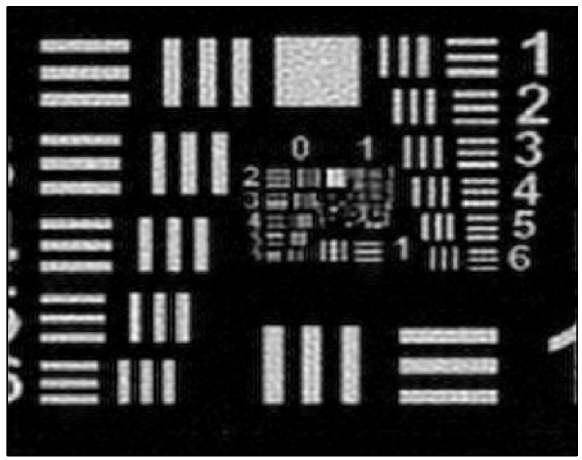
No resolution enhancement



With non-linear structured illumination



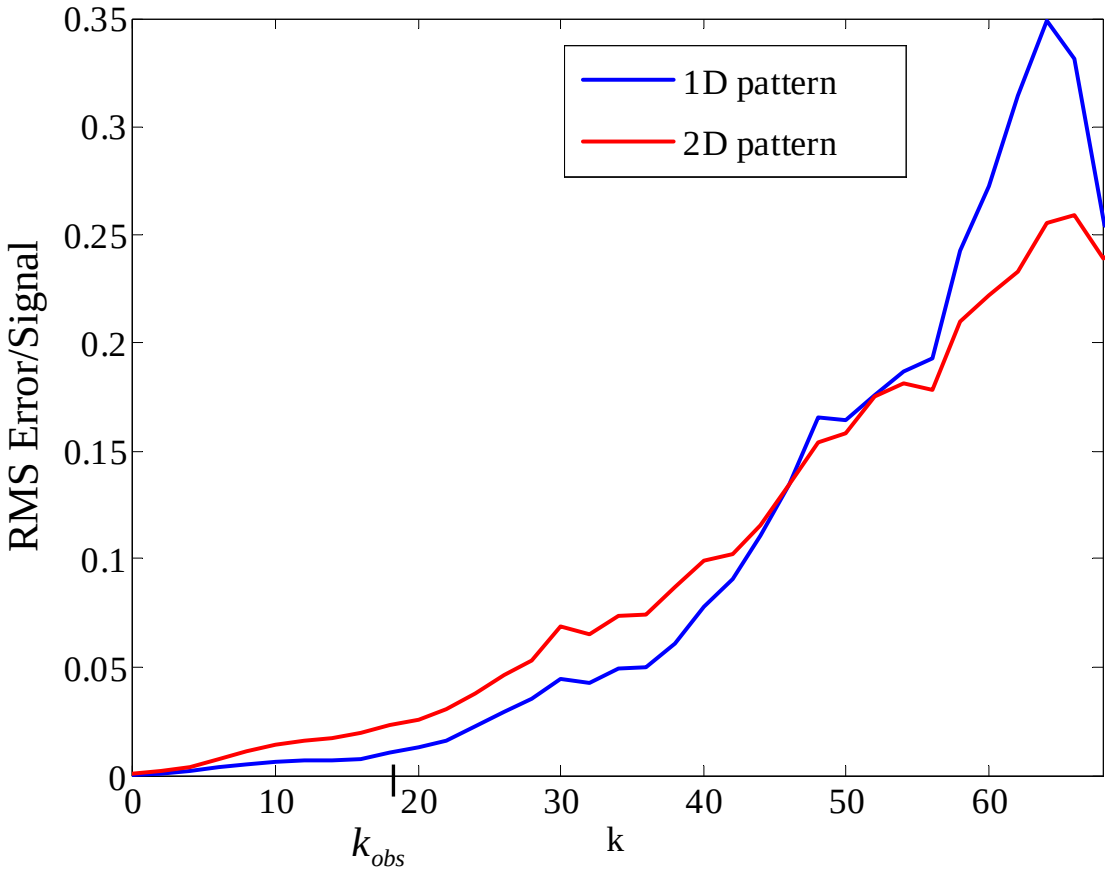
1D pattern



2D pattern

Reconstructed from 49 images

What is the resolution limit?



Error in reciprocal space as a function of wavenumber for 1D and 2D illumination. Assumed 4% fluorophore degradation and ~6000 expected photons in the brightest pixel.

Conclusions

- Noise limits the resolution in non-linear SI
- Resolution limits are similar with 1D and 2D patterns
- Future work will consider resolution dependence on the experimental parameters

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