SIM, biosensors
Administrative

• HW 7 due 11/17
• Final 12/19 – 4:30 – 7:00 in this room
• Grad student presentations
  • Teams of 2 (one team of 3)
  • Read a modern (previous 2 years) paper on optics
  • Prepare 12 minute presentation
  • Paper can not be from your current or previous lab
  • Let me know teams by 11/29
• Last class
  • 2D Fourier transforms
  • Intro to SIM

• This class
  • More SIM
  • Biosensors
Beats move the invisible frequencies lower, so we can detect them with our scope.

\[ f_{\text{beat}} = |f_1 - f_2| \]

\[ f = \frac{c}{\lambda} \]
Spatial frequencies

\[ k_0 = \frac{1}{\text{diffraction limit}} \]

\[ k_0 = \frac{2NA}{\lambda_{em}} \text{ - Maximum observable frequency} \]

Moiré fringes can take two high frequencies that are offset, and make a lower freq appear.

Similar to beat frequencies – two high frequency waves sum together for a low frequency output.
We can pattern the illumination to convolve higher order frequencies

- Super imposing the same frequency at an angle results in Moiré’ fringes
- We can pattern our illumination to have a sinusoid on the sample
- The frequency of our patterned sinusoid will move the resulting information in frequency space to the edges
- We can add in higher frequency information contained in the sinusoidal illumination pattern
- Repeating this process at many angles enables full reconstruction with increased frequency content
SIM Theory

- Structured illumination adds higher order frequencies
- In the frequency domain, those higher orders are now transmitted through the NA
- All processing is carried out in frequency domain, then IFFT to convert back to the image
Repeat pattern many times to complete Fourier space

• To increase the resolution along one dimension, take the three sine wave patterns that will fill the entire image (3 translations of grating)

• To improve along another axis, rotate the grating, and take another 3 translations

• Total of at least 9 images to build 1 SIM frame
Generating SIM grids

- Bring polarized light onto a diffraction grating
- Allow the 2 first order peaks through
- Focus on back aperture of objective
- Forms an image of a grating in the sample plane
- Rotate and translate diffraction grating
Resolution of SIM

- Axial resolution enhanced by factor of 2

<table>
<thead>
<tr>
<th></th>
<th>CLSM</th>
<th>STED</th>
<th>CW-STED</th>
<th>3D-SIM</th>
<th>PALM/STORM</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda_{em}$ [nm]</td>
<td>460-670</td>
<td>670</td>
<td>520</td>
<td>620</td>
<td>460</td>
</tr>
<tr>
<td>$D_{ax}$ [nm]</td>
<td>180-250</td>
<td>60</td>
<td>70</td>
<td>130</td>
<td>110</td>
</tr>
<tr>
<td>$D_{xy}$ [nm]</td>
<td>500-700</td>
<td>700</td>
<td>560</td>
<td>340</td>
<td>280</td>
</tr>
<tr>
<td>$V_{xy}$ [$\times10^{-3} \mu m^3$]</td>
<td>10-23</td>
<td>1.3</td>
<td>1.5</td>
<td>3.0</td>
<td>1.8</td>
</tr>
</tbody>
</table>

(Localization precision)

(PSF)

(PSF, reconstructed)
Structured illumination imaging

- Works with any dyes
- Requires repeated exposures with multiple gratings (9 times)
- Only need normal excitation levels (x9 for repeated exposures)
- Easy to grab multi channel data
- Improved axial resolution
Cautions with SIM

- Thick samples are a no go, scattering ruins pattern
- Dyes require high SNR and low photobleaching (9 exposures per 1 image)
- If there is motion happening faster than the time of 1 SIM frame, it will contaminate image
- Need to ensure higher order diffraction peaks are blocked

Photobleaching during SIM capture will result in “square edges”
2 Color SIM

- Gratings are optimal to 1 wavelength
- Can either change gratings, or live with sub-optimal reconstruction
- $PCC_{\text{widefield}} = .76$
- $PCC_{2\text{ColorSIM}} = .48$
3D SIM

- Add in 0\textsuperscript{th} order beam
- Sets up 3D lattice
- Need 15 images to from 3D SIM frame
- Improves axial resolution at the expense of acquisition time
Saturated SIM – Super resolution

• Relies on non-linear fluorescence of samples

• Illumination pattern is no longer sinusoidal, also contains even higher frequencies

• Fluorescence increases with excitation intensity up to a point

• After saturation, it can not emit faster due to physical limitations
Due to the saturation of fluorophores, if we apply enough intensity, we won’t get a pure sine wave.

The clipped fluorescence will add additional frequencies into the spectrum.

We can use these frequencies to go even higher in frequency space -> better resolution.
SSIM

• Additional harmonics enable even higher frequency reconstruction

• Requires many individual exposures to generate 1 SSIM frame (36 frames to double resolution, 50 nm)

• Theoretically unlimited resolution if you can apply higher powers
SSIM

Widefield          WF-deconvolved

SIM

CHO cell expressing Dronpa-Lifeact
On to Matlab...