## Fourier transforms, SIM

- Last class
  - More STED
  - Minflux
  - Fourier transforms
- This class
  - More FTs
  - 2D FTs
  - SIM



### Why do we convert to frequency domain

- Some problems are very hard to solve in the time domain
- Imagine a repeating source of noise
- There may be a very easy solution in the frequency domain
- It's easy (in Matlab) to convert to frequency, apply the correction, and invert back to time domain



# Fourier transforms useful to pick out signal in

## noise

- If you think you have an oscillating signal, but it is too noisy, you can often pick it out in frequency space
- Noise is often distributed randomly in frequency space, but your signal remains at one peak
- Conversion from time to frequency domain can clear things up
- Frequency space is heavily used in audio processing



## DC components of signals

- If the mean of your signal is ≠ 0, then you will have a DC component (freq = 0)
- This can overwhelm your lower frequencies
- Common to subtract the mean off your signal first
- Can also take the log of the frequencies to display nicely



## Fourier filtering

- If we know that the signal we care about is going to fall within some range
- It is easy to:
  - convert to frequency space
  - Set unwanted frequencies to 0
  - Inverse Fourier transform
- Very easy to set low pass, high pass, or band pass filters



#### More Fourier filtering





## Fourier transforms encode phase as complex number $y_1 = sin(2*pi*t + deg2rad(0))$

- Consider 3 sine waves with different frequencies and phases
- The FFT will look exactly the same independent of the phases
- In order to reconstruct the time varying signal, we need the phases



#### 2D Fourier transform

- In exactly an analogous way, linear combinations of 2d sine functions can be combined to form any image
- If you can calculate the amplitude and phase of each set, you can reconstruct any arbitrary image
- Matlab has a 2D FFT function that allows you to calculate amplitudes very quickly
- Allows image processing in the frequency domain



#### 2D Fourier transforms

















## 2D Fourier Transform of Images

- Now frequencies are represented by amplitude in space
- X axis (frequency) is periodic signal in y dimension, and vice versa
- The center of the image is the DC signal
- Diagonal points represent diagonal periodicity
- Edges represent the highest frequencies encoded









## 2D Fourier transform meaning

- In space, each pixel represents an intensity
- In frequency, each pixel represents the amount of that spatial frequency
- In the camera man, there are vertical lines (buildings), horizontal lines (skyline), and diagonal lines (tripod)



## Amplitude spectra

#### Images



Fourier Transforms



## FFT and filtering

- The more frequencies encoded in an image, the sharper the detail you can resolve
- By restricting higher frequencies you can filter the image



#### Applications: Noise removal in images

- Similar to the 1D case, periodic noise can be easily suppressed
- Regular noise appears as points
- Set those points in frequency space = 0
- IFFT to convert back to spatial coordinates



#### Applications: recognition of textures

- Repeating textures will have distinct
  Fourier components
- Easy to pick them out in frequency space







Drosophila eye and it's 2D FFT

## Frequency sensitivity of visual system

- Campbell Robson sensitivity curve
- The U shape is a pattern of your visual system, not the image
- The computer doesn't care, but you (and readers) can misinterpret images that contain too high or low of frequencies



#### SIM

• 3<sup>rd</sup> class of superresolution

## Origins of diffraction limit

- The airy disk size is determined by the wavelength and NA
- The resolution between two objects is set by Rayleigh criterion
- It is the maximum angle that sets our size

$$R = 1.22 \frac{\lambda}{2\mathrm{nsin}(\theta)} = .61 \frac{\lambda}{NA}$$

Smallest distance at which we can resolve 2 points









#### Airy Disk

## Limit is result of sample diffracting light

- Abbe realized we can think of the sample diffracting the light
- The lens re-images those diffraction patterns back onto the sample plane
- The angle of diffraction is proportional to the spacing

Image Objective  $\sin(\theta) = \frac{\lambda}{2d}$ d = sample structural distance

Sample

### Effects of finite objective size

- The fact that we can't capture all the diffracted rays means that we lose the largest angles
- These angles correspond to the smallest features in the sample
- To resolve smaller features, we need to capture these higher frequencies



## Optical transfer function

- Optical transfer function is the fourier transform of the PSF
- Frequency space representation of how the image is formed on the camera
- Convolution in image space = multiplication in frequency space

#### k = spatial frequency k<sub>0</sub> = 2NA/ $\lambda_{em}$ = maximum observable spatial frequency

The Process of Image Formation in Fluorescence Microscopy



### On to Matlab...

### Applications: Help with anti-aliasing

\*

- Aliasing is a feature of sampling two slowly
- Adds frequencies not present in original signal
- Shows up in images as blotchy regions



#### Example



down sample by factor of 4











down sample by factor of 4