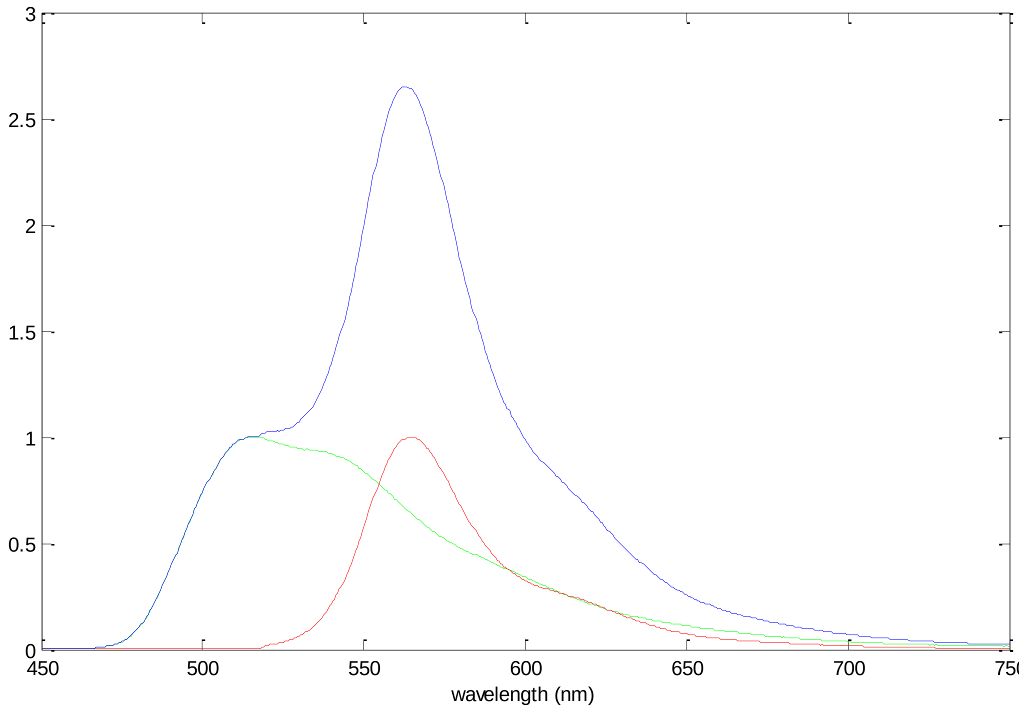
20.309 / 2.673 / MAS.402
Biological Instrumentation and Measurement, Fall 2008
Department of Biological Engineering
Massachusetts Institute of Technology

Problem Set #7 Due: Tuesday, November 4

1. **Spectral resolved imaging using Fourier transfer interferometry** In the lecture, Fourier transfer interferometry is shown to be a very powerful technique to measure infrared absorption signature of biochemical species. Within the past decade, the same approach has been used for resolving different labels in fluorescence microscopy. Consider a pixel in a fluorescent image that has contribution from two probes: fluorescein and rhodamin. The spectra from the pixel and the spectra of the pure species are shown below (green: fluroescein, red: rhodamin, blue: composite spectrum from the pixel).



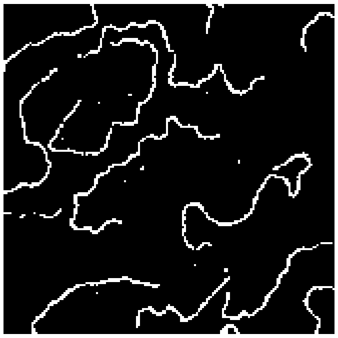
Please calculate the following:

(a) The interferrograms of the pure species (fluorescein.mat and rhodamin.mat).
(b) The interferrogram from the pixel (composit.mat).
(c) What is the chemical composition of the pixel? Can you deduce that from both the spectrum domain and the interferrogram domain?

2 **Point spread function (PSF) and Image resolution** The following is an AFM image of DNA strands (extracted and modified from www.ntmdt-tips.com, stored as AFM_DNA.mat). The image is 1 micron on each side. What would the image look like if the DNA is uniformly labeled? For simplicity, we will approximate the point spread function as Gaussians for the ease of computation:

$$PSF(r) = \mathrm{e}^{-(\frac{\mathrm{r}}{\mathrm{r0}})^2}$$

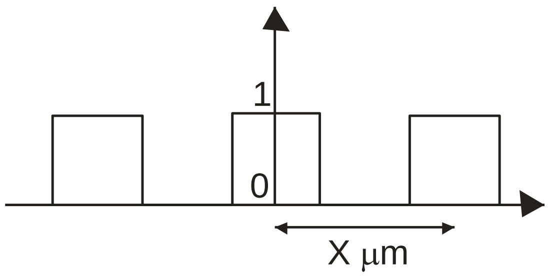
Consider two PSFs, one with r0 = 40 nm and one with r0=400 nm.



3. **Optical transfer function (OTF) and contrast** As discussed in the lecture, the OTF is the Fourier transform of the point spread function and can be viewed as a low pass filter function. While the real optical transfer function drops to zero at high frequency, we will approximate the OTF as Gaussians for the ease of computation in this exercise:

$$OTF(f) = \mathrm{e}^{-(\frac{f}{f0})^2}$$

Consider two OTFs, one with f0 = 2 μm^{-1} and one with f0=0.1 μm^{-1}.



You are given four images of square waves with periods of 10 μm, 5 μm, 1 μm, and 0.1 μm (see figure). The maximum and minimum values of these square waves are 1 and 0 respectively.

(a) Use Fourier transform to calculate the spectra of these four images (1D images).
(b) Apply the two OTFs on the spectra of these bar graphs as low pass filters. How are the spectra modified? What are the resultant final images?

4. **Numerical aperture and light collection efficiency** Recall that the numerical aperture is defined as:

$$NA = n\ sin\theta$$

where n is the index of refraction of the medium and θ is the half angle subtended by the lens. For this exercise, consider the index of refraction to be 1 (in air). If there is an isotropically emitting point source at the microscope focus, plot the amount of power collected by the objective as a function of increasing NA.