Dialogues of Civilization 2018 - Biomedical Imaging Homework 4

Due date: 6/13/2018

Please solve the following problems and email the solutions to *kercher.e@husky.neu.edu*.

1 Sampling and the Nyquist Theorem

(a) Write a function in Matlab that takes an input signal, samples it at a specified frequency, and returns the Fourier transform of the sampled signal. Use this function to "measure" a 100 Hz sine wave. Generate this signal for 1 second and use your function to sample it at various frequencies (e.g., your measurements). What is the minimum frequency needed to fully resolve this signal? Check that your results are consistent with the Nyquist theorem. Plot the FFT of this measurement, make sure your frequency axes are correct! What happens if your sampling frequency does not obey Nyquist? (**Note:** Use the 1D interpolation function `interp1` to do your sampling, not `resample`.)

(b) Generate the 100 Hz sine wave for .1, 1, and 10 seconds and sample each signal using a few frequencies that obey Nyquist. Pick one sampling frequency and plot the 3 FT spectra on the same graph. Use your results to explain how the sampling frequency and sampling time both affect the measurement.

(c) Modulate the 100 Hz sine wave with a 10 Hz sine wave at a ratio of 70%/30%. Measure this signal at a frequency that obeys Nyquist. How do the amplitudes of the FT relate to the ratio of the two waves?

(d) Now generate a 100 Hz `square` wave for 1 second. Measure this signal at 2001 Hz. Explain what is happening in the spectrum.

(e) Now measure the same square wave at 2050 Hz. Can you explain the spectrum using the same equation/reasoning from part (d)? If not, what else is happening in the spectrum? To give you a clue, repeat the measurement using the `resample` function (instead of `interp1`). Plot the two spectra and compare.

(f) Sample the square wave at a few more frequencies and explore the aliasing phenomenon. Come up with an equation that predicts an aliased frequency as a function of the signal and sampling frequencies. Justify your equation using an example from your graph.

2 Fluorescence Microscopy

(a) Define the following terms, in your own words, in the context of fluorescence microscopy:

- Excitation wavelength
- Emission wavelength
- Stokes shift.

Draw a two-level Jablonski (ladder) diagram that includes these three elements.

(b) You are interested in using a fluorescence microscope to investigate and visualize 3 elements of a biological sample. Your microscope has 2 lasers at $\lambda = 405nm$ and $\lambda = 488nm$. Use the AAT Bioquest Spectrum Viewer: `https://www.aatbio.com/spectrum/` to design your 3-color experiment, and fill out the table below. Also include an image of the graph that includes your laser lines. (Hint: you can save the graph as a png using one of the tabs directly above the graph).

Fluorophore Name	Peak Excitation	Peak Emission	Emission color

(c) Now add EGFP as a 4th dye to your experiment and examine the graph. Would this dye work with the three you chose previously? Why or why not? If not, select a different dye to add as a 4th option and include an image of the new plot.