**Gen 575 Winter 2022**

**Problem Set 2**

*Due by 11:59 pm May-03-2022 to beliveau [at] uw.edu*

**Name:**

**Problem 1 (50 points)**

You are starting a new technology development project that involves *in situ* sequencing. You intend to do four color imaging by hacking a 4-color Illumina MiSeq kit. While information about the dyes used in the kit are proprietary, your internet searching fortuitously leads you to the information in the table below:

|  |  |  |
| --- | --- | --- |
| **Label** | **Absorbance Max (nm)** | **Emission Max (nm)** |
| Dye A | 530 | 550 |
| Dye B | 580 | 600 |
| Dye C | 650 | 670 |
| Dye D | 700 | 720 |

(*Source*: https://dspace.mit.edu/bitstream/handle/1721.1/113770/1022284037-MIT.pdf?sequence=1)

**1.1 (9 points)** Your first thought is to design some custom filter sets to use in your project. In the cells below, enter ***ideal*** excitation filter, dichroic mirror, and emission filter choices for each dye. You can assume that in this ideal case you will have narrow linewidth, coherent (ie, laser) light sources available for all four dyes. You may enter your answers in shorthand form—e.g., “650/30” to indicate a bandpass filter with a 30-nm wide transmission band centered at 650 nm, “555dm” to indicate a longpass dichroic mirror that reflects light ≤555 nm and transmits lights >555 nm, and “550lp” to indicate a longpass filter that transmits light with a wavelength >550 nm. An example “488 cube” is provided.

|  |
| --- |
| **488 cube** |
| Ex: 490/10 |
| DM: 495bs |
| Em: 520/30 |

|  |  |  |  |
| --- | --- | --- | --- |
| **Dye A cube** | **Dye B cube** | **Dye C cube** | **Dye D cube** |
| Ex: | Ex: | Ex: | Ex: |
| DM: | DM: | DM: | DM: |
| Em: | Em: | Em: | Em: |

**1.2 (9 points)** You check in with your PI and learn that the only microscope you have access to is a Keyence BZ-X800E. Alas, this microscope does not support lasers, so you will need to rethink your filter set choices. You ask around and find a copy of the BZ-X800E manual, which includes details about its white light lamp (shown below).

Graphical user interface, application

Description automatically generated

Given the spectral properties of this light source, redesign you filter set from ***1.1***:

|  |  |  |  |
| --- | --- | --- | --- |
| **Dye A cube** | **Dye B cube** | **Dye C cube** | **Dye D cube** |
| Ex: | Ex: | Ex: | Ex: |
| DM: | DM: | DM: | DM: |
| Em: | Em: | Em: | Em: |

**1.3 (12 points)** Your PI suggests you start with a simpler, 2-color experiment using Dye A and Dye B. Using your answer from ***1.2*** as a starting point, go to Chroma’s website and pick out individual optical filters (<https://www.chroma.com/products/optical-filters>) to populate custom cubes for Dye A and Dye B. Record the Chroma part numbers (eg, “ET470/40x”) of your choices in the table below. You only need to consider the spectral properties of the filters and dichroic mirrors when choosing:

|  |  |
| --- | --- |
| **Dye A cube** | **Dye B cube** |
| Ex: | Ex: |
| DM: | DM: |
| Em: | Em: |

**1.4 (20 points)** You place your Chroma order but find out soon after that that your custom cubes will not ship for several months due to supply chain issues. Sad. In order to keep the project moving, your PI suggests that you practice doing some multicolor imaging experiments on the BZ-X800E using the existing filter cubes and a different set of dyes. Fortunately, your lab has experience with nucleic acid chemistry, so you will be able to order essentially any dye you want and chemically conjugate it to dNTPs to test out the *in situ* synthesis process your project depends on. The four filters currently in the BZ-X800E are listed below:

|  |  |  |  |
| --- | --- | --- | --- |
| **DAPI** | **EGFP** | **TRITC** | **Cy5** |
| Ex: 350/50 | Ex: 470/40 | Ex: 545/25 | Ex: 620/60 |
| DM: 400bs | DM: 495bs | DM: 565bs | DM: 660bs |
| Em: 460/50 | Em: 525/50 | Em: 605/70 | Em: 700/75 |

For simplicity, your PI suggests you pick either an ATTO or Alexa Fluor dye for each channel. Add the filter sets above to FPbase (<https://www.fpbase.org/spectra/>) and select a dye for each channel. Record your dye choices below:

* DAPI channel:

* EGFP channel:
* TRITC channel:
* Cy5 channel:

For each channel, take a screenshot of the collection efficiency of the emission light from the FPbase ‘Efficiency’ tab and paste it below. You can enter “X-Cite 120Q Metal Halide” as the light source in order approximate the lamp on the BZ-X800E and assume your filters/mirrors have 100% transmission:

DAPI channel

EGFP channel

TRITC channel

Cy5 channel