Sample Preparation

1. Find your sample from the Eppendorf box.
2. Vortex your sample for 10 seconds and spin it down with the micro-centrifuge.
3. Take a new disposable cuvette from the box and unwrap it.
4. Remove the old Parafilm on the top of your Eppendorf.
5. Carefully pipette all of your sample (should be 60 µL) into the channel of the cuvette at the very bottom.
6. Throw away the old Eppendorf tube.
7. Measure your samples using TCSPC and steady-state.
    - **Note: Upload your TCSPC measurements to your Sakai Dropbox immediately after taking them.**
8. After your measurements, pipette your sample from the cuvette into a new Eppendorf tube labeled with your group's initials. Place this in the Eppendorf box and dispose of the cuvette.

Data Analysis

1. Download your TCSPC .asc file from your Sakai Dropbox.
2. Download the readTCSPC.m file from the Sakai->Resources->Labs->Lifetime Analysis folder.
3. Using MATLAB and the provided script, parse your TCSPC data. The resulting data should be a two column matrix. The first column represents your time bins. The second column represents the number of photons collected for that bin.
    - data = readTCSPC('mydata');
    - plot(data(:,1),data(:,2)); % just to visualize the data
4. Your data takes on the form of a decaying exponential, $e^{-t/\tau}$. Your goal is to find the lifetime, τ. The easiest way to do this is to first take the log of your data.
    - newData = log(data(:,2));
    - plot(data(:,1),newData);
5. The slope of the resulting line from step 4 will ultimately tell you your lifetime. To find this slope, you first need to choose a region of your data to analyze. Avoid the initial peak and the end of your data. Once you've chosen your region, find the starting and ending indices of this region, using the variable editor in MATLAB. For example, if you chose time= 1.0016 as your starting point, then your initial index (ind1) should be 328.
6. Using MATLAB's polyfit function, find the slope of your log(data) from the starting index to the final index.
    - fit = polyfit(data(ind1:ind2,1),newData(ind1:ind2),1);
7. Calculate the lifetime of your donor in the presence of the acceptor (τ) using this slope.
    - τ = -1/fit(1)
8. Assume that the lifetime of your donor (ATTO 520) in the absence of any acceptor (τ_0) is 4.5 ns.
9. Using the lifetime of your donor (step 7) and the donor in the absence of the acceptor (step 8), calculate the transfer efficiency for your RET pair.
    - TE = $1-\tau/\tau_0$