Comparison of rhodamine, cyanine and fluorescein

We will compare the overlap integral part of FRET pairs to make predictions about their relative R_0 values. One goal of this comparison is to use a FRET pair that is easily excited by 509 nm. It appears that the best choice for us is to use cyanine-3 as the acceptor. The figure below shows data obtained from photochemCAD for both rhodamine 6G and 1,1'-Diethyl-2,2'-carbocyanine iodide.

The absorption and emission spectra of each of these dyes is shown in the figure below. The Franck-Condon analysis is also possible on the cyanine dyes/ However. photochemCAD gives you a good starting point as shown in the figure below.
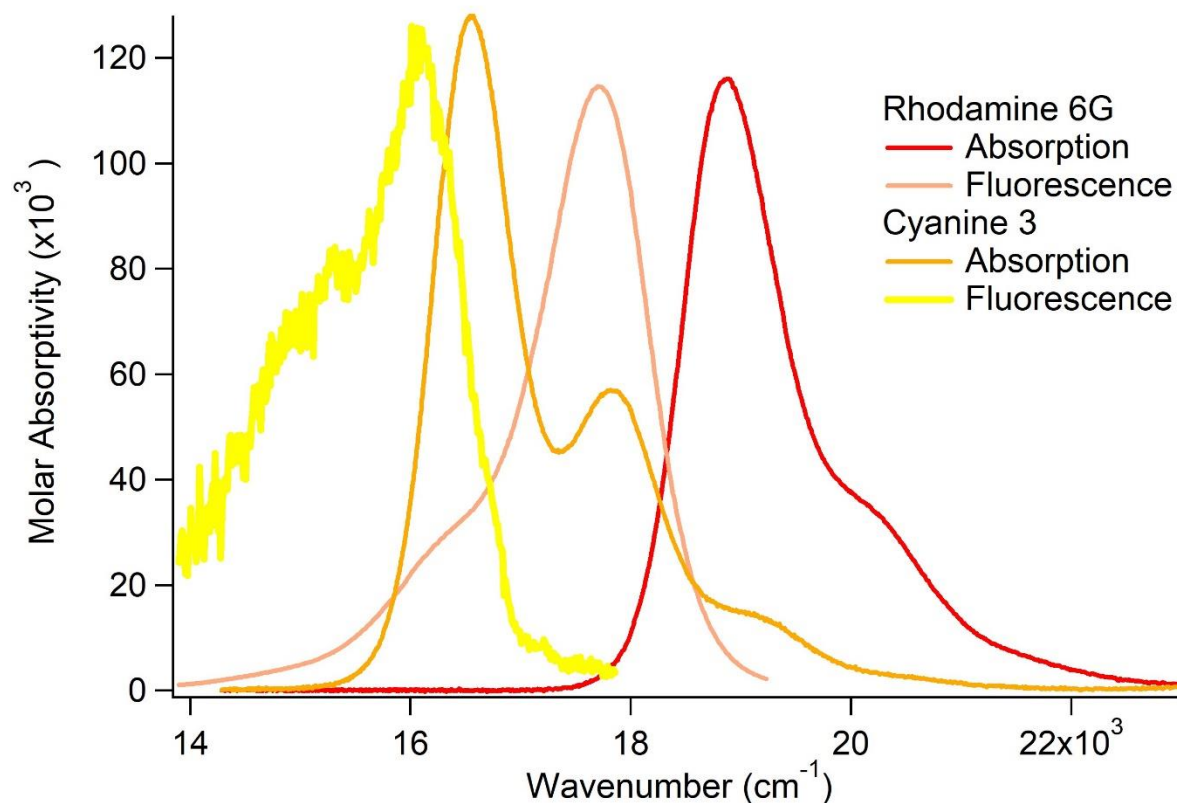
In order to carry out overlap integrals numerically, we need for the points to align on the abscissa. Unfortunately, each of these data were obtained under different wavelength ranges and the wavenumbers are not even because they were all converted from wavelength.

There is a power and easy interpolate routine on Igor. I could not find anything like this on Excel. See the interpolated files below. Their energy spacing is now 1 cm-1 and you can multiply and integrate to obtain an overlap.
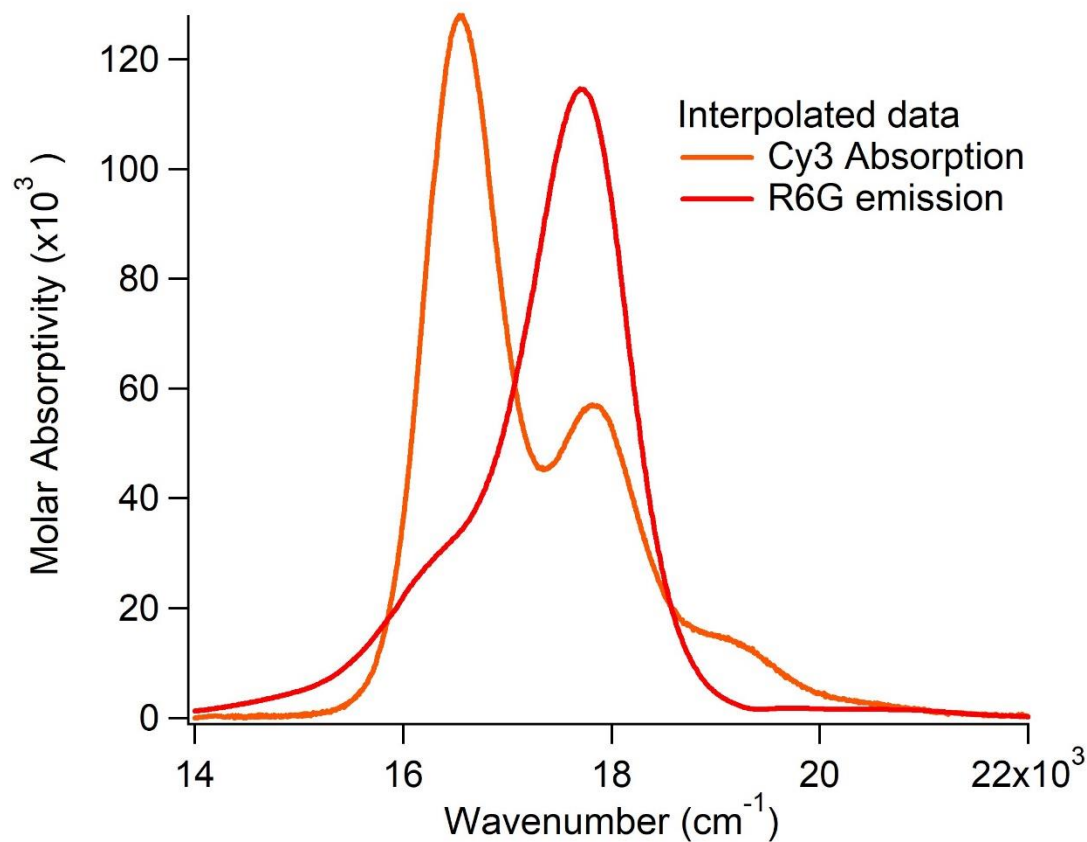
Figure 2. Interpolated files for Cy3 and R6G

Using this approach we can compare the overlaps for the fluorescein-rhodamine pair compared to the rhodamine-cyanine FRET pair to see how different the overlaps are. We can then compare this to observed energy transfer.

If we compare this to interpolated data for rhodamine 6G and fluorescein, we find that there is less overlap for the famous pair. The reasons why rhodamine 6G and fluorescein are of such great interest arises from their convenience for common lasers (argon ion, NdYAG etc.) in the green portion of the spectrum. One can see by eye that the overlap is not nearly as good for the Fl/R6G pair as it is for the R6G/Cy3 pair.
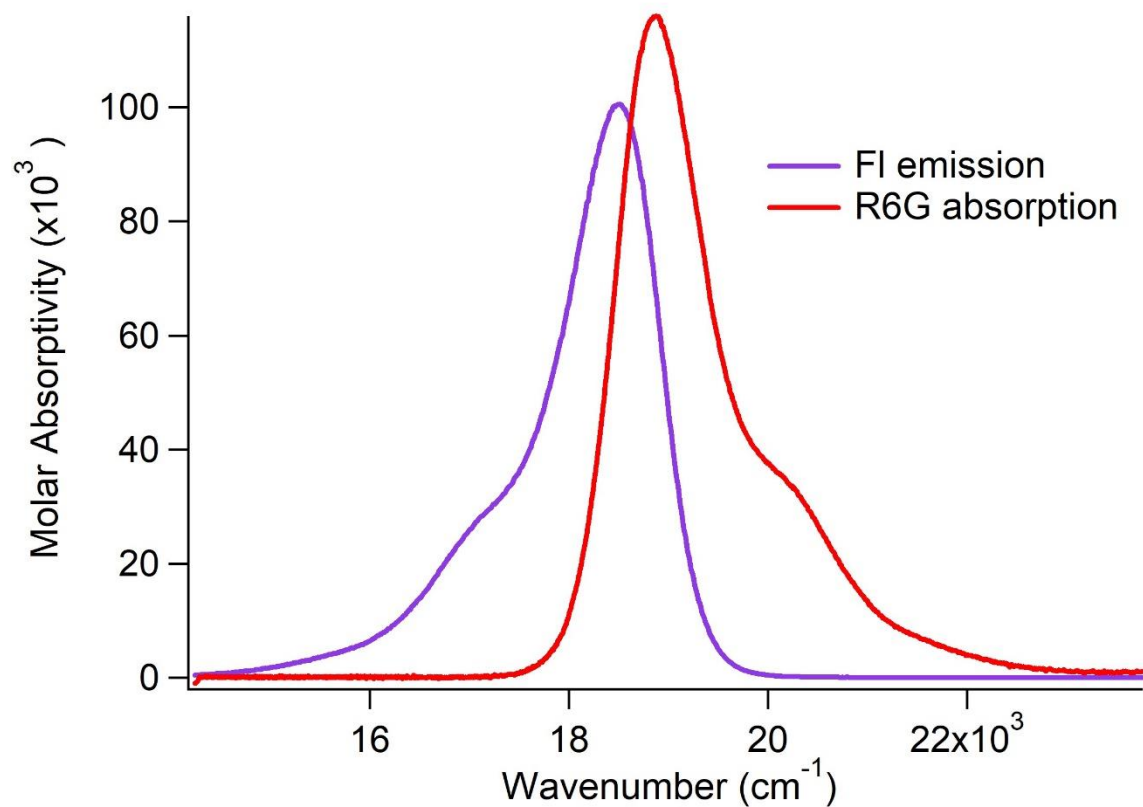
Figure 3. Interpolated files for R6G and Fl.

Use the interpolated files provided to calculate overlap products and then integrals. This procedure is shown in the pdf on treating Photochem CAD data. See "Steps involved in spreadsheet or computer calculation of spectral overlap." Compare the two spectra above and predict whether R6G-Cy3 or Fl-R6G has the greater rate, and smaller R_0.