Measure the fluorescence excitation and emission spectrum

1. Turn on the instrument
    a. Turn on the lamp power
    b. Turn on the computer, monochromater, detector.
    c. Open the software Felix
2. Excitation measurement procedure
    a. In the menu ‘Acquisition’, select ‘New Acquisition/excitation scan’
    b. Choose the appropriate excitation wavelength range and the emission wavelength. (The emission and excitation wavelength cannot overlap)
    c. Click the button ‘More’, select ‘Real time spectrum correction’ and select both ‘Excitation correction’ and ‘emission correction’
    d. Click ‘Acquire’, then ‘Start’
3. Emission measurement procedure
    a. In the menu ‘Acquisition’, select ‘New Acquisition/Emission scan’
    b. Choose the appropriate emission wavelength range and the excitation wavelength. (The emission and excitation wavelength cannot overlap)
    c. Click the button ‘More’, select ‘Real time spectrum correction’ and select both ‘Excitation correction’ and ‘emission correction’
    d. Click ‘Acquire’, then ‘Start’
4. Measure Fluorescein excitation and emission spectrum
    a. Measure the buffer excitation spectrum (excitation: 400nm-510nm, emission: 530nm) and emission spectrum (excitation: 460nm, emission: 480nm-600nm)
    b. Dilute the fluorescein stock solution by a factor 100 x 50 (16nM)
    c. Measure the excitation and emission spectra using the same settings as in a.
    d. Subtract the measured fluorescein spectrum from the background spectrum, normalize; you now have the corrected spectrum.
    e. Now take the emission spectrum at a different excitation wavelength (440nm). Compare the emission spectra at different excitation wavelengths.
    f. Dilute the fluorescein stock solution by a factor of 100x100 (8nm), measure the emission spectrum. Compare the fluorescence spectra and show that the concentration and fluorescence signal are proportional to each other.
5. Measure Fluorescence anisotropy
    a. Put the polarizer in the excitation and emission path
    b. In the ‘Acquisition’, select ‘New Acquisition/Emission Scan with Polarizer’. Choose appropriate excitation and emission wavelengths for fluorescein.
    c. Calibrate the G-factor: Use horizontal excitation light, measure vertical and horizontal emission fluorescence. Take the ratio between vertical and horizontal fluorescence.

d. Measure the anisotropy of the fluorescein sample. Use vertical excitation light, measure the vertical and horizontal emission fluorescence. Calculate the anisotropy.
e. Add 4420 antibody and measure the anisotropy.