

Outline for fluorescence class at DMC-- 22 October 2012

Hands-on: meet in Perry lab and prepare to sample on the DMC dock

- Measure chlorophyll fluorescence in the Damariscotta River Estuary, with a WET Labs fluorometer
- Collect water samples for measurement of chlorophyll concentration (see JGOFS_chl_method.pdf). You will collect water, filter particles in the lab onto Whatman G/FF filters, place filters in acetone to extract the chlorophyll pigment, sonicate the filters in acetone to enhance rate of extraction, read fluorescence of samples on a Turner Designs AU-10 bench-top fluorometer before you head back to Orono or elsewhere.
- Demo of chlorophyll extract – red fluorescence
- Epifluorescence microscope (Mayer lab) – ‘see’ fluorescence of living cells

Lecture/discussion:

- 1) big picture - why the interest in chlorophyll fluorescence? what do we really want to know? what does fluorescence actually provide? do coupled measurements get us closer?
- 2) brief review of fluorescence in general and chlorophyll fluorescence in particular
- 3) BRIEF overview and history of measurement of chlorophyll – or different aspects of chlorophyll
 - *in vivo*: by eye (color of the water) and by smell
 - *in vivo*: by eye (Munsell color chart)
 - *in vitro*: spectrophometry of extracts
 - *in vitro*: fluorescence of extracts
 - *in vitro*: HPLC of extracts
 - *in vivo*: chlorophyll fluorescence profiles by pump, then *in situ* fluorometry
 - *in vivo*: epifluorescence
 - *in vitro*: spectrophometry of filter pads (a_676)
 - *in vivo*: spectrophometry of filter pads (a_676), profiles and underway
 - remote sensing: ocean color remote sensing reflectance
 - remote sensing: ocean color remote sensing fluorescence line height
 - *in vivo*: pump and probe fluorescence for physiology (profiles and bench top)
- 4) general principles of fluorometric measurement - excitation/emission, light source, filters, detectors, geometry, temporal resolution.
- 5) specifics of chlorophyll fluorometry
 - bench top and in situ manufacturers
 - choice of wavelength (absorption spectra, excitation/emission spectra)
 - calibration of fluorimeters – *in vitro* (extracts) and *in vivo* (of living cells)
 - drift
 - temperature effects
- 6) interpretation of data, and challenges therein, and potential workarounds
- 7) synthesis