VARIABLE FLUORESCENCE/PHOTOSYNTHETIC EFFICIENCY

1) for the DCMU stock you need to make up a solution such that your final concentration (in the fluorometer) is between 1-10 micromolar DCMU. I usually make up a 10,000 micromolar DCMU solution in 70% ethanol, then you can add a few drops to your cuvette and it'll be about the right concentration. You'll also want disposable cuvettes, or clean the cuvette really well between uses.

2) Collect a whole-water sample. Dark adapt it for about 30 minutes at ambient temperature (this gets rid of the first phase of non-photochemical quenching). If you don't dark adapt, you get a slightly different value, which complicates the interpretation. By dark-adapting, you can collect water samples at any time of the day. You also want to filter some of the water through a 0.2 micron filter, so that you can use it as a blank. Ideally, you'd have a filter blank for each sample. In reality, I usually do a blank a few times per day, and if it's not changing, you're fine.

3) Measure the raw fluorescence on your fluorometer (doesn't particularly matter what type of fluorometer...we've used both a TD-10AU and a TD-700, with both acidification and non-acidification filter sets). If you are using the 10AU, it's better to disable the auto-scale feature. Also measure the blank, on the same scale.

4) add a few drops of DCMU (or to 10 micromolar), mix, and read the fluorescence again immediately. It won't be particularly stable...different people use different protocols. I usually use the * feature on the fluorometer, which measures an average over some period of time. Other people take the reading when it first levels off (but then it will keep going up, eventually). Again, doesn't matter too much as long as you're consistent. Add DCMU to the blank, and measure that also.

5) The second reading is Fm, and should be much higher than the first reading, which is Fo. The variable fluorescence is simply (Fm-Fo)/Fm, and should be between zero (dead) and about 0.7 (completely healthy). You should subtract the blank and the DCMU-blank from Fo and Fm respectively.

If you're using the PAM instead, you would collect your sample, dark adapt, and then measure Fo, followed by Fm using a saturating (actinic) light pulse (again blanking with the filtered water). One word of caution... PAMs have different sensitivities, so if you're using a PAM 101/102/103, you either need to pre-concentrate the cells or develop a biomass curve. If you're using a Phyto-PAM or Water-PAM, they work with pretty low levels of biomass.