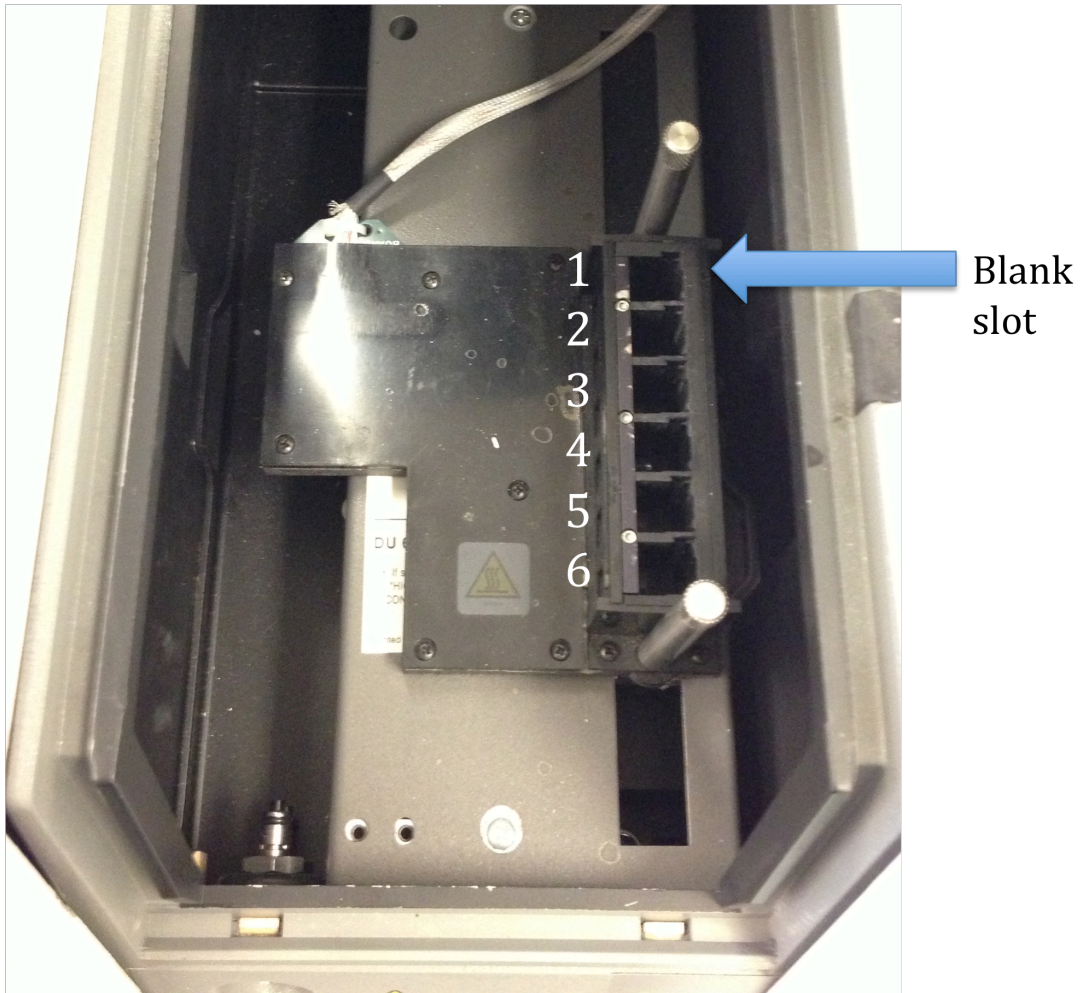


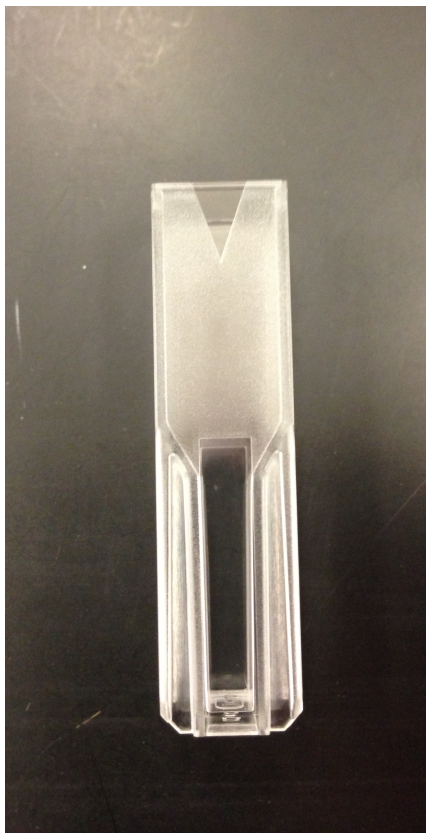
Protocol for DU 800 Spectrophotometer

Make sure that computer is on. The computer is old and will take a long time to start up. System is on if monitor, computer tower and DU 800 have green LED signals by their power buttons.

1. DU 800 Spectrophotometer program icon is located on the left side of the computer screen. Double click to start program.
2. On the upper left-hand of the DU 800 spectrophotometer program, you can choose from a series of programs to run. The default program is "Fixed Wavelength". That is the suggested program to use for finding spec readings.
3. Under the Fixed Wavelength lab, a second tab allows you to choose the default method vs the yeast OD600 method of analyzing the spec. **For all yeast spec analysis, use the yeast OD600 protocol.**
4. Cuvettes used in the spectrophotometer can be found in the top drawer directly behind the machine, along the wall.
5. You must **BLANK** all procedures before starting readings. Readings without a blank do not mean anything. To create a blank, place 1 ml of the solvent used in preparing samples into the plastic cuvette (ex. Distilled water, YPR/YPD liquid media, LB liquid media).

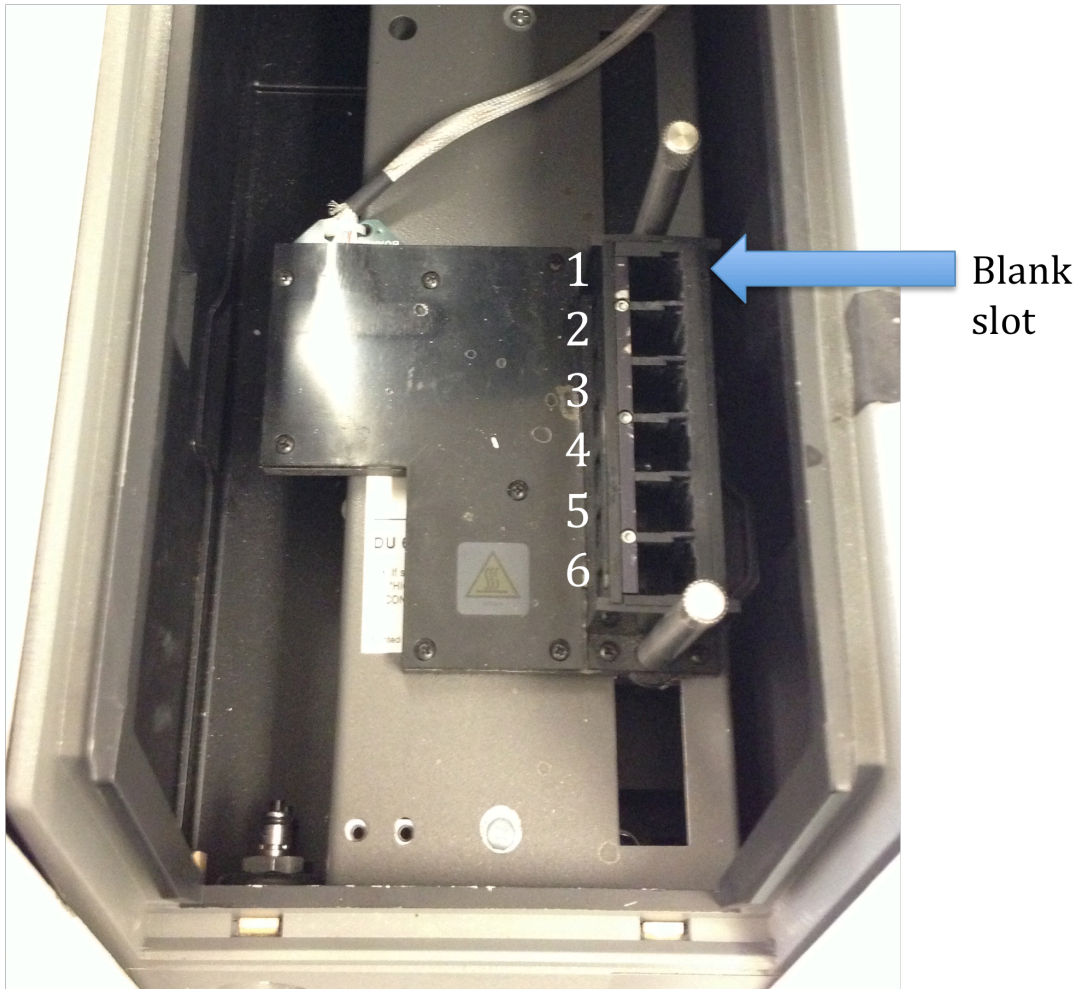


6. Prepare a BLANK as well as up to 5 samples to be analyzed. There are only 6 sample wells. The blank must be inserted into the top well.
7. Each cuvette contains a triangle on the top of one side. This triangle should point toward the left of the machine when inserted into the reading well. Avoid touching the bottom half of the plastic cuvettes to reduce the amount of fingerprints that could interfere with correct wavelength reading.



8. Place all cuvettes into reading wells before use.
NOTE: you do not have to fill all the wells to run the machine. If you only have one sample to analyze, then fill the blank and first sample well directly below. The other wells can stay empty.
9. Once sample cuvettes have been inserted into wells, close the machine lid.
10. You must turn on the lamps before reading. To turn on the lamps, click on the visible or UV lamp tabs located on the bottom left of the program window. You can use an individual lamp, or both at the same time. Lamps that are on will have RED writing in the program window and orange indicator lights will illuminate on the spectrophotometer below the lid and power indicator.
 - a. TUNGSTEN LAMP=visible
 - b. D2 LAMP=UVNOTE: Lamps should only be on during analysis. After completion of spectrophotometer use, make sure that lamps are turned off. Turn off lamps by clicking on the red lamp tabs. Orange indicator lights should turn off on spectrophotometer.
11. Once all samples, including blank, are inserted into wells and lights have been turned on, CLICK on the BLUE BLK octagon located on the top left of the program window. This blanks the machine to whatever solvent you are using. After

blanking is complete, CLICK on the YELLOW READ SIGN for wavelength reading of your samples. The blank should read out to a really small number (it will not be 0.000) The output will show the readings of each sample. 1-1 represents the blank, and each sample after corresponds to the vertical well.



12. You can re-blank at any time by pressing the BLUE BLK octagon.
13. You can save the output readings by clicking on the Save File tab located on the top of the program window, Before leaving the program, please clear all data by pressing the Clear tab located on the top of the program window.
- 14. After you have cleared your data, Turn off all lamps.**
15. Take out cuvettes from machine, you can rinse and reuse cuvettes if you have multiple readings, SAVE YOUR BLANK CUVETTE if further readings are necessary. Dispose of biohazard liquid waste into the liquid waste collection flasks at your workbench and place cuvettes into the orange-bagged steel containers after use.

16. Before leaving spectrophotometer work area, make sure all cuvettes are out of machine, and that the lid is closed. Make sure all lamps have been turned off. Leave DU800 spectrophotometer program window open and cleared of any data. Leave computer running. Monitor will go into sleep mode after a period of time.