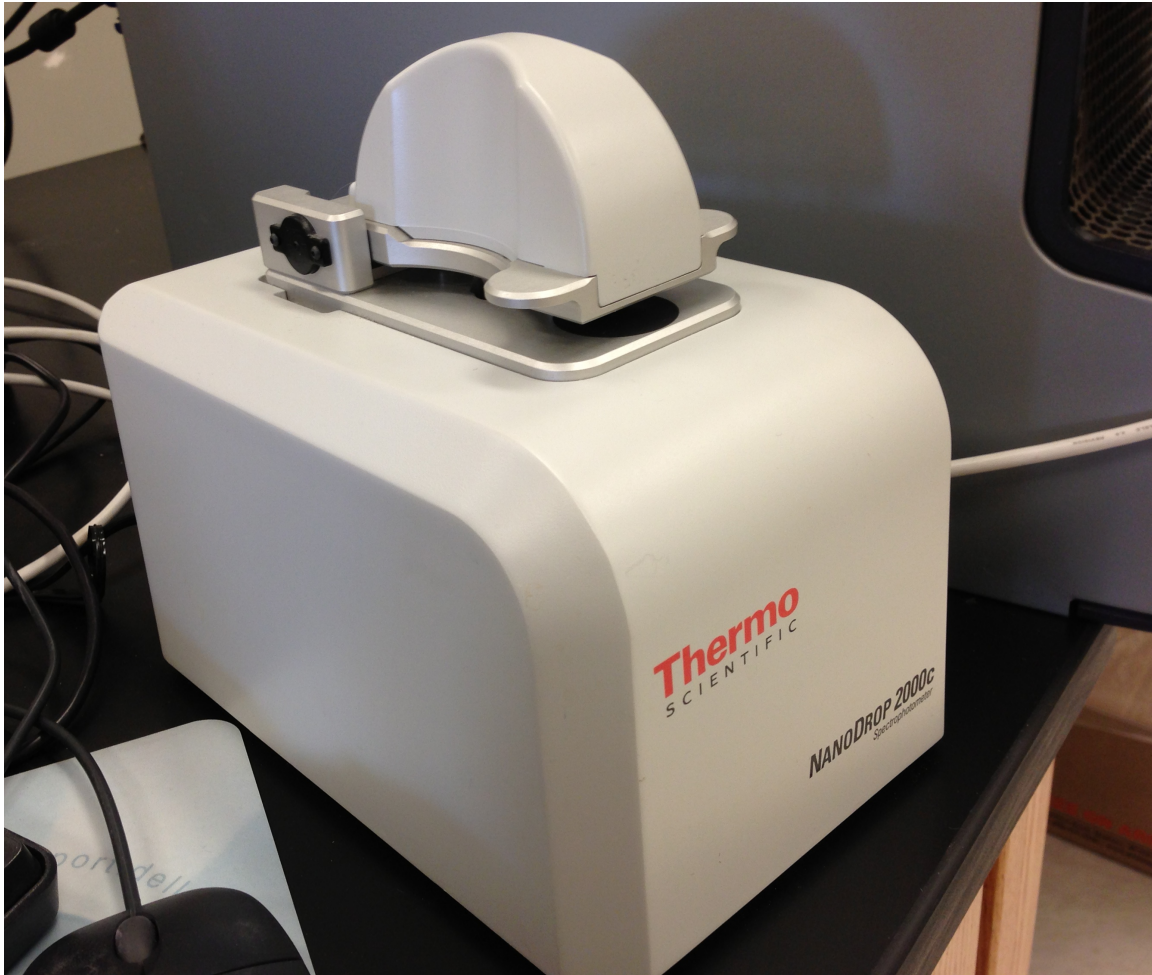


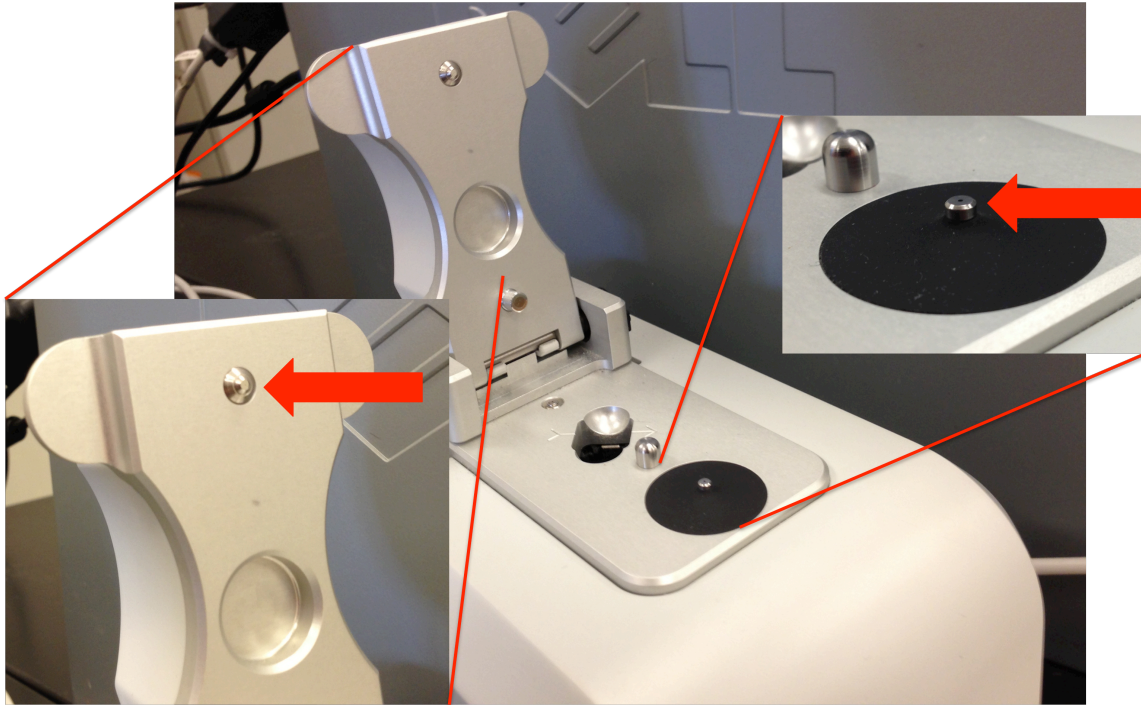
## Protocol for NanoDrop 2000c Spectrophotometer



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

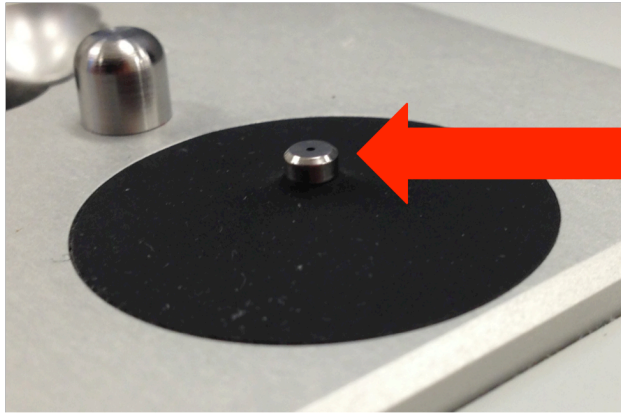
1. NanoDrop 2000 spectrophotometer program icon is located on the left side of the computer screen. Double click to start program.
2. Once program opens, select the tab of the type of an analysis you would like to run (ex. Nucleic Acid tab calculates concentration of DNA or RNA in a sample). Click on appropriate tab. **The majority of NanoDrop use within the CMBB lab is to obtain DNA concentrations.** The Nanodrop can also be used as a spectrophotometer of cell cultures (Cell Cultures Tab) but is much more time consuming compared to the DU 800 spectrophotometer protocol.
3. For DNA and RNA concentrations, click on the **Nucleic Acid Tab.**

4. Once the program opens, a pop-up window will alert the user that a routine wavelength verification test must be completed. Before allowing program to complete wavelength check, first make sure that the NanoDrop sensor is free of any debris. Open the NanoDrop arm. Take a kim wipe located at the work area and clean the sensor in two locations shown below.



5. After cleaning, lower the NanoDrop arm gently (Do not slam the arm back down or allow to drop).
6. After you have cleaned the NanoDrop, and have lowered the arm, click the **OK** button for the routine wavelength verification to commence. You will hear the machine run for a few seconds.

7. You must blank the machine before measuring concentrations. Lift the NanoDrop Arm and dispense 1.5  $\mu\text{l}$  of blanking solution directly on top of the Nanodrop sensor (The blanking solution should be the solvent used to resuspend the nucleic acid, ex. water, 1x TE).



8. Once you have loaded the blanking solvent onto the sensor, lower the arm and click the **Blank button** at the top left of the computer program.
9. The machine will then run again while it is blanking the solvent. Once the blank has completed, the **Green Measure button** will illuminate at the top left of the computer program. Lift the NanoDrop arm, and use a kim wipe to remove any remaining solvent from the sensor as well as the metal cap located on the arm. This will reduce contamination between samples.
10. Make sure that the sample to be read is properly suspended. Add 1.5  $\mu\text{l}$  of the sample onto the NanoDrop sensor and close the arm.
11. At the top right for the computer program, select the type of nucleic acid to be read. Keep the box directly to the right displaying 50.00. Below, select the units to be used in the concentration calculation (The majority of the time, use  $\text{ng}/\mu\text{l}$ ). Click on the **Green Measure button** located at the top left of the computer program.
12. The program will complete the measurement calculation and will pop-up a window to allow you to save your data. Select your folder (you can create a folder to store information in **My Documents**). If you do not want to store the information, simply select cancel. The concentration of the sample will be displayed in the Conc. Window located in the top right of the program. A series of calculations are also displayed below, the most important of which is the 260/280 calculation. DNA should have a 260/280 of between 1.8-2.0, RNA should be above 2.0. Contaminated DNA will have a low 260/280 of below 1.8.

13. The measurement will give an output of wavelength vs absorbance. Below, the program temporarily stores the information from the sample including the concentration and the 260/280.
14. Take two measurements of each sample to make sure that you get a correct read of the true concentration of the samples. Make sure to clean the sensor and cap between each sample reading and redo.
15. When complete, make sure that the NanoDrop sensor and metal cap are cleaned of residue using a kim wipe. Make sure you leave the arm down. Save any data you want and then close the program. Leave the computer logged in and running for future use.