Spectrophotometers

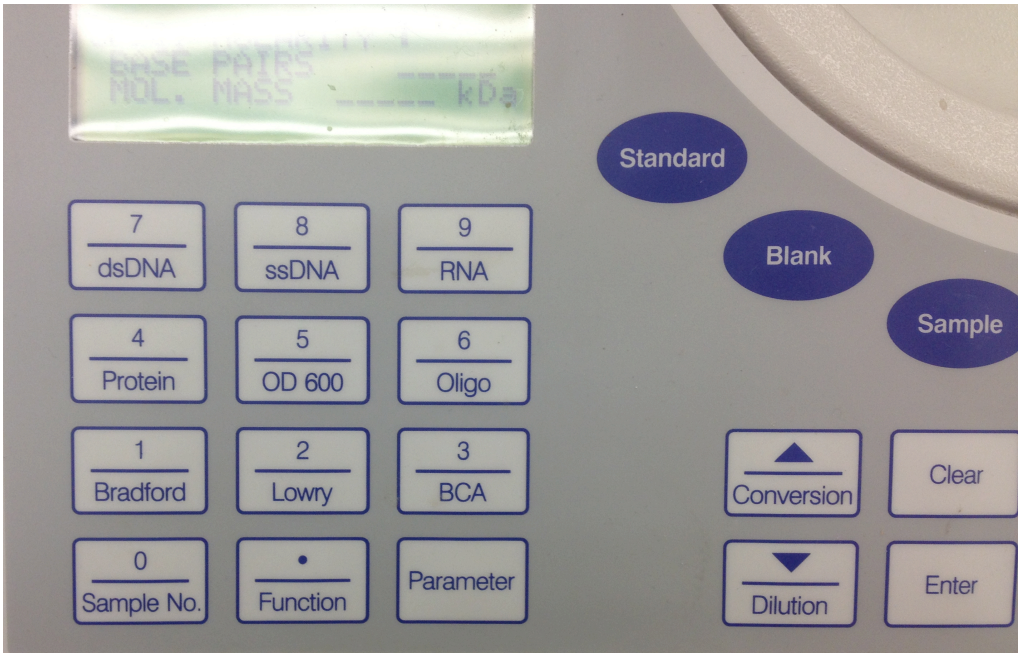
Eppendorf Biophotometer (Kell 405)
Eppendorf Biophotometer Plus (PSC 555)
GE Nanovue (PSC 535)
Implen P360 (PSC 533)
ThermoFisher NanoDrop Plus (PSC 533)

Every time you use the Spectrophotometers, you MUST sign the log book.

Contact: Sonja Young (404) 413-5363; sstovall@gsu.edu for help with the Biophotometer
Gemeia Cameron (404) 413-5379; gmcameron@gsu.edu for help with the Nanovue
Hyuk Kyu Seoh (404) 413-5379; hseoh@gsu.edu for help with the Implen
Ping Jiang (404) 413-5370; pjiang@gsu.edu for help with the Nanodrop

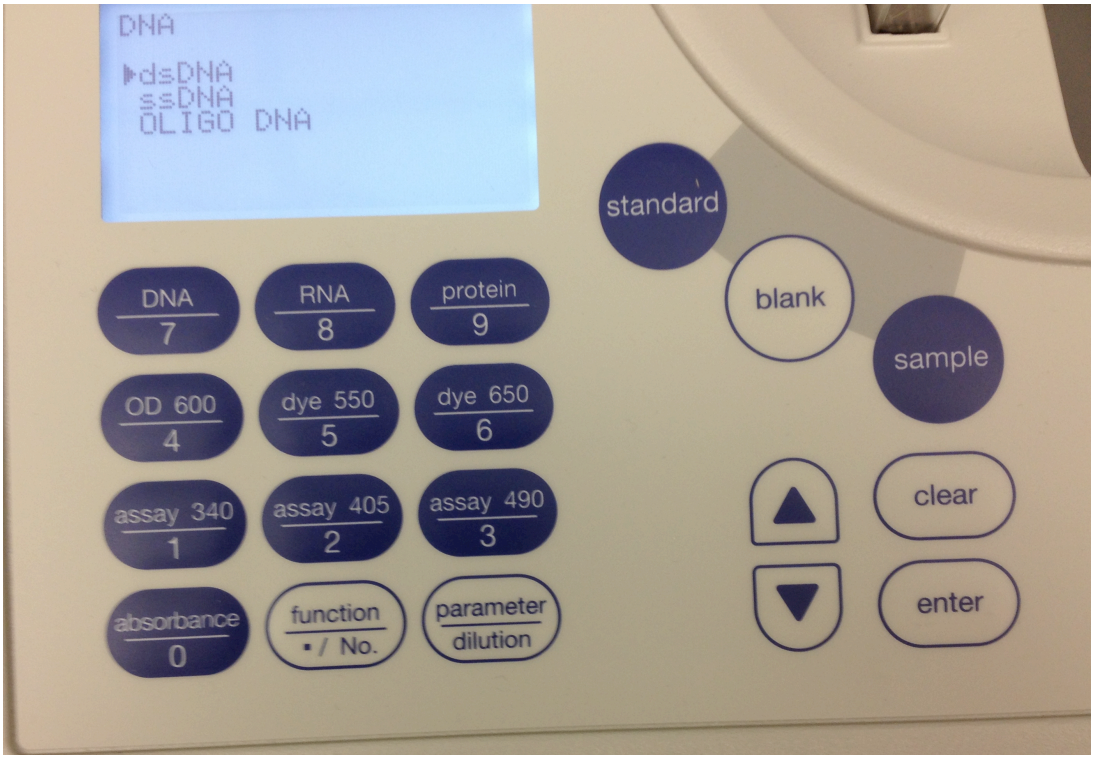
Eppendorf Biophotometer

1. Warm up not needed
2. Turn on instrument, back of instrument, just above power cord.
3. Press button for what you need to do. DNA, RNA, Protein, O.D. 600, Assay type.
4. Remove dust cover from cuvette holder.
5. Hold cuvette by grooved sides not clear sides. After sample loaded (at least 50 ul) in cuvette, place cuvette in slot with clear sides lined up with the arrow (direction of light path). Push firmly. Check to make sure no bubbles.
6. First blank with water or buffer. Select Blank button.
7. Enter dilutuon. ul sample Press enter. ul diluent Press enter.
8. Load sample into cuvette. Place in cuvette holder. Press Sample button.
9. For DNA samples, the window will show 230, 260, 280 and ratios 260/280 (1:2) and 260/230.
10. Turn instrument off.
11. Put dust cover back on cuvette slot.

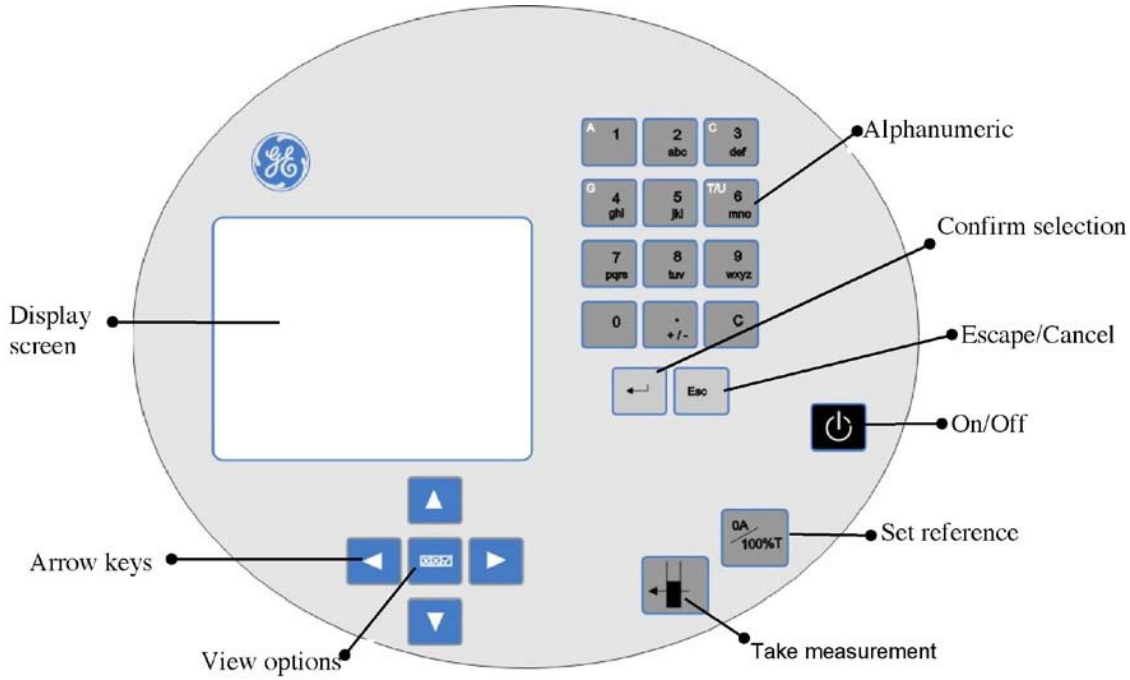


Biophotometer Plus

1. Warm up not needed
2. Turn on instrument, back of instrument, just above power cord.
3. Press button for what you need to do. DNA, RNA, Protein, O.D. 600, etc.
4. Using arrows and enter buttons, scroll to whatever submenu you want. DNA/RNA – select type of DNA or RNA; Proteins – select assay type, Absorbance – select wavelength you want.
5. Slide blue cover back.
6. Hold cuvette by grooved sides not clear sides. After sample loaded (at least 50 ul) in cuvette, place cuvette in slot with clear sides lined up with the arrow (direction of light path). Push firmly. Check to make sure no bubbles.
7. First blank with water or buffer. Select Blank button.
8. Enter dilutuon. Select Parameter/Dilution button. Cuvette size – Enter; Unit – select unit using arrows, Enter; Factor – leave as is, Enter.
9. Load sample into cuvette. Place in cuvette holder. Press Sample button.
10. For DNA samples, the window will show 230, 260, 280, 320 and ratios 260/280 (1:2) and 260/230.
11. Turn instrument off.
12. Put dust cover back on cuvette slot or slide cover over slot for Plus

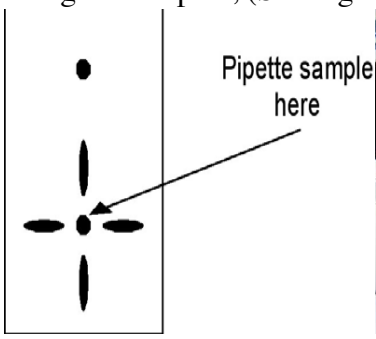


GE Nanovue Plus



1. Turn the **power on** by pressing On/Off key on the top far-right side of the spec face.
2. **Select** "**1**" on the touchpad to select the *Life Science* folder.

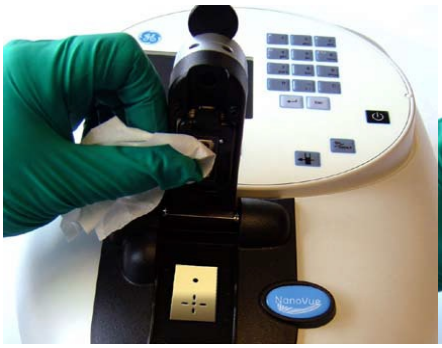
3. **Select** the specific life science **application** you require (6 types: DNA, RNA, Oligo, Tm Calculation, Cy Dye, Protein).
4. **Lift** the **sample head** to the vertical position & **pipet 2 uL** of **sample** volume onto the hydrophobic gold-plated surface.
   *NOTE: Be sure the pipetted sample sits over the black spot between the four alignment spots, (See Fig. Below) & do not introduce bubbles into the sample.



5. **Gently lower** the **sample head** onto the top of the sample. Be sure the pipetted sample resembles a uniform droplet & is NOT spread-out. A noticeably spread-out sample indicates the target area may be contaminated & requires cleaning (see below).
6. Use the **0A/100%T &**  buttons to **take reference & measurement** scans respectively.
   *NOTE: It is not recommended to raise & lower the head repeatedly to take multiple measurements of one sample; this can cause the droplet to disperse.

   If repeat measurements are required, use the  button.

7. If you prefer, **Select** "**2**" on the keypad to **Print** your results.
8. After the reading has been taken, **gently lift** the **sample head** & **Recover** the **sample** using a pipette.
9. **Clean BOTH** top & bottom **plates** by wiping the surface with a kimwipe. Wipe the bottom plate towards you & the top plate upwards. This avoids contaminating the return light path located on the rear of the bottom plate. (See above fig.)

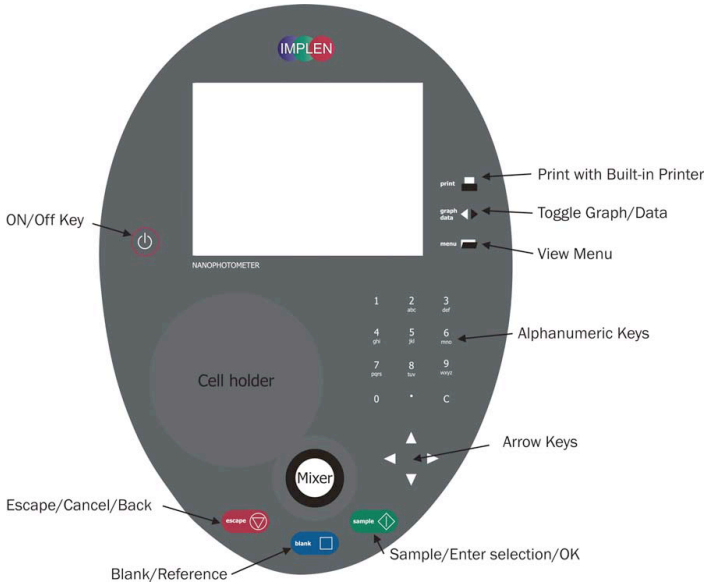



For more in-depth use of NanoVue Plus options & applications, contact Gemeia (gmcameron@gsu.edu) or Ping (pjiang@gsu.edu).

Implen Nanophotometer P-360

1. Applications and Overview

Nucleic Acids- dsDNA, ss DNA, ssRNA, oligonucleotides
Proteins- Protein UV (A280), Protein Dye, Bradford, Lowry, BCA
Cell Density- for bacterial cell cultures
General Functions- Specific wavelengths, wavescan



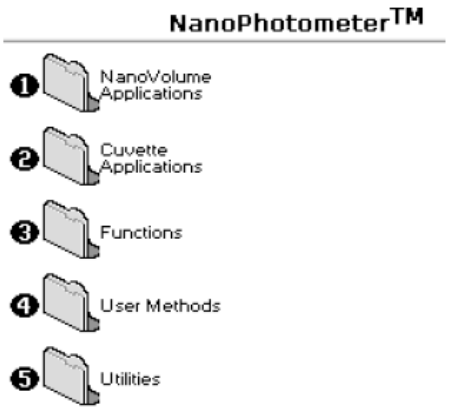
Key	Action
On/Off Key	Turns the instrument on/off.
Arrow Keys	Use the four arrow keys to navigate around the display and select the required setting from the active (highlighted) option.
View Menu	View menu for that application mode. Some of these are common to all applications and described on page 8. Menu unique to an application are described in the relevant section of the NanoPhotometer® P-Class User Manual.
Alphanumeric Keys	Use these to enter parameters and to write text descriptions where appropriate, or required. Use repeated key presses to cycle through lower case, number and upper case. Leave for 1 second before entering next character. Use C button to backspace and 1 to enter a space.
Escape/Cancel/Back: ▽	Escape from a selection and return to the previous folder. Cancel a selection. Stop making measurements.
Blank/Reference	Set reference to 0.000 A or 100%T on a reference solution at the current wavelength in the mode selected. When in scan mode, does a reference scan.
Sample/Enter Selection/OK: ◇	Enter, or confirm a selection. Take a measurement.
Print (P 330 and P 360 only)	Prints the results shown on the screen on the built-in printer, if a built-in printer is connected to the NanoPhotometer®.
Graph/Data (P 330 and P 360 only)	Toggle graph on/off. The graph shows a wavescan plot across the range 220 nm to 400 nm (for Dye methods 220 nm to 750 nm) with cursors denoting 230, 260, 280 and 320 nm (Nucleic Acid methods) and 260, 280 and 320 nm (Protein methods).

2. Start and Menu Options:

Turn power on and wait.

Choose application fold with numeric pad (ex, #1 for Protein)

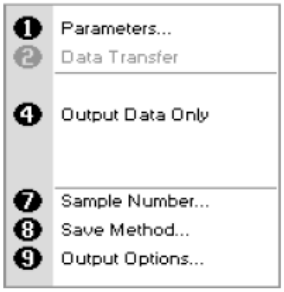
The user interface is built around folders which are displayed on the main screen when the instrument is switched on. Different folders are numbered and opened by using the associated number key on the keypad. After switching on the NanoPhotometer® a self-calibration check is performed and the default main screen "NanoPhotometer®" is offering the choice of:



Keypad number	Description
1	Life Science methods such as nucleic acid assays and protein assays using the NanoPhotometer® P-Class Submicroliter Cell
2	Life Science methods such as nucleic acid assays, protein assays and cell density using cuvettes
3	General spectroscopic methods
4	Contains nine folders that can store user adapted methods (up to 81)
5	Instrument set up (date, time, number format), Output Options and Baseline Compensation set up.

Menu Options:

Menu options for P 330/P 360:



After each measurement the following options are possible in the Menu:

1) Return to parameter screen.
2) Transfer the results via selected Output Option.
4) Toggle on/off the graph in the print-out or saved file.
7) Define the sample number you wish to start from.
8) Save the parameters as a method.
9) Open Output Options settings, possibility to change the Output Options settings within the method as described in 7.3 Output Options / Printer

Exit ***Menu*** by pressing ***Escape*** ⊘, **OR** wait

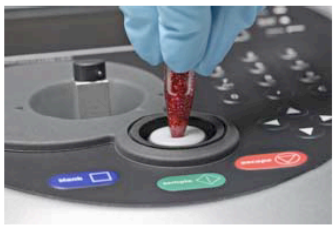
3. THE NANOPHOTOMETER® P-CLASS SUBMICROLITER CELL

With its innovative optical pathway the cell is designed for optimum measurement results with submicroliter samples ranging from 0.3 µl up to 5 µl of undiluted sample. Due to a pathlength of 0.04 mm, 0.1 mm, 0.2 mm, 1 mm and 2 mm the cell is offering an automatic dilution of 1/250, 1/100, 1/50, 1/10 and 1/5 in comparison to a standard cuvette measurement. Because the measurements are processed with undiluted samples, the reproducibility of the results is extremely high. If desired, samples can be retrieved after the measurement for further processing. The NanoPhotometer® P-Class Submicroliter Cell can be used for all UV/Vis analysis utilizing the wavelength range of 190 nm to 1,100 nm.

The NanoPhotometer® P-Class Submicroliter Cell is delivered for version P 300 with one lid with a pathlength of 0.2 mm (Lid 50), for version P 330 with two lids pathlength 0.2 mm (Lid 50) and 1 mm (Lid 10) and for version P 360 with three lids pathlength 0.04 mm (Lid 250), 0.2 mm (Lid 50) and 0.1 mm (Lid 10). Lid 5 (2 mm pathlength), Lid 100 (0.1 mm pathlength) and Lid 250 (0.04 mm) can be ordered optionally. The dilution factor (lid factor) is printed on the lid. Please make sure that you use the appropriate lid for your sample.

3.1 Technical instructions

Step 1 Insert the NanoPhotometer® P-Class Submicroliter Cell into the cell holder with the cell windows facing the light beam. We recommend facing the Implen logo to the front. The light beam is directed from RIGHT to LEFT as indicated with small arrows. Insert the NanoPhotometer® P-Class Submicroliter Cell always in the same direction.

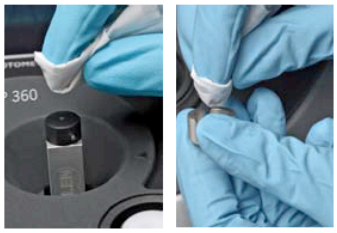


Step 2 Use the integrated vortexer (P 330 / P 360 only) to mix your sample well to achieve an accurate homogeneity of the sample.

Step 3 Pipette the appropriate sample volume onto the centre of the measuring window. **Warning!!** Do not overfill the well.

Lid	Sample volume	Pathlength	Dilution
5 (optional)	3.5 – 5 µl	2 mm	1:5
10 (optional for P300)	1 – 3 µl	1 mm	1:10
50	0.3 – 2 µl	0.2 mm	1:50
100 (optional)	0.3 – 2 µl	0.1 mm	1:100
250 (optional)	0.3 – 2 µl	0.04 mm	1:250

Step 4 Make sure that for the measurements the lid fits exactly onto the positioning supports mounted to the body of the cell. Take measurement. **Remember** to consider the lid factor in your instrument software. Please refer to the NanoPhotometer® P-Class User Manual for detailed information.



Step 4 Take the lid off and retrieve the sample with a pipette for further applications if desired. Remove sample residues from the measurement window and the mirror in the lid. Clean the measurement window and mirror in the lid well with a slightly wet fluff-free tissue. Use water, 70% ethanol or isopropanol. Do not use aggressive solvents like strong acids or bases or organic solvents at any time.
Important Note: Residual fluffs must be removed for optimum performance

Your cell is ready for the next sample.

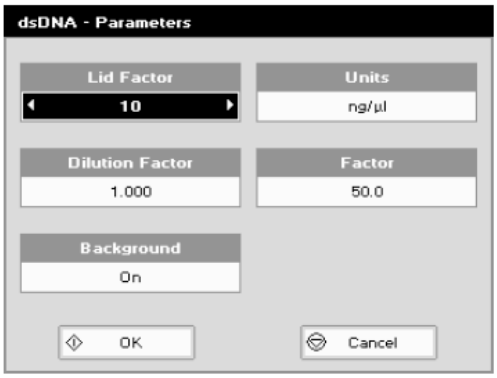
Operation Limitations: Do not autoclave the unit! Do not use an ultrasound bath to clean! Do not drop in water or solvent bath. The unit is water resistant, but not water proof!

3.2 Software instructions

The ***NanoVolume Applications*** and ***Cuvette Applications*** are very similar concerning the analysis of dsDNA, ssDNA, RNA, Oligonucleotides, protein UV and protein dye analysis. This section describes the specific features which have to be considered using the NanoPhotometer® P-Class Submicroliter Cell. For general information please follow the detailed instructions under Nanovolume Applications and Cuvette Applications.

The procedure is as follows:

Exemplary Parameter Screen



Parameter Screen

Step 1 Press 1 to select ***NanoVolume Applications*** folder
Step 2 Press 1 to select ***Nucleic Acids*** folder **OR** 2 to select ***Protein*** folder.
Step 3 Select the method you want to use by pressing the corresponding number.
Step 4 Select the ***Lid Factor*** using the left and right arrows.

Lid	Sample volume	Pathlength	Dilution
5 (optional)	3.5 – 5 µl	2 mm	1:5
10 (optional for P 300)	1 – 3 µl	1 mm	1:10
50	0.3 – 2 µl	0.2 mm	1:50
100 (optional)	0.3 – 2 µl	0.1 mm	1:100
250 (optional)	0.3 – 2 µl	0.04 mm	1:250

Step 5 Select subsequent parameters and specifications as described under **4.** ***Nanovolume Applications and Cuvette Applications.***

After the selections are confirmed the results screen displays in top left corner the chosen Lid and the required sample volume.

Nanodrop 2000/2000c

Field experience indicates that the following volumes are sufficient to ensure reproducibility:

Aqueous solutions of nucleic acids: 1 µL
Purified protein: 2 µL
Bradford, BCA, Lowry or Protein Pierce 660 nm assays: 2 µL
Microbial cell suspensions: 2 µL
It is best to use a precision pipettor (0-2 µL) with precision tips to ensure that sufficient sample (1-2 µL) is delivered.

Lower precision pipettors (0-10 µL and larger) are not as good at delivering 1 µL volumes to the measurement pedestal.
If the user is unsure about the sample characteristics or pipettor accuracy, a 2 µL sample volume is recommended.

Quick Start

1. **Double**-click the software icon and select the software application of interest from the right pane.

2. **Establish** a blank using the appropriate buffer or Water.
- Pedestal Option: Pipette 1-2 µL of the appropriate blanking solution onto the bottom pedestal, lower the arm and click **Blank**.
Note: The arm must be down for all measurements.

3. **Wipe** away the blank from the measurement pedestals using a dry, lint free laboratory wipe. Enter the sample ID in the appropriate field. **Pipette 1 µL** of sample and click **Measure**.
Note: A fresh aliquot of sample should be used for each measurement.

After the measurement:
- Simply wipe the upper and lower pedestals using a dry lint free-laboratory wipe and the unit is ready for the next sample.
- It is recommended that a new blank be taken every 30 minutes when measuring many samples in one measurement session. After 30 minutes, the time since the last blank measurement will be displayed in the bottom status bar.

Pedestal Basic Use

1. Raise the sampling arm and pipette the sample onto the lower measurement pedestal.

2. Lower the sampling arm and initiate a spectral measurement using the software on the PC. The sample column is automatically drawn between the upper and lower pedestals and the measurement is made.

3. When the measurement is complete, raise the sampling arm and wipe the sample from both the upper and lower pedestals using a dry, lint-free laboratory wipe. Simple wiping prevents sample carryover in
Subsequent measurements for samples varying by more than 1000 fold in concentration.

4. Put dry, lint-free laboratory wipe in between pedestal. Close the software.