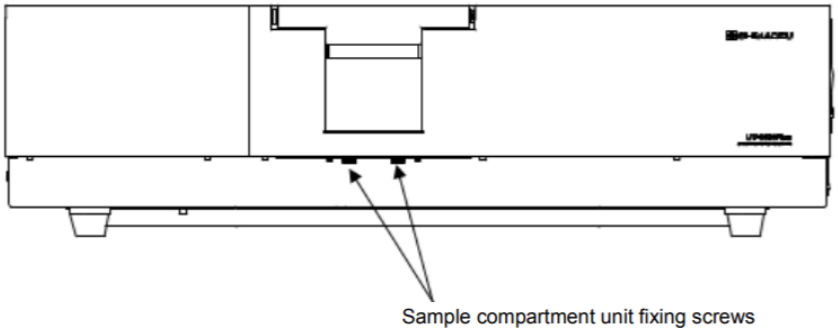
OPT4 Operating Procedure for Temperature Analysis using the 8-Position Micro Multi Cell Shimadzu UV-3600 UV-Vis-NIR Spectrophotometer

See Shimadzu Instruction Manual for TMSPC-8

1. Create or Start a Reservation for OPT4 in CoreResearch
2. Remove the sample holder assembly from the sample compartment
3. Install the TMSPC-8 8-cell holder into the sample compartment
    a. Unscrew the front two screws until the screws drop
    b. Remove the standard sample accessory



    c. Place the TMSPC-8 8-cell holder into the sample compartment. Make sure the slot at the back of the 8 cell holder goes under the screw at the back of the holder.
    d. Screw in the front two screws
    e. Connect the communication cable to the socket on the inside of the UV-3600 sample chamber and the two external cables to the temperature controller. ***Never connect the communication cable to the socket on the inside of the UV-3600 sample chamber unless the green power switch is off on the side of the UV-3600 instrument.***
4. Circulating cooling water is required if you want to take measurements below room temperature or if you want to include a ramped cooling cycle in your measurement program. If using cooling water:
    a. Cooling water lines from the 8-cell holder compartment should be connected to the Fisher Isotemp Refrigerated Circulator
    b. Verify the water level in the bath. If low, fill with tap water (**not DI water)**. A tap water faucet can be found on the safety shower in the Sample Prep lab.
    c. Turn on the power switch to the circulator (located on the side of the controller)
    d. The typical set point is 22C. The set point should be no higher than the maximum temperature you wish to achieve while collecting data. To change the set point, press the set/enter button and use the arrow keys to select the desired temperature. Press set/enter to accept the new set point.
    e. Verify that there are no leaks in the water lines from the circulator to the cell holder compartment
5. Turn on the green power switch located on the side of the UV-3600 instrument

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6. Turn on the temperature controller power switch located on the back of the controller

There are two choices of software to use with the TMSPC-8 8-cell holder:

- LabSolutions UV-Vis should be used when collecting Absorption or Transmission versus Wavelength at a single set temperature (This Page)
- Tm Analysis should be used when collecting Absorption or Transmission versus Temperature at up to 3 set wavelengths (Page 4)

Using LabSolutions UV-Vis Software (Absorption/Transmission versus Wavelength)

1. Open the "Lab Solutions UV-vis" on the computer.
2. Click "spectrum".
    a. Environmental settings are where the number of significant figures can be changed. Click number of digits and set values to change this.
3. Click "instrument control".
    a. Load your previously saved method file by clicking the "Read" button. If you do not have a saved method file, load the "8cell default method".
4. To create or edit a method file, click "edit".
    a. Enter starting and ending wavelength.
    b. Set desired data interval (typical value is 1 nm).
    c. Set desired scan speed (typical is medium or slow).
    d. Set the value type/measurement type to Absorbance or Reflectance.
5. Click on "Advanced".
    a. Set the desired slit width to 5 nm.
    b. Slit program should be "standard".
    c. Light source should be "automatic".
    d. Detector unit, sample side should be "Direct Receiving of Light".
    e. S/R switch should be "standard".
    f. Ensure that "perform stair correction" is checked.
    g. Click "OK".
6. Click on "Accessories"
    a. Select "8-Series Multi-Cell" in the left window
    b. Select the number of cells (samples) you will be using from the Number of Cell pull down menu
    c. Click "OK"
7. Click on "close after creating new parameter file".
    a. Enter the filename of the new parameter file and save it in parameter folder.
    b. Click "close".
8. Enter a file name.
9. Load the empty 8-cell holder with the dot facing the front. Make sure that no samples are present in the cells. Screw on the cover for the 8-cell holder and close the sample chamber cover on the UV-Vis instrument.
10. Click on the "Connect" button. The system will go through several initialization checks that will take 10 minutes. When completed, click the OK button.
11. Click on the Initialize button. Make sure the sample chamber cover is closed and then click OK. It will take a couple of minutes for the holder to initialize.
12. When initialization is complete, the buttons for manually moving the holder to the various sample positions will be activated.

13. Perform a baseline correction
    a. A baseline correction should be performed at start-up, and anytime you change the parameter settings.
    b. Make sure that no samples are present by selecting an empty cell position using the cell next button and make sure the sample chamber cover is closed.
    c. Click “baseline”. Confirm wavelength range. Click “OK”.
14. Load the samples into the in the 8-cell holder, and if desired, load a reference into the reference compartment.
    a. Load your samples in the 8-cell holder. Each well holds approximately 125 microliters of liquid.
        i. Click the Preset button to enter a Sample Name and a File Name for each sample. The front compartment (closest to the dot) is position 1.B. The number of samples should match the number of cells selected previously in the method file.
    b. Move to cell position 1if not already in that position.
    c. To manually set the desired measurement temperature (see pages 2-6 and 2-7 in the Instruction Manual for TMSPC-8), use the front panel of the temperature controller to:
        i. Press Mode button to select Normal mode
        ii. Use the Up/Down arrows to select the desired temperature
        iii. Press Start button
15. If you are cooling your sample below room temperature, turn on the flow of nitrogen gas. This will prevent condensation accumulating on the outside of the 8-cell holder.
16. Click the “Start” button to initiate the measurement. *Note – The Start button will measure all specified sample cell positions automatically. The “1 Cell” button will only measure the sample cell position that is currently selected.*
17. Adjust the overlay graph Y axis values as desired (the X axis will scale automatically)
18. When the scan is complete ***the data is only stored in temporary memory, but not to the computer disk.***
19. To save the data to the computer disk:
    a. On “data print table”, there is an excel button. Open a blank excel book and then click the “excel” button. Your date should populate the open excel book. Save this file to your folder in the user data folder.
    b. Click “text out” to save data as a .txt file.
20. To workup your data, click the “active tab” in the top left corner of graph.
    a. “Peak” will peak pick for you. Click “execute” on the right side.
    b. “Peak area” will give a peak area for you when you select a desired wavelength range.
    c. “Peak pick” will let you select a peak and it will read its absorbance.
21. When finished, click “disconnect” in the top right of the software.
22. Close the “Lab Solutions UV vis” software.
23. Go to **Shutting Down the Instrument** (Page 5)

Using Tm Analysis Software (Absorption/Transmission versus Temperature)

1. Open the LabSolutions Tm Analysis software on the computer
2. Load the empty 8-cell holder with the dot facing the front, make sure that no samples are present in the cells, and close the sample chamber cover.
3. Click on the "Connect" button at the bottom of the screen. The system will go through several initialization checks that will take about 10 minutes. When completed:
    a. Click the OK button.
    b. A dialog box will appear that asks if you want to initialize the multi-cell unit. Click yes. It will take a couple of minutes for the initialization to finish.
4. Create a data collection method or load a previously saved method
    a. To load a previously saved method, choose "Open Method" from the File pull down menu and choose the desired method file
    b. To create a new data collection method, click on the Edit Method icon. See Chapter 5 (pages 5-1 to 5-8) in the Shimadzu Instruction Manual for TMSPC-8 for instructions on setting up a measurement method.
5. Save the data collection method by selecting "Save Method" under the File pull down menu. Choose your personal directory to save the file
6. Perform Autozero, Baseline Correction and/or Cell Blank
    a. Autozero or baseline correction should be performed at start-up, and anytime you change the wavelength range or slit width. Autozero is used when taking measurements at a single wavelength value and Baseline Correction is used when taking measurements at multiple wavelengths.
    b. Make sure that no samples are present in the reference holder and select an empty compartment position in the 8-cell holder using the cell move button on the photometer button bar. Make sure that the sample chamber cover is closed
    c. *If measuring at a single wavelength*:
        Click Autozero on the photometer button bar to autozero the reading between the reference compartment and the selected sample compartment.
    d. *If measuring at multiple wavelengths:*
        Click Baseline on the photometer button bar and verify or modify the displayed wavelength range. Click OK to zero the reading between the reference compartment and the selected sample compartment
    e. *Cell Blank:*
        Using the Cell Blank function will correct the absorbance affected by differences in dirt, residue or scratches of the individual cells. When multiple sample cells are used, this is the effective tool to correct the absorbance variation among the cells.
7. Use the front panel of the temperature controller to:
    a. Set the mode to 'external'. This will allow the software to control the temperature setting.
    b. Toggle the 'start/stop' setting to 'stop'. The software will automatically toggle the controller to 'start' when it begins data collection.

8. Load the sample(s) into the 8-cell holder and if desired, load a reference into the reference compartment. The samples should be loaded into the cell positions that were selected in the method file.
9. If you are cooling your sample below room temperature, turn on the flow of nitrogen gas. This will prevent condensation accumulating on the outside of the 8-cell holder.
10. Click the "Start" button on the instrument bar to initiate the measurement
11. Graph display settings can be modified by selecting "Settings" from the Graph pull down menu
12. To save the data as X-Y pairs:
    a. From the file pull down menu choose "Save As"
    b. From the "Save as Type" menu choose "Text File (*.txt)"
    c. Click on the "Select" button and choose the dataset you want to save. (Each sample and each wavelength will be saved to a separate dataset).
    d. Enter a filename and click "Save"
    e. Repeat for each dataset that you want to save as a text file
13. To perform analysis of the data (calculate the melting temperature Tm), click on the Tm-Analysis Icon.
    a. See Chapter 6 (pages 6-1 to 6-4) in the Shimadzu Instruction Manual for TMSPC-8 for instructions on performing data analysis.
    b. To save a table of the analysis results, select "Save Tm Table" from the "File" pull down menu.
14. When finished, Click "disconnect" in the bottom panel of the software.
15. Close the "Tm Analysis" software.
16. Go to **Shutting Down the Instrument** (Below)

Shutting Down the Instrument

1. Unload the 8-cell holder containing your samples
2. Turn off the flow of nitrogen to the instrument (if you were cooling below room temperature)
3. Turn off the power switch on the temperature control unit
4. Turn off the power switch on the Fisher water circulator (if used)
5. Turn off the green power switch on the side of the UV-3600
6. Unload the 8-cell holder sample compartment and install the sample holder assembly. ***Never unplug the communication cable from the socket on the inside of the UV-3600 sample chamber unless the green power switch is off on the side of the UV-3600 instrument.***
7. Stop or Update Actual Usage for your OPT4 reservation in CoreResearch
8. Remove your samples from the 8-cell holder and clean the 8-cell holder using DI water and/or alcohol. (DI water and alcohols can be found in the sample prep lab). When clean, return the 8-cell holder to the storage box.