

Hitachi U-4100 UV-vis-NIR spectrophotometer (341-F)

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Hardware

Our spectrophotometer is made up of these key components:

- Light sources: tungsten lamp (UV, visible and near IR) and deuterium lamp (deep UV)
- Monochromators, beam splitters, mirrors and focusing lenses
- Sample stage with holder: liquid (cuvettes) or solid (films)
- Detector: PMT detector (liquid) or integrating sphere (solid)

****Before you turn on the instrument, ensure that the appropriate sample stage with detector is installed. Make sure the instrument is off BEFORE you begin changing sample holders. See pages 8-9 for how to change detectors.****

Start-up Procedure

- 1) Install appropriate sample stage before turning the spectrophotometer on.
- 2) Flip the black switch on the upper left hand side of the front of the spectrophotometer box. You will hear the lamp ignite, followed by a high-pitched buzz. (If you don't hear this sound, please contact an MCF scientist for assistance!) The lamp will need about 30 minutes to warm up before good-quality data can be obtained.
- 3) Open the “**UV Solutions**” program by clicking on the icon on the computer desktop. This will initialize and synch the spectrophotometer with the program. A **Measurement window** will appear on the main screen. The status in the bottom right hand side of the Measurement window will read “Ready” when initialization is complete.

Data Acquisition (wavelength scan)

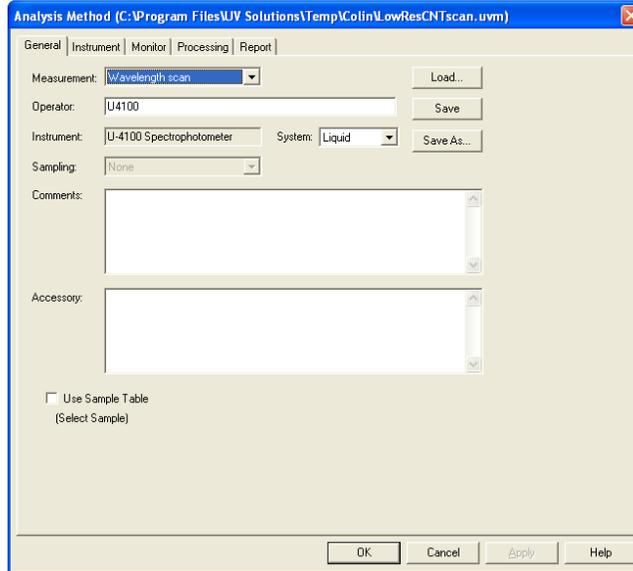
The vertical toolbar on the right hand side of the screen has four buttons. The easiest way to ensure that you don't forget any key parameters for data acquisition and don't save over someone else's data is to go through the functions of each button sequentially.



- 1) **Method** → opens **Analysis Method** window
 - a. **General** tab:
 - i. **Measurement**: choose measurement type (wavelength scan, time scan, or photometry)
 - ii. Enter the operator's name



- iii. *System*: select liquid or solid **this should correlate with sample stage installed**
- iv. Insert any comments about the sample and data acquisition; uncheck “Use Sample Table”
- v. A saved analytical method can be opened by clicking the “load” button
- vi. The inputted analytical method can be saved by clicking the “save” or “save as” buttons



b. **Instrument** tab

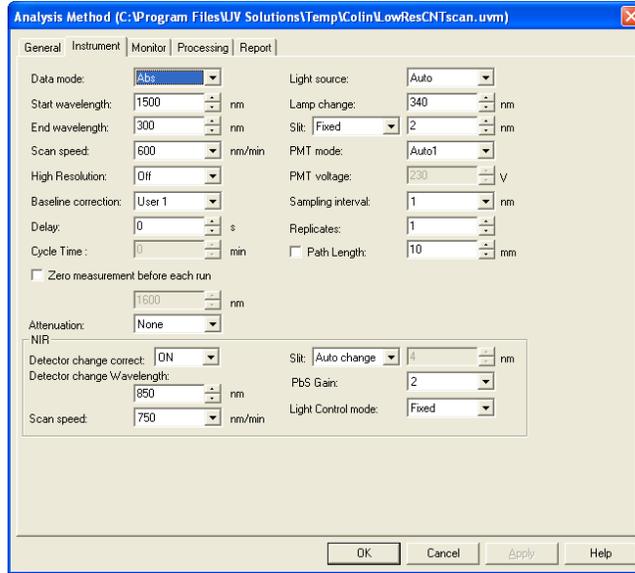
- i. *Data mode*: chose to collect transmittance (%T), absorbance (Abs), energy on sample side E(S), energy on the reference side E(R), or reflectance (%R) data
- ii. *Start wavelength*: 176.0-3300.00 nm
- iii. *End wavelength*: 175.0-3299.00 nm
- iv. *Scan speed*: select a scanning speed (nm/min); for general measurements, 120-600 nm/min works well
- v. *High resolution*: usually turn this off so that automatic smoothing is performed (Savitsky-Golay method) to eliminate noise; if you have a very steep signal, it may be helpful to turn it on
- vi. *Baseline correction*: select “User1”
- vii. *Delay*: can be set at 0-3600 seconds; time during which the spectrophotometer will sit idle prior to collecting a measurement; this is useful if a sample needs time for temperature stabilization, etc. prior to data collection
- viii. *Cycle time*: only relevant if you set Replicates at 2 or more; this is the amount of time that will elapse between the start of a measurement to the beginning of the next (see xvii)
- ix. *Zero measurement before each run*: usually leave box unchecked
- x. *Attenuation*: none
- xi. *Light source*: generally select Auto

Liquid Detector: 190-3300 nm
Integrating Sphere: 220-2600 nm

- xii. *Lamp change*: 340 nm is default wavelength where deuterium and tungsten lamps will change; wavelength can be changed to 325.0-370.0 if 340 nm overlaps an important signal in your sample
- xiii. *Slit*: use fixed or auto (adjusts slit width automatically in response to optical energy intensity at the detector); for “fixed” input slit-width
- xiv. *PMT mode*: Auto1
- xv. *PMT voltage*: can be changed to remain at a constant value when “auto” is chosen for slit width
- xvi. *Sampling interval*: input the desired interval between data points (there are limitations based on scanning speed)
- xvii. *Replicates*: number of times you would like to repeat the measurements; usually set at 1 unless you have a spectrum that will change over time and would like to monitor it (replicate measurements will be performed using the Cycle Time in viii)
- xviii. *Path length*: when checked, a value for the sample’s pathlength can be entered; if the chosen pathlength is not 10 mm, the Abs spectrum will automatically scale the data to pathlength = 10 mm

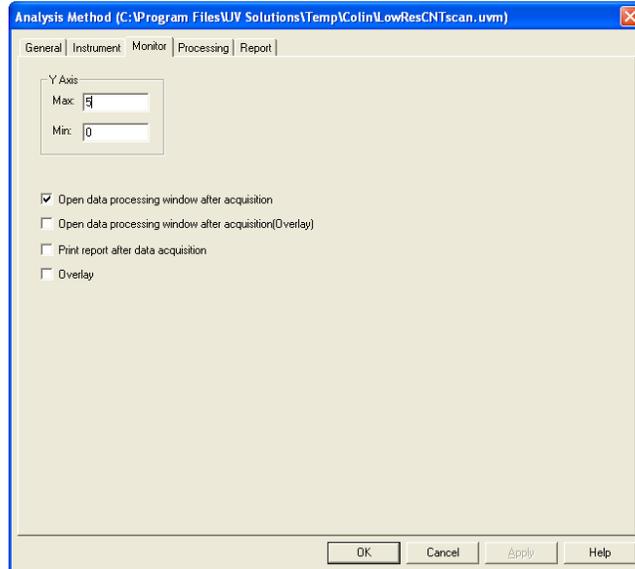
In NIR box

- xix. *Detector change correct*: ON automatically corrects for a difference in the photometric value at each detector
- xx. *Detector change wavelength*: 850 nm is default wavelength; can be adjusted at 700.00-900.00 nm if an important signal in your spectrum occurs at 850 nm
- xxi. *Scan speed*: can be changed, but default generally is fine
- xxii. *Slit*: as for the PMT detector above (xiii), Auto change allows the slit to be adjusted to maintain a constant optical energy intensity at the detector
- xxiii. *PbS Gain*: usually select 2, unless you need higher resolution measurements in NIR (select 3 or 4; however, there will be increased noise)
- xxiv. *Light control mode*: generally use “fixed” unless you chose a fixed slit width (then choose “auto change”)



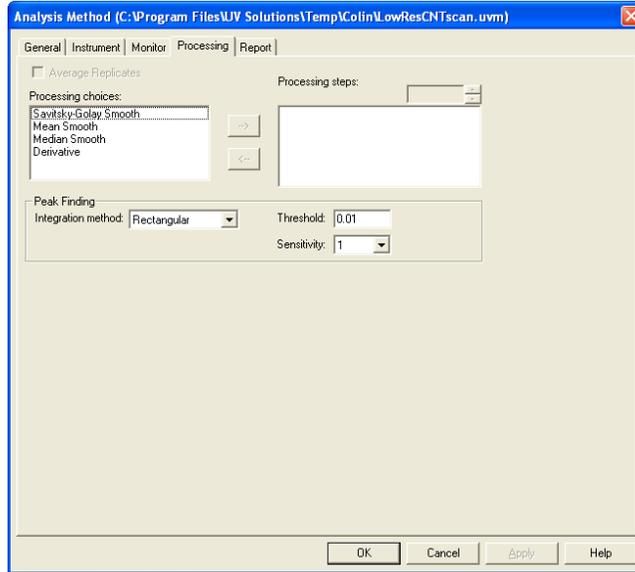
c. **Monitor** tab

- i. *Y-Axis*: allows you to set the scale for the Measurement window (which can also be autoscaled to fit data during collection)
- ii. Check the “Open data processing window after acquisition” box and generally leave the other boxes unchecked



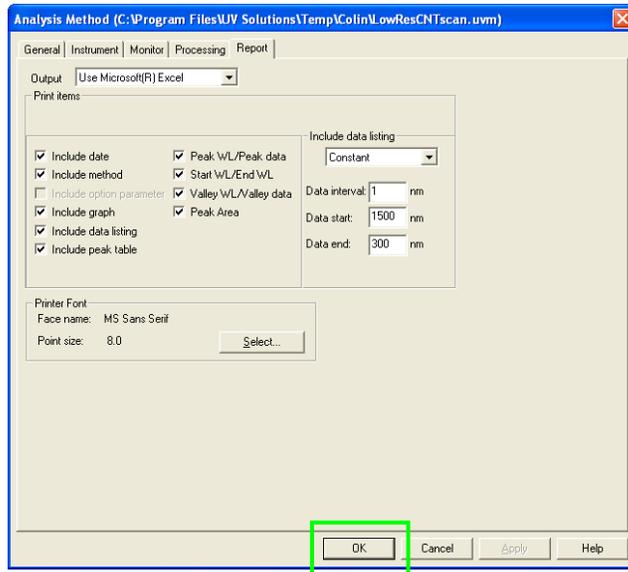
d. **Processing** tab

- i. You can choose to average the replicates together if “Replicates” were selected in the Instrument tab
- ii. You can also choose smoothing or derivative functions to apply to your data; generally no smoothing is necessary if you have turned the High Resolution off.
- iii. *Peak finding*: select Rectangular and adjust Threshold and Sensitivity as needed (generally defaults are fine)



e. **Report** tab

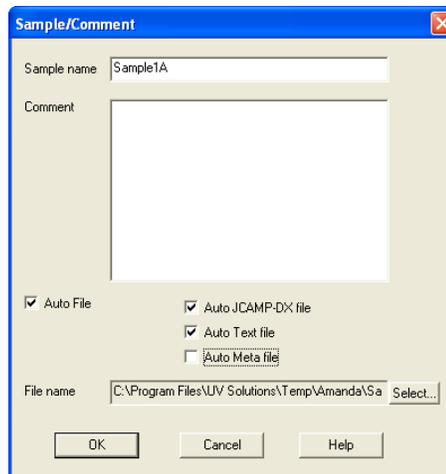
- i. *Output*: usually select “Use Microsoft (R) Excel”
- ii. Check the boxes for the data parameters you would like to include in the report
- iii. If you checked “include peak table,” you can input the data range and interval you wish include *****Generally, this should match your Sampling Interval and Start/End wavelengths from the Instrument***



tab**

- f. Click OK at the bottom of the **Analysis Method** window to save your acquisition parameters

- 2)  **Sample** → allows you to name your sample and choose the directory and filename it will be saved in
- Name your sample
 - Check boxes for which types of files you would like to generate (usually use default setting)
 - Select or create a directory and folder to save your files in and name the file
 - Click OK



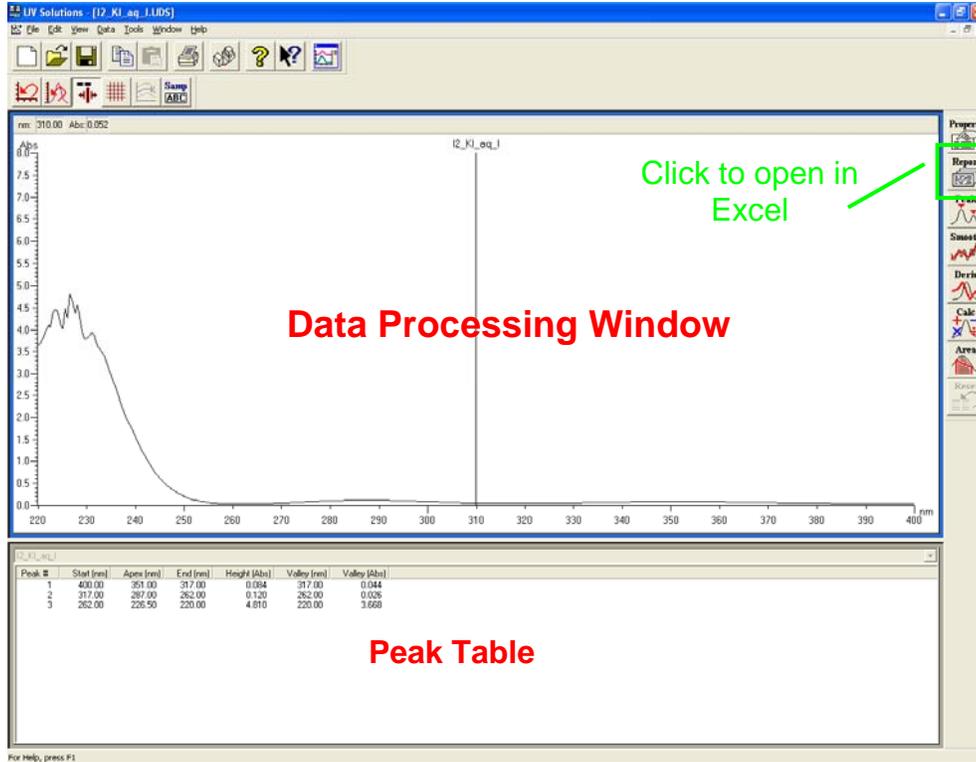
- 3) Insert cuvetts both filled with only your chosen solvent into the reference and sample positions; for solids, insert empty quartz slide “sandwiches” in the reference and sample positions

- 4)  **Baseline** → collects a baseline reading with “blank” samples in both the reference and sample positions



- 5) Place your sample in the sample position

- 6) Press the  **Measurement** button to acquire data; upon completion of measurements, data will be saved and a **Data Processing Window** will open containing your spectrum (and peak table if chosen)
- 7) In **Data Processing** window, click the **Report** button on the right hand side to open an Excel spreadsheet file containing your data.
- 8) You can print both the spectrum and peak table from the Data Processing Window by clicking the **Print** button on the top tool bar.



Shut-Down Procedure

- 1) Remove samples from spectrophotometer
- 2) Save all data and close UV Solutions program
- 3) If there is a user coming immediately after you, leave the instrument on
- 4) If there is no user immediately after you or the next user needs to change the sample stage, turn the instrument off.

Please be very careful...

- **BE GENTLE** when changing one sampling stage to the other.
- The liquid stage has several lenses and windows in it that can get scratched or misaligned if it is not handled gently.
- The integrating sphere is made of **INCREDIBLY** fragile material. Take **extreme** care not to handle it gently (ie, do not slam it down onto the table or drop into place inside the spectrophotometer as this could crack the barium sulfate coating inside it).
- Be careful not to drop or break barium sulfate wafers used at the sample and reference ports of the integrating sphere.
- **ALWAYS** gently place the unused sample stage/detector into the box provided on the lab bench.
- **ALWAYS** cover the integrating sphere and the windows of the liquid sample holder with foil when not in use.
- **ALWAYS** close the box to prevent dust from settling getting into the detectors.

Changing Detectors

Liquid Detector to Integrating Sphere

1. Ensure instrument is off
2. Remove two screws in base plate and loosen thumb screw
3. Detach all three cables (black connector, silver connector and white connector)
4. Open front door of instrument
5. Grab two handles and carefully lift liquid detector out and place on the table
6. From the storage box, grab the large base plate and place into instrument by aligning the screw holes with the gold threaded screw ports in the instrument. (Make sure not to trap white connector cable underneath.)
7. Secure with the two screws from the liquid detector's base plate and the additional screw inside the blue toolbox
8. VERY gently and carefully remove the foil from the integrating sphere base plate and place into instrument on the right side. You will have to slide it underneath the lip of the large base plate. Ensure the open holes on the integrating sphere base plate fit over the "peg" screws on the sample chamber's rails. ***If these "pegs" are missing, please contact me immediately! They should NEVER be removed!***
9. Plug in the silver connector (match up the shapes) and the black connector (dot on black connector goes down; if there is any resistance, do not force this cable in)
10. Tuck the cables behind the integrating sphere and the mirror so they are not in the beam path
11. Put the sample holder onto the large base plate (pegs fit into holes). It will be touching the integrating sphere when it's properly positioned.
12. Return the liquid detector to the storage box and cover the quartz windows with the foil pieces.
13. Turn on the instrument

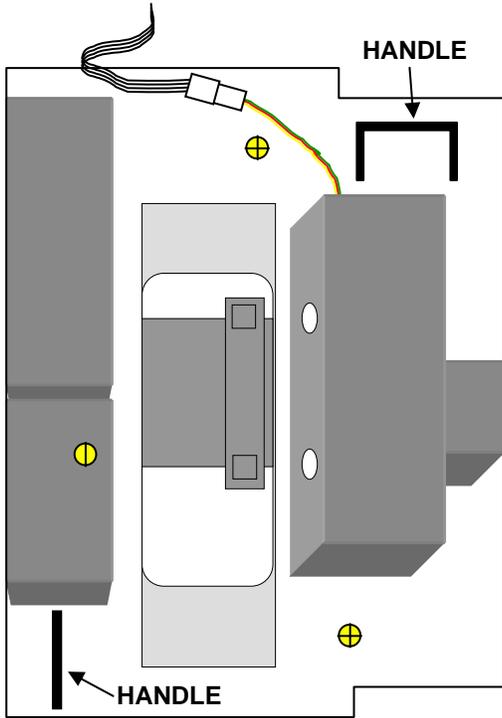
Integrating Sphere to Liquid Detector

1. Ensure the instrument is off
2. Remove the sample holder from the large base plate and place on the table
3. Remove the three screws securing the large base plate
4. Place the large base plate on the table
5. Detach the two cables (silver connector and black connector)
6. Remove the cables from the clips on the back and right walls
7. VERY gently and carefully lift out the integrating sphere base plate and GENTLY place on the table
8. Open the front door of the instrument
9. From the storage box on the desk, grab the liquid detector (remove foil) by the two handles and place into instrument by aligning the screw holes with the gold threaded screw ports in the instrument. (Make sure not to trap the white connector cable underneath.)
10. Secure the liquid detector with the thumb screw on the left side and two of the screws that were removed from the integrating sphere's large base plate. Place the remaining screw inside the blue toolbox.
11. Plug in the three cables: white connector, silver connector and black connector (dot goes down)
12. Place the integrating sphere's large base plate into the storage box first

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13. VERY gently and carefully place the integrating sphere into the storage box and cover both the sphere and mirror with the foil pieces
14. Place the sample holder inside the storage box
15. Turn on the instrument

LIQUID DETECTOR



INTEGRATING SPHERE

