SPR User Manual

Sample Cell:

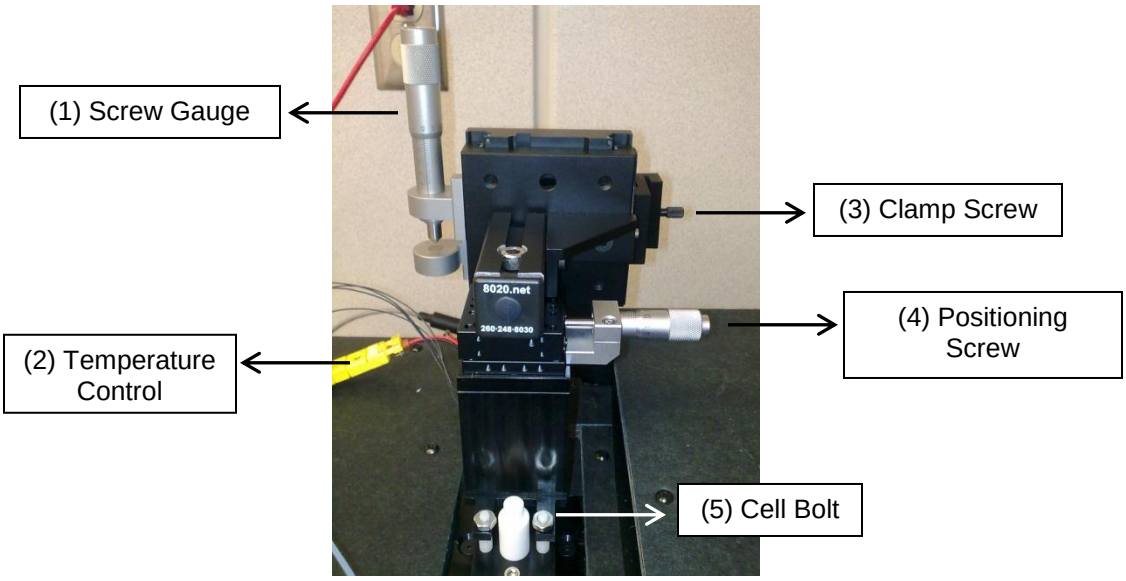

Fig. 1: Sample Cell properly attached to the stage

1. Turn the screw gauge **(1.1)** to raise the stage off the glass slide
2. Loosen the screw on the right side of the stage **(1.3)** thereby separating the stage from the vertical clamp stand
3. To change cells, loosen the two bolts **(1.5)** holding the cell to the stage. Tighten the bolts with the desired cell in place
4. Clean the prism with isopropyl alcohol or ethanol
5. Completely cover the surface of the prism with 1.52 refractive index liquid
6. Gently place the new gold coated glass slide onto the prism with the gold side facing up ensuring it is held tightly in place by the built-in grooves on the slide holder
7. Attach the stage onto the vertical stand and lower the stage so that the O-ring of the cell presses on the slide and is held in place by the weight of the stage and the built-in spring system. (The screw gauge **(1.1)** completely lifts off the base)
8. Attach the temperature control cable of the cell to the controller **(1.2)**.

Experimental Apparatus & Equipment:

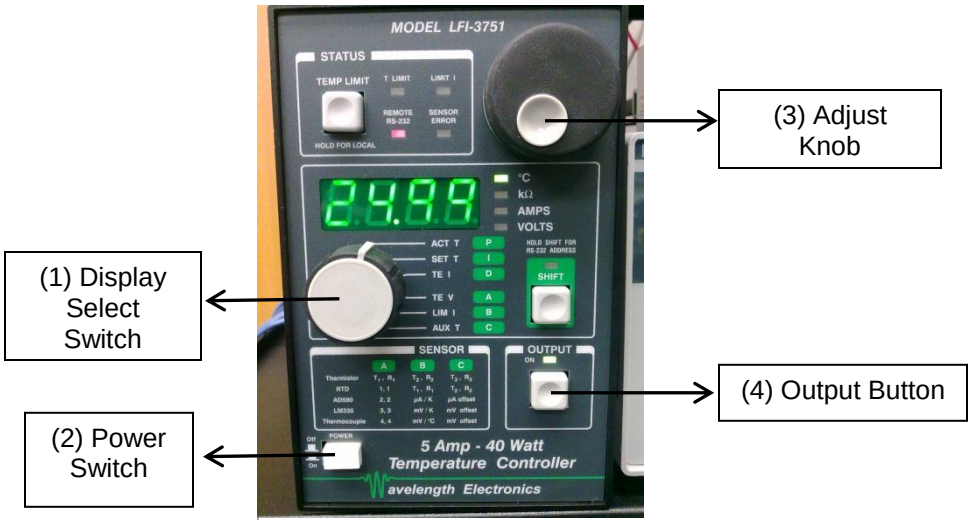

Fig. 2: Temperature Controller

1. Turn on the power strip
2. Turn on the temperature controller **(2.2)**
3. Turn the display select switch **(2.1)** to "SET T" and rotate the Adjust Knob **(2.3)** to choose the desired setpoint temperature (between 14C and 45C).
4. Turn the select switch **(2.1)** back to "ACT T" and press the "Output" button **(2.4)**.

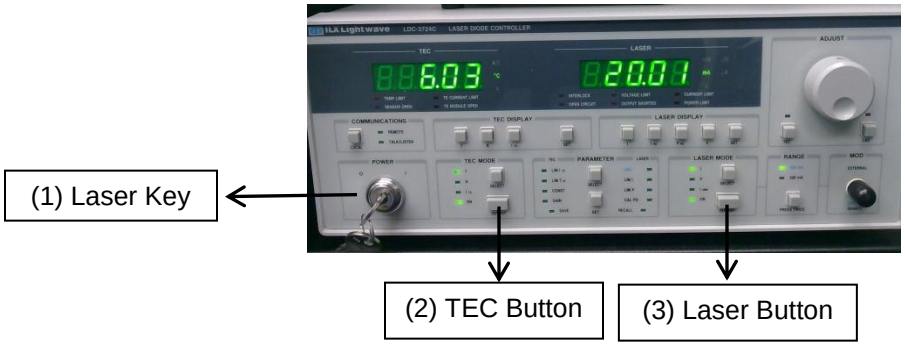

Fig. 3: Laser Controller

5. Turn the key of the laser control to on **(3.1)**
6. Turn on the TEC **(3.2)**
7. Turn on the Laser **(3.3)**
8. Wait for both to stabilize (usually 30mins) at 6C and 20mA

Fig. 4: Hamamatsu CCD Controller

9. Turn on the Hamamatsu CCD **(Fig. 4)**

Software:

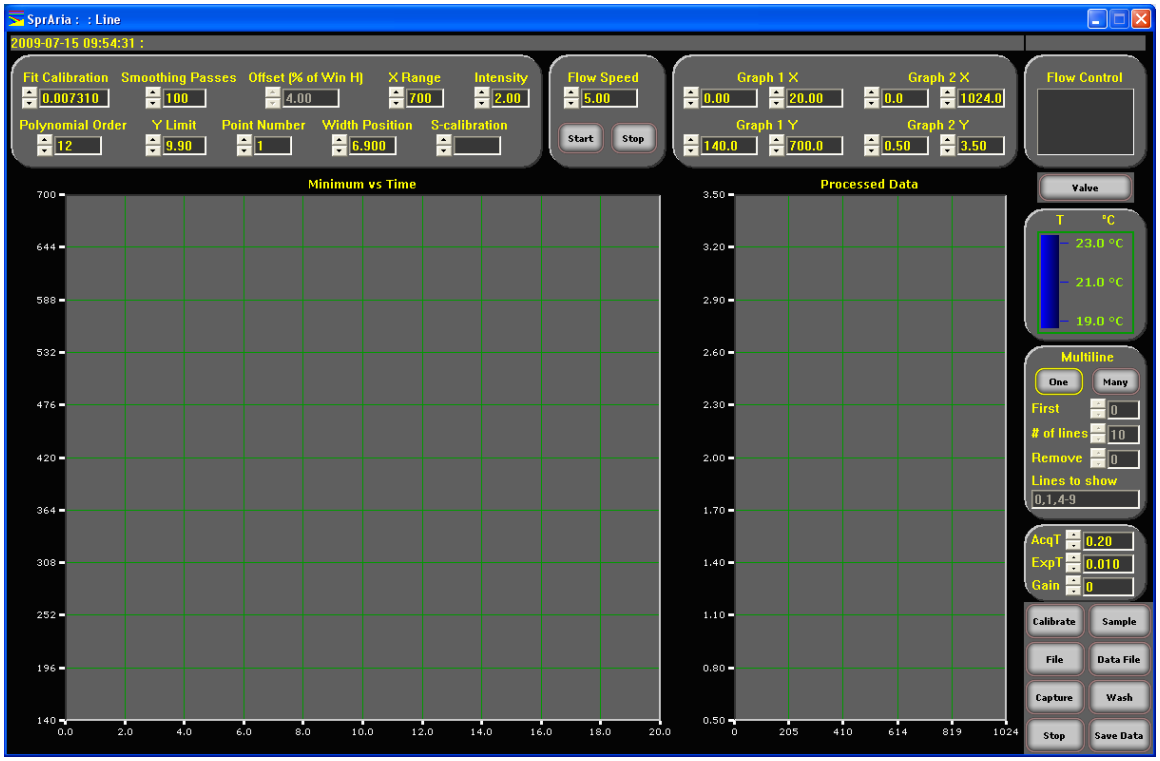

Fig. 5: SPR ARIA

1. Start the SPR Aria Software

Fig. 6: Multiline Control

2. Choose "one" or "many" in the multiline option, depending on the situation **(Fig. 6)**

Fig. 7: Acquisition Control

3. Choose the AcqT, ExpT and Gain (default: 0.5, 0.02, 0) **(Fig. 7)**

Fig. 8: Action Panel

4. Press "Sample" in the Action Panel **(Fig. 8)** to see a real-time curve of the sample

Fig. 9: SPR Curve Fitting Panel

5. Choose "Y-limit" in the Curve Fitting Panel **(Fig. 9)** to be the Y value below which you want to fit the SPR curve

6. Choose "Width Position" in the Curve Fitting Panel **(Fig. 9)** to be below the curve

7. Click "File" in the Action Panel **(Fig. 8)** and choose a file name for the SPR Minimum vs time plot to be saved

8. Click "Data File" in the Action Panel **(Fig. 8)** and choose a file name for the SPR Curve to be saved (a suffix with timestamp is added everytime the curve is captured)

9. Click "Capture" in the Action Panel **(Fig. 8)** to start taking SPR minimum vs time data. At any point press the "Save Data" option to save the current SPR curve

10. When done, press "Stop" in the Action Panel **(Fig. 8)**

11. All data is stored in Desktop\SPRARIA\DAT folder

Software (Flow Cell):

Pump control

Fig. 10: Flow Speed

The pump is controlled through the center option panel. The speed of the pump is the value of the dial in ml / min. The pump works in withdrawal mode when the sign of number is +. The pump will stop if the speed is set to 0, if less (-) than 0 it pumps in opposite direction, and when the Stop button is pressed - stops. Pump will NOT automatically start when Capture starts.

FLOW CONTROL

Fig.11: Flow Control Display

Flow control display window **(Fig. 11)** shows the order of the execution of the experiment. After double click on flow control display window (black space) the window of flow control modification **(Fig. 12)** opens. You can use flow control window to program your experiment: In position ("Posit") column of the table you can type the position (number from 1 to 5) of flow valve. Valve connects the volumes of reagents solutions that should be pumped through the SPR cell. In time column ("Time") type the time interval for how long this solution will interact with chip surface. In comments you can type information about this particular solution (protein, concentration, buffer, etc.). After typing protocol for experiment (sequence of solutions with time and comments) press "Apply" button and "OK ". The window **(Fig.12)** will be closed and in the window **(Fig. 11)** will be seen protocol in real time and it execution.

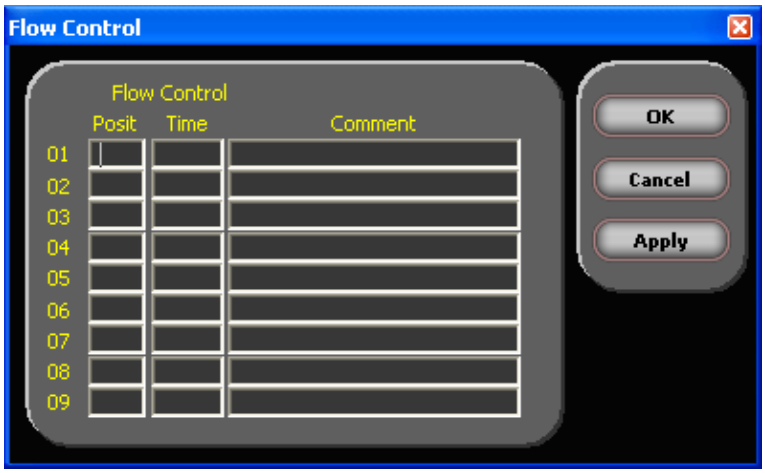

Fig. 12: Flow Control Modification

Support Valve

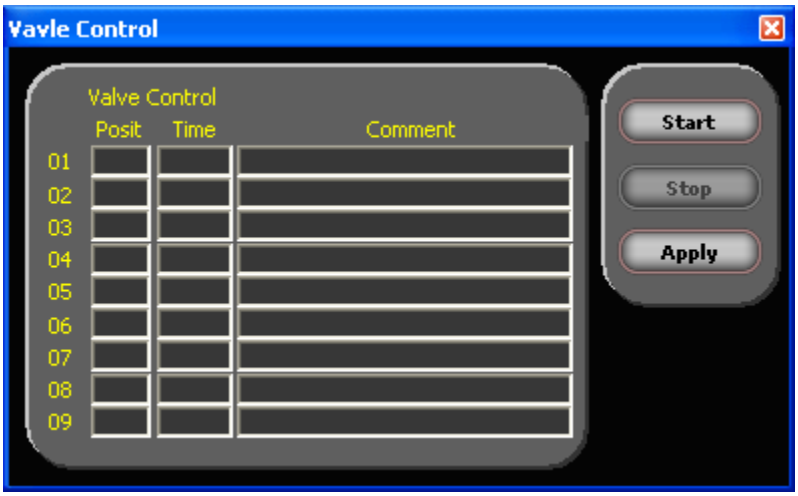

Fig. 13: Support Valve

Support valve is used to fill out empty tubing before experiment start and for washing or substituting reagents connected to particular input of flow control valve. To open the support valve window double click on the "Valve" button of flow control window **(Fig. 11)**. Use the same procedure to put information in the table as for flow control valve. Support valve has 8 ports. Numbers 8, 7 and 6 are closed for output. Ports from 1 to 5 are connected to the flow valve. Always return to the closed port after using support valve.

Appendix

Fitting Options

Fitting options window is used to submit the fitting, calculation and miscellaneous parameters that software uses to determine minima positions of SPR curve.

Fig. 14: Fitting Control

- Fit calibration is the conversion factor between pixels and angle. angle = calibration * pixel.
- Smoothing passes dictates the number of smoothing passes which the smoothing function will use.
- Offset (% of Win H) specifies the value of offset for curves plotted in the graph windows in Multiline regime.
- Polynomial Order specifies the order of the polynomial used to fit the data.
- Y Limit is the upper boundary for data used in polynomial fitting. Data with Y values greater than Y Limit are not used in the fitting function.
- Point Number: The fitting function will use every nth data point where n is the Point Number value.
- Width Position specifies where to determine the half-width.
- S-calibration is the factor used to accommodate the S polarization. All data values are multiplied by this factor.

Action Panel

Fig. 15: Action Panel

Action panel is used for experiment preparation and execution.

- Clicking “File” button the dialog box **(Fig.16)** opens, allowing set a file name for sensogram data (the data plotted in the graph 1 will be stored in this file with supported information.

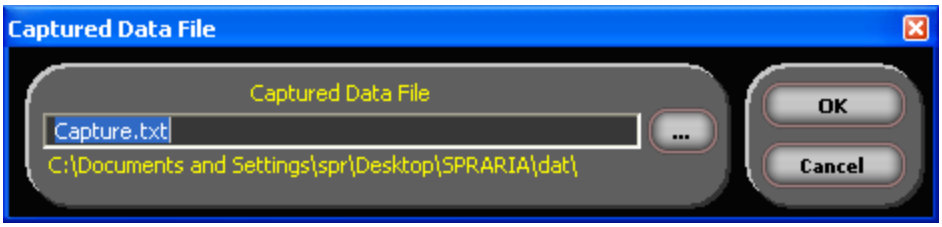

Fig.16: Captured Data File

- Clicking “Data File” button the dialog box **(Fig. 17)** opens, allowing set file name for row data presented in the graph. These data will be put in the file only when button “Save Data” is pressed any time during experiment. This procedure allows to store row data during experiment if necessary, especially on critical points of experiment say during background detection, when analyte is introduced in the cell, during adsorption and washing, in the same time avoiding to collect big files of unprocessed data.

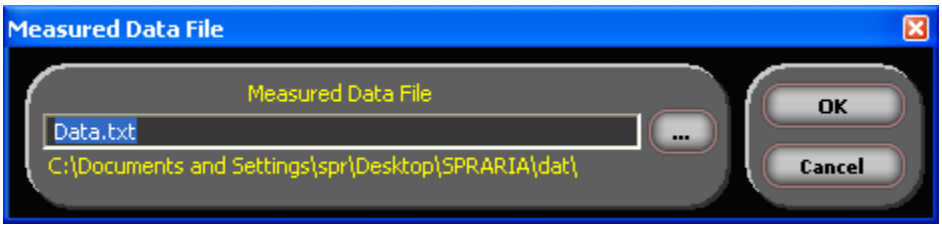

Fig. 17: Measured Data File

- Clicking “Capture” button the experiment will start, executing in the sequence protocol that you put in the flow control window. In the left top corner of the UI appears word “Capture” indicating that experiment is running.

- Clicking “Stop” button will stop the experiment running.

- Clicking “Wash” button will start procedure that flushes and wash flow system: all tubing, valves and pump using the protocol that you put in the flow control window. It similar to capture mode but runs without data measurement. In this case in the left top corner of the UI appears word “Wash” indicating that washing procedure is running.

- Clicking “Sample” button allows detection of the SPR signal in the graph 2 without processing data. Useful for preparation of the experiment. In the left top corner of the UI appears word “Sample” indicating sampling mode is running.

- Clicking “Calibrate” button allows change polarization from *p* to *s* and acquires the calibration curve. Calibration curve will be stored in special file and in the graph 2 will be displayed reflectivity curve $R=I_p/I_s$. In the left top corner of the UI appears word “Calibrate” indicating that calibration mode is running.

Linear Array Options

Fig.18: Line Control

Linear array options **(Fig.18)** allow switching between single line (button "One") and linear array or multiline (button "Many) measurements. By the default, program works in single line mode.

To switch to multiline mode - click on the button "Many" **(Fig.18)**. This will activate dimmed numbers in the dials in linear array options panel Fig. 14 and "Offset (% of Win H)" dial in fitting options panel **(Fig. 14)**.

The dials "First", "# of lines" and "Remove" are used to set the number of CCD lines to be measured and processed. Total number of CCD lines are 255 numbered from 0 to 254. Size of every pixel of CCD is 24x24µm.

- Dial "First" specifies what will be the first line used for measurements. For example, if number will be set for 8 – all data for lines between 0 and 8 will be omitted.

- Dial "# of lines" specifies how many lines you like to measure. For example, if you specify 10 lines and "First" equal 5 you will have 255-5= 250 CCD lines available for measurements. Every line on the CCR chip will correspond to 25 lines on CCD array and total width of the line measured will be 24 µm x 25 lines= 600 µm. Thus you will have 10 lines with width 600 µm.

- Dial "Remove" specifies how many lines should be skipped from processing. If the pattern on the SPR linear array is not sharp and SPR response is overlapping you can remove specified number of lines from the end of every line. For example, If "Remove" is set for 2 , then 2 CCD lines from boundary of 2 closest lines (total 4 lines) will be removed from processing. If we recall example with 600 µm wide line, - the measuring width will be reduced to 4 x 24 µm= 96µm or by 48 µm from each side of 600 µm line reducing it to 504 µm.

"Lines to show" will specify data that will be displayed in the graph 1 and 2 windows. This allows to see the process that goes at all lines or selected.