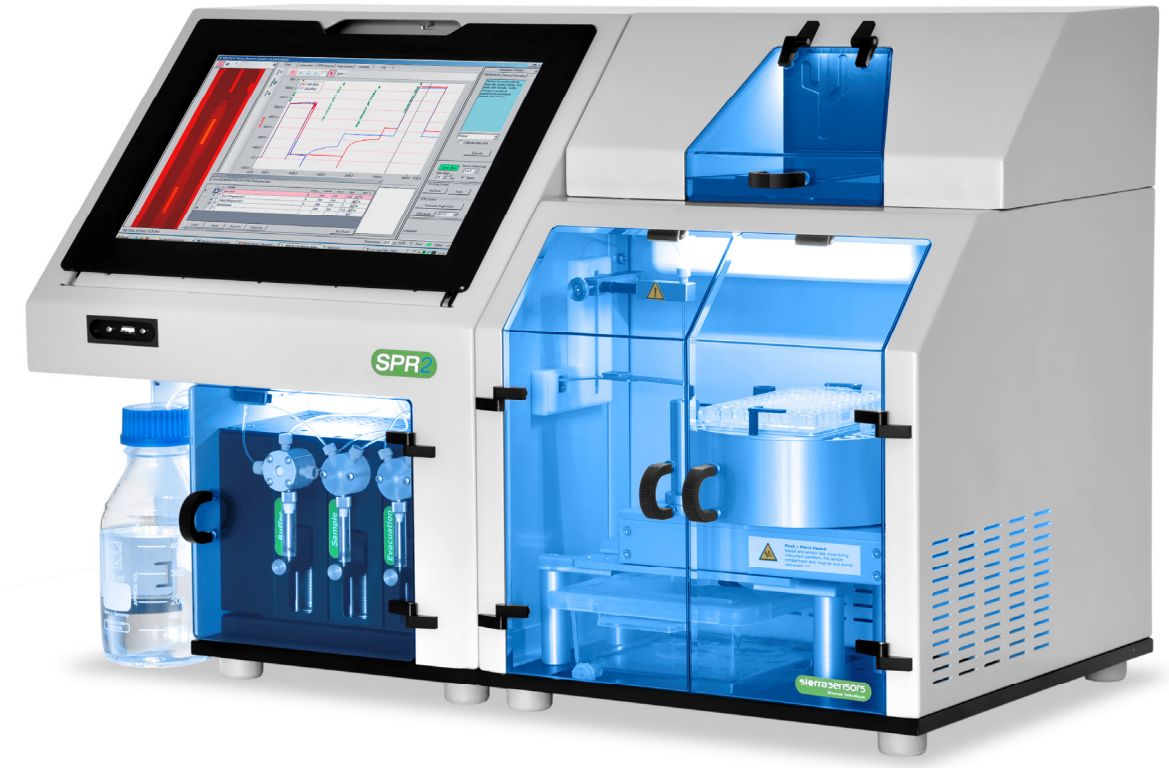
SPR-2 Control Manual



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Content:

1 Instrument Safety Notes

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1.1 Introduction

This instrument has been designed for the analysis of molecular interactions and must be operated in a dry and clean laboratory environment. Do not use the instrument outside its conceived purpose. Before operating the instrument please read the following safety instructions.

Hazardous Voltages

The SPR-2 analysis system runs on mains voltage between 100 and 240V. Always disconnect mains supply before replace fuses or opening covers.

Pinch / Pierce Hazard

Needle and sample rack move during instrument operation. The sample compartment door must be shut during instrument run.

Moving Parts

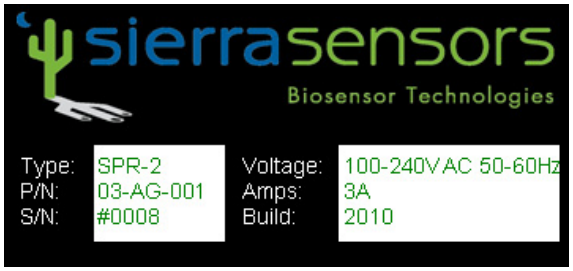
Injections needle and sample carousel move during experimental runs. The sample compartment door must not be opened during active instrument operation.

Heavy Object

The SPR-2 instrument weight approximately 35kg.

1.2 Operating Conditions / Mains supply

Instrument ratings are printed next to the mains input connector. Do not operate the instrument outside this specification.



Fuse:
2 x 3.15 A FAST

2 System Start-up

The control software can be started by double-clicking on the control software icon: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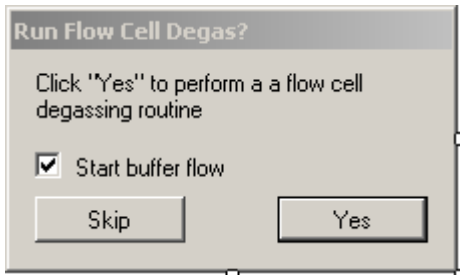
At first the system is initialized in the following order:

1. General software and driver initialisation
2. Syringe pumps and valves reset & initialisation
    a. reset evacuation valve move to position #1 (waste)
    b. reset sample valve and move to position #2 (waste)
    c. reset sample pump
    d. reset evacuation pump
    e. reset buffer pump (syringe is emptied through waste connection)
3. Sample pickup unit reset & initialisation
    a. reset and initialize the pickup needle, move to "0" position (up)
    b. reset and initialize the translational positioning of the carousel, move to washing position
    c. reset and initialize the vial positioning of the carousel
4. SPR optical unit reset and initialisation
    a. reset and initialize the sensor chip clamping unit ("*CoverLift*")
    b. reset and initialize the sensor loading unit ("*PrimsMount*")
    c. reset und initialize the SPR angle unit ("*AngleAdjust*")

The above sequence takes about 10-30 seconds to perform. Upon completion the user is asked whether a more sophisticated fluidics system flush and subsequent SPR angle scan should be performed:



"**Skip**" will just start the buffer (if checkbox is selected)

"**Yes**" will run a flow cell degas procedure and subsequent angle scan.

Choose "Skip" only, if you know the system is in a well defined and clean configuration.

Figure 1: Post initialisation dialog window. The same dialog is shown after a sensor chip is docked.

3 Control Software

3.1 Overview

The main window is separated in 3 parts:

SPR real time image: The raw image of the flow cell area as seen by the SPR detection system; the detection sports are highlighted

Data / main window: Binding data output from the SPR detector, alternatively more advance functions can be accessed though this control

Maintenance and manual injection: Automation control to operate maintenance commands and perform manual injections

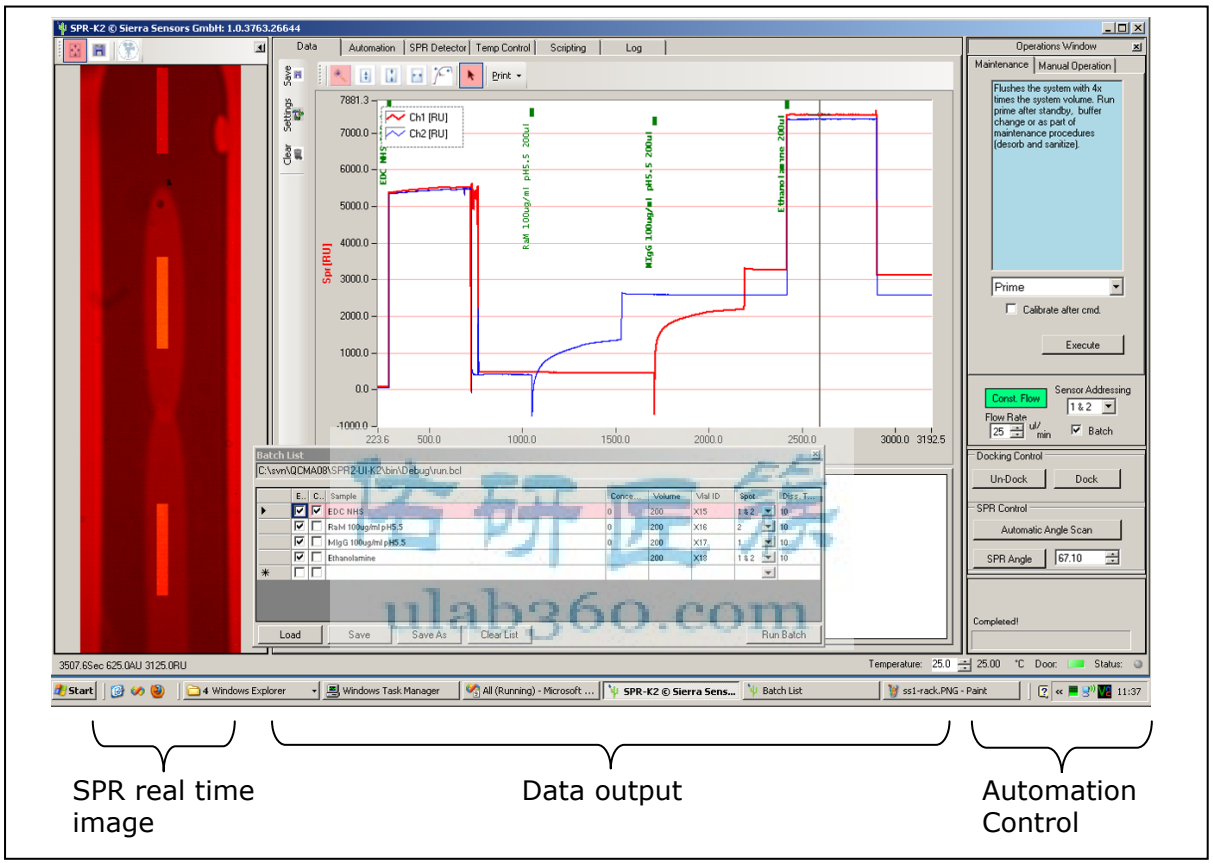


Figure 2: SPR Control software

3.1.1 Output Data Window

The main output window presents the real time binding measurement. Graph specific functions can be accessed through icons around the graph. More specific features are configured through the settings window (see Figure 4).

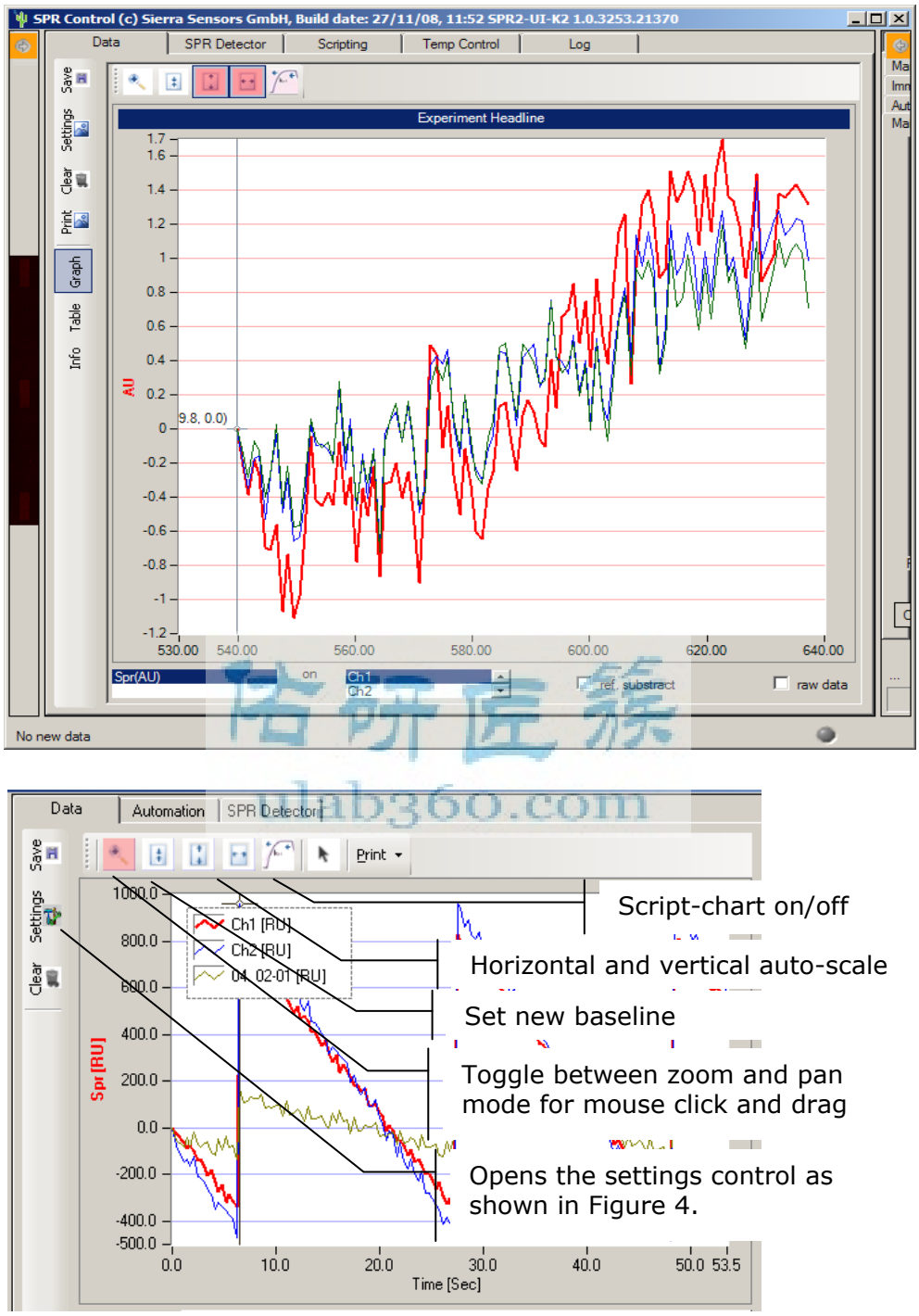


Figure 3: Output graph window

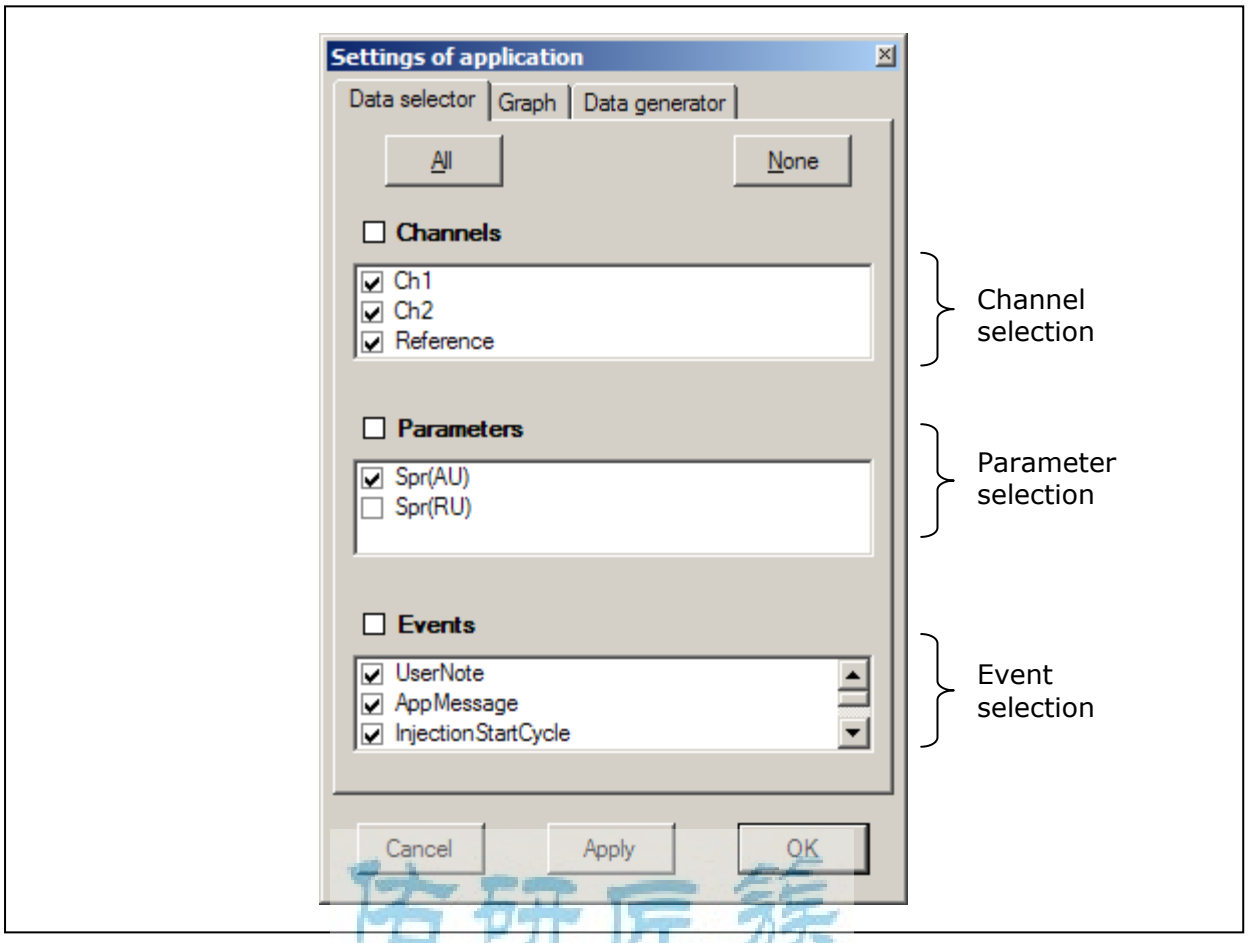


Figure 4: Settings window for the output graph

3.1.2 SPR Real time image

On the right side of the control software the real-time image of the flow cell is shown. In the default configuration a red colour-map with highlighted detection spots is used.

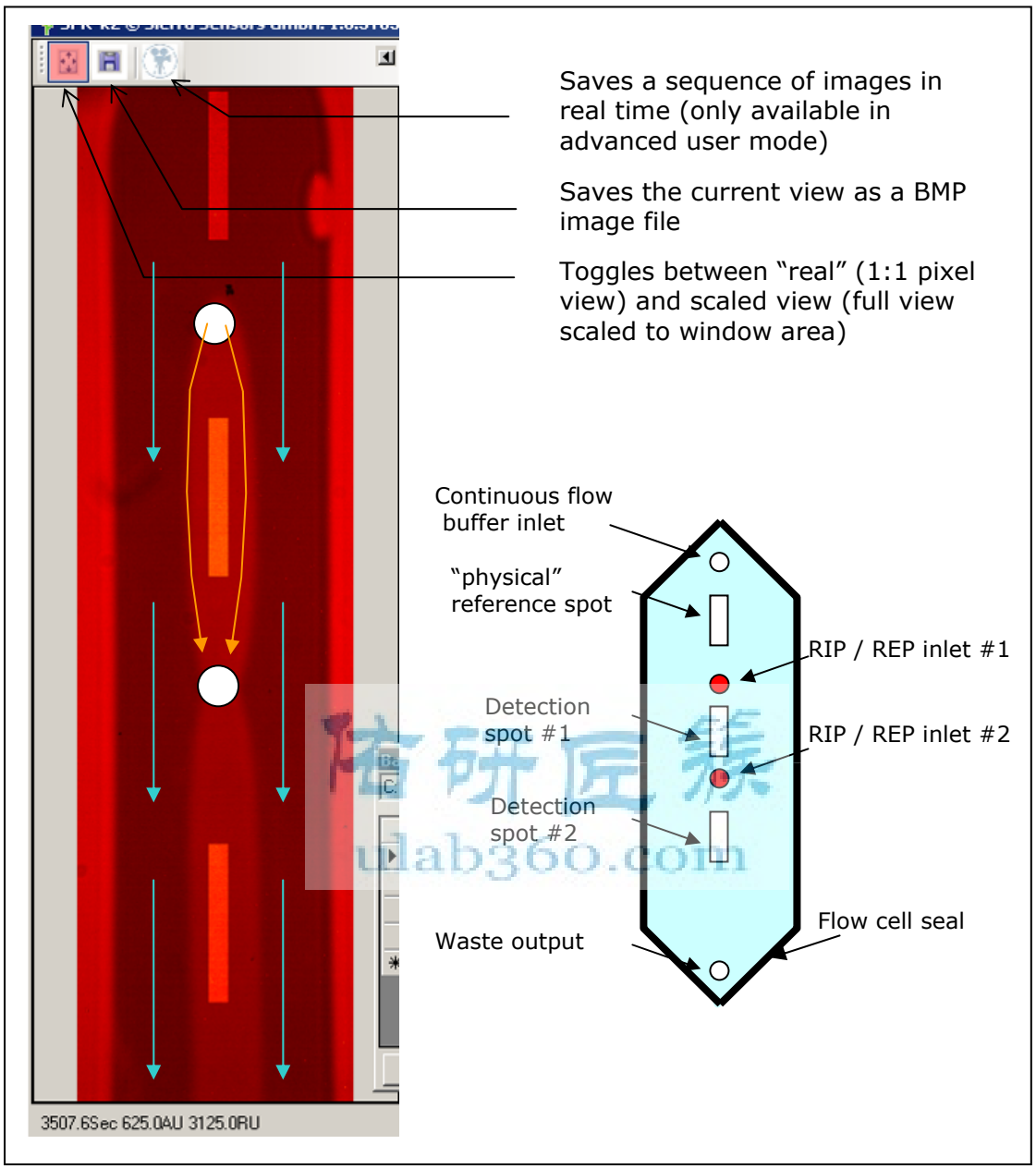


Figure 5: Real time view of the flow cell in "low" sensitivity mode. On the right an illustration of the flow cell including inlet/outlets

3.1.3 Maintenance and manual injection control

This is a frame on the right side of the main window and it is used to run maintenance routines including docking, un-docking and system clean commands as well as manual injections. The control itself is separated into the maintenance commands and a control to run manual injections.

3.1.3.1 Maintenance functions

System maintenance functions are mostly used to run the scheduled maintenance routines and to dock and un-dock new sensor chips. Figure 6 documents the key functions of Maintenance window.

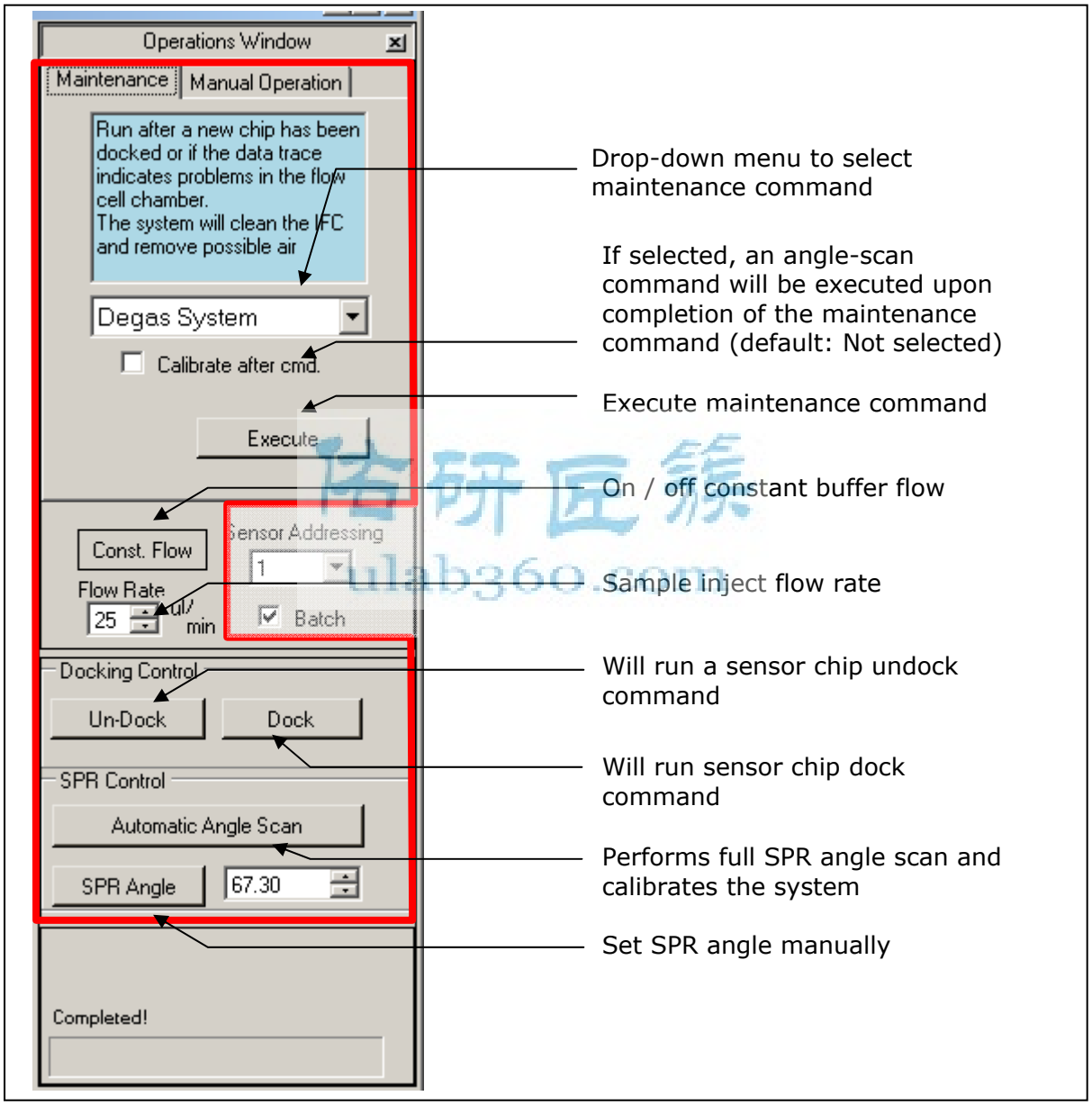


Figure 6: Maintenance control window overview

3.1.3.2 Manual Injections

Manual sample injections can be started from within the Operations Window. The following Figure explains the usage.

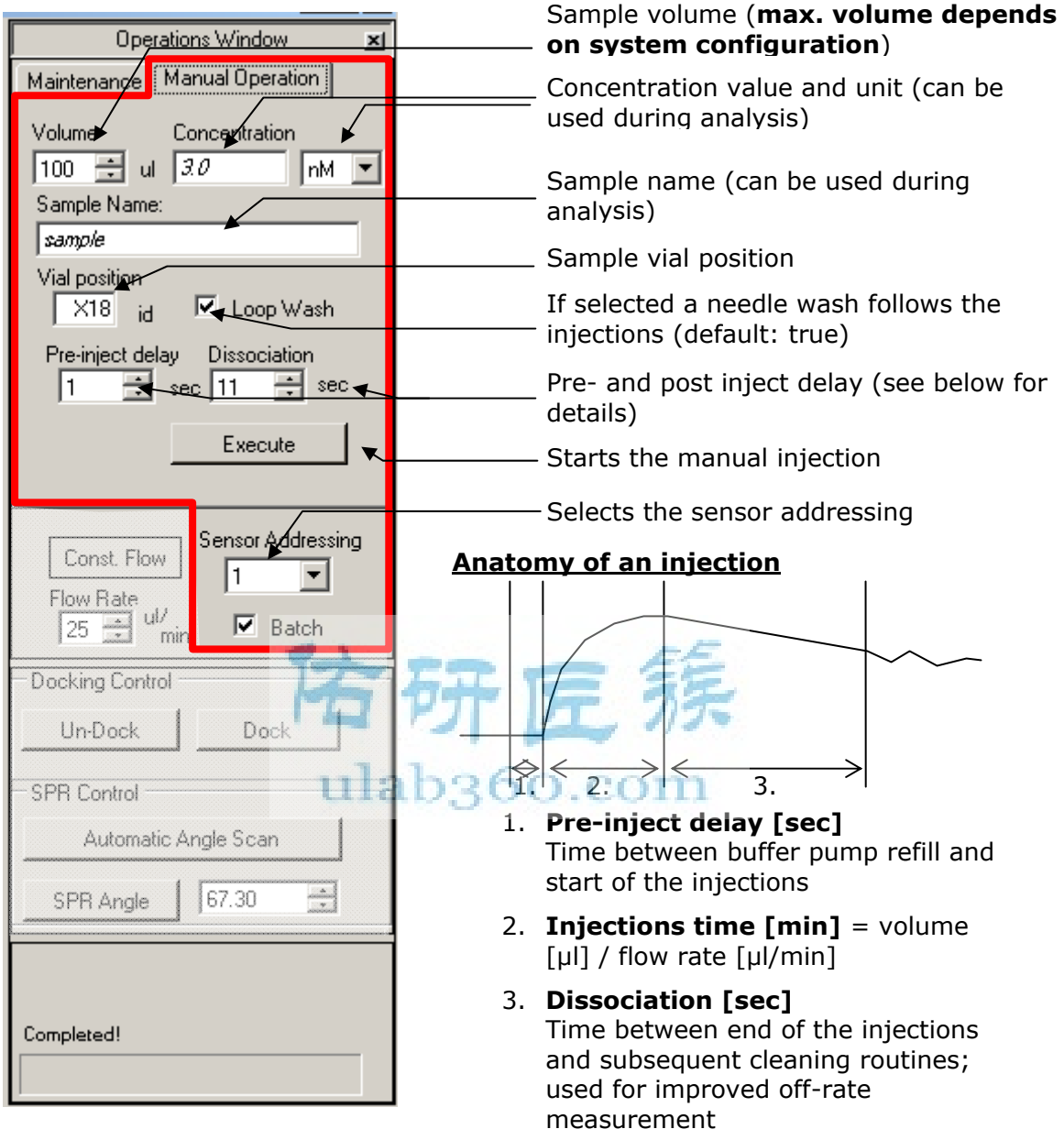


Figure 7: Manual injection control

3.1.4 SPR 2 controller configuration

The SPR control tab provides a lot of expert level access to the real time view of the flow cell. Under normal assay conditions the only function which is changed here is the resolution / sensitivity of the detector. By clicking either the "High" or "Low"-button the detector is put in the high sensitive or low sensitivity mode respectively. The "low"-mode should be used during maintenance (system start-up, sensor chip docking) and also during the immobilisation stage. In this mode the full flow cell including the sealing is shown. Potential issues (air bubbles) could be identified easily and thus rectified through maintenance functions.

Figure 8 explains the individual functions of the control.

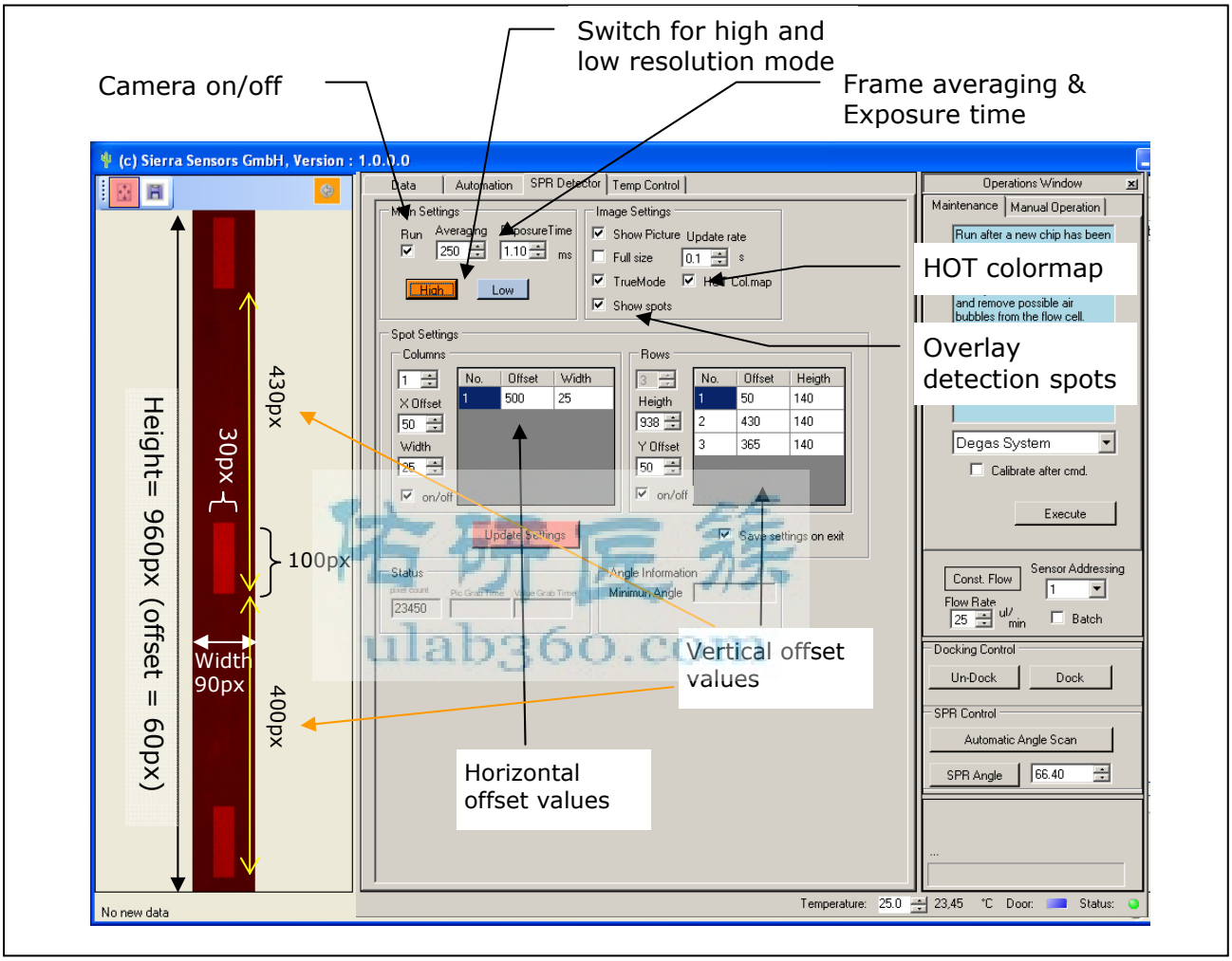


Figure 8: Software configuration for the SPR camera control

Note:

Click "***Update Settings***" to apply new settings

See also section 17.3 ("How to adjust the SPR detection spots").

4 Sample Automation and Automated Methods

4.1 Introduction and Background Information

The "Automation" window provides high-level access to the scripting environment of the system. Scripts are low level command sequences which can accessed through the advanced user mode (see section 6.1).

The following definitions are used for the automation control:

Rack (file extension: *.rcf)

A rack holds information about sample vials and their contents including specific information which relate to the injection parameters of a certain sample. I.e. a regeneration solution might have only a very short injection volume whereas a specific analyte might have a longer injection time / volume.
Racks can be saved and loaded. The content of the rack is modified through the table view within the automation control window.
Expert users can also directly access the XML-format of the *.rcf data files.

Batch List / Automated Methods (file extension: *.bcl)

A batch control list is a sequence of commands, usually injections with a few scattered control commands such as save or clear data. Each row of a batch list has a certain command template associated. When a batch list is executed, the batch list rows are compiled to a sequence of script commands based on these script's file templates. Batch lists can be modified through either the batch list table at the bottom of the automation control window or through the individual batch list control window which is used to execute the method.

Script (file extension: *.ppa)

A script is a sequence of low level commands which control the hardware and some software components of the system. A script can be edited through the advances user mode (see section 6.1).

Notes:

All files MUST reside in the file-type specific folder

"*BatchJobs*" for batch control list files (*.bcl)

"Racks" for rack definition files (*.rcf)

"Scripts" for script files (*.ppa)

4.2 Data structure

The data is saved using a binary format which holds the mass-binding data and the meta data which is required for an efficient analysis of the data. The data is stored in the following structure:

0. Top level containing all information inside the *.SDF file
1. Each ***.sdf-file**/experiment contains one or more cycles
2. Each **cycle** contains one ore more injections
3. Each **injection** contains one ore more channels
4. Each **channel** contains one or more parameter

Cycles are defined by selecting the "Cycle Start" checkbox within the batch control list (see Figure 9 and section 4.2.1 for more details on the cycle feature).

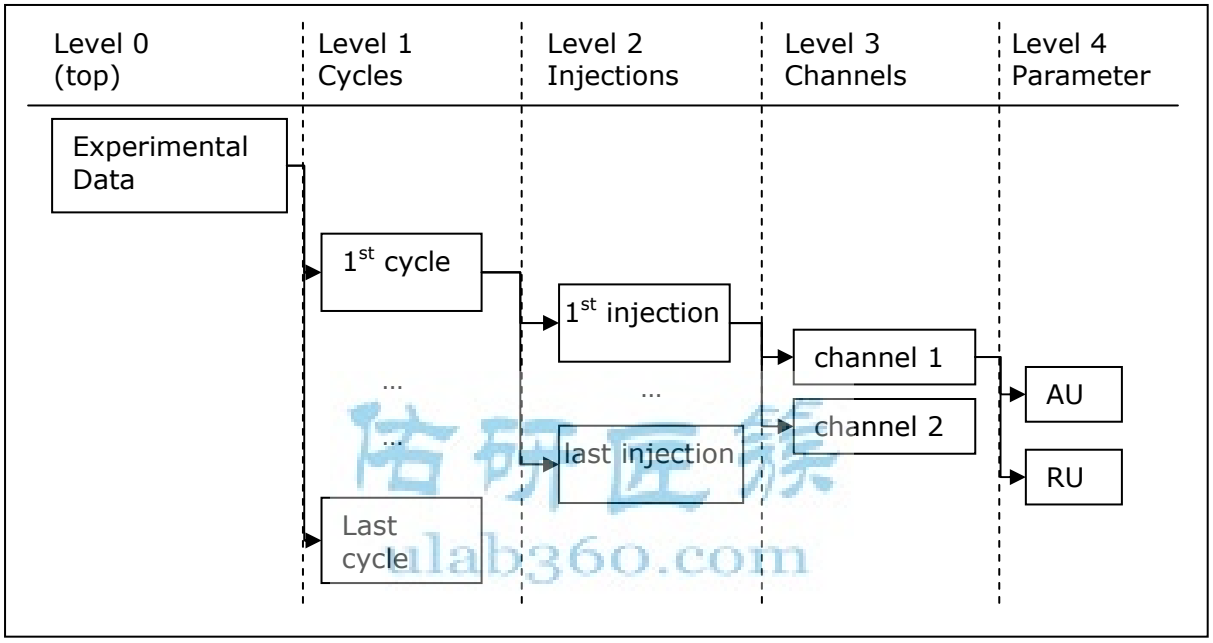


Figure 9: Hierarchical data structure used for SDF file (Sierra Data Files)

4.2.1 Data Structure Examples

The following 2 examples should illustrate the use of the cycle option.

4.2.1.1 Capture Assay

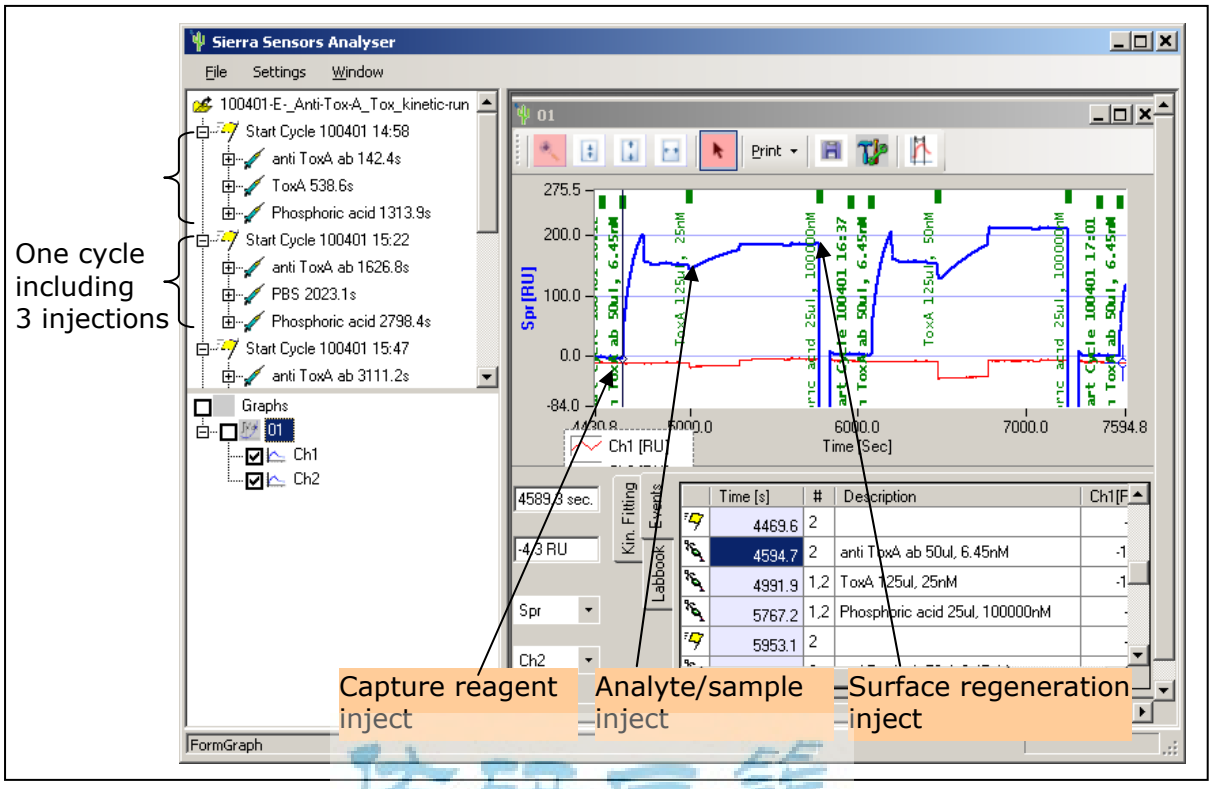


Figure 10: Example for a cycle definition in a capture style assay

4.2.1.2 Direct binding assay

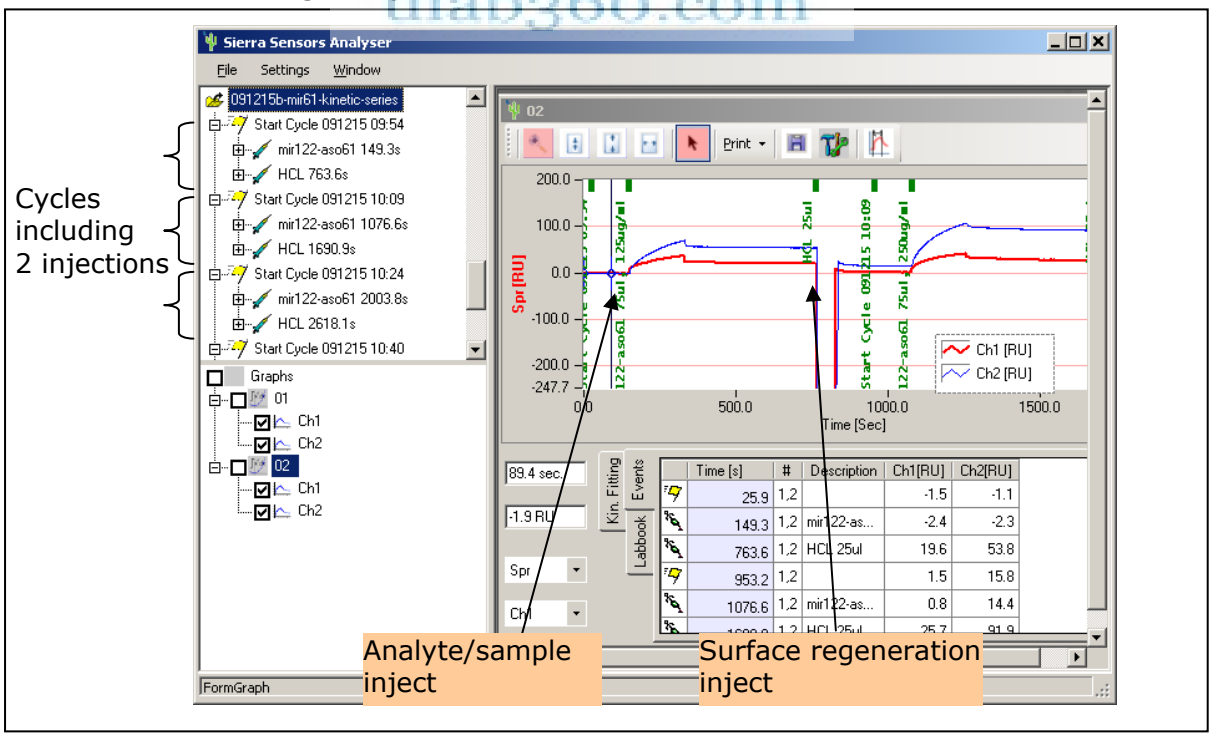


Figure 11: Example for a cycle definition in a direct binding assay

4.3 Automated Methods

Automated methods, or batch control lists (BCL), are put together following the illustration in Figure 12. Once a batch list is built it can be executed in the "Batch List" execution window. The individual columns for each command hold the relevant command parameter:

Row name	Description	Comments
Enable	If checked, the row will be executed	
Cycle Start	If checked a new cycle will be generated with this command first	
Cmd	Type or command. 1. Inject sample injection 2. Data Save saves data, file name must be provided in the sample column 3. Data Clear: Clears the data 4. Main. Degas: Performs a flow cell degas routine 5. Main. Standby: Puts the system in to standby mode	Only in executable control batch list window Default command data will be deleted without further confirmation
Sample	Name of the sample, or filename	
Concentration [nM]	Analyte concentration of the sample liquid	
Vial ID	Vial position	Double click will open rack
Volume [µl]	Sample volume in µl	
Spot	Detection spot to be addressed: 1 – 1st spot only 2 – 2nd spot only 1&2 – both spots	
Diss. Time [sec]	Time the system delays the post-injection cleaning routine; this is required for a detailed off-rate analysis	

Table 1: Definitions for items in the batch list command row

How to build an automated method

1. Define rack content for individual vials and well plate (optional)
2. Build sequence of injections through drag&drop from the visual representation of the rack to the batch list table
3. Save and/or forward core batch list to executable batch list ("to Batch"-button)
4. Add control commands
5. Execute sequence

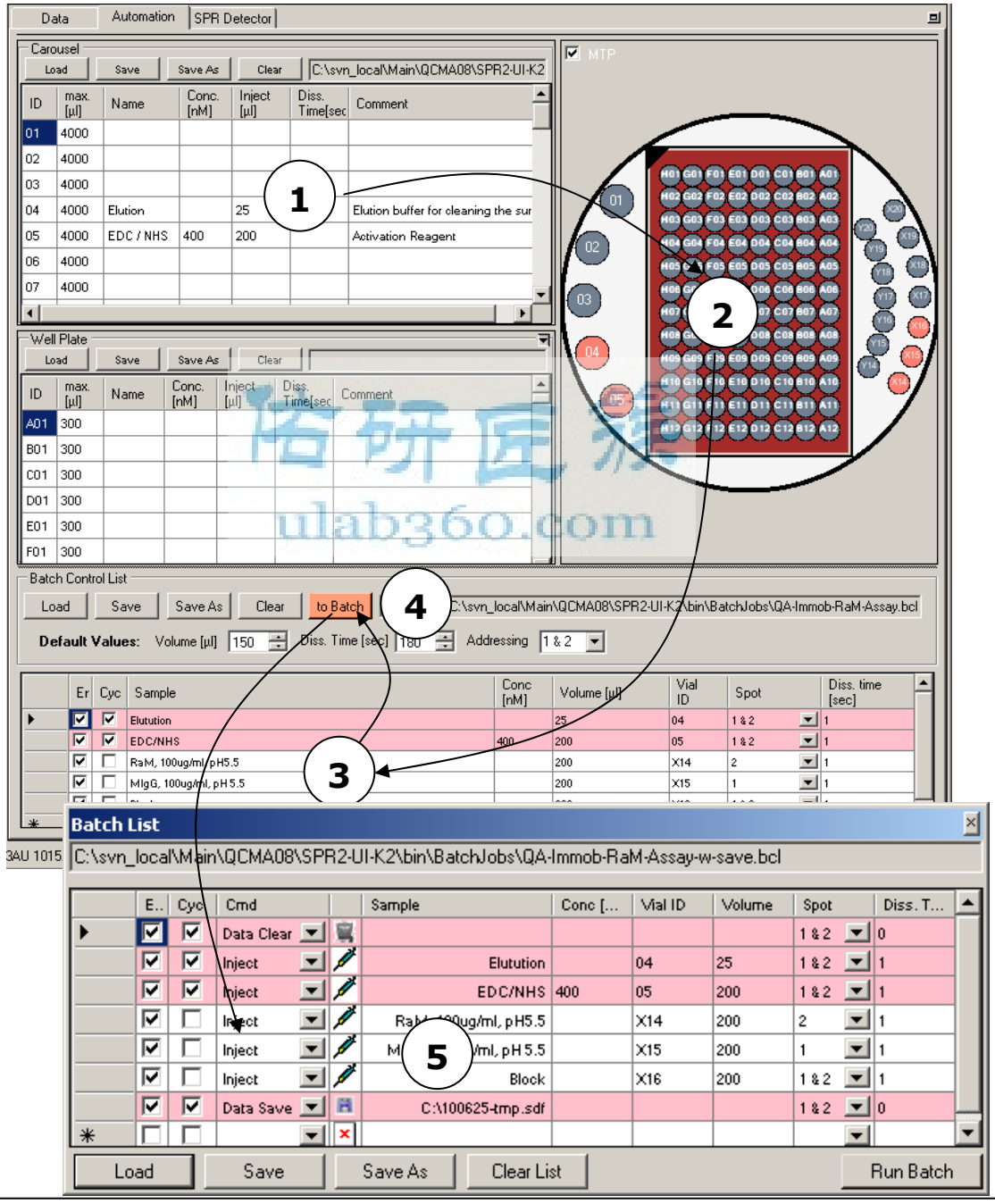


Figure 12: How to create a batch list

5 Sensor Chip Docking

5.1 Introduction and Background

The sensor chip is loaded into the system by placing the sensor chip on the sensor loading tray. A flow cell is formed by pushing the sensor chip on to a micro gasket surrounding the Hydodynamic Isolation™ area of the microfluidic manifold.

Notes:

1. Please make sure the tray area is kept clean and dust free
2. New sensor chips should be allowed to come to ambient temperature before being docked (in case a cold sensor chip is docked, condensation will occur which will disturb the SPR detector).
3. Keep a sensor chip inside the system at all times.

5.2 Undocking and Docking

1. **Save the data**
2. Make sure **no command is active**
3. Choose "**Un-Dock**" from the maintenance control
   To prevent liquid spill inside the detection unit, this command will clean and inject a certain amount of air in to the flow cell. After that the docking unit will un-clamp, open and eject the sensor chip.

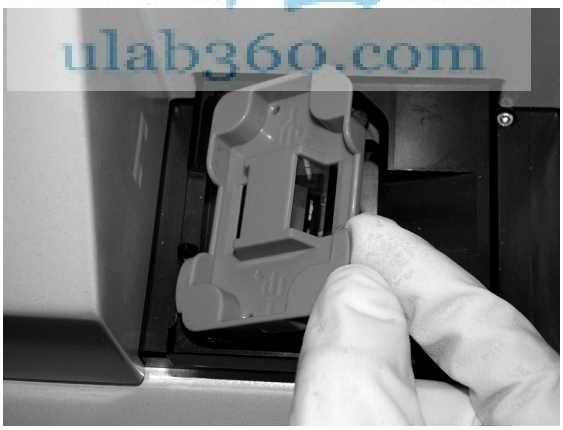


Figure 13: Docking of the sensor chip

4. Put new chip in the tray
   Be aware of the chip orientation
5. Choose "**Dock**" from the maintenance control
   If the chip can't be docked, the system will report an error and eject the chip.
6. After the chip has been successfully docked, the same dialog as during the system start-up will show up (see Figure 1).

5.3 Sensor Chip Storage

Used Sensor chips can be stored in the fridge, by keeping them moist inside the plastic box. This can be achieved by putting a small moist tissue into the plastic box together with the sensor chip. The case should be sealed off using Parafilm or a similar seal.

6 "Advanced User" Mode

The advance user mode is available through pressing <Ctrl>+<Shift>+<P> key combination. It will enable 3 more basic user interfaces which allow access and/or monitoring of lower level instrument functionality. The description given here is only very brief. These controls must NOT be used without in-depth instrument knowledge.

6.1 Native Scripting Control

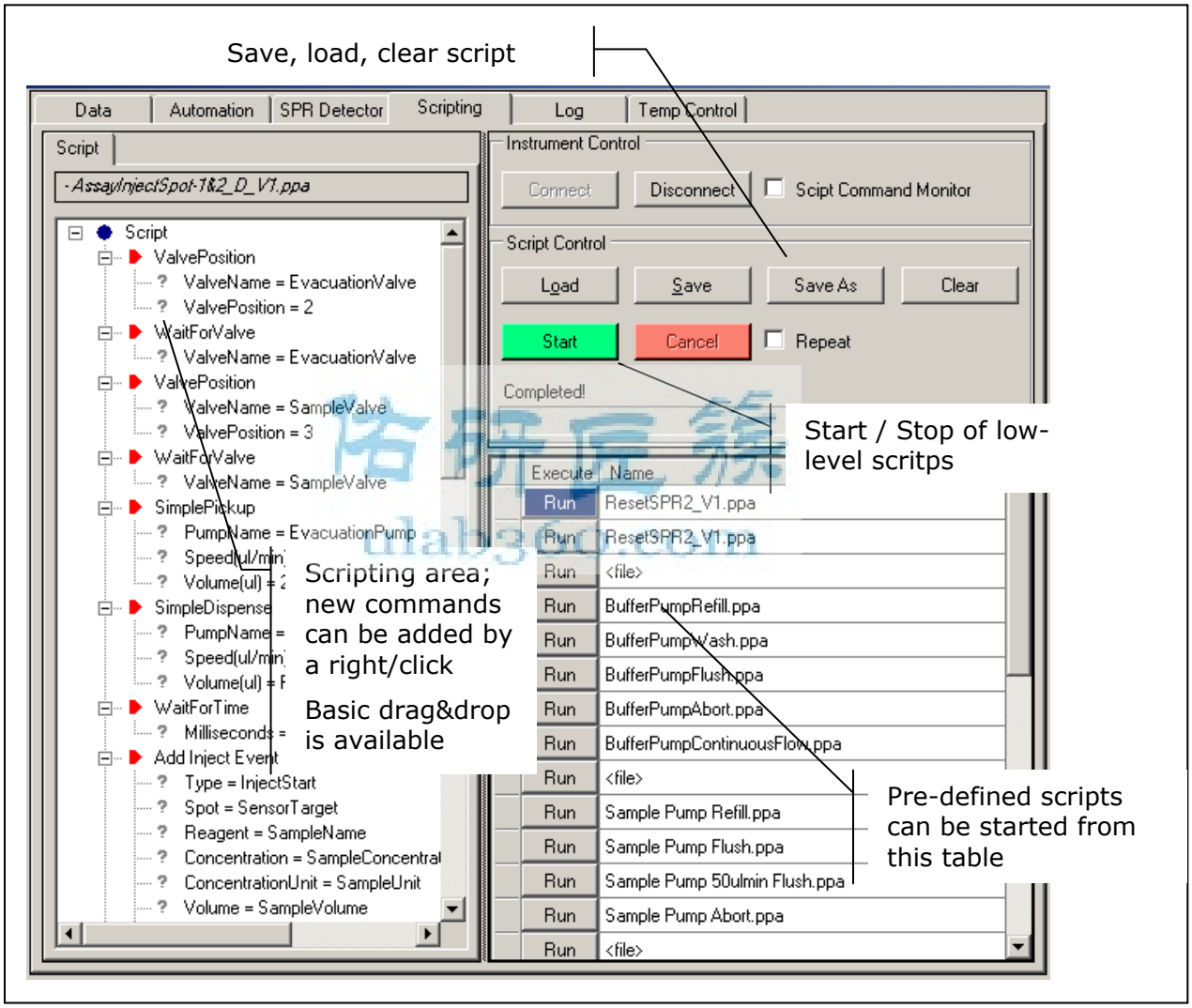
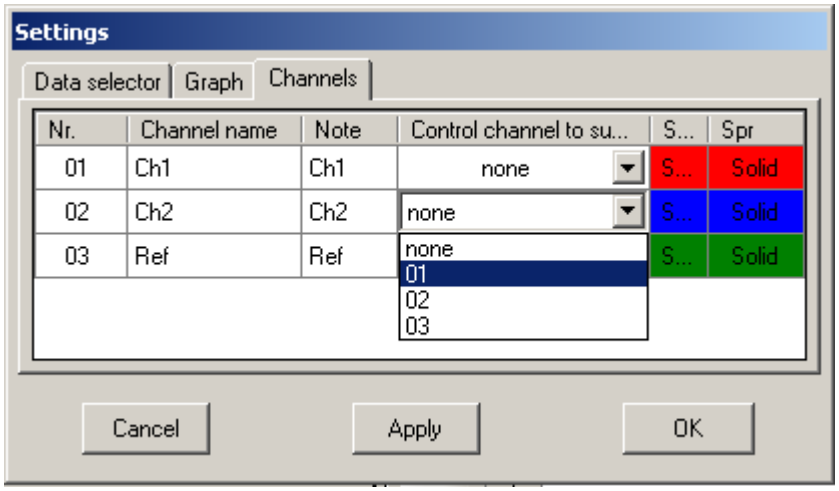


Figure 14: Low level scripting access

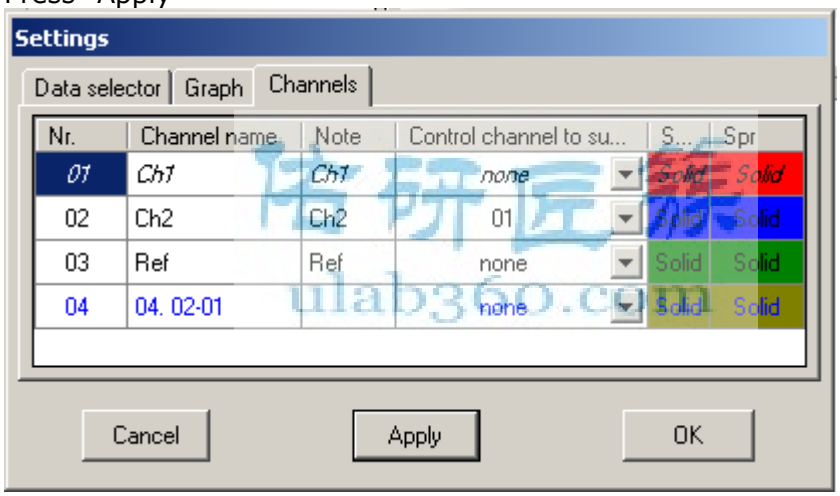
7 FAQ

7.1 How to add a real time control channel in the output window?

1. Open the settings dialog in the output window (see section 3.1.1 on page 6).
2. Choose the control channel for the active channel:



3. Press "Apply"



4. Go to the "Data selector" page, and enable the new control subtracted channel and click "Apply".

7.2 How do I detect and remove air bubbles from the flow cell?

Air bubbles in the flow cell show up as bright spots. The system should completely air free, but overtime air might build up at particular locations in the liquid handling system (i.e. dirt in tubes, inter connections, needle, etc.).

Irregularities during the injection such as:

- Smooth or disturbed transition between buffer and analyte
- "Weird" flow path inside the flow cell
- Carry-over between the spots or at the end of the injection

most likely indicate air inside the system.

Smaller amounts which have build up can be removed using the "Degas" routine from the maintenance tab. If larger amounts of air are in the system a "Prime" command should be executed.

Notes:

1. Use 0.01% Tween in running buffer
2. Make sure system is clean (i.e. desorbed, and/or sanatized)
3. A 70% ethanol flush will dislodge trapped air in the system

7.3 How to adjust the SPR detection spots

It could be necessary to re-adjust the detection spot positioning after a longer of time of use or other changes to the settings (i.e. different buffer, etc.).

All distances are defined as relative positions with respect to its predecessor.

Horizontal positioning

See (1) and (2) in Figure 15. The 1st column is 395 pixel away from the right border, the 2nd column is 80px right of the 1st column.

Vertical positioning

See (3) and (4) in Figure 15. The pixel axes origin is at the bottom of the picture. The distance of the 1row from the border is 200px, the 2nd row is 350px further.

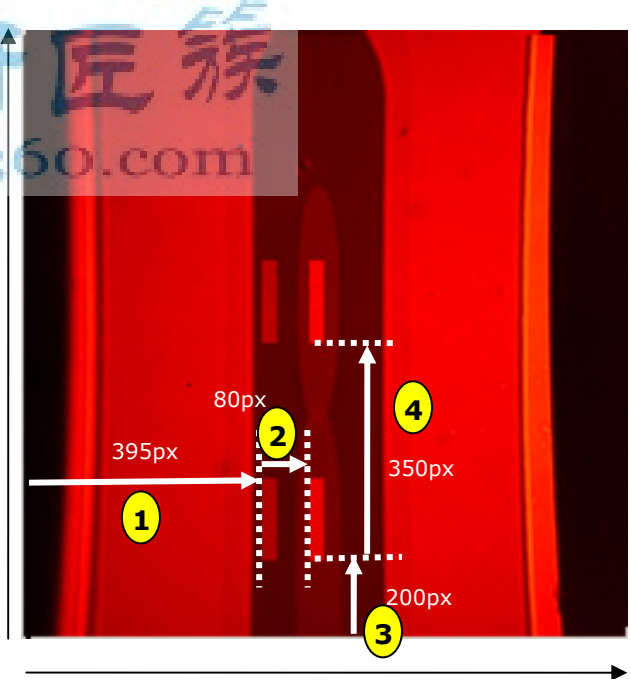


Figure 15: Spot positioning

Figure 16 shows a screenshot of the corresponding SPR Detector tab in "Advanced user mode".

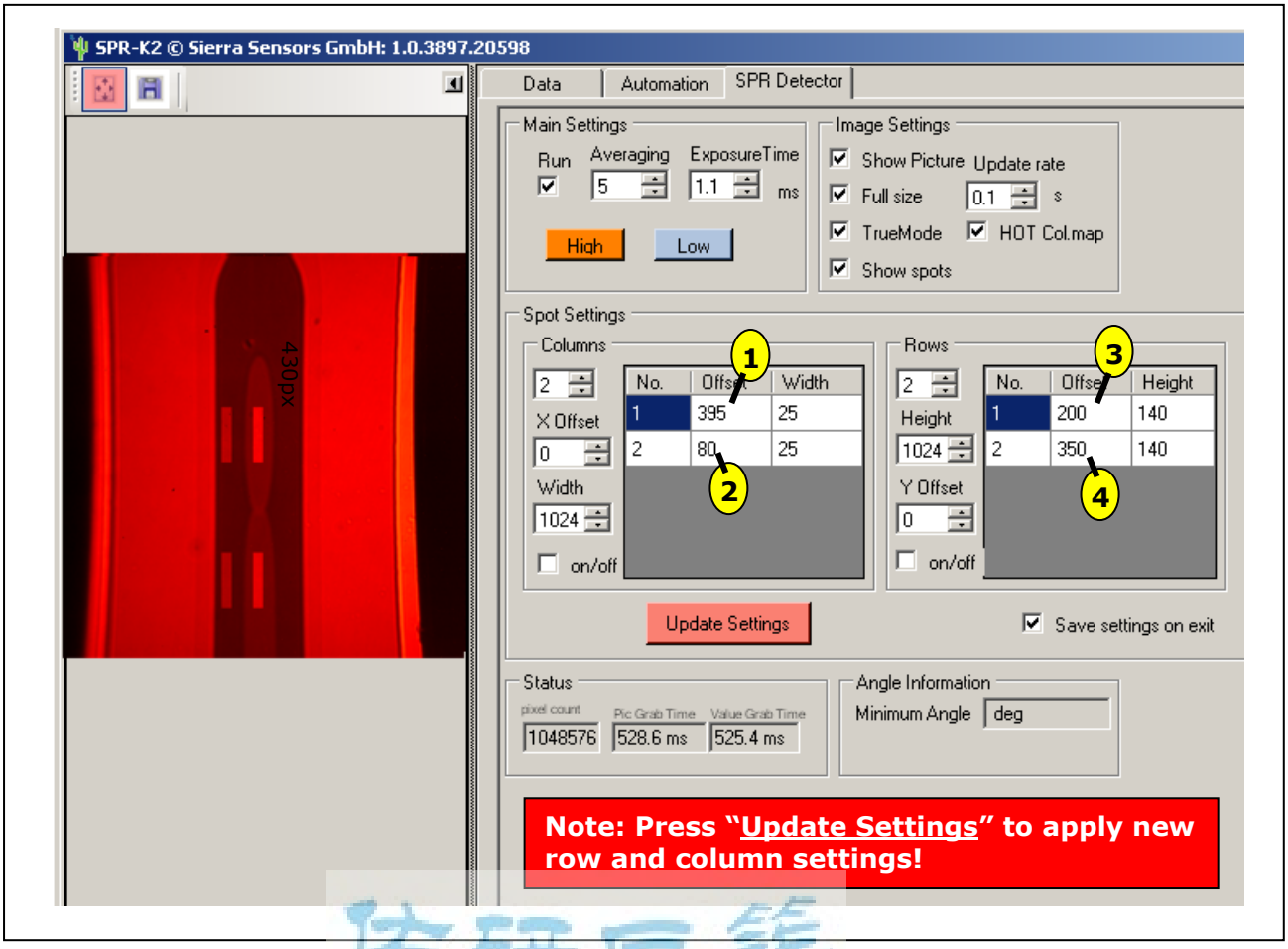


Figure 16: SPR Detector page with highlighted spot settings

7.4 Data recovery after system crash

An `SPR2-UI-K2_autosave.sdf` file is saved every 300 seconds in the control software directory (default file location is `C:\Program Files\Sierra Sensors\SPR-2 Control\bin`).

Notes:

Recover the file immediately and before the system is restarted, otherwise the autosave file will be overwritten

8 Support & Contact

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Revision History:

Ver. 1-99	Issue [A-Z]	Description of Change	Date	Author
1		Initial version created from SPR2-SystemCoreManual-V4.doc	15/4/10	KW
2		Docking info merged	28/6/10	KW
2.1		Safty Note merged	5/7/10	*
2.1	b	typos	31/8/10	KW
2.1	c	FAQ for spot positioning adde	3/9/10	KW