RI. SE | Research Institutes of Sweden

Approved/Godkänd	Date/Datum	Rev	Page/Sida
Dag Ilver	2022-08-31	R1	1(16)
Issued by/Utfärdare	Type of document/Dokumenttyp		
Christian Jonasson, Dag Ilver	User Guide		
Distribution	Reference/Referens		
	V-FC UserGuide R1		
	For information only/För kännedom		

Video-based Flow Cytometer

User Guide

Contents

1 Introduction

This document describes how to use the second release of the Video-based Flow Cytometer (V-FC) developed by RISE AB. The instrument is designed to detect contamination of drinking water by sewage water containing *E.coil*, chosen as faecal indicator bacteria. In other applications the instrument is used for detection of other bacteria or auto-fluorescent microalgae.

2 Sensor system

In this integrated version, the instrument will be working as a stand-alone instrument, able to perform measurements in either automatic mode or manually operated. The sensor system consists of three major parts:

1) **Biochemical part**; specific labelling of *E.coli* using IgY antibodies labelled with fluorescent nanoparticles (quantum dots, 655 nm emission peak)

2) **Instrument hardware;** Pumps, mixer, incubators, electronics and optical detection unit for fluorescent bacteria (laser and CCD camera).

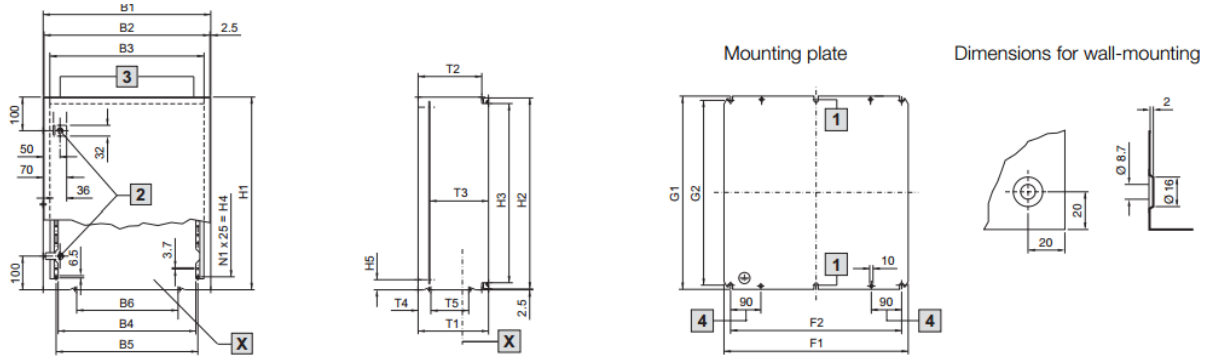
3) **Embedded computer;** with software for measurement control, data analysis, and communication

3 Instrument

This section describes the front panel and the included parts inside.

3.1 Dimensions

The outer dimensions of the instrument are 700x500x250 mm.



Model No. AE	Width dimensions mm						Height dimensions mm						Depth dimensions mm					Mounting plates mm			
Spray-finished	B1	B2	B3	B4	B5	B6	H1	H2	H3	H4	H5	N1	T1	T2	T3	T4	T5	F1	F2	G1	G2
1057.500	500	495	460	411	423	303	700	695	660	625	30	25	250	230	208 – 224	38	113	449	410	670	645

Figure 1. Dimensions of the instrument box.

3.2 Front panel

The instrument front panel, showed in Figure 2, has one ON/OFF-switch, two buttons and seven LEDs. All the components are described in table below. The front door can be open using a key.

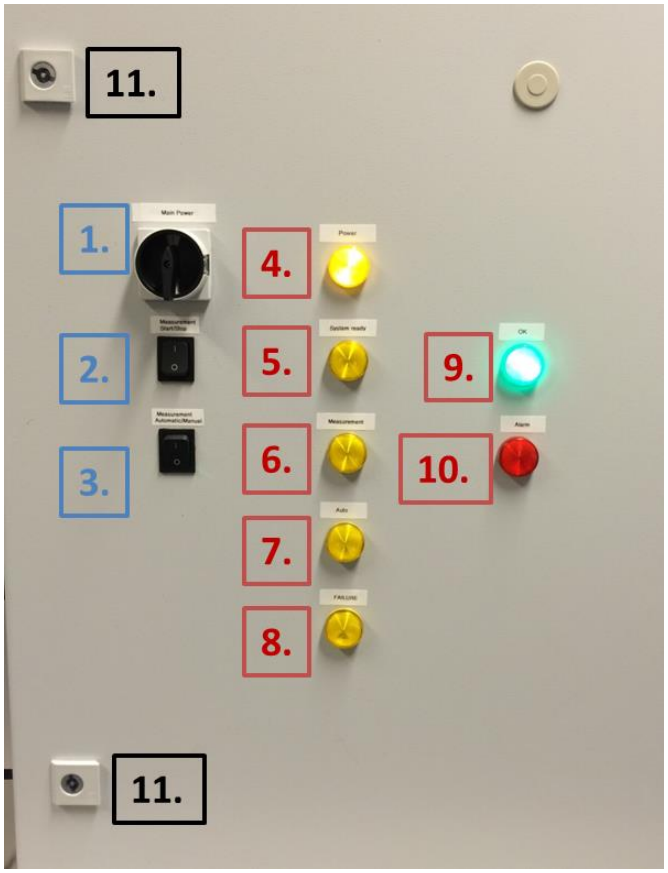


Figure 2. Instrument front panel.

Table 1. Components on the front panel.

#	Component	Function	Comment
		Buttons and switch	
1.	**Power ON/OFF switch**	Main power switch	Before switch main power OFF: 1. Wait for system to finish an ongoing measurement. 2. Turn “Auto mode” button to “0” (OFF) and the Embedded Computer button to “0” (OFF) and wait for two min before switch the main power OFF
2.	**Embedded Computer ON/OFF**	Button is used to perform a controlled shut down of the embedded computer.	Button should be set to “0” (OFF) before turning the main power OFF. During startup this button must be set to “1” (ON).
3.	**Auto Mode ON/OFF**	Toggles the system state between ”Manual” mode (= 0) and ”Auto/Remote” mode (=1).	First time the system is set in “Auto mode” (incl. start-up) a measurement is performed. Next measurement will then start according to the time set interval. In “Auto mode” the system is reachable via the Modbus protocol, see separate section.
		LED indicators	
4.	**Power**	Main power indicator	
5.	**System ready**	System ready for measurement	LED turns on after system has passed the initial start-up phase and is ready for measurement
6.	**Measuring**	Indicates that a measurement is running	

7.	**Auto**	Indicates the current state of the system is.	LED on = “Auto mode” LED off = “Manual mode”
8.	**Failure**	System error indicator	

3.3 Built-in hardware

Figure 3 shows an overview of the hardware inside the instrument, including AC/DC converters, embedded computer (PC), I/O signal device, mixing stage, incubator (holding a ~39° C), optical detection module, a three channel peristaltic pump, temperature controllers and a container for the reagents cooled down to about ~5° C. Some of the parts will be described in more detail in following sections.

The water sample to be analyzed is pumped into the system from the outside.

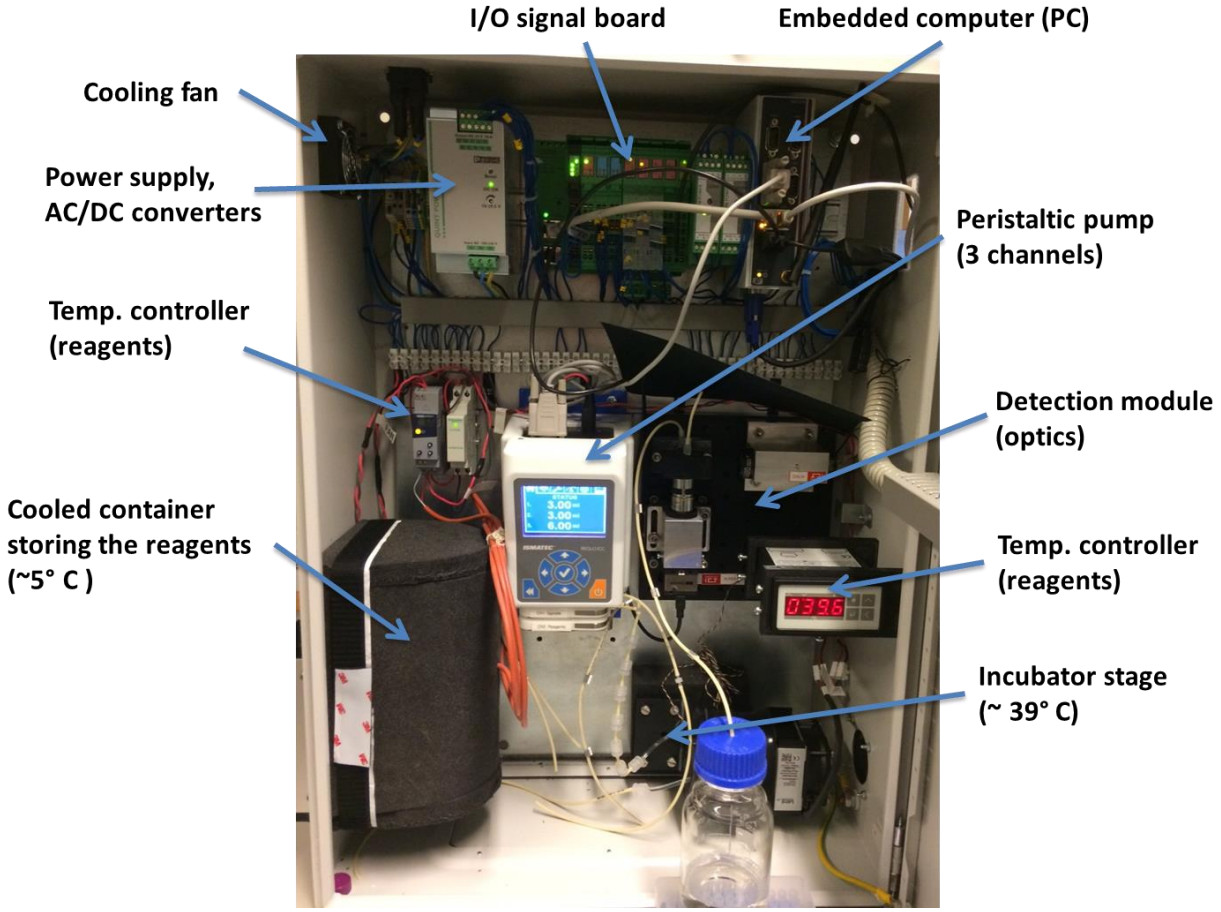


Figure 3. Picture showing the inside of the instruments and where the different components are located.

3.3.1 Optical detection module

Figure 4 shows detection module and its optical components. The water sample that is analyzed is flowing through the cuvette hidden between the black plastic holder, but the sample inlet and outlet are visible at the upper side.

A 100mW laser (532 nm) is used as excitation source. On the detection side a CCD camera is used in combination with a 10x magnification objective and long pass emission filter with cut-off wavelength at 550 nm. The camera is connected to the embedded PC via a USB cable.

Warning! Do not open the instrument front door when a measurement is running. The laser has high power and could be dangerous to the eyes. The laser is only on during measurements. If the door needs to be open during measurement, use protective goggles.

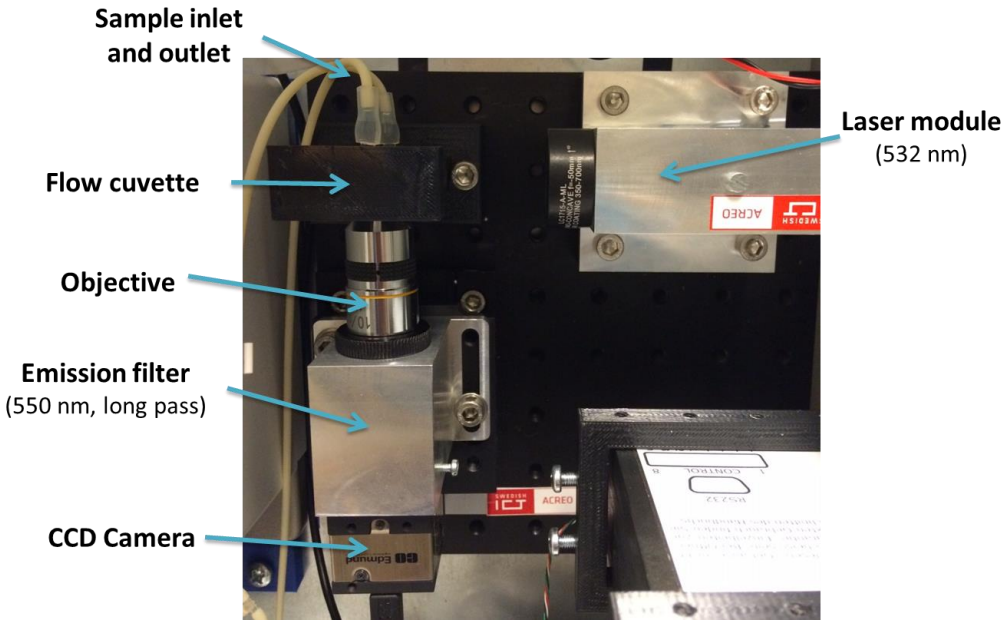


Figure 4. The optical detection module inside the instrument consists of a laser source (532 nm) at the excitation side and a CCD camera with emission filter on the detection side.

3.4 Flow diagram

The flow diagram in Figure 5 shows how the water sample is pumped into the system and mixed with the reagents before it flows further into the incubator. After incubation (~ 10-15 min) the sample is pumped into the optical module to be analyzed.

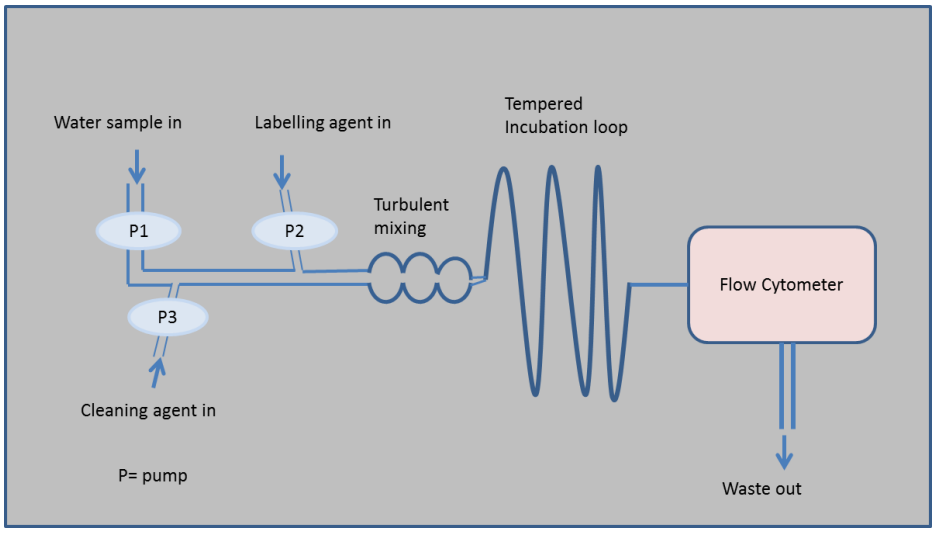


Figure 5. Flow diagram describing how the water sample flows through the system.

3.5 Computer

The instrument has a built-in PC running Windows Embedded 7. The main program (firmware) controlling the instrument, “SafeWater_R2011b.exe”, starts automatically when the system starts up.

If needed a screen can be connected to the PC using the VGA port and mouse and keyboard can be connected via USB.

3.5.1 Software User Interface

The built-in software has a graphical user interface (GUI), which enables the user to control the measurement and visualize the results and show the progress of the video processing. This requires a screen and mouse/keyboard are connected.

From the GUI the user can manually start/stop the pumps, control the video capture and start the pre-programmed measurement sequence.

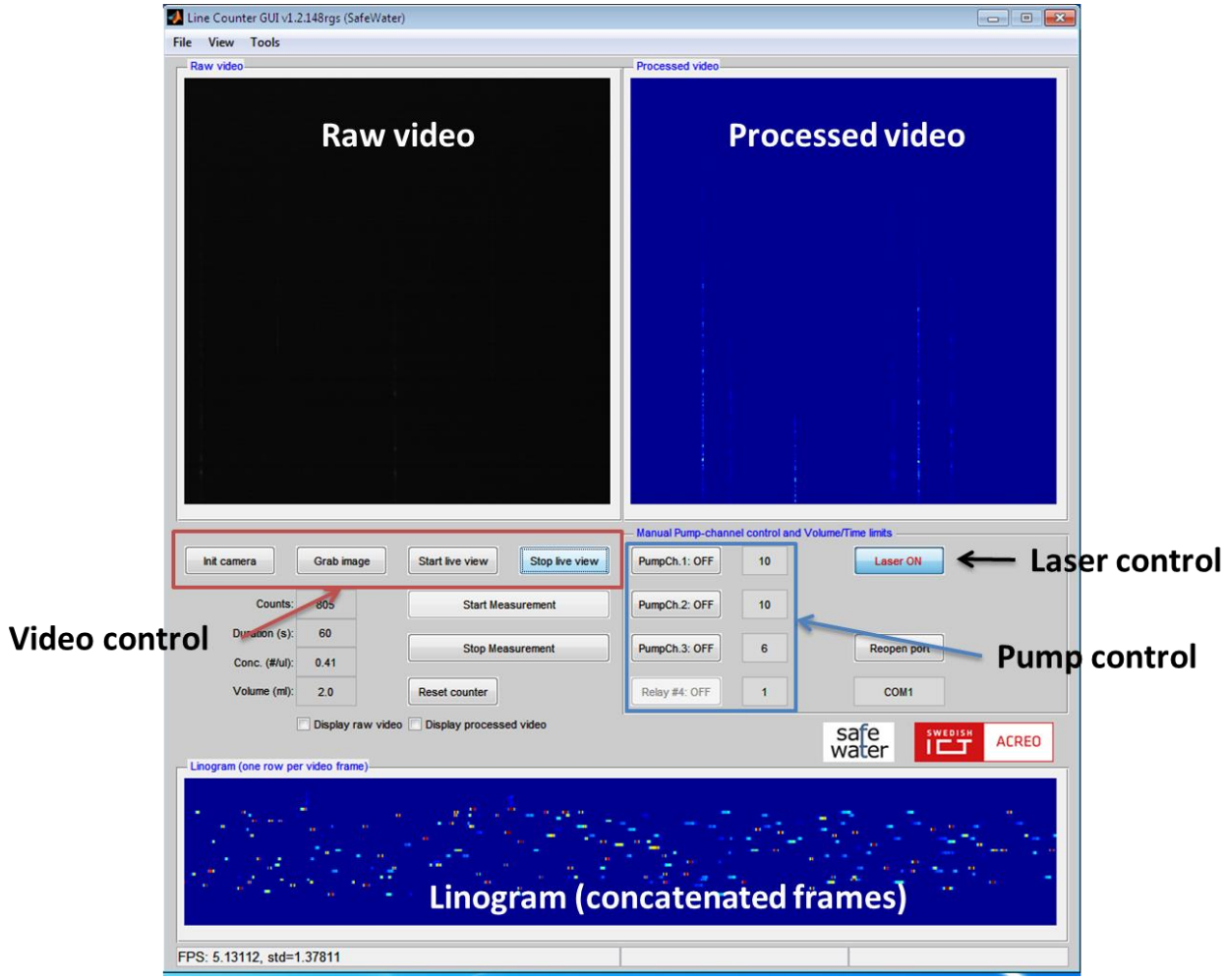


Figure 6. The GUI includes buttons for pump control, video control, raw video display, processed video and linogram (concatenated frames).

3.5.2 Buttons

Table 2. GUI Buttons

Button	Function
Init camera	Starts the raw video stream
Grab image	Grabs a raw image (not to be used)
Start live view	Starts video processing and particle counting
Stop live view	Stops video processing
Reset counter	Resets the indicators: number of counts, duration time, volume and concentration
Pump #1	Start/stop Pump 1 (Water sample)

Pump #2	Start/stop Pump 2 (Reagent)
Pump #3	Start/stop Pump 3 (Mixing)
Pump #4	*Not in use*
Re-open port	Opens specified COM-port for pump control (Only to be used if computer fails to connect to the pump control board during program start-up)

3.5.3 Graphs

Table 3. GUI graphs

Graph	Function
Raw video	Displays the raw video stream
Processed video	Shows the processed video after background reduction and thresholding. The color indicates the intensity of the detected fluorescence light (from blue to red).
Linogram	Shows the linogram of the last 50 (?) analyzed frames, one line per frame. Number of islands in the linogram are counted and added to the total number of counts showed in the “Counts” indicator.

3.5.4 Program Menu

Table 4. GUI program menu

Menu item	Function
File / Load video from file	Load video from file: Loads and analysis a pre-recorded video file (.avi). To record a video file a separate software must be used which is installed on the computer (uEye Cockpit).
File / Start batch analysis	Start batch analysis: Start a batch analysis of video files stored in a selected directory.
View / Show accumulated linogram	Plots the linogram of all analyzed video frames in a 2D image.
View / Show thresholded linogram	Show thresholded linogram: Plots the thresholded linogram of all analyzed video frames in a 2D image.
View / Plots counts vs. threshold	Plots counts vs. threshold: Plots the number of counts versus the threshold value
Tools / Debug console	*Not in use*

3.5.5 Input parameters

Table 5. GUI Input parameters

Parameter	Function
Pump #1 hold time	Pump 1 (sample) duration (min) during measurement sequence
Pump #2 hold time	Pump 2 (reagent) duration (min) during measurement sequence

Pump #3 hold time	Pump 3 (measurement) duration (min) during measurement sequence
Incubation time	Time of the incubation step (min)
COM-port	Pump COM-port

3.5.6 Settings file

Default settings (Figure 7) are loaded from file (*settings.ini*) during program start-up. The file is located in the program directory and contains default settings for example; COM-port number, pump volumes, flow rates, incubation time, camera settings and alarm levels.

```
[Serial]
pump_if      = COM1   ; Pump comms via USB or RS232/485

[Timing]
incubation   = 10     ; Incubation time (min)
video_delay      = 300    ; Delay (s) between time of pump ch. 1 start and video processing start

[Main Application]
period_sec   = 18000      ; Minimum measurement repetition period (sec)

[Pump]
pump_id      = 1      ; For Reglo-ICC's shared commands
channel_count    = 3

; NB: flow rates are computed by the Reglo-ICC pump itself.
speed_rpm    = 10.0, 10.0, 5.0    ; (RPM) for ch.1,2; range=[0.1, 100]; 100 rpm --> 10ml/s for ID=1.3mm
volume_ml    = 3.00, 3.00, 4.50   ; (ml) for ch.1,2
diameter_mm      = 1.30, 1.30, 1.30   ; (mm) tubing for ch.1,2,3

speed_flush_rpm      = 100    ;               ; (RPM) for ch. 1
volume_flush_ml      = 20     ;               ; (ml) for ch.1

; Allowed standard tube diameters (mm) are:
;    0.13 0.19 0.25 0.38 0.44 0.51 0.57 0.64 0.76 0.89 0.95 1.02 1.09
;    1.14 1.22 1.30 1.42 1.52 1.65 1.75 1.85 2.06 2.29 2.54 2.79 3.17

[Processing]
pthr     = 1e-30      ; threshold from p standard (ztest)

[Camera]
bl_offset    = 150    ; Black-level offset for the camera (uint8)
master_gain      = 50     ; (%) of the maximum gain for a B/W uEye camera

[Modbus interface]
StationId    = 46
ServerPortTCP    = 502

[Phoenix server]
IPaddress1   = 192.168.3.1
DeviceId1    = 1

[Latest measurement]
RegFile_length   = 14
RegFile      = 14, 2016, 9, 10, 10, 10, 10, 10, 0, 0, 0, 0, 0, 16
TimeStamp    = 10-Sep-2016 10:10:10

[Alarm]
alarm_level      = 0.2    ; (/ul) Alarm level for setting instrument indicator

[Current Output]
conc_max     = 1      ; (/ul) Value corresponding to maximum output current (20 mA)
```

Figure 7. Settings.ini-file

4 Starting up the instrument

1. Make sure the instrument is connected to the mains (220 V).
2. Make sure the button “Embedded Computer” is set to “1” (ON)
3. Use the power switch in the front panel to turn on the system power → The “Power” LED will now shine
4. The embedded computer will now start up and automatically start the software controlling the instrument.
5. Wait a few minutes…
6. The program will do a system check during its start-up phase. Connections the built-in pump, I/O modules and camera will be established. If something goes
7. After a successful start-up → The “System Ready” LED will shine.
8. The system is now ready for measurements.
9. If the button “Auto Mode” is set to “1” (ON) the system will enter auto/remote state and perform one measurement directly after start-up. Next measurement will start according to schedule, which is controlled by “time between measurements” in the settings.
10. If the button “Auto Mode” is set to “0” (OFF) the system will enter manual mode.

5 Turning of the instrument

1. Wait for any active measurement to finish
2. Set the “Auto mode” button to “0” (OFF)
3. Set the “Embedded Computer” button to “0” (OFF)
4. Wait approx. 1 min..
5. Turn off the main power using the power switch

6 Measurement sequence

The pre-programmed measurement sequence will automatically pump the water sample into the mixing container and add the reagent. After the incubation the mixture will be pumped into the detection module and the video analysis will be started. When the measurement is finished data will be stored into the "data" folder located in the program directory. The pump duration and incubation time can be set in the program, see the input parameter section.

6.1 Start a measurement from the front panel

1. Make sure the instrument is powered up and the "System ready" LED is on
2. Start the measurement by setting the "Auto mode" button to "1" (ON).

   First time the system is set in "Auto mode" (incl. start-up) a measurement is performed. Next measurement will then start according to the time set interval.
3. To exit "Auto mode" set "Auto mode" button to "0" (OFF).

6.2 Start a manual measurement from GUI

1. Make sure the instrument is powered up and the "System ready" LED is on
2. Make sure the "Auto mode" button is set to "0" (OFF)
3. Press "Start measurement" button in the GUI

6.3 Log files

When the measurement sequence is finished the results will be stored to a log file (.txt) as shown in the example below:

```
-----------------------------------
Program version: v1.0 (SafeWater)
Measurement started: 2015-03-24 10:30:15
File saved: 2015-03-24 10:48:24

Video duration [s]: 600
Volume analyzed [ml]: 18
Number of particles counted: 1650
Estimated concentration [#/ul]: 0.092
-----------------------------------
```

The log file will be saved in the folder called data located in program directory. A couple of plots showing the results of the video processing will also be stored in the same location. All saved files will be given default names including the date and timestamp of the measurement start; e.g. 2015-03-24 103219.txt, 2015-03-24 103219 thresholded linogram.png, 2015-03-24 103219 counts vs threshold.png. Examples of these figures are shown in Figure 8 and Figure 9.

A data flow diagram explaining the pre-programmed measurement sequence is shown in **Error! Reference source not found.** below.

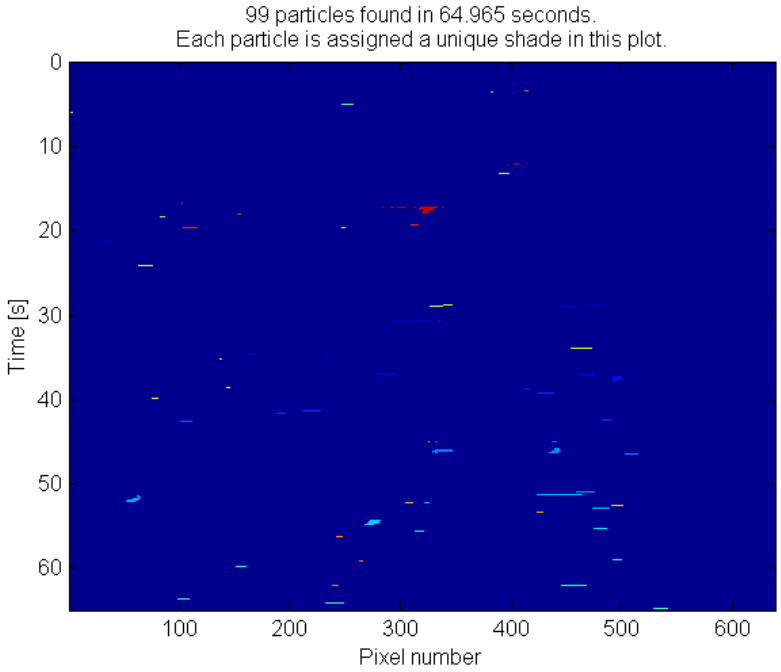


Figure 8. Thresholded linogram showing a summary of all processed video data for the last measurement. Each island is counted as one particle/bacteria.

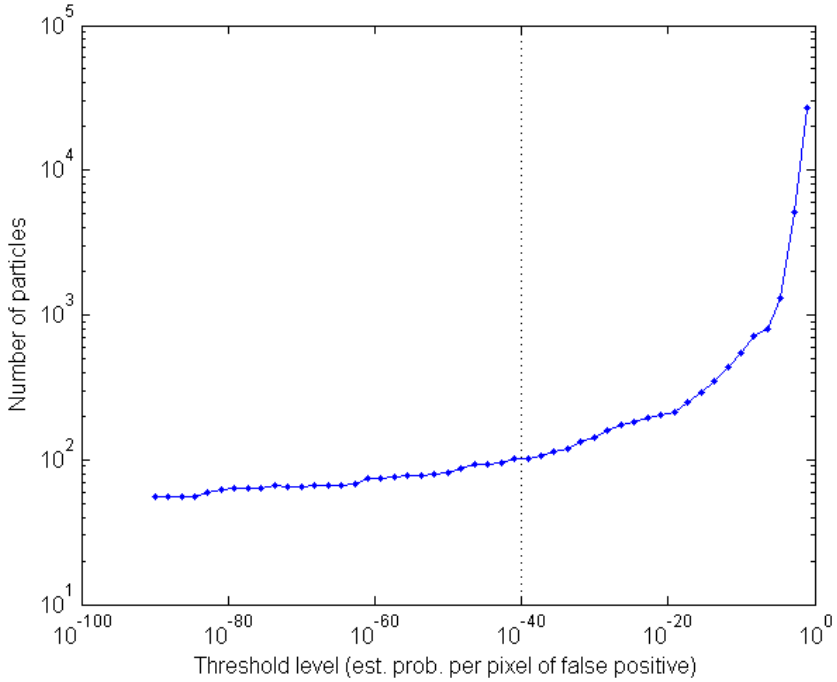


Figure 9. Plot showing the number of counts versus the threshold value.

6.4 Analog signal out

The instrument has an analog current output, 4-20 mA.

- 4 mA corresponds to 0 /ul.
- 20 mA corresponds to the maximum concentration set in the Settings.ini-file, e.g. 1 / ul (1000/ml).

7 Remote connection via Modbus

The IP address for the Flow Cytometer (FC#3) was assigned to 192.168.221.111 in order to be accessible locally over an Ethernet cable. The user should be able to connect to the instrument whose Station-ID is chosen to be 46 using the IPv4 protocol. The FC firmware listens to port 502, and provides access to the holding registers from register addresses 0 to 13, decimal.

At present, the instrument stores 14 registers (of type int16), of which only four are meant to contain measurement data. Their meaning is related to the latest complete measurement, as summarized in the following list:

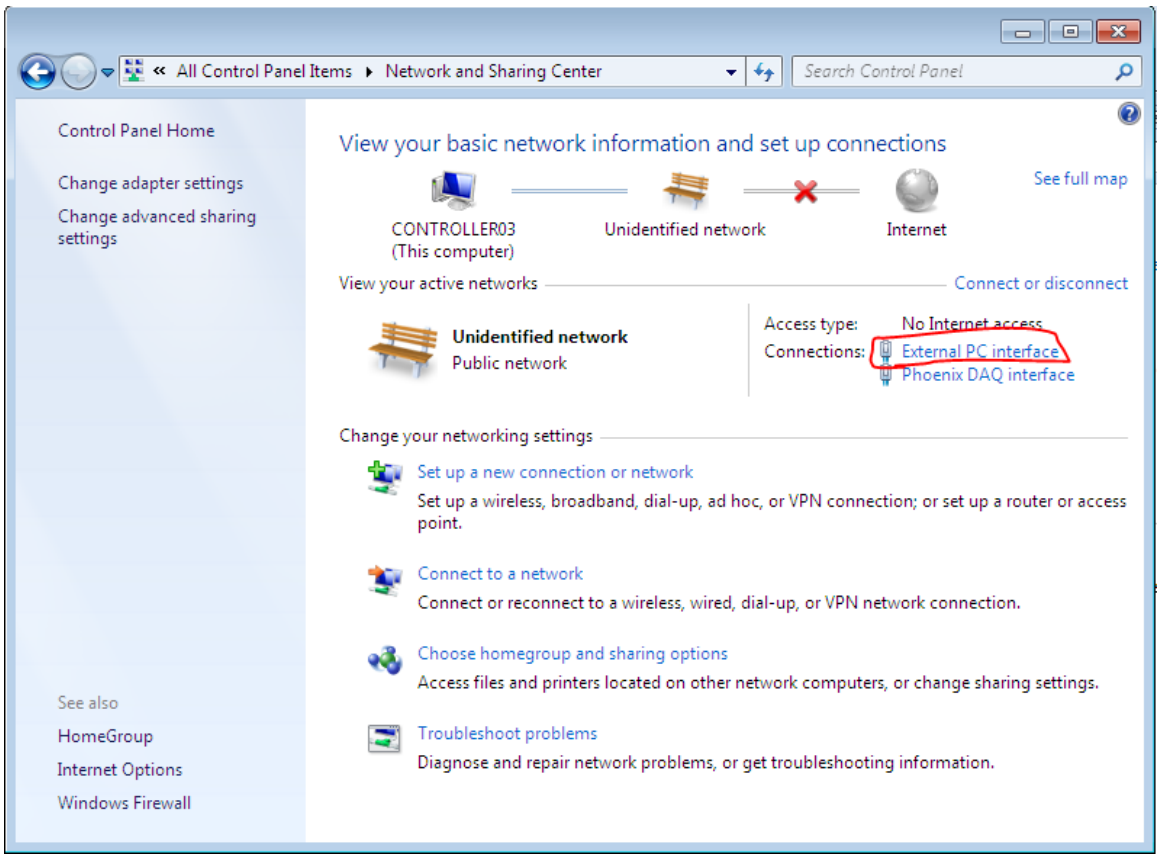
Table 6.

#	Addr	Register name	Typical value	Additional comment
1.	0	Register Count	14	This number may increase in future firmware releases
2.	1	Latest Year	2016	
3.	2	Latest Month	1 … 12	
4.	3	Latest Day	1 … 31	
5.	4	Latest Hour	0 … 24	
6.	5	Latest Minute	0 … 59	
7.	6	Latest Second	0 … 59	
8.	7	Latest Duration	0 … 32767	Measurement duration in seconds
9.	8	Latest Data Word1	0 … 32767	Particle count in the latest completed measurement
10.	9	Latest Data Word2	0 … 32767	Volume (µl) processed in the latest measurement
11.	10	Latest Data Word3	–32768 … 32767	Unused, set to -1
12.	11	Latest Data Word4	–32768 … 32767	Unused, set to -1
13.	12	Phoenix Mirror DI	0 … 3	Front-panel switches: current configuration
14.	13	Phoenix Mirror DO	0 … 511	Phoenix module’s discrete outputs: current configuration

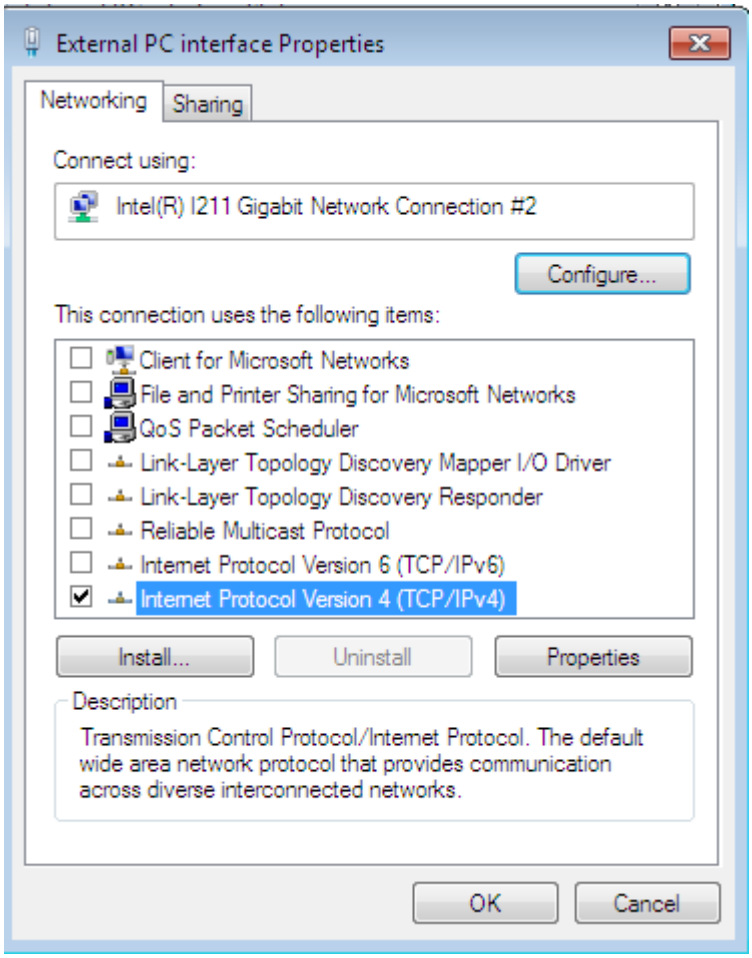
Register #8 above provides the duration of the latest completed measurement sequence. This is useful for the case when a remote user (Master) initiates a non-scheduled measurement via ModbusTCP. The result of a newly started measurement will become available in registers #9 – #11 after approximately so many seconds.

8 How to Change the Instrument IP Address

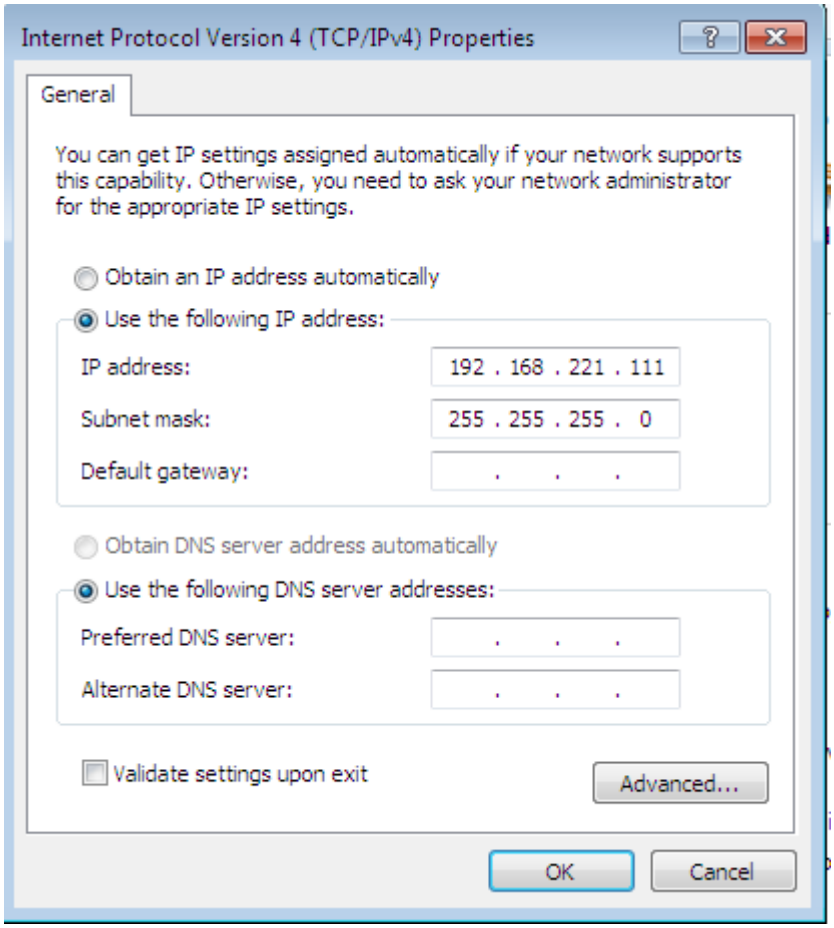
1. Make sure the ethernet cable is connected to the 2nd LAN port on the embedded PC
2. On the embedded PC: Open "Network and Sharing Center" from Control Panel
3. Click on the "External PC interface



4. Click on "Properties" button
5. Mark "Internet Protocol Ver 4" and click "Properties"



6. Choose "Use the following IP address" and enter the IP address and netmask:



8.1 To enable internet access (if network allows)

1. Do all above, 1-5
2. Choose: "Obtain an IP address automatically"
3. Click "OK"