PamStation®12

Workstation Processing Flow-Through PamChip® arrays

PamGene

PamStation12 Operator Manual

Version 13, 2022

C E

PamStation®12 instrument meets the Essential Requirements of the EMC Directives 98/37/EC, 73/23/EEC and 89/336/EEC for an EG statement type IIA.

Contents

1   SYMBOLS USED IN THIS MANUAL

Symbols are ANSI/IEC approved (American National Standard Institute and International Electro technical Commission) International Standardization Organization.

Read this carefully

Caution: Risk of personal injury to the user or a safety hazard.

Caution: Lift carefully. Heavy item could cause injury

Caution: Hot surface

Caution: High Voltage

Protect from direct sun light.

2   INTRODUCTION

2.1   Intended use

The PamStation®12 instrument is solely intended for the processing of up to 3 x PamChip®4 microarrays and is intended for research purposes only.

2.2   Description of the instrument

The PamStation®12 is an integrated, microfluidic workstation consisting of an incubator, dispensing and aspiration needle(s) and an imaging module. It is designed and optimized for PamGene's flow-through microarray technology.

The PamStation®12 is specifically intended for the processing of up to 3 x PamChip®4 microarrays. Both the instrument and the PamChip® microarrays are products of PamGene International BV.

Figure 1: PamChip®4 microarray

2.3   Principles of operation

In the PamStation®12, experiments are carried out in PamChip®4 microarrays. The surface of these microarrays is composed of a substrate consisting of aluminium oxide. This material is approximately 60 μm thick and has a porous structure with long branched interconnected capillaries with a diameter of about 200 nm.

The sample, typically proteins, react with the peptide immobilized on the chip. A fluorescently labelled antibody to detect the phosphorylated peptides on the arrays is added to the sample. The sample is then dispensed onto the PamChip® and the chip placed in the PamStation®12. Once inside the instrument the sample is incubated at a pre-determined temperature. During incubation the sample is pumped back and forth through the substrate to maximize reaction kinetics and minimize analysis time. On traditional microarrays the rate-limiting factor of the phosphorylation reaction is diffusion. Due to the size of the pores, the maximum distance of the target and probe molecules is reduced to 100 nm, thereby eliminating diffusion as a factor in the phosphorylation. Furthermore, due to the reaction-by-pumping, a continuous mixing of the sample is achieved. Typically, incubation is achieved in 0.5-2 hours.

Each PamChip®4 consists of 4 separate microarrays. Each array is spotted with up to 400 peptides molecules. Upon wetting of the surface material of the PamChip® microarrays it becomes translucent. This allows for imaging during, or at the end of, incubation using a CCD digital camera of the system. Up to three different wavelengths can be selected to suit the dyes used. Because the light is of low intensity, no bleaching occurs with normal exposure times. During the assay the temperature of the reaction can be varied. The PamStation®12 allows for aspiration and automated washing of the arrays. Up to four different washing buffers can be loaded, two of which can be heated to the assay temperature. All of these processes are controlled by Evolve12 (software version 3)

3   PAMSTATION®12 SYSTEM

3.1   General principle

The PamStation®12 is an integrated processor for up to 3 PamChip® 4 arrays. It consists of several modules linked by a transport system, which delivers the chip to each module as required. This is achieved by the use of a fast stepper motor. Typically wagon movement from one position to the next is carried out in less than 10 seconds. The instrument is capable of processing 1, 2 or 3 chips (4, 8 or 12 microarrays respectively) at a time.

Figure 2: PamStation®12

The modules within the instrument are:

- Incubator (Wagon)
- Dispensing syringe and aspiration needle(s)
- Imager

3.2   Incubator/ wagon system

Figure 3: Incubator in load position

The incubator, also referred to as the wagon, is the heart of the instrument. It carries the chips between the loading, incubation, washing, and reading positions. It also incubates the chip and facilitates the pressure pumping action during hybridization and washing.

The incubator comprises a heating block, a lid, three cover glasses and three fluid barriers.

The incubator is capable of operating at a temperature range of 20[1]*- 80 °C.

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[1] **The instrument can be set at 20°C but cannot cool below ambient temperature. The software allows the temperature to be set at 20°C however the minimum operating temperature during an experiment is ambient + 3°.**

The incubator may be hot when it is outside the instrument

A cover glass covers each PamChip® array during hybridization or incubation to prevent evaporation of fluid. The lid of the incubator heats these cover glasses to prevent condensation. The cover glasses can be taken out easily to facilitate cleaning. The sample is made to flow back and forth through the array by changing the pressure. Pressure sensors inside the incubator detect broken arrays.

A consumable fluid barrier in the incubator protects the valves, tubing and pumps from liquids. These fluid barriers comprise an absorbent material with a blue colouring agent. A colour change of blue to white will be noticeable if the fluid barrier comes into contact with fluids.

3.3   Dispensing and aspiration system

The dispensing unit is located behind the door at the front of the instrument. It consists of four motor driven syringes. Only position 2 is used for both STK and PTK protocols.

Liquid level is detected by a sensor, which detects the top flange of the syringe as it descends. Each syringe can hold up to 5 ml. At the rear of the compartment is an aspirating needle that removes waste fluid into a 100 ml glass bottle.

Figure 4: The wash head unit

3.4 Imaging system

The excitation light required for detection is provided by one LED. The life of the light source is in excess of 60,000 hours. The light is guided to a position above one array of the PamChip® through an angled mirror, which also allows reading by a 12-bit CCD camera focused on the array. The light of the LED is guided through a filter. The LED can detect fluorescein.

Table 1: Overview of installed filter characteristics

Designation	Dye	Excitation wavelength	Emission wavelength
Filter 1	FITC	460-490	515-550

3.5 Other system parts

3.5.1 Syringes

If an automated dispensing step or an automated wash step is part of the protocol, a syringe filled with the appropriate buffer has to be placed inside the instrument at the position mentioned in the Evolve protocol. **Multiple syringes are supplied with the instrument and more syringes can be acquired at PamGene. Needles are included for free within a box of PamChips**

3.5.2 Waste bottle

The waste bottle can be found outside the instrument at the right-hand side. The waste bottle is connected to the instrument with two tubes: a small one going to the aspiration needle and a bigger one to the aspiration pump. The waste bottle should always be placed in the holder in an upright position. It is advised that the waste bottle must be emptied at the end of the day.

3.5.3 Fluid barrier

The fluid barrier comprises of three different parts; a rubber casing, an absorption powder and a lid that is coloured with a colouring agent. The fluid barrier is placed in the incubator and protects the underlying valves, tubes and pumps. If an array breaks, the fluid will fall onto the fluid barrier and will be absorbed and an error message will be displayed. If this happens the top of the fluid barrier will change colour, from light blue to white. One of the positions has turned white the fluid barrier should be replaced. There are ten fluid barriers supplied with the instrument. More can be ordered from PamGene.

Figure 6: Fluid barrier

Figure 7: Fluid barrier in incubator

4   PREPARATION BEFORE RUNNING AN EXPERIMENT

Make sure the instrument and the PC are connected properly as described in chapter 8.4: Installation of the PamStation®12 and PC.

4.1   Starting up instrument and PC

- Turn on the instrument by switching on the power supply using the switch at the back-left hand side of the instrument above the power cord.

Figure 8: Power switch at left side of the instrument

- Turn on the PC and the Monitor. Log into the system using the standard Microsoft log-in prompt.

    Log in as   pamstation
    Password is   pamgene

4.2 Syringes

If an automated dispense step or an automated (quick) wash step is part of the protocol, a syringe filled with the appropriate buffer has to be placed, at the position described in the Evolve protocol, inside the instrument.

Preparation of the syringes

Take a clean syringe.

Select a wash-needle (art. # 32155) which was used less than 5 times.

Connect the wash-needle to the glass part of the syringe.

Pour the buffer (either 1 x PK or PBS-T buffer) into the syringe Insert the metal piston approximately 1 mm into the barrel.

Turn the complete syringe upside down (needle must point upwards).

Remove all air bubbles (only air bubbles smaller than +/- 0,5 mm are allowed).

Remove remaining buffer from the outside of the syringe and needle with a tissue.

Take the complete syringe and push the metal piston to dispense a small amount of buffer, make sure the buffer can exit the needle without much effort.

Cleaning of Syringes

Remove any remaining wash buffer from the syringe.

Remove the metal piston from the glass part of the syringe.

Fill the syringe with MQ water.

Insert the metal piston back in the syringe.

Dispense the MQ water through the needle by pushing the piston.

Repeat step 2 till 5, 3 times.

Remove the metal piston and disconnect the needle from the glass part of the syringe.

Place the syringe parts in an open box for several hours to enable any remaining water to evaporate.

Mark the storage box to registry the times the needle is used.

Placement of syringes in the PamStation®12

Open the front door of the PamStation12 instrument.

Place the complete syringe into slot #2 of the PamStation12.

Gently tighten the rollers against the metal piston by turning the tension screw of slot #2 a quarter against the clock.

Check from the top if the two rollers nicely surround the metal piston.

Remove the droplet collected in the needle tip with a tissue.

⚠️ **Don't apply vertical pressure on the opened door.**

Behind the door at the front, four slots are visible, labelled 1 to 4. Generally, for kinase assays only slots 2 is used! Turn the switch above the slot 90 degrees clockwise to open it. Make sure that the flat side of the syringe is pointing towards the instrument and between the two metal holders of the slot.

Turn the switch to its original position, the slot will close and the syringe will be held in place.

⚠️ **Position of waste fluid tubing.**

The waste tubing should not be blocked by a syringe which is positioned in position 3 or 4 but should move in front of the white block as indicated in the figure below.

Figure 9: Inside door of the PamStation®12 and placement of a syringe.

4.3 Waste bottle

Before starting an assay, check that the waste bottle is attached in the proper way to the instrument (screwed to the red cap) and check that the bottle is empty. The bottle needs to be positioned straight in the dedicated holder at the right-hand side of the instrument. If needed, empty the waste bottle by unscrewing the glass part of the bottle. Dispose of the contents in the appropriate way.

There is no warning if the waste bottle is full. A full bottle can cause aspiration errors and damage the aspiration pump.

4.4   Fluid barrier

If an array breaks, the fluid will fall onto the fluid barrier and will be absorbed. A fluid barrier has to be placed in the incubator before starting an experiment. The fluid barrier will protect the underlying valves, tubes and pumps. An error message will be displayed when the system detects a broken array. This array will no longer be used by the system.

Placement of a fluid barrier

Before running an experiment, a fluid barrier has to be inserted in the incubator. This is normally done during the load step of a protocol when the wagon is moved outside the PamStation®12.

To insert a fluid barrier, move the incubator outside the instrument (during a disposable load), open the lid of the incubator by turning the big metal button at the front of the wagon clockwise and place the fluid barrier in the incubator as shown on the pictures below. A PamChip® can be used to press the fluid barrier deeper into the incubator.

Quality control of fluid barrier

At the end of every experiment, when the PamChip® has been removed, the fluid barrier has to be checked for a colour change. If fluid has been in contact with the fluid barrier the colour of the fluid barrier will change from blue to white. If this occurs the fluid barrier must to be replaced by a new one. It is advised to check the fluid barrier also prior to the start of an experiment.

Removal of fluid barrier

This can easily be done with the aid of tweezers.

Figure 10: Fluid barrier in incubator

5 EVOLVE12 SOFTWARE

The PamStation®12 uses Evolve12 software (version 3) for the creation and running of protocols.

Open Evolve12 by clicking on the Evolve shortcut icon on the desktop.

The following pop-up window will open:
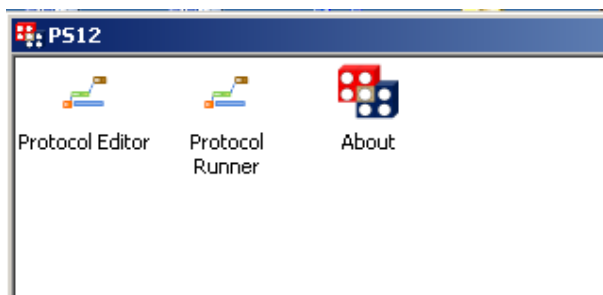
Evolve12 comprises of two different parts; Evolve editor for creating a protocol, Evolve runner for the running of a protocol. The 'About'-box contains version and author information.

Table 2: Evolve software parts

Activity	Program
Creating a protocol	Evolve12 Protocol Editor
Running of Experiment	Evolve12 Protocol Runner

Chapter 5.1 will describe the usage of the editor and the making of a protocol while chapter 5.2 will describe the protocol runner.

5.1 Evolve12 protocol editor

This section of the manual is to help scientists and engineers in designing and making protocols.

5.1.1 Starting up the protocol editor

Open evolve by double clicking on the 'Evolve12 Protocol Editor' icon.

Protocol Editor

5.1.2 Protocol editor screen

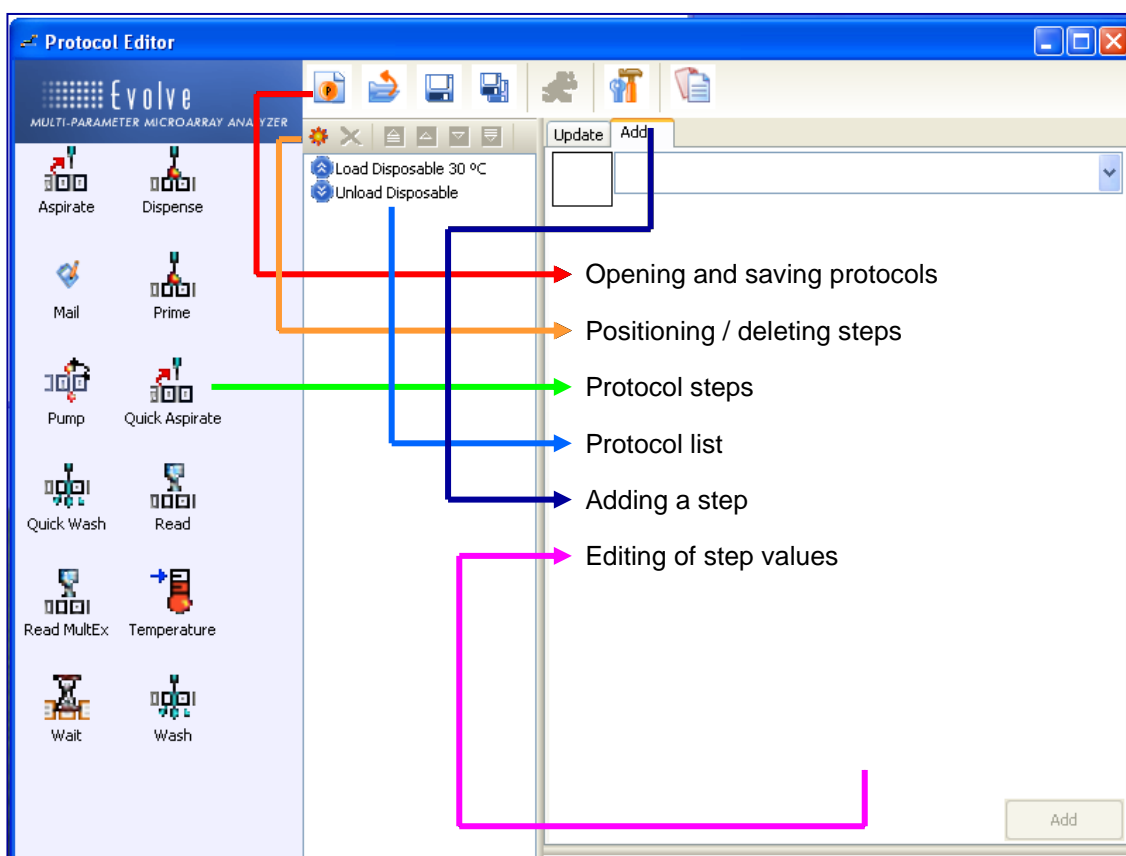The protocol editor screen comprises of 6 different areas as shown in figure 11.

Figure 11: Evolve Protocol Editor window

Open/saving a protocol

Located at the top middle of the protocol editor screen (see figure 12) are 7 buttons. With these buttons it is possible to start a completely new protocol, open an existing one, save the current protocol, save the current protocol with a different name (save as), view the default parameter setting of the PamStation12 or copy the protocol to the clipboard.

After saving a protocol and clicking anywhere on the screen a seventh button will appear, This so called 'running man'-run button will provide easy access to the protocol runner.

The 'advance protocol parameters' icon allows to view the default parameter settings of the PamStation (these parameters can be modified by a PamGene representative).

The 'copy to clipboard' symbol allows the user to copy the protocol to the clipboard.

Open a completely new protocol

Open an existing protocol

Save current protocol

Save current protocol as

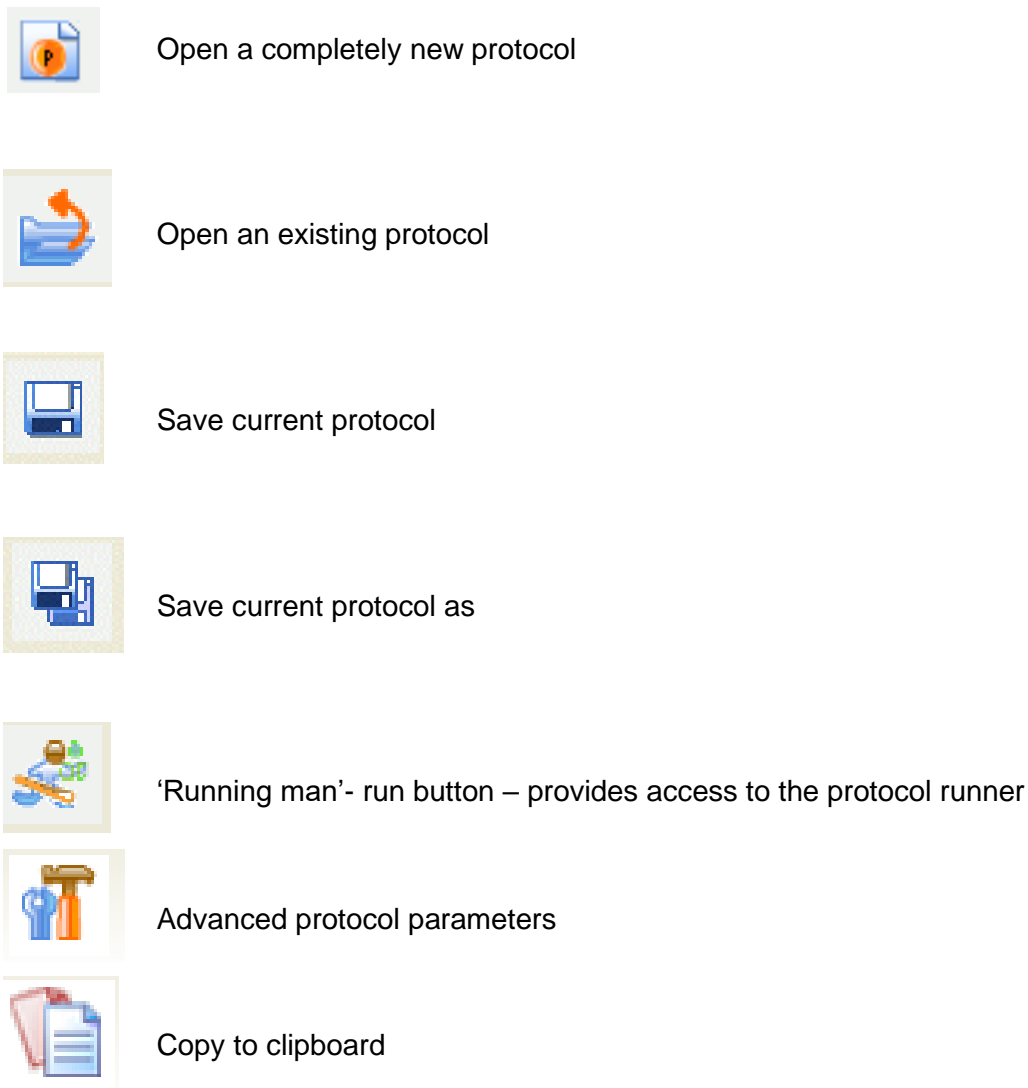'Running man'- run button – provides access to the protocol runner

Advanced protocol parameters

Copy to clipboard

Figure 12: Menu-bar protocol editor

Protocol steps

On the left side of the protocol editor screen are 12 symbols. These symbols represent the different steps of the protocol. These steps can be added to form the desired protocol. The steps are described in a short reference table and in more depth in chapter 5.1.3, 'Description of protocol steps.'

5.1.3 Description of protocol steps

Short descriptions of the 12 protocol steps are given in the reference table below. Also a recommended value for each step is given for optimal results of the assay. Please note that the terms '**disposable**' and '**PamChip®**' have the same meaning.

Table 3: Description of Protocol Steps

Step	Variable Parameters	Recommended values	Explanation
Aspirate	Duration	5-10 sec Depending on amount	Removes fluid from arrays
Quick Aspirate	Duration	5-10 sec Depending on amount	Removes fluid from arrays in a quick way.
Dispense	Dispense (Manual)	(option to add solution manually)	Dispenses specified fluid onto the arrays.
	Fluid	1-4	
	Temperature	35-60°C	
	Amount of dispense	25 µl	
Pump	Number of Cycles	Any amount	Incubates the array for a specified time and controls pressure cycles
	Cycle time	20-60 sec depending on amount and viscosity of fluid and application	
	Cycle frequency	See above	
Read	Once / read by cycle	Assay dependent	Records images, once or kinetically (only one exposure time per filter type)
	Filter	1-3	
	Exposure time (ms)	Assay and filter dependence	
Read MultEx	Once / read by cycle	Assay dependent	Records images, once or kinetically. (multiple exposure times are possible with the same filter type)
	Filter	1-3	
	Exposure time (ms)	Assay and filter dependence	

	Temperature	Assay dependent	Changes temperature of the incubator
	Dispensing, aspiration and pumping		The wash step is a combination of the dispensing, pumping and aspiration steps.
	Dispensing, aspiration and pumping		The wash step is a combination of the dispensing, pumping and a quick aspiration step.
	Wait	e.g. 10 seconds	Provides the option to pause in a protocol
	Prime	25-50 µl Select Fluid 1 - 4	Primes a dispense needle
	Mail	(see section 8 and consult ICT to setup the system)	The mail step can be used to inform the user of the processing status of the instrument

Loading and unloading disposable

Every protocol starts with a 'load disposable' (load PamChip®) step. This step initializes the instrument to a set pressure and predetermined variable temperature. The temperature is variable between 20 and 80 degrees Celsius[2]. During this step the instrument will ask the operator to press the load button (located on the left bottom of the screen). After pressing this button, the wagon will move outside the instrument and the operator can place the PamChip® into the incubator. (The green button turns into a red stop button). Every protocol ends with an 'Unload disposable' step. During this step it is possible and recommended to remove the disposable from the instrument.

Figure 13: Load and Unload window

Aspiration step

The aspiration step removes liquids from the arrays manually or by use of the aspiration needle. By selecting the manual check box the wagon will move outside and the operator can remove the fluid manually. The default option is to remove fluids by use of the aspiration needle. The aspiration time is the time it takes the aspiration needle removes fluids from the array. The time needed depends on the amount of fluid and its viscosity. The recommended value is 10 seconds for 25 µl of water per array.

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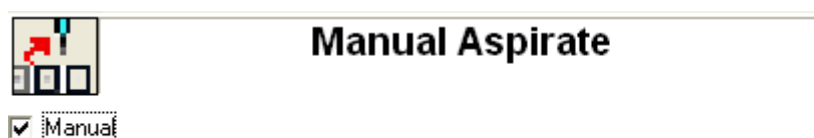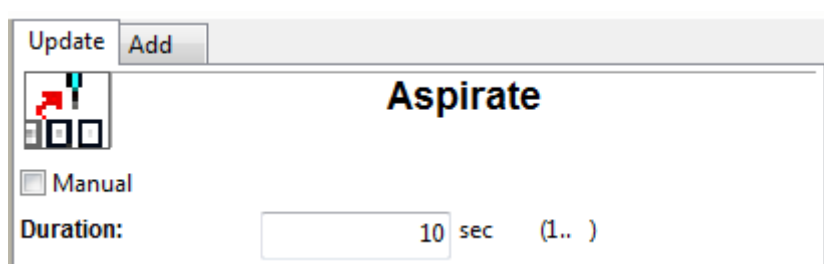
[2] The instrument can be set at 20°C but cannot cool below ambient temperature. The software allows the temperature to be set at 20°C however the minimum operating temperature during an experiment is ambient + 3°.

Quick aspiration step

The quick aspiration step is a quicker version of the normal aspirate step. By selecting the manual check box, the wagon will move outside and the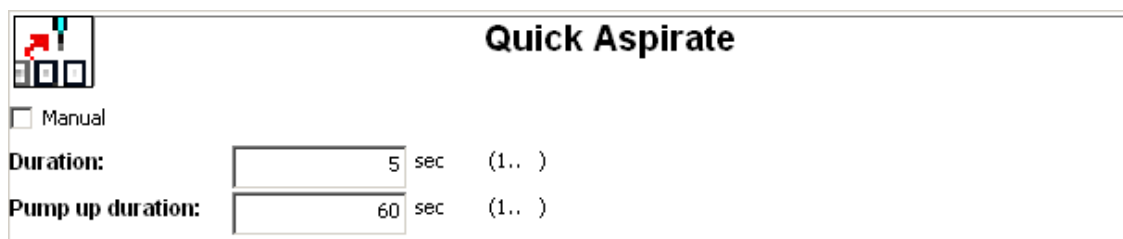 operator can remove the fluid manually. The default option is to remove fluids by use of the aspiration needle. The 'duration' is the time it takes the aspiration needle removes fluids from the array. The 'pump up' duration is the time needed to pump up all fluid prior to aspiration. The pump-up duration depends on the amount of fluid and its viscosity. The recommended value is around 60 seconds for 40 µl of fluid per array.

Figure 14: Aspiration window

Dispensing step

The dispensing step adds fluids to the arrays manually or automatically. The operator has several options in this step. The first option is to dispense manually or automatically. If the manual box is checked the wagon will move outside the instrument. The operator can then manually add fluids.

The default option is to let the instrument dispense fluids by using up to four different syringes. As described in section Dispensing and aspiration system, there is room for up to four different syringes with different buffers. Placement of the syringes is described in Syringes. Syringe numbers 1 and 2 (also called fluid 1 and 2) can only be operated at room temperature while numbers 3 and 4 can be heated.

If syringes 3 or 4 are selected the option to enter the preferred temperature opens up (grey temperature field turns white). There is no active cooling on any syringe. The amount of liquid that can be added to an array is between 5-50 µl, although 40 µl is recommended. After dispensing, manually or automatically, the fluid is always pumped down. (See section 4.2 for addition information about syringes)

Dispense

☐ Manual

Fluid Name: fluid1

Fluid Temperature: 45 ºC   (45..60)

Volume: 25 µl   (5..50)

☐ Temperature control

Manual Dispense

☑ Manual

Figure 15: Dispense window

The manual dispense will bring the wagon outside of the instrument and allows the user to add sample to each of the arrays. The manual dispense step can also be used to add reagents to the arrays or wash the arrays manually.

Pump step

Using the pump step, it is possible to incubate the disposable for a specified time and to control the pressure cycles. There are three variables in this step:

- Number of cycles
- Cycle time
- Cycle frequency

A cycle consists of pumping the liquid up through the array one time then down one time. This is done by opening a valve for a very short time (less than 1 sec), closing the valve and then waiting for a given amount of time (e.g., 15 cycles mean pumping the fluid 15 times up then 15 times down through the array. The cycle time is the time that a complete cycle will take. The cycle frequency is the number of cycles per minute. The cycle time and the cycle frequency are linked. If a kinetic read is required use the read step with the read by cycle option (explained in the read step) instead of this step.
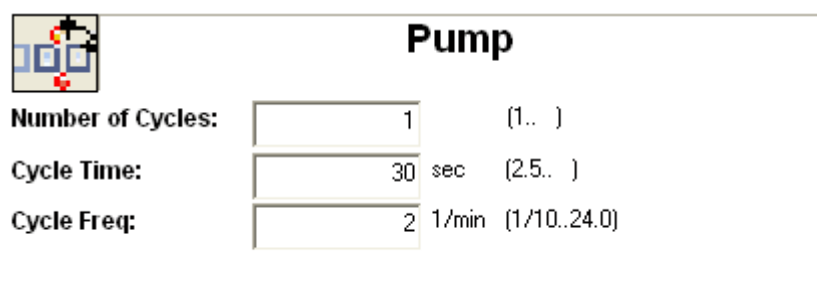
Figure 16: Pump window

Read step

The read step is the step that takes the pictures. This can be done once or kinetically (every few cycles). This is determined by checking the box of your choice.

If the once box is checked the only other option available is the selection of filter or filters and the desired shutter time. This selection is done by double clicking on 'filter 1'. A pop-up screen with scroll down function will appear. In the scroll down function the selection of filters (one to three) can be found and at the bottom of the pop-up screen. The desired shutter time can be programmed.

It is possible to add different filters, this is done by pressing the add button shown as a small sun button. This will activate the aforementioned pop-up menu
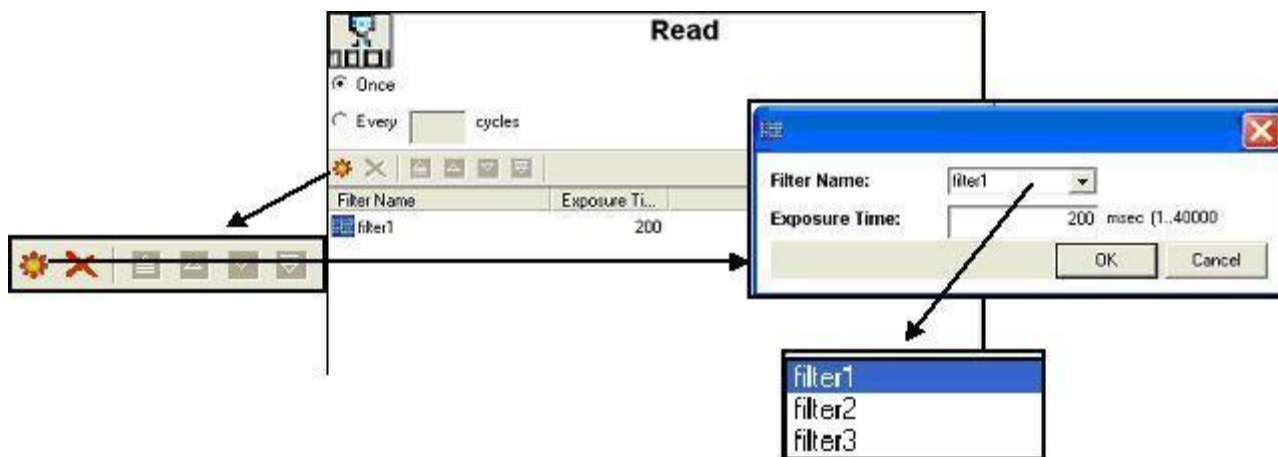
Figure 17: Read window

If a kinetic read is chosen, an addition to the variables is shown. This addition includes all the variables of the pump step as described in the aforementioned pump step as array as the now open option available when a picture has to be taken; 'every (number) cycles. With the read step it is not necessary to program in a pump step as one is already automatically included.
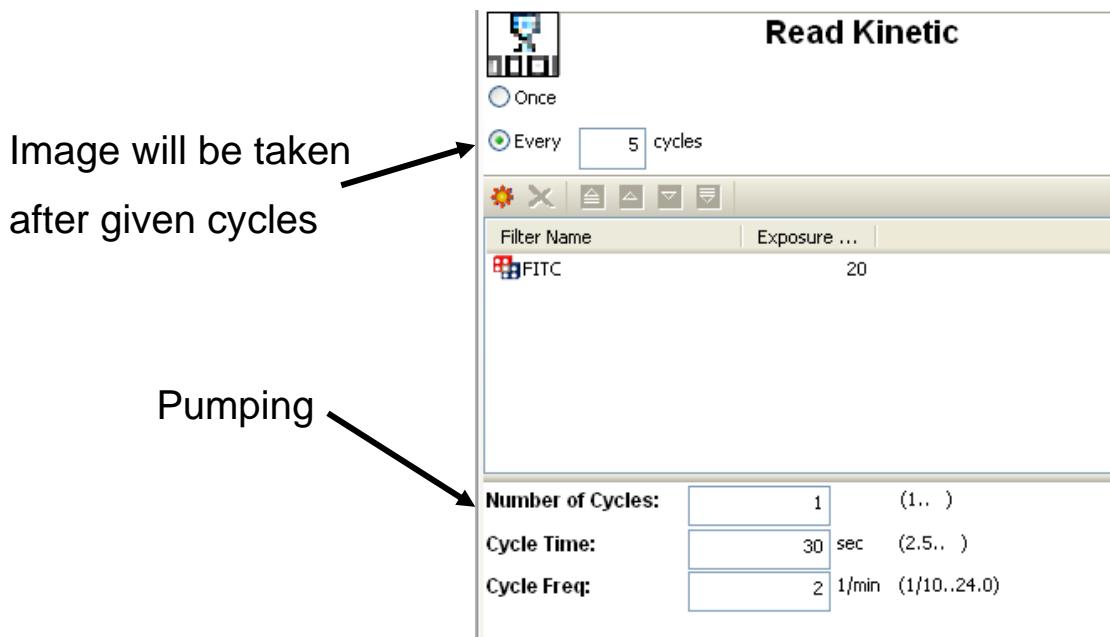
Figure 18: Read kinetic window

The imaging system is optimised for reading when the arrays contain fluid. Do not perform a read step after an aspiration or washing step since the array will not contain sufficient fluid for optimal imaging.

Read MultEx

The read MultEx is almost identical to the normal read step but the read MultEx enables the user to take images with different exposure times made with the same filter within one read step.
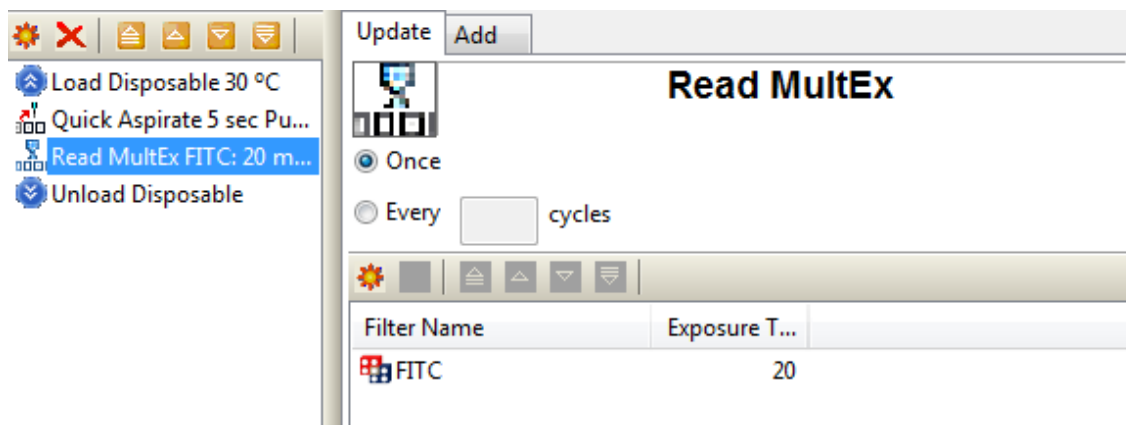
Temperature step

This step sets the temperature of the assay between (20-80°C)
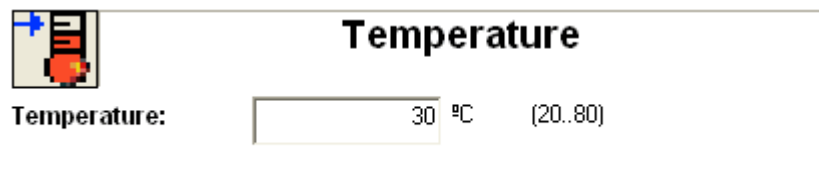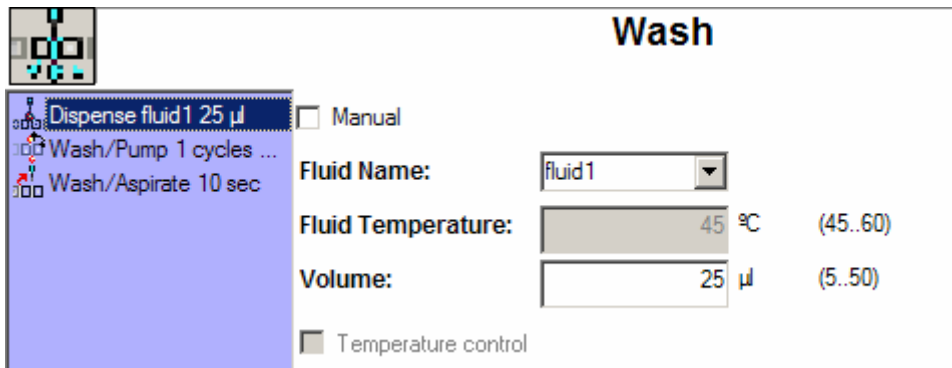
Figure 19: Temperature window

Wash step

The wash step consists of a dispense step, a pump step and an aspirating step. Information about these steps is given in the appropriate chapters earlier in this manual.
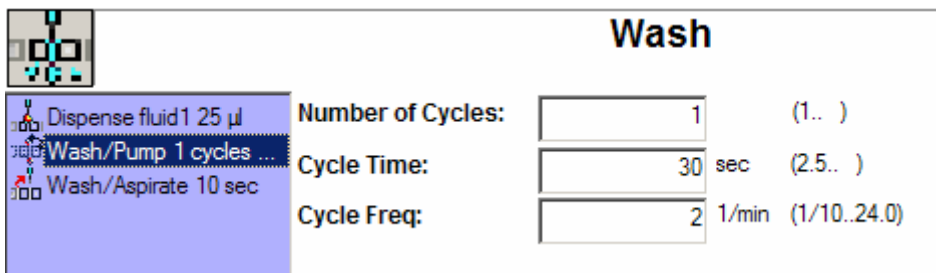
The cycle counter in the protocol runner does not count pumping being done in the wash step.

Dispense settings in Wash step

Pump settings in Wash step

Aspirate settings in Wash step

Figure 20: Wash window

Quick wash step

The quick wash step consists of a dispensing step, a pumping step and a quick aspiration step. Information about these steps is given in the corresponding chapters in this manual.

The cycle counter in the protocol runner does not count pumping being done in the wash step.
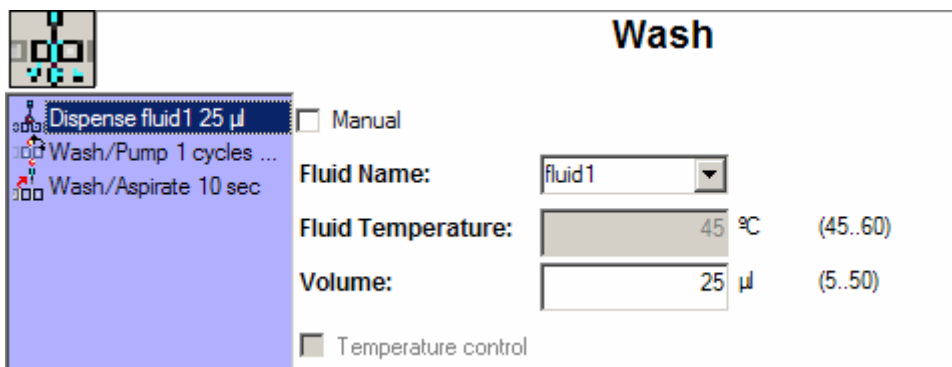
Dispense settings in Wash step
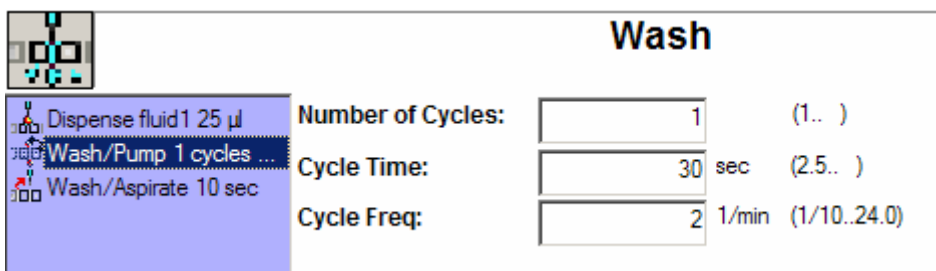
Pump settings in Wash step
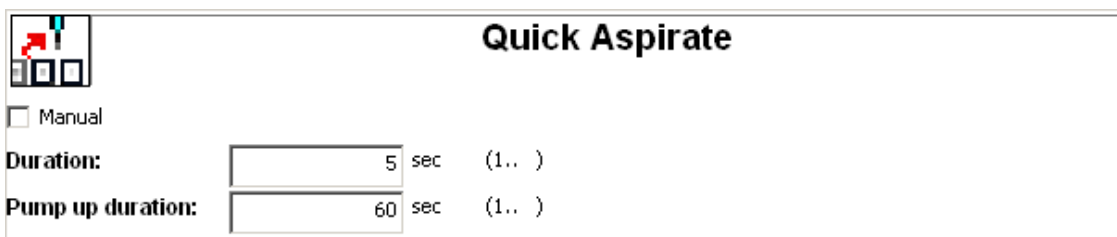
Aspirate settings in Wash step

Figure 21: Quick-wash window

Wait Step

The pausing of a protocol for a specified time in seconds (minimum 1 sec) is accomplished with the wait step.
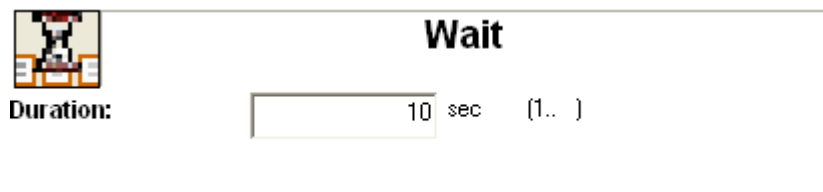
Figure 21: Wait window

Priming step

Prior to a dispensing or wash step it is recommended to run a priming step for the specific dispensing needles. Fluid is dispensed in small tubes at the front corners of the incubator. These tubes have to be cleaned/emptied regularly.
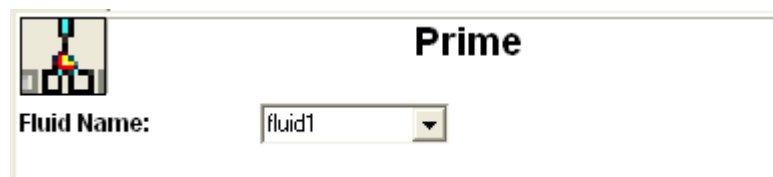
Figure 22: Prime window

Mail Step

The mail step can be used when the user needs to do something else then standing by the instrument waiting for every step when it is going to be finished. The mail step sends a mail to the user mail address telling on which step the current protocol runs.
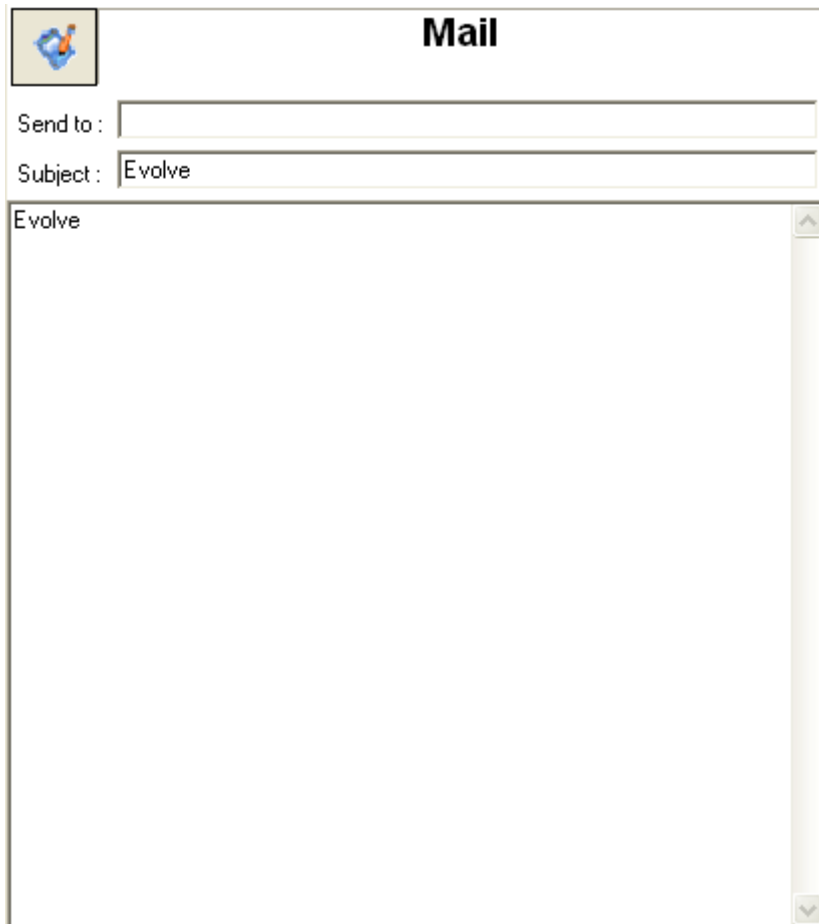
Figure 23: Mail window

Adding steps

Steps can be added to the protocol list by clicking on the preferred step, holding the mouse button and dragging the step to its desired location in the protocol list. A second option is to click on the 'add' button, whereupon a scroll down menu with the names of the different steps will appear. Click on the name of the preferred step and it will appear in the value-editing screen. After inserting the relevant values for the preferred step, the operator can click on the 'add' button located at the bottom right of the value-editing window.

5.1.4 Protocol steps

Located in the middle of the screen is the current protocol with a listing of all the current protocol steps. By clicking on a step, the information relating to that step will be shown in the value-editing window at the right-hand side of the screen.

5.1.5 Positioning of steps

Located above the list of protocol steps are 6 small buttons.

These buttons can be used to move a step to a different position in the list of protocol steps or to delete the step.

New step

Delete step

Move step to the top of the protocol

Move step one place up in the protocol

Move step one place down in the protocol

Move step to the bottom of the protocol

If the evolve screen is minimized these buttons can disappear; just enlarge the screen and they will reappear.

5.1.6 Editing of step values

If a step is added to the protocol list, the options / variables of that step will be shown in the editing window on the right side of the protocol screen. By clicking on an option / value this can be changed. Every step has different options and variables; these are described in chapter 5.1.3, 'Description of steps.' After changing an option or variable click on the update button located on the bottom right side of the screen.

5.1.7 Advanced protocol parameters

Use the 'Tools' button to select the protocol to be edited. This button is located at the top of the screen, in the centre.

In the advanced protocol parameters, it is possible to set some of the instrument operating parameters. These parameters are protected and can't be changed by the user. For changes to these parameters please contact PamGene. The advanced protocol settings are saved with the protocol.
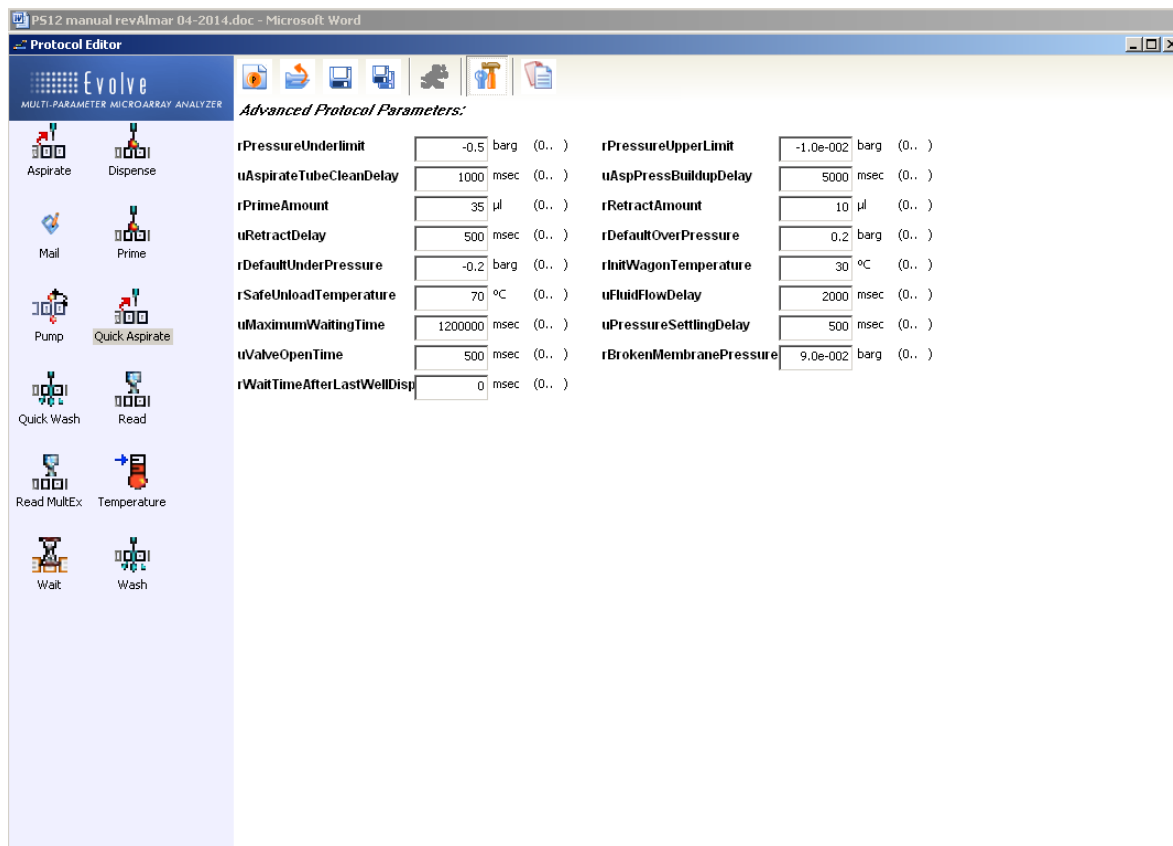
Figure 24: Advanced protocol settings

5.1.8 'Running man'- run button

Located next to the 'save' button at the top middle of the screen is the 'running man' run button. This button is only visible if the protocol is saved and when it is selected from the protocol list.

By clicking on this button, a quick link to the protocol runner is opened, so it is not necessary to open the protocol runner separately.

5.1.9 Editing an existing protocol

Use the 'open a protocol' button to select the protocol to be edited. This button is located at the top of the screen, in the centre.

A screen with a list of protocols will open. Select the protocol to be edited and press the open button.
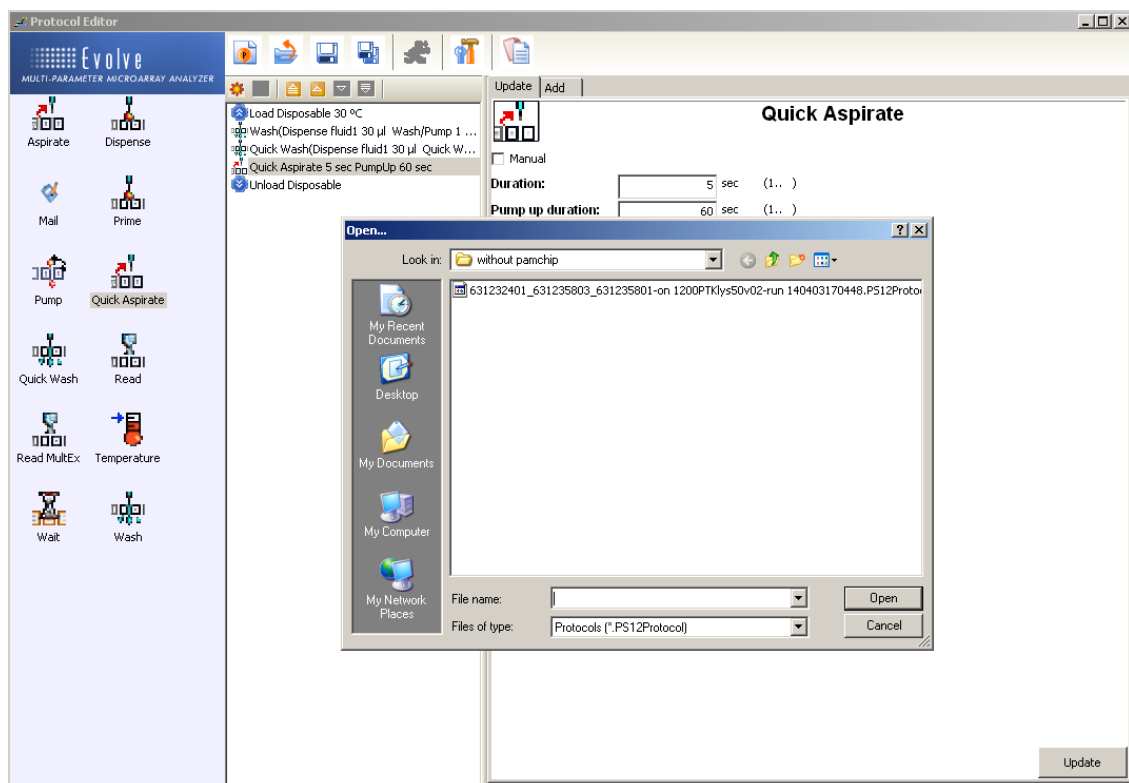
Figure 25: Editing an existing protocol

Steps can be added, removed and edited as described in chapter 5.1.2 to 5.1.6. The steps can be moved up and down the protocol list by using the positioning buttons as described in 5.1.5. Saving the protocol is described in 5.1.11.

5.1.10 Saving a protocol

A protocol can be saved using the 'save' or 'save as' buttons located at the top of the screen in the centre.

'Save' button; saves the protocol with the name and place already chosen

'Save as' button; saves the protocol and defines a new name and place

5.1.11 Loading a protocol

Open the Evolve protocol editor as described in chapter 5.1.1, 'Starting up the protocol editor.' Double click on the 'New Protocol' icon at the top middle of the screen.

The protocol will consist of a combination of the 9 steps shown on the left-hand side of the protocol screen. These pre-programmed steps, their functions and parameters involved are described in chapter 5.1.3, 'Description of protocol steps'. A protocol will consist of some, but not necessarily all, of the steps described. The different steps can be integrated in the protocol by clicking, holding the mouse button and dragging them to their right position.

If a step is not in the right position or the step has to be removed use then the 'position buttons' that are located above the list of protocol steps as described in 5.1.5, 'Positioning of steps.'

After a step is positioned correctly, it can be edited to achieve the desired values. Select the step by clicking on it with the mouse. An editing screen will appear on the right side of the screen. After editing the step to the desired values press the update button located at the bottom right of the screen. If the protocol is finished it can be saved by using the 'save' or the 'save as' buttons that are located at the top middle of the screen.

5.2 Protocol runner

Open Evolve protocol runner by double clicking on the 'Evolve' symbol.

Protocol
Runner

It is also possible to open the protocol runner from the evolve protocol runner screen by pressing the 'running man'-run button located next to the save buttons at the top middle of the screen. See chapter 5.1.8, 'Running man-run button.'

5.2.1 Protocol runner screen
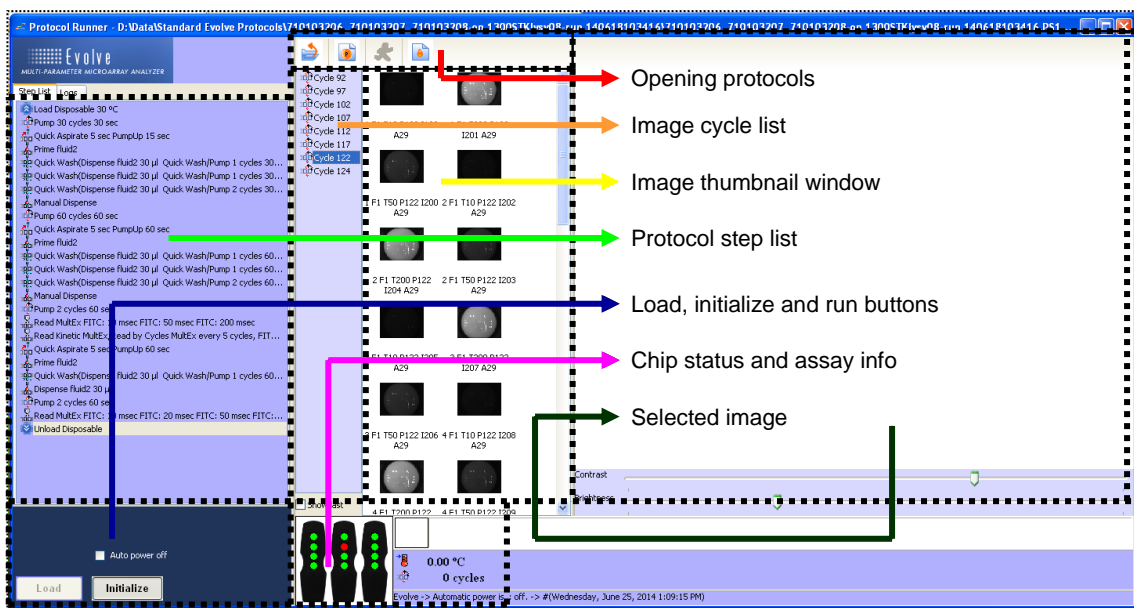
Figure 26: Protocol runner window

Open protocol

You can now select a protocol to run. Click on the 'Open' icon found at the top of the screen:

The following screen will appear:
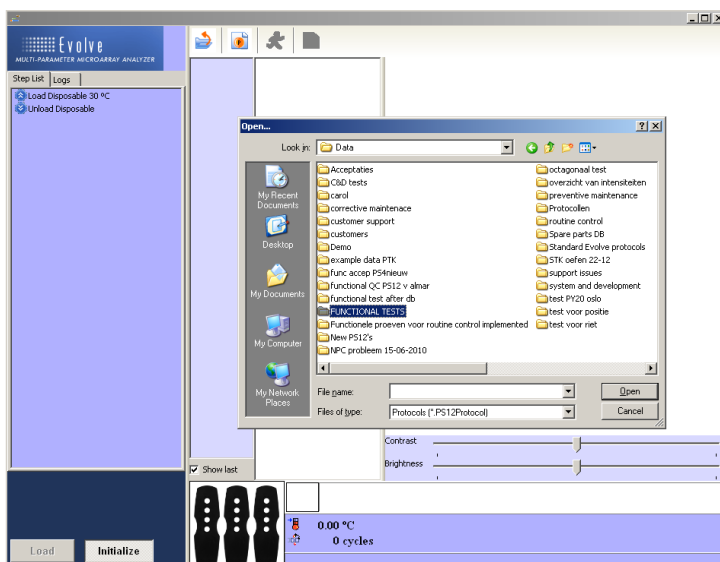
Figure 27: Open protocol runner window

Select your protocol file. Click on 'open'. You have now successfully loaded your protocol into the protocol runner.

Image list
The rectangle next to the protocol list shows a list of pump cycle numbers.
During these pump cycles the PamStation12 has taken images. By clicking on one cycle numbers, the images taken will be shown in the Image thumbnail window.

Image thumbnail window
When a pump cycle is chosen from the list, all the images taken at that specific cycle are shown in the Image thumbnail window. Each of the images has a unique name.

Protocol list

Located on the left side of the screen in the complete list of the protocol steps in this protocol. To view a step more completely, leave the mouse pointer for a few seconds and the information will automatically pop-up.

Load, initialize and run buttons

At the bottom left of the screen, under the protocol list, are two buttons; the 'load' button that has to be pressed to move the wagon outside (the instrument will ask the operator when this is needed) and the 'initialize' button to make contact between the software and the instrument. After pressing the 'initialize' button the green 'run' button will appear. This button has to be pressed to start running the protocol. (Chapter 5.2.2., figure 28, point 3)

If the green 'run' button is pressed it will change to a red 'stop' button. This red button should be used if a run suddenly needs to be aborted. Should it be pressed, an error-handling screen will pop-up. See chapter 7, 'Error handling'.

Chip status and assay information

At the bottom of the screen, in the centre, the chip status display is shown. The little circles represent the arrays on the chip. Each circle can have 3 colours:

- White: The array is not selected, and not used for the experiment;
- Green: The array is selected and assay is in progress without problems;
- Red: A problem is detected with the array. The array is turned off for the remaining of the experiment.

Next to the chip status display the actual incubator temperature is shown. This does not always reflect the temperature of your sample, which remains at the one programmed into your 'temperature' or 'load' step. The amount of pumping cycles completed is also registered here. Pumping cycles carried out during a wash step are not counted. (Chapter 5.2.2., figure 28, point 4.

Images list

Images taken during a protocol are grouped according to the number of cycles being pumped. This image-list is shown in the middle of the protocol screen. When clicking on a 'cycle number' all the images with that number of pumping cycles will be shown

as thumbnails. It is possible to select one thumbnail to get a bigger picture on the screen. (Chapter 5.2.2., figure 28, point 6 and 7)

5.2.2 Running a protocol

After opening the protocol runner and loading the desired protocol (see chapter 5.2.1, 'Protocol runner screen'), the following screen will be shown. The name of the protocol will appear at the top of the screen. The steps of your protocol will appear at the left-hand side of the screen.
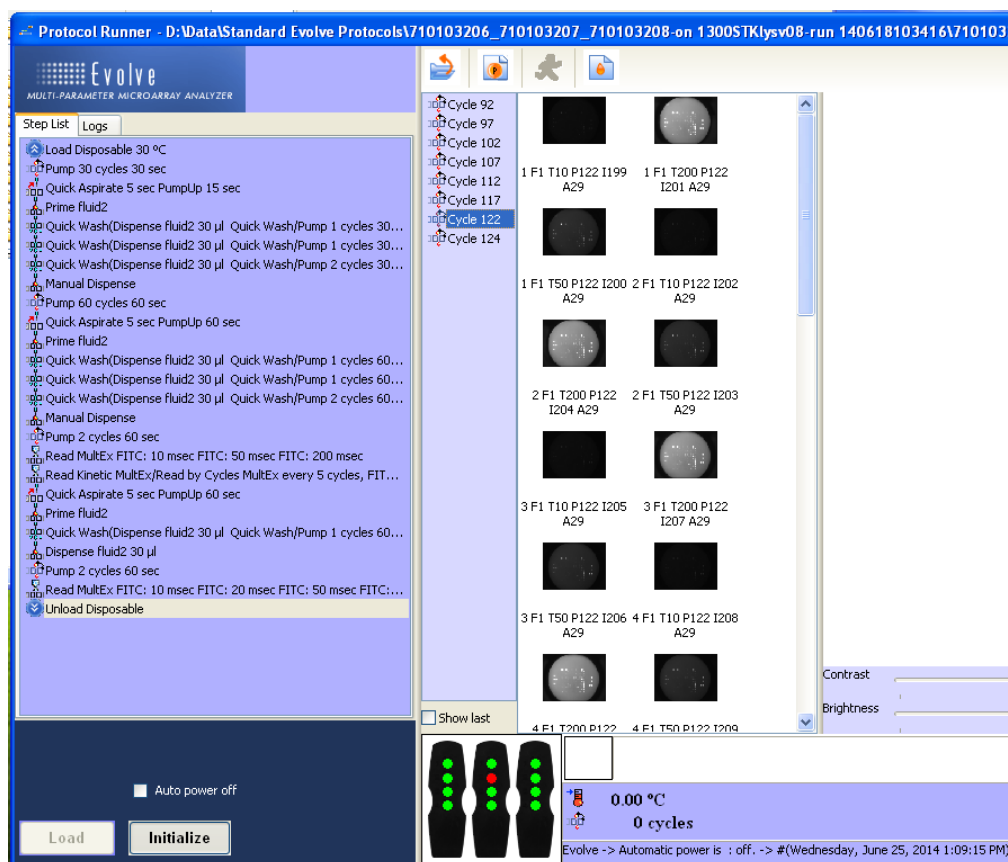
Figure 28: Protocol runner window

Click on the 'initialize' button. The computer will now connect itself to the instrument. Make sure that the instrument is connected and turned on. A large green 'run' button will pop-up.

Click on the green RUN button to activate and start the protocol. This button will now change into a red stop button.

While this red button can be used to stop a protocol at any time, it should only be used when absolutely necessary.

The next screen which appears is
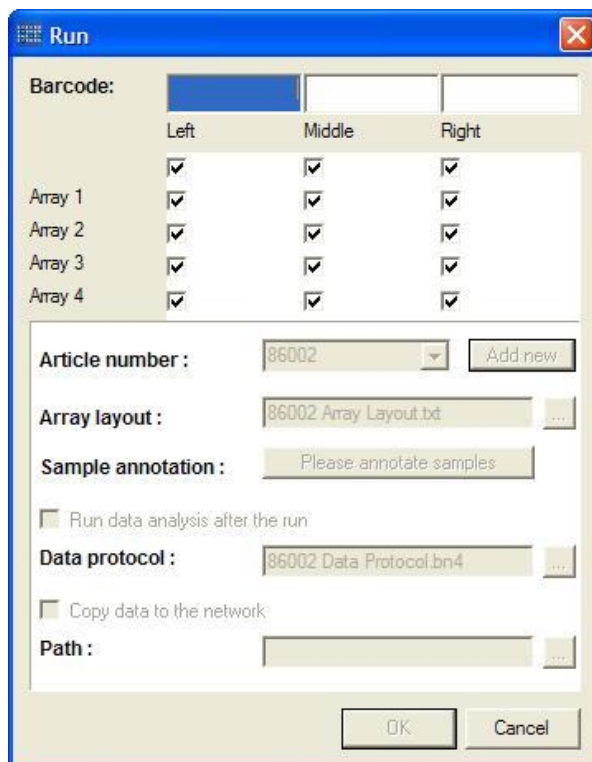
Figure 29: Run entry window

A dialog window appears where the following requires to be filled in

Barcode

This will automatically be filled in if you use the barcode reader and point to the barcode on the PamChip®, otherwise you can manually enter the 9 digit or 12-digit lot number of the PamChip®.

Arrays

The user can select which arrays will be employed in the run.

Article number

The user requires to select which article number associated with the PamChip®.

Array layout

The array layout will be automatically selected based on the article number selected. However, the user may override this article array layout.

Sample Annotation

The user needs to create a sample annotation file will be used in the run. It will be automatically filled in, otherwise can be overridden by the user.

Run data analysis after the run

The user can select whether to automatically run data analysis after a run.

Copy data to the network

The user can select whether to copy the results to a network drive.

The OK button will only activate if you have filled in Barcode, Article number and clicked on the "Please annotate samples".

Now the protocol has begun and will move to the first step.

The protocol will always start with a 'load' step. During the 'load' step the instrument will ask the operator to press the 'load' button.

Please press the "Load" button...
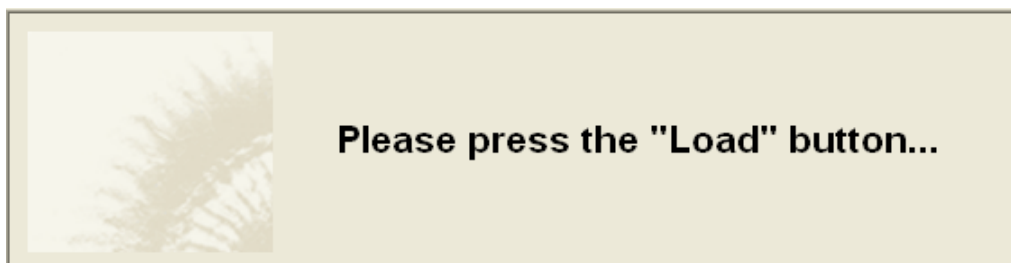
The 'load' button is situated at the bottom left of your screen. After pressing the 'load' button the wagon will move outside the instrument.

The wagon will move outside the instrument so make sure the area in front of the PamStation® is empty.

Open the lid to place the disposable into the incubator by turning the big metal button at the front of the wagon.

The incubator can be hot.

Before placing the disposable into the incubator, check that there is a fluid barrier located at the bottom of the incubator. If not, place a fluid barrier as described in chapter 4.4, 'Fluid barrier.' If there is a fluid barrier check its colour. This should be light blue. If the colour is white replace the fluid barrier.

Place the disposable in the incubator, close the lid and turn the big button anti-clockwise and press the 'load' button. The wagon will move back into the instrument and the assay will continue with the next step.

5.2.3 Stopping a protocol

A protocol can be stopped or aborted at any point during the experiment by clicking on the red stop button. **This should only be done when absolutely necessary**.

The error-handling screen will appear as shown below:
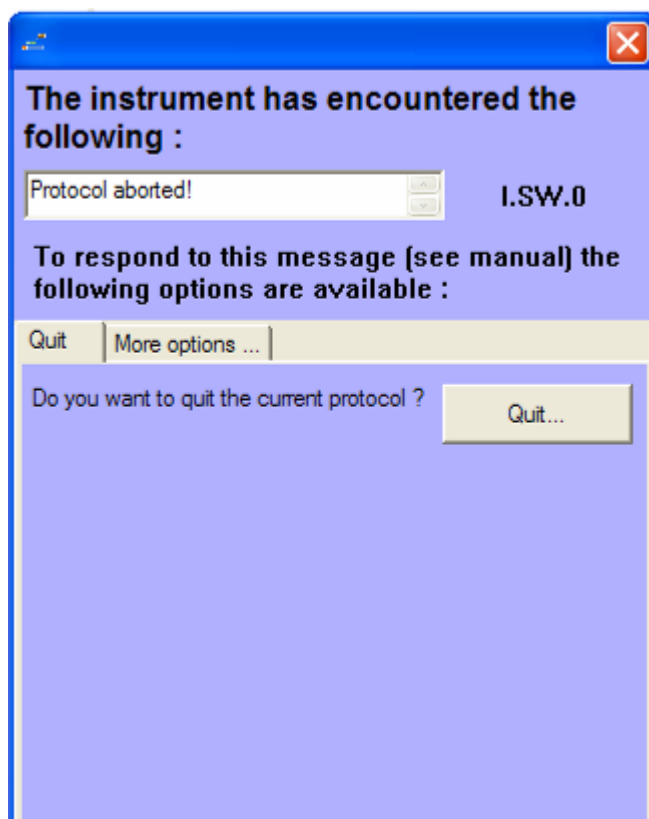
Figure 30: Error window

Use the unload option to unload the disposable and/or use the shutdown button to terminate the protocol. The error-handling screen is handled in more detail in chapter 7, 'Error handling.'

5.2.4   End of a protocol

Unload disposable

Before the end of an experiment the disposable must be removed from the incubator. This is normally done during the 'unload' step. Press the 'load' button when requested and the incubator will move outside the instrument.

The wagon will move outside the instrument so make sure the area in front of the PamStation® is empty.

The disposable can be removed by opening the lid of the incubator.

The incubator can be hot.

When removing the disposable, check the fluid barriers for a colour change. If any are white, replace it as described in chapter 4.4, 'Fluid barrier.'

Close the lid before pressing the load button for the second time.

After pressing the 'load' button for the second time the incubator will move inside again and red 'stop' button will become a green 'run' button.

6   PAMSTATION®12 MAINTAINANCE AND SHUT DOWN

6.1   Maintenance

Syringes

If syringes were used during the experiment, then it is very important to clean the needles as soon as possible after usage to prevent clogging by crystallization of salts. Please follow the cleaning of syringes procedure on page 6 of this manual for proper handling.

Fluid barrier

At the end of every experiment, when the disposable is removed, the fluid barriers have to be checked for a colour change. If any fluid has come into contact with a fluid barrier the colour of the fluid barrier will change from blue to white. If this occurs the fluid barrier has to be replaced by a new one.

After every experiment the fluid barrier has to be inspected for a change in colour.

Replacing the fluid barrier can easily be done by using a set of pliers and gently lifting the old fluid barrier out of the incubator.

The inside of the fluid barrier consists of an absorbent powder, which can be spilled if the fluid barrier is not gently removed.

The new fluid barrier can be placed into the incubator as described in chapter 4.4, 'Fluid barrier.' The fluid barrier should be changed once a month for optimal effect.

Cover glass

After every experiment the cover glass has to be removed from its position in the wagon and inspected for dirt. When the wagon is in the unload position, pull the cover glass gently from the wagon to remove it. Clean it with water and a piece of optical quality cloth. Replace the cover glass with the right side up. This side contains the word 'top'.

6.2   Shutdown

After an experiment has finished and no additional experiments are planned, press the shutdown button as indicated with the black arrow in the picture below. Switch off the

power button, at the back right hand side of the instrument. The computer and monitor can be turned off in the normal way.
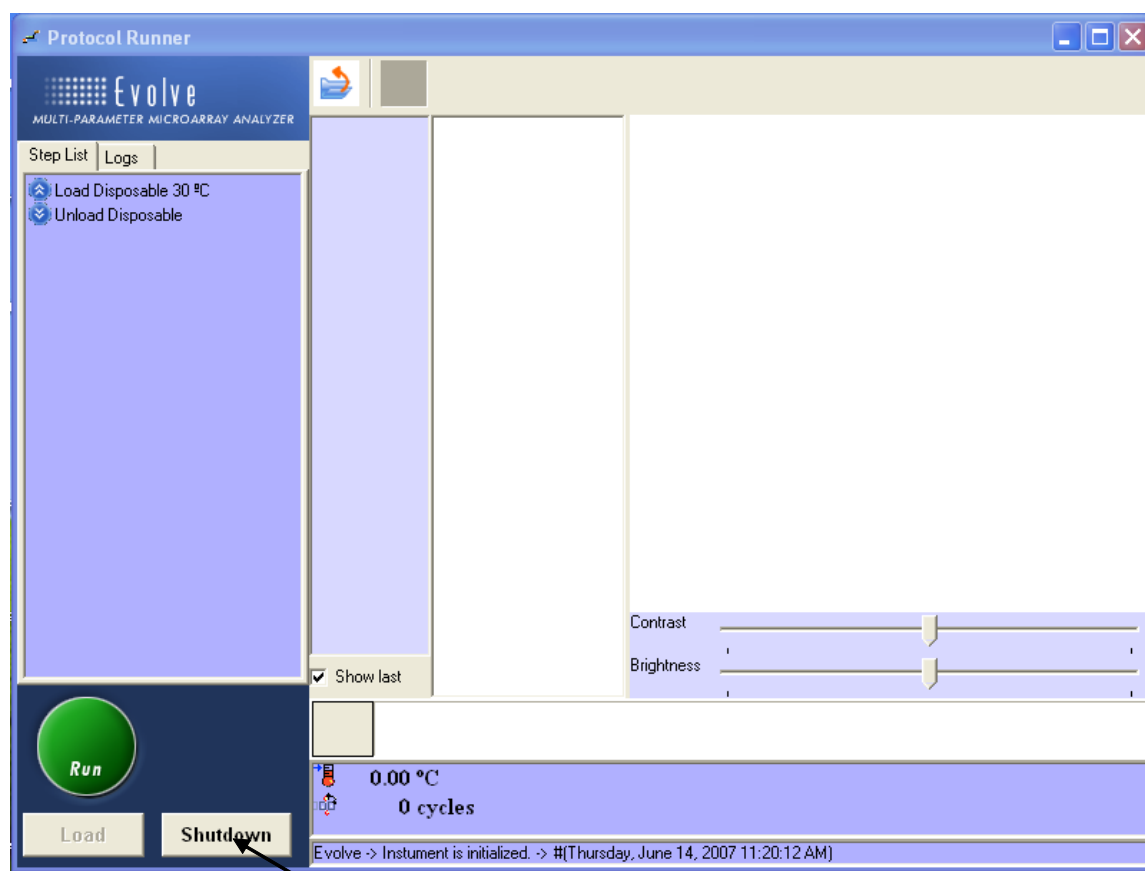
Shutdown button

Figure 31: Shutdown window

7 ERROR HANDLING

7.1 Status Lights

On the PamStation®12 there two lights. A red light and a green light.

These lights indicate the status of related to the instrument control software (called Evolve).

Green, Red	*Off,*	instrument control software not active
Green,	*On,*	instrument control software is ready to run a protocol
Red,	*Off,*	instrument control software is currently running a protocol

7.2 Error messages

When an error occurs the error screen will appear as shown below:
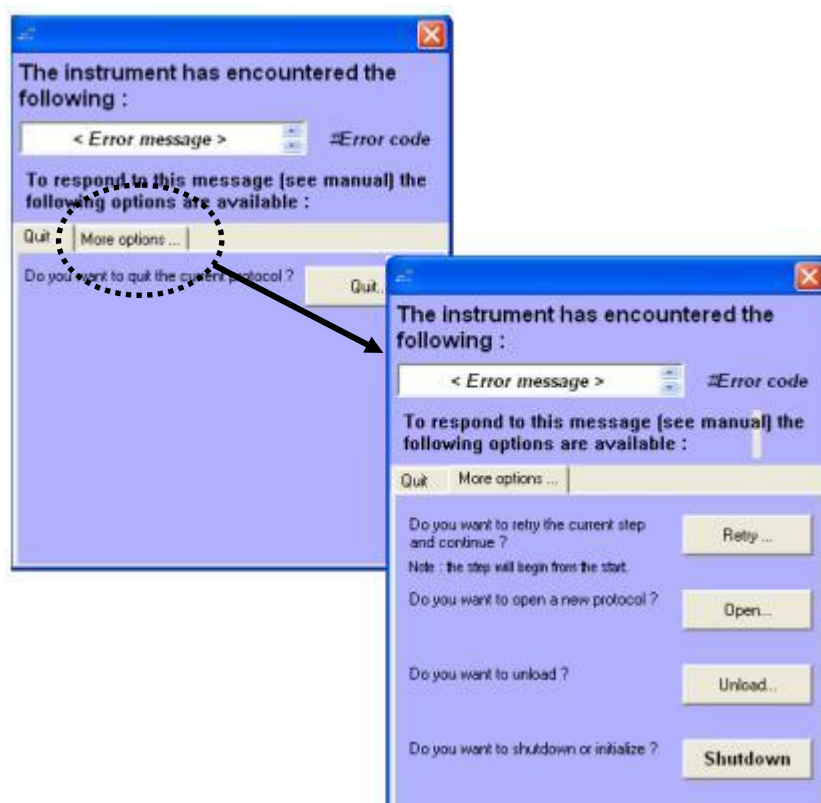
Figure 32: Error message window

There are five different options available:

Quit...	Quit the current protocol. The disposable will stay inside the instrument.
Retry...	Resumes the current protocol. **Caution** the step will start completely afresh. So, if the protocol is aborted during aspiration of the fourth array, the other arrays will be aspirated again!
Open...	Open an existing protocol. You can also create a new protocol or edit an existing one in the protocol editor, save it and open it with this option.
Unload...	Unload the disposable and shutdown the experiment.
Shutdown	Shutdown the instrument. The disposable will stay inside the instrument.

For the handling of the errors see chapter 7.3 troubleshooting.

7.3   Troubleshooting

7.3.1   Error codes

Here is a list of error message which can be generated by Evolve12. Each error has a Solution suggestion. Check chapter 7.3.2. for solutions related to the errors in this section.

Aspiration errors

E.HW.2101	*Aspirate pressure not ok*	For Solution see E.HW.2101

CMOS / CCD Camera errors

E.HW.2203	CCD get device list error
E.HW.2204	CCD get device count error

Dispense head errors

E.HW.2401	Syringe almost empty
E.HW.2402	Syringe not present

Disposable errors

E.HW.2503	Broken membrane(s) detected
E.HW.2504	Disposable(s) not present

Wagon errors

E.HW.3301	Wagon cover not closed
E.HW.3201	Wagon temperature above safety limit

Can errors

E.HW.4000 4100	**-** Can errors

Sound error

E.HW.2400	Sound error in combination with a wait time error

Other problems without error numbers

No **error** **number**	Pictures are out of focus

7.3.2 Error Handling

E.HW.2101

Message	"Aspiration pressure not ok"
Category	Error
Number	2101

Explanation: The pressure monitored during aspirating is too high or too low

Solution: There are multiple causes for this error:

If pressure was too high(Blockage of tubing)	If viscose fluids are used increase 'dry aspirating time' in protocol.
	Check tubing between aspiration head and wasted bottle for blockage. Change tubing if a blockage is present.
	Check that waste bottle is not too full.
If pressure was too low(Missing tubes)	Check if tubing is connected properly between aspirating head and waste bottle
	Check tubing between aspiration head and waste bottle for holes.
	Check if waste bottle is attached in the proper manner.
	Call Support.

Note: In most cases Evolve can be resumed and the protocol can be continued. However, if this error occurred during aspirating and one or more arrays were already aspirated, there is a small chance that a 'broken membrane error' will occur because there is no fluid left on the microarray. If this happens a new protocol can be opened, which comprises only of an aspiration step at the temperature of the previous protocol, and can be run with the wet microarrays selected. After this, edit the current protocol and remove previous steps and with the aspirate step (set load step at desired assay temperature) and continue the assay.

E.HW.2203

Message	CCD get device list error
Category	Error
Number	2203

Explanation: CCD camera can't communicate with the computer.

Solution :

USB3.0-cable	
	Check that the USB3.0-cable is connected to the computer in the proper socket and to the instrument.
	Check the can-card cable.
	Restart computer and instrument.
	Call Support.

Note: If this error is given during a protocol and the problem was a loose USB-cable, there is a small possibility that the protocol can be resumed. Otherwise, the computer and instrument will have to be restarted and the protocol run again.

E.HW.2204

Message	CCD get device count error
Category	Error
Number	2204

Explanation: CCD camera can't communicate with the computer.

Solution :

USB3.0-cable	
	Check that the USB3.0-cable is connected to the computer in the proper socket and to the instrument.
	Check the can-card cable.
	Restart computer and instrument.
	Call Support.

Note: If this error is given during a protocol and the problem was a loose firewire-cable, there is a small possibility that the protocol can be resumed. Otherwise, the computer and instrument will have to be restarted and the protocol run again.

E.HW.2400

Message	"Sound error in combination with a wait time error"
Category	Error
Number	2400

Explanation: The syringe used for automated dispensing is completely empty.

Solution :

Syringe empty	Fill syringe with the appropriate buffer.
	Call Support.

Note: If this error is given at the start of a dispense step, then the experiment can be resumed. If fluid has been dispensed into some of the arrays, it has to be removed first. Use a new protocol with an aspiration step (set the temperature load step at the assay temperature) and select only the wetted microarrays. After that the original protocol can be resumed.

E.HW.2401

Message	"Syringe almost empty"
Category	Error
Number	2401

Explanation: This error is given during a load step when there is between 150 and 100 ul of fluid left in a syringe (automated dispense).

Solution: There are multiple causes for this error:

Syringe empty	Remove the syringe and fill it with the appropriate buffer.
	Call Support.

Note: Use the resume protocol option in Evolve.

E.HW.2402

Message	"Syringe not present"
Category	Error
Number	2402

Explanation: This error is given when the syringe is not present at the correct position.

Solution: There are multiple causes for this error:

Syringe not present	Place the syringe in the correct slot.
	Call Support.

Note: Use the resume protocol option in Evolve.

E.HW.2503

Message	"Broken membrane(s) detected"
Category	Error
Number	2503

Explanation: There is an unexplained pressure difference in the given array number(s). Reasons; broken microarray, no fluid on given microarray, problem with the fluid barrier

Solution: There are multiple causes for this error:

Broken microarray	Use the unload option to inspect the array that gives an error.
No fluids	Use the unload option to inspect the array that gives an error.
Fluid barrier	Use the unload option to move wagon outside.
	Open wagon-lid.
	Remove disposable.
	Inspect fluid barrier (colour change from blue to white).
	Change fluid barrier.
	Call Support.

Note*: Broken microarray:* Edit current protocol; remove all steps (except load) before the failed step. Edit current protocol step if needed. Set load step temperature at desired assay temperature. Open the new protocol and run the new protocol without the broken microarray.

No fluids on the microarray: Use the unload step to move wagon outside. Add fluids manually. Resume the protocol. Edit the protocol for next the assay.

Fluid barrier: Change the fluid barrier. Edit protocol if needed. Run edit protocol or resume current protocol.

E.HW.2504

Message	"Disposable(s) not present"
Category	Error
Number	2504

Explanation: No all disposables, specified at the beginning of the run are present at the correct positions.

Solution :

Disposable(s) not present	Use the unload option to change the disposable position

E.HW.3201

Message	"Wagon temperature above safety limit"
Category	Error
Number	3301

Explanation: There is a maximum temperature for unloading the wagon, 68 degrees.

Solution :

	No hardware adjustments.

Note: Edit the protocol to prevent unloading of the wagon. Then run that protocol (open new protocol).

E.HW.3301

Message	"Wagon cover not closed"
Category	Error
Number	3301

Explanation: The cover of the wagon was not closed properly.

Solution:

Wagon cover	Close the wagon cover.
	Call Support.

Note: Use the resume option. The protocol should resume normally.

E.HW.4000 - 4100

Message	"Can errors "
Category	Error
Numbers	E.HW.4000 – 4100

Explanation: There is a problem with the can card in the computer or with the communication between the can card and the instrument.

Solution :

Cable problem	Check that all cables between computer and instrument are connected properly.
	Turn off computer and instrument and turn on again.
	Call Support.

Note : This error will normally only occur before running a protocol. Run the protocol again.

No error numbers

Message	"Pictures are out of focus"
Category	Error
Number	No error number

Explanation: The cover of the wagon was not closed properly.

Solution :

Liquid was removed from array in the step prior to the read step	The imaging system is optimized for reading when the arrays contain fluid. Do not perform a read step after an aspiration or washing step since the array will not contain sufficient fluid for optimal imaging.
	Call Support.

Note: Adapt the protocol and retest the sample.

8   INSTALLATION

The following information is supplied for guidance in selecting a location for the instrument.

8.1   Safety and Electrical Compliance

8.1.1   Safety Considerations

- The PamStation®12 **weighs approximately 80kg** and therefore must be placed on a level, stable, bench that is capable of supporting its weight.

- Protect from direct sun light.

- The back of the instrument should be at least 30cm from any walls to prevent overheating

- There are high voltage circuits within the instrument associated with the light source. The instrument case should only be opened and the light source changed by qualified engineers.

- There are moving parts within the instrument which may be damaged or cause injury if touched whilst in motion. Do not touch them until they have stopped moving. This particularly applies to the incubator, which moves outside the instrument for loading

- The incubator may be hot and should be handled with caution when outside the instrument

- As the instrument will be used with biological materials or inflammable or toxic substances, good laboratory practice for handling such materials should be observed.

- When the instrument is in use, all casing panels and doors should be kept closed

8.1.2 Electrical Compliance

The European configuration of the instrument is designed to be compatible with the following standards:

- CE Mark

8.2 Recommended Environmental Conditions

Operating temperature:

- o At humidity range 0 – 54%, non-condensing: 15-30°C
- o At humidity of range 55-80%, non-condensing: 15-23°C
- Operation is preferred in an'air-conditioned' environment (dust-free, controlled humidity and temperature).
- Temperature storage: 0 - 40°C
- Humidity operating and storage maximum 80% relative humidity, non-condensing
- The instrument can be set at 20°C but cannot cool below ambient temperature. The software allows the temperature to be set at 20°C however the minimum operating temperature during an experiment is ambient + 3°.

Other atmospheric conditions:

- The instrument is for indoor use only.
- Avoid placing in direct sunlight.
- Place the instrument on a level surface without vibration.
- Do not expose to inflammable gases or solvents, or to corrosive liquids or vapours.
- Do not place close to devices that emit electrical noise.
- The maximum altitude for use of the instrument is 2000m above sea level.

8.2.1 Electrical requirements

Place close to a well-grounded electrical supply capable of handling at least 1500W. Three outlets are required for the instrument and associated computer equipment.

8.2.2 Space requirements

The dimensions of PamStation®12 are approximately 66 cm (width) x 59 cm (depth) x 49 cm (height). On the back, sides and top of the PamStation®12 approximately 10-20 cm of space is needed for ventilation. On the front of the instrument additional space is needed for opening of the door, and experimental handling. In addition, space needs to be available for a PC and monitor.

8.3 List of consumab;es

Article	Number of items	Article number for reordering
Cover glasses, 3 already inserted in the incubator	4	32154
Syringes	4	32153
Needles	4	32152
Fluid barriers, type C	10	41005

8.4 Installation of the PamStation®12 and PC

- It is recommended that the PamStation12 is installed by PamGene.
- Before installing the instrument, make sure that there are no abnormalities outside the instrument (damage, contamination, etc).
- Make sure that the place of installation is in accordance to PamGene guidelines.

A computer, a monitor, a mouse and keyboard are normally shipped with the instrument. The computer and monitor are supplied with their own power cable. Installation of these can be done in the normal manner, according to guidelines of the computer manufacturer. A standard power cable (the same as for the computer and the monitor), a USB-cable and a canbus-cable are supplied for the instrument as well as a Linux board.

Make sure that the instrument is switched off when the power- cable is connected.

The power cable can be plugged in at the back of the instrument, on the right side just below the power-switch.

Make sure that the PC and the instrument are switched off when the firewire-cable and the canbus-cable are attached.

The firewire-cable and the canbus-cable can be attached at the appropriate places at the left-hand side at the back of the instrument and at the back of the computer. The locations of these cables are labelled with their names at the back of the instrument and on the back of the Linux board.
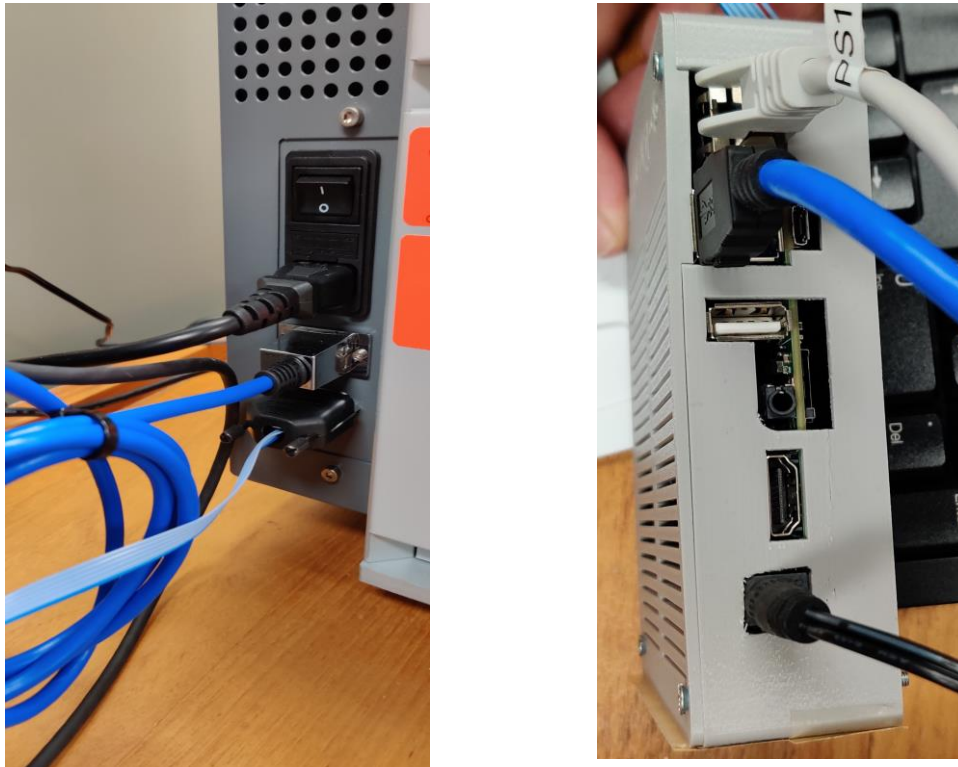

Figure 33: (left) Placement of canbus and firewire cable at back of instrument. (right) Placement of USB 3.0 and firewire cable at back of the Linux board

9 PAMSTATION®12 SPECIFICATIONS

9.1 Instrument specifications

Dimensions and Weight of Instrument

Dimensions	49cm (height) x 66cm (width) x 58cm (depth)
Weight	82 kg

Optical Properties

Filter specifications	Filter1 = Fluorescein (FITC)
	Filter 2 = Cy3
	Filter 3 = Cy5
CCD camera	Basler aca1920-40um, CMOS/CCD sensors IMX249
Data output	TIFF,
Dynamic range	12 bit
Sensitivity	0.0002 lux / 10 sec integration
Pixel resolution	5.86 x 5.86 μm

Incubator Properties

Temperature range	Ambient + 3°C – 80°C (minimum 20°C)
Temperature accuracy	<0.5°C from set value
Temperature uniformity across disposable	<0.5°C SD across the PamChip® disposable
Heating rate	Maximum 6°C/min from 30°C to 80°C
Cooling rate	Maximum 3°C/min from 80°C to 30°C

Washing Module

Dispense accuracy	<5% deviation at 10 µl/array, <5% at 50 µl/array
Dispense reproducibility	<5% CV over 10 µl – 50 µl range
Thermal regulation	Up to 65°C, accuracy ± 5°C, no active cooling possible
Syringe capacity	5 ml
Wash bottle	100 ml, glass
Waste Liquid Disposal	As according to local rules

Power Supply Requirements

Voltage	220V/240V – 110V/115V
Frequency	50-60Hz
Maximum Power*	1500W
Average Power requirement	800W

As is standard, a surge-protected, constant power supply should be used. Up to three outlets may be required for the instrument and associated computer equipment.

9.2 Software Information

Networking Software	Windows 10
Network Card	Intel Pro
Instrument Control Software	Evolve

NB. Alternative PCs require validation by PamGene

10   SUPPORT

PamGene aims to provide total support to enable customers to fully realize the benefits of the PamGene technology and to maximize use of the PamStation®12 system.

The software components and data handling are essential interfaces to the system and are supported by a dedicated software support team through email, telephone and on-site visits, if required.

Please contact support on:

+31(0) 73 615 8900

support@pamgene.com

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Wolvenhoek 10

5211 HH Den Bosch

The Netherlands

Phone +31 73 615 80 80 (General number)

Fax +31 73 615 80 81

11   RIGHTS AND RESTRICTIONS

11.1 Intellectual property rights

The use of The PamChip® microarrays is licensed among others under patent and patent applications issued to PamGene B.V.

Any patents, patent applications and other proprietary rights named in this manual are included for reference purposes only. No licenses to use the processes or products covered by such patents, patent applications or proprietary rights are granted by or implied by PamGene International B.V. It is the responsibility of the user to determine whether a license is required and obtained.

11.2 Trademarks

PamStation®12 and PamChip® are trademarks of PamGene BV, registered in one or more countries.

11.3 Disclaimer

PamGene International B.V. reserves the rights to change its products and services at any time to incorporate technological developments. This manual is subject to change without notice.

Although this manual has been carefully prepared with every precaution to ensure accuracy, **PamGene International B.V.** can assume no liability for any errors or omissions, or for any direct of indirect damages resulting from application of this information.

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