

## User manual

# **WALLAC** 1420

## Software version 2.00 release 9





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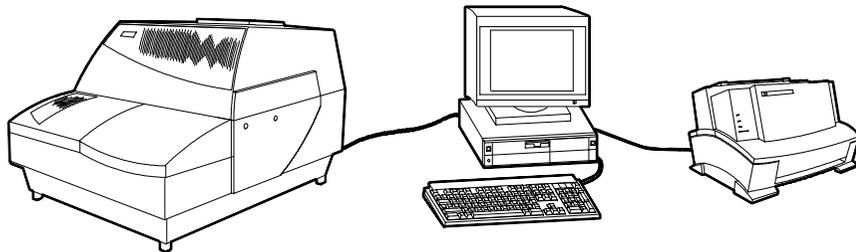
## **Introduction**



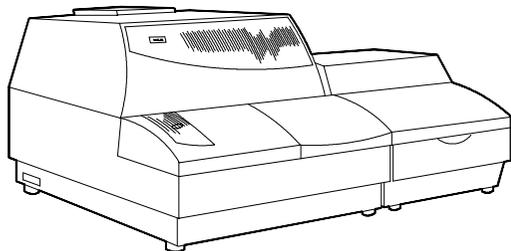
## System overview

*Note: This manual describes all the features of VICTOR2. Some versions of the instrument do not have one or more of these features. You can ignore the parts of this manual not relevant to the version of VICTOR2 you have.*

Wallac 1420 multilabel counter is a complete platform for quantitative detection of light-emitting, or light absorbing markers. Depending on the model of the instrument you have, it is suitable for flash or glow luminometry, fluorometry, fluorescence polarization, high-sensitivity time-resolved fluorometry, homogeneous time-resolved fluorometry (LANCE option), flash absorbance and photometry. It is a compact bench top unit with features such as dispensers, shaking, temperature control, top or bottom reading and scanning. The software is a 32-bit application running under Windows 95, Windows 98 or Windows NT. Up to 1536-well plates can be counted (depending on the technology and model) as well as Petri dishes, slide filters and Terasaki plates. Plates can be loaded manually or with stackers for automatic operation. Output can be to a file on the PC and/or to a laser printer. The following figure shows the Wallac 1420 system with its PC and laser printer.



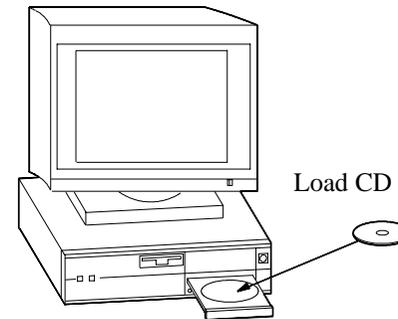
If the dispenser unit is connected to Wallac 1420 it looks as follows:



### Software installation

Note: the following instructions assume that the counter, PC and printer have been connected up and switched on (see the installation instructions in the Instrument manual).

1. Load the software CD.



2. The setup loader will start automatically and give you a choice of either selecting the Installation Wizard to guide you through the installation or reading files from the CD.
3. When you have completed the installation you should remove the CD from the PC, otherwise the setup loader will start again next time you boot the PC.

### **Operation overview**

Parameters must first be set using the protocol editor. This is activated by selecting a protocol in the Explorer.

A sample plate can then be loaded and measured using the protocol set for it.

Other parameters affecting the whole system e.g. labels, filters etc. can be set in the Tools menu.



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## **Operating Wallac 1420**



## Starting up Wallac 1420

When you boot the system the following window appears:



Wallac 1420 is operated by moving the mouse cursor to a point in this window (a menu title, a button or an icon) and clicking the left mouse button. If you click an icon or a button, an operation will be started. If you click a menu title, the menu will appear. You can then move the cursor to the item you want in the menu and click the left mouse button again to activate it.

### Instrument control

The main window has three tabs: Instrument Control, Live Display and Temperature. The instrument control tab enables you to control the instrument by starting, stopping or ending counting. It shows you the protocol being used to control the measurement and it also shows status of the instrument.

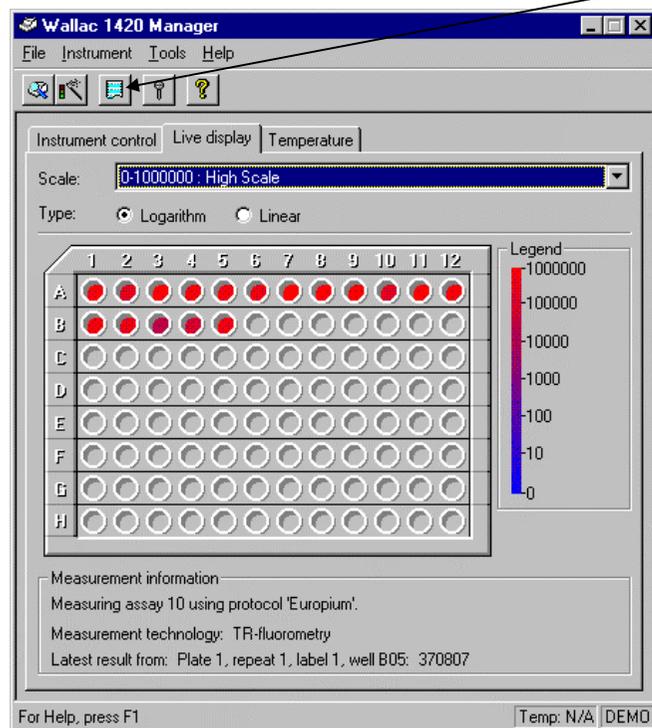
If the system supports plate heating, the status bar will display the current temperature of the plate and samples. When run with a system not supporting plate heating this will not be displayed.

When the workstation is used without an instrument (i.e. running in demo mode), the status bar includes the text "DEMO". When Wallac 1420 workstation is connected to an instrument (running in normal mode), the demo mode indicator is blank. You can switch between normal and demo modes using the Options selection in the **Tools** menu.

## Live display

The **Live display** shows you the live results in the form of a plate map with colour coding to indicate the size of the results. If you move the cursor on to a well that has been measured, the numerical value will appear. At the bottom of the window there is information about the sample being measured including its current value.

Note: in multiple label, scanning and kinetics measurements, only results from the first measurement of the label are visible in the Live display. You can see results from all labels in the Latest results display which you can get by clicking the **Latest assay run** icon.

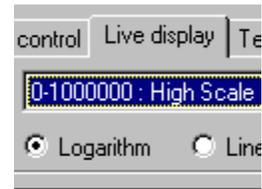


You can choose the result range you want used to determine the colour code of the wells. The closer a result is to the upper limit of the range, the more red it will appear. The closer to the lower limit it is, the more blue it will appear. These colours will correspond to the

## Operating Wallac 1420

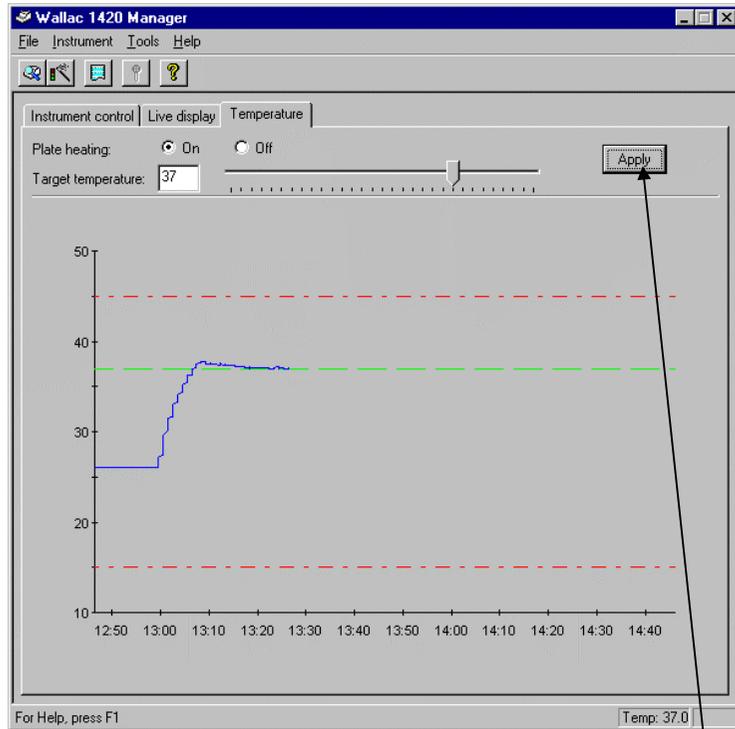
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values shown in the legend appearing beside the plate map. Choose the range that is appropriate for the samples you are measuring so that you get the greatest variation in sample colours.



You can choose whether you want the scale to be **logarithmic** (which means that the difference between smaller values will be more visible whereas the difference between higher values will be less visible), or a **linear** scale in which every part of the range is treated equally.

## Temperature



In the **Temperature** tab of the main window you select plate heating and the target temperature. The latter can be set with 0.1 °C precision within the interval 15 – 45 °C. The minimum temperature is 15 °C or room temperature plus 2 °C depending on which is higher. If the target is changed, the new value is only effective when the **Apply** button has been clicked. The figure shows the temperature rising rapidly to its new level and then stabilizing there. If the plate heating option is not installed, these controls are disabled.

The current temperature is shown in the bottom right hand corner of the window irrespective of which tab is selected.

If the plate heating option is not installed, these controls are disabled.

## Barcode options

An optional barcode reader can be included. This allows you to load barcode labelled plates, which are identified by the barcode reader. Codabar, Code39, Interleaved 2 of 5, Code 128, UPC and EAN barcodes can be read.

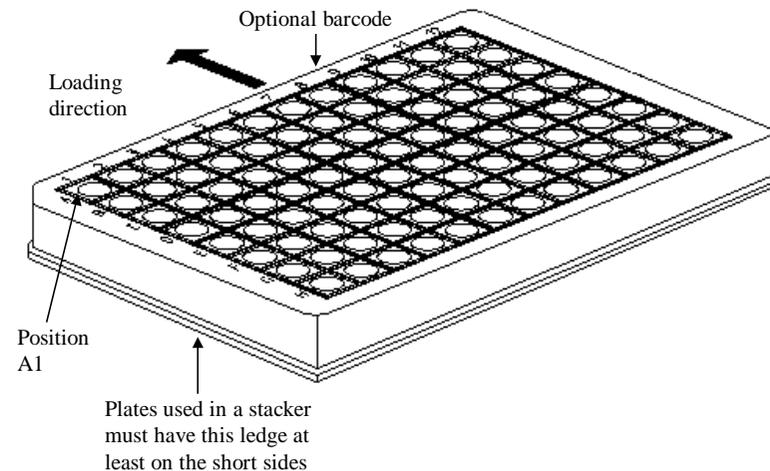
There are two sorts of identification that can be used:

This identification can be by plate number, in which case you must select the protocol as part of the start up procedure.

Alternatively, the barcode can be the number of the protocol to be used for measuring that plate. This system is especially useful for models using stackers. In this case the first plate to be loaded from the stacker must have a barcode. Measurement can be started simply by briefly pressing the button on the counter. See page 118 for more details on barcode usage.

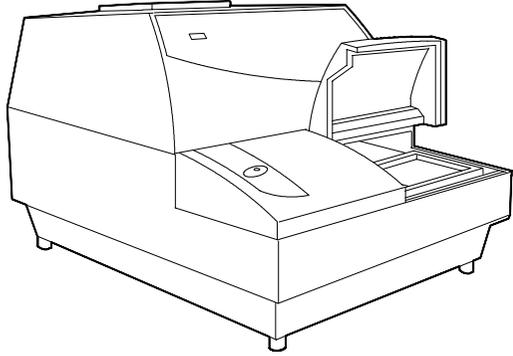
## Plate orientation

The figure shows a typical 96-well plate and how it is to be orientated when loading it either directly into Wallac 1420 or into an input stacker. The A1 position must be in the left corner furthest from you i.e. the side that enters the counter first. If a barcode is used, it should also be on the side that enters the counter first. The barcode can then be used to identify the plate or to select the protocol.

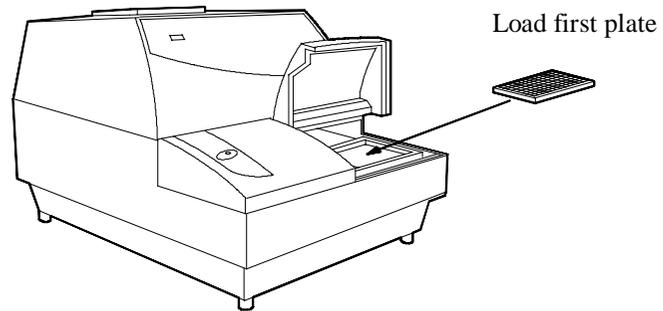


## Starting operation with the Start button

1. Open the loading position lid.

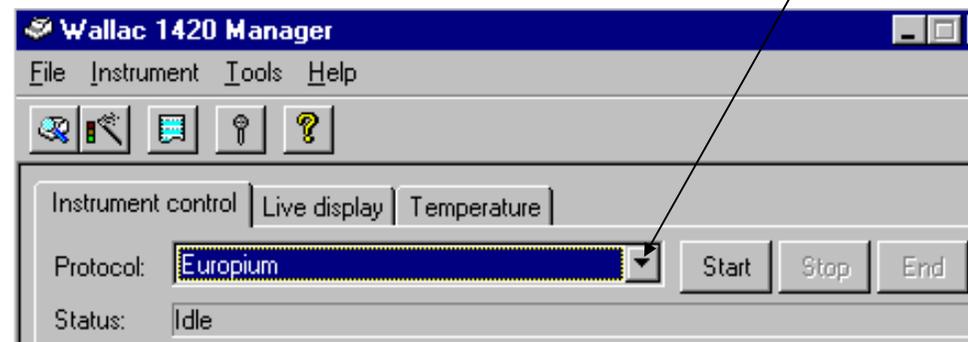


2. Load your first plate and close the lid. See the picture on page 20 for correct plate orientation.

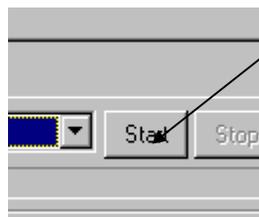


## Operating Wallac 1420

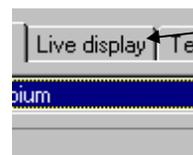
3. Select the protocol you want to use by scrolling through the list of active protocols.



4. Use the mouse to click the **Start** button on the user interface

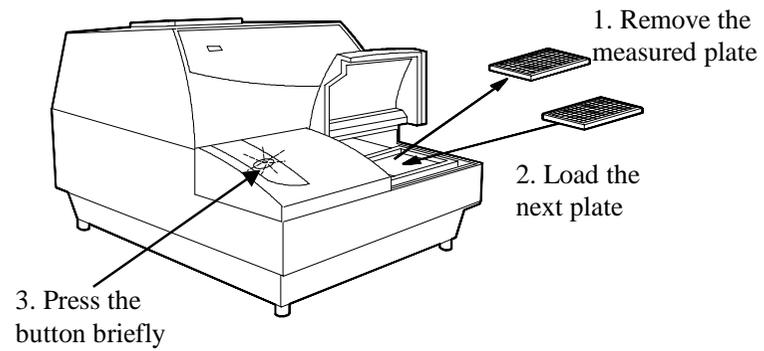


You can follow the measurement process by selecting the **Live display**.

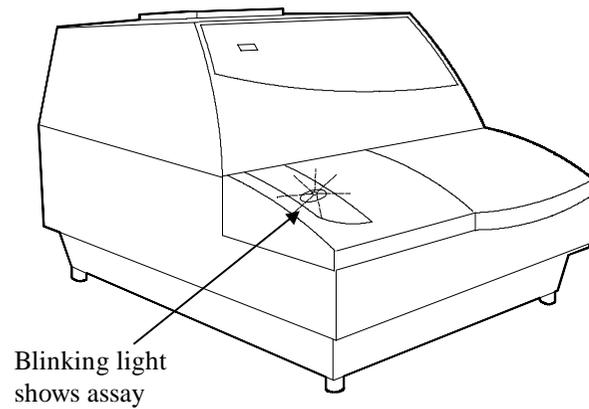


## Operating Wallac 1420

5. When the plate has been measured, remove it and load the next plate.



6. Briefly press the button on the counter, this will be blinking if there are more plates to be loaded for this assay. If it is not blinking then the assay has finished.



Continue until all plates have been measured.

## Starting operation with the Start Wizard

An alternative way to start counting is to use the Start Wizard. This allows you to define how many plates you are going to use and to give the positions of samples. Proceed as follows:

1. Click on the **Start Wizard** icon and follow the instructions that appear.



The Start Wizard will appear to guide you through the procedure for starting.



The steps are:

**Introduction** to the wizard. (You can select the introduction not to appear next time if you want).

**Protocol selection** - click the protocol you want to use for measurement.

**Definition** of the sample names and number of plates. See Samples tab on page 39 for a description of the principles involved in sample definition.

*Note: Errors in protocol selection or plate layout definition will lead to incorrect results.*

**Comments** that you want added to the results - just type in what you want

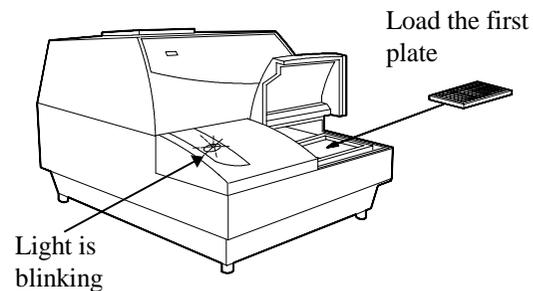
**Load** the first plate - the protocol name and comments appear in order to confirm that it is the one you want. Note: the A1 position must be in the left corner furthest from the user i.e. the side that enters the counter first. If a barcode is used it should also be on the side that enters the counter first.

**Click Finish** to start measurement. See the Live display during measurement.

When the plate has been measured, remove it, load the next plate and briefly press the button on the counter.

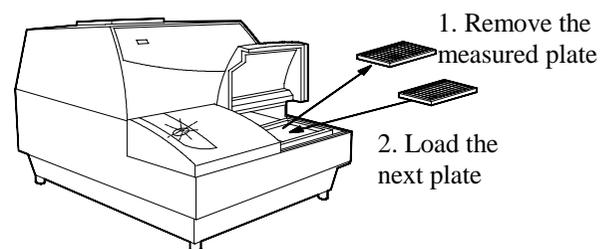
## Starting operation and using a protocol number barcode

1. When you load a plate, the button will be blinking. See the picture on page 20 for correct plate orientation.



2. Press the button briefly. Operation will start. The button light will be lit without blinking. The barcode on the plate will be read and the protocol specified by the barcode will be selected and used for measuring the plate.

When the measurement has finished and the light starts blinking again, remove the plate and load the next one and briefly click the button. If the plate belongs to the same assay as the previous one, no barcode is needed. If it is barcoded for a new assay then the appropriate protocol will be used for measuring the plate.



Continue measuring plates this way. Note that the button light will remain blinking as long as the protocol number barcode option is selected.

## Stacker operation

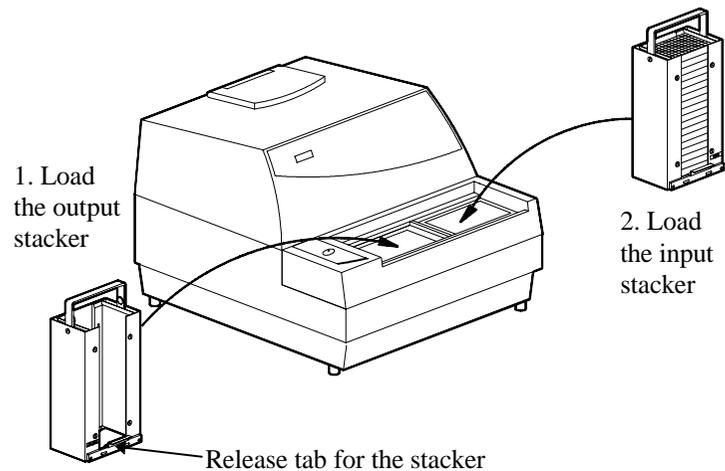
A stacker is able to hold up to 20 (or 40 in the larger version) microplates. No lids, loose frames or very loose strips are acceptable. See Appendix 2 for a list of plates that fit in the stackers.

### Starting operation, no protocol number barcode

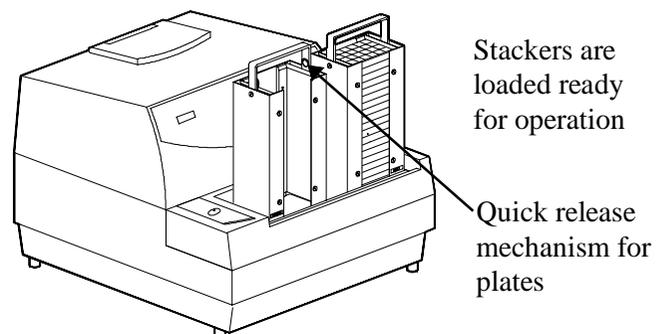


**Caution** *Do not put your fingers into the sample loading area.* In the stacker model the loading area is uncovered when the stackers are not in place so you must avoid the danger of getting your fingers trapped by the plate lift mechanism. If you need to do something in that area, **switch power off first.**

1. In stacker operation, plates are loaded into the input stacker with the first plate to be measured at the bottom. See the picture on page 20 for correct plate orientation.
2. The empty output stacker (the one with the tabs for the quick release mechanism) should be fitted first into the multilabel counter; it occupies the left-hand position.
3. Fit the input stacker (with plates) in the right hand position.



*Note: Make sure the stackers have clicked into position properly.*



4. Select the protocol and click the **Start** button or use the **Start Wizard**.

5. The plates will be moved one by one from the input stacker to the measurement position. After each plate has been measured, it is moved to the output stacker. When the assay has been completed operation will stop.

If there are still plates in the input stacker it means that you must start a new assay by selecting the protocol and clicking **Start** as described above.

6. When all plates have been moved to the output stacker, it can be removed. To do this, press the release tab at the bottom of the stacker, then lift it up.

After you have removed the stacker, pull up the handles of the quick release mechanism to allow the plates to be emptied easily from the stacker. **Note: make sure you do not operate this mechanism when the stacker is loaded in Wallac 1420 because you may jam the conveyor.**

If the button on Wallac 1420 is blinking, it means that the assay has not finished and you must remove the empty input stacker, load an empty output stacker and then an input stacker which has the remaining plates of the assay (plus plates for other assays if you want).

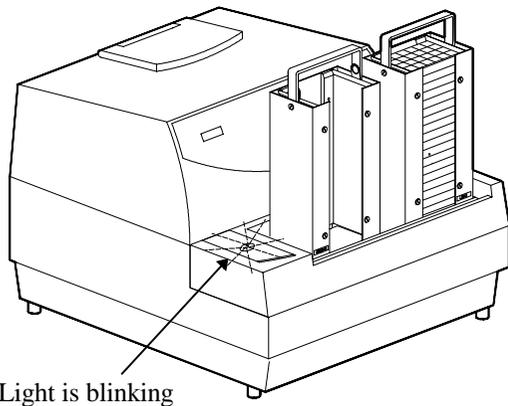
## Starting operation and using a protocol number barcode



**Caution** *Do not put your fingers into the sample loading area.* In the stacker model the loading area is uncovered when the stackers are not in place so you must avoid the danger of getting your fingers trapped by the plate lift mechanism. If you need to do something in that area, ***switch power off first.***

This configuration makes best use of the stackers; operation is automatic.

1. Load the stackers. The button light will be blinking.



2. Click the button briefly.

One by one all the plates will be loaded and measured. If a plate has a barcode, then it will be measured using the protocol specified by the barcode. If it has no barcode, it will be measured using the protocol specified by the previously read barcode.

3. Only when the input stacker is empty will operation stop. The button light will be blinking. Remove the stackers and load new ones if required.

4. Click the button briefly to start operation again.

## Ending operation

### Stop button



Clicking the **Stop** button in the workstation interrupts the measuring process immediately. The plate being measured is brought out to the loading position. You cannot resume measurement after clicking **Stop** but must begin the assay from the beginning if required.

### End button



Clicking the **End** button in the workstation ends the assay after the current plate has been completely measured, including any possible repeat measurements. The plate is brought out as described above.

## Button on Wallac 1420

This button has different functions depending on how long you press it for and what is happening when you press it.

If a plate has been measured, you can remove it, load a new plate and *briefly* press this button to continue the assay with the same protocol as the plate just measured. However, if you press this button for more than 2 s, the current assay will be stopped and a new assay will be started with the current protocol. This will not work if the protocol barcode option is selected.

If a plate is being measured and you press this button, nothing happens.

If no assay is running and you press the button for more than 2 s, then a new assay will be started with the current protocol. This will not work if the protocol barcode option is selected.

Note: make sure in all cases that you have loaded the correct plate before pressing the button because the plate in the loading position (or the empty plate holder) will be taken into the counter.

## Light on the button

The following table shows you what the light on the button will be like depending on what is happening with Wallac 1420 and whether or not the protocol barcode option is used.

<b>Condition</b>	<b>Barcode none or plate number</b>	<b>Barcode for protocol number</b>
Not measuring, no assay	Off	Blinking
Not measuring, waiting next plate	Blinking	Blinking
Measuring, loading or unloading	On	On



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## **Protocol editing**

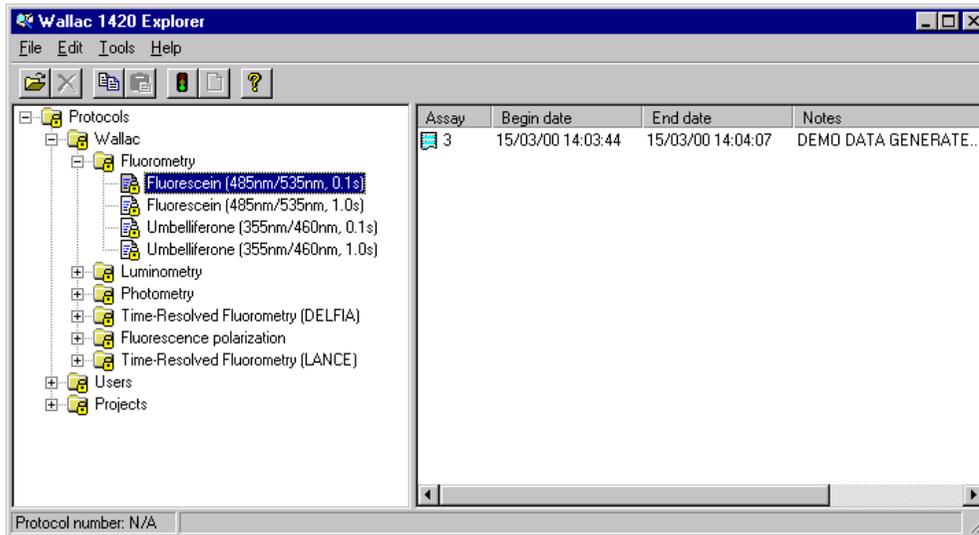


## Explorer

Through the Explorer you can access the Protocol Editor and the Result Viewer. Click the **Explorer** icon to open the Explorer.



The Explorer will open.



### Explorer folders

In the Wallac folder there are, as a default, folders named according to the technologies installed. In each of these folders there are protocols using the appropriate technology.

On the same level as the Wallac folder there are two other folders: Users and Projects.

To open a folder, click on it and then select **Open** from the **File** menu. Alternatively you can open a folder by double-clicking the left mouse button when the cursor is on the folder icon. You can also click once on the + mark next to the folder to see the protocols contained in it.

To close a folder, either double-click on it or click the - sign next to it.

If you are working on the Advanced or Service level, you can **create**, **rename** or **delete** folders. A sub folder of **Users** or **Projects** can be renamed by selecting it and clicking the right mouse button and selecting **Rename**.

### Explorer icons

Factory pre-set protocols and groups have a lock symbol to show they cannot be changed. User made protocols and groups do not include the lock because the user can change them. See the examples below:



The icon for a factory pre-set folder



The icon for a factory pre-set protocol



The icon for a user-made folder



The icon for a user-made protocol

### Protocol selection

You can: **Open**, **copy**, **paste**, **start** or **create** a protocol, **delete** a user made protocol (plus its associated results).

To open an existing protocol you can do one of the following:

- a) Move the mouse cursor onto it and then click the left mouse button to select it, then click the **File** menu and click **Open**.
- b) Move the cursor on to it, click the right mouse button and select **Open** from the menu that appears.
- c) Double-click the mouse when the cursor is on the icon.

At the bottom of the window on the status line the protocol ID will appear (if defined, otherwise it is not available, N/A) and also any notes that have been included in the protocol.

When you create a protocol you can save it e.g. as a user protocol in the **Users** folder, or you can save it according to the technology. The choice is yours, but the default structure gives you a starting point and helps you to find the pre-set protocols.

You can move a user made protocol to a different folder by selecting it, holding down the mouse button, dragging it to the target folder and releasing the mouse button.

Note: The dual label protocol (only available if TR-FIA is installed) should not be copied and edited e.g. to make a single label europium or samarium protocol. You should begin with an existing single label protocol. For dual label operation, you must select the europium label first and then the samarium label. After you have selected the second label, a third, samarium in the europium window, will be created automatically. When running with these three labels you must have a valid Eu/Sm normalization, see the **Tools** menu for how to make such a normalization.

For information about results see the chapter on "Result viewing".

### Protocol editor

*Note: changes to protocols should only be made by authorized persons.*

The protocol editor window contains seven tabs:

**Samples** - shows a plate map to allow you to edit wells and plates

**ID** - you can identify the protocol

**Measurement** - you can select the labels used for measurement

**Plate** - you can select the plate type and other parameters

**Outputs** - you can define the output

**Events** - you can give a command to be followed after an assay has been run with this protocol

**General** - information about the protocol

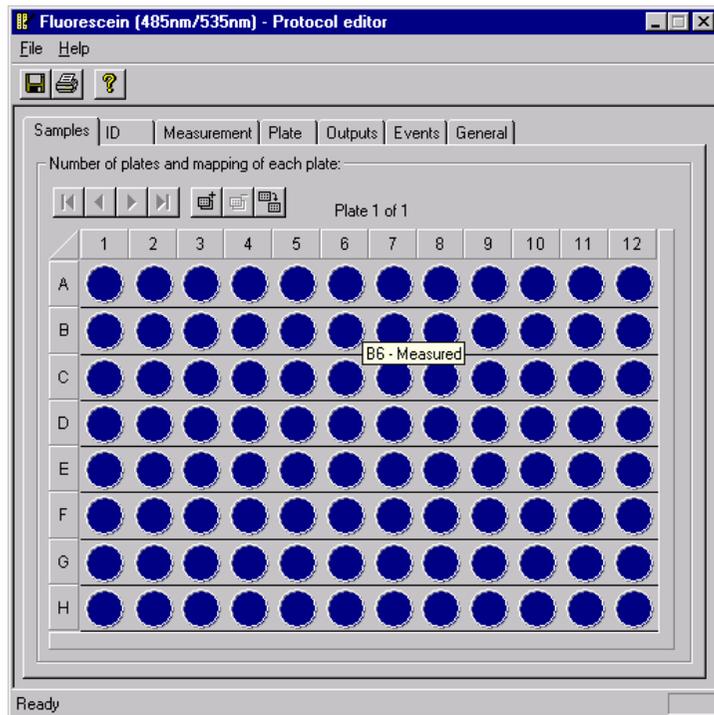
You can set parameters for up to 40 plates in one protocol.

Protocol entries are checked for validity by the software and invalid entries are informed when you try to switch from one view to another.

When you have edited a protocol you can use the command line or the button bar to save or print the protocol.

**Note** - if you want to change the plate type, *do this before setting other plate parameters.* This is because when the plate type is changed all the previous plates will be deleted and one plate of the selected type will appear.

## Samples



Selecting this tab causes a plate map to be displayed to allow you to edit samples. Buttons allow you to select the **first plate**, **previous plate**, **next plate** or **last plate**.

You can also add a new plate, delete the currently selected plate or duplicate a plate. An added plate has all the positions marked as Measured and will become the last plate in the sequence. When you delete a plate it is the one currently visible that is deleted. A duplicated plate is a copy of the current plate and will become the last plate in the sequence.

A map of all the wells appears. An empty well (i.e. one that will not be measured) will be grey, a well with a sample (i.e. one that will be measured) will be coloured. If you move the cursor onto a well the sample type will appear. If you click on the well a list of available well types will appear.

## Protocol editing

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To change the markings for the individual wells in a plate you can select a group of wells by using the mouse. To start the selection, click the left mouse button at the desired starting position and then drag the mouse cursor to the desired ending position on the plate without releasing the left mouse button.

**Complete rows or columns** can be selected by clicking on the character/number on the plate frame corresponding to the row/column. More than one row/column can be selected by clicking the mouse and dragging in the same way as when selecting free well areas.

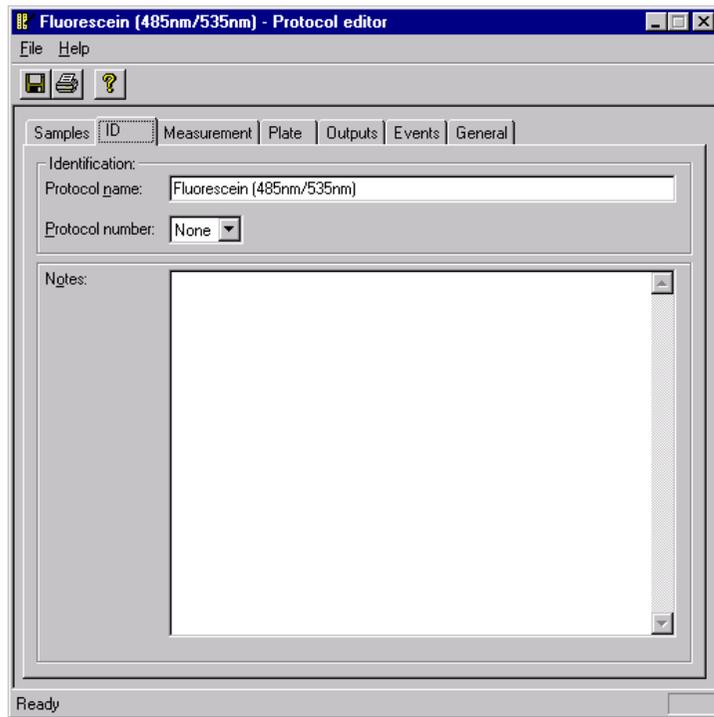
**The whole plate** can be selected by clicking on the upper left corner of the plate frame.

**To select a rectangular area of wells** on the plate, hold down the Shift key while dragging with the mouse.

The number of wells selected is shown in the plate indicator text after the plate order number.

When you have selected the wells and released the left mouse button, a drop down menu of sample types will appear. These will be the types defined in the Wallac 1420 Manager **Tools** menu item **Miscellaneous settings**. By selecting one of these types you specify what the type of the selected area is to be.

## ID

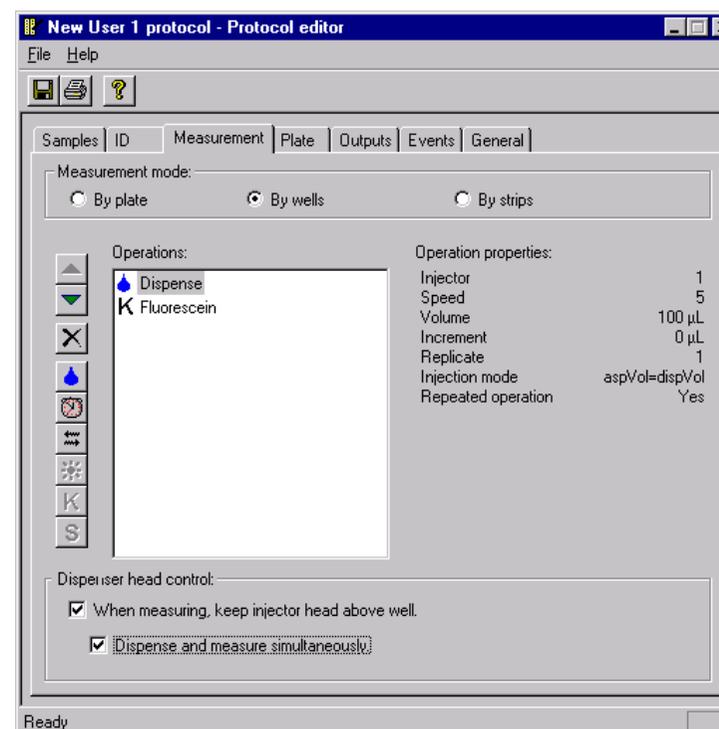


This tab allows you to select the protocol number and name. The number can be selected from a drop down list of available numbers. The protocol name has to be typed in. In the case of a factory pre-set protocol you cannot change the number.

Notes or additional comments can be connected with the protocol. These appear at the bottom of the Explorer window.

## Measurement

The measurement tab specifies the measurement sequence to be used in the protocol.



Note: If the protocol being edited is pre-set, the measurement sequence cannot be changed.

### Measurement mode

This field allows you to specify on what unit the complete measurement sequence should be performed before moving on to the next similar unit. The possible unit is: a single well, a strip or a plate.

Plate and strips modes are disabled when using a fast kinetic or scan measurement in the operation sequence. Well mode will be disabled when using more than one label in the operation sequence. However, copying and pasting of the same label is possible. Plate mode

can be used for slow kinetics by making repeated counting of the plate with delays in between.

### Measurement operations

This field lists the operations that have been specified to be used in the assay. The operations are listed in the order they will be performed.

If you select an item in the list and then click the right mouse button, a menu will appear to allow you to **Copy**, **Delete** or see the **Properties** of the selected item. A row of buttons beside the Operations list enables you to add items to the list. Click the button referring to the operation you want. Set the parameters and click **OK** to add the item to the list.

#### Down button

This moves the selected operation in the operation list one step closer to the end. This button is enabled when the operation currently selected in the measurement operations list is not the last operation in the list.

#### Up button

This moves the selected operation in the operation list one step closer to the beginning. This button is enabled when the operation currently selected in the measurement operations list is not the first operation in the list.

#### Delete button

This deletes the operation selected in the operation list. This button is enabled when there is an operation selected in the measurement operations list.

#### Dispense button

Clicking this causes a dialogue to appear that lets you specify the parameters for a dispense operation. This button is enabled when the dimensions of the plate type used in the protocol are 8x12 or less. You can select

- the number of **injectors** to be used (from 1 to 4 depending on how many are installed)

- the **volume** to be dispensed (in microlitres). A slide bar helps you to select this volume, 350 microlitres is the maximum

- the **speed** from 1 to 5 (where 5 is the fastest and 4 the default)

- the **increment** (using a slide bar with a range from plus 100 microlitres to minus 100 microlitres), this is the volume added to or subtracted from the previous volume dispensed before the next dispensing

## Protocol editing

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the number of **replicates** (from 1 to 3)

the **injection mode**. You can either aspirate and then dispense the same amount ( $\text{aspVol}=\text{dispVol}$ ) or you can fill the whole syringe and then dispense several times ( $\text{aspVol}=\text{syringeVol}$ ). The former is more accurate but the latter is faster.

At the bottom of the dialogue is a check box “Perform on first plate repeat only”. If you only want dispensing for the first of the plate repeats, check this box. If you want dispensing for every repeat then leave it unchecked. Clicking the **OK** button inserts a dispense operation in the measurement operations list.

### Delay button

Clicking this causes a dialogue to appear that lets you specify the duration of the delay between the completion of the preceding operation and the start of the one succeeding the delay operation in the operation list. You use a slide bar to do this. Clicking the **OK** button inserts the delay operation into the measurement operations list. A single delay can be from 0.1s to 3600 s.

At the bottom of the dialogue is a check box “Perform on first plate repeat only”. If you only want delay for the first of the plate repeats, check this box. If you want delay for every repeat then leave it unchecked.

### Shake button

Clicking this causes a dialogue to appear that lets you specify the parameters for a shake operation. You can specify the duration of the operation, the speed (slow, normal or fast), the extent the shaker moves and the path that it follows - straight line, elliptical or figure of eight (linear, orbital or double orbital respectively). Clicking the **OK** button inserts the shake operation into the measurement operations list.

At the bottom of the dialogue is a check box “Perform on first plate repeat only”. If you only want shaking for the first of the plate repeats, check this box. If you want shaking for every repeat then leave it unchecked.

### Label button

Opens the Label Selector to let you specify a label to be inserted in the measurement operations list. There can be up to 10 labels in the list. Note: for LANCE measurements two labels must be selected. If only one is selected then the measurement will be treated as normal time-resolved fluorescence.

When using well or strips mode, the label button will be disabled when there is already a label in the measurement sequence. However, copying and pasting of the same label is possible. For detailed information about labels see Labels on page 67.

### **Kinetics button**

Clicking this causes a dialogue to appear that lets you specify the parameters for a kinetic measurement. You can give the number of repeat measurements (up to 100) and the delay between each measurement (0 to 600 s). You need to also select the label by clicking the label button and then selecting from the label dialogue.

If the repeat is less than 26 and the delay between repeats is 0, results will come in one group. The kinetics button is enabled only when using well mode and there are no other labels in the measurement sequence. However, copying and pasting of the same kinetic label is possible.

### **Scanning button**

Clicking this causes a dialogue to appear that lets you specify the parameters for a scan measurement. This means that several measurements are made for a single well and the measurements are made from different points of the well. These points are in the form of an array for which you can give the number of horizontal steps, the number of vertical steps and the point displacement i.e. the distance between each point on the well where the measurement is made. The total number of points can be a maximum of 100. The distribution of points can be square or rounded.

You also need to select the label by clicking the **Label** button and then selecting from the label dialogue.

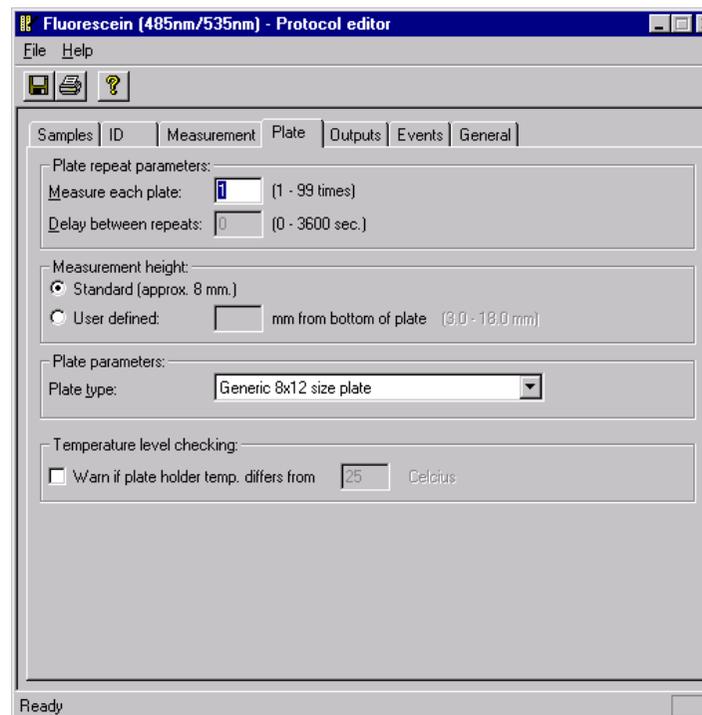
The scanning button is enabled only when using well mode and there are no other labels in the measurement sequence. However, copying and pasting of the same scanning label is possible.

### **Injector position during measurement**

At the bottom of the Measurement tab are two check boxes for controlling the injector head. These will only be enabled if you have selected the “by wells “ mode You can select if you want the measuring head to be kept above the well during measurement. This means that measurement can be started immediately after injection without waiting for the injector head to move aside.

If you check this box then a second check box is enabled where you can select if you want injecting and measurement to happen simultaneously. This is for use in very fast kinetic measurements.

## Plate



### Plate repeat parameters

The number of times each plate is to be measured (1-99). If this is more than 1 then you must give the time between the end of one measurement and the beginning of the next (0-3600 secs.). This allows you to do slow kinetic measurements.

### Measurement height

**Standard** - This selection is only enabled when using a plate type that has its dimension data stored in the instrument.

The default height set is about 8 mm above the bottom of the plate.

**User defined** - If you do not want to use the standard measurement, you can give a new value here. You must click the “**user defined**” button. The minimum value is 3.0 mm (8.0 mm if the dispenser is used) and the maximum 18.0 mm depending on the plate type and if the dispenser is used or not. The height is measured from the bottom of the plate. This is useful if you are measuring samples on filter paper and you need to optimise for the sample being at the top or at the bottom of the wells.

### Plate type

Select the one you want from the list of available plate types under the scroll bar. If the plate size and/or format is different from the current one all the plates in the plate map will be deleted and one plate of the selected type will appear. Make sure you select the plate type before defining the plate map to avoid losing your settings.

Plate types are read from the Wallac 1420 database when the Protocol Editor is started so it will not recognize plate types added while the Protocol Editor is open.

Note: the generic 8 x 12 plate selection does not check the height of the plate before a measurement.

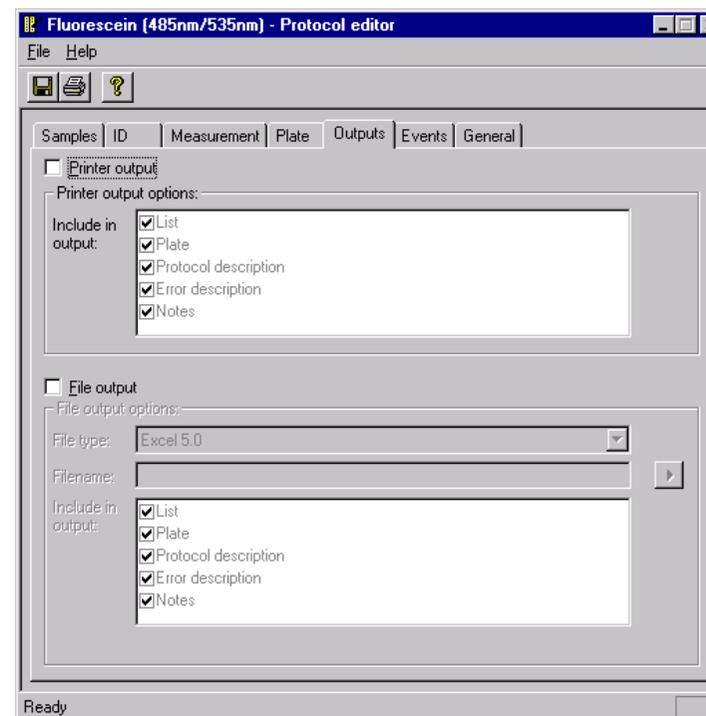
*Note: If the type you selected has a different number of wells than the current one, all the previous plates will be deleted and one plate of the selected type will appear.*

### Temperature level checking

This setting allows you to define what the target temperature should be when a plate is measured. The plate heating controls are only enabled when used with a system that supports the plate heating option.

Note: Setting this option will not activate the plate heating automatically, but it will cause a warning to appear if the temperature differs significantly from what has been specified in the protocol.

## Outputs



The **Outputs** tab in the **Protocol editor** allows you to select where results will be sent at the end of a run. This can be to a printer and/or file. Click the check box of the output device(s) you want.

If you have selected **Printer output**, you must select the format, either plate format (results are in a table format with rows and columns in the order shown in the plate map) and/or list format (results are in a single column starting with those from the first well). When this option is not set, the "Include in output" checked list box is disabled. This list box defines which parts of the assay run data should be included in the printed output: List, Plate, Protocol description, Error description, Notes.

## Protocol editing

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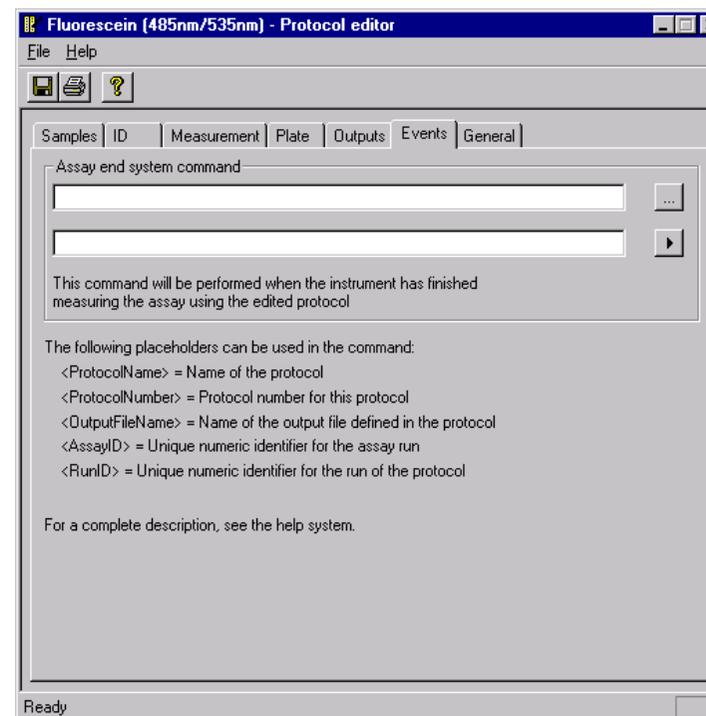
If you have selected **File output** you must specify the file type from the drop down menu of possible types. The types you can save files in are Excel 5 or later versions, Tab delimited text or MultiCalc.

If the **Include in output** list box is checked, then it specifies which optional parts of the assay run information should be included in the file saved when the measurement is completed. These are the same as for printer output: List, Plate, Protocol description, Error description, Notes.

You must also type in the file name. This can include keywords connected with the file as described in Events. The button to the right of the filename box gives you a list of all valid placeholder arguments to choose from. Other filenames possibly used in other protocols are also available through this button.

Note: you will not be allowed to change to another tab if an invalid placeholder argument is found. However, if a previous file with the same file name exists, it will be overwritten by the new file without warning.

## Events



This tab allows you to give a command that will be executed when a protocol has been run. The keyword should be in triangular brackets < > exactly as the examples given in the tab show. These commands are then automatically replaced by the appropriate name or number etc. after the assay run ends.

The first box specifies the executable to run, while the second box specifies the command-line arguments to use. The button to the right of the first box lets you browse to find the executable file you want to use. The button to the right of the second box again lists all the valid keyword arguments that you can choose from, see Keywords below.

For example, you can start an external spreadsheet running and have it execute the file produced by the run. This file will be identified by e.g. protocol name or number. There are also unique internal identifiers that the system gives for each protocol and assay run.

### Keywords

The following keywords can be used for file names in the protocol editor Outputs tab and in Assay end system command in the Events tab:

<ProtocolName>, the name of the protocol. Specified in the ID tab in the protocol name edit box.

<ProtocolNumber>, the protocol number for the protocol. Specified on the ID tab in the protocol number box.

<ProtocolID>, a unique numeric identifier for the protocol.

<AssayID>, a unique numeric identifier for the assay run.

<RunID>, a numeric value identifying the specific run of a certain protocol.

<RunDate>, date when the assay was run in the format YYYYMMDD.

<Plate barcode>, the barcode of the first plate of the assay. If there is no barcode on a plate, you should define an explicit file name for the plate.

<Output file name>, the name of the output file given in the Outputs tab.

In some cases the numeric value of the AssayID or the RunID may not be suitable for the program handling the file. You can add a function “pad” to allow you to decrease or increase the number. You can specify <AssayIDpadX> where X=1..7 or <RunIDpadX> where X=1..4.

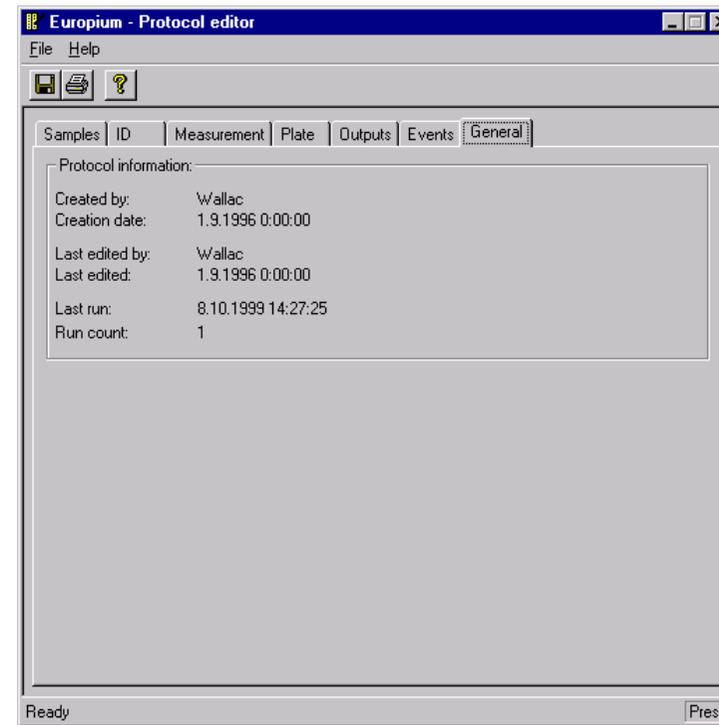
### Examples

If <AssayID> = 1234 and you add pad 2 to get <AssayIDpad2> the result will be 34. This would be useful with MultiCalc which could not accept an assay ID as large as 1234.

If <AssayID> = 234 and you add pad 4 to <AssayIDpad4> the result would be 0234. This would be useful if you needed a four digits identifier.

The validity of possible keyword arguments is checked when you try to switch from this view to another. If an invalid argument is found you are notified and the page switch is cancelled.

## General



This tab gives you information about the protocol, when it was created, when it was edited most recently, by whom it was created and edited and when an assay was last run with it.

The user names used come from the identifier given when Windows was booted. Pre-set protocols are created in the factory.

### File menu

The protocol editor file menu contains the following items of which **Save** and **Print** have corresponding buttons.

#### Save

When you select this item or click the button the current protocol information is saved.

#### Print

When you select this item or click the button a dialogue opens up for you to select the printer type and other details of the printout.

#### Print preview

This item allows you to see what will be printed out. You can select whether you want a side by side two page view or a single page view. You can also zoom in (magnify the page) to see more details, alternatively you can zoom out (reduce the magnification) if the page is already magnified. You can step to the next page or back to the previous one. When you have finished viewing the pages that are going to be printed out you can click the **Print** button to get the printout or **Close** to return to the protocol editor.

#### Print setup

You can define the printer type that will be selected. You can also set the paper size and orientation.

#### Start

Starts measurement with the currently selected protocol.

Note: if the protocol has been changed you will be prompted to save the protocol before measurement is started.

#### Exit

This closes the protocol editor. If the protocol has been changed it will prompt you to save it first.

### Tools menu

The **Tools** command line menu has one item **Options**. When you click this a dialogue with a check box appears. If you do *not* check it, then, whenever you click the **Start** icon, measurement of the selected protocol will begin directly. If you do check it, then, whenever you click the **Start** icon, the Start Wizard will be used to start the measurement of the selected protocol.



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## **Result viewing**



### Latest results

The results from the latest assay can be viewed by clicking the **Latest assay run** icon.



The **Result Viewer** is started and the latest results shown after the measurement has been completed. This command performs following actions:

1. If there are no assay runs in the database yet, a warning is given and the Result Viewer is not invoked.
2. If this button is clicked while an assay run is already in progress, that assay is displayed in the Viewer.
3. If this button is clicked and there is no current assay running, the latest assay run is displayed on the Viewer.

### Results in the Explorer

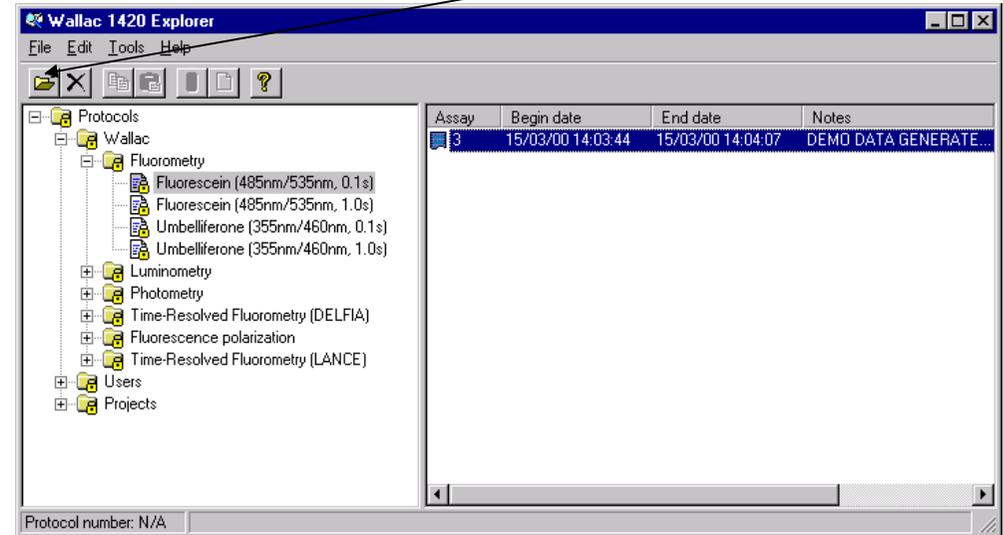
Alternatively, once results have been received and stored in the computer, you can view them by means of the Wallac 1420 Explorer. See the picture on the next page.

The protocol connected to the latest results will be selected automatically, but you can select any protocol that was used to make the measurements that you want to view. The results files associated with the protocol will then appear on the right side of the window. Each entry for a results file comprises the following items Assay ID, the date and time when the assay began and when it ended, Notes and Errors.

Note: to change a column width in the Results Viewer, move the mouse cursor to the line between two columns. The cursor form will change to a vertical bar with two arrows. Hold down the left mouse button and move the column division in the direction you choose. The information in a column is complete if no “.....” are shown at the end.

## Result viewing

Click on the result file and then click the **Open** icon.



Note: these results are in the form of raw numerical data

The Result Viewer gives details of the actual measurement. There are five tabs and these described below.

The Result Viewer also provides you the following functions by means of command line menus or, in most cases, buttons:

**File** - Export (Button), Page setup, Print (Button) and Exit.

**View** - List (Button), Plate (Button), Protocol (Button), Errors(Button), Notes (Button), View all result data (only when List is selected), Toolbar, Status bar

**Plate** - first plate, previous plate, next plate, last plate (all with corresponding buttons). These buttons or menu items that allow you to step between plates only appear when the plate mode is selected.

**Help** - information about Result Viewer.

## List

The screenshot shows a software window titled "Assay 3, 2.11.1999 12:39:59 - Wallace 1420 Result Viewer". The window contains a data table with the following columns: Plate, Repeat, Well, Type, Time, and Fluorescence. The data is organized into rows, with the first row (row 1) containing the following values: Plate: 1, Repeat: 1, Well: A01, Type: M, Time: 00:00:00,20, Fluorescence: 567349. The table continues with rows 2 through 31, each containing similar data for different wells (A02 through C06). The software interface includes a menu bar (File, View, Plate, Help), a toolbar with various icons, and a status bar at the bottom showing "Ready".

1	A	B	C	D	E	F	G	H	I	J	K	L	M
1	Plate	Repeat	Well	Type	Time	Fluoresc							
2	1	1	A01	M	00:00:00,20	567349							
3	1	1	A02	M	00:00:00,40	201371							
4	1	1	A03	M	00:00:00,60	810853							
5	1	1	A04	M	00:00:00,80	589159							
6	1	1	A05	M	00:00:01,00	485074							
7	1	1	A06	M	00:00:01,20	356788							
8	1	1	A07	M	00:00:01,40	897002							
9	1	1	A08	M	00:00:01,60	824611							
10	1	1	A09	M	00:00:01,80	749138							
11	1	1	A10	M	00:00:02,00	182367							
12	1	1	A11	M	00:00:02,20	860354							
13	1	1	A12	M	00:00:02,40	713396							
14	1	1	B01	M	00:00:02,60	518399							
15	1	1	B02	M	00:00:02,80	310954							
16	1	1	B03	M	00:00:03,00	24834							
17	1	1	B04	M	00:00:03,20	100488							
18	1	1	B05	M	00:00:03,40	370807							
19	1	1	B06	M	00:00:03,60	155639							
20	1	1	B07	M	00:00:03,80	174239							
21	1	1	B08	M	00:00:04,00	988639							
22	1	1	B09	M	00:00:04,20	451235							
23	1	1	B10	M	00:00:04,40	127892							
24	1	1	B11	M	00:00:04,60	14622							
25	1	1	B12	M	00:00:04,80	18822							
26	1	1	C01	M	00:00:05,00	384101							
27	1	1	C02	M	00:00:05,20	536346							
28	1	1	C03	M	00:00:05,40	575472							
29	1	1	C04	M	00:00:05,60	605746							
30	1	1	C05	M	00:00:05,80	611094							
31	1	1	C06	M	00:00:06,00	174571							

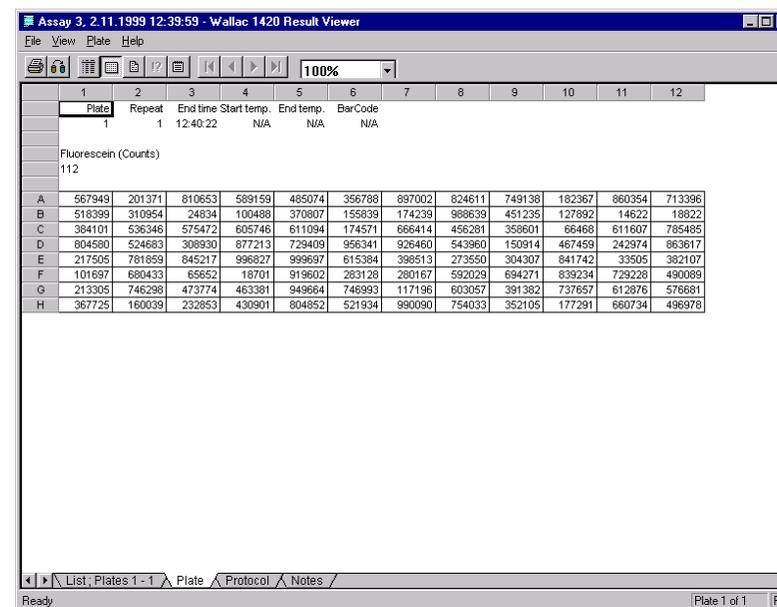
The following information appears: Plate ID, Number of repeats, Well number and Sample type abbreviation (e.g. M= Measured). In addition there are pairs of columns. The number of pairs depends on what you have set in protocol editing parameters. The first column of each pair is the measurement time and the other the result value. You can change column widths to get more information. In the case of high-density plates results can be split over several tabs.

**View all result data** - This item in the View menu is only active if List view is selected. It causes an extra column to appear in the result list giving the following extra information:  
 Time-resolved fluorometry – number of flashes  
 Fluorescence – measurement time  
 Luminometry – counting time  
 Photometry – A/D converter value and the number of readings

## Result viewing

### Plate map

This plate map shows you the results arranged from top to bottom in the order they appeared in the list from left to right. See List format for the alternative way of viewing results.



The screenshot shows the 'Assay 3, 2.11.1999 12:39:59 - Wallac 1420 Result Viewer' window. The interface includes a menu bar (File, View, Plate, Help), a toolbar with navigation icons, and a zoom level of 100%. The main display area contains a table with the following data:

	1	2	3	4	5	6	7	8	9	10	11	12	
Plate	Repeat	End time	Start temp.	End temp.	BarCode								
1	1	12:40:22	N/A	N/A	N/A								
Fluorescein (Counts)													
112													
A	567949	201371	810653	599159	485074	356788	897002	824611	749138	182367	860354	713396	
B	518399	310954	24834	100488	370807	155839	174239	988639	451235	127892	14622	18822	
C	384101	536346	575472	605746	611094	174571	666414	456281	358601	66468	611607	785485	
D	804580	524683	308930	877213	729409	956341	926480	543960	150914	467459	242974	863617	
E	217505	781859	845217	996827	999697	615384	398513	273550	304307	841742	33505	382107	
F	101697	680433	65652	18701	919602	283128	280167	592029	694271	839234	729228	490089	
G	213305	746298	473774	463381	949664	748993	117196	603057	391382	737657	612876	576681	
H	367725	160039	232853	430901	804852	521934	990090	754033	352105	177291	660734	496978	

The status bar at the bottom shows 'Ready' and 'Plate 1 of 1'.

The cells in the two first rows displayed include information about the currently displayed plate. The information included is:

**Plate:** The number of the physical plate currently being displayed.

**Repeat:** The number of the repeat of the physical plate currently being displayed.

**Measurement end time:** Date and time when the measurement of the result plate was finished.

**Start temperature:** Plate holder temperature when the measurement of the plate was started.

**End temperature:** Plate holder temperature when the measurement of the plate ended.

**Barcode:** Barcode of the plate if used, if not used it displays the text 'N/A'.

## Result viewing

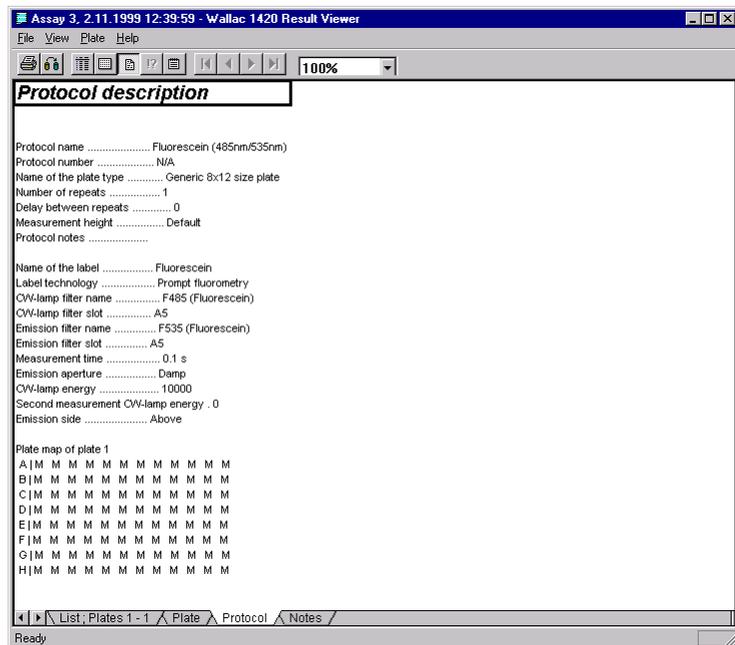
The cells displayed in the rows directly above each result plate gives information directly relating to the label or result type displayed in the plate grid below it. The information included is:

**Name** of the label or result type displayed in the result plate

**Unit** for the results from the result plate

**Instrument Background** value measured for the label or result type.

## Protocol



Selecting Protocol causes a list of protocol parameters to appear. There is also a plate map showing which sample types were on the plate. Any notes written in the protocol editor Notes box will appear here.

## Result viewing

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### Notes

This tab allows you to see any notes that are connected with the results being viewed.

### Error

Error gives you information about the general error situation and specific information about any errors that occurred for each measurement type.

### Print

Clicking this icon leads to an extended print dialogue which in addition to the normal print options gives you a choice of printing **selected sheets** or the **entire workbook**. The former is the default. You can also click the **Preview** button to get a preview of the pages to be printed.

In the case of high-density plates the results from the first columns (1 to 18 or 1 to 24) appear on one page and the rest (19-36 or 25 to 48) appear on the next page. Only rows of one plate will be printed on one page.

If you press **OK** in the print dialogue, the selected parts of the run information will be printed on the printer that has been selected in the print dialogue.

### Export results

If you select **Export** in Result Viewer it allows you to export the results of the active plate. A window appears in which you can give the path, the file name (type it in) the file type, (select it from the list), a **Save** button and a **Cancel** button.

The file types you can save files in are Excel 5.0 or later, Tab delimited text or MultiCalc .

To help you select where you want the file to be saved, you can browse to find the folder you want. Buttons allow you to go up one level, create a new folder, and display the folder information as a list just showing folder names or with additional details. The white area in the centre of the window is where the folders appear when you start to browse to find the path.

---

## **Tools menu**



### Tools menu

*Note: These operations can affect the whole system and should only be undertaken by someone qualified to do them.*

There are up to twelve items in this menu (depending on the options installed):

**Explorer**- for protocol editing and result viewing, see pages 35 and 57.

**Start Wizard** - for starting when you first need to define plates and wells, see page 24.

**Results of latest assay run** - the result viewer is started and the latest results shown, see page 57.

**Labels** – for defining labels used in measurements.

**Filters** – for defining filter types.

**EuSm dual label normalization wizard**, - (requires TR-FIA to be installed).

**LANCE normalization wizard** – (requires LANCE to be installed).

**Plate dimension wizard** – for fine-tuning the plate position when measuring high density plates

**Dispenser maintenance** – described in Appendix 3.

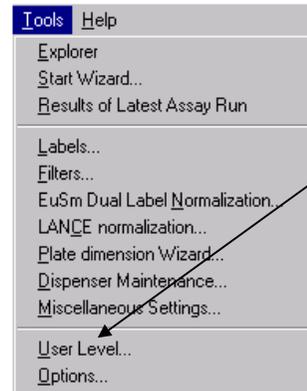
**Miscellaneous settings** – including plate types and well names.

**User level** – selecting the features accessible to the user.

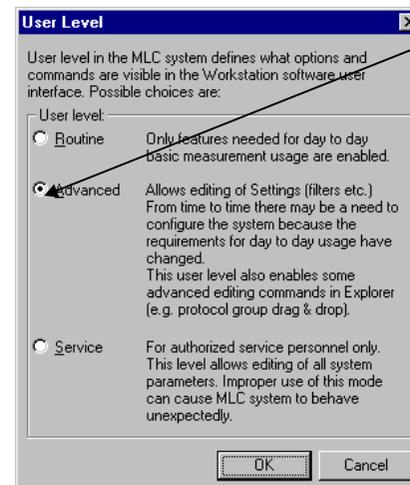
**Options** – includes demo mode selection, barcode and plate map features.

## User level

User level in the Tools menu allows you to select the user level: **Routine**, **Advanced** or **Service**.



If the **User Level** is selected to be **Routine**, this will automatically disable any system operations. To enable these operations you must select the **Advanced** level. **Service** is only for qualified service specialists. It gives access to e.g. Instrument Options.



## Labels



In this window there are tabs corresponding to the measurement technologies (labels) in the instrument. Each tab includes the names of the currently defined labels. Names of user defined labels can be freely defined by the user but names of factory pre-set labels cannot be changed. See the sections below for details.

There are four buttons at the bottom of the labels window:

**Add** - add a new item to the list. Give the name and edit the properties.

**Copy** - (select an item to activate this). Make a copy of an item. It will appear with a new icon showing it is different from a default item, and the name will be preceded by the words "Copy of". You can give it a different name and edit other parameters by selecting Properties.

**Remove** - (select a user created item to activate this). Remove the selected item from the list. Preset items and labels used in protocols cannot be removed. This command requires confirmation because you cannot undo Remove.

## Tools menu

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**Properties** - (select an item to activate this). View the properties of the selected item. If the item is user defined then you can edit the properties, but if it is a factory pre-set, you cannot edit them.

## Time-resolved fluorometry

Note: to be enabled this requires the TR-FIA option to be installed.

In time-resolved fluorometry a flash lamp is used to illuminate the sample, different lanthanides are used as labels. The picture shows the parameters that can be set.

The screenshot shows a dialog box titled "TR-Fluorometry Label Properties". It is divided into several sections:

- Name:** A text field containing "Europium 1" and a "Password..." button. Below it, "Last Edited: GT 15/03/00 14:35:57".
- Parameters:**
  - Flash Energy Area: Radio buttons for "Low" and "High" (selected).
  - Flash Energy Level: A numeric input field with "245" and a slider.
  - Excitation Filter: Radio buttons for "D340" (selected), "D320", and "Optional".
  - Light Integr Capacitors: Radio buttons for "1", "2" (selected), and "3".
  - Light Integr Ref Level: A numeric input field with "157" and a slider.
  - Emission Filter: A dropdown menu showing "D615 (Europium, LANCE\_615nm) - Slo".
  - Emission Aperture: Radio buttons for "Normal" (selected), "Damp", and "Band".
  - Counting Delay: Two numeric input fields (1: 400, 2: 0) with a range "(0 - 65515 us)".
  - Counting Window: Two numeric input fields (1: 400, 2: 0) with a range "(0 - 65515 us)".
  - Counting Cycle: A numeric input field with "1000" and a range "(1000 - 65535 us)".
  - Flash absorbance: Radio buttons for "Yes" and "No" (selected).
  - Beam size: Radio buttons for "Normal" (selected) and "Narrow".
- Second Measurement:** A checkbox that is unchecked, with an "Emission Filter" dropdown menu showing "D615 (Europium, LANCE\_615nm) - Slot".

At the bottom are "OK", "Cancel", and "Help" buttons.

### Protocol name

This is the name by which the protocol is identified. A password can be associated with it to prevent parameters being changed by an unauthorized person.

### Flash Energy Area

There are two options for this parameter “Low” and “High”. The energy of the flash lamp used is dependent on the discharge capacitor value. The “Low” Flash Energy Area selects a basic capacitor of 10 nF. The “High” Flash Energy Area selects a second 10 nF capacitor in the lamp’s discharge circuit thus doubling the energy. If you select "High" you will reduce the counting time (number of flashes), but the counts will remain about the same.

### Flash Energy Level

The Flash Energy Level sets the voltage of the discharge capacitor; the range is from 1 to 255 (corresponding to 500 to 800 volts). Note that although the lamp input energy depends on the squared power of the discharge voltage, the output optical power is not linearly dependent on the input energy. By changing this parameter you will fine-tune the Flash Energy. If you increase the number, you will reduce counting time, but counts from your sample will remain about the same.

***CAUTION: If software version 2.0 release 8 or higher is installed on an instrument having a serial number less than 4201396, then you should avoid the combination of settings: Flash Energy Area = "High" and Flash Energy Level = high values (e.g.255) along with 384-well or higher plates. The reason is that this "high power/continuous usage" combination may damage the older type of flash lamp. The cooled flash excitation system available for newer instruments is especially designed to cope with this.***

### Excitation filter

There are two non-changeable excitation filters in the flash excitation path. Both are colour glass filters. The main filter is D 340, which has a maximum transmittance at 340 and half-bandwidth of about 35 nm. This filter is used for factory-set protocols. The second filter, D320, has a maximum transmittance at 320 but its bandwidth is much broader; it includes the same excitation band as D340 but its lower edge goes down to 280nm allowing excitation of some special Tb-chelates.

### Light integration capacitor

A reference circuit monitors the total excitation energy. When the selected total amount of energy has been reached the flashes stop. The reference circuit makes use of three light integration capacitors, numbered 1, 2 and 3, any one of which can be selected. The capacitor selected determines the total excitation energy in the same proportions as the capacitor numbers (within component tolerances). This means that selecting capacitor 2 instead of capacitor 1, approximately doubles the excitation energy for one measurement, i.e. the number of flashes for one measurement doubles, hence the time used for one measurement doubles and the number of counts doubles. Selecting capacitor 3 instead of capacitor 1

triples the excitation energy, the measurement time and the number of counts. The number of flashes can be seen from the result viewer by selecting **View all result data**.

### **Note**

Each flash contributes to the total excitation energy. Flashes keep on occurring until the required total excitation energy is achieved. However, because the energy comes in discrete flashes the last flash will cause the total excitation energy to exceed the required amount by a maximum of not more than the energy in one flash. This will not be significant if there are many flashes but could be significant if there are only a few flashes. E.g. in standard measurements the amount of flashes is about 1000, the variation in the total excitation energy is thus 1/1000 or 0.1 %. If the amount of flashes is about 100, the variation in excitation energies is 1/100 or 1 %.

### **Light Integration Reference Level**

These values change the excitation energy approximately linearly. Changing this value from 50 to 100 has about the same effect as changing the capacitor from 1 to 2. The number of flashes can be seen from the result viewer by selecting **View all result data**.

### **Emission filters**

You can select the emission filter from the drop-down list of available filters. See the chapter on Filters for more information about how to define filters.

Factory pre-set emission filter names begin with a letter indicating the technology for which they are to be used: D is for time-resolved fluorometry. After the letter comes the wavelength.

### **Emission aperture**

In front of the photomultiplier tube there is a 4-position aperture slide, which has three different apertures and a shutter position.

The normal aperture is circular with a diameter of about 4 mm and it is used for factory set labels.

The damped aperture is also circular but only 1 mm in diameter. This allows you to shift the dynamic range of the emission signal for a particular user selected label which may otherwise exceed the linear range of the instrument. This damped aperture is used for the top fluorometry factory set labels.

The band aperture is an approximately 1 mm x 3 mm rectangle and it reserved mainly for future applications. Its size and alignment are about the same as the excitation area from the flash lamp.

## Tools menu

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The shutter is used at all times when no measurement is occurring to prevent stray light getting to the photomultiplier tube.

### Counting parameters

These determine the time factors involved in the measurement process, all times are in microseconds:

**Counting delay** - the time after the excitation pulse at which counting of the emission signal begins

**Counting window** - the time period within which counting occurs

**Counting cycle** - the time between excitation pulses within a measurement.

The second or additional counting window is fully independent of the first one and it useful when optimizing time-resolved parameters or for calculations of the decay time of the label.

### Factory-set values for the main counting window

Label	Delay	Window	Cycle
Eu	400	400	1000
Sm	50	100	1000
Tb	500	1400	2000
Dy	30	30	1000

Factory set values for the second counting window are always 0/0. The counts in this window are displayed only if the values deviate from zero

### Flash absorbance

If you want there to be an absorption measurement as well as the usual time-resolved fluorescence emission measurement, select this parameter to be **Yes**. Both measurements will then be made simultaneously.

### Beam size

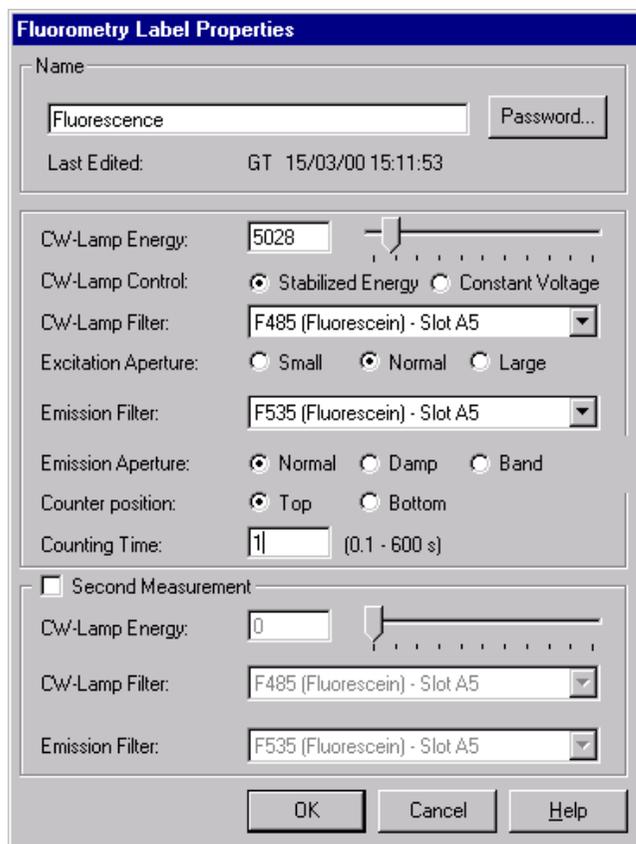
This parameter can be selected to be **Normal** or **Narrow** provided the Adjustable beam size option has been installed. It enables measurements on plates with very small well sizes by focusing the excitation light beam to a smaller size.

### Second measurement parameters

In the case where you want to make measurements with two filters, you can use this parameter to select the type of the second emission filter. The options are the same as for the first measurement emission filter. Two measurements are made, first with the normal filter and then with the second, which the system automatically moves into place.

## Fluorometry

Fluorometry - light of a particular wavelength is selected with a filter from the spectrum of a continuous wave lamp and used to excite the fluorochrome in the sample. The parameters that can be set for fluorometry are the following:



The screenshot shows a dialog box titled "Fluorometry Label Properties". It contains several sections for configuring the instrument. The "Name" section has a text field with "Fluorescence" and a "Password..." button. Below it, "Last Edited:" is "GT 15/03/00 15:11:53". The "CW-Lamp Energy:" section has a text field with "5028" and a slider. "CW-Lamp Control:" has radio buttons for "Stabilized Energy" (selected) and "Constant Voltage". "CW-Lamp Filter:" is a dropdown menu showing "F485 (Fluorescein) - Slot A5". "Excitation Aperture:" has radio buttons for "Small", "Normal" (selected), and "Large". "Emission Filter:" is a dropdown menu showing "F535 (Fluorescein) - Slot A5". "Emission Aperture:" has radio buttons for "Normal" (selected), "Damp", and "Band". "Counter position:" has radio buttons for "Top" (selected) and "Bottom". "Counting Time:" is a text field with "1" and a range "(0.1 - 600 s)". There is a checkbox for "Second Measurement" which is unchecked. Below this, there is another "CW-Lamp Energy:" section with a text field "0" and a slider. "CW-Lamp Filter:" is a dropdown menu showing "F485 (Fluorescein) - Slot A5". "Emission Filter:" is a dropdown menu showing "F535 (Fluorescein) - Slot A5". At the bottom are "OK", "Cancel", and "Help" buttons.

### Protocol name

This is the name by which the protocol is identified. A password can be associated with it to prevent parameters being changed by an unauthorized person.

## Tools menu

---

### **CW-Lamp energy**

Using this parameter you are able to change the excitation energy supplied by the CW-Lamp.

### **CW-Lamp Control Mode**

There are two ways of ensuring that the light incident on the plate from the CW-Lamp remains constant – stabilized energy mode or constant voltage mode. In the stabilized energy mode a photodiode close to the light path has part of the incident light directed onto it. This sends feedback to the lamp controller which alters the lamp voltage to adjust the intensity of the light produced so that it remains constant. In the constant voltage mode the lamp voltage is not changed but the software corrects for changes in the lamp output. You can select the control mode you want from the option buttons. The default mode is stabilized energy. The use of the constant voltage mode allows the CW-Lamp lamp to be run continuously at maximum power and it is faster than the stabilized energy mode. This enables optimum performance in cases where the light intensity is critical, as in fluorescence polarization or where speed is critical as in fast kinetics.

### **CW-Lamp filters**

You can select the CW-lamp excitation filter from the drop-down list of available filters. See the chapter on Filters for more information about how to define filters.

Factory pre-set CW-lamp filter names begin with a letter indicating the technology for which they are to be used:

F is for fluorometry. After the letter comes the wavelength.

### **Excitation aperture (Wallac 1420-040/041/042/043 only)**

There are three sizes of excitation aperture controlling the size of the incident light beam. You can select with the option buttons Small, Normal or Large. Normal is the default.

### **Emission filters**

You can select the emission filter from the drop-down list of available filters. See the chapter on Filters for more information about how to define filters.

Factory preset emission filter names begin with a letter indicating the technology for which they are to be used:

F is for fluorometry. After the letter comes the wavelength.

### Emission aperture

In front of the photomultiplier tube there is a 4-position aperture slide, which has three different apertures and a shutter position.

The normal aperture is circular with a diameter of about 4 mm and it is used for factory set labels.

The damped aperture is also circular but only 1 mm in diameter. This allows you to shift the dynamic range of the emission signal for a particular user selected label that may otherwise exceed the linear range of the instrument. This damped aperture is used for the top fluorometry factory set labels.

The band aperture is an approximately 1 mm x 3 mm rectangle and it reserved mainly for future applications. Its size and alignment is about the same as the excitation area from the flash lamp.

The shutter is used at all times when no measurement is occurring to prevent stray light getting to the photomultiplier tube.

### Counter position

The counter position can be selected to be **Top** or **Bottom**.

### Counting time

This parameter determines how long the excitation continues.

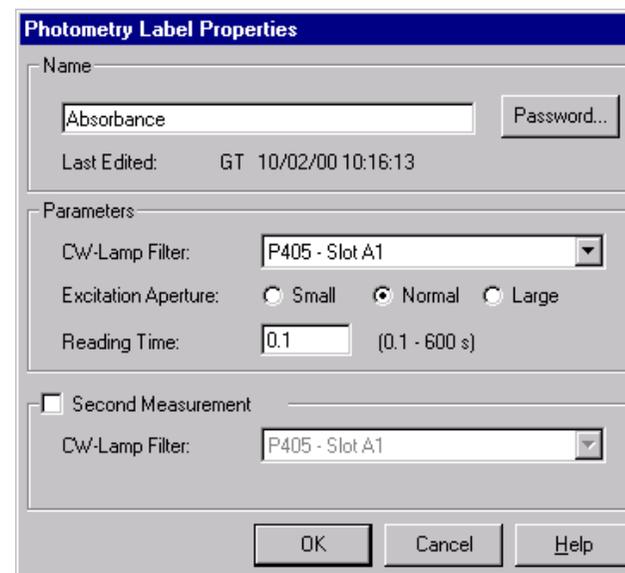
### Second measurement parameters

In the case where you want to make measurements with two filters and/or two different energies, you can set these parameters in this field. Click the check box **Second measurement** to activate them.

There are three of them: CW-Lamp energy , CW-Lamp Filter and Emission Filter. See the corresponding sections for the first measurement for more details. When you select one of more of these parameters two measurements are made, the first with the normal settings and the second with these new settings. The change is automatic.

### Photometry

A reference measurement is made before the plate is moved to the measurement position. This enables the absorbance to be calculated.



The image shows a dialog box titled "Photometry Label Properties". It is divided into three sections: "Name", "Parameters", and "Second Measurement".

- Name:** A text field containing "Absorbance" and a "Password..." button. Below it, "Last Edited: GT 10/02/00 10:16:13".
- Parameters:** A "CW-Lamp Filter:" dropdown menu set to "P405 - Slot A1". Below it, "Excitation Aperture:" with radio buttons for "Small", "Normal" (selected), and "Large". Below that, "Reading Time:" a text field with "0.1" and "(0.1 - 600 s)".
- Second Measurement:** A checkbox labeled "Second Measurement" which is unchecked. Below it, a "CW-Lamp Filter:" dropdown menu set to "P405 - Slot A1".

At the bottom are three buttons: "OK", "Cancel", and "Help".

The parameters that can be set for photometry are the following:

#### Protocol name

This is the name by which the protocol is identified. A password can be associated with it to prevent parameters being changed by an unauthorized person.

#### CW-Lamp filters

You can select the CW-lamp filter from the drop-down list of available filters. See the chapter on Filters for more information about how to define filters.

Factory pre-set CW-lamp filter names begin with a letter indicating the technology for which they are to be used:

P is for photometry. After the letter comes the wavelength.

### **Excitation aperture (Wallac 1420-040/041 only)**

There are three sizes of excitation aperture controlling the size of the incident light beam. You can select with the option buttons Small, Normal or Large. Normal is the default.

### **Reading time**

This parameter determines how long the sample is read to determine the absorbance.

### **Second measurement**

In the case where you want to make measurements with two filters, you can click this check box and then set the parameter **CW-Lamp filter** - It has the same options as the parameter with the same name above. When you select this, two measurements are made, the first with the normal settings and the second with this new setting. The change is automatic.

### Luminometry

Luminescence in the sample is detected.

The parameters that can be set for luminometry are shown in the picture and described below.

**Luminometry Label Properties [Factory preset]**

Name:  Password...

Last Edited: Wallac 30/05/96 14:20:00

Parameters:

Emission Filter:

Emission Aperture:  Normal  Damp  Band

Counting Time:  (0.1 - 600 s)

Second Measurement

Emission Filter:

OK Cancel Help

#### Protocol name

This is the name by which the protocol is identified. A password can be associated with it to prevent parameters being changed by an unauthorized person.

#### Emission filters

You can select the emission filter from the drop-down list of available filters. See the chapter on Filters for more information about how to define filters.

Factory preset emission filter names begin with a letter indicating the technology for which they are to be used:

L is for luminescence. After the letter comes the wavelength.

#### Emission aperture

In front of the photomultiplier tube there is a 4-position aperture slide, which has three different apertures and a shutter position.

The normal aperture is circular with a diameter of about 4 mm and it is used for factory set labels.

The damped aperture is also circular but only 1 mm in diameter. This allows you to shift the dynamic range of the emission signal for a particular user selected label which may otherwise exceed the linear range of the instrument. This damped aperture is used for the top fluorometry factory set labels.

The band aperture is an approximately 1 mm x 3 mm rectangle and it reserved mainly for future applications. Its size and alignment is about the same as the excitation area from the flash lamp.

The shutter is used at all times when no measurement is occurring to prevent stray light getting to the photomultiplier tube.

### **Counting time**

This parameter determines how long the luminescence is counted for.

### **Second Measurement**

In the case where you want to make measurements with two filters, you can click the **Second measurement** check box and then set this emission filter. It has the same options as the normal emission filter. When you select this, two measurements are made, the first with the normal settings and the second with this new setting. The change is automatic.

### Fluorescence polarization (Wallac 1420-040/041/042/043 only)

Fluorescence polarization - light of a particular wavelength is selected with a filter from the spectrum of a continuous wave lamp. This light is then polarized and used to excite the fluorochrome in the sample. The emission light is then viewed through two polarizers, one parallel to the incident polarization (S-plane) and one perpendicular to it (P-plane). These are, each in turn, moved automatically into the emission light path. The ratio:

$$1000 * (\text{Parallel} - G * \text{Perpendicular}) / (\text{Parallel} + G * \text{Perpendicular})$$

is calculated to get the polarization in units of mP. G is a correction factor, see the end of this section. The parameters that can be set for fluorescence polarization are the following:

**Fluorescence polarization Label Properties**

Name: FP-Fluorescein Password...

Last Edited: GT 15/03/00 15:21:42

Parameters:

CW-Lamp Energy: 65535

CW-Lamp Filter: F485 (Fluorescein) - Slot A5

Polarizer Aperture:  Small  Normal

Emission Filter: F535 (Fluorescein) - Slot A5

Emission Aperture:  Normal  Damp  Band

Counting Time: 1 (0.1 - 600 s)

G factor: 1

OK Cancel Help

### Protocol name

This is the name by which the protocol is identified. A password can be associated with it to prevent parameters being changed by an unauthorized person.

**CW-Lamp energy**

In fluorescence polarization constant voltage mode is used and the light source is driven with high power. The software corrects for changes in energy of the CW-lamp to ensure that results are calculated for a constant excitation energy. *For fluorescence polarization you should always use maximum power, 65535.*

**CW-Lamp filter**

You can select the CW-lamp excitation filter from the drop-down list of available filters. See the chapter on Filters for more information about how to define filters. Factory pre-set CW-lamp filter names begin with a letter indicating the technology for which they are to be used: F is for fluorometry. After the letter comes the wavelength.

**Polarizer aperture**

There are two sizes for the aperture of the S-plane polarizer in the excitation light path. You can select by option buttons whether this is Small (for high-density plates) or Normal (96 or 384-well plates).

**Emission filter**

You can select the emission filter from the drop-down list of available filters. See the chapter on Filters for more information about how to define filters. Factory preset emission filter names begin with a letter indicating the technology for which they are to be used: F is for fluorometry. After the letter comes the wavelength.

**Emission aperture**

In front of the photomultiplier tube there is a 4-position aperture slide, which has three different apertures and a shutter position. The normal aperture, which is circular with a diameter of about 4 mm, should be used for fluorescence polarization.

**Counting time**

This parameter determines how long the excitation continues. The following times are recommended for 96 and 384-well plates:

Concentration (nM)	Measuring time (s)
2 or more	0.1
1	0.2
0.5	0.3
0.1	1.0

## Tools menu

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### G factor

In fluorescence polarization assays a G-factor is used to correct for the bias in polarization values caused by differences in instruments and assay conditions. To calculate the G-factor prepare buffer and 1 nM fluorescein samples (replicates should be at least 24). The formula used is:

$$G = \frac{\text{CorrectedS}}{\text{CorrectedP}} * \frac{\left(1 - \frac{L}{1000}\right)}{\left(1 + \frac{L}{1000}\right)}$$

where the corrected parallel and perpendicular S- and P-counts are background subtracted fluorescence count rates obtained from a pure fluorophore solution e.g.:

CorrectedS = averageS(1nM)-averageS(buffer) and

CorrectedP = averageP(1nM)-averageP(buffer)

L is the literature polarization value for the fluorophore in mP. E.g. the value for 1 nM fluorescein is 27 mP in aqueous buffer at room temperature. The G-factor is typically between 0.8 and 1.2.

The G factor is used in the calculation of the corrected mP for samples as:

$$mP = 1000 \times \frac{\text{CorrectedS} - G \times \text{CorrectedP}}{\text{CorrectedS} + G \times \text{CorrectedP}}$$

where CorrectedS = S(sample)-S(buffer) and

CorrectedP = P(sample)-P(buffer)

## LANCE

Note: this requires the LANCE option to be installed.

In LANCE homogenous time-resolved fluorescence a flash lamp is used to illuminate the sample. In the most common form of LANCE this excites the donor molecule which, after a delay, transfers the energy to the acceptor molecule which then emits light. Two labels must be defined, one for the donor and the other for the acceptor. The picture shows the parameters that can be set for a LANCE label.

The screenshot shows the 'Lance Label Properties' dialog box. It has a title bar with the text 'Lance Label Properties'. Below the title bar, there is a 'Name' field containing 'LANCE' and a 'Password...' button. Below that, it says 'Last Edited: GT 15/03/00 15:27:03'. The main section is titled 'Parameters' and contains several settings:

- Flash Energy Area:  Low  High
- Flash Energy Level: 245 (with a slider)
- Excitation Filter:  D340  D320  Optional
- Light Integr Capacitors:  1  2  3
- Light Integr Ref Level: 157 (with a slider)
- Emission Filter: D545 (Terbium) - Slot A3 (dropdown menu)
- Emission Aperture:  Normal  Damp  Band
- Counting Delay: 1: 50 2: 0 (0 - 65515 us)
- Counting Window: 1: 100 2: 0 (0 - 65515 us)
- Counting Cycle: 3000 (1000 - 65535 us)
- Flash absorbance:  Yes  No
- Beam size:  Normal  Narrow

At the bottom, there is a checkbox for 'Second Measurement' which is unchecked. Below it, the 'Emission Filter' is set to 'D615 (Europium, LANCE\_615nm) - Slot'. At the very bottom are three buttons: 'OK', 'Cancel', and 'Help'.

### Protocol name

This is the name by which the protocol is identified. A password can be associated with it to prevent parameters being changed by an unauthorized person.

### Flash Energy Area

There are two options for this parameter “Low” and “High”. The energy of the flash lamp used is dependent on the discharge capacitor value. The “Low” Flash Energy Area selects a basic capacitor of 10 nF. The “High” Flash Energy Area selects a second 10 nF capacitor in the lamp’s discharge circuit thus doubling the energy. If you select "High" you will reduce the counting time (number of flashes), but the counts will remain about the same.

### Flash Energy Level

The Flash Energy Level sets the voltage of the discharge capacitor; the range is from 1 to 255 (corresponding to 500 to 800 volts). Note that although the lamp input energy depends on the squared power of the discharge voltage, the output optical power is not linearly dependent on the input energy. By changing this parameter you will fine-tune the Flash Energy. If you increase the number, you will reduce counting time, but counts from your sample will remain about the same.

***CAUTION: If software version 2.0 release 8 or higher is installed on an instrument having a serial number less than 4201396, then you should avoid the combination of settings: Flash Energy Area = "High" and Flash Energy Level = high values (e.g.255) along with 384-well or higher plates. The reason is that this "high power/continuous usage" combination may damage the older type of flash lamp. The cooled flash excitation system available for newer instruments is especially designed to cope with this.***

### Excitation filter

There are two non-changeable excitation filters in the flash excitation path. Both are colour glass filters. The main filter is D 340, which has a maximum transmittance at 340 and half-bandwidth of about 35 nm. This filter is used for factory-set protocols. The second filter, D320, has a maximum transmittance at 320 but its bandwidth is much broader; it includes the same excitation band as D340 but its lower edge goes down to 280nm allowing excitation of some special Tb-chelates.

### Light integration capacitors

A reference circuit monitors the total excitation energy. When the selected total amount of energy has been reached the flashes stop. The reference circuit makes use of three light integration capacitors, numbered 1, 2 and 3, any one of which can be selected. The capacitor selected determines the total excitation energy in the same proportions as the capacitor numbers (within component tolerances). This means that selecting capacitor 2 instead of capacitor 1, approximately doubles the excitation energy for one measurement, i.e. the number of flashes for one measurement doubles, hence the time used for one measurement doubles and the number of counts doubles. Selecting capacitor 3 instead of capacitor 1

triples the excitation energy, the measurement time and the number of counts. The number of flashes can be seen from the result viewer by selecting **View all result data**.

### Note

Each flash contributes to the total excitation energy. Flashes keep on occurring until the required total excitation energy is achieved. However, because the energy comes in discrete flashes the last flash will cause the total excitation energy to exceed the required amount by a maximum of not more than the energy in one flash. This will not be significant if there are many flashes but could be significant if there are only a few flashes. E.g. in standard measurements the amount of flashes is about 1000, the variation in the total excitation energy is thus 1/1000 or 0.1 %. If the amount of flashes is about 100, the variation in excitation energies is 1/100 or 1 %.

### Light Integration Reference Level

These values change the excitation energy approximately linearly. Changing this value from 50 to 100 has about the same effect as changing the capacitor from 1 to 2. The number of flashes can be seen from the result viewer by selecting **View all result data**.

### Emission filter

You can select the emission filter from the drop-down list of available filters. See the chapter on Filters for more information about how to define filters.

Factory pre-set emission filter names begin with a letter indicating the technology for which they are to be used: D is for time-resolved fluorometry. After the letter comes the wavelength.

### Emission aperture

In front of the photomultiplier tube there is a 4-position aperture slide, which has three different apertures and a shutter position.

The normal aperture is circular with a diameter of about 4 mm and it is used for factory set labels.

The damped aperture is also circular but only 1 mm in diameter. This allows you to shift the dynamic range of the emission signal for a particular user selected label which may otherwise exceed the linear range of the instrument. This damped aperture is used for the top fluorometry factory set labels.

The band aperture is an approximately 1 mm x 3 mm rectangle and it reserved mainly for future applications. Its size and alignment are about the same as the excitation area from the flash lamp.

## Tools menu

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The shutter is used at all times when no measurement is occurring to prevent stray light getting to the photomultiplier tube.

### Counting parameters

These determine the time factors involved in the measurement process, all times are in microseconds:

**Counting delay** - the time after the excitation pulse at which counting of the emission signal begins

**Counting window** - the time period within which counting occurs

**Counting cycle** - the time between excitation pulses within a measurement.

The second or additional counting window is fully independent of the first one and it useful when optimizing time-resolved parameters or for calculations of the decay time of the label.

### Factory-set values for the main counting window

Label	Delay 1	Delay 2	Window 1	Window 2	Cycle
High count 615	50	0	100	0	1000
High count 665	50	0	100	0	1000
Long decay 615	50	0	100	0	3000
Long decay 665	50	0	100	0	3000
545	50	0	100	0	3000
615	50	0	100	0	1000
665	50	0	100	0	1000
572	50	0	100	0	3000
FQA 615	50	200	100	100	1000
FQA 545	50	200	100	100	1000

### Flash absorbance

If you want there to be an absorption measurement as well as the usual time-resolved fluorescence emission measurement, select this parameter to be **Yes**. Both measurements will then be made simultaneously.

### Beam size

This parameter can be selected to be **Normal** or **Narrow** provided the Adjustable beam size option has been installed. It enables measurements on plates with very small well sizes by focusing the excitation light beam to a smaller size.

### Second measurement parameters

In the case where you want to make measurements with two filters, you can use this parameter to select the type of the second emission filter. The options are the same as for the first measurement emission filter. Two measurements are made, first with the normal filter and then with the second, which the system automatically moves into place.

### LANCE Label Properties

- **LANCE 615:** 100 flashes, window 50/100/1000, 615nm. This label is for fast decay Eu-chelates (for example Eu-W1024) in a TR-FRET assays.
- 
- **LANCE 665:** 100 flashes, window 50/100/1000, 665nm. This label is for fast decay Eu-chelates (for example Eu-W1024) in a TR-FRET assays.
- 
- **LANCE High Count 615:** (same as **LANCE 615** but 1000 flashes). Higher count rate.
- 
- **LANCE High Count 665:** (same as **LANCE 665** but 1000 flashes). Higher count rate.
- 
- **LANCE Long Decay 615:** (same as **LANCE 615** but 3000 cycle time). This label is for long decay Eu-chelates (for example Eu-W8044) in a TR-FRET assays.
- 
- **LANCE Long Decay 665:** (same as **LANCE 665** but 3000 cycle time). This label is for long decay Eu-chelates (for example Eu-W8044) in a TR-FRET assays.
- 
- **LANCE 545** (same as **LANCE Long Decay 615** but 545nm emission).
- 
- **LANCE 572**(same as **LANCE Long Decay 665** but 572nm emission).
- 
- **LANCE FQA 615** (same as **LANCE 615** but including 2<sup>nd</sup> window 200/100) This is a label for Eu-TRF-Quenching assays.
- 
- **LANCE FQA 545** (same as **LANCE 615** but with 545 emission filter and 2<sup>nd</sup> window 200/100) This is a label for Tb-TRF-Quenching assays.

### Filters

In this window there are three tabs: "Emission Filters", "CW-Lamp Filters" (i.e. continuous wave lamp excitation filters) and "Filter Slides" (including filter wheels). "Filter Slides" allows you to specify the slides for emission and CW-lamp filters. The names of the filters or slides in the currently selected tab are displayed. Names of user defined filters can be freely defined by the user but names of factory preset filters cannot be changed.

There are four buttons at the bottom of the window:

**Add** - add a new item to the list available e.g. a new filter (not available for slides). Give the name and edit the properties. It will appear with a new icon showing it is different from a default item.

**Copy** - (select an item to activate this, not available for slides). Make a copy of an item. It will appear with a new icon showing it is different from a default item, and the name will be preceded by the words "Copy of". You can give it a different name and edit other parameters by selecting **Properties**.

**Remove** - (select a user created item to activate this; not available for slides). Remove the selected item from the list. You cannot remove a pre-set item from the list nor can you remove a filter that has been assigned a position in a slide. This command requires confirmation because you cannot undo **Remove**.

**Properties** - (select an item to activate this). View the properties of the selected item. If the item is user defined then you can edit the properties, but if it is a factory pre-set, you cannot edit them.

## Emission filters



If you select properties of an emission filter or you want to define the properties of a new filter, a further dialogue appears where the name of the filter and the technology for its uses can be defined. There is also a section where you can add additional information that you can connect with the filter properties.

Factory pre-set emission filter names begin with a letter indicating the technology for which they are to be used:

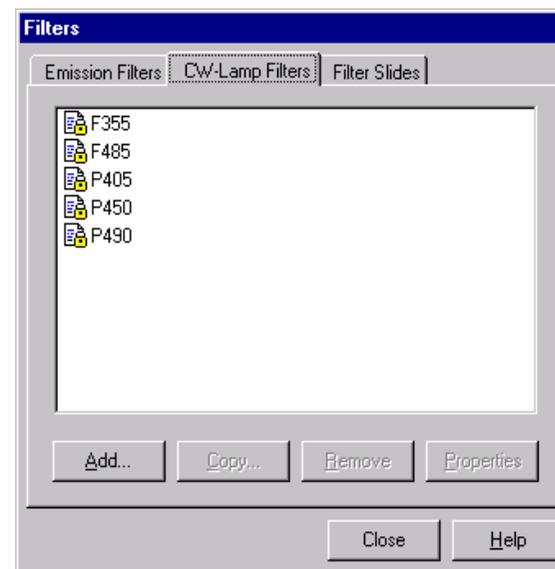
D is for time-resolved fluorometry (LANCE filters are marked as such).

F is for fluorometry.

L is for luminescence.

After the letter comes the wavelength.

## CW-Lamp filters



If you select properties of a CW-lamp filter or you want to define the properties of a new excitation filter, a further dialogue appears where the name of the filter and the technology for its use can be defined. There is also a section where you can add additional information that you can connect with the filter properties.

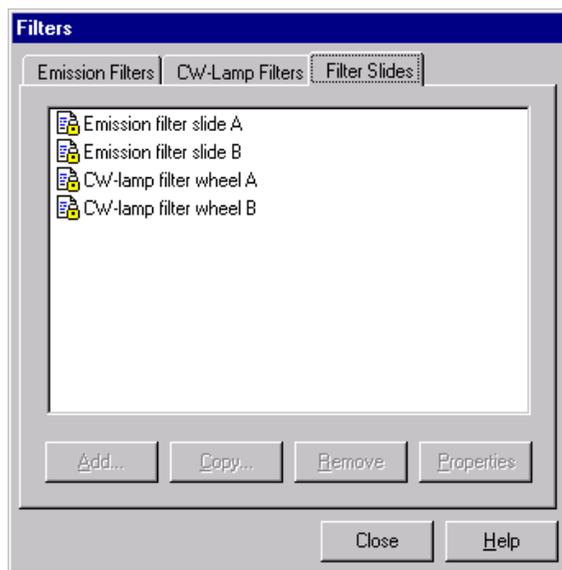
Factory pre-set CW-lamp filter names begin with a letter indicating the technology for which they are to be use:

F is for fluorometry

P is for photometry (absorption)

After the letter comes the wavelength.

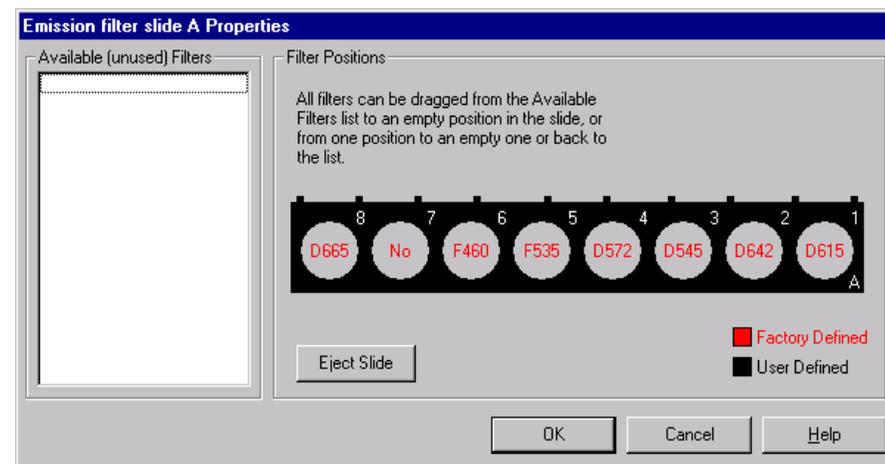
## Filter slides



There are two types of slide: Emission filter and CW-lamp excitation filter. The former is a strip and the latter a wheel. The instrument is loaded with slide A in each case but an empty slide B is also available for user filters as described below.

## Tools menu

### Emission filter slide



There are 8 positions in emission filter slides A and B. The physical dimensions of the filter slots are:

diameter	25.5 mm
depth	11 mm
clear aperture	22 mm.

All standard filters with diameter of 25.4 mm (1 inch) and thickness of 10 mm fit in the emission filter slides.

In emission filter slide A, positions 1 to 4 and 8 are used for emission filters for time-resolved fluorescent chelates (if this option is included), position 5 and 6 for emission filters for fluorometry labels umbelliferone and fluorescein, respectively. Position 7 is kept open for luminometry measurement. If you want to add your own filters you must either use slide B which is fully customizable, or change some of the existing filters.

Factory-pre-set filter names begin with a letter indicating the technology.

D is for Time resolved fluorometry, including LANCE

F is for fluorometry

P is for photometry (not used for emission filters)

L is for luminometry

After the letter comes the wavelength:

User-defined filters can be named freely but there is only space for five letters to be shown in the figure of the filter position. The full name will be shown if you move the mouse cursor onto the slot where the filter is.

### **Physically changing the emission filter slide**

Click the **Eject slide** button on the Emission filter slide dialogue. A message will appear telling you to unscrew and remove the plate on the side of the counter. When you have removed the plate, click **OK** to cause the slide to be ejected. Pull the slide out carefully.

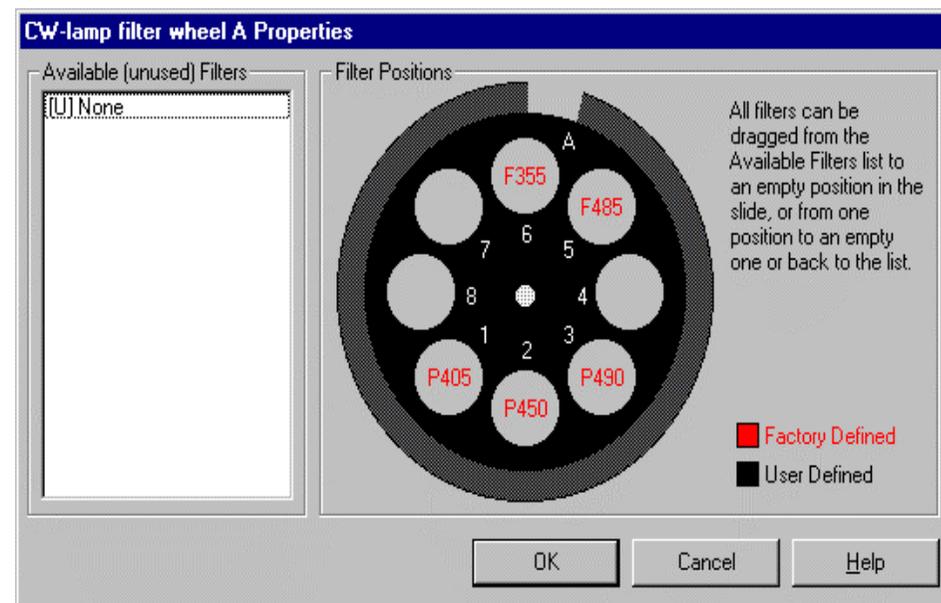
*Avoid touching the filters with your fingers.*

When you have got the slide out you can then change the filters or load filters into emission filter slide B.

When the slide is ready, carefully return the slide to its position. Push it in as far as it will go. Replace the filter slide cover and screw it in place, then click **OK** on the dialogue on the screen. After this you can operate the instrument.

## Tools menu

### CW-Lamp filter wheel



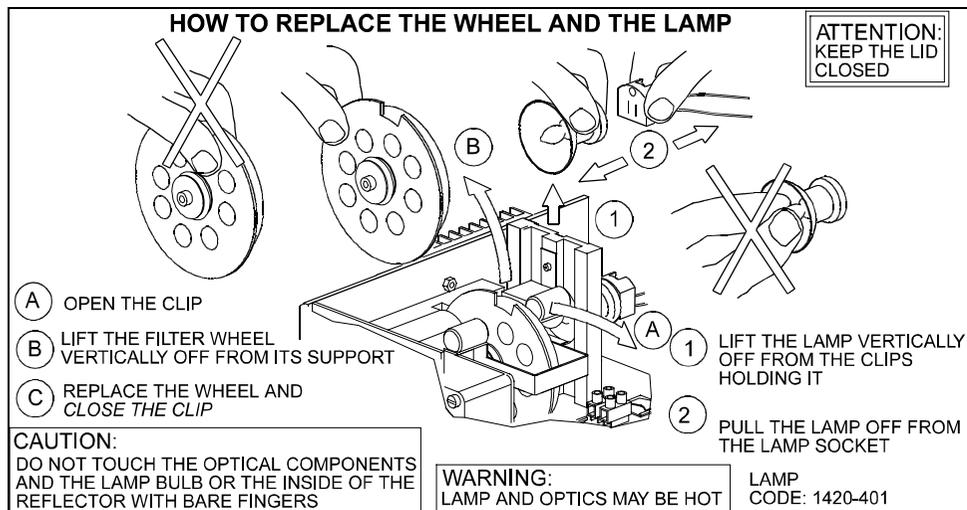
Excitation filters fit into a wheel positioned in front of the CW lamp. There are eight positions on this wheel and two are reserved by default for fluorometry and three for photometry. The remaining positions can have filters added by the user.

Note: default filters can be removed and replaced by user filters.

There is also a second filter wheel (B) which has only four positions but these are for the most common size of commercial filters i.e. “round one inch” and the wheel is supplied empty with locking rings.

### Physically changing the CW-Lamp filter wheel

If you want to change a filter in the CW-Lamp filter wheel or you want to install wheel B then you must open the cover and lift out the filter wheel as shown in the picture. Either change the filters you want or take a new filter wheel B. Replace the filter wheel and make sure you close the cover before starting a measurement.



### Changing the CW-Lamp

If the CW-Lamp needs replacing - lift the cover to get access to the lamp. *Make sure the lamp is switched off and give the lamp chance to cool before removing it and replacing it.* Follow the instructions on the inside of the cover, see the picture on the next page. You will probably find it easier to use your left hand to remove the lamp from the clips holding it. When you have replaced the lamp, close the cover and switch power on.

### Changing filters in the software

*Note: if you change a filter in the software you must also change it physically in the filter slide or wheel.*

When you select **Properties**, a picture of the selected slide appears with the filters that are loaded in it marked in red or black as shown previously. Those that are in red are factory pre-set. The excitation filter slide is in the form of a wheel and the emission filter slide is a strip.

To **move a filter from the list to a place on the slide**, move the cursor to the filter name on the list, drag it keeping the mouse button pressed down until it is over the empty slide position that you want it to occupy, then release the mouse button. The name of the filter will appear in black in the slide position.

To **move a filter from one position to another**, click on it and drag it to the empty place you want it to occupy.

To **remove a filter from the slide**, click on it and drag it back to anywhere in the area of the **Available filters** list, then release the mouse button.

**Filter properties**

The filters used in the instrument have the properties shown in the table below.

The following abbreviations are used:

CWL = Centre wavelength, BW = Bandwidth (Full Width Half Maximum), Tpk = Maximum transmittance at the peak wavelength (%)

Filter type	Name	Centre Wavelength (CWL)	Bandwidth (BW) (Full Width Half Maximum)	Maximum transmittance at peak wavelength (Tpk) (%)
Tr-fluorescence excitation	D340	340 nm,	ca 50 nm	ca 60%
Tr-fluorescence excitation	D320	320 nm,	ca 80 nm	ca 70%
Europium chelate emission (LANCE option installed)	D615	615 nm,	ca 8.5 nm	ca 80%
Europium chelate emission (LANCE option installed)	D665	665 nm	ca 7.5 nm	ca 80%
Samarium chelate emission	D642	642 nm,	ca 6.5 nm	ca 80%
Terbium chelate emission	D545	545 nm,	ca 7.5 nm	ca 70%
Dysprosium chelate emission	D572	572 nm,	ca 7.5 nm	ca 70%
Colorimetric label emission	P405	405 nm,	ca 10 nm	ca 45%
Colorimetric label emission	P450	450 nm,	ca 6 nm	ca 30%
Colorimetric label emission	P490	490 nm,	ca 6 nm	ca 30%
Fluorescein excitation	F485	485 nm	ca 15 nm	ca 60%
Fluorescein emission	F535	535 nm	ca 25 nm	ca 50%
Umbelliferone excitation	F355	355 nm	ca 40 nm	ca 35%
Umbelliferone emission	F460	460 nm	ca 25 nm	ca 50%

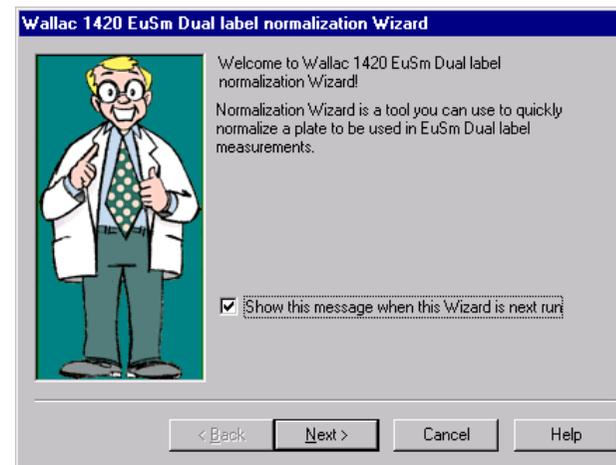
Note: the time-resolved filters are available only if this option is included with the instrument.

## EuSm Dual label normalization

Note: TR-FIA must be installed for this to be enabled.

Before dual label europium samarium time-resolved fluorescence measurements can be made, the instrument must be normalized for the plate type(s) to be used. To begin this procedure select EuSm Dual label normalization from the Wallac 1420 Manager Tools menu. This will activate a wizard that will guide you through the normalization procedure.

First comes a welcome to the wizard page.



You can select that this does not appear in the future. After this the main steps in the normalization are:

### Plate type selection

You must select from the list the plate type you are going to use. This is necessary because normalization is plate specific i.e. *you must have the same size and type of plate as you plan to use for the actual dual label measurement*. The list shows:

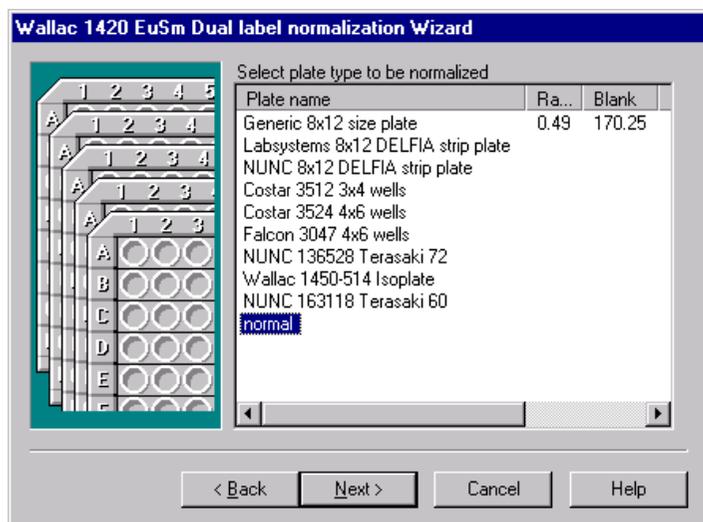
#### Plate type name.

**Date** when the normalization for this plate type was last done.

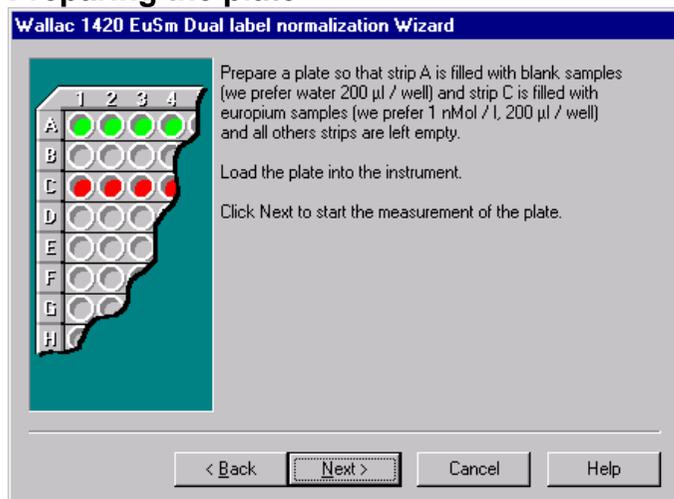
**Name** of the user who ran the last normalization.

**Correction** and **background** values from the last normalization run.

## Tools menu



## Preparing the plate

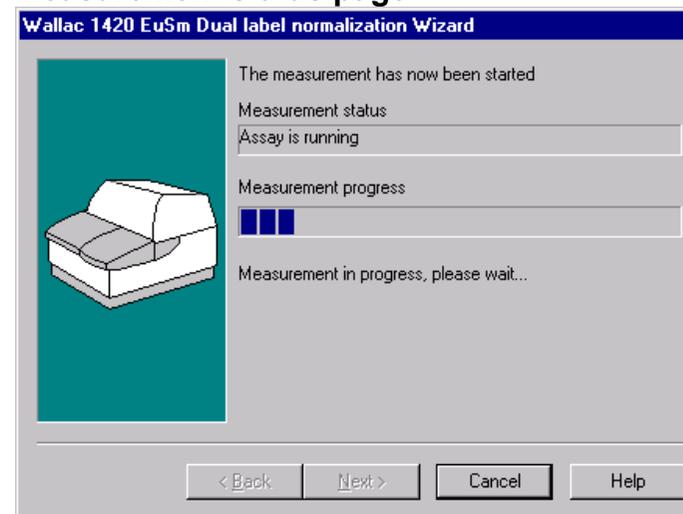


The wizard explains what samples you should put in the plate and where you should put them. Plates must have 3 or more strips, (maximum of 8), with 1 - 12 wells per strip. The strips must be horizontal e.g. A1 to A12 etc.

## Tools menu

Strip A should be full of blank samples, Strip B should be empty and Strip C should be full of europium samples.

### Measurement status page



This is shown during the actual normalization measurement and it gives you feedback about the measurement. The measurement status pane shows the current status of the measurement. The progress of the measurement is shown by the progress bar. The **Next** button becomes available when the measurement has been completed. When the measurement is completed click **Next** to move the wizard directly to the Normalization confirmation page, otherwise the wizard moves to the Measurement warning page.

### Measurement warning page

This is used to inform you that something out of the ordinary has happened during the measurement of the normalization assay. This page gives you enough information to decide whether the results can be accepted regardless of the problem that caused the warnings. Clicking **Next** on this page advances the wizard to the normalization confirmation page. Clicking **Cancel** closes the wizard and the normalization factors are not recorded in the database.

## Normalization confirmation page

Wallac 1420 EuSm Dual label normalization Wizard

The normalization measurement is now completed. Check the results and press Finish to accept the new normalization factors or Cancel to ignore them.

Normalization factors:

Ratio:

Blank:

Supplementary values:

Sample	Label	Mean	CV%
Blank	Samarium	2255	6.5
Blank	Samarium in Europium window	5159	5.6
Europium	Samarium	2130	7.1
Europium	Samarium in Europium window	4836	7.9

This appears after normalization has been successfully completed. It shows the measured normalization values and requires you to confirm that you accept these values before they are stored in the database. The two values produced by the normalization are the background and the europium contribution to the samarium window. During actual dual label measurements the europium counts in the europium windows are first calculated by subtracting the background from the measured counts. Knowing the europium counts and the normalization ratio the actual samarium counts in the samarium window can be calculated by subtracting the europium contribution to the measured counts.

Additional information about the reliability of the factors is also shown so that you can be sure that the results of the measurement are correct.

Note: to avoid accidental clicking of the **Finish** button, it only becomes visible after five seconds have elapsed since the page was opened. During the time the **Finish** button is disabled the text of the button is 'Wait t', where t is the remaining waiting time and it is updated every second. When you click **Finish**, the new values replace the old values in the database.

The **Back** button is enabled only if the wizard moved to this page from the Measurement warning page.

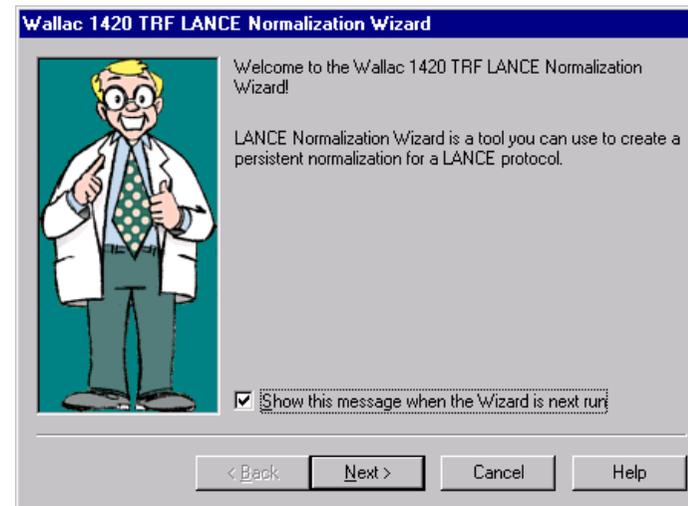
## LANCE normalization

Note: LANCE must be installed for this to be enabled

### Using the LANCE normalization wizard

Before LANCE time-resolved measurements can be made, the instrument must be normalized for the protocol to be used. This procedure corrects for the following effects in LANCE measurements: background from the plate, crosstalk of the donor signal into the acceptor window, and quenching. The final result is normalized to the maximum donor intensity. To begin this procedure select LANCE normalization from the Wallac 1420 Manager Tools menu. This will activate a wizard that will guide you through the normalization procedure.

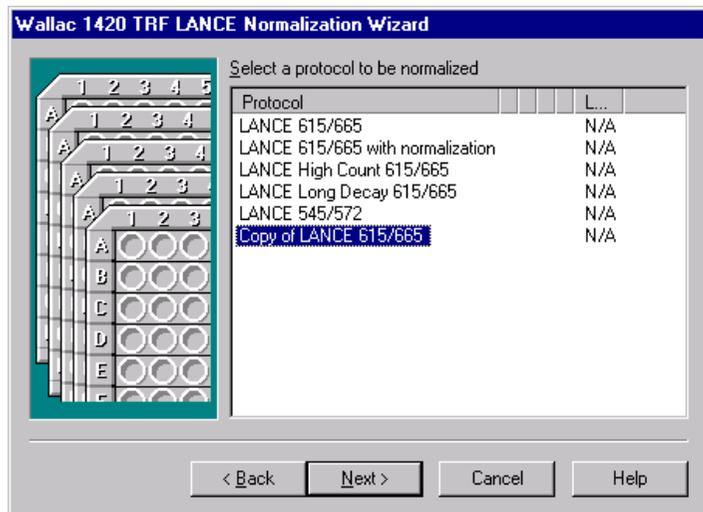
First comes a welcome to the wizard page.



You can select that this does not appear in the future. Click **Next** to proceed. After this the main steps in the normalization are:

## Protocol selection

You must select from the list of LANCE protocols the one you want to do the normalization for. Highlight the one you want and then click the **Next** button.



### Preparing the plate

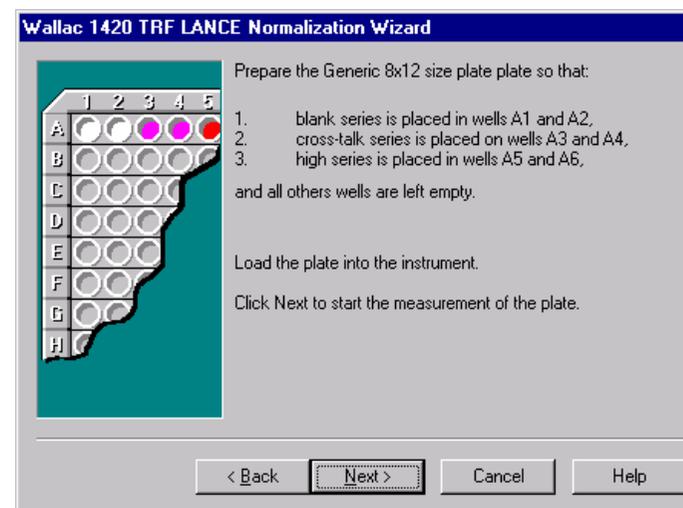
The wizard explains what samples you should put in the plate and where you should put them. The strip to be used must be horizontal e.g. A1 to A12 etc. The plate used is the one defined in the protocol you previously selected.

Blanks should be in A1 and A2; these are used to correct for the plate background in the acceptor window. These are made by dispensing buffer only.

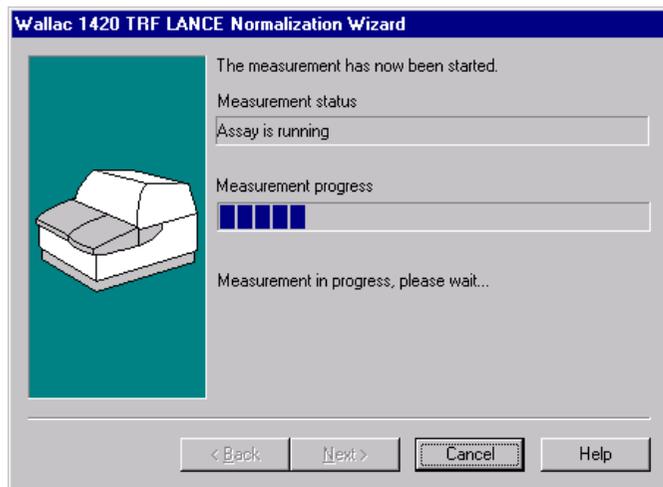
Spectral crosstalk samples (i.e. donor only samples) in A3 and A4; these are used to calculate the amount of the blank corrected donor signal in the acceptor window as a proportion of the donor signal. Only donor and buffer should be dispensed to these wells.

High intensity samples in A5 and A6; these are to normalize the final result to the maximum donor value. The ratio of the high intensity donor value to the sample donor value corrects for possible quenching in the sample. These samples should include all the reagents to give the maximum acceptor signal.

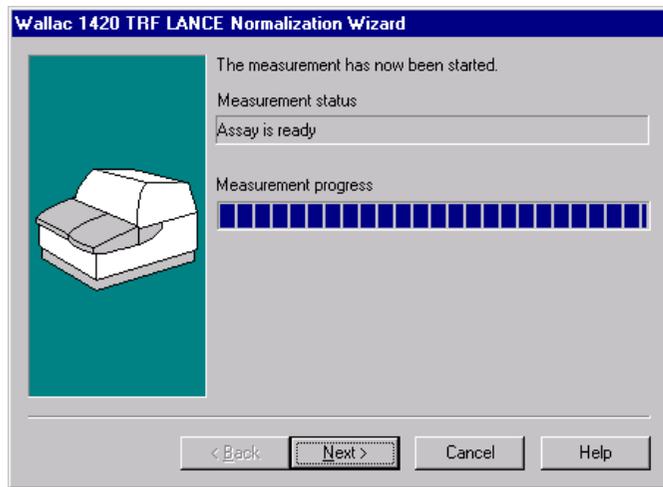
All other wells should be left empty. Then load the plate and click **Next**.



## Measurement status page



This is shown during the actual normalization measurement and it gives you feedback about the measurement. The measurement status pane shows the current status of the measurement. The progress of the measurement is shown by the progress bar.



## Tools menu

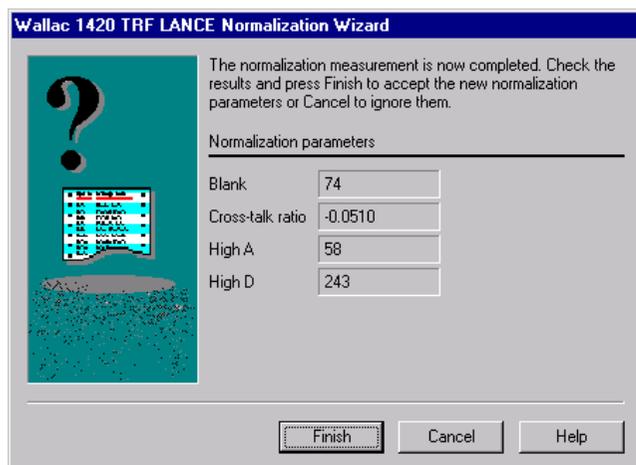
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The **Next** button becomes available when the measurement has been completed. If the measurement was completed without any warnings the window shows the text "Assay is ready". Click **Next** to move to the Normalization confirmation page otherwise the wizard moves to the Measurement warning page.

### Measurement warning page

This is used to inform you that something out of the ordinary has happened during the measurement of the normalization assay. This page gives you enough information to decide whether the results can be accepted regardless of the problem that caused the warnings. Clicking **Next** on this page advances the wizard to the normalization confirmation page. Clicking **Cancel** closes the wizard and the normalization factors are not recorded in the database.

## Normalization confirmation page



This is the last page of the LANCE normalization wizard and appears after normalization has been successfully completed. It shows you the measured normalization values and requires you to confirm that you accept these values before they are stored in the database.

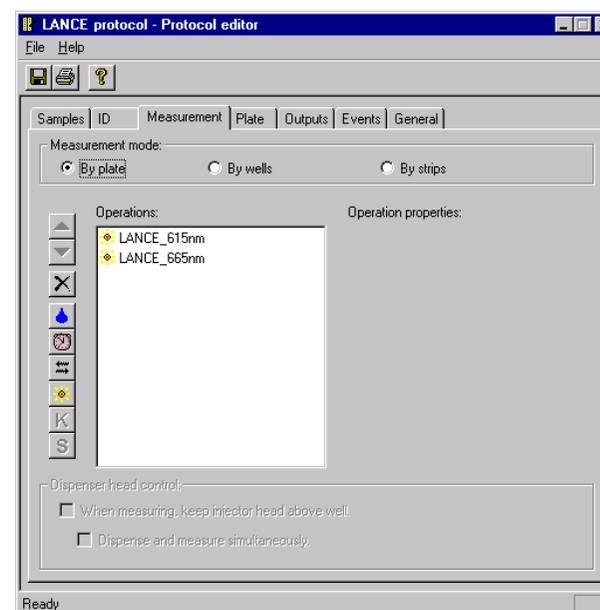
The values obtained are the blank measurement in the acceptor window, crosstalk, i.e. the blank corrected contribution of the donor to the acceptor window signal as a proportion of the donor signal, the maximum acceptor signal in the acceptor window and the maximum donor signal in the donor window. These values are then used in a measuring sample to obtain the actual acceptor value i.e. the blank and crosstalk corrected acceptor signal normalized to the maximum donor signal (thus eliminating quenching effects). See the Calculation methods in the instrument manual for more details.

Note: to avoid accidental clicking of the **Finish** button, it only becomes visible after five seconds have elapsed since the page was opened. During the time the **Finish** button is disabled the text of the button is 'Wait t', where t is the remaining waiting time and it is updated every second. When you click **Finish**, the new values replace the old values in the database.

### Using LANCE normalization samples in the assay

Instead of running the LANCE normalization wizard and saving values that can be used for any subsequent LANCE assay with that particular protocol, you can include LANCE normalization samples on the plate as part of the assay samples and thus obtain corrected values for that particular plate.

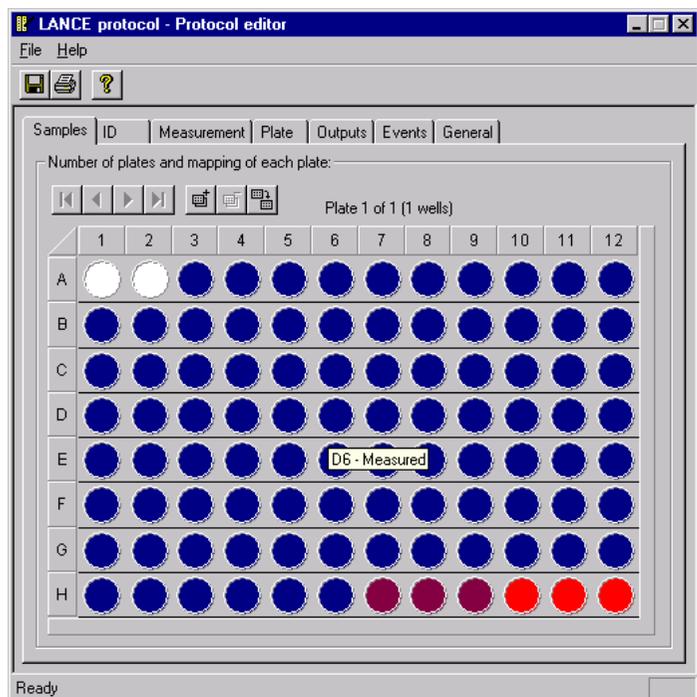
When you define the protocol make sure that both LANCE labels are defined in the Measurement tab as shown below. If only one label is selected then the measurement will be treated as normal time-resolved fluorometry.



When you set up the plate you can select the LANCE well types from the menu that appears when you click the left mouse button. Use this feature to include at least one of each of the three LANCE well types on the plate.

You can choose the number of replicates of each well type and you can choose where they are located on the plate as the following example shows:

## Tools menu

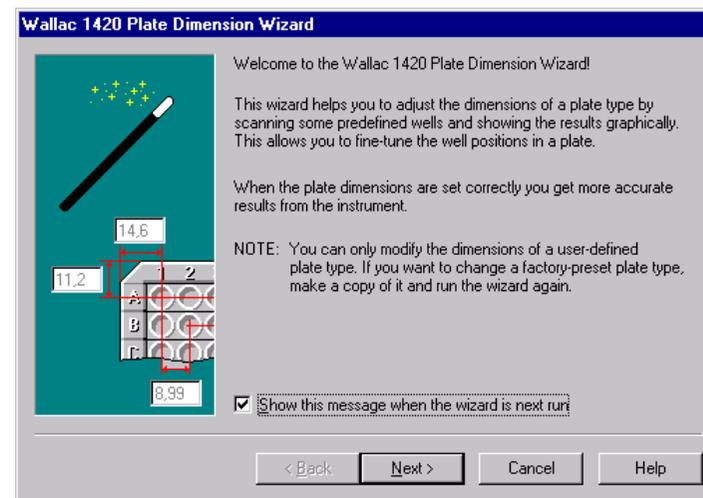


When you have defined the plate and saved it, you can run the assay in the normal way. When the LANCE samples are detected on the plate, they will automatically be used to make the necessary corrections for this particular LANCE measurement. They will overrule any values that may have been stored in the instrument memory from a normalization with the LANCE wizard. However, these "on the plate" normalization values will not be stored and used for any other assays than the current one. Only values resulting from use of the LANCE wizard are stored.

## Plate dimension wizard

A description is given here of how the Plate dimension wizard can be used to fine-tune the plate dimensions. This is needed in the case of *user -defined* high-density plates (384 or higher) to ensure exact positioning of the plate. The factory default plates cannot be changed. If the same plate type is used for more than one technology, it is recommended that you repeat this procedure for each technology. In this case, when defining plates give each plate type a different name for each technology.

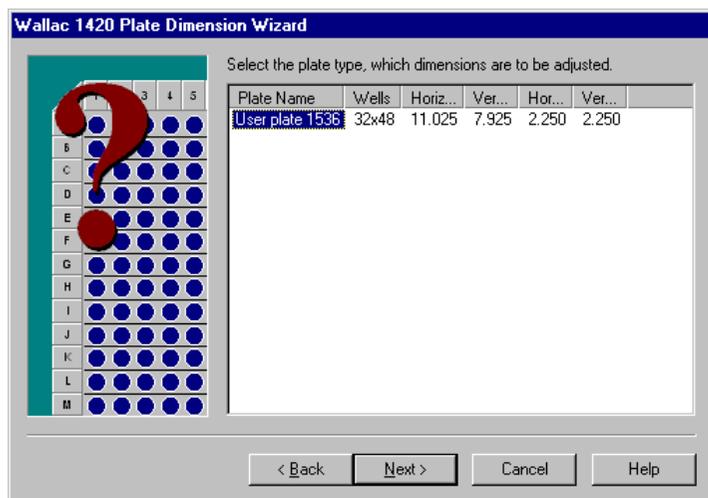
First a welcome page appears:



You can select that this page does not appear any other time by removing the check mark from the check box. Then click the **Next** button.

## Plate selection

Note: This step requires that there is at least one user defined plate.



Select the plate whose dimensions you want to fine-tune. Remember that this should be the plate type with the name you chose specifically for the technology to be used.

Note that you must click in the Plate name field to select the plate. When you have done this **Next** will be enabled. Click it to go to the next dialogue.

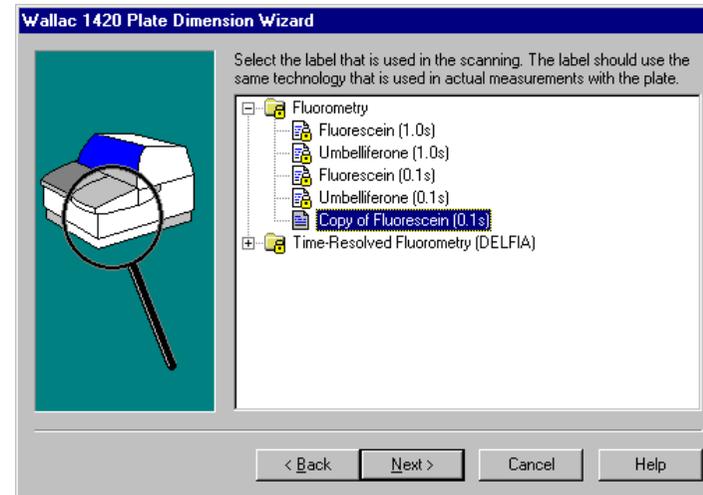
## Technology selection

You must select the technology to be used to count the plate.

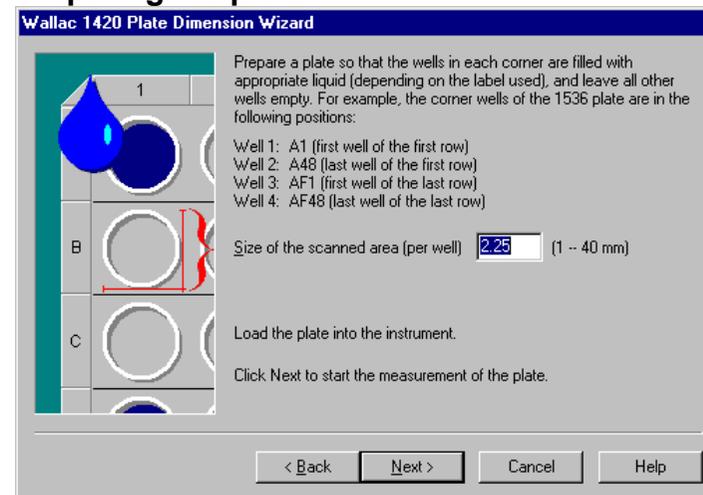
Note: the options that appear will depend on what is installed in the instrument.

Open the folder and select the actual protocol for which the plate dimensions are to be fine-tuned. The **Next** button will then be enabled and you can click it to go to the next dialogue of the wizard.

## Tools menu

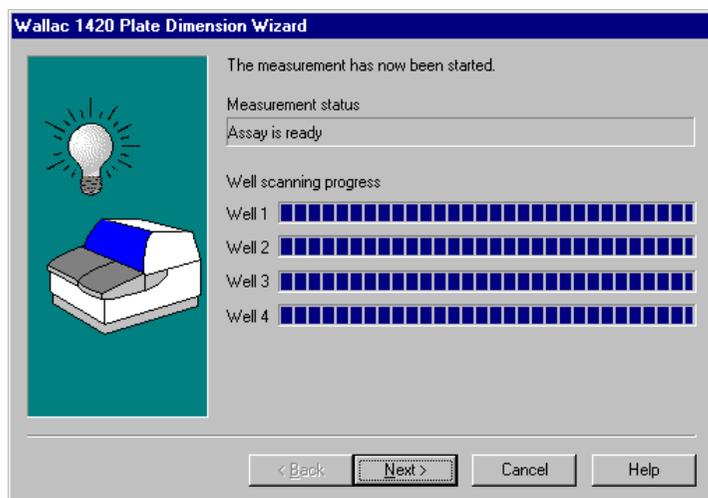


## Preparing the plate



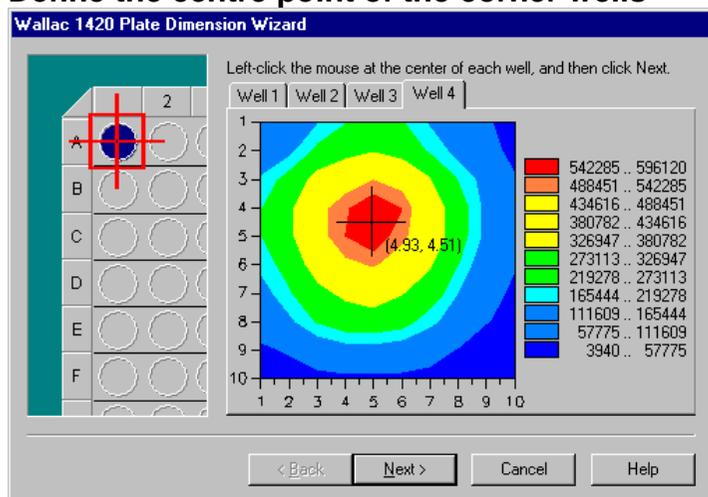
Prepare the plate by dispensing appropriately labelled sample to each of the four corner wells of the plate. All the other wells should be empty. Specify the area of the well to be scanned. The example shows the default value for a 1536-well plate.

When the plate has been prepared, load it and click **Next**.



After scanning is completed the **Next** button will be activated. Click it to continue.

### Define the centre point of the corner wells



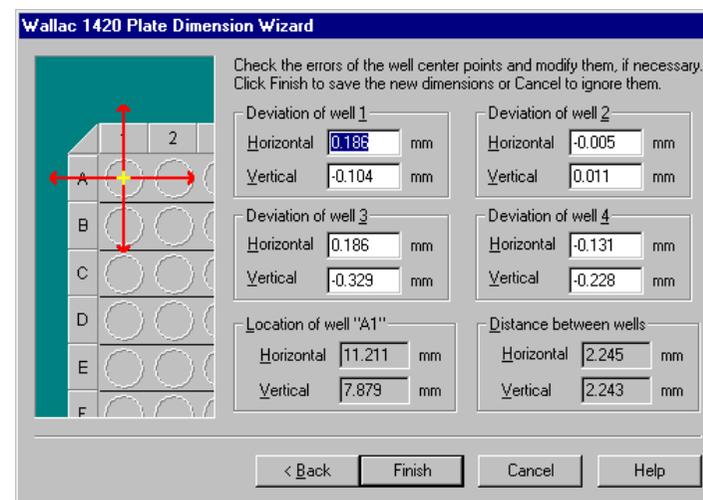
## Tools menu

The window that appears allows you to define the centre point of each of the four corner wells.

Each tab shows one of the four wells with a plot of the intensity of the light from the well. This is colour coded with red showing the highest intensity. Position the mouse cursor where you think the centre of the well is and click the left mouse button. A black cross will appear centred at the point of the cursor. If you are not satisfied with the position of the cross move the mouse cursor and click the left button again.

Repeat this for each of the four wells.

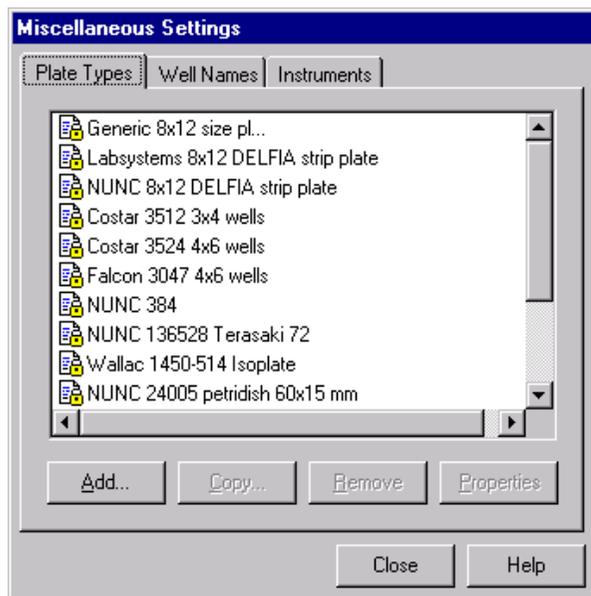
When the centre of all four wells has been marked, the **Next** button will be enabled. Click this to continue.



Numerical values are shown in the final window. If you approve these, click **Finish** to save the values and end the wizard. If you feel the deviations are too large you can click **Back** to reposition the well centres. You can alternatively click **Cancel** to end the wizard without saving the values.

Note: for even better adjustment it is recommended that you repeat the scan with a smaller area selected, see the lower figure on page 112, e.g. for a 1536-well plate this could be 1 mm per well.

## Miscellaneous settings



In the dialogue that appears when you click this item in the **Tools** menu, there are three tabs for:

**Plate types** the available plate types are listed including a number of default types.

**Well names** the available names are listed. There are up to five default types: two **Empty** and to be **Measured** that are generally valid, and three **LanceBlank**, **LanceCrosstalk** and **LanceHigh** for "on plate" LANCE normalization measurements (these require that LANCE is installed).  
Note: well names cannot be copied.

**Instruments** when you select this tab you can see names of instruments. Selecting a name and properties gives more information.

The following buttons appear in Miscellaneous:

## Tools menu

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**Add** - add a new item to the list available e.g. a new plate type. Give the name and edit the properties. It will appear with a new icon showing it is different from a default item.

**Copy** - (select an item to activate this). Make a copy of an item. It will appear with a new icon showing it is different from a default item, and the name will be preceded by the words "Copy of". You can give it a different name and edit other parameters by selecting **Properties**.

**Remove** - (select a user created item to activate this). Remove the selected item from the list. You cannot remove a preset item from the list nor an item that is used in a protocol. This command requires confirmation because you cannot undo **Remove**.

**Properties** - (select an item to activate this). View the properties of the selected item. If the item is user defined then you can edit the properties, but if it is a factory pre-set, you cannot edit them.

## Plate type

When you click the **Plate types** tab the available plate types are listed. This list includes a number of default types. To define a new plate click **Add**. You can also click on an existing plate type and then click **Copy** to save the plate type with a new name. If you wish, you can edit the parameters to make a new plate type. Click **Properties** to see the plate properties.

**Plate Properties**

Name

User plate 1536

Parameters

Number of well rows: 32 (1 - 32)

Number of well columns: 48 (1 - 48)

Height of the plate (mm): 10.4

Strip orientation:  Horizontal  Vertical

Dimensions from the corner of the plate to the center of the first well and from the center of a well to the center of the next well (mm):

Tune ...

OK Cancel Help

In the case of **Properties**, the dialogue that appears gives information about the plate. Each of the parameters in this dialogue must be defined when you add a plate. The software does not allow you to give unreasonable values e.g. a well separation that would mean that all wells do not fit in the plate. The maximum number of rows and columns are shown.

### Plate name

This is the name that will appear on the list of plate types. You can change this name by editing the current name or by marking it and deleting it and typing a new name.

## Tools menu

---

### **Number of well rows**

This is the number of wells in a row. The product of this with the number of well columns gives you the number of wells in the plate.

### **Number of well columns**

This is the number of wells in a column. The product of this with the number of well rows gives you the number of wells in the plate.

### **Height of the plate**

This defines the height of the plate from its base to its top. The units are millimetres.

### **Strip orientation**

You select whether the strips are aligned **horizontally** i.e. in rows so that the first strip enters the instrument first, or **vertically**, i.e. in columns so that first well of each of the strips enters the instrument first. The normal setting is Horizontal. The order of the measurement is according to the strip definition.

### **Plate dimensions**

The picture shows what dimensions are needed to define a plate. The units are millimetres and you can specify the dimension to the nearest hundredth of a millimetre.

Note: the distances should be measured from the outermost part of the plate.

### **Tune**

When you click the Tune button the Plate dimension wizard is started. See the description starting on page 108 for how this works.

### **Well names**

In the dialogue that appears, you can give a name for a well for identification purposes e.g. standard, unknown etc. You can define a short form (single letter) for the name and select a colour for it from a scroll bar of colours. The short form is checked to see that it is not used for any other well name. The default names are “Empty” and “Measured”.

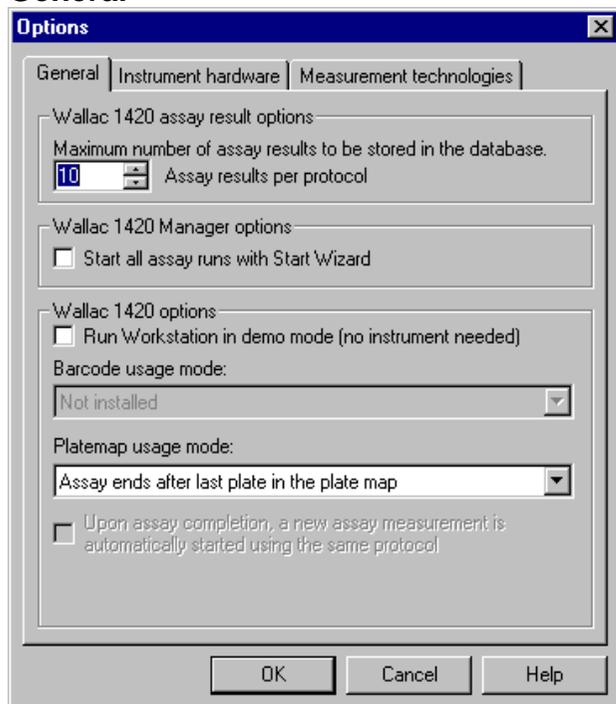
### **Instrument properties**

In the **Instrument tab** of Miscellaneous settings, when you select properties of an instrument, the nickname you selected appears and can be changed. Also the official name and product number of the instrument as well as its serial number will be displayed. You cannot change these.

## Options

This can only be accessed if you have **Advanced level** selected. There can be four tabs but only three show on **Advanced level**, the fourth requires **Service level**.

### General



You can define the number of results to be held in the database for one protocol. When this number is exceeded the oldest results for that protocol will be automatically deleted from the database to make room for new ones. The maximum number of results is 100.

If you want the start to be always by means of the Start Wizard, check this box. This means that when you click **Start** the Start Wizard will be activated. This is useful if e.g. you need to define the number of plates before each measurement.

If you just want to run the software as a demo without having the counter connected, check the “Run Workstation in demo mode” check box.

## Tools menu

---

Note: if you change to Demo mode, your PC has to be rebooted before the change takes effect. Make sure you have no other programs running when you make this selection because they will be closed during the shutdown procedure.

If do not have the barcode reader option, then the line "Barcode reader switched off" will be selected. If you have the reader and want to use the barcode as a plate number to be connected with your results, select "Barcode is stored to results". In both these cases you must select the protocol manually, see Start or Start Wizard for more information.

If you have the reader and you select "Barcode is used as a protocol number" then the barcode will determine the protocol used to measure the plate. If a plate does not have a barcode then the protocol used to measure the previous plate will be used.

See the Plate orientation picture on page 20 for where to fix the barcode.

You can specify what happens when the end of the plate map definitions is reached for a protocol. There are three possibilities:

"Assay ends after last plate in the plate map" - no more plates will be measured until a new start instruction is given or a new barcode read. If there are more plates an error message will appear.

"Additional plates possible (latest plate map is used)" - further plates will be measured using the last defined plate map.

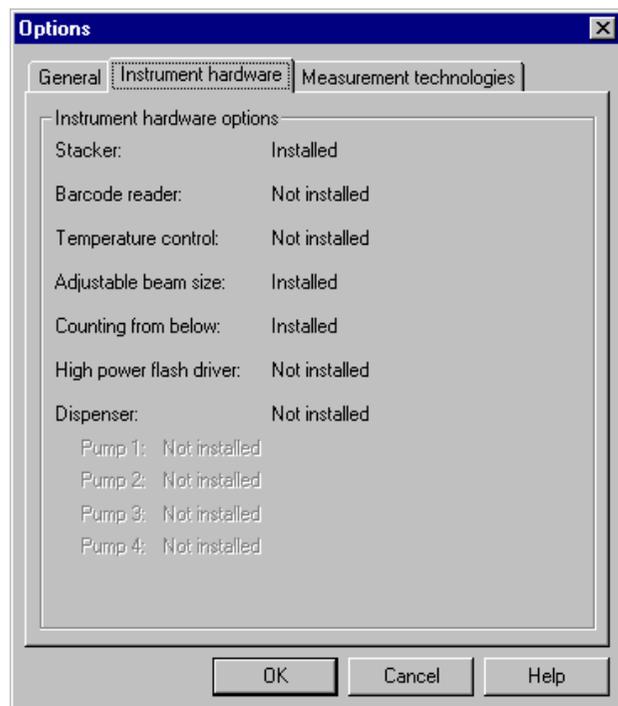
"Additional plates possible (all wells will be measured)" - further plates will be measured with no plate map taken into consideration i.e. every well will be measured.

There is also a check box "Upon assay completion, a new assay measurement is automatically started using the same protocol". This check box is only enabled if the "Barcode stored to results" option has been selected.

If you are using stackers, then measurement of the next plate with the previously used protocol will begin automatically. This feature is useful for automatically producing a separate export file for each plate. Define your protocol to have only one plate and select this feature. Each plate will be run as a separate assay producing its own export file. The external software can then process these files as from the same assay because each will have been produced by the same protocol.

If you are not using stackers, the instrument waits until the button on the instrument is pressed and then begins measuring the current plate with the previously used protocol.

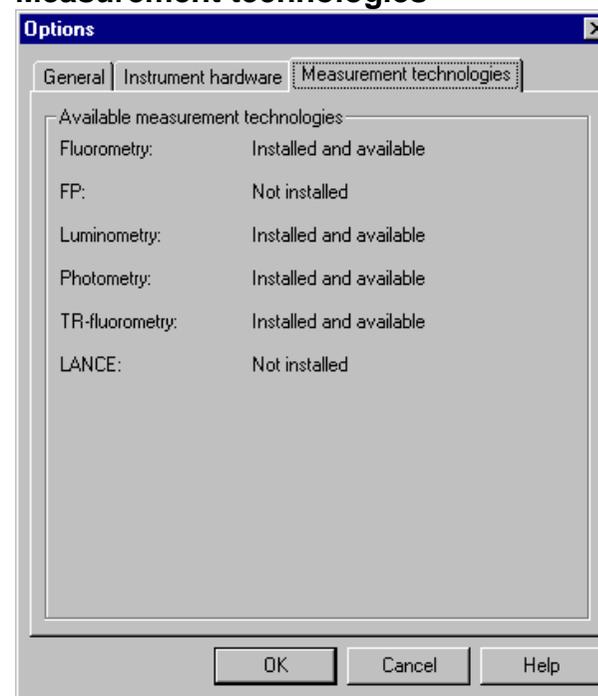
## Instrument hardware



This tab can only be accessed if **Advanced level** has been selected in User Level. It shows which options are installed. These include:

- Stacker option
- Barcode reader options
- Temperature control
- Adjustable beam size
- Counting from below
- High power flash driver
- Dispenser and pumps

## Measurement technologies



The final section of this tab tells what measurement technologies are installed and available:

Fluorometry

FP (Fluorescence polarization)

Luminometry

Photometry

TR-Fluorometry (dissociation enhanced time-resolved fluorometry)

LANCE (homogeneous time-resolved fluorometry)

### Communication

This tab can only be accessed if Service level has been selected in User Level. The Instrument communication interface card settings (Interrupt request and Input /Output port base address) can be selected from the scroll bar options provided. You must reboot before these new selections become effective.

---

**Exiting**



### File menu

The File menu in the main window contains one item, **Exit**. This allows you to exit from the workstation software. You need to confirm the operation.

If you try to exit while an assay run is taking place, you will get an error message telling that this is not possible.



---

## **Troubleshooting**



### Out of memory

If free disk space for the database drops below a specified limit, a warning message appears in the top right corner of the Manager window. A warning sound is also made once a minute. These warnings are continued until enough free disk space is available again. An assay run cannot be started from the user interface while the disk space is below the limit, however the **Start** button on the instrument still starts the run.

Please try one or more of the following to correct the situation:

Check that Windows is set to handle your virtual memory settings. Do the following to check this:

Open the Control Panel in Settings in Windows.

Double-click on the System icon.

Switch to the Performance tab.

Click the Virtual Memory button.

Check that the option “Let Windows manage my virtual memory settings (recommended)” is set.

See Out of memory picture.

Other things to check:

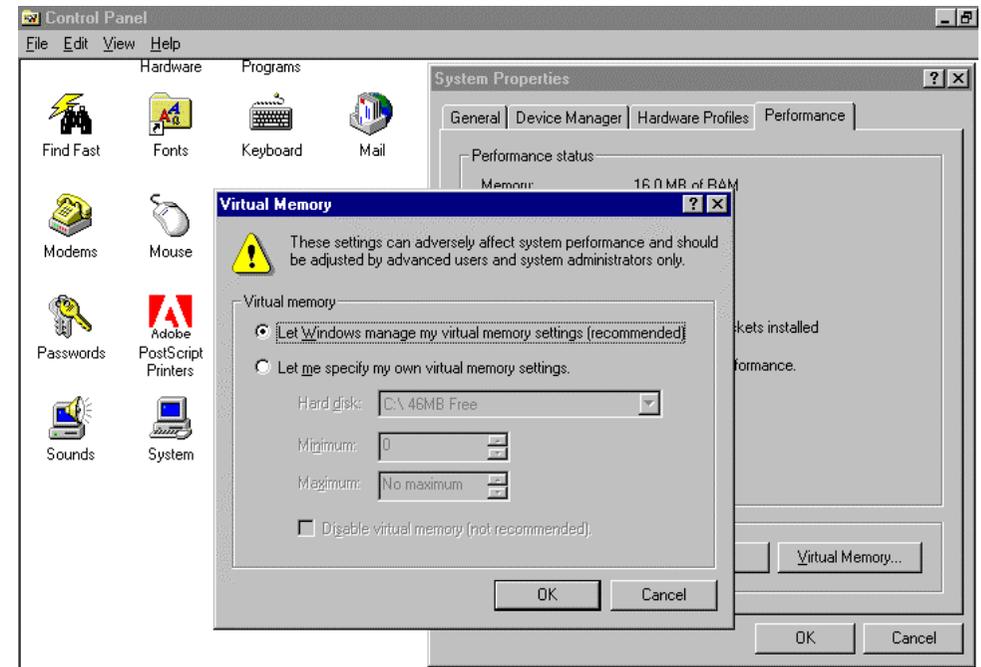
Check that there is at least 20 MB available on your C: drive.

Try running with 256 colours.

See System Requirements.

This picture shows the windows you need to open to get to the Virtual memory dialogue.

## Troubleshooting



### Display problems

If you get display problems when running a DOS application in graphics mode while Wallac 1420 software is running, you might have a problem with your display driver. Please contact your display driver manufacturer for an upgrade.

### Performance degradation

If you find your Wallac 1420 software is not running as fast as it used to, please run the Wallac 1420 **Database Maintenance** program. You should anyway do this on a regular basis. This program will compact your database and will in some situations increase the software performance drastically.

You can find this program by clicking the Wallac 1420 icon in your Windows 95 Start menu. It is called Wallac 1420 Database maintenance.

Check that no other major resource-consuming programs are running while using the Wallace 1420 software.

Check that there is at least 20 MB available on your C: drive.

Defragment your hard disk. You can find this function in your Windows 95 Start menu under Programs\Accessories\System tools\Disk Defragmenter.

### Processing error

This dialogue will be displayed if the Instrument server goes to an error state or if the Manager cannot communicate with the Instrument server.

Right after the header there is text telling to user that Instrument server has stopped processing of the Workstation software because of an error. After that there is a description about the error given by Instrument server or explanation that Manager cannot communicate with the Instrument server if that is the case. When you click the **OK** button, the reboot sequence is activated and the PC will be restarted.

### System requirements not met

This dialogue will be displayed during Manager startup if “/NoSystemCheckAtStartup” command line option was *not* used and the minimum system requirements for Manager were not fulfilled.

The “Current System” panel is used to display information about the current system parameters. The “System requirements” panel is used to display the minimum system parameters for the Manager to start. The “Suggestions panel” will list the reasons why the Manager cannot be started and a description about what must be done to fix the situation. When the **OK** button is clicked, the dialogue together with the manager is closed.

The minimum system requirements are:

Pentium processor

16 MB or more memory

Screen size (800x600 or more)

256 or more screen colours



---

## **Appendices**



## Appendix 1: Connecting MultiCalc to Wallac 1420

### Introduction

MultiCalc can read assay text files from Wallac 1420. Evaluation of assays can be fully automated. Measured wells are read sequentially. Plate printout and re-order work only for full plates.

### MultiCalc version 2.6 or higher is required

Note: The Windows properties setting for MultiCalc should have:

Misc/Background/Always suspend = NOT CHECKED

Idle sensitivity = Low.

To see what the situation is, click the MultiCalc shortcut icon with the right mouse button and select **Properties** from the menu. In the dialogue that appears, select the **Misc** tab and then in the **Background** field make sure that **Always suspend** is not checked.

See also Installation in the Instrument Manual for how to install this option in MultiCalc.

### Wallac 1420 MultiCalc assay protocols

Create or edit a protocol using the Explorer and Protocol Editor.

In the protocol editor dialogue select the **ID** tab. Select the protocol number to be the same as the MultiCalc protocol ID number. Then, under the **Outputs** tab, include all items in output and select the **File type** as 'MultiCalc'. The **File Name** should be as follows:

```
c:\wiacalc\0com\1420_2\<<ProtocolNumber>.<RunIDpad2>
```

Note:

Max 3 labels can be selected.

Plate repeat must be 1.

Kinetics or scanning measurements are not possible.

Plates having more than 144 wells are treated sequentially in MultiCalc

### Installation of Wallac 1420 output option in MultiCalc

Copy the files 1420\_2.C02 and 1420\_2.h2 from the distribution CD-ROM and paste them into c:\wiacalc\0com.

Set the properties for 1420\_2 in MultiCalc to AOSPMTV, by selecting (on level-5) ETC; SYSTEM; ETC; COMM.PROT; PROPERTY; select 1420\_2; type the letters AOSPMTV and press **Enter**.

Escape to the main menu and then restart MultiCalc.

Note:

Property A stands for Automatic evaluation, whenever an assay file is available.

Property O is for the connection type Offline.

### MultiCalc assay protocols

All assays with Wallac 1420 have to be of the FLUORO type for all measurement technologies used in Wallac 1420. The response from labels 1 and 2 will appear as COUNTS and COUNTSB and from label 3 as COUNT.

### Usage of MultiCalc

Leave MultiCalc in the counter mode; evaluation will occur automatically if property A has been set as described above.

Evaluation can also be started manually by selecting COUNTER:1420\_2: in MultiCalc and pressing Enter.

Re-evaluation is also possible there after the Recover function has been used.

## Appendix 2: Plate types for stackers

All stackers are tested with following plates (until further notice given).

Note: Lids or loose frames can not be used in the stacker

### Wallac

1244-550	DELFLIA microtitration strip plates
C554-160	Rigid low fluorescence plate
C120-105	Anti-mouse coated plates
C122-105	Streptavidin coated plates
1450-510	Rigid sample plate
1450-410	Rigid sample plate
1450-514/515	Isoplate

### Nunc

475515	FluoroNunc Black strip plate
437591	FluoroNunc White strip plate
45161	FluoroNunc Clear non-strip
477178	8 x 12 strip plate clear
242757-93	384 well white
242757-	384 well clear

### Costar

White, clear bottom  
Black, clear bottom  
Black  
White

### Greiner

655101	Clear, non-strip	
655074	White, non-strip	
655077	Black, non-strip	
655180	Clear, sterile	Only without lids!

### Falcon

3915	Clear non-strip
	White non-strip

## Appendix 2: Plate types for stackers

---

### Labsystems

95029550	Combiplate, white
9502817	Cliniplate, black

Acceptable range of plate dimensions for plates in a 1420-216 Stacker:

Length:	127.2 - 128.2 mm
Width:	84.5 - 86.0 mm
Height:	14.0 - 25.0 mm
Ledge height:	1.5 - 6.5 mm

Note: the length and width include the size of the ledge.

## Appendix 3: Dispenser operation

### Introduction

The optional dispenser allows you to make measurements that require e.g. the addition of reagent to start the process, as in the case of flash luminescence, or start and stop an enzyme reaction. It can also be used for dispensing Enhancement Solution for a DELFIA time-resolved fluorometry measurement. It can be used with any of the technologies supported by Wallac 1420.

The dispenser can have up to four pumps fitted. This allows you to have separate pumps dedicated for specific purposes. All the needles are directed to the same well allowing dispensing of more than one reagent to the same well. The number of pumps fitted and the total volume of each pump are set during installation. This information then appears when dispenser maintenance is selected. The default setting for the dispensed volume can be between 5 to 350 microlitres.

Note: dispensing to 384-well plates is possible if just *one* pump is installed.

The optional dispenser is a separate unit from Wallac 1420 itself making for convenient maintenance and flexibility in syringe selection.

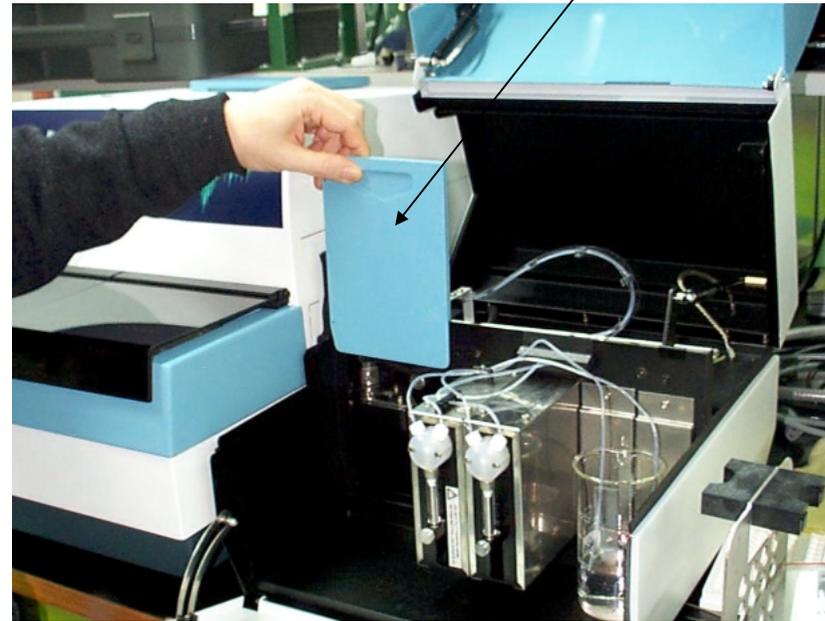
The information about installing the dispenser unit will be found in the Instrument manual as part of the installation instructions.

The following instructions tell how to use the dispenser.

### Emptying the waste vial

Note: the waste vial must be empty when operation starts.

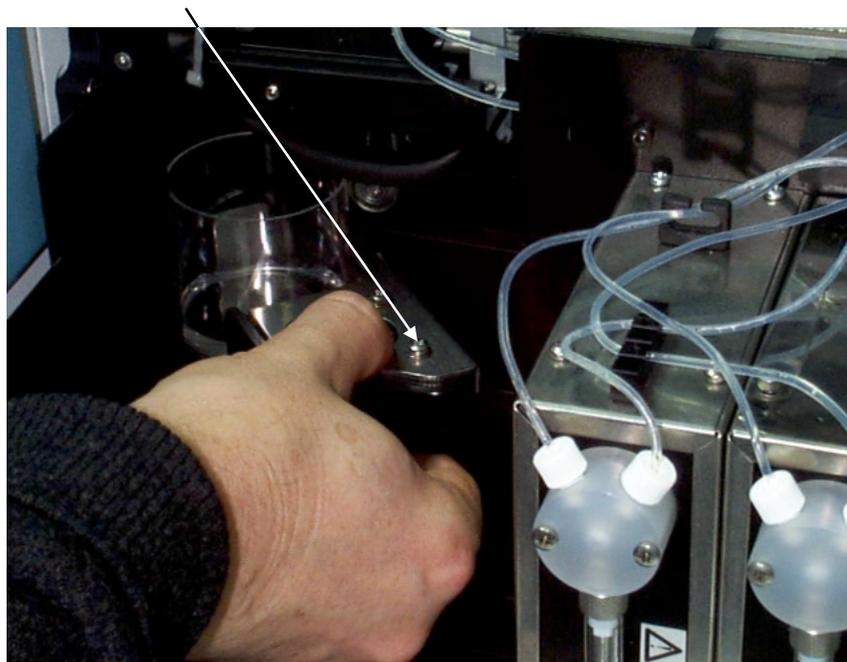
To check that the waste vial is empty and if not, to empty it, proceed as follows. Open the top and front cover of the dispenser and then lift off the blue plate at the back of the dispenser.



### Appendix 3: Dispenser operation

---

Pull out the holder for the waste vial.

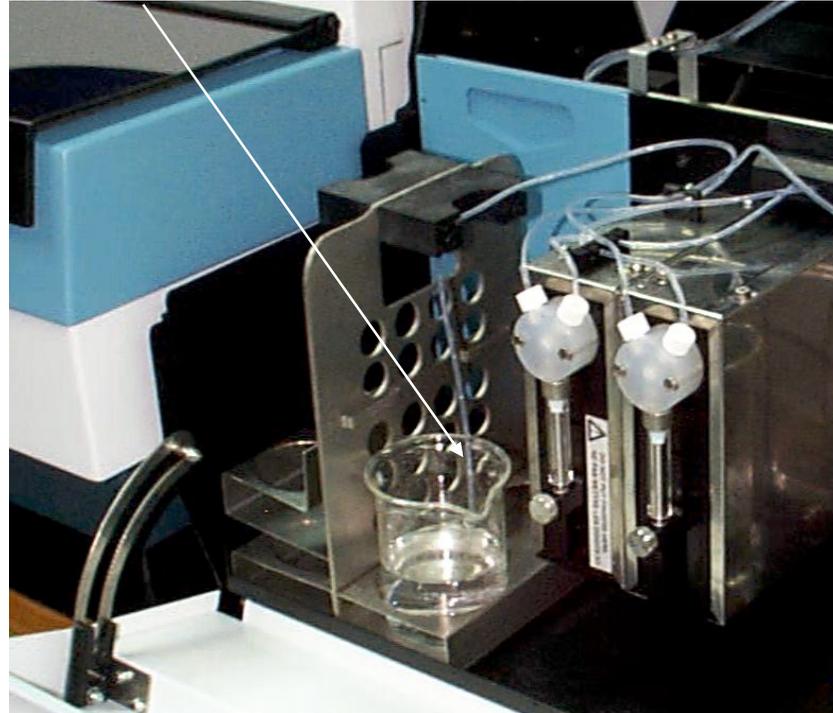


Empty out any liquid, *following the disposal procedure recommended by the manufacturer of the reagent you have been using.*

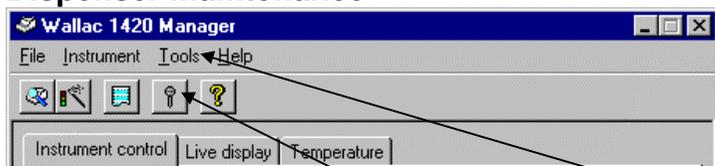
Then replace the waste vial on its holder and slide it back into place. ***Replace the blue plate.*** This is important because it prevents light leakage to Wallac 1420.

### Preparing the tubing

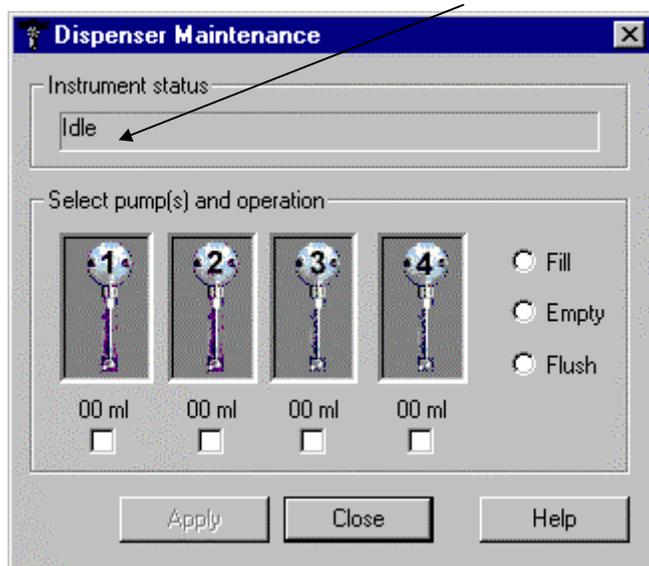
Put the tubing into a container of water.



## Dispenser maintenance



Clicking the Dispenser maintenance icon or the item in the Tools menu invokes the **Dispenser Maintenance Setup** page to perform common dispenser maintenance operations. This is enabled only when working with a system that has the dispenser installed. The current state of the instrument server is shown at the top of the page.



The setup page presents you with a graphical representation of the number of pumps (1 - 4) available when performing an operation. You can select the pump(s) which are going to perform the operation by checking boxes under the respective pump pictures. The operation to be performed can be either **Fill**, **Empty** or **Flush**, and you can select which of these you wish to perform. The operation is then performed on *all* the selected pumps.

Note: before performing these operations make sure the dispenser has been prepared, as described earlier.

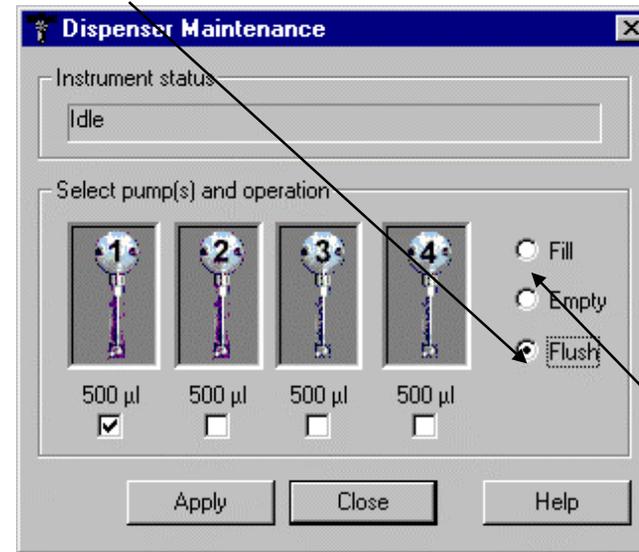
### Appendix 3: Dispenser operation

**Fill** is used for filling the syringe and tubing before operation begins.

**Empty** is used after operation ends to empty the contents of the syringe and tubing back into the bottle.

**Flush** is used for cleaning the syringe and tubing after it has been emptied.

Click **Flush**.

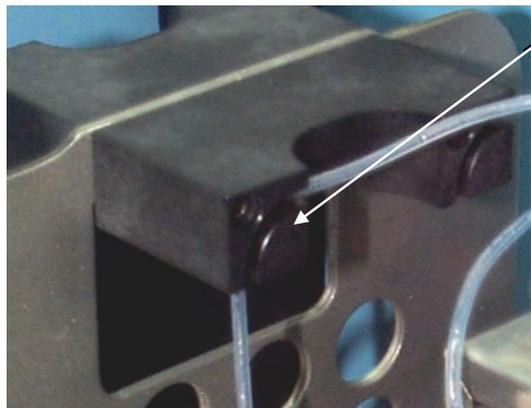


Take the tubing out from the container of water. Empty the tubing by clicking **Fill**.

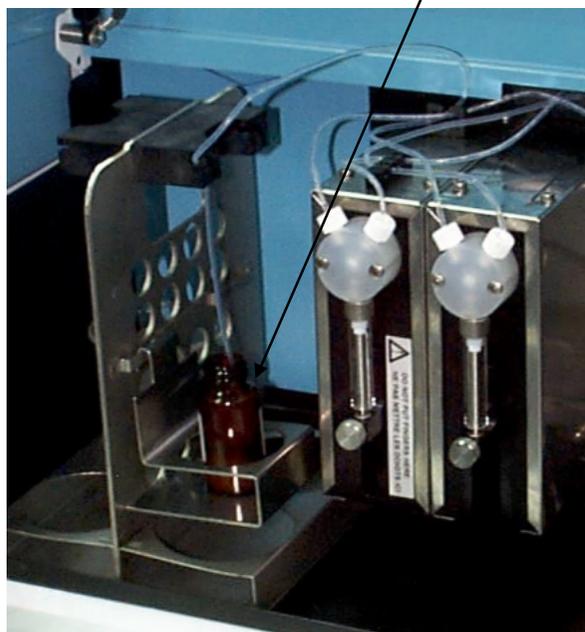
### Appendix 3: Dispenser operation

---

Put a reagent bottle into the holder. Fit the tubing into the grip.



Fit the end of the tubing into the reagent bottle. Click **Fill** to fill the tubing with reagent.

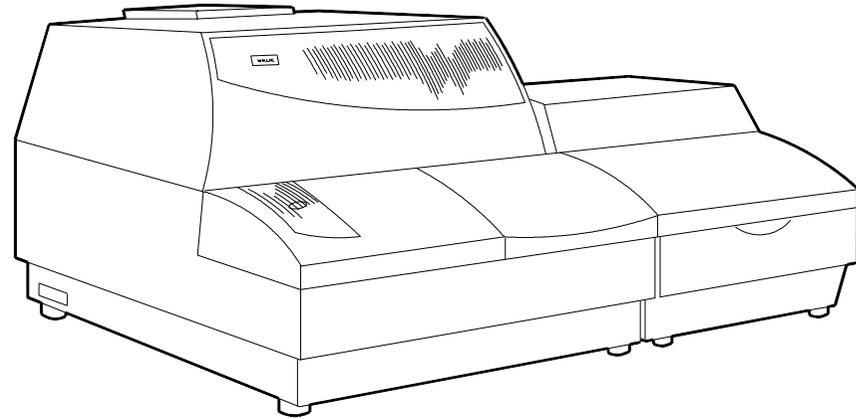


## Appendix 3: Dispenser operation

---

### Measurement

Close the covers of the dispenser. The system should now be ready for operation.

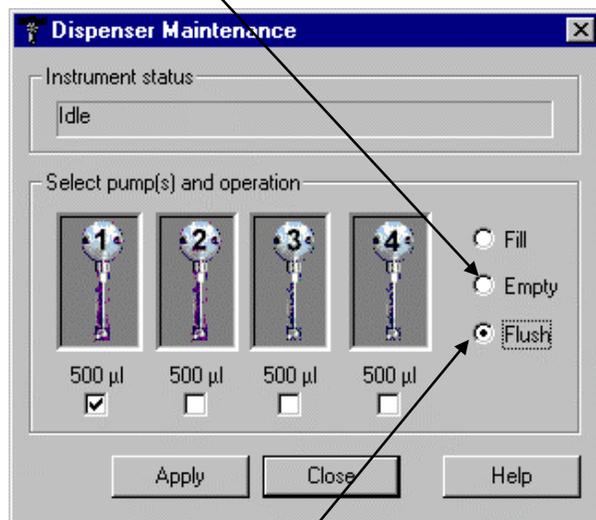


Proceed with the measurements.

### Maintenance

After measurement has been completed, perform the following maintenance operations:

Click the **Empty** command. This will cause the reagent to be sucked back into the reagent bottle so that none is wasted.



Open the covers. Remove the tubing from the reagent bottle and put it into a container of water and select the **Flush** command again.

Note: it may be necessary to use a solution of 70% ethanol rather than water for rinsing the tubing. See the reagent manufacturer's instructions.

### Needle maintenance

Every time the dispenser has been used, the tubing must be flushed properly. When this is done, the needles are rinsed at the same time. However, it is good to regularly clean the outside of the needles.

### Appendix 3: Dispenser operation

---

*Avoid touching the needles with your fingers.* To clean the needles use e.g. a stick with a tip of soft material dipped in ethanol, or use a spray bottle filled with ethanol. Be very gentle when touching the needles so that they are not bent out of shape. Rinse with water.

The position of the needles is very critical for high quality performance. If a needle is bent, call the service engineer to get the needle replaced. *Do not try to re-adjust the damaged needle because the quality of dispensing could be affected.* Both the needle and the tubing will be replaced as a set.

It is good to check the flow of liquid dispensed (during flushing). If the flow is not normal it might indicate that the needle is partly blocked. If this has happened due to dried reagents, use water and e.g. ethanol to dissolve the plug. If that does not help, the needle needs to be changed; contact the service.

---

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