VINCI -

Multidimensional Fluorescence Spectroscopy

Reference Manual

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Table of Contents

1 Introduction

Vinci is a comprehensive software package utilized to acquire and analyze fluorescence data using ISS spectrofluorometers. The package is divided in three parts

Instrument Control	This part of Vinci is related to the control of automated devices (shutters, polarizers, sample holder, monochromators) and the control of external devices (stopped-flow apparatus, titrator, temperature bath, microwell plate reader) of the spectrofluorometer.
Data Acquisition	Data Acquisition assists the experimenter with setting up a simple data acquisition routine (spectra, kinetics) and with custom-built data acquisition protocols that can be stored and used at a later time.
Data Analysis	This portion of Vinci deals with the manipulation of data files and the display of data. It includes operations (smoothing, derivative, calculation of areas), arithmetic between files and data display routines.

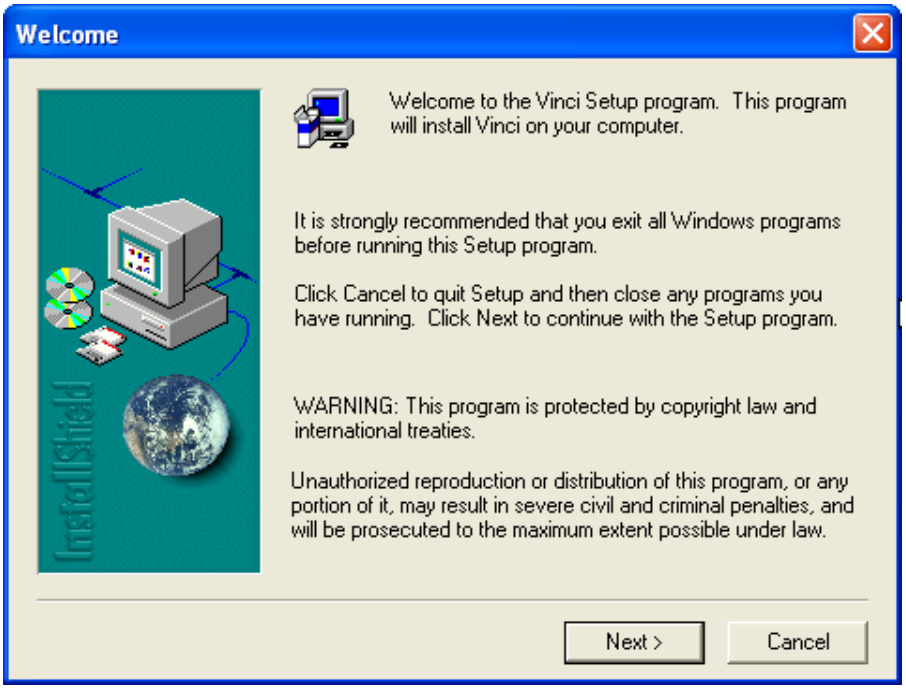
The Reference Manual will guide the user through the different parts of the software.

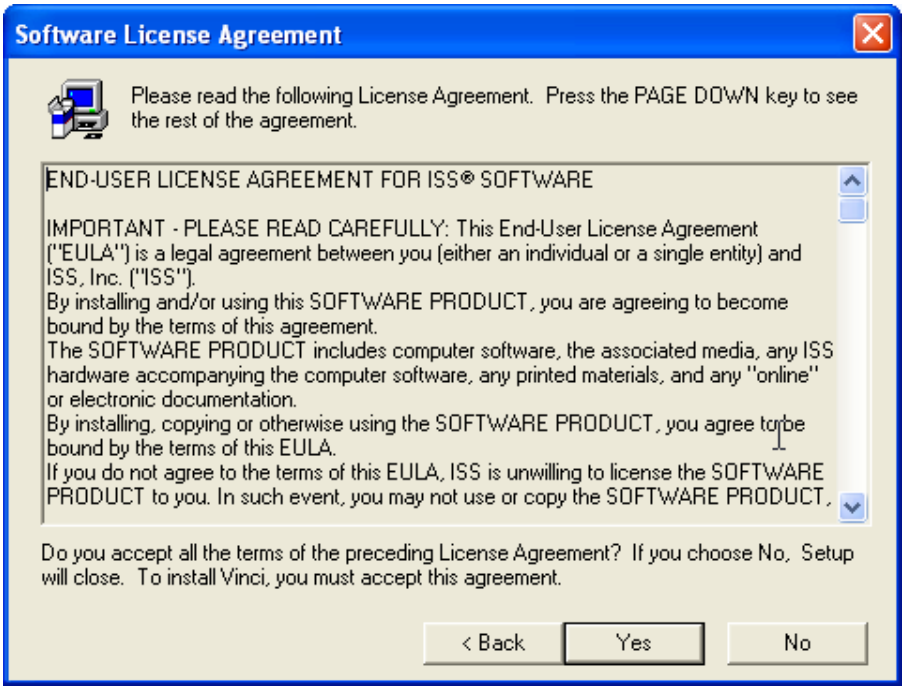
Instrument Control	Chapters 4 through 7 deal with “Instrument Control”. Consult Chapters 6 and 7 when adding devices to the spectrofluorometer or when changing the configuration of the instrument.
Data Acquisition	Chapter 3 gives a quick start for the acquisition of an emission spectrum. Chapters 8 through 10 explain in detail how “Data Acquisition” is performed.
Data Analysis	Chapters 15 and 16 are devoted to “Data Manipulation” and “Data Analysis”.

2 Starting Vinci

2.1 Installation

Insert the CD-ROM into the computer reader and the auto-installation program starts. The following window will come up briefly after the software installation procedure starts.

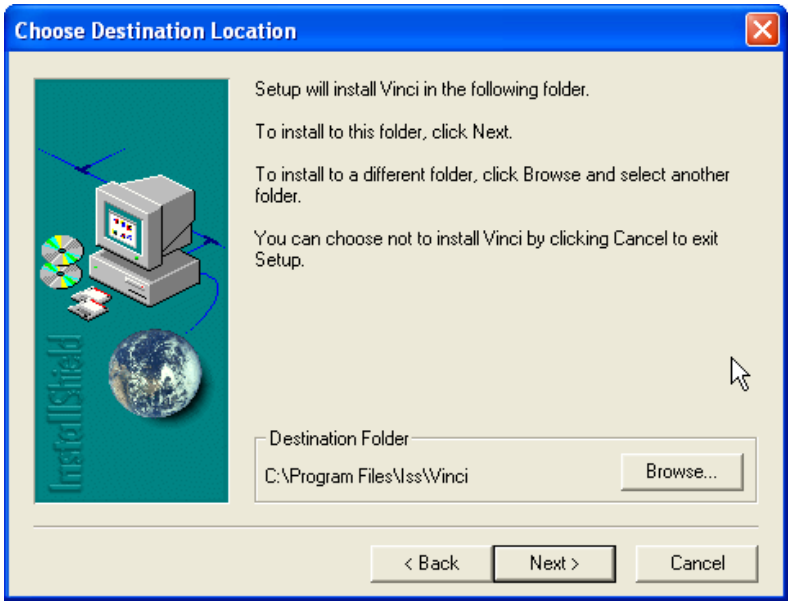




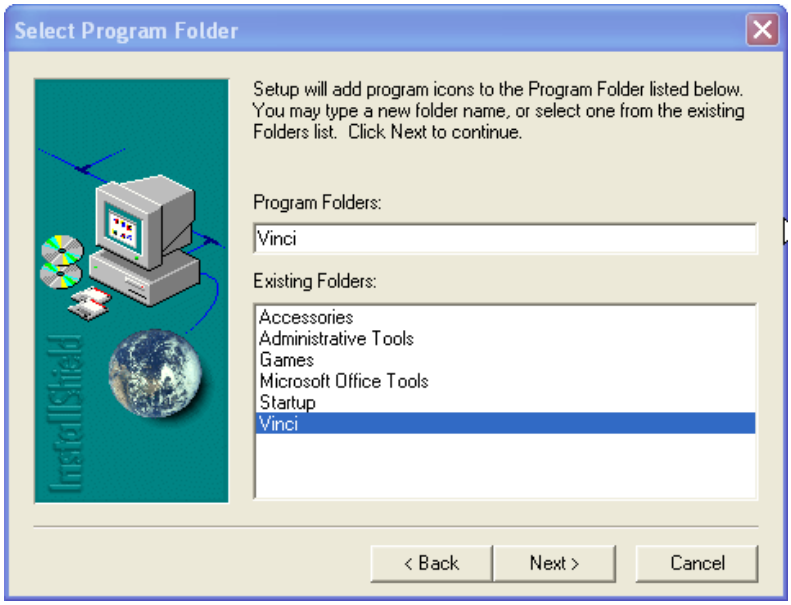
The installation proceeds only after the <Yes> button on the license agreement has been selected.

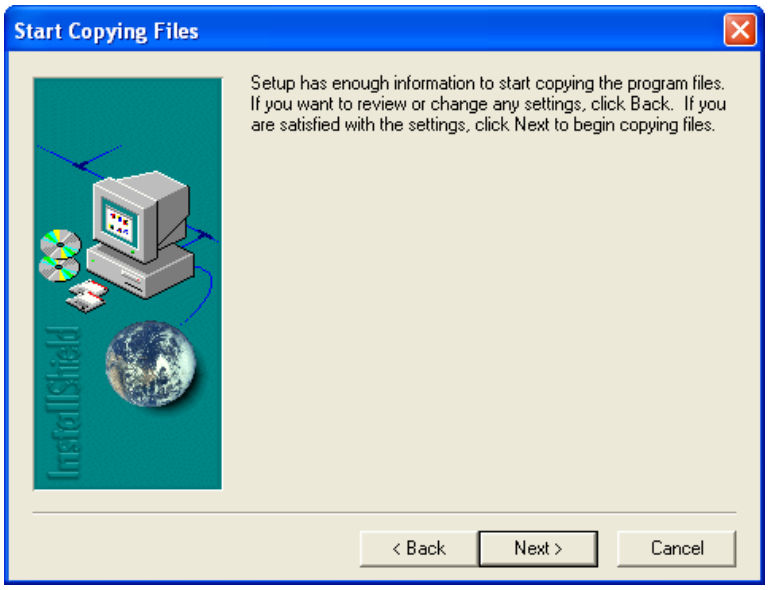
By default the software will be installed in the Program Files directory.

The user can select a different directory.

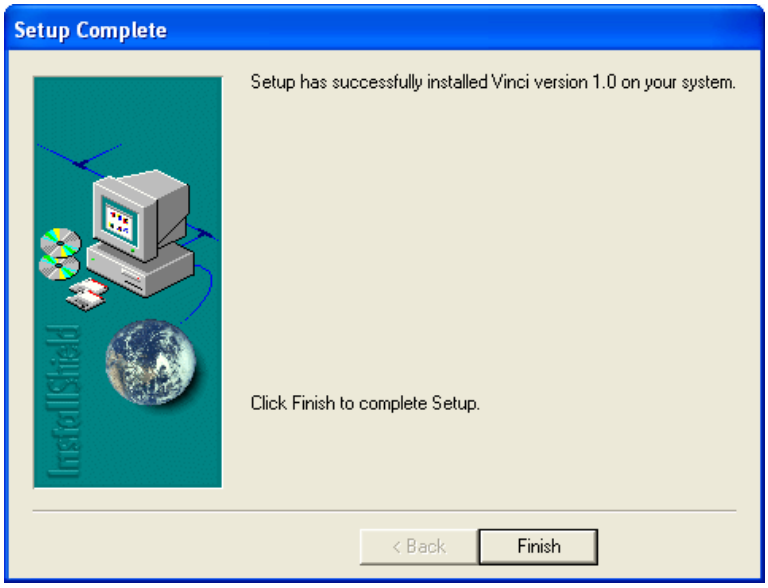


The installation procedure will guide the user through each step.

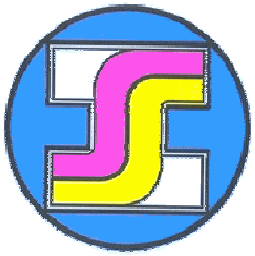

Select Program Folder
Setup will add program icons to the Program Folder listed below. You may type a new folder name, or select one from the existing Folders list. Click Next to continue.
Program Folders:
Vinci
Existing Folders:
Accessories
Administrative Tools
Games
Microsoft Office Tools
Startup
Vinci
InstallShield
< Back
Next >
Cancel


Start Copying Files
Setup has enough information to start copying the program files. If you want to review or change any settings, click Back. If you are satisfied with the settings, click Next to begin copying files.
InstallShield
< Back
Next >
Cancel

The Setup is completed after pressing <Finish>.



At completion, the ISS icon is displayed on the computer desktop and Vinci is started by left clicking the icon.



2.2 Registration

While the analysis portion of the software can be loaded and utilized on several computers, the data acquisition portion of the Vinci software is licensed for use on one computer only. The Vinci software requires registration with ISS.

When entering the <Experiment> part of Vinci, the following screen is displayed.

The software can be utilized with no registration for a 30 days period. Just click on <Try!> to start the software.

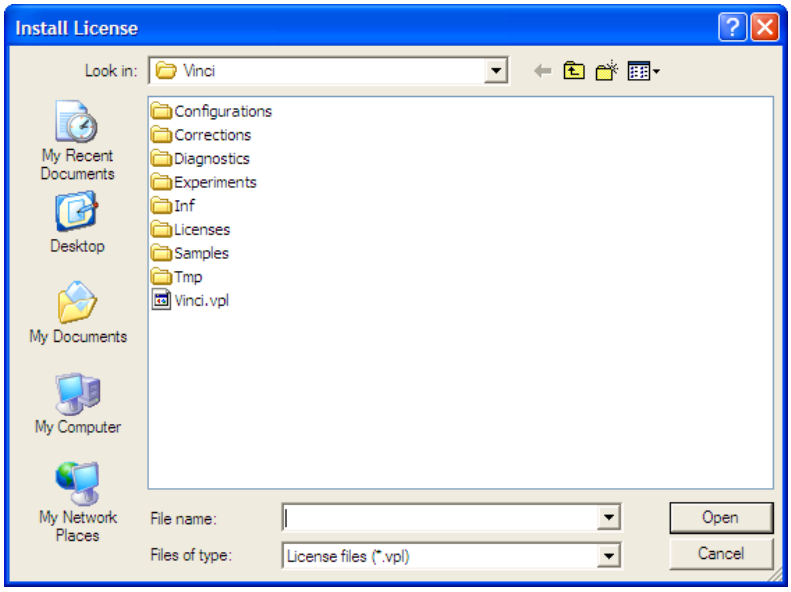
2.1.1 Obtaining a License

Communicate to ISS the 16-digit alphanumeric code displayed on the top row of the monitor. Email is the preferred communication; just send the request to:

support@iss.com

ISS personnel will email a file back to you (the file name is *vinci.vpl*). Insert the disk with the file in the computer and click on <Enter license> button. The Install License screen will request the location of the license file; the file will be loaded and the license activated. Once a license has been issued and installed, the dialog box will not be displayed anymore.

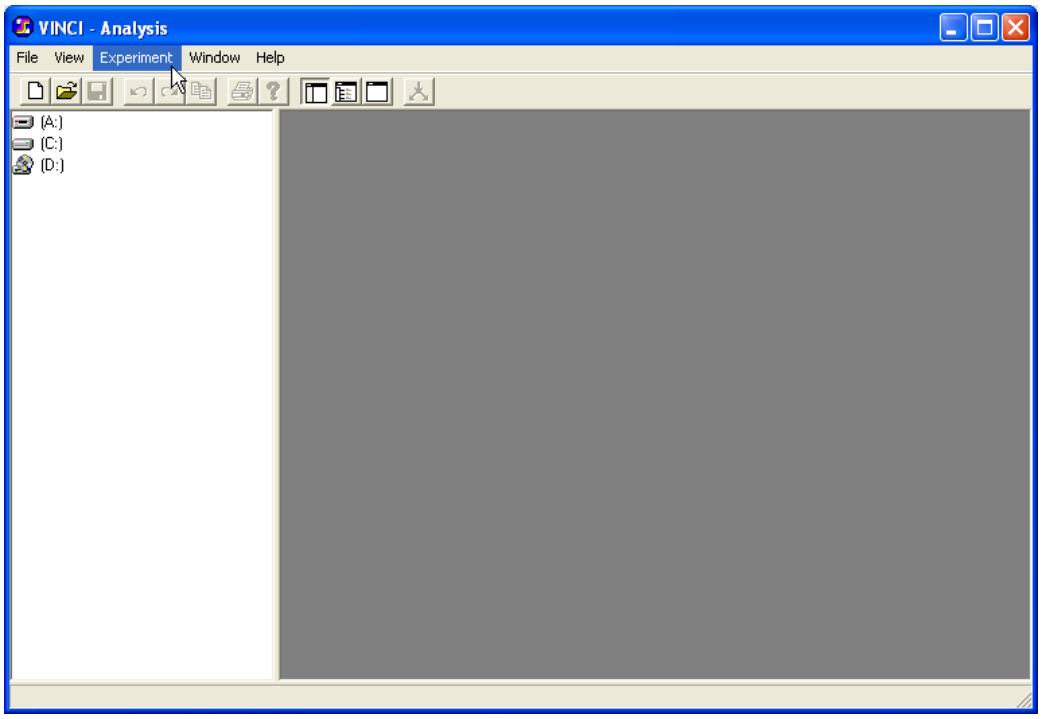
Note that if you install the software onto a different computer, a new license number is requested. Contact Customer Support department at ISS for instructions.


Install License
Look in:
Vinci
Configurations
Corrections
Diagnostics
Experiments
Inf
Licenses
Samples
Tmp
Vinci.vpl
My Recent Documents
Desktop
My Documents
My Computer
My Network Places
File name:
Files of type:
License files (*.vpl)
Open
Cancel

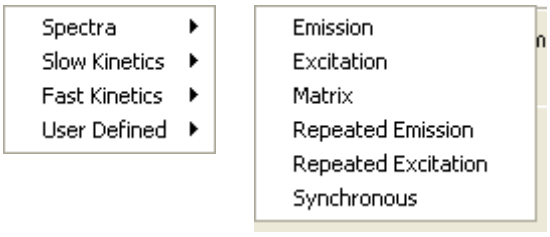
3 Quick Start: Acquisition of an Emission Spectrum

The acquisition of an emission spectrum (as well as the acquisition of other parameters) is five clicks away from the Vinci desktop icon of the computer. In the following paragraph, we will show how to acquire the emission spectrum on ovalene (ovalene in PMMA is available from several suppliers). The monochromator slit bandwidths on the PC1 monochromators are 8 nm (1 mm) in excitation and 4 nm (0.5 mm) in emission, respectively.

Click on the Vinci icon on the desktop and the following screen is displayed



Select <Experiment> and then, in the order, <Spectra> and <Emission>.

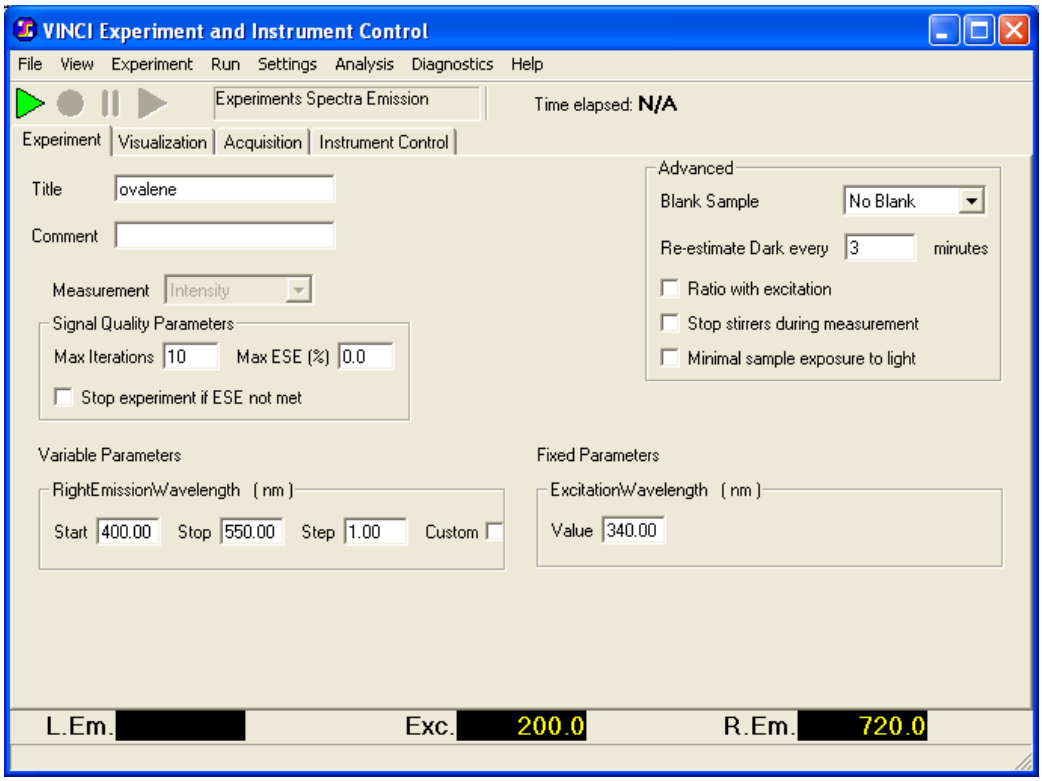


In the experiment window enter the following values:

- ✓ **340**, as the value (in nm) of the excitation wavelength;
- ✓ **400**, for the starting point of the emission wavelength
- ✓ **550** for the ending point of the emission wavelength
- ✓ **1** (in nm), for the step size of the emission monochromator (this value can vary from 0.25 nm to 10 nm).

Finally, a Title and a Comment can be entered as well.

Other parameters can be varied for instance, the time window for acquiring data but for this experiment, we will keep the default values. The record will be stored in the *C:\Data* folder.

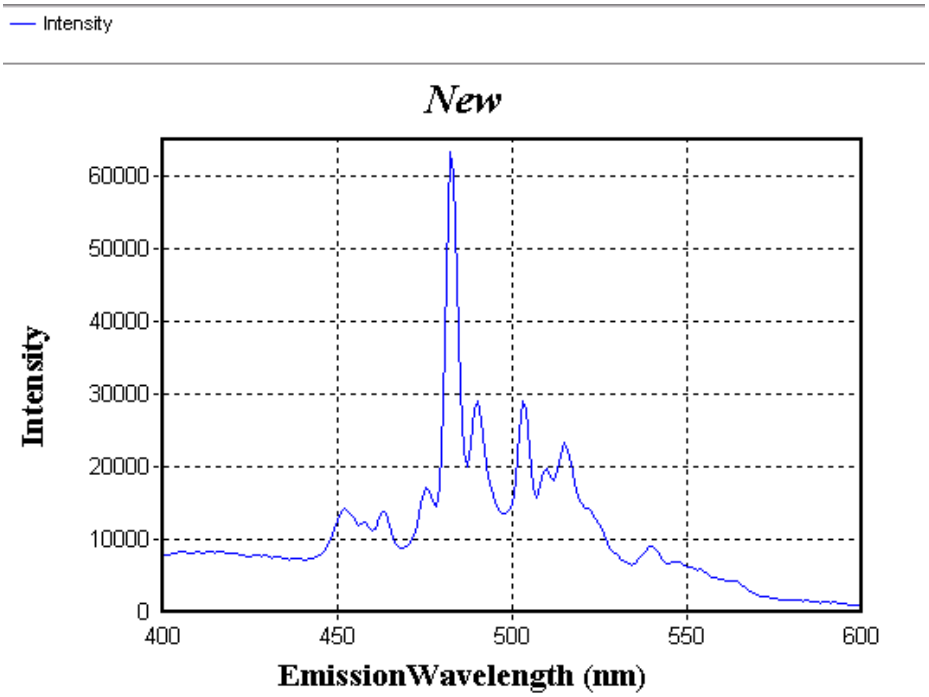


When done, click the green arrow to start data acquisition.

Shutters will be closed and the monochromators will be moved to the starting position. The acquisition starts; Vinci will show the spectrum as it is being acquired in real-time. Upon completion, the plot shown below will be displayed.

Once acquired, the spectrum can be saved. The default folder for saving is *C:\Data*, although the user can define a different folder.

The experiment is completed.


Intensity
New
60000
50000
40000
30000
20000
10000
0
Intensity
400
450
500
550
600
EmissionWavelength (nm)

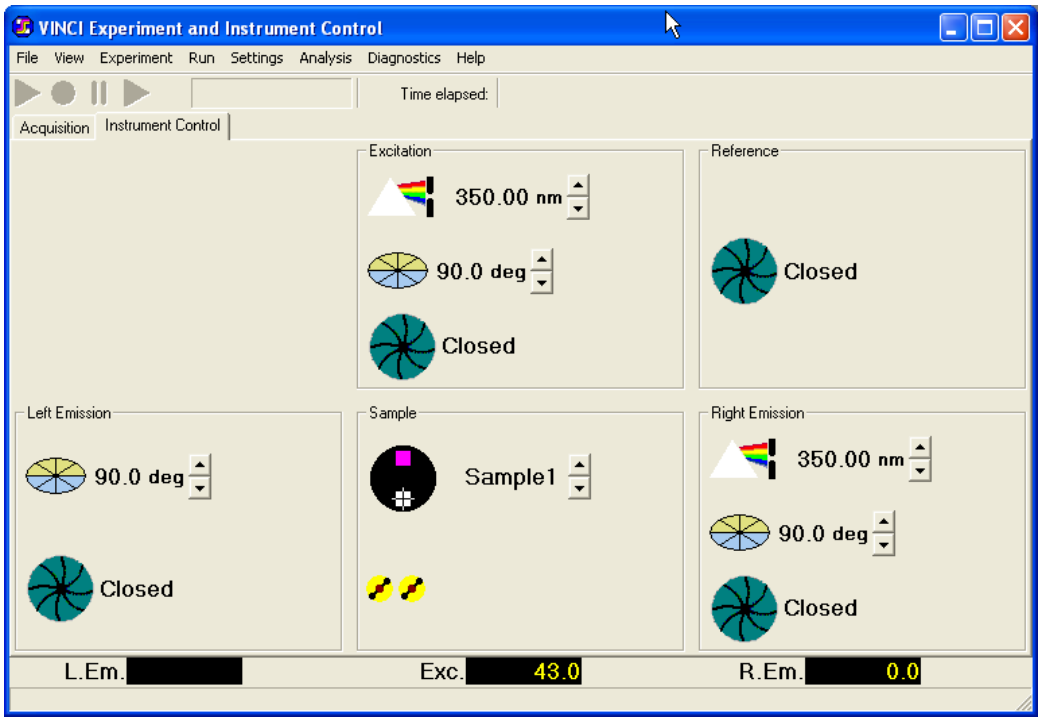
4 Instrument Control Panel and Acquisition Parameters

4.1 Instrument Control Panel

The Instrument Control Panel display is a tool for easy control of all automated devices of the instrument. The instrument is schematically divided into five sections: the Excitation and Reference channels, the Left and Right Emission channels, and the Sample compartment area.

In each section the devices are represented by specific icons and; the user can control each device by clicking on the respective icon. The figure below represents a T-format instrument equipped with a 2-cuvette sample holder and 2 stirrers. The configuration of an instrument is set at the factory and loaded in the software delivered with the instrument.

Vinci includes configurations for the most common instruments marketed by ISS; additional devices can be added to a configuration, as explained in section 3.4 below. The user is able to change/edit the instrument's configuration when other devices have to be added to and/or interfaced with the instrument.



The bottom row of the display reports the signal value from the three acquisition channels; respectively:

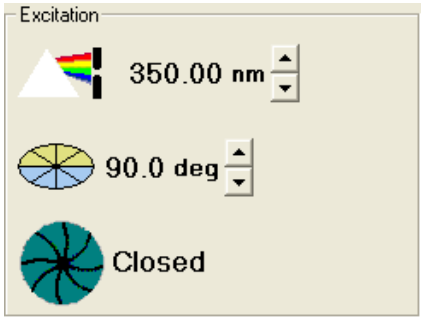
L.EM, the left emission channel;
R.EM, the right emission channel;
Exc., the excitation (or reference) channel.

The signal displayed in the bottom row is updated at a 10 Hz rate. The units utilized are counts per second (c/s) in the photon counting acquisition mode or arbitrary units when using the analog acquisition mode .

4.2 Excitation and Emission

The three icons displayed in the Excitation channel section represent the monochromator, the polarizer and the shutter. Each icon has different levels of control.

There are several ways to have control over the devices:



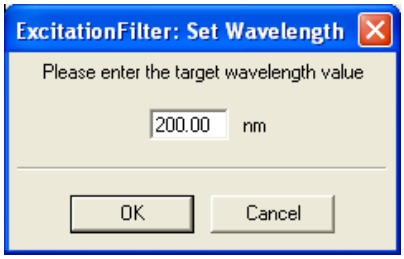
Pressing on the up/down arrows (where it applies) allows for a step-wise control of a device.
By right-clicking on each image, the action menu is accessed for specific control of each of the devices.
By left clicking on any of the devices icons, one can move them to a set position.

4.2.1 Monochromators

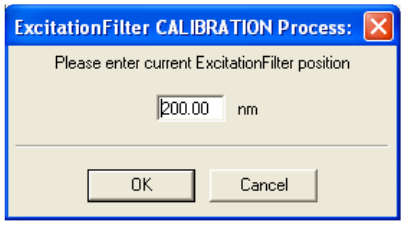
The monochromator settings can be moved up or down in 1 nm steps by clicking on the up/down arrows located on the right hand side of the icon.

By right-clicking on the icons (with the exception of the shutter icon) one can choose between the following options:

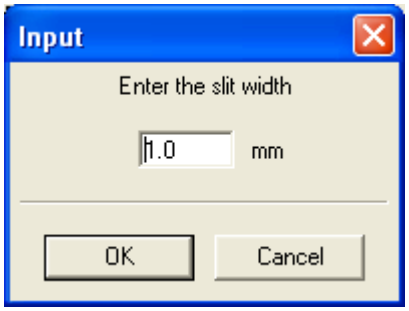
Clicking on <Move> and entering the destination wavelength allows to move the monochromator to a desired wavelength. The <Move> option can also be accessed directly in an alternative way by left-clicking on the icon.



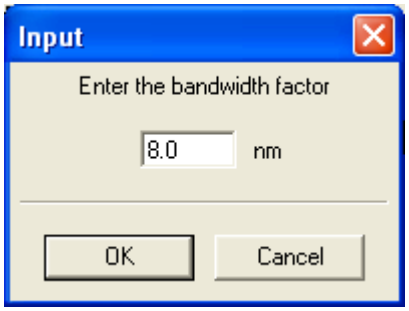
The <Calibrate> option allows the user to enter the position of the excitation monochromator, as read on the dial. This menu is not used on self-calibrating monochromators utilized on some spectrofluorometers.



<Set slit width> allows the user to enter the value of the slits utilized in the excitation channel monochromator.



The <Select Bandwidth Factor> parameter is the bandwidth in nanometers of the slits utilized in the monochromator (linear resolution of the monochromator in nanometers per millimeter).

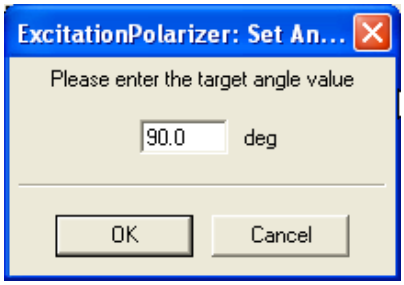


4.2.2 Polarizers

Clicking the up/down arrows to the right of the icon moves the polarizer between the zero/ninety positions.

Right-clicking on each of the icons (with the exception of the shutter icon) allows to select between the following options:

For moving the device to fixed positions left-click on <Move> and enter the numerical value (in degrees). Alternatively the <Move> menu can be directly accessed by left-clicking on the icon.



4.2.3 Shutters

The shutters can be opened/closed by directly clicking on the icon.

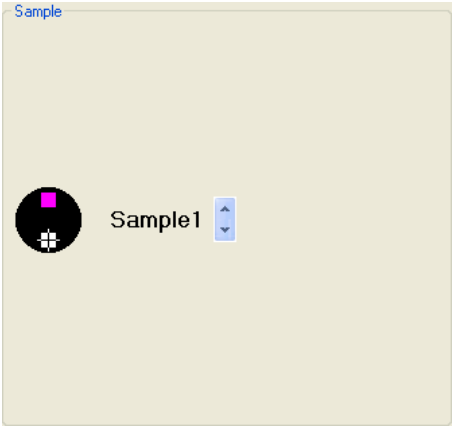
Right-clicking on the icon will display the menu.

Select <Move> and the shutter will OPEN/CLOSE, depending upon the initial position.
The OPEN/CLOSE operation also can be directly attained by left-clicking on the icon.

4.3 Sample Compartment

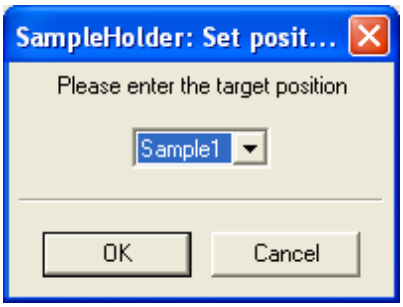
4.3.1 Sample Holder

The Sample Compartment area includes icons for the Sample Holder and Stirrers, Temperature, Pressure etc.



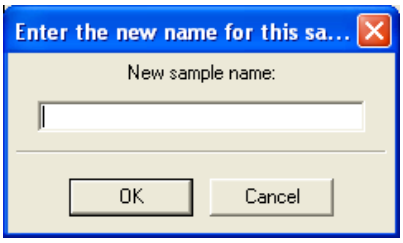
Right-clicking on the Sample Holder icon will display the 'Action Window'.

Select <Move> to move the Sample Holder to a different position.
The <Move> option can be accessed also by left-clicking the icon itself.



The <Calibrate> option is used for some sample compartment models equipped with an electronic limit switch to determine their absolute position.

<Change Sample Name> allows to change the name of the sample.



4.3.2 Stirrers

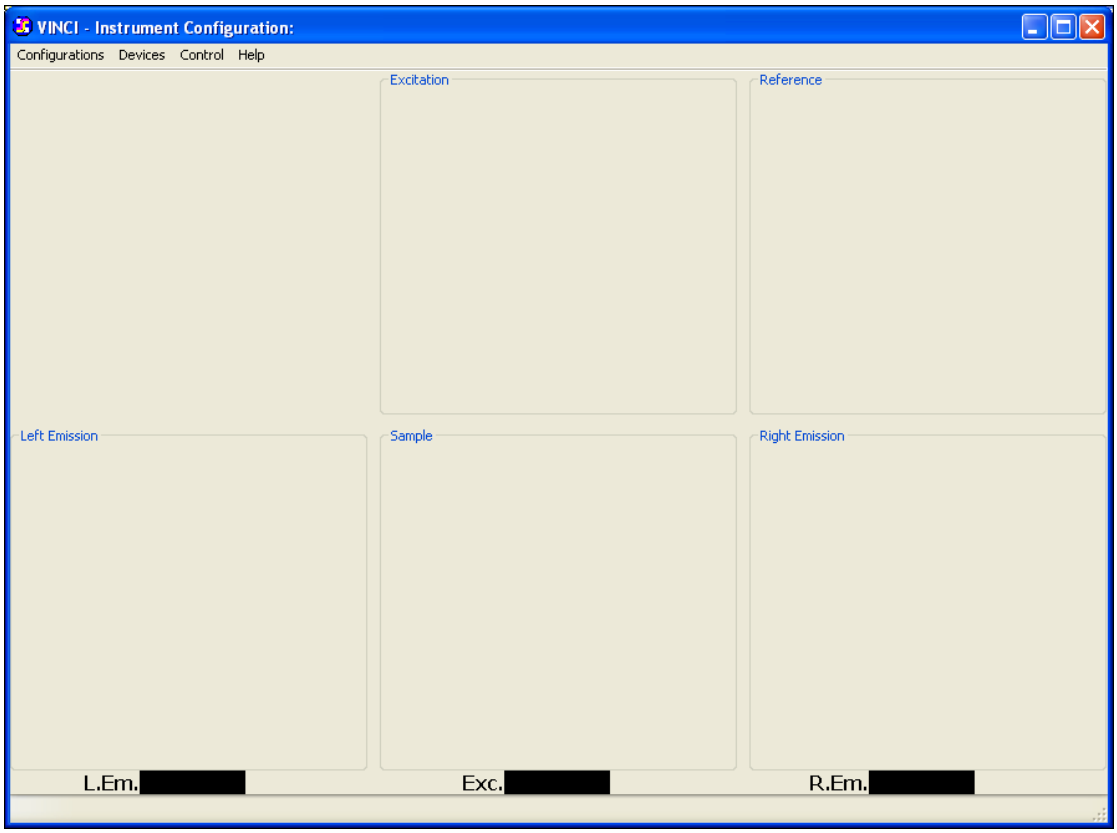
Right-clicking the Stirrers icon will display the 'action window'. <Move> will activate the Stirrers.

Alternatively, the stirrers can be independently activated and/or stopped by left clicking on the icon.

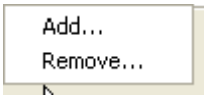
Note that the speed of rotation of the stirrers is controlled through the knob located on the outside wall of the sample compartment. While each stirrer can be independently turned ON/OFF, the speed for the stirrers cannot be independently controlled.

5 Adding Devices to the Spectrofluorometer

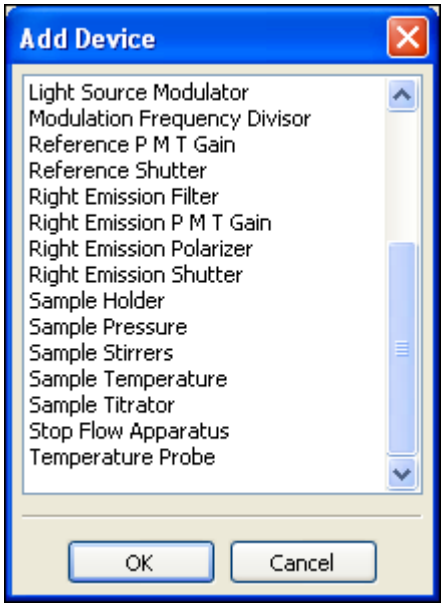
In order to modify an instrument configuration, the Vinci Instrument Configuration Editor has to be opened. This executable file (*VinciConfig.exe*) is located in the main Vinci folder. Open that folder, and identify the file. Upon clicking on the file and the following blank screen will be displayed:



Click on <Devices> on the menu list on top and then select <Add>.

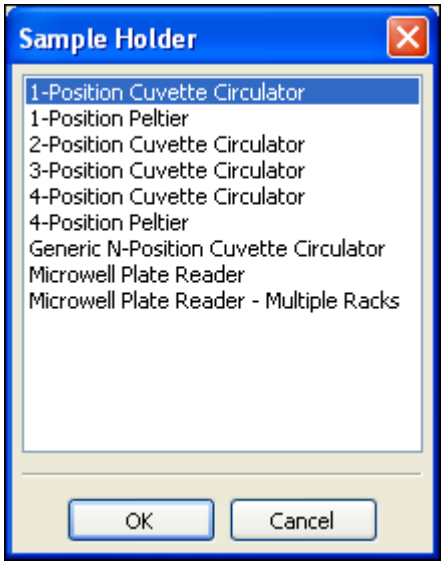


A list of the available devices is displayed. If the instrument configuration is not defined, the list includes all of the available devices.

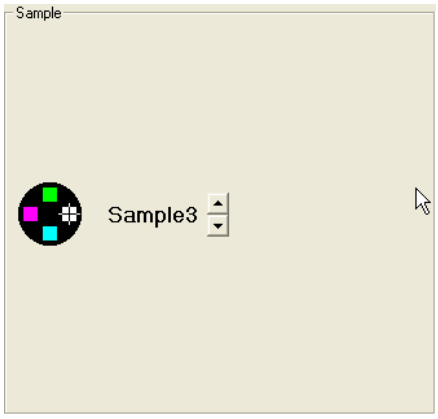


5.1 Adding a Sample Holder

By clicking on <Sample Holder> the following list of Sample Holders is displayed. Select the desired Sample Holder from the list.

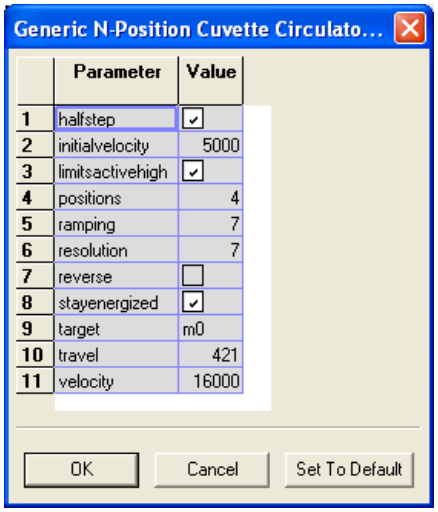


Once a Sample Holder has been selected, and the installation has been completed, the Sample Holder icon will appear in the Device Configuration.



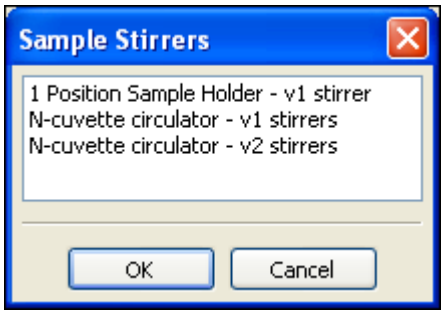
Right-clicking on the icon will display the action window. This action window includes an additional option: <Adjust Parameters>.

The <Adjust Parameters> option allows setting the proper values for the stepper motor driving the Sample Holder.



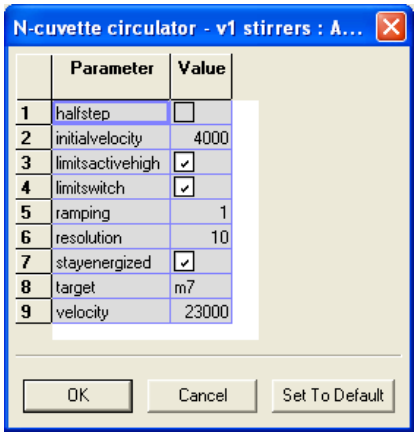
5.2 Adding Computer-Controlled Stirrers

Each sample compartment is equipped with computer-controlled stirrers, which can be activated and added to the instrument's configuration.



Again right-clicking on the icon will display the 'action window'. This window includes an additional option: <Adjust Parameters>. Checking this option will bring up the motor configuration menu.

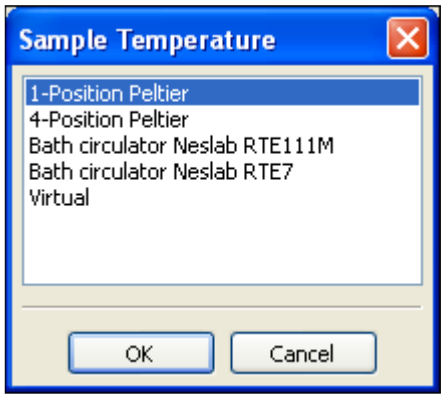
The stirrer motor configuration is shown in the table on the right.



5.3 Peltier Holders and Bath Circulators: Controlling the Temperature of the Sample

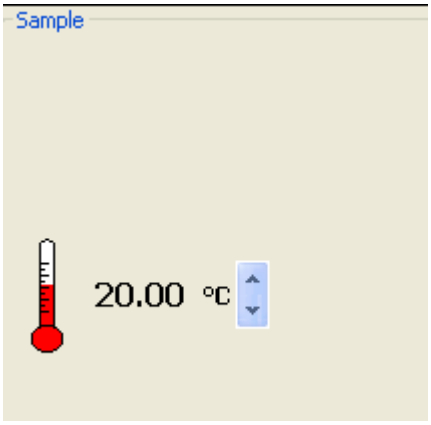
Devices for temperature control that are supported by Vinci are listed under 'Sample Temperature'.

These devices are controlled through the RS-232 port of the computer (COM ports). The user has to select a port for each device.



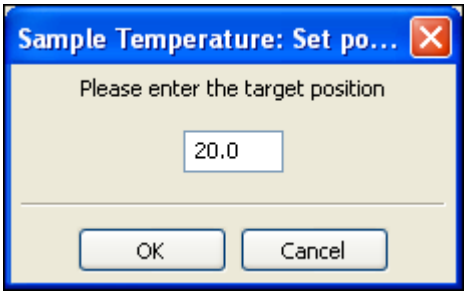
The selection of the COM port is shown in the following display.

Once the selection of the port is completed, the temperature icon will be added to the other icons in the sample compartment.

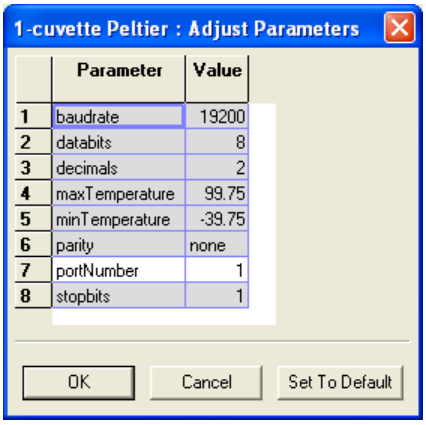


Right-clicking on the icon will display the 'action window'. Also this action window includes the additional option: <Adjust Parameters>.

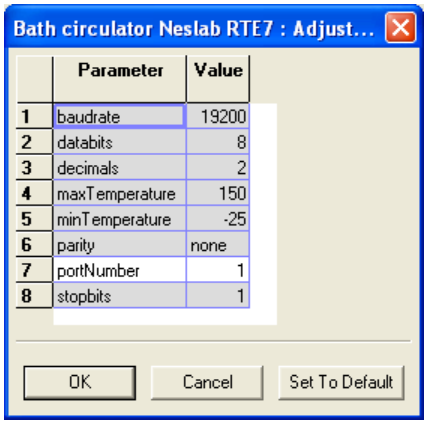
By selecting <Move>, the user can enter a temperature value (in Celsius) to be set for the sample compartment.



Each device has its own set of parameters. Shown on the right are the parameters for the '1-Cuvette Peltier-Controlled Sample Holder'.



The window on the right shows the parameters for the bath circulator model Neslab RTE7



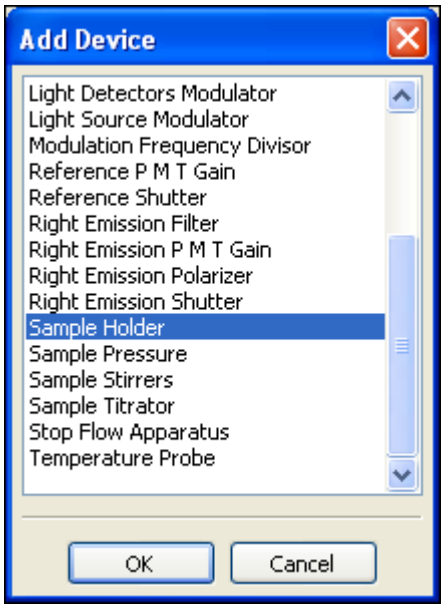
These parameters are to be left unchanged by the user. The <Set To Default> button helps the user to restore the proper values if, for any reasons, they have been changed.

5.4 Microwell Plate Reader

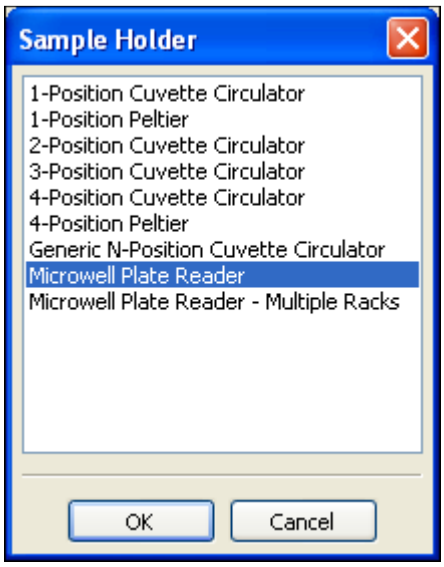
The Microwell Plate Reader offered by ISS (Model K432) accommodates up to four (4) plates at a time; light is brought to the wells by using a fiber optics bundle, which is part of the package offered by ISS. Each well in the plates can be read sequentially for a total of up to 96x4=384 wells.

The Microwell Plate Reader is controlled through the RS232 port of the computer. To add the device to the instrument configuration, follow these steps:

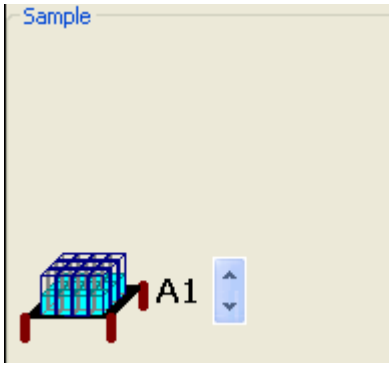
a. Remove any sample holder present in the instrument configuration;
b. Select the 'Add Device' field and select 'Sample Holder'.



Under ‘Sample Holder’ select ‘Microwell Plate Reader’ or ‘Microwell Plate Reader – Multiple Racks’ depending on your experimental set up.

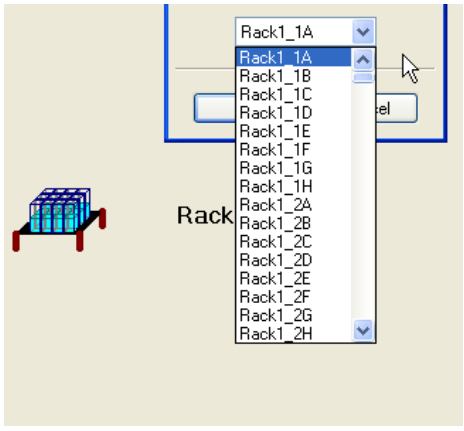


After checking ‘OK’ the Microwell Plate Reader icon is displayed in the Instrument Configuration.



Right-clicking the icon displays the ‘action window’.

Selecting <Move> enables to access a certain position in the rack. The letters and numbers identify the exact position of the wells of a 96-well plate: twelve numbers identify the rows; the eight letters from A to H identify the columns. For instance, H12 identifies the well located in the lower right corner.
The multi-rack option sample holder may accommodate up to four (4) plates. In this case the *Rack number* precedes the microwell numbers. Racks are numbered from one to four starting with the upper left side and proceeding clockwise.



When <Calibrate> is selected, the fiber bundle is moved to the *Home* Position. Any additional step is counted from the *Home* position.

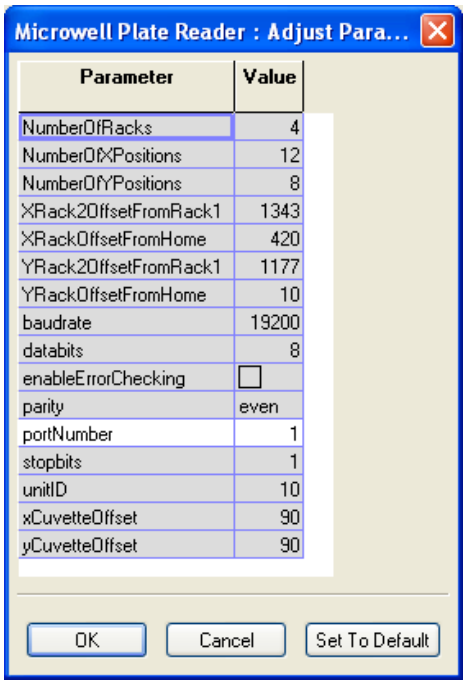
An individual well can be renamed by selecting the <Enter a new name for this sample>; the well that is being renamed is the one that is located in the measuring position.

<Adjust Parameters> lists the default values pertinent to Model no. K432. Only some of these parameters can be changed by the user; specifically:

The 'Number of Racks' specifies the number of plates loaded in the device. If the user is planning to measure one plate only, the number should be "1".

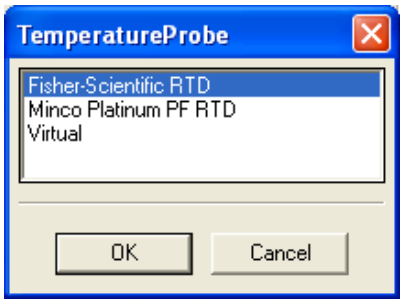
The 'Number of X positions' and the 'Number of Y positions' relates to the type of microwell plate utilized. For a 96-well plate these numbers are 12 and 8, respectively. For a 384 well plate, the numbers are 24 and 16, respectively.

Note: The capability of measuring a 384-plate depends upon the diameter of the fiber bundle utilized in the measurements.

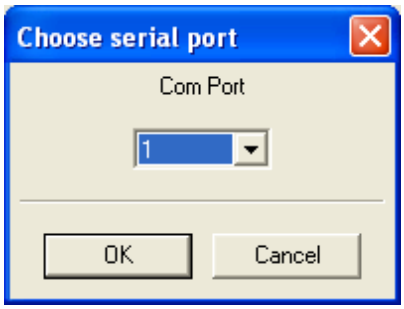


5.5 Thermometer: Measuring and Recording the Temperature of a Sample

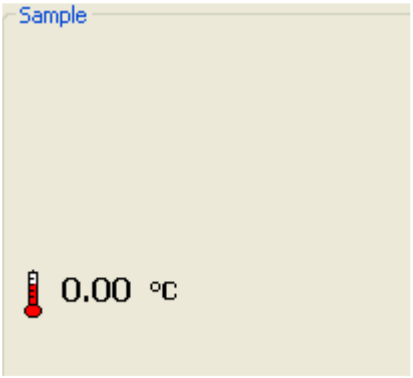
A temperature probe can be conveniently added to the instrument to record the temperature of the sample. The temperature probe is a passive device and only monitors and records the value of the temperature.



If the device is read through the RS-232 port of the computer, as it is the case for the Fisher-Scientific RTD, the COM port communicating with the device has to be selected by the user.

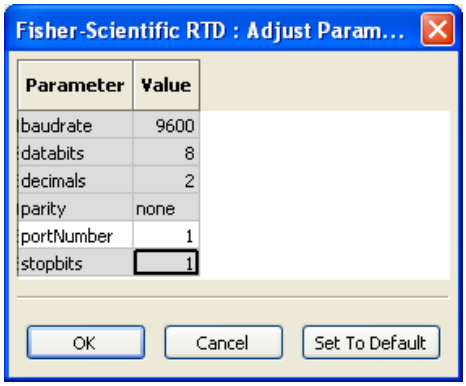


The icon for the temperature probe is a thermometer.



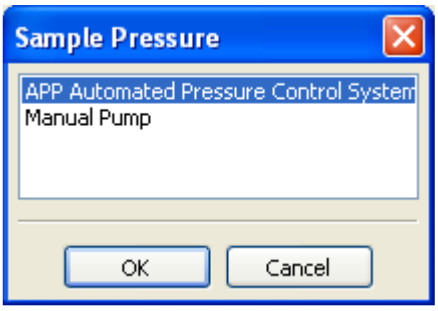
Clicking the icon shows 2 options for this accessory:

The <Adjust Parameters> window contains all parameters pertinent to the probe:

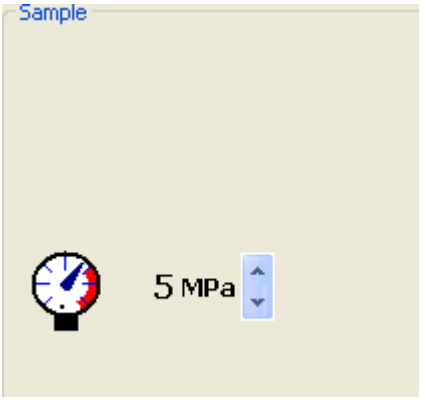


5.6 Pressure Cell

The ISS Pressure Cell is controlled through a manual pump. Adding the pressure pump to the devices in Vinci allows the user to set up a series of experiments where the pressure is automatically recorded with the other experimental parameters.

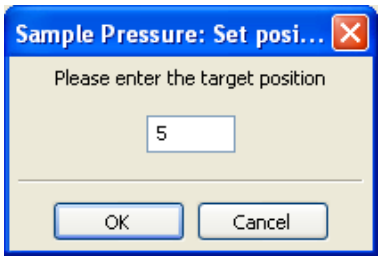


Once the pressure pump is added to the sample compartment, the pressure icon is displayed. The pressure is measured in Mega Pascal [MPa] units.

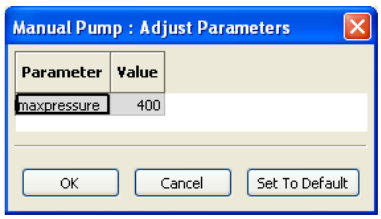


Clicking on its icon will show two choices for the interacting with the Pressure Pump:

With a manual pump, a target pressure can be entered in the software by right-clicking on the icon and selecting <Move>.

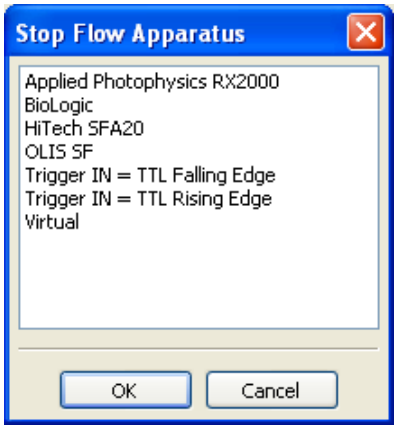


The <Adjust Parameters> fields contain default values specific to the ISS pressure gauge.



5.7 Stopped-Flow Apparatus for Fast Kinetics Measurements

Vinci supports stopped-flow devices for fast kinetics studies made by several manufacturers. The list is displayed on the right.



No icon will appear for the stopped flow apparatus in the Instrument Control display.

Note: The stopped-flow apparatus is coupled to the ISS data acquisition card using the kinetics module. Data acquisition can be done in analog mode using the A2D or A2D200K data acquisition cards. Alternatively, fast kinetics data acquisition can be done in photon counting mode using the PCMC version 2 card. Consult with ISS Customer Support for details about data acquisition using this device.

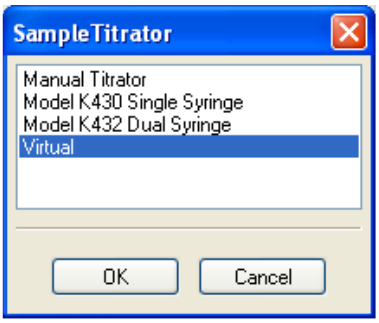
Most of the stopped-flow devices trigger the ISS data acquisition card on a raising TTL pulse. Devices such as the OLIS SF and the BioLogic models require a TTL pulse. The Vinci software, in conjunction with the data acquisition card delivers both signals.

5.8 Titrator

Vinci supports the following devices marketed by ISS:

a. Manual titrator (manually activated syringe)
b. Model K430 Single Syringe Titrator
c. Model 432 Dual Syringe Titrator (Hamilton Model 500)

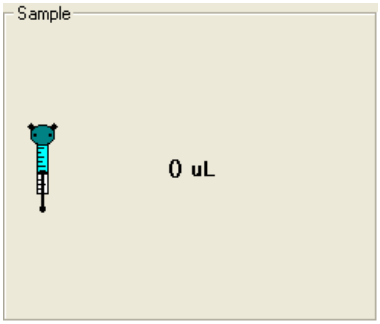
Note: the "Virtual" titrator driver is utilized for demo purposes only.



5.8.1 Manual Titrator

The activation of the manual titrator is suggested when acquiring a series of measurements (fluorescence intensity, kinetics, polarization), each at a different fluorophore and/or titrant concentration. Data plots can be generated with the value of the added volume on one of the axis.

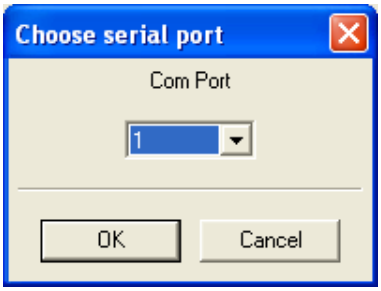
When the Manual Titrator is activated, the syringe icon is displayed in the Sample Compartment area.



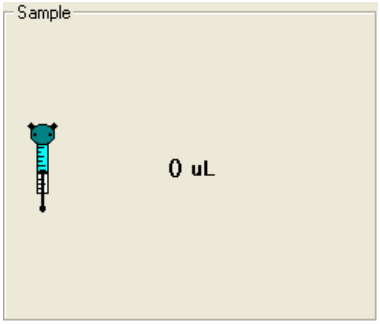
5.8.2 Computer-controlled Titrators

Models K430 and K432 are controlled remotely and after the experiment is set no further intervention of the operator is required. Both models K430 and K432 are controlled through the RS-232 port.

The COM port has to be selected for the communication.



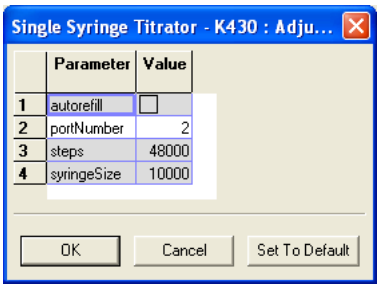
If the activation is successful, the syringe icon is displayed in the Sample Compartment area.



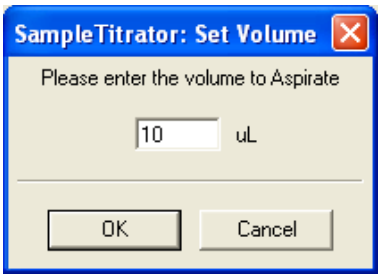
Right-clicking on the icon will display the action window.

For programming the titrator the <Adjust Parameter> window has to be accessed first. In this window, the user has to enter the volume of the syringe utilized in the experiment.

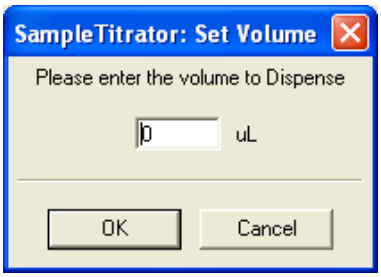
In the default configuration, the <autorefill> box is not checked. When the box is checked, the syringe is automatically refilled if the remaining volume of titrant is not sufficient to complete the experiment.



Several operation options for the titrator can be entered via the Instrument Control panel. When <Aspirate> is selected (where?), the user enters the volume requested (in µl).

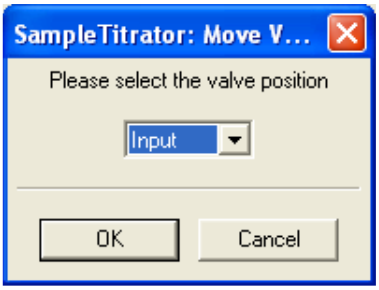


Similarly, to <Dispense> a volume into the cuvette.



The <Move Valve> button allows to move the valve to either of three positions:

- input (input port connected to syringe)
- output (output port connected to syringe)
- bypass (connection between input and output ports)

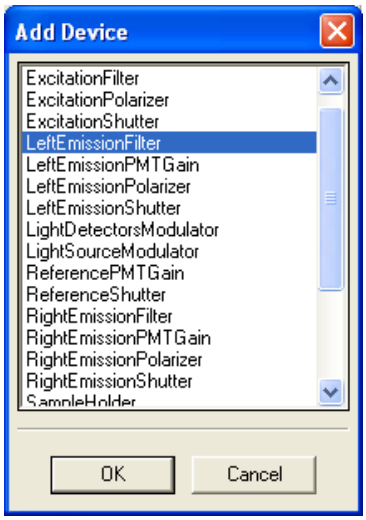


When <Aspirate> and <Dispense> are used, the software automatically moves the syringe to the proper position.

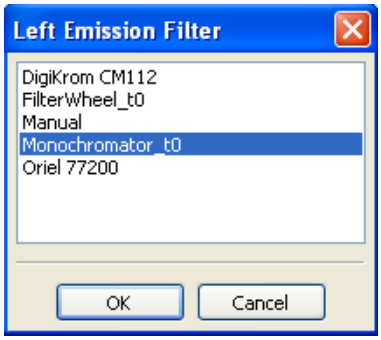
5.9 Monochromator

A third monochromator can be added to the left emission channel of an ISS spectrofluorometer, or a higher-resolution monochromator can replace the standard monochromator mounted on the instrument. Whenever a monochromator is added or replaced, changes in the Instrument Configuration are required.

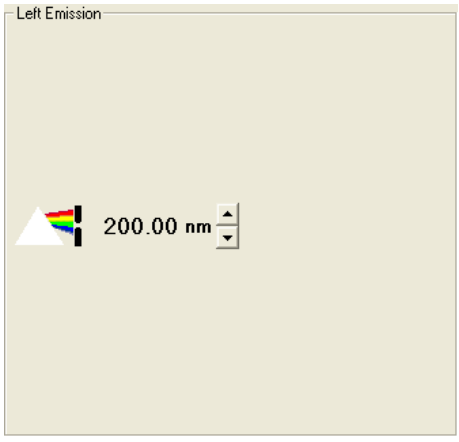
Click on <Left Emission Filter> when adding a monochromator in the left emission channel of the instrument.



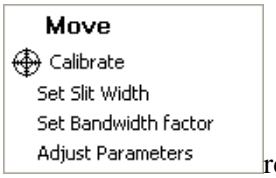
Select <Monochromator_t0> for monochromator.



Upon checking the OK box the monochromator icon is displayed in the Left Emission Channel area.

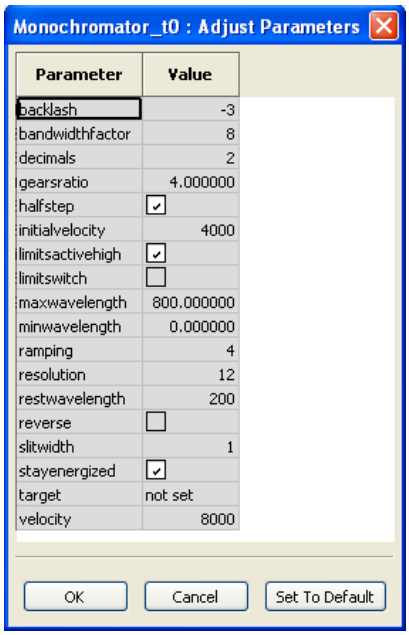


The action table is displayed after right-clicking on the icon There are several options to choose from:



replace

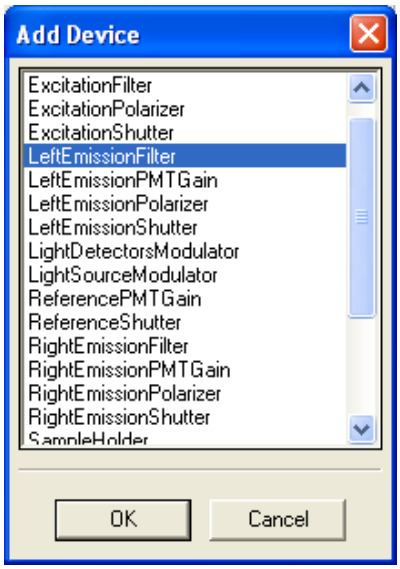
The <Adjust Parameter> window includes the relevant parameters to be changed to drive the stepper motor of the specific monochromator.



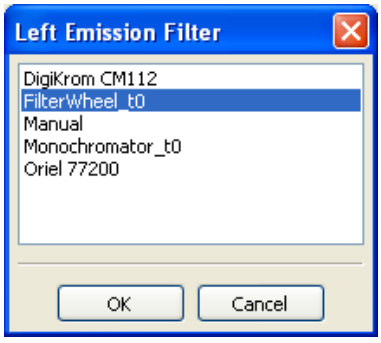
5.10 Filterwheel

A 4-position filterwheel can be added on the left emission channel of the ISS spectrofluorometers.

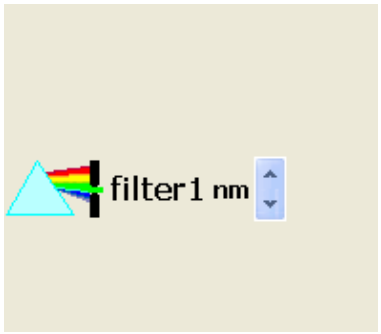
Click on <Left Emission Filter> when adding a filterwheel in the left emission channel of the instrument.



Select <FilterWheel_t0>.



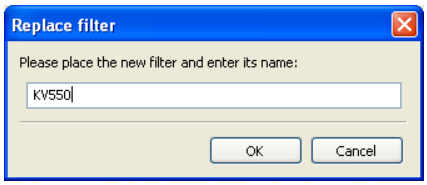
The Filterwheel icon is displayed in the Left Emission Channel area after checking the OK box.



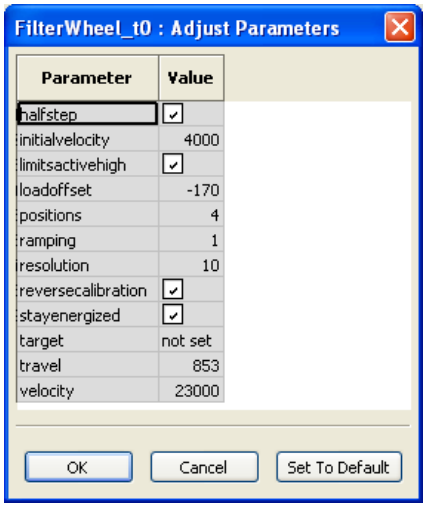
The action table is displayed after right-clicking on the icon There are several options to choose from:

change

Select the <Replace Filters> option if it is required to enter the name for a specific filter. Example: KV550.

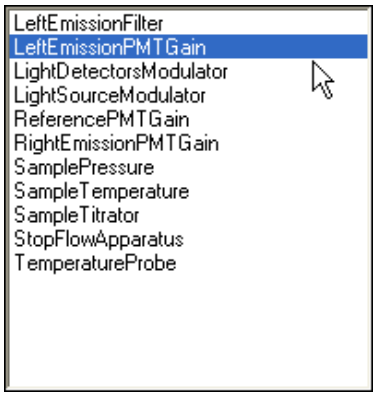


The <Adjust Parameter> window includes the relevant parameters specific to the stepper motor of the filterwheel.



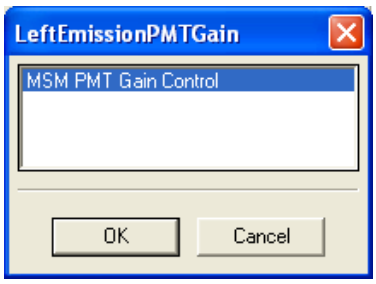
5.11 Controlling the Gain of Photomultiplier Tubes

In photon-counting mode operation maximum-level voltage is applied to the photomultiplier while in analog mode the voltage settings can be changed. The change can be done manually, using the knob located on the photomultiplier tube housing, or remotely through the computer. When remote gain control is chosen, the switch on the PMT housing has to be in the REM (remote) position.

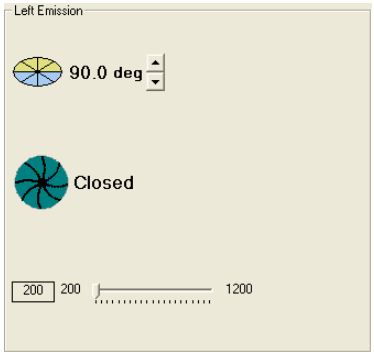


In order to add the automatic gain control, select the location of the PMT.

Click on <OK> button.

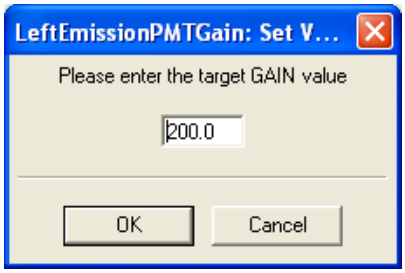


A slide bar will appear in the Left Emission channel area. The slide bar allows the user to change the gain remotely via the computer screen.

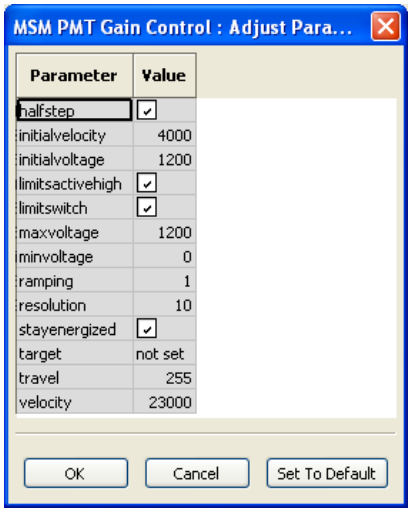


The gain can also be set by right-clicking on the slide bar and entering the voltage gain value (in Volts) that should be applied to the PMT.

Checking the OK button will set the PMT gain remotely to the required voltage value.



The <Adjust Parameters> window includes parameters that specific to the control of the ISS PMT housings.



6 Adding ISS Cards Drivers to the Computer Configuration

The Vinci Software supports the following data acquisition cards produced by ISS and installed in the instruments:

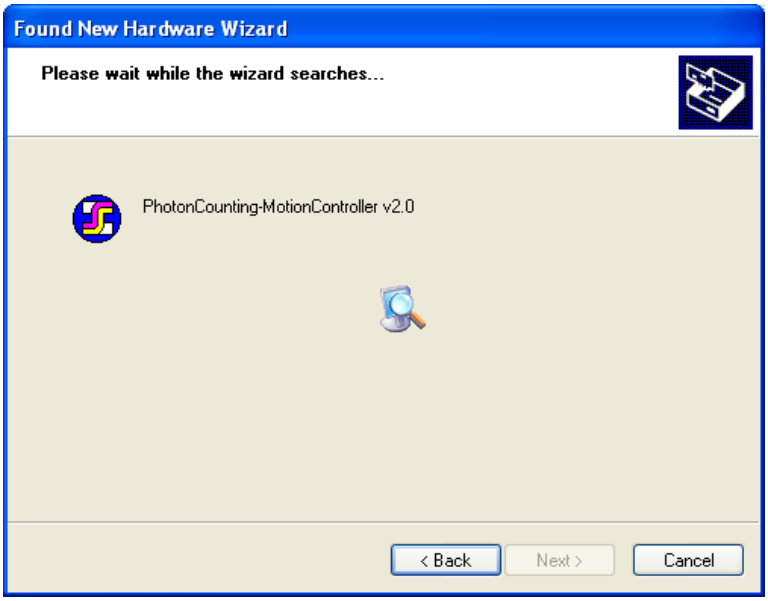
Card	Bus	Description
PCMC version 1	EISA	Photon Counting Data acquisition card
A2D	EISA	Analog Data Acquisition card
PCMC version 2	PCI	Photon Counting Data acquisition card
A2D200K	PCI	2-Channel Analog Data Acquisition card

The drivers for these cards are stored in the CD-ROM supplied with the software package in the folder named <inf>.

6.1 PCI-Based Cards

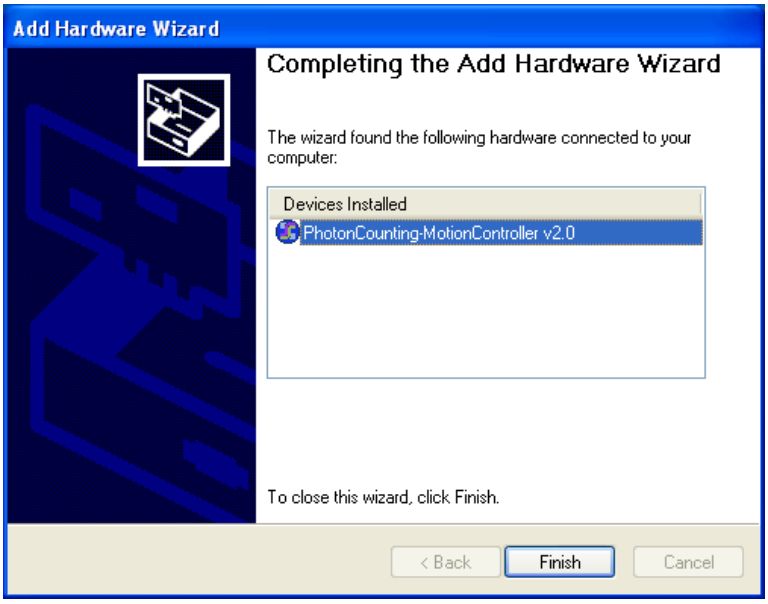
Insert the card in the computer and a message display will request for the location of the driver. The drivers are located in the folder <inf> stored in the Vinci software folder. The user will point to the <inf> folder and the installation procedures starts.

During the installation process, the ISS icon will be displayed in the dialog windows.



The driver's installation takes a few seconds. Once the installation is completed, the following screen is displayed.

Click finish when the window is displayed.



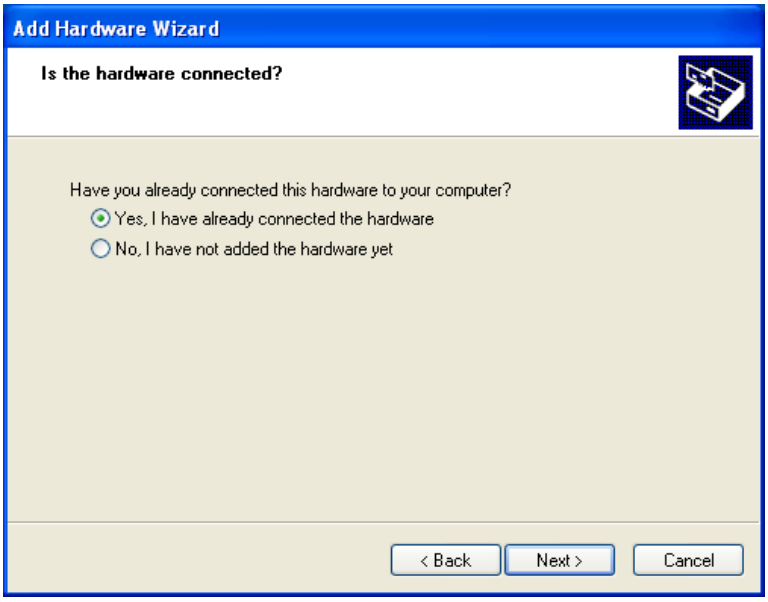
6.2 EISA-Based Cards

We assume that the cards are already installed in the computer. The drivers for these cards have to be installed manually:

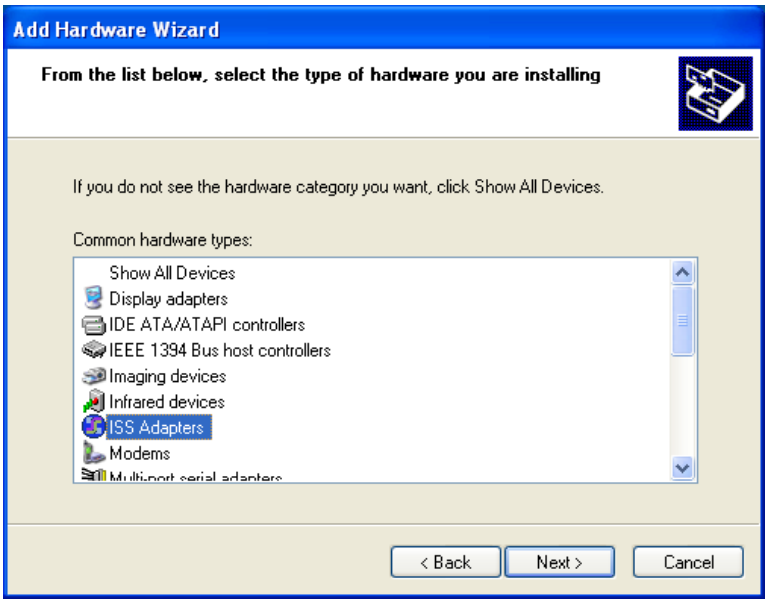
To do it, insert the Vinci CD-ROM in the appropriate computer drive, press <Start>, then select <Settings> and <Control Panel>.

Select the <Add New Hardware> option and the wizard will guide you through the installation process.

Select <Yes> to proceed with the installation.



Select <ISS Adapter> from the list.

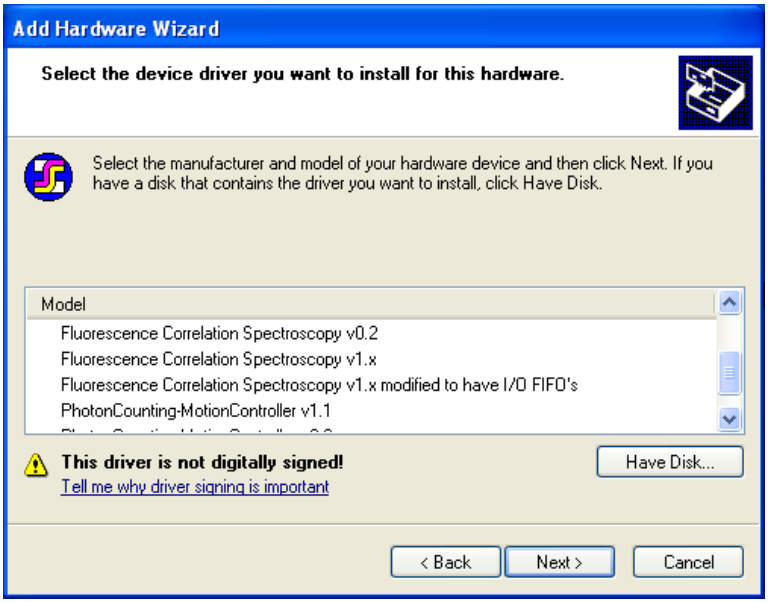


When the <Browse> button is pressed, select the location of the CD-ROM or the location of the Vinci software installed on the hard drive of the computer.

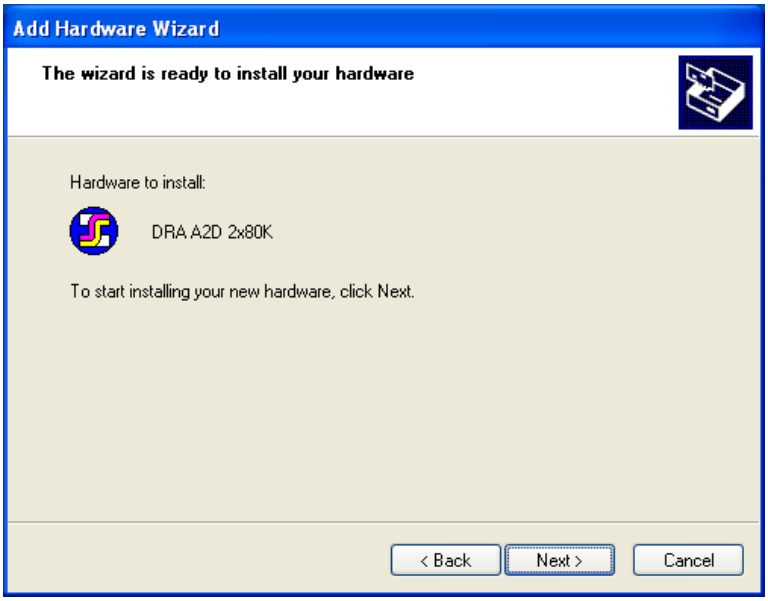
Select on the *issdrvx.inf* file.

A list of the known drivers will be displayed.

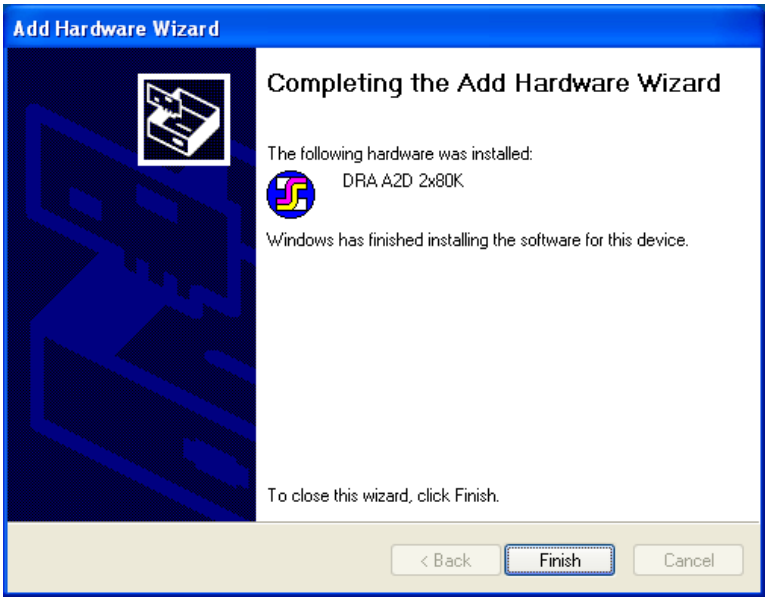
Select the driver(s) pertinent to the ISS acquisition cards installed in the computer.



Follow the instructions given in the installation window.

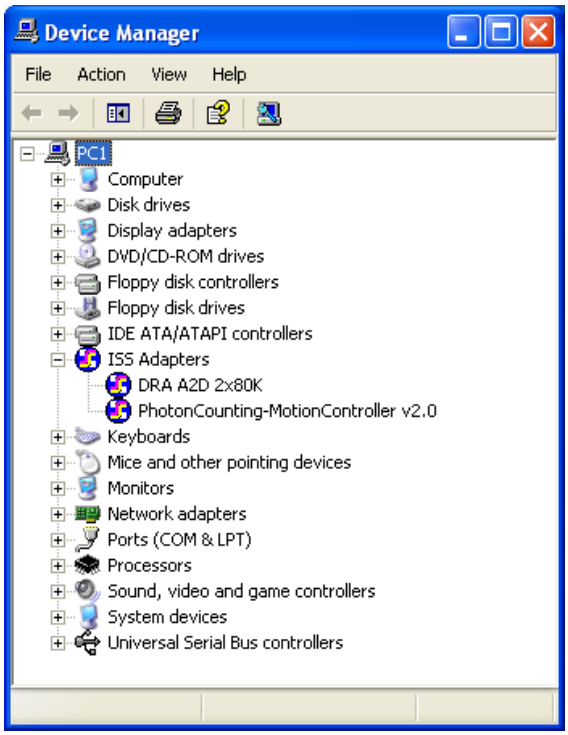


After completion of the installation, this window will be displayed. Check the Finish button to finalize the installation operation.



Once the files are installed, they are listed in the Device Manager of the computer. Open the <Device Manager> panel under <System Properties>.

The ISS icon will appear in the list of the installed devices close to the <ISS Adapters>; the name of each installed data acquisition card is displayed as well.

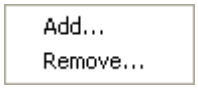


7 Modifying the Instrument Configuration

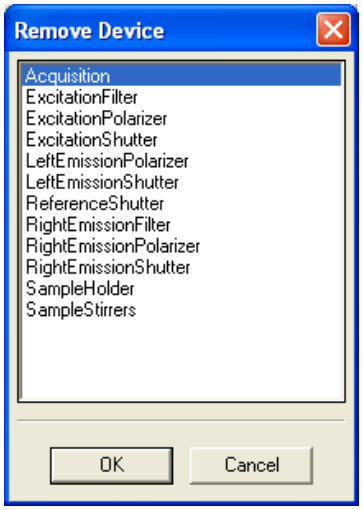
7.1 How to Remove a Device from the Instrument Configuration

Devices can be removed as well from Instrument Configuration.

To add/remove devices to your instrument configuration in the Instrument Configuration Editor click on <Devices>, then "Remove":



Select from the list the devices that need to be removed from the configuration.



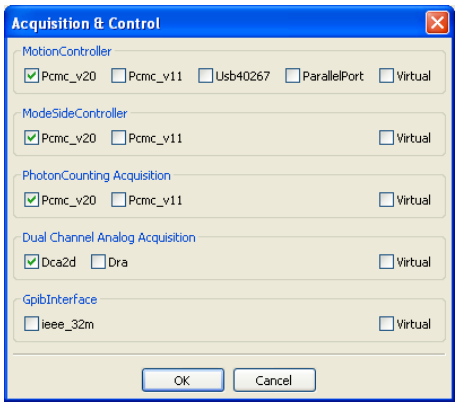
Once a device has been selected, it will be removed from the Instrument Configuration file if the OK button is checked. The new file has to be saved for the new configuration to take effect (see section 3.8).

7.2 Virtual and Real Acquisition Modes

By checking <Virtual>, the user can select between the demo mode of operation or the <Real> Data Acquisition mode.

The Virtual mode is selected when using the software on a workstation for data manipulation and data analysis. The Virtual mode can also be used to simulate data: the computer to check and test acquisition routines provides values.

The <Real…> feature allows the user to select the proper data acquisition card(s) utilized in the instrument as well as the proper controller of the instrument automation features. When checking <Real…>, the following window is displayed:



The following five cards supported by the Vinci software are:

A. PCMC versions 20 and 11 (ISA bus) and 20 (PCI bus). These cards are utilized for the instrument control and for data acquisition in photon counting mode.
Note: These cards are used to control the stepper motors on the instrument (MotionController) as well as to set the left/right channel for data acquisition in analog mode.

B. The A2D card (ISA bus) and the A2D200K card (PCI bus) are utilized for analog data acquisition.

Note: If all the cards are selected, Vinci proceeds to check first for the presence of PCI-based cards; if these cards are not found, then it assumes that the ISA-bus cards are present.

7.3 Loading a Predefined Instrument Configuration

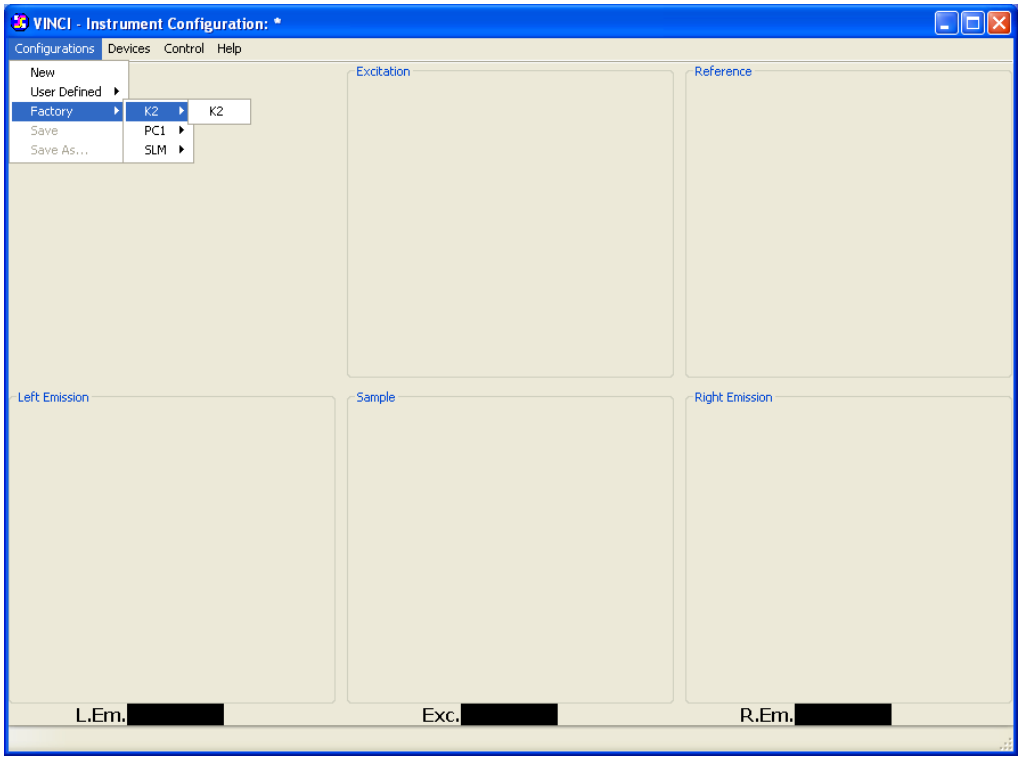
Vinci includes the default parameters of the instruments offered by ISS; one can simply load the configuration for a set instrument. The following instrument configurations are included in the software:

Default Instrument Configurations included in Vinci
PC1 - Photon Counting Spectrofluorimeter
K2 - Multi-frequency Phase Fluorometer
Chronos - Lifetime Spectrometer
Phoenix - Upgrade of the SLM8000, SLM8100, SLM48000

As shown below, the user can add a custom-configuration and save it as a default configuration. This feature is useful when interfacing the instrument with external devices such as a stopped-flow apparatus, a titrator or a computer-controlled temperature bath, or other devices controllable through the RS-232 port of the computer. The software includes proper drivers for controlling some of these devices (see updated list on www.iss.com) and for synchronizing their operations with the data acquisition.

7.3.1 How to Select a Pre-defined Instrument Configuration

Open the *Instrument Configuration* file and select <Configurations> on the top row of the screen.

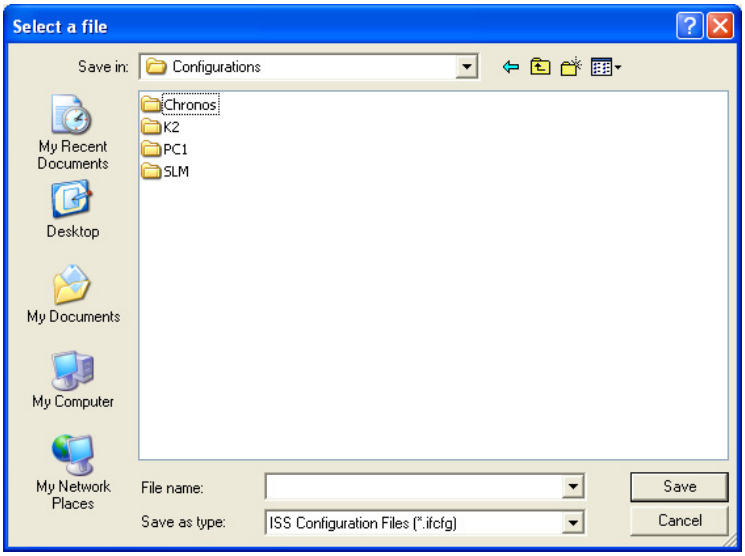


There are 3 configuration choices to choose from:

a) New

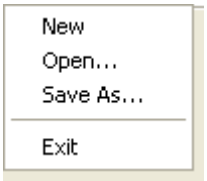
b) User-defined
c) Factory

Configuration files are stored in the Configuration folder, which is one the folders included in the Vinci software. Once generated configuration files can be stored directly in this folder.



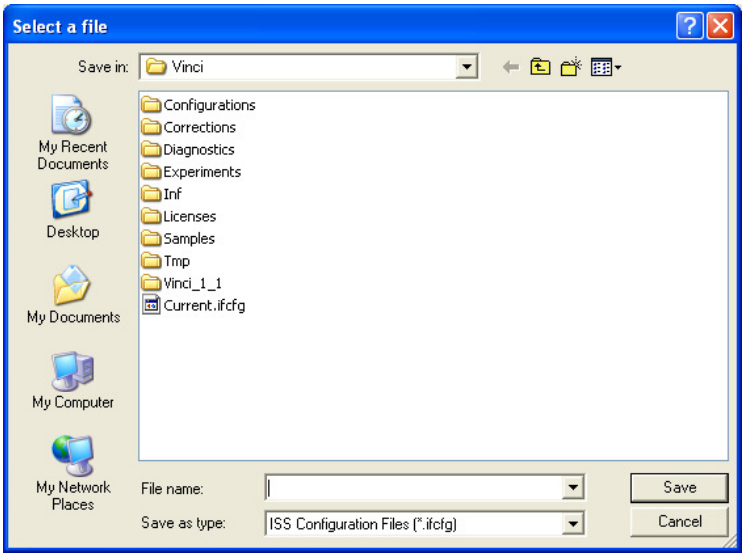
7.4 Saving a Configuration

Once an instrument configuration file has been generated, click on <File> and select <Save As>.



Select the Configurations folder in the Vinci folder. This folder includes the various instrument configurations that are available. A new configuration can be saved with a new name in the specific folder.

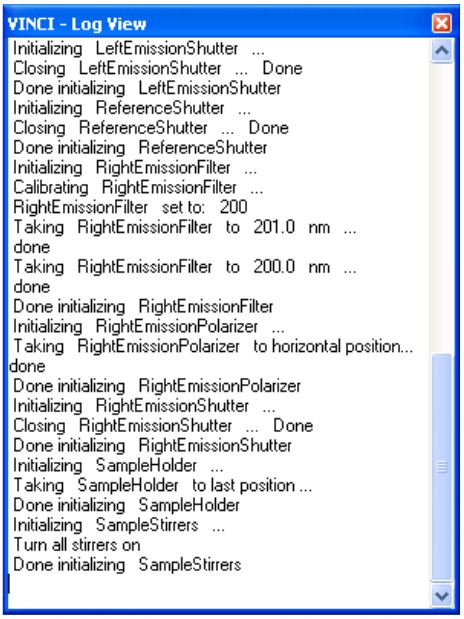
Note that a file named "*Current.ifcfg*" is present in the Vinci folder. Any new configuration has to be saved under this filename for the configuration to be active when the acquisition software starts.



7.5 Details of the Instrument Configuration File

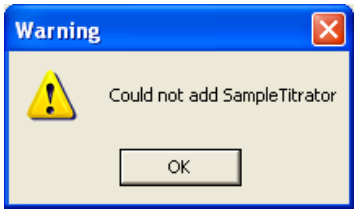
When pressing <View> in Experiment and Instrument Control, the button is displayed.

Clicking on it will list all automated and activated devices on the instrument in a separate window on the right side of the screen.
The list is used for verification purposes only. If any of the devices fails during the initialization process, an error message is displayed.



7.6 Error Messages for Failing to Add a Device

If a device is not connected during the installation procedure, a warning message is displayed on the screen. The warning message on the right alerts the user about a failure to add the Titrator.

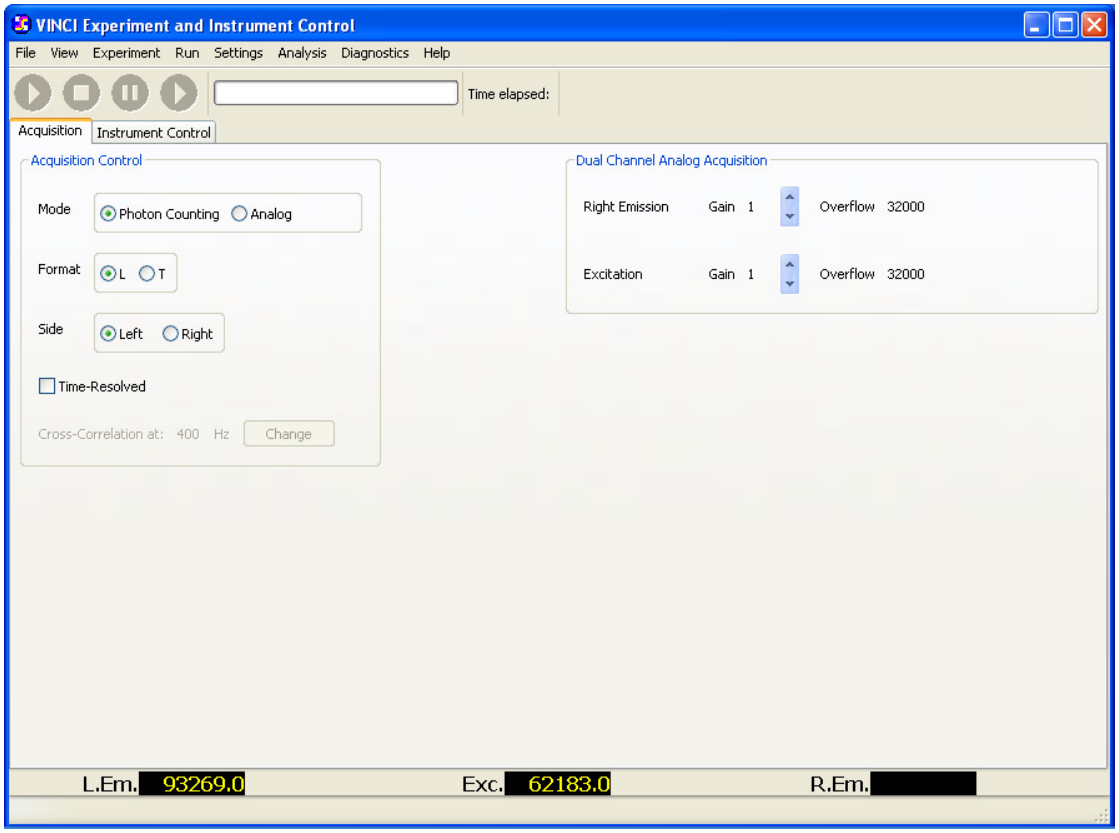


7.7 Acquisition Parameters

The Acquisition Parameters page deals with the configuration of each instrument. Some instruments only acquire in Photon Counting mode; others only in Analog mode; some instruments acquire data in both modes.

The term “format” refers to the geometric configuration of an instrument. Instruments configured in L-format have one emission channel only, either the Left or the Right. Instruments configured in T-format feature two emission channels.

The measurement set up is specified under Acquisition in Vinci Experiment and Instrument Control



7.7.1 Acquisition Controls

Mode Refers to the type of data acquisition. Choices are:
<Photon Counting> or <Analog>

Format Refers to the instrument configuration geometry:
<L-format> is used for measurements utilizing one emission channel;
<T-format> is used for measurements utilizing two emission channels.

Side Defines which emission channel is used for the

measurement. In case of T-format measurements this option is redundant.

7.7.2 Dual Channel Analog Acquisition Controls

Lifetime measurements are performed utilizing the Analog acquisition mode. Upon selecting this mode the user has the option to control two additional parameters:

Gain The analog data acquisition cards supported by Vinci feature on-board gain controls accessible through the up/down arrows in this window.

Overflow Is the maximum signal digitized by the analog data acquisition card;

a. For A2D card (12-bit data acquisition card) the overflow value is 4,000;
b. For A2D200K card (16-bit data acquisition card) the overflow value is 32,000.

8 Experiments: A Detailed Exposition

Experiments can be selected in Vinci-Experiment and Instrument Control. Click on “Experiment” and the following list of experiments will appear:

If “Spectra” is selected, a menu of the various types of spectra that can be executed will be available.

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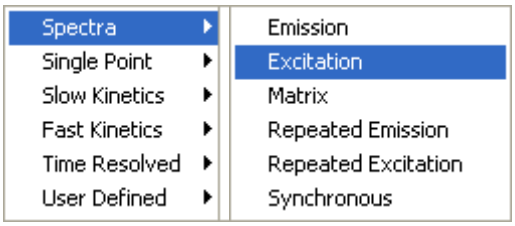
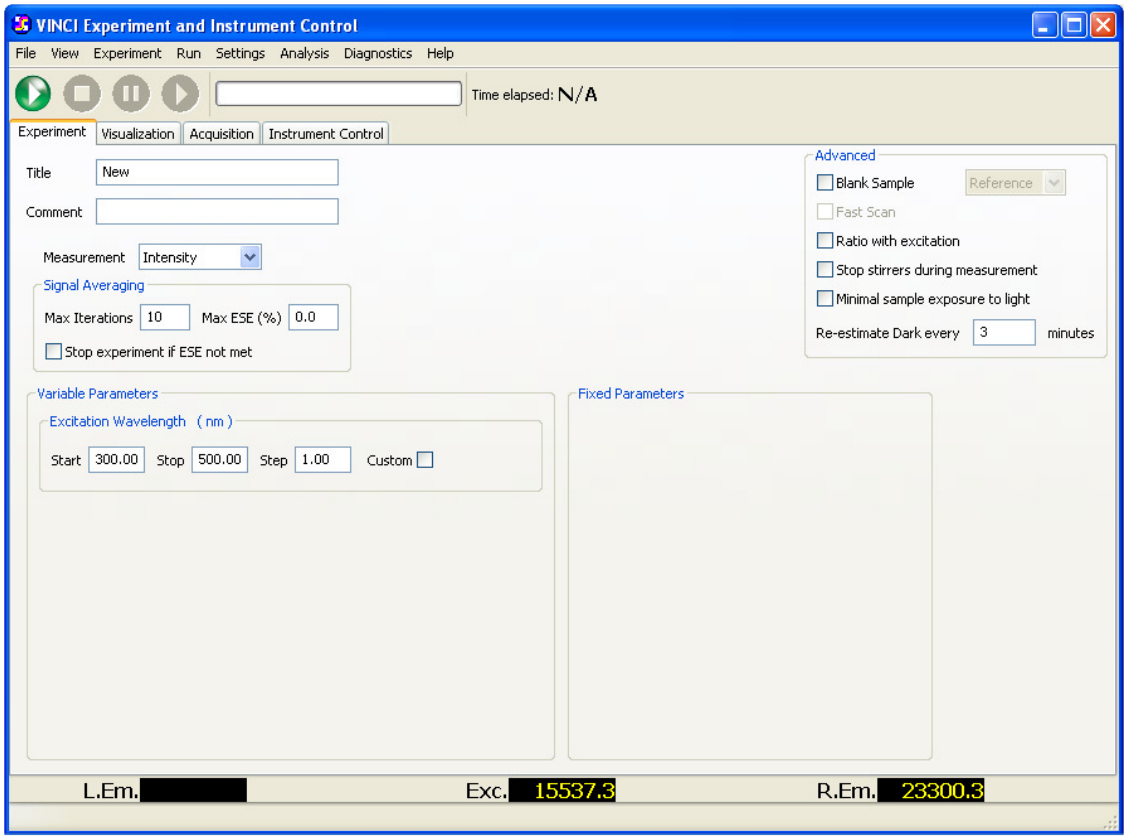


8.1 Spectra

8.1.1 Emission Spectra

For the acquisition of emission spectra the option “Emission” is selected from the menu above. When selecting “Emission” the following page is displayed:



This page contains the following parameter

Title Enter an alphanumeric title for the measurement. The title is displayed when the file is opened.

Comment Enter an additional alphanumeric text line

Measurement This field shows the measurement parameter that was selected but it also allows to choose to another parameter at this stage if necessary

Signal Averaging

Max Iterations This field indicates the time used for signal averaging. The intensity is collected with a 0.1 sec integration time. If e.g. a value of "5" is entered, the measurement of each data point will last 0.5 seconds.

Max ESE (%) Maximum Estimated Standard Error of each data point.

- If ESE = 0, the data acquisition time is the time specified in the Max Iterations (see above).
- If ESE ≠ 0, Vinci acquires data until the value defined by the user is reached; once the ESE value is reached, the program will start acquiring the next data point.

Please see Chapter 10 for more details on this issue.

Stop experiment if ESE not met If ESE ≠ 0 and the ESE value is not reached within the time set in <Max Iterations>, the program will move to the next data point unless the <Stop experiment if ESE is not met> box is checked.

Variable Parameters

Start Starting wavelength of the emission monochromator (in nanometers)

Stop Ending wavelength of the emission monochromator (in nanometers)

Step Indicates the step-size of the emission monochromator in nanometers

Custom Select this box for editing the predefined numerical values to be measured (see below)

Fixed Parameters

Value Position (in nanometers) of the excitation monochromator

Advanced Parameters

Blank sample	When this option is checked, a blank is measured under the same conditions as the sample for blank correction
Ratio with excitation	When this option is checked the signal in the emission channel is referenced with the signal in the reference channel. (see also 8.1.1).
Stop stirrers during measurements	When this option is checked, stirrers stop during the actual time of data acquisition to avoid fluctuations of the fluorescence signal
Minimal sample exposure to light	The excitation shutter is open only during the time of actual data acquisition and closed the remaining time
Re-estimate dark every ... minutes	The value of the dark current is determined every "t" minutes, where "t" is the value entered in this field.

Once the input of parameters is completed, click on the green triangle to start the data acquisition.

8.1.1.1 Emission Spectra with and without Reference Channel

In some instances it might be of interest to the user to acquire corrected emission spectra using the reference channel. Suppose we want to acquire an emission spectrum between the wavelengths λ_1 and λ_2; the intensity at each wavelength is corrected by the intensity monitored through the reference channel as in the following relation:

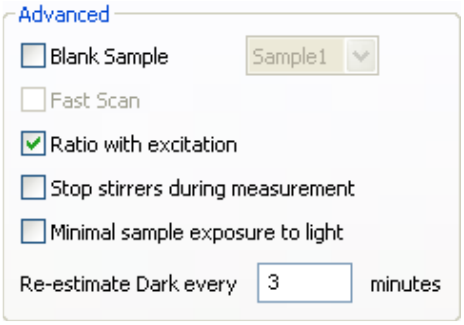
$$I_\lambda = \frac{I_{E\lambda 1}}{I_{E\lambda}} \; I_{F\lambda}$$

where:

I_λ	Corrected fluorescence intensity at wavelength λ
$I_{F\lambda}$	Fluorescence intensity at wavelength λ
$I_{E\lambda 1}$	Intensity at λ1 monitored by the reference channel
$I_{E\lambda}$	Intensity at λ monitored by the reference channel

If for some reasons the intensity of the excitation light changes (for instance fluctuations in the light source) the fluorescence intensity is expected to change correspondingly. Using the option "Ratio with excitation" helps to correct for these variations by dividing the two intensities, we get a number that is independent of these variations; this number is multiplied by the intensity of the excitation at λ1, which is used as a normalization factor.

In order to have the excitation channel correction made, check the box “Ratio with excitation” in the Advanced Parameters field.

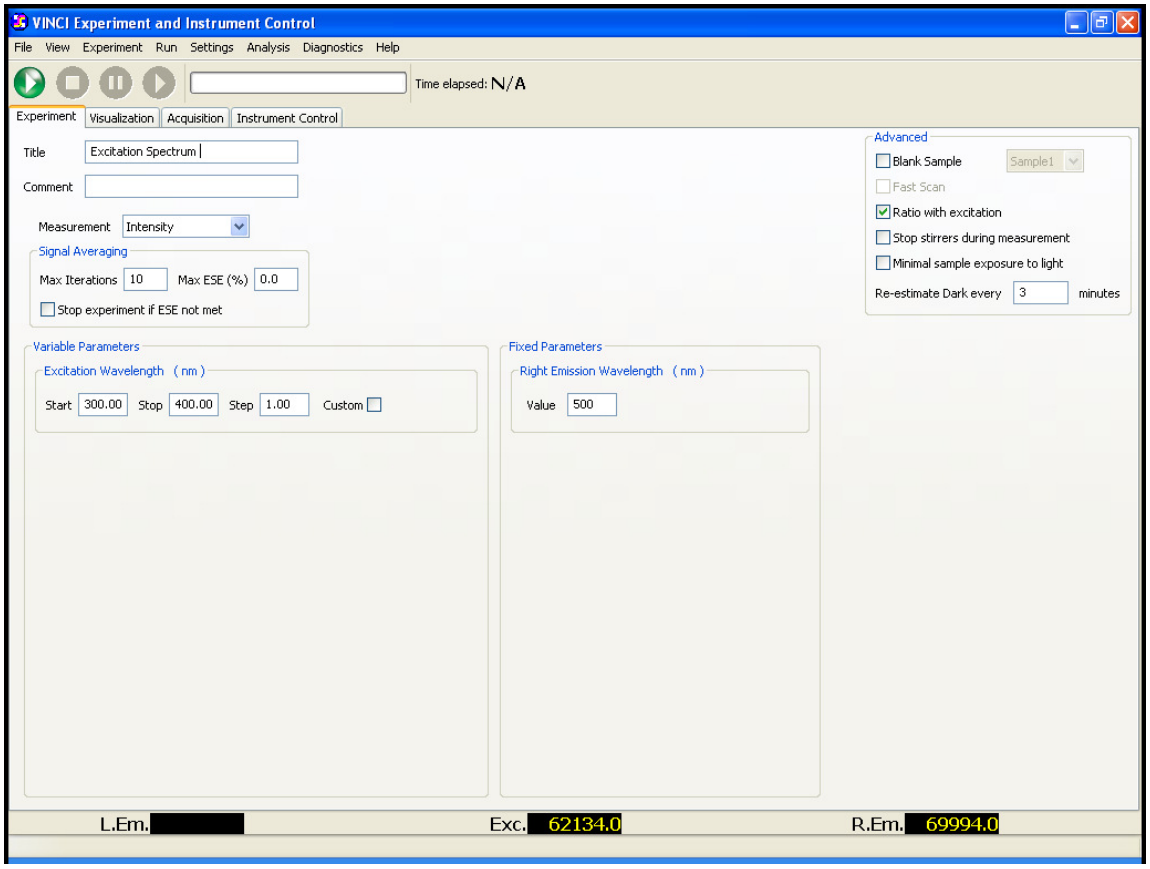


8.1.2 Excitation Spectra

In this experiment, the excitation monochromator is scanned between two wavelengths, while the emission monochromator is set at a fixed wavelength; typically, the set wavelength of the emission monochromator is higher than the maximum wavelength reached by the excitation monochromator.

Excitation spectra require the use of a quantum counter to correct for variations in the light output of the utilized light source. Excitation spectra are therefore always acquired using the “ratio with excitation” option, which is set as a default in the software.

The following page is displayed when this type of measurement is selected,:



General Parameters

Title Enter an alphanumeric title for the measurement. The title is displayed when the file is opened.

Comment Enter an additional alphanumeric text line

Measurement This field shows the measurement parameter that was selected but it also allows to choose another parameter at this stage if necessary

Signal Averaging

Max Iterations This field indicates the time used for signal averaging. The intensity is collected with a 0.1 sec integration time. If e.g. a value "5" is entered, measurement of each data point will last 0.5 seconds.

Max ESE (%) Maximum Estimated Standard Error of each data point.

- If ESE = 0, the data acquisition time is the time specified in the Max Iterations (see above).
- If ESE ≠ 0, Vinci acquires data until the value defined by the user is reached; once the ESE value is reached, the program will start acquiring the next data point.

Please see Chapter 10 for more details on this issue.

Stop experiment if ESE is not met If ESE ≠ 0 and the ESE value is not reached within the time set in <Max Iterations>, the program will move to the next data point unless the <Stop experiment if ESE is not met> box is checked.

Variable Parameters

Start Starting wavelength of the emission monochromator (in nanometers)

Stop Ending wavelength of the emission monochromator (in nanometers)

Step-Size In nanometers, the difference between successive locations of the emission monochromator

Custom Select this box for editing the values defining the position of the emission monochromator (see below)

Fixed Parameters

Value Position (in nanometers) of the emission

monochromator

Advanced Parameters

Blank sample	When this option is checked, a blank is measured under the same conditions as the sample for blank correction
Ratio with excitation	When this option is checked the signal in the emission channel is referenced with the signal in the reference channel. (see also 8.1.1).
Stop stirrers during measurements	When this option is checked, stirrers stop during the actual time of data acquisition to avoid fluctuations of the fluorescence signal
Minimal sample exposure to light	The excitation shutter is open only during the time of actual data acquisition and closed the remaining time
Re-estimate dark every ... minutes	The value of the dark current is determined every "t" minutes, where "t" is the value entered in this field.

When done entering the parameters, click onto the green arrow to start the data acquisition.

8.1.2.1 Excitation and Emission Spectra with Asymmetric Movement of the Excitation Monochromator

This feature allows the user to acquire excitation and emission spectra with asymmetric position of the monochromator. Some examples of measurements that can be acquired include the following:

- An excitation spectrum is scanned every 5 nm in the region from 300 to 400 nm; within this region, the scan step-size is 1 nm from 350 to 370 nm.
- An excitation spectrum is scanned from 400nm to 300 nm (reversed scanning)

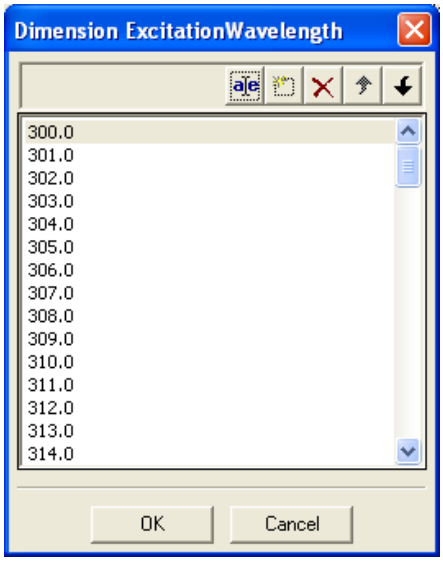
In the Experiment page, check the <Custom> box. The <Edit List> button is displayed.

For Excitation spectra

For Emission spectra

The list displays the positions of the excitation (or emission) monochromator at which the intensity of fluorescence will be recorded. When the spectrum is recorded from 300 nm to 400 nm with a 1 nm step-size, such list includes the values 300, 301, 3 02, etc.

This list can be edited by pressing the  button on top. Items on the list can be deleted by pressing the  button.



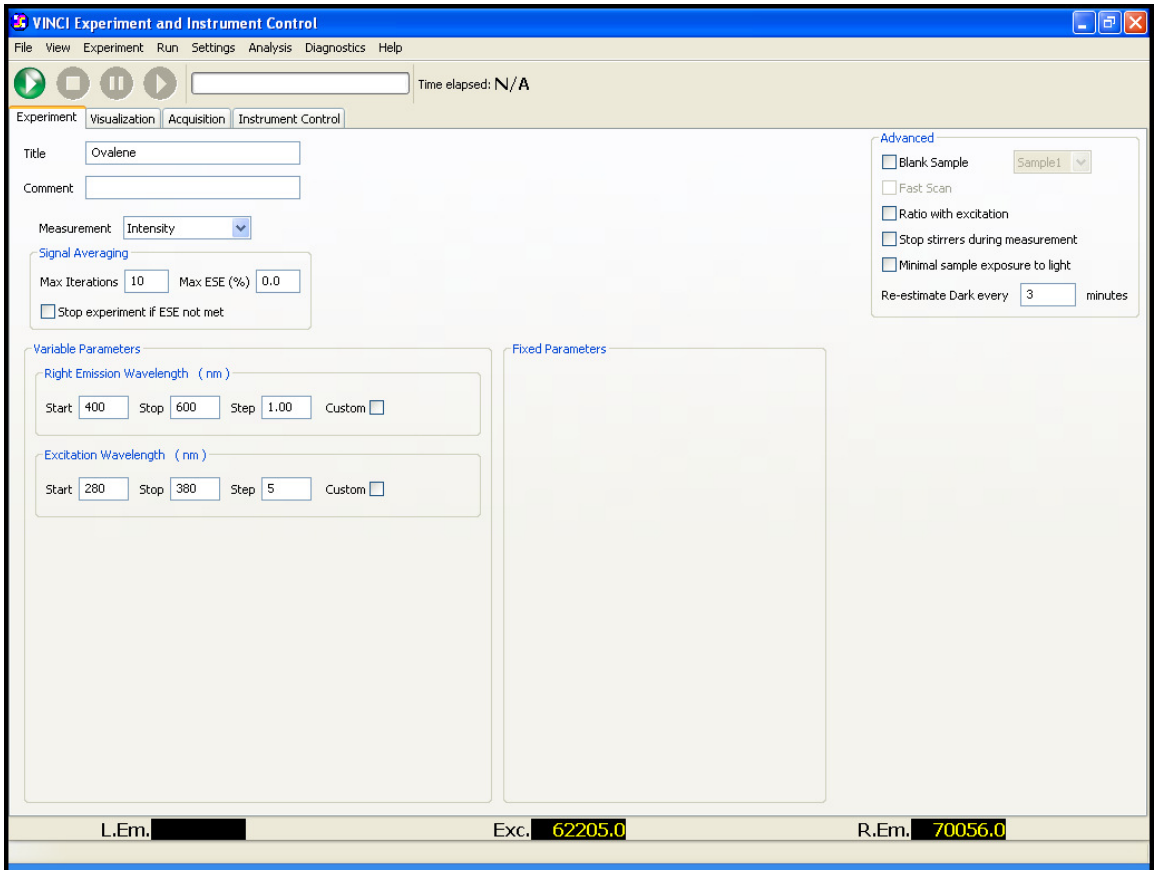
For instance, we want to acquire data from 300 nm to 400 nm but want to skip the region from 350 nm to 360 nm all the is required is to delete the values 350, 351, …, 359 and 360 from the list. The emission monochromator will move only to the points specified in the list.

8.1.3 Excitation-Emission Matrices

In this data acquisition routine, the excitation monochromator is scanned between two wavelengths with a set step size. At each position of the excitation monochromator, the emission monochromator is scanned between two wavelengths.

As an example, we will acquire spectra on ovalene. The excitation monochromator varies between 280 nm and 380 nm, with 10 nm intervals. At each interval, the emission monochromator is scanned between 400 nm and 550 nm, with 1 nm steps.

The setting is reported in the following screen. The positions of both the excitation monochromator and the emission monochromator can be edited.



General Parameters

Title Enter an alphanumeric title for the measurement. The title is displayed when the file is opened.

Comment Enter an additional alphanumeric text line

Measurement This field shows the measurement parameter that was selected

Signal Averaging

Max Iterations This field indicates the time used for signal averaging. The intensity is collected with a 0.1 sec integration time. If e.g. a value "5" is entered, measurement of each data point will last 0.5 seconds.

Max ESE (%) Maximum Estimated Standard Error of each data point.

- If ESE = 0, the data acquisition time is the time specified in the Max Iterations (see above).
- If ESE ≠ 0, Vinci acquires data until the value defined by the user is reached; once the ESE value is reached, the program will start

acquiring the next data point.
Please see Chapter 10 for more details on this issue.

Stop experiment if ESE is not met	If ESE ≠ 0 and the ESE value is not reached within the time set in <Max Iterations>, the program will move to the next data point unless the <Stop experiment if ESE is not met> box is checked.

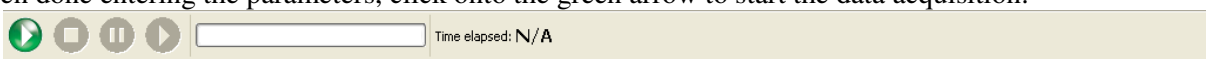
Variable Parameters for Right Emission and Excitation monochromators

Start	Starting wavelength of the monochromators (in nanometers)
Stop	Ending wavelength of the monochromators (in nanometers)
Step-Size	In nanometers, the difference between successive locations of the emission and excitation monochromators
Custom	Select this box for editing the values defining the position of the emission monochromator (see below)

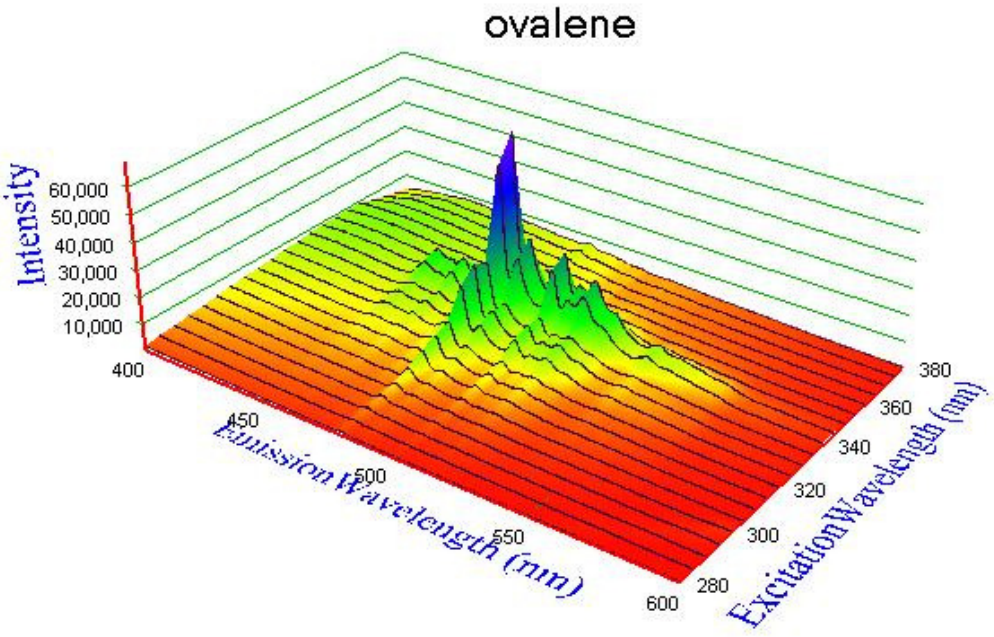
Advanced Parameters

Blank sample	When checked, the measurement on a blank is acquired and subtracted from the sample measurement data
Re-estimate dark	The value of the dark current is determined every "t" minutes
Ratio with excitation	When this option is checked the signal in the emission channel is referenced with the signal in the reference channel
Stop stirrers during measurements	When this option is checked, stirrers stop during the actual time of data acquisition to avoid fluctuations of the fluorescence signal
Minimal sample exposure to light	The excitation shutter is open only during the time of actual data acquisition and closed the remaining time

When done entering the parameters, click onto the green arrow to start the data acquisition.



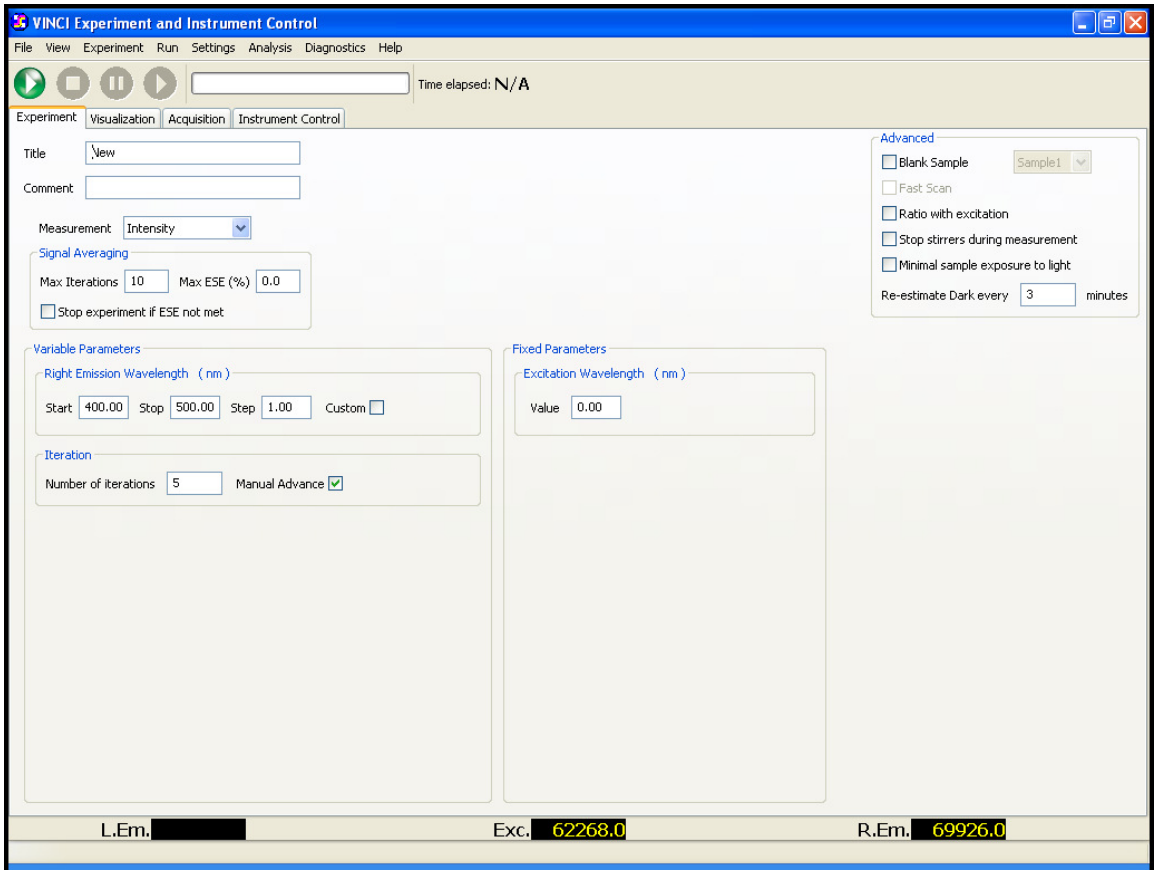
The acquired plot will look like the one reported in the following picture.



A detailed discussion of the 3D plot properties will follow in the section covering the data display. The graphic routine utilized by Vinci features several options such as zooming, rotation, and editing of the spectra colors.

8.1.4 Repeated Excitation and Emission Acquisition Spectra

This routine allows the user to acquire an emission or excitation spectrum for a specified number of times. The repetition can be automatic or manually activated by the operator. The screen-shot below shows the window, which is displayed when <Repeated Emission> is selected. The measurement option <Repeated Excitation> produces a similar screen.



Information Parameters

Title Enter an alphanumeric title for the measurement. The title is displayed when the file is opened.

Comment Enter an additional alphanumeric text line

Measurement This field shows the measurement parameter that was selected

Signal Averaging

Max Iterations This field indicates the time used for signal averaging. The intensity is collected with a 0.1 sec integration time. If e.g. a value "5" is entered, measurement of each data point will last 0.5 seconds.

Max ESE (%) Maximum Estimated Standard Error of each data point.

- If ESE = 0, the data acquisition time is the time specified in the Max Iterations (see above).
- If ESE ≠ 0, Vinci acquires data until the value defined by the user is reached; once the ESE value is reached, the program will start acquiring the next data point.

Please see Chapter 10 for more details on this issue.

Stop experiment if ESE is not met — If ESE ≠ 0 and the ESE value is not reached within the time set in <Max Iterations>, the program will move to the next data point unless the <Stop experiment if ESE is not met> box is checked.

Variable Parameters

Start — Starting wavelength of the emission monochromator (in nanometers)

Stop — Ending wavelength of the emission monochromator (in nanometers)

Step-Size — In nanometers, the difference between successive locations of the emission monochromator

Custom — Select this box for editing the values defining the position of the emission monochromator (see below)

Fixed Parameters

Value — Position (in nanometers) of the excitation monochromator

Iterations

Number of Iterations — Number of times the acquisition of the emission spectrum is desired.

Manual Advance — When the box is checked, a new acquisition is started by the experimenter by clicking on the <Continue> button.

Advanced Parameters

Blank sample — When checked, the measurement on a blank is acquired and subtracted from the sample measurement data

Re-estimate dark — The value of the dark current is determined every "t" minutes, where "t" is the value entered in this field.

Ratio with excitation — When this option is checked the signal in the emission channel is referenced with the signal in the reference channel

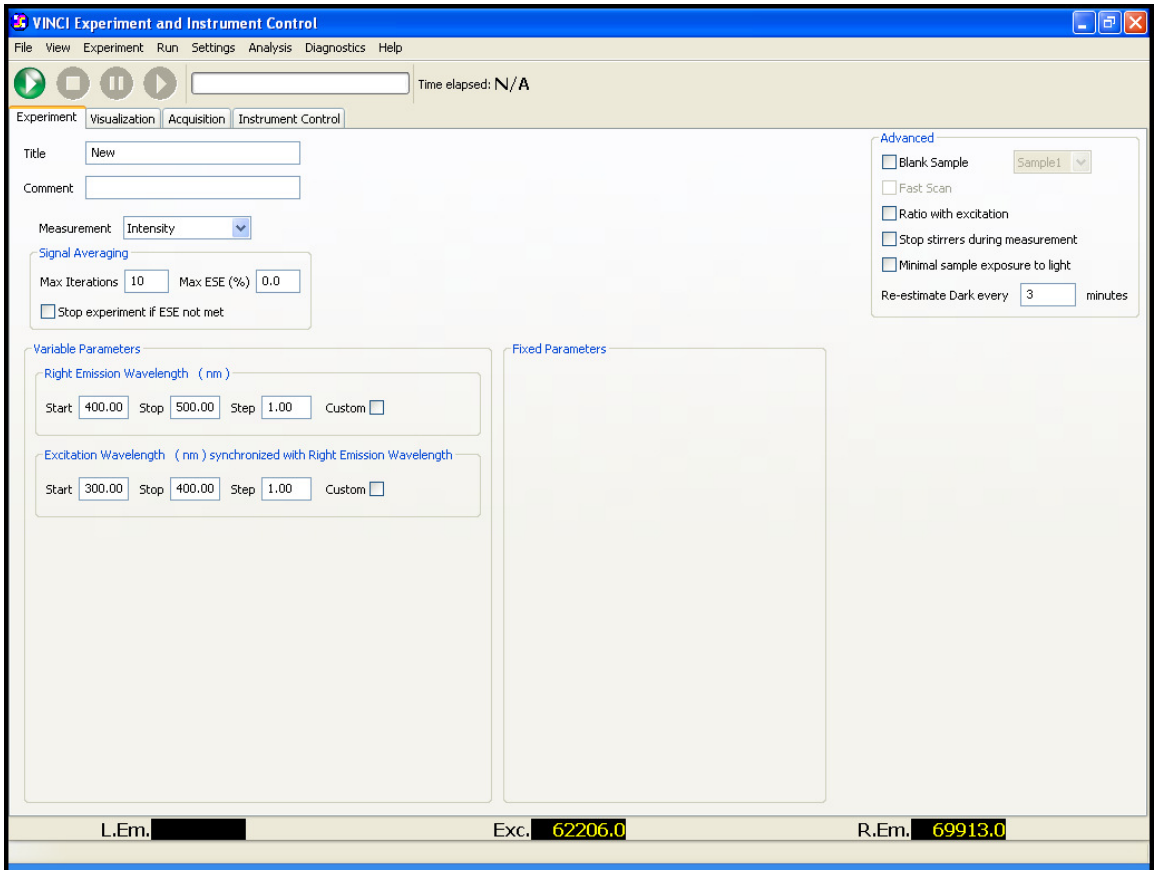
Stop stirrers during measurements	When this option is checked, stirrers stop during the actual time of data acquisition to avoid fluctuations of the fluorescence signal
Minimal sample exposure to light	The excitation shutter is open only during the time of actual data acquisition and closed the remaining time

When done entering the parameters, click onto the green arrow to start the data acquisition.

8.1.5 Synchronos Spectra

Synchronous spectra are a useful tool to investigate the range of excitation and emission wavelengths for a compound. Typically, the two monochromators are scanned in sequence with the emission monochromator starting at a longer wavelength than the excitation monochromator.

The following figure displays the window for synchronous spectra acquisition. The user is requested to enter the starting and ending wavelengths of each monochromator, along with the step size. The position of each monochromator can be edited.



General Parameters

Title Enter an alphanumeric title for the measurement. The title is displayed when the file is opened.

Comment Enter an additional alphanumeric text line

Measurement This field shows the measurement parameter that was selected

Signal Averaging

Max Iterations This field indicates the time used for signal averaging. The intensity is collected with a 0.1 sec integration time. If e.g. a value "5" is entered, measurement of each data point will last 0.5 seconds.

Max ESE (%) Maximum Estimated Standard Error of each data point.

- If ESE = 0, the data acquisition time is the time specified in the Max Iterations (see above).
- If ESE ≠ 0, Vinci acquires data until the value defined by the user is reached; once the ESE value is reached, the program will start acquiring the next data point.

Please see Chapter 10 for more details on this issue.

Stop experiment if ESE is not met If ESE ≠ 0 and the ESE value is not reached within the time set in <Max Iterations>, the program will move to the next data point unless the <Stop experiment if ESE is not met> box is checked.

Variable Parameters

Start Starting wavelength of the emission monochromator (in nanometers)

Stop Ending wavelength of the emission monochromator (in nanometers)

Step-Size In nanometers, the difference between successive locations of the excitation and emission monochromators

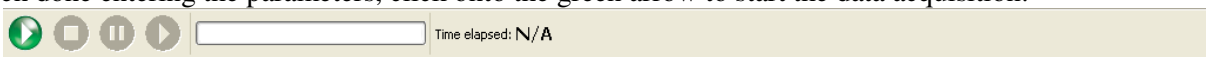
Custom Select this box for editing the values defining the position of the emission monochromator (see below)

Advanced Parameters

Blank sample When checked, the measurement on a blank is acquired and subtracted from the sample measurement data

Re-estimate dark	The value of the dark current is determined every "t" minutes
Ratio with excitation	When this option is checked the signal in the emission channel is referenced with the signal in the reference channel
Stop stirrers during measurements	When this option is checked, stirrers stop during the actual time of data acquisition to avoid fluctuations of the fluorescence signal
Minimal sample exposure to light	The excitation shutter is open only during the time of actual data acquisition and closed the remaining time

When done entering the parameters, click onto the green arrow to start the data acquisition.



8.2 Single Point Measurement

Single Point Measurements are aimed at measuring fluorescence parameters at a specific, fixed excitation or emission wavelength or if it is a ratiometric measurement at two distinct excitation or emission wavelengths.

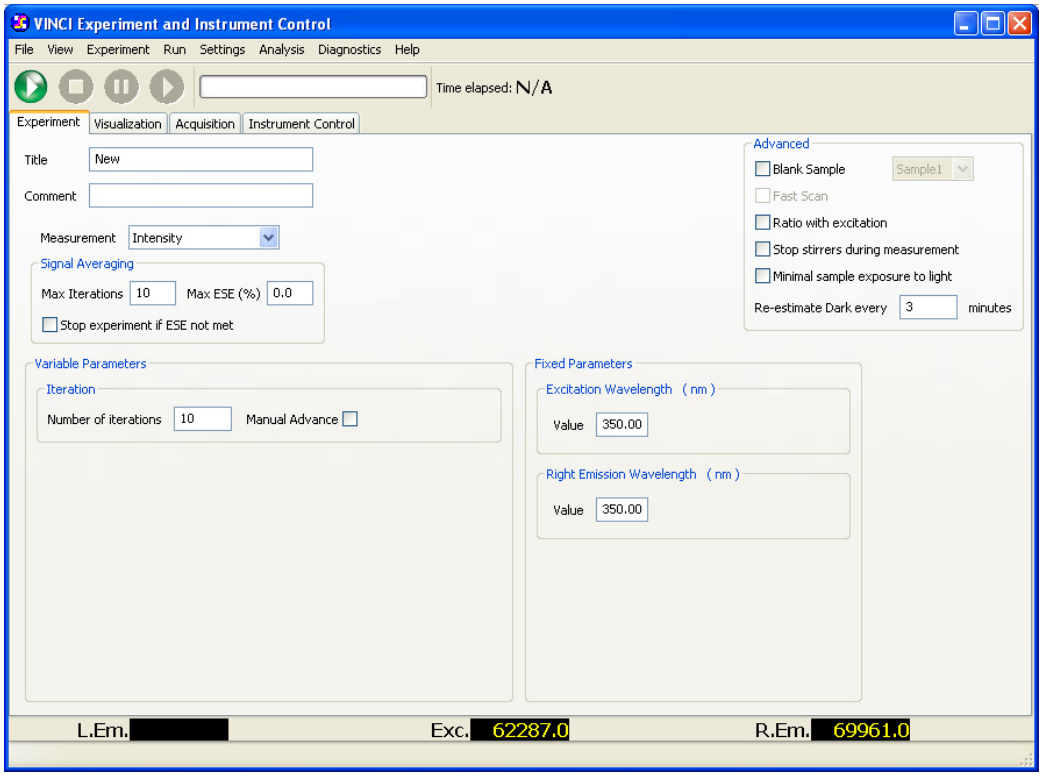
Three types of fluorescence measurements can be conducted:

- Fluorescence intensity at a specific wavelength
- Fluorescence polarization at a specific wavelength
- Ratiometric measurements at selected wavelengths

These three options are displayed when selecting <Single Point Measurements> from the <Experiment> menu.

8.2.1 Single Point Intensity Measurements

When selecting <Intensity>, the following window appears:



Information Parameters

Title Enter an alphanumeric title for the measurement. The title is displayed when the file is opened.

Comment Enter an additional alphanumeric text line

Measurement This field shows the measurement parameter that was selected

Signal Averaging

Max Iterations This field indicates the time used for signal averaging. The intensity is collected with a 0.1 sec integration time. If e.g. a value "5" is entered, measurement of each data point will last 0.5 seconds.

Max ESE (%) Maximum Estimated Standard Error of each data point.

- If ESE = 0, the data acquisition time is the time specified in the Max Iterations (see above).
- If ESE ≠ 0, Vinci acquires data until the value defined by the user is reached; once the ESE value is reached, the program will start acquiring the next data point.

Please see Chapter 10 for more details on this issue.

Stop experiment if ESE is not met If ESE ≠ 0 and the ESE value is not reached within the time set in <Max Iterations>, the program will move to the next data point unless the <Stop experiment if ESE is not met> box is checked.

Variable Parameters

# of Iterations This field indicates how many times a data point is re-measured

Manual advance If this field is checked the user initiates remeasurement of the data point with a mouse-click

Fixed Parameters

Value Position (in nanometers) of the excitation mono-chromator

Value Position (in nanometers) of the emission mono-chromator

Advanced Parameters

Blank sample	Position (in nanometers) of the excitation mono-chromator
Ratio with excitation	When this option is checked the signal in the emission channel is referenced with the signal in the reference channel
Stop stirrers during measurements	When this option is checked, stirrers stop during the actual time of data acquisition to avoid fluctuations of the fluorescence signal
Minimal sample exposure to light	The excitation shutter is open only during the time of actual data acquisition and closed the remaining time
Re-estimate dark	Number of minutes "t" between dark current checks

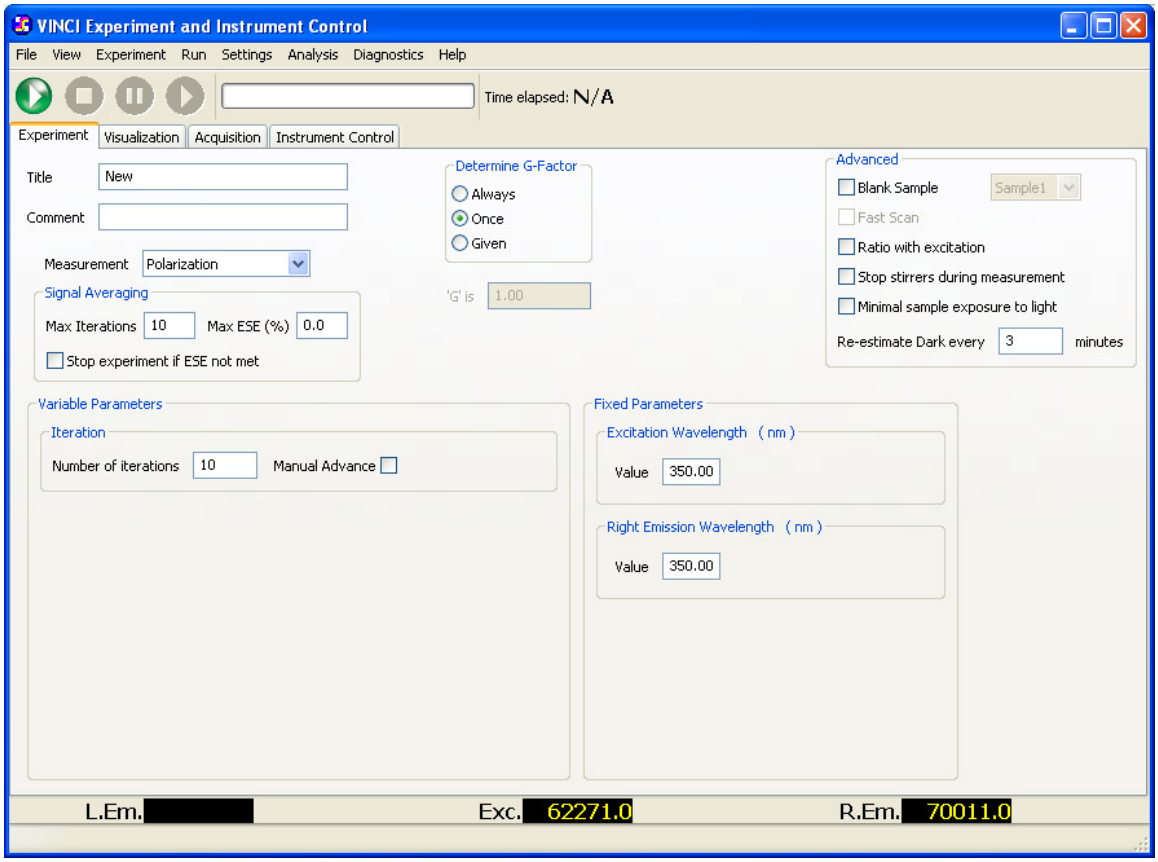
When done entering the parameters, click onto the green arrow to start the data acquisition.

8.2.2 Single Point Measurement of Fluorescence Polarization

In Single Point Polarization measurements the polarization (or anisotropy) of a sample is measured at a specific wavelength. In this type of measurements the excitation and emission wavelengths are fixed.

Left/Right channel acquisition is selected in the <Acquisition> menu. The selection is done prior to starting the data acquisition.

Upon selecting <Polarization> from the <Single Point Measurement> menu the following screen will be displayed:



Information Parameters

Title Enter an alphanumeric title for the measurement. The title is displayed when the file is opened.

Comment Enter an additional alphanumeric text line

Measurement This field shows the measurement parameter that was selected

Signal Averaging

Max Iterations This field indicates the time used for signal averaging. The intensity is collected with a 0.1 sec integration time. If e.g. a value "5" is entered, measurement of each data point will last 0.5 seconds.

Max ESE (%) Maximum Estimated Standard Error of each data point.

- If ESE = 0, the data acquisition time is the time specified in the Max Iterations (see above).
- If ESE ≠ 0, Vinci acquires data until the value defined by the user is reached; once the ESE value is reached, the program will start

acquiring the next data point.
Please see Chapter 10 for more details on this issue.

Stop experiment if ESE is not met — If ESE ≠ 0 and the ESE value is not reached within the time set in <Max Iterations>, the program will move to the next data point unless the <Stop experiment if ESE is not met> box is checked.

Variable Parameters

# of Iterations — This field indicates how many times a data point is re-measured

Manual advance — If this field is checked the user initiates remeasurement of the data point with a mouse-click

Fixed Parameters

Value — Position (in nanometers) of the excitation monochromator

Value — Position (in nanometers) of the emission monochromator

Advanced Parameters

Blank sample — Position (in nanometers) of the excitation monochromator

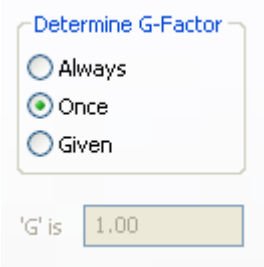
Ratio with excitation — When this option is checked the signal in the emission channel is referenced with the signal in the reference channel

Stop stirrers during measurements — When this option is checked, stirrers stop during the actual time of data acquisition to avoid fluctuations of the fluorescence signal

Minimal sample exposure to light — The excitation shutter is open only during the time of actual data acquisition and closed the remaining time

Re-estimate dark — The value of the dark current is determined every "t" minutes

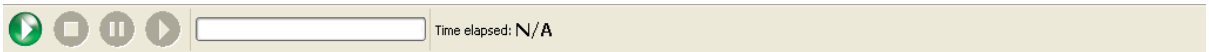
One last parameter that needs to be addressed by the user is the g-factor.



There are three choices available for the measurement of the G-factor:

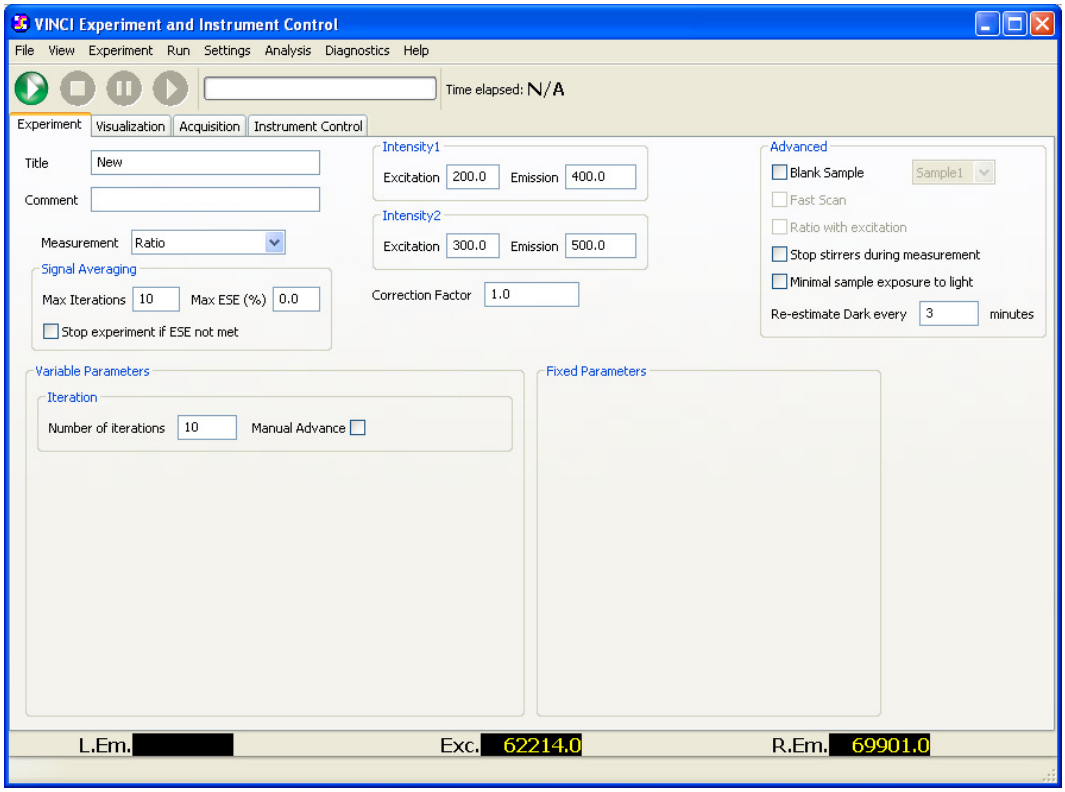
a. The G-factor is measured each time;
b. The G-factor is measured once (default value);
c. The G-factor is not measured but is directly entered in the field 'G' below

When done entering the parameters, click the green arrow to start the data acquisition.



8.2.3 Single Point Ratiometric Measurement

In this type of experiment the fluorescence intensity is measured at two fixed positions of the emission monochromator (with the excitation monochromator fixed) – emission ratiometric measurements - or at two, fixed position of the excitation monochromator (with the emission monochromator fixed) –excitation ratiometric measurements.



General Parameters

Title Enter an alphanumeric title for the measurement. The title is displayed when the file is opened.

Comment Enter an additional alphanumeric text line

Measurement This field shows the measurement parameter that was selected

Signal Averaging

Max Iterations This field indicates the time used for signal averaging. The intensity is collected with a 0.1 sec integration time. If e.g. a value "5" is entered, measurement of each data point will last 0.5 seconds.

Max ESE (%) Maximum Estimated Standard Error of each data point.

- If ESE = 0, the data acquisition time is the time specified in the Max Iterations (see above).
- If ESE ≠ 0, Vinci acquires data until the value defined by the user is reached; once the ESE value is reached, the program will start acquiring the next data point.

Please see Chapter 10 for more details on this issue.

Stop experiment if ESE is not met If ESE ≠ 0 and the ESE value is not reached within the time set in <Max Iterations>, the program will move to the next data point unless the <Stop experiment if ESE is not met> box is checked.

Variable Parameters

# of Iterations This field indicates how many times a data point is re-measured

Manual advance If this field is checked the user initiates remeasurement of the data point with a mouse-click

Fixed Parameters

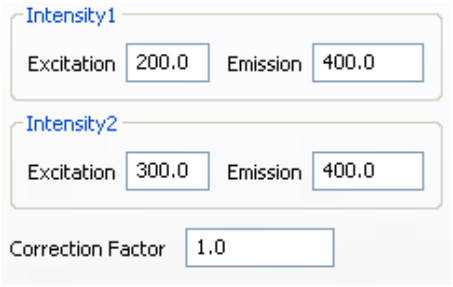
Value Position (in nanometers) of the excitation mono-chromator

Value	Position (in nanometers) of the emission mono-chromator

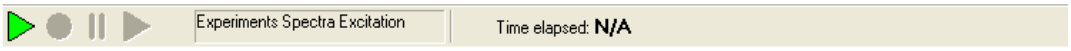
Advanced Parameters

Blank sample	Position (in nanometers) of the excitation monochromator
Stop stirrers during measurements	When this option is checked, stirrers stop during the actual time of data acquisition to avoid fluctuations of the fluorescence signal
Minimal sample exposure to light	The excitation shutter is open only during the time of actual data acquisition and closed the remaining time
Re-estimate dark	The value of the dark current is determined every "t" minutes

Ratiometric measurements require the input of additional parameters: positions of the excitation and emission monochromators and if applicable a correction factor that allows correcting for any instrument related asymmetries.



When done entering the parameters, click onto the green arrow to start the data acquisition.



8.3 Slow Kinetics

In this series of experiment, the fluorescence is measured versus time. Three types of fluorescence measurements are feasible:

- Fluorescence intensity at selected wavelengths
- Fluorescence polarization at selected wavelengths
- Ratiometric measurements at selected wavelengths

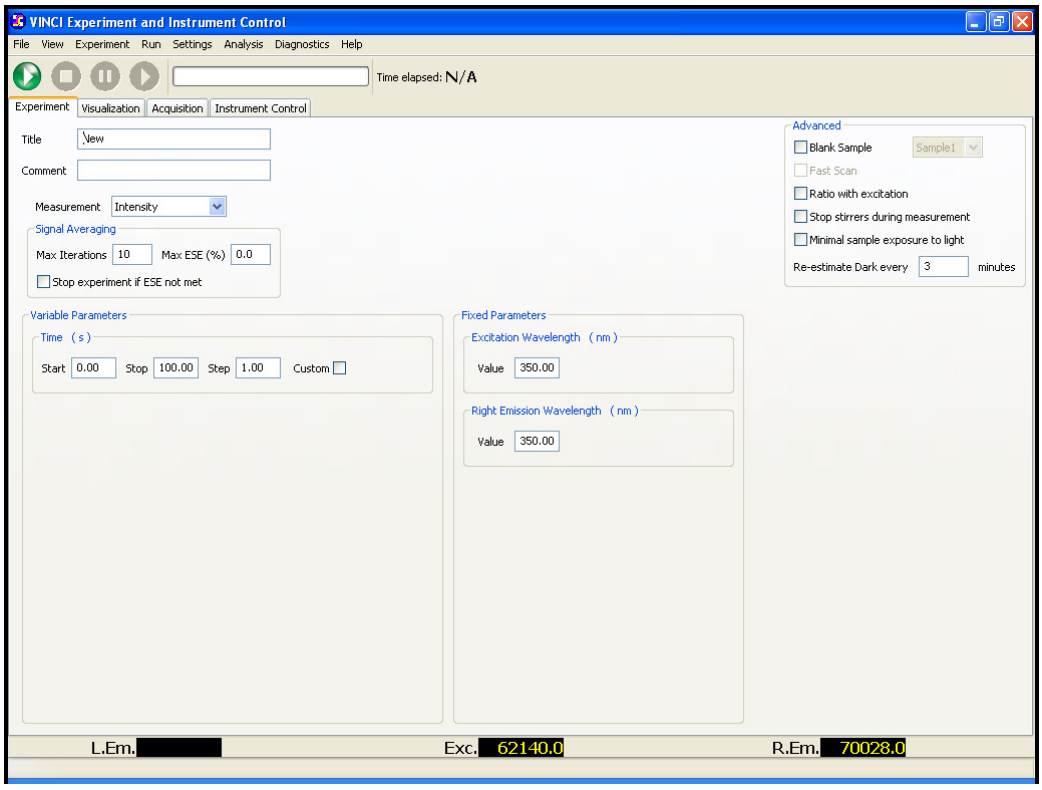
These three options are displayed when selecting <Slow Kinetics> in the Experiment list.

The shortest time interval (time resolution) that can be resolved in this type of measurements is 300 milliseconds. To acquire data with shorter time resolution the <Fast Kinetics> option has to be selected (see below).

The Left/Right channel acquisition is selected in the <Acquisition> menu. The selection is done prior to starting the data acquisition.

8.3.1 Slow Kinetics: Intensity

When selecting <Intensity>, the following window appears:



Information Parameters

Title Enter an alphanumeric title for the measurement. The title is displayed when the file is opened.

Comment Enter an additional alphanumeric text line

Measurement This field shows the measurement parameter that was selected

Signal Averaging

Max Iterations This field indicates the time used for signal averaging. The intensity is collected with a 0.1 sec integration time. If e.g. a value "5" is entered, measurement of each data point will last 0.5 seconds.

Max ESE (%) Maximum Estimated Standard Error of each data point.

- If ESE = 0, the data acquisition time is the time specified in the Max Iterations (see above).
- If ESE ≠ 0, Vinci acquires data until the value defined by the user is reached; once the ESE value is reached, the program will start acquiring the next data point.

Please see Chapter 10 for more details on this issue.

Stop experiment if ESE is not met If ESE ≠ 0 and the ESE value is not reached within the time set in <Max Iterations>, the program will move to the next data point unless the <Stop experiment if ESE is not met> box is checked.

Variable Parameters

Start Starting time of the measurement. Zero is the default value

Stop Duration time (in seconds) of the experiment. Maximum time is 99999 seconds

Step-size Time interval (in seconds) between measurements.

Custom Select this box for editing the times where data are acquired (see below)

Fixed Parameters

Value	Position (in nanometers) of the excitation mono-chromator
Value	Position (in nanometers) of the emission mono-chromator

Advanced Parameters

Blank sample	Position (in nanometers) of the excitation mono-chromator
Re-estimate dark	Number of minutes "t" between dark current checks
Ratio with excitation	When this option is checked the signal in the emission channel is referenced with the signal in the reference channel
Stop stirrers during measurements	When this option is checked, stirrers stop during the actual time of data acquisition to avoid fluctuations of the fluorescence signal
Minimal sample exposure to light	The excitation shutter is open only during the time of actual data acquisition and closed the remaining time

When done entering the parameters, click onto the green arrow to start the data acquisition.

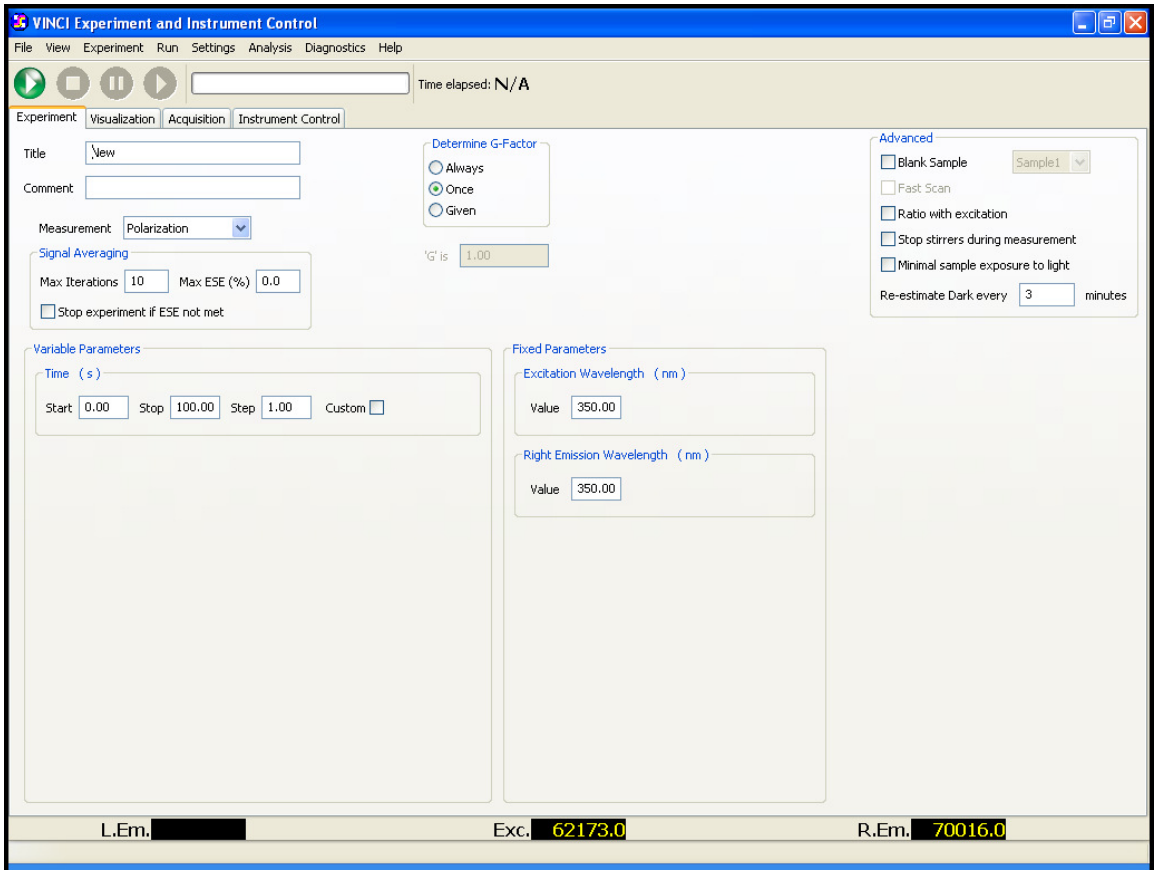
8.3.2 Slow Kinetics: Polarization

In slow polarization kinetics measurements the polarization (or anisotropy) of a sample is measured at selected time intervals. In this type of measurements the excitation and emission wavelengths are fixed.

The shortest measurable time interval (time resolution) for this type of experiments is **300 milliseconds**. To acquire data with shorter time resolution the <Fast Kinetics> option has to be selected (see below).

The Left/Right channel acquisition is selected in the <Acquisition> menu. The selection is done prior to starting the data acquisition.

Selecting <Polarization> in the <Slow Kinetics> experiment option will bring up the following screen:



Information Parameters

Title Enter an alphanumeric title for the measurement. The title is displayed when the file is opened.

Comment Enter an additional alphanumeric text line

Measurement This field shows the measurement parameter that was selected

Signal Averaging

Max Iterations This field indicates the time used for signal averaging. The intensity is collected with a 0.1 sec integration time. If e.g. a value "5" is entered, measurement of each data point will last 0.5 seconds.

Max ESE (%) Maximum Estimated Standard Error of each data point.

- If ESE = 0, the data acquisition time is the time specified in the Max Iterations (see above).
- If ESE ≠ 0, Vinci acquires data until the value defined by the user is reached; once the ESE

value is reached, the program will start acquiring the next data point.
Please see Chapter 10 for more details on this issue.

Stop experiment if ESE is not met — If ESE ≠ 0 and the ESE value is not reached within the time set in <Max Iterations>, the program will move to the next data point unless the <Stop experiment if ESE is not met> box is checked.

Variable Parameters

Start — Starting time of the measurement. Zero is the default value

Stop — Duration time (in seconds) of the experiment. Maximum time is 99999 seconds

Step-size — Time interval (in seconds) between measurements.

Custom — Select this box for editing the times where data are acquired (see below)

Fixed Parameters

Value — Position (in nanometers) of the excitation monochromator

Value — Position (in nanometers) of the emission monochromator

Advanced Parameters

Blank sample — Position (in nanometers) of the excitation monochromator

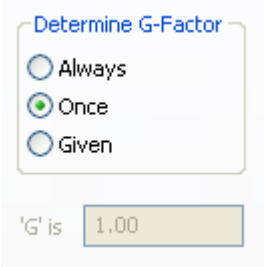
Re-estimate dark — The value of the dark current is determined every "t" minutes

Ratio with excitation — When this option is checked the signal in the emission channel is referenced with the signal in the reference channel

Stop stirrers during measurements — When this option is checked, stirrers stop during the actual time of data acquisition to avoid fluctuations of the fluorescence signal

Minimal sample exposure to light — The excitation shutter is open only during the time of actual data acquisition and closed the remaining time

One last parameter for the user is the g-factor.

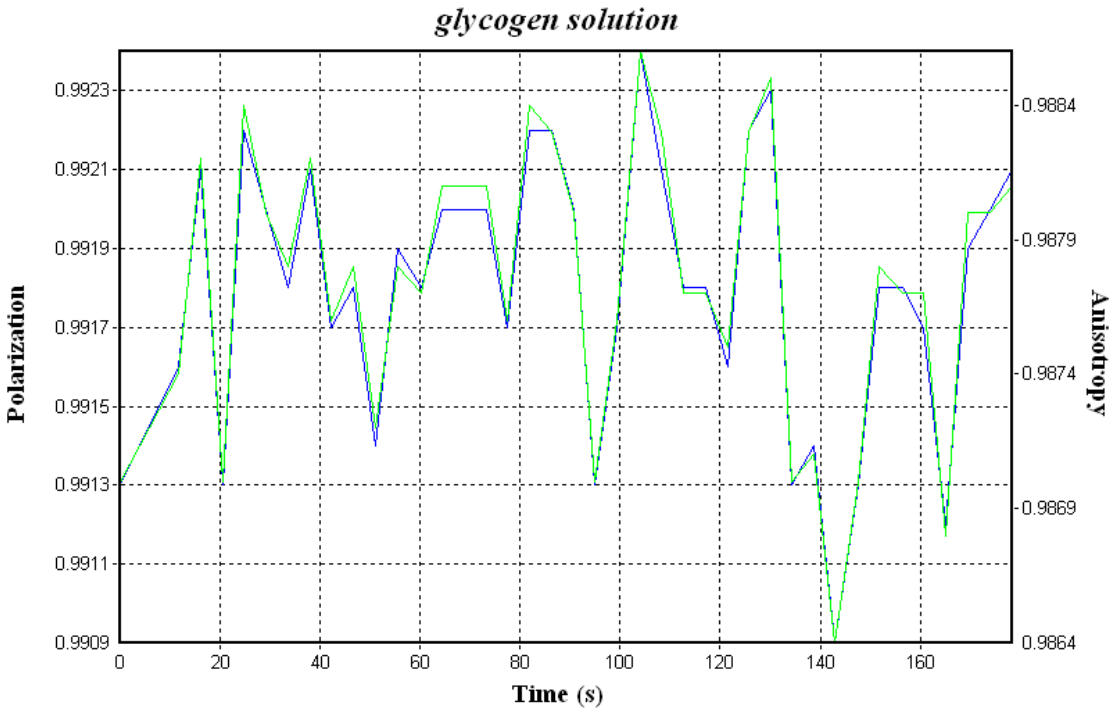


There are three choices are available:

d. The G-factor is measured each time;
e. The G-factor is measured once (default value);
f. The G-factor is never measured

When done entering the parameters, click the green arrow to start the data acquisition.

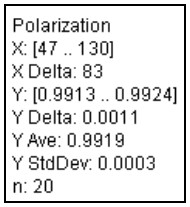
Vinci opens the "Visualization Window" and the polarization (left axis)/anisotropy (right axis) versus time plot is displayed in real-time.



Upon completion of the data acquisition, Signal Quality 

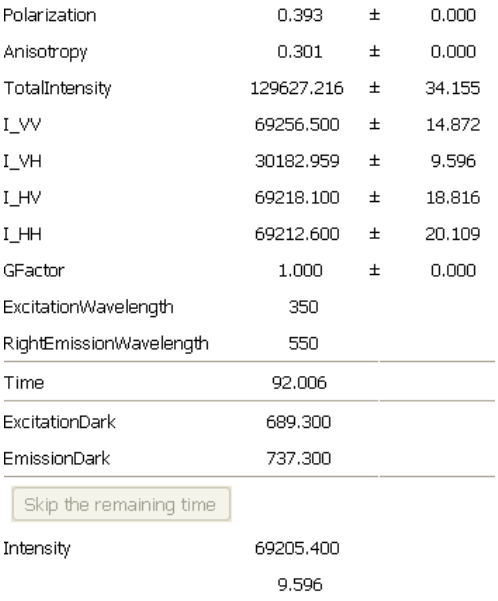
Parameters for the measurement can be calculated by clicking on the statistical symbol on the top of the plot area. The following values are displayed:

- Spread (width between minimum and maximum values) on the X and Y axes;
- Average value of Y
- Standard deviation



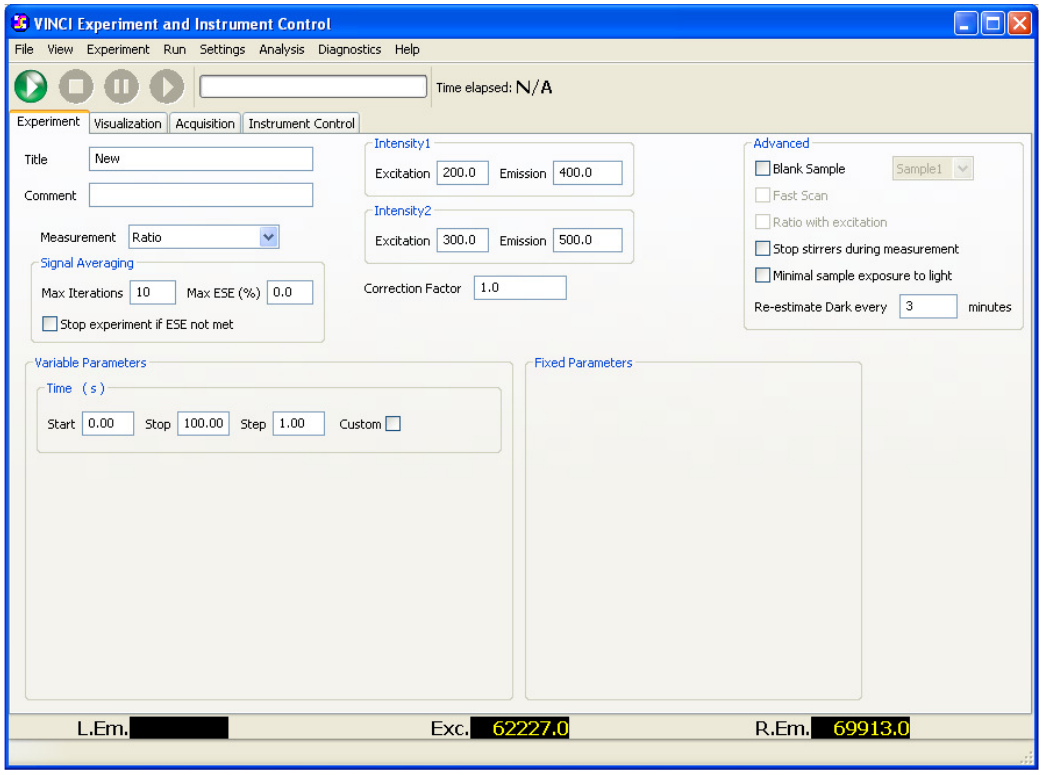
During data acquisition a dynamic window is shown on the left side of the polarization plot. The reported values are the values measured during the specified time interval.

Data acquisition can be interrupted and data are saved by pressing the <Skip Remaining Time> button in the window.



8.3.3 Ratiometric Measurements in Slow Kinetics

In this experiment the intensity of the fluorescence is measured at two locations of the emission monochromator (with the excitation monochromator fixed) or at two locations of the excitation monochromator (with the emission monochromator at a fixed position).



General Parameters

Title Enter an alphanumeric title for the measurement. The title is displayed when the file is opened.

Comment Enter an additional alphanumeric text line

Measurement This field shows the measurement parameter that was selected

Signal Averaging

Max Iterations This field indicates the time used for signal averaging. The intensity is collected with a 0.1 sec integration time. If e.g. a value "5" is entered, measurement of each data point will last 0.5 seconds.

Max ESE (%) Maximum Estimated Standard Error of each data point.

- If ESE = 0, the data acquisition time is the time specified in the Max Iterations (see above).

- If ESE ≠ 0, Vinci acquires data until the value defined by the user is reached; once the ESE value is reached, the program will start acquiring the next data point.

Please see Chapter 10 for more details on this issue.

Stop experiment if ESE is not met If ESE ≠ 0 and the ESE value is not reached within the time set in <Max Iterations>, the program will move to the next data point unless the <Stop experiment if ESE is not met> box is checked.

Variable Parameters

Start Starting time of the measurement. Zero is the default value

Stop Duration time (in seconds) of the experiment. Maximum time is 99999 seconds

Step-size Time interval (in seconds) between measurements.

Custom Select this box for editing the times where data are acquired (see below)

Advanced Parameters

Blank sample Position (in nanometers) of the excitation monochromator

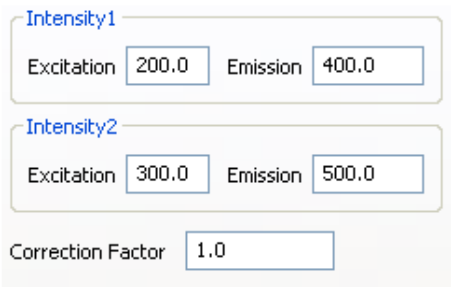
Re-estimate dark The value of the dark current is determined every "t" minutes

Ratio with excitation When this option is checked the signal in the emission channel is referenced with the signal in the reference channel

Stop stirrers during measurements When this option is checked, stirrers stop during the actual time of data acquisition to avoid fluctuations of the fluorescence signal

Minimal sample exposure to light The excitation shutter is open only during the time of actual data acquisition and closed the remaining time

Additional parameters are required for this measurement. They are the positions of the monochromators; if one monochromator is intended to stay put at a location, the same value of the wavelength is entered for both the Excitation and Emission fields.



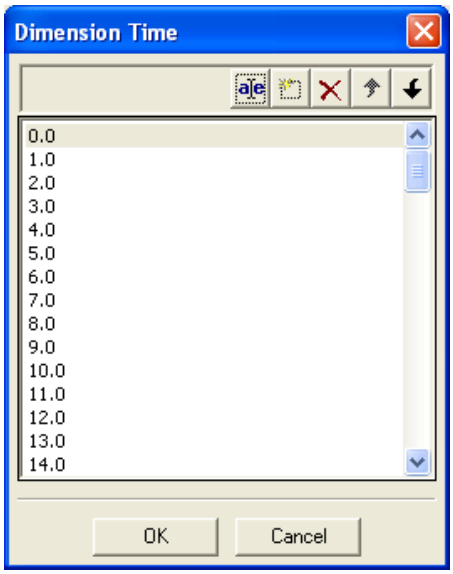
When done entering the parameters, click onto the green arrow to start the data acquisition.

8.3.4 Slow Kinetics Experiments Using an Asymmetric Time Scale

The time dimension utilized in the Slow Kinetics experiments is linearly spaced: the duration time is divided in as many time intervals as the number of measurements. Vinci allows the user to select an asymmetric time scale for each of the three measurements.

In the Experiment page, when the custom box is checked, the <Edit List> button is displayed.

The list displays the time intervals at which the intensity of fluorescence will be recorded. When stepping from 0 s to 1000 s in 1 s steps, such list includes the values 1, 2, 3, etc.



This list can be edited by pressing the  button on the top.

Items on the list can be deleted by pressing the  button.

After editing data is only acquired at the times specified in the table.

8.4 Fast Kinetics

Fast kinetics acquisition routines allow to record events from a one millisecond up to about a ten seconds time scale. In order to reach the millisecond resolution, the instrument is coupled to a stopped-flow accessory that triggers the data acquisition.
In these experiments the chosen fluorescence parameter is measured versus time and each data acquisition time interval can be as short as 100 µs. Data are acquired in photon counting mode in the time interval and displayed. Three types of fluorescence measurements are feasible:

- Fluorescence intensity at selected wavelengths
- Polarization at selected wavelengths (in T-format: simultaneous acquisition on the left and right channels)
- Ratiometric measurements at selected wavelengths

These three options are displayed when selecting <Fast Kinetics> in the Experiment list.

The Left/Right channel acquisition is selected in the <Acquisition> menu. The selection is done prior to starting the data acquisition. Note that polarization and ratio measurements require data acquisition in T-format (simultaneous acquisition on the left and right channels).

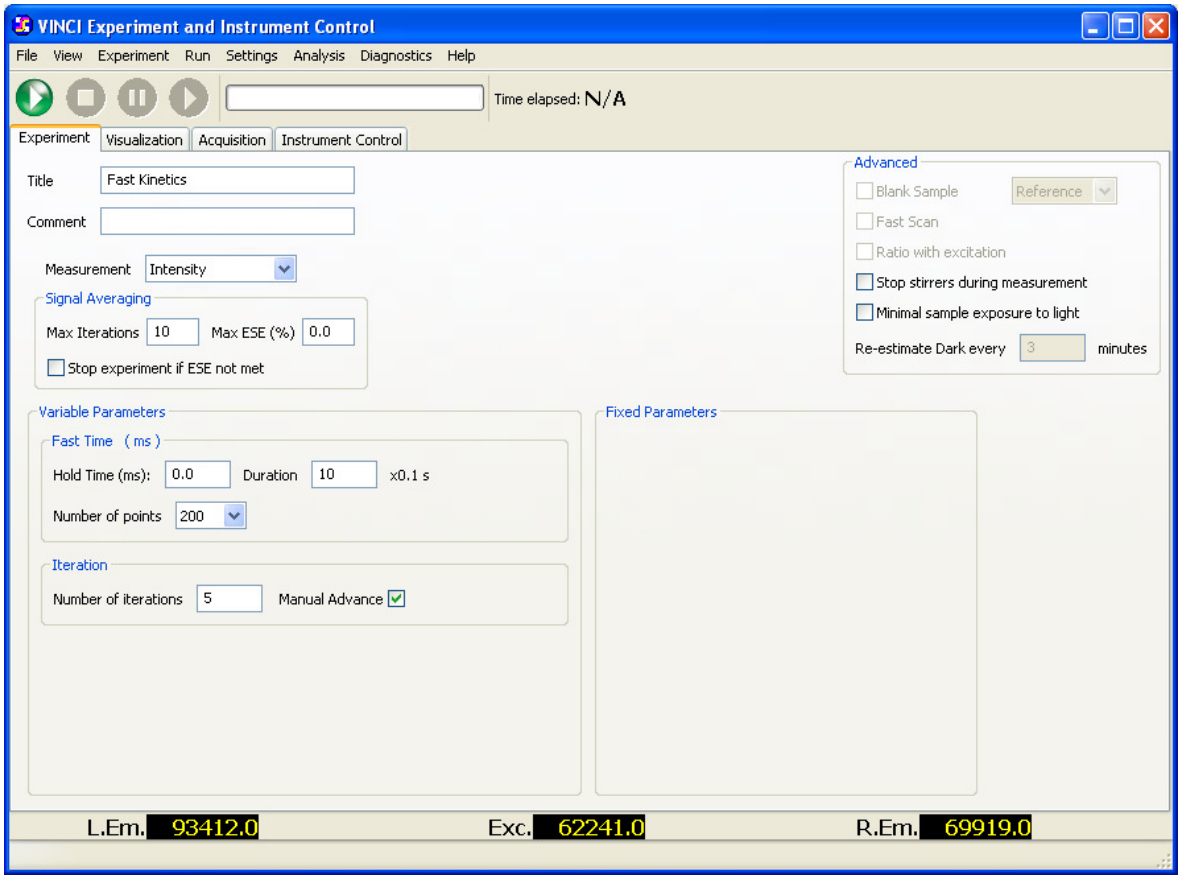
Before starting any of these experiments, please check if the stopped-flow apparatus is properly connected to the spectrofluorometer and activated in the Instrument Configuration file. As a result the stopped-flow apparatus accessory symbol is shown in the Sample Compartment in Instrument Control.

8.4.1 Data display in Fast Kinetics

In data acquisition routines that encompass the recording of each point in relatively long times (longer than 100 milliseconds) data are displayed and stored as number of counts per second. In Fast Kinetics data acquisition, data are displayed and stored in “absolute counts” the number of counts in the time window utilized for the data acquisition. When using analog acquisition instead of photon counting acquisition for Fast Kinetics, the signal displayed is the signal provided by the analog-to-digital converter.

8.4.2 Intensity

When intensity is selected, the following screen is displayed.



General Parameters

Title Enter an alphanumeric title for the measurement. The title is displayed when the file is opened.

Comment Enter an additional alphanumeric text line

Measurement This field shows the measurement parameter that was selected

Signal Averaging

Max Iterations This field indicates the time used for signal averaging. The intensity is collected with a 0.1 sec integration time. If e.g. a value "5" is entered, measurement of each data point will last 0.5 seconds.

Max ESE (%) Maximum Estimated Standard Error of each data point.

- If ESE = 0, the data acquisition time is the time specified in the Max Iterations (see above).
- If ESE ≠ 0, Vinci acquires data until the value defined by the user is reached; once the ESE value is reached, the program will start

acquiring the next data point.
Please see Chapter 10 for more details on this issue.

Stop experiment if ESE is not met — If ESE ≠ 0 and the ESE value is not reached within the time set in <Max Iterations>, the program will move to the next data point unless the <Stop experiment if ESE is not met> box is checked.

Variable Parameters

Hold time — The time is given in milliseconds. Data acquired during this time are discarded. The operator may decide to have a "zero" Hold Time and in this case data can be purged manually.

Duration — Duration of the experiment (in multiples of 0.1 seconds)

Number of points — The number of data points to be acquired during the experiment. The maximum number is 1,000 data points.

Fixed Parameters

Number of Iterations — Number of times the experiment is repeated. When using a computer-controlled stopped-flow device, the injection of reagent can be repeated automatically for the number of times specified in this field.

Manual advance — When this option is checked, the injection is activated manually.

Advanced Parameters

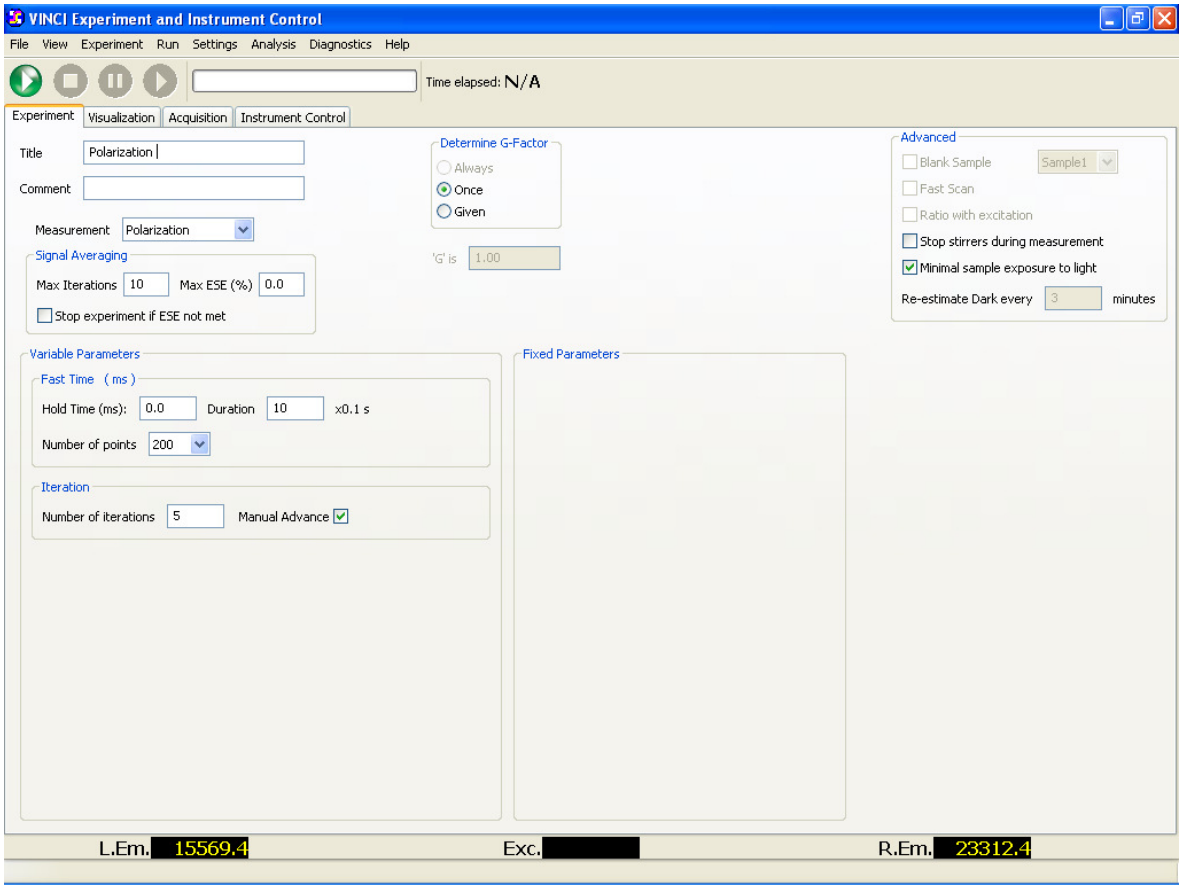
Stop stirrers during measurements — When this option is checked, stirrers stop during the actual time of data acquisition to avoid fluctuations of the fluorescence signal

Minimal sample exposure to light — The excitation shutter is open only during the time of actual data acquisition and closed the remaining time

When done entering the parameters, click onto the green arrow to start the data acquisition.

8.4.3 Fast Kinetics Polarization

This experiment is acquired in the T-format instrument set up using both the left and right emission channels with fixed polarizer settings (one at the horizontal and one at the vertical position).



Information Parameters

Title Enter an alphanumeric title for the measurement. The title is displayed when the file is opened.

Comment Enter an additional alphanumeric text line

Measurement This field shows the measurement parameter that was selected

Signal Averaging

Max Iterations This field indicates the time used for signal averaging. The intensity is collected with a 0.1 sec integration time. If e.g. a value "5" is entered, measurement of each data point will last 0.5 seconds.

Max ESE (%) Maximum Estimated Standard Error of each data point.

- If ESE = 0, the data acquisition time is the time specified in the Max Iterations (see above).
- If ESE ≠ 0, Vinci acquires data until the value defined by the user is reached; once the ESE value is reached, the program will start acquiring the next data point.

Please see Chapter 10 for more details on this issue.

Stop experiment if ESE is not met If ESE ≠ 0 and the ESE value is not reached within the time set in <Max Iterations>, the program will move to the next data point unless the <Stop experiment if ESE is not met> box is checked.

Variable Parameters

Hold time The time is given in milliseconds. Data acquired during this time are discarded. The operator may decide to have a "zero" Hold Time and in this case data can be purged manually.

Duration Duration of the experiment (in multiples of 0.1 seconds)

Number of points The number of data points to be acquired during the experiment. The maximum number is 1,000 data points.

Fixed Parameters

Number of Iterations Number of times the experiment is repeated. When using a computer-controlled stopped-flow device, the injection of reagent can be repeated automatically for the number of times specified in this field.

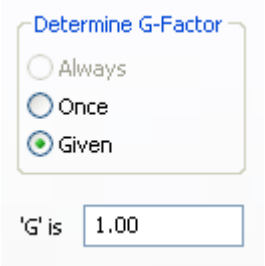
Manual advance When this option is checked, the injection is activated manually.

Advanced Parameters

Stop stirrers during measurements When this option is checked, stirrers stop during the actual time of data acquisition to avoid fluctuations of the fluorescence signal

Minimal sample exposure to light The excitation shutter is open only during the time of actual data acquisition and closed the remaining time

The final parameter for the user to decide on is the g-factor.



There are two choices:

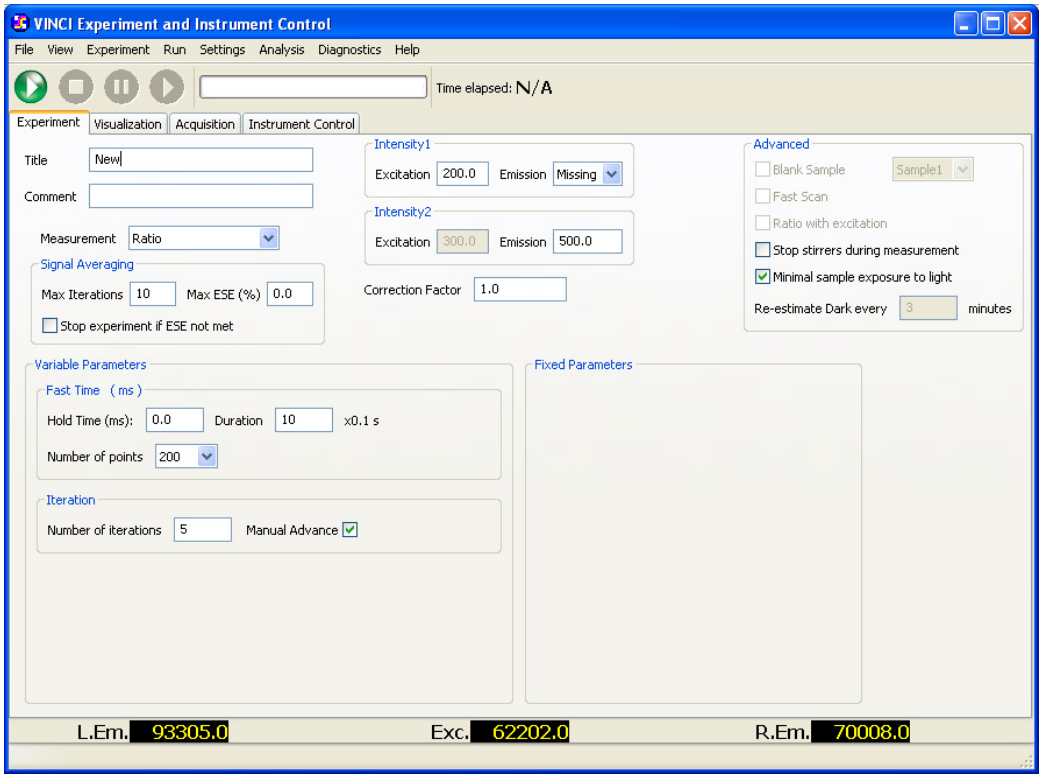
a. The G-factor is measured once (default value);
b. The G-factor is never measured

The g-factor can be measured using the statistics built in the acquisition software.

After completing the input of the required parameters, click the green arrow to start data acquisition.

8.4.4 Ratiometric Measurements

This experiment is acquired with a T-format instrument only; the fluorescence signal at two different wavelengths is acquired simultaneously on the left and right emission channels. A second emission monochromator is required in the left emission channel of the instrument; alternatively, a bandpass filter can be utilized to select the wavelength in the left emission channel.



Information Parameters

Title Enter an alphanumeric title for the measurement. The title is displayed when the file is opened.

Comment Enter an additional alphanumeric text line

Measurement This field shows the measurement parameter that was selected

Signal Averaging

Max Iterations This field indicates the time used for signal averaging. The intensity is collected with a 0.1 sec integration time. If e.g. a value "5" is entered, measurement of each data point will last 0.5 seconds.

Max ESE (%) Maximum Estimated Standard Error of each data point.

- If ESE = 0, the data acquisition time is the time specified in the Max Iterations (see above).
- If ESE ≠ 0, Vinci acquires data until the value defined by the user is reached; once the ESE value is reached, the program will start acquiring the next data point.

Please see Chapter 10 for more details on this issue.

Stop experiment if ESE is not met — If ESE ≠ 0 and the ESE value is not reached within the time set in <Max Iterations>, the program will move to the next data point unless the <Stop experiment if ESE is not met> box is checked.

Variable Parameters

Hold time — The time is given in milliseconds. Data acquired during this time are discarded. The operator may decide to have a "zero" Hold Time and in this case data can be purged manually.

Duration — Duration of the experiment (in multiples of 0.1 seconds)

Number of points — The number of data points to be acquired during the experiment. The maximum number is 1,000 data points.

Fixed Parameters

Number of Iterations — Number of times the experiment is repeated. When using a computer-controlled stopped-flow device, the injection of reagent can be repeated automatically for the number of times specified in this field.

Manual advance — When this option is checked, the injection is activated manually.

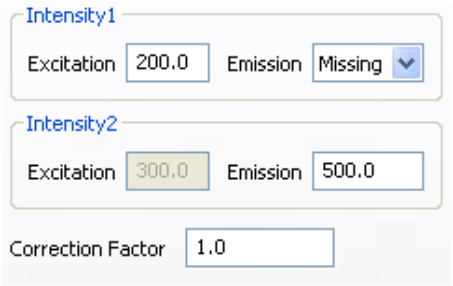
Advanced Parameters

Stop stirrers during measurements — When this option is checked, stirrers stop during the actual time of data acquisition to avoid fluctuations of the fluorescence signal

Minimal sample exposure to light — The excitation shutter is open only during the time of actual data acquisition and closed the remaining time

Re-estimate dark — The value of the dark current is determined every "t" minutes

Additional parameters are required for this measurement: the positions of the monochromators; depending on the type of measurement the excitation or the emission monochromator is intended to stay at one location. In this case the same value of the wavelength is entered for both, the Excitation or Emission.



After completing the input of the required parameters, click the green arrow to start data acquisition.

8.4.5 Summary of Measurements Options Available in Fast Kinetics

Measurement	*Dark current*	*Ratio with excitation*	*Blank*	*G-factor*
Intensity	*	√	√	Only in T-format
Polarization	*	√	√	√
Ratio	*		√	√

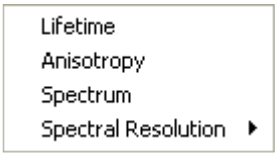
Symbols explanation	
If <Max ESE(%)> ≠ 0	*
Optional (if box is checked)	√

8.5 Frequency Domain Time-Resolved Measurements

Time-resolved measurements convey valuable information about a fluorescent molecule. There are two methods that can be utilized to measure time-resolved parameters: time domain and frequency domain. ISS has pioneered the development of frequency domain technology. Several time-resolved measurements can be performed using Vinci:

- Lifetime
- Anisotropy decays
- Time-resolved spectra
- Phase-resolved spectra

These options are displayed when selecting <Time Resolved> in the Experiment list if you have a frequency domain Chronos.

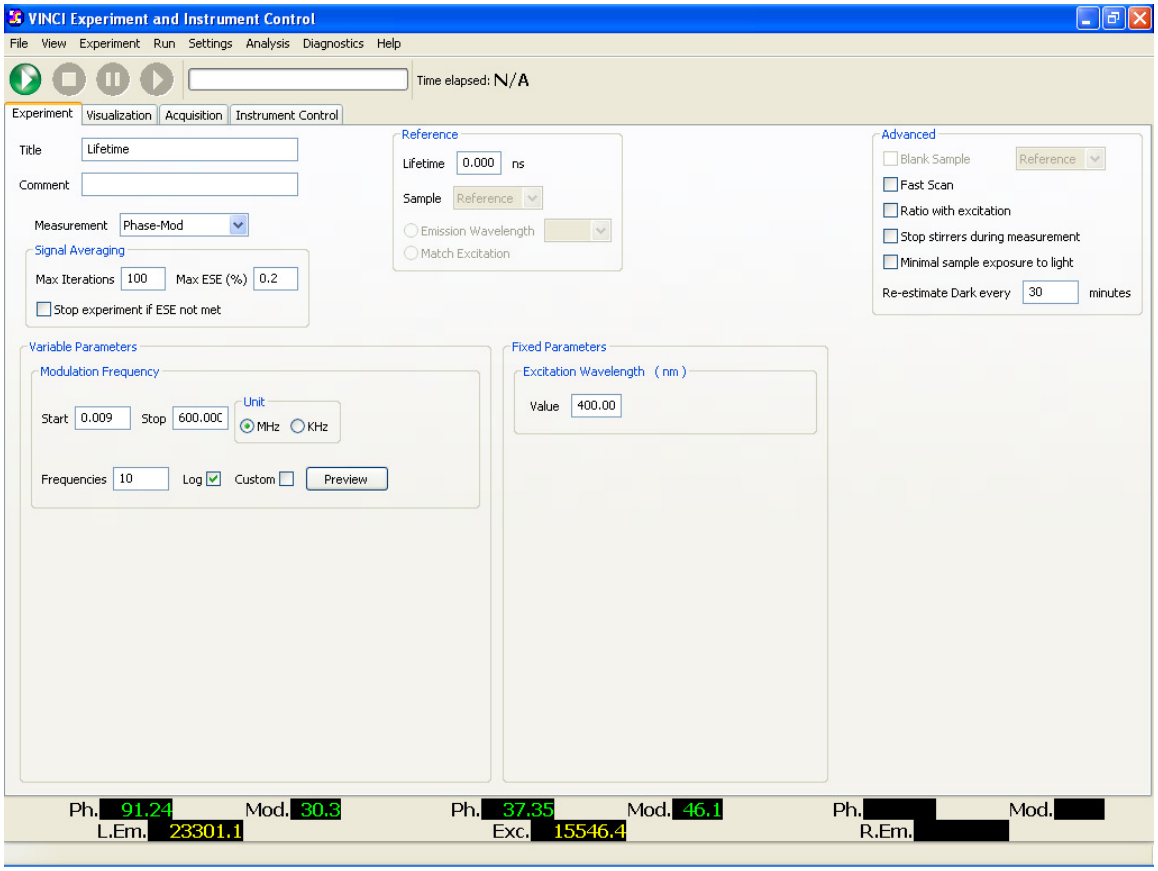


Depending on the type of measurement the Left/Right channel acquisition option needs to be selected in the <Acquisition> menu. Lifetime and anisotropy measurements are performed using a filter in the left emission channel while time-resolved spectra or phase-resolved spectra require a monochromator (typically installed on the right channel). The selection needs to be done prior to starting the data acquisition.

8.5.1 Lifetime Measurements in Frequency Domain

Fluorescence Lifetime measurements in the frequency domain require to measure the phase angle and modulation of a sample. This is typically done by using a reference compound with a known single exponential fluorescence decay time and measuring the unknown compound against this "Reference". Data on lifetime standards are given on the ISS website http://www.iss.com/resources/lifetime.html.

When checking "Lifetime" from the time-resolved measurement option in the "Experiment" menu the following screen will be retrieved:



General Parameters

Title Enter an alphanumeric title for the measurement. The title is displayed when the file is opened.

Comment Enter an additional alphanumeric text line

Measurement This field shows the measurement parameter that was selected

Signal Averaging

Max Iterations This field indicates the time used for signal averaging. The intensity is collected with a 0.1 sec integration time. If e.g. a value "5" is entered, measurement of each data point will last 0.5 seconds.

Max ESE (%) Maximum Estimated Standard Error of each data point.

- If ESE = 0, the data acquisition time is the time specified in the Max Iterations (see above).
- If ESE ≠ 0, Vinci acquires data until the value defined by the user is reached; once the ESE value is reached, the program will start

acquiring the next data point.
Please see Chapter 10 for more details on this issue.

Stop experiment if ESE is not met — If ESE ≠ 0 and the ESE value is not reached within the time set in <Max Iterations>, the program will move to the next data point unless the <Stop experiment if ESE is not met> box is checked.

Reference

Lifetime — Lifetime of the "Standard" in [ns]

Variable Parameters
Modulation Frequency

Start — Numerical value of starting frequency

Stop — Numerical value of final frequency to be measured

Unit — MHz or KHz. This selection is required depending on the expected lifetime of the sample. Short lifetime samples require selection of MHz. Long-lifetime (phosphorescent) samples require selection of KHz.

Frequencies — Number of frequencies for measurement. The higher the number of frequencies the better but practically 15 to 20 frequencies will suffice for reliable measurement of a multi-exponential decay time.

Log — Frequencies are measured in a logarithmic scale

Custom — User-defined frequency scale

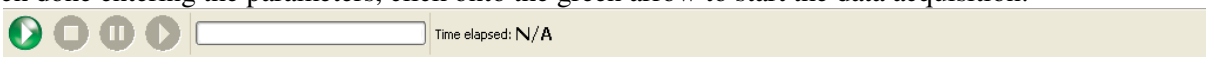
Advanced Parameters

Blank Sample — Shows the location of the blank in the sample compartment. This option only applies to 3 and 4 position sample compartments. When this option is checked, blank subtraction will be performed during measurement. Blank subtraction will correct any kind of fluorescence background by directly subtract the background phasor from sample. A blank that will properly represent the background of sample should be placed in the location of the blank in the sample compartment. This is a new feature added to Vinci later. If you don't have this option available, please contact ISS for a software update.

Fast Scan — When this option is checked, frequency domain data will be acquired in an expedited fashion and this option

	allows to measure lifetime data (full frequency responses) on standard fluorophores in less than a minute
Ratio with excitation	Referencing with the reference channel
Stop stirrers during measurements	When this option is checked, stirrers stop during the actual time of data acquisition to avoid fluctuations of the fluorescence signal
Minimal sample exposure to light	The excitation shutter is open only during the time of actual data acquisition and closed the remaining time
Re-estimate Dark	Allows to enter a time in minutes for re-estimation of dark counts

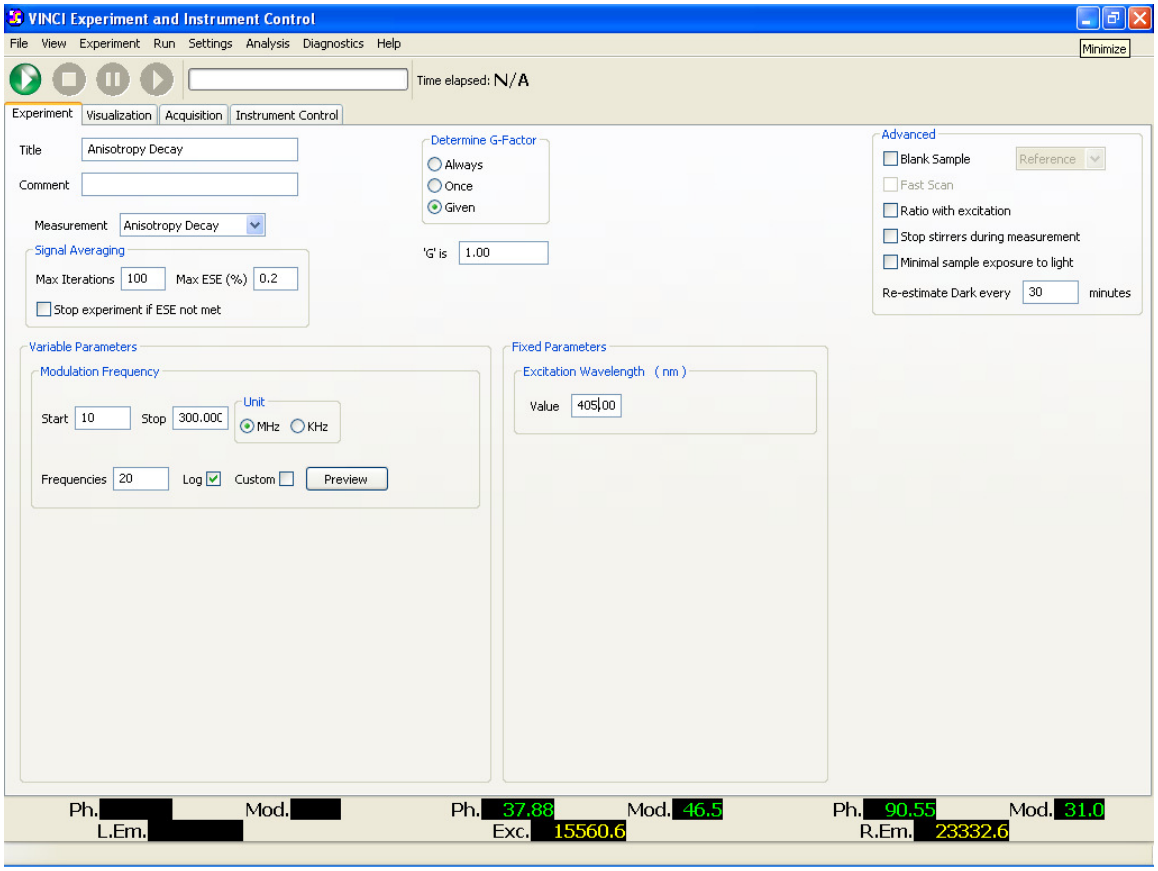
When done entering the parameters, click onto the green arrow to start the data acquisition.



8.5.2 Anisotropy Decay Measurements in Frequency Domain: Delta Phase and Amplitude Ratio

These measurements are the frequency domain analog of anisotropy decays. In frequency domain there are two quantities, which characterize an anisotropy decay: differential polarized phase angle or **delta phase** Δ_ω (between the perpendicular $\phi_\perp$ and parallel $\phi_\parallel$ components of the emission) at modulation frequency ω, and the ratio of the parallel $m_\parallel$ and perpendicular $m_\perp$ components of the modulated emission (Λ_ω) – **amplitude ratio**.

When checking the option anisotropy in the time-resolved measurements menu the following screen will be retrieved:



Please note that in those cases where an emission filter (left emission channel) rather than a monochromator is used in the experiment the G-factor = 1.

General Parameters

Title Enter an alphanumeric title for the measurement. The title is displayed when the file is opened.

Comment Enter an additional alphanumeric text line

Measurement This field shows the measurement parameter that was selected

Signal Averaging

Max Iterations This field indicates the time used for signal averaging. The intensity is collected with a 0.1 sec integration time. If e.g. a value "5" is entered, measurement of each data point will last 0.5 seconds.

Max ESE (%) Maximum Estimated Standard Error of each data point.

- If ESE = 0, the data acquisition time is the time specified in the Max Iterations (see above).

- If ESE ≠ 0, Vinci acquires data until the value defined by the user is reached; once the ESE value is reached, the program will start acquiring the next data point.

Please see Chapter 10 for more details on this issue.

Stop experiment if ESE is not met — If ESE ≠ 0 and the ESE value is not reached within the time set in <Max Iterations>, the program will move to the next data point unless the <Stop experiment if ESE is not met> box is checked.

Variable Parameters

Modulation Frequency

Start — Numerical value of starting frequency

Stop — Numerical value of final frequency to be measured

Unit — MHz or KHz. This selection is required depending on the expected lifetime of the sample. Short lifetime samples require selection of MHz. Long-lifetime (phosphorescent) samples require selection of KHz.

Frequencies — Number of frequencies for measurement. The higher the number of frequencies the better but practically 15 to 20 frequencies will suffice for reliable measurement of a multi-exponential decay time.

Log — Frequencies are spaced on a logarithmic scale

Custom — User-defined frequency scale

Advanced Parameters

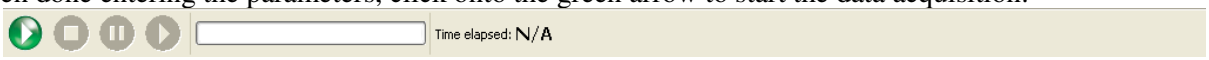
Blank Sample — Shows the location of the blank in the sample compartment. When this option is checked, blank subtraction will be performed during measurement. Blank subtraction will correct any kind of fluorescence background by directly subtract the background phasor from sample. A blank that will properly represent the background of sample should be placed in the location of the blank in the sample compartment. This is a new feature that is only available from Vinci ver 1.7. If you don't have this option available, please contact ISS for a software update.

Stop stirrers during measurements — When this option is checked, stirrers stop during the actual time of data acquisition to avoid fluctuations of the fluorescence signal

Minimal sample — The excitation shutter is open only during the time of

exposure to light	actual data acquisition and closed the remaining time
Re-estimate Dark	Allows to enter a time in minutes for re-estimation of dark counts

When done entering the parameters, click onto the green arrow to start the data acquisition.

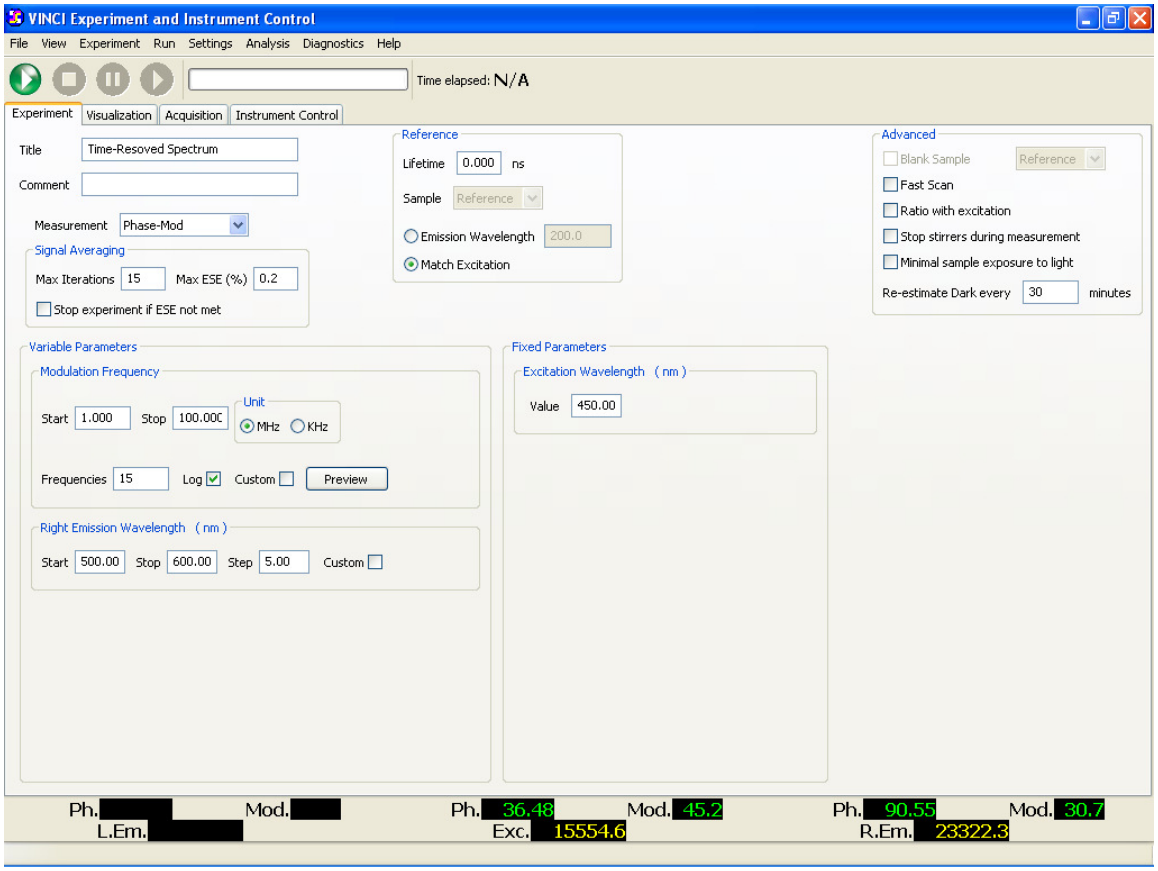


8.5.3 Time-Resolved Spectra

When checking the option “Spectrum” in the time-resolved measurements menu the following screen will be retrieved:

General Parameters

Title	Enter an alphanumeric title for the measurement. The title is displayed when the file is opened.
Comment	Enter an additional alphanumeric text line
Measurement	This field shows the measurement parameter that was selected



Signal Averaging

Max Iterations This field indicates the time used for signal averaging. The intensity is collected with a 0.1 sec integration time. If e.g. a value "5" is entered, measurement of each data point will last 0.5 seconds.

Max ESE (%) Maximum Estimated Standard Error of each data point.

- If ESE = 0, the data acquisition time is the time specified in the Max Iterations (see above).
- If ESE ≠ 0, Vinci acquires data until the value defined by the user is reached; once the ESE value is reached, the program will start acquiring the next data point.

Please see Chapter 10 for more details on this issue.

Stop experiment if ESE is not met If ESE ≠ 0 and the ESE value is not reached within the time set in <Max Iterations>, the program will move to the next data point unless the <Stop experiment if ESE is not met> box is checked.

Reference

Lifetime	Lifetime of the "Standard" in ns
Sample	Defines the location of the Lifetime Standard in sample compartment (only applies if the position the standard in the sample compartment is not pre-defined)
Emission Wavelength	Wavelength in [nm] of the emission monochromator for measuring the reference
Match Excitation	Is chosen if in case the lifetime standard is a scattering solution (e.g. Ludox or glycogen)

Variable Parameters

Modulation Frequency

Start	Numerical value of starting frequency
Stop	Numerical value of final frequency to be measured
Unit	MHz or KHz. This selection is required depending on the expected lifetime of the sample. Short lifetime samples require selection of MHz. Long-lifetime (phosphorescent) samples require selection of KHz.
Frequencies	Number of frequencies for measurement. The higher the number of frequencies the better but practically 15 to 20 frequencies will suffice for reliable measurement of a multi-exponential decay time.
Log	Frequencies are measured in a logarithmic scale
Custom	User-defined frequency scale

Right Emission Wavelength [nm]

Start	Starting wavelength of the emission monochromator (in nanometers)
Stop	Ending wavelength of the emission monochromator (in nanometers)
Step-Size	In nanometers, the difference between successive locations of the emission monochromator
Custom	Select this box for editing the values defining the position of the emission monochromator (see below)

Advanced Parameters

Fast Scan	When this option is checked, frequency domain data will be acquired in an expedited fashion and this option allows to measure lifetime data (full frequency responses) on standard fluorophores in less than a minute
Ratio with excitation	When this option is checked the signal in the emission channel is referenced with the signal in the reference channel
Stop stirrers during measurements	When this option is checked, stirrers stop during the actual time of data acquisition to avoid fluctuations of the fluorescence signal
Minimal sample exposure to light	The excitation shutter is open only during the time of actual data acquisition and closed the remaining time
Re-estimate Dark	Allows to enter a time in minutes for re-estimation of dark counts

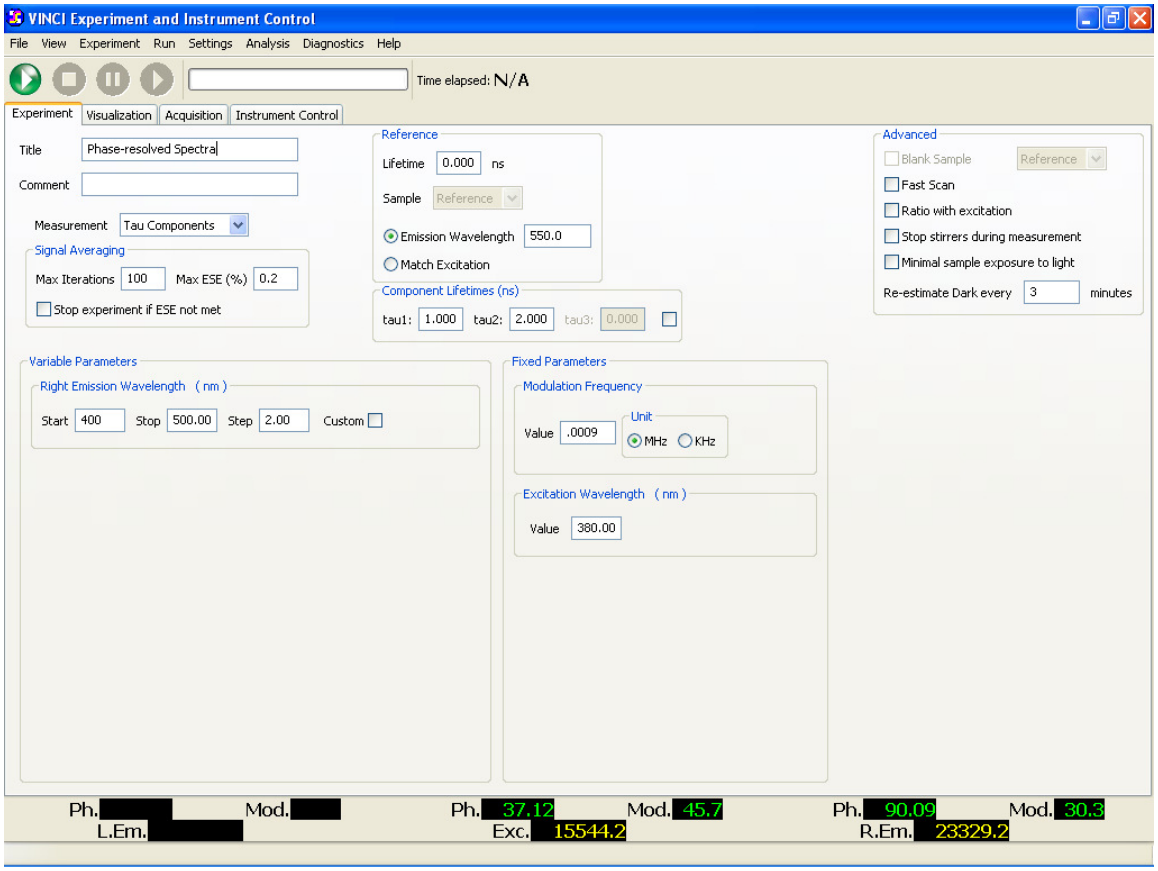
When done entering the parameters, click onto the green arrow to start the data acquisition.

8.5.4 Phase-Resolved Spectra

When checking the option “Spectrum” in the time-resolved measurements menu the following screen will be retrieved:

General Parameters

Title	Enter an alphanumeric title for the measurement. The title is displayed when the file is opened.
Comment	Enter an additional alphanumeric text line
Measurement	This field shows the measurement parameter that was selected



Signal Averaging

Max Iterations This field indicates the time used for signal averaging. The intensity is collected with a 0.1 sec integration time. If e.g. a value "5" is entered, measurement of each data point will last 0.5 seconds.

Max ESE (%) Maximum Estimated Standard Error of each data point.

- If ESE = 0, the data acquisition time is the time specified in the Max Iterations (see above).
- If ESE ≠ 0, Vinci acquires data until the value defined by the user is reached; once the ESE value is reached, the program will start acquiring the next data point.

Please see Chapter 10 for more details on this issue.

Stop experiment if ESE is not met If ESE ≠ 0 and the ESE value is not reached within the time set in <Max Iterations>, the program will move to the next data point unless the <Stop experiment if ESE is not met> box is checked.

Reference

Lifetime	Lifetime of the "Standard" in ns
Sample	Location of the Lifetime Standard in sample compartment
Emission Wavelength	Wavelength in [nm] of the emission monochromator for measuring the reference
Match Excitation	Is chosen if in case the lifetime standard is a scattering solution (e.g. Ludox or glycogen)

Component Lifetime [ns]

Tau 1	Lifetime in [ns] of Component 1 in the mixture
Tau 2	Lifetime in [ns] of Component 2 in the mixture

Variable Parameters

Right Emission Wavelength [nm]

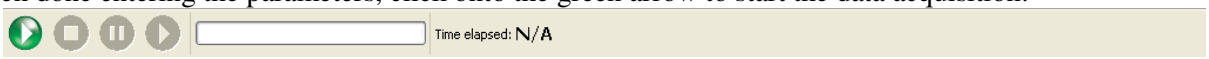
Start	Starting wavelength of the emission monochromator (in nanometers)
Stop	Ending wavelength of the emission monochromator (in nanometers)
Step-Size	In nanometers, the difference between successive locations of the emission monochromator
Custom	Select this box for editing the values defining the position of the emission monochromator (see below)

Advanced Parameters

Fast Scan	When this option is checked, frequency domain data will be acquired in an expedited fashion and this option allows to measure lifetime data (full frequency responses) on standard fluorophores in less than a minute
Ratio with excitation	When this option is checked the signal in the emission channel is referenced with the signal in the reference channel
Stop stirrers during	When this option is checked, stirrers stop during the

measurements	actual time of data acquisition to avoid fluctuations of the fluorescence signal
Minimal sample exposure to light	The excitation shutter is open only during the time of actual data acquisition and closed the remaining time
Re-estimate Dark	Allows to enter a time in minutes for re-estimation of dark counts

When done entering the parameters, click onto the green arrow to start the data acquisition.

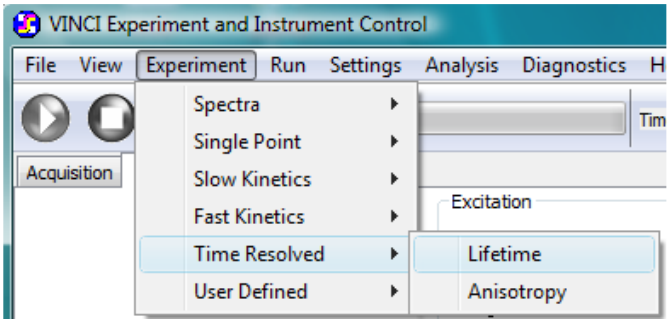


8.6 Acquiring Time-Domain Data

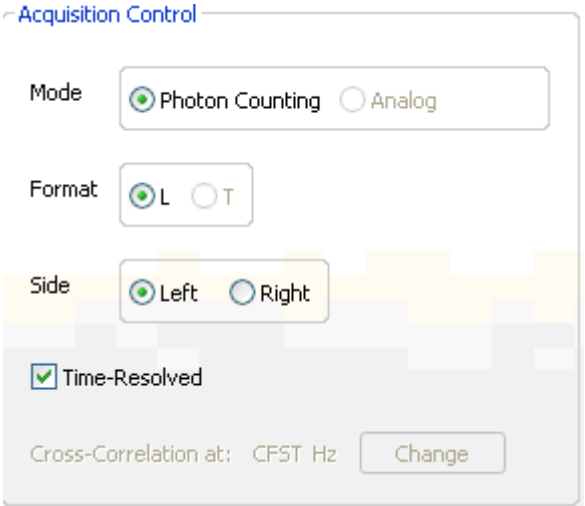
The following time-resolved, time-domain measurements can be performed using Vinci:

- Lifetime
- Anisotropy decays

These options are displayed when selecting <Time Resolved> in the Experiment list on ChronosBH.



Lifetime and anisotropy measurements are typically performed using a filter in the left emission channel. Some customer may have different configurations. Depending on your configuration the Left/Right channel acquisition option needs to be selected in the <Acquisition> menu. The selection needs to be done prior to starting the data acquisition.



A time-domain lifetime and anisotropy measurement requires measurement of both a sample and a reference (scatter solution). The decay curve of the sample in a time-domain measurement represents the convolution of the sample decay and the instrument response function (IRF). The instrument response

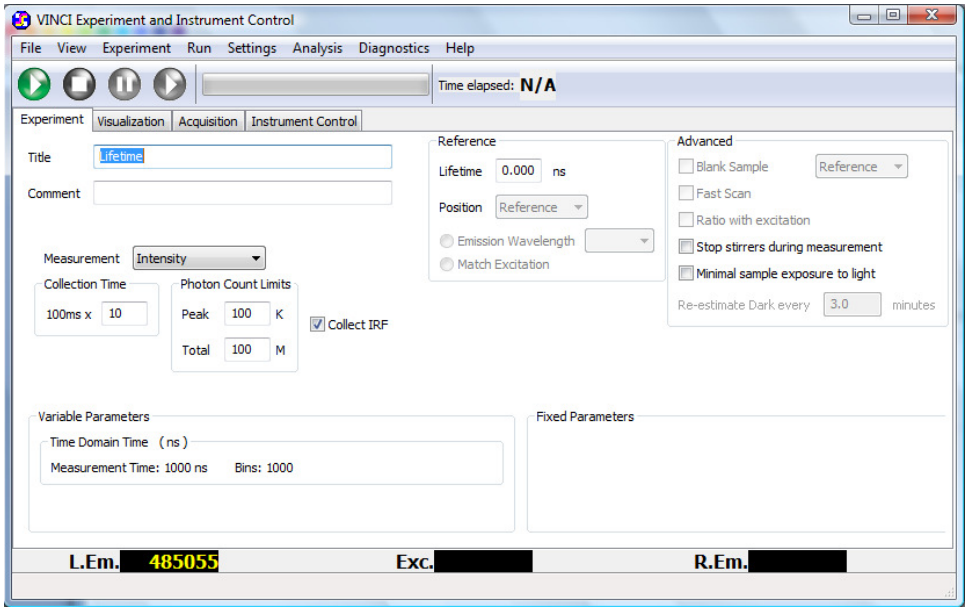
function (IRF) can be measured with a scattering solution, for example, glycogen/water solution (~0.1 mg/ml). The lifetime of the sample can also be measured against a single exponential lifetime standard excited at the same wavelength. Note: Vinci only works when the lifetime of the reference standard is shorter (by a least a factor of 3) than the sample.

When a glycogen solution is used as the reference, the reference lifetime should be set to zero in experimental setup. If a short lifetime fluorescence standard is used, enter the proper reference lifetime of the standard.

Before proceeding with any measurement, one should set up the proper gain for your detector. There are several different detectors used in ChronosBH, please refer to the ChronosBH manual for instruction on how to set up your detector.

8.6.1 Intensity Decay Measurements

Select Experiment->Time Resolved->Lifetime to bring up lifetime experiment menu.
The following lifetime experiment window will be displayed:



General Parameters

Title Enter an alphanumeric title for the measurement. The title is displayed when the file is opened.

Comment Enter an additional alphanumeric text line

Measurement This field shows the measurement parameter that was selected

Data Collection Patameters

Collection Time This field indicates the time used for collecting a decay curve. If the value is "10", collection time is 1s

(100ms*10).

Photon Count Limits	***Peak***	This field defines the peak intensity of decay curve.
	Total	This field defines the integrated intensity of decay curve.

Note: "Collection Time", "Photon Count Limits" provide variable choice for data collection. They all have the same priority. The data collection will stop as soon as one parameters satisfies a chosen condition.

Variable Parameters

Measurement Time	is the TAC "**Range**" which is the maximum time for photon detection
Bins	are the "**ADC Resolution**" which is the time resolution or signal density.

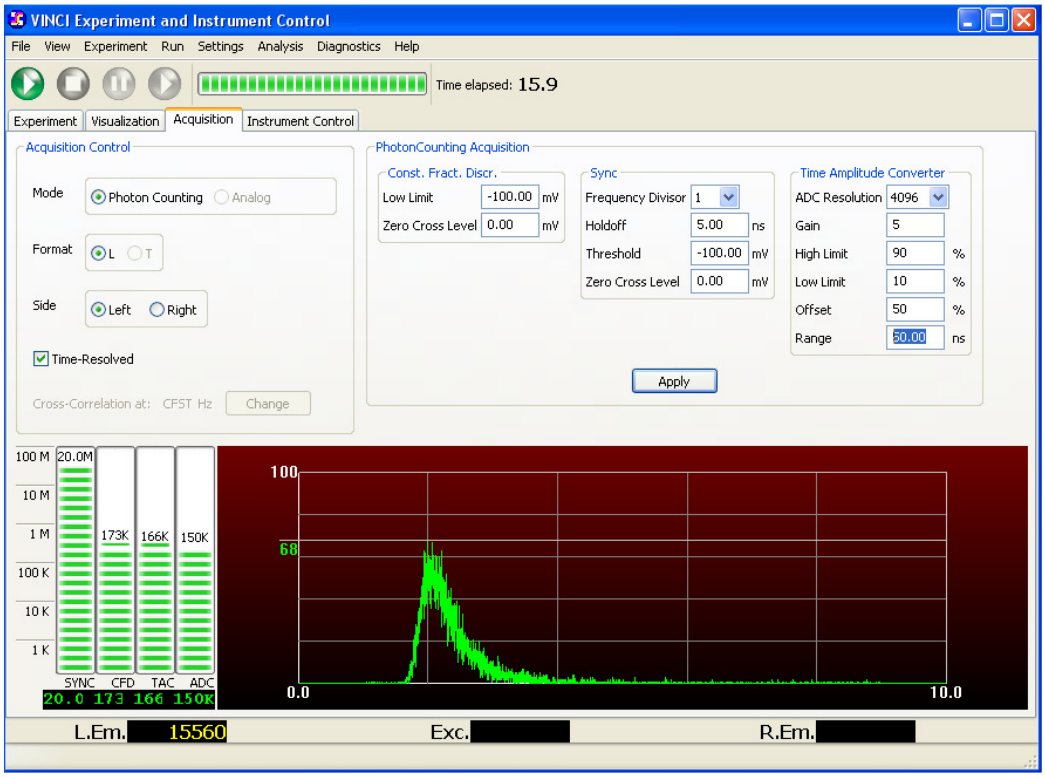
Reference

Lifetime	of the "Standard" in ns.
Position	Location of the Lifetime Standard in sample compartment
Emission Wavelength	in [nm] of the emission monochromator for measuring the reference
Match Excitation	is chosen if in case the lifetime standard is a scattering solution (e.g. Ludox or glycogen)

Advanced Parameters

Blank Sample	Shows the location of the blank in the sample compartment. When this option is checked, blank subtraction will be performed during measurement. Blank subtraction will correct any kind of fluorescence background by directly subtracting the background phasor from the sample. A blank that will properly represent the background of sample should be placed in the location of the blank in the sample compartment. This is a new feature that is only available in Vinci version 1.7. If you don't have this option available, please contact ISS for a software update.
Stop stirrers during measurements	When this option is checked, stirrers stop during the actual time of data acquisition to avoid fluctuations of the fluorescence signal
Minimal sample exposure to light	The excitation shutter is open only during the time of actual data acquisition and closed the remaining time
Re-estimate Dark	Allows to enter a time in minutes for re-estimation of dark counts

For time-domain lifetime measurement, a real-time display of the decay curve is shown in acquisition panel. Photon counting acquisition parameters in acquisition panel are described in the ChronoBH user manual.

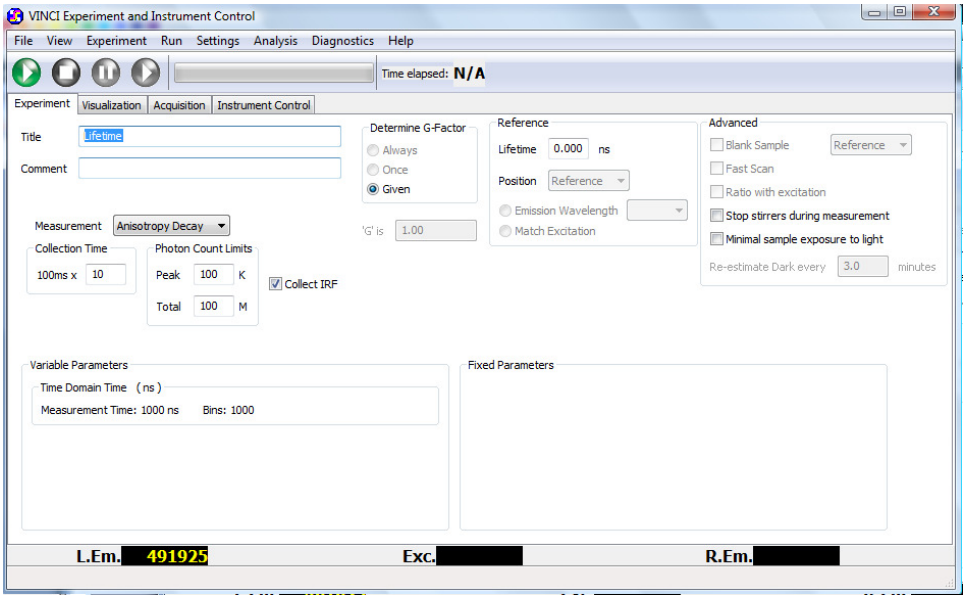


Once you have a proper signal level for the sample and the reference, go to the experiment panel, set a proper collection time or a desired intensity and start the experiment by clicking green button.

Vinci will collect the decay curve of sample and reference. After the measurement is finished the measured intensity decay can be saved and opened in “Vinci Analysis”.

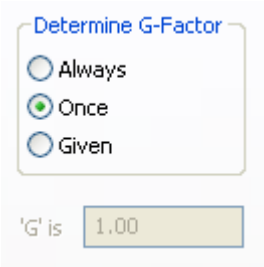
8.6.2 Anisotropy Decay Measurements

Select Experiment -> Time-Resolved -> Anisotropy in menu, and the following experimental setup window will be displayed.



Since most time-domain anisotropy decay parameters are the same as for time domain intensity decay measurements we refer you to the time-domain intensity decay measurement section for an explanation of the parameters.

One parameter that is not used in time domain lifetime measurements is the G-Factor



There are three choices available for the measurement of the G-factor:

g. The G-factor is measured each time;
h. The G-factor is measured once (default value);
i. The G-factor is not measured but is directly entered in the field ‘G’ below

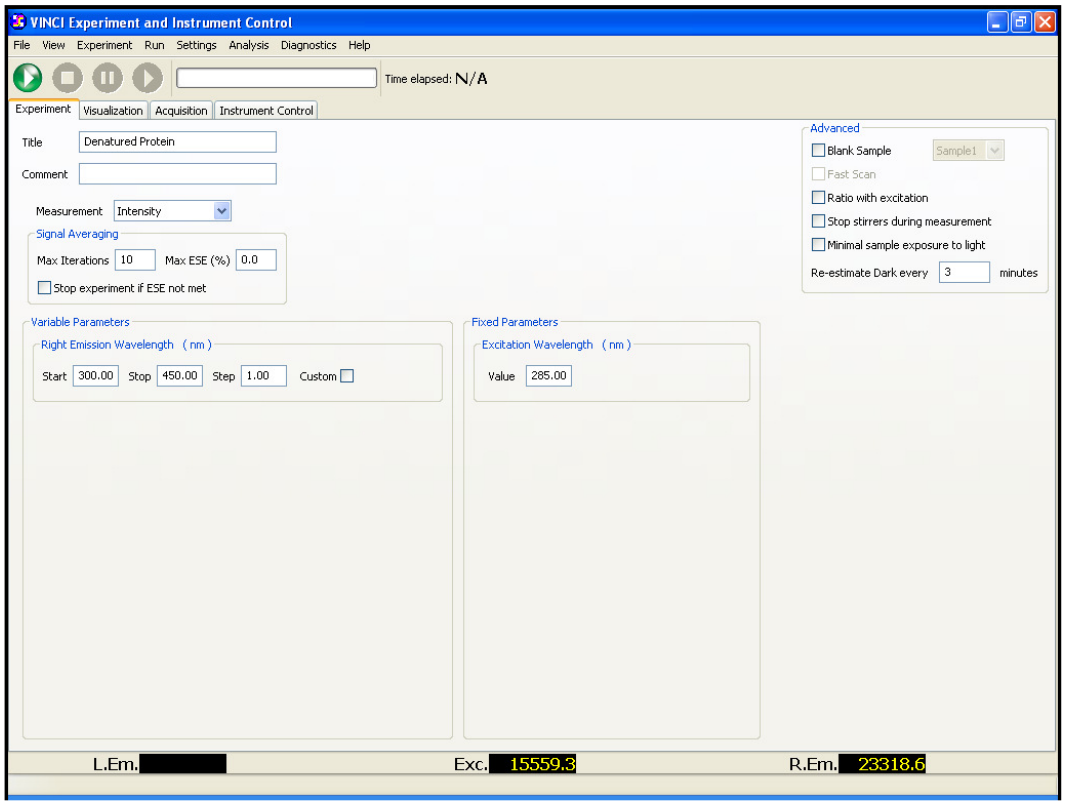
In anisotropy decay measurements, Vinci measures the sample decay curve at vertical-vertical and vertical-horizontal polarizer positions, then measures the reference decay curve at magic angle conditions. Once you have a proper signal level for the sample and the reference, go to the experiment panel, set a proper collection time or a desired upper intensity level and start the experiment by clicking on the green start button.

Vinci will collect the decay curve of sample and reference. After the measurement is finished the measured intensity decay can be saved and opened in “Vinci Analysis”.

8.7 User Defined Menu

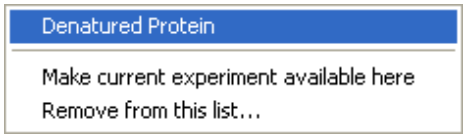
The user can build a customized experiment protocol and save it in <User Defined Menu>. The experiment can then be re-loaded and used in the future. The user-defined experiment can be built on a standard measurement that is selected form the Experiment menu:

As an example let us establish a protocol for the acquisition of emission spectra called "Denatured Protein". The excitation wavelength is set at 285 nm and the emission is acquired between 300 and 450 nm with a 1 nm step-size and a 1 second integration time.



This protocol can be saved by selecting the option <Make current experiment available here> under <User defined> in the Experiment menu.

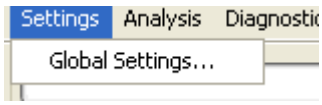
The "Denatured Protein" experiment will be added to the list of user-defined experiments.



A series of experiments can be added to the list. After saving the experimental set up under <User defined> the title of the new experiment is displayed under this category. Each experiment including all set up parameters can be re-loaded in a matter of seconds.

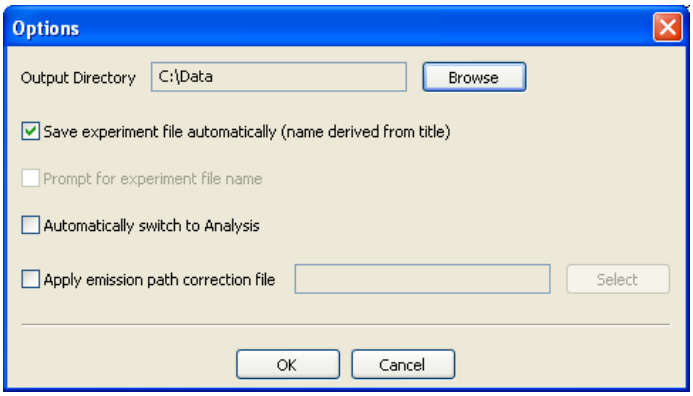
9 Setting Experimental Parameters

Select <Settings> to define experimental parameters related to the data acquisition and choose “Global Settings...”



9.1 Global Settings

This page includes parameters related to the measurement.



Output directory	The field shows the folder where the records are stored. A different folder can be selected by clicking on <Browse>. Folders have to be pre-opened.

The following list of fields allows the user to select the preferred method for storing the data files. Respectively:

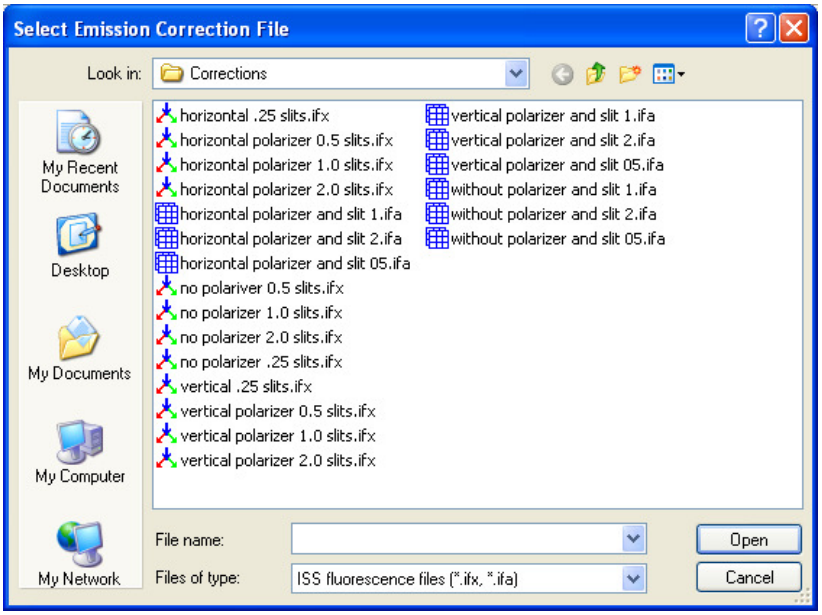
Save experiment file automatically

Prompt for experiment file

Automatically switch to analysis

At the start of each experiment, put the sample holder in the first position

The last field of the list allows the user to select the correction factors for the automatic acquisition of correction emission spectra. Whenever the box is checked, the following list is displayed:



Correction files are acquired at ISS periodically and stored in the Vinci software. The files are specific to the type of monochromator and photomultiplier tube utilized in an instrument configuration; the correction is also a function of the slit bandwidth used in the monochromator and to the plane of polarization of the light collected. A series of files are stored in the Vinci software:

a. No polarizer in the acquisition channel and slit sizes from 0.25 to 2 mm
b. Polarizer in vertical position in the acquisition
c. Polarizer in vertical position

Each file is acquired for three bandwidths: 4 nm, 8 nm, and 16 nm.

10 Experiments: Advanced Applications

When acquiring an emission spectrum, the spectrofluorimeter measures the fluorescence intensity at a given emission wavelength through the emission monochromator. The user may be interested in more complex experiments, such as:

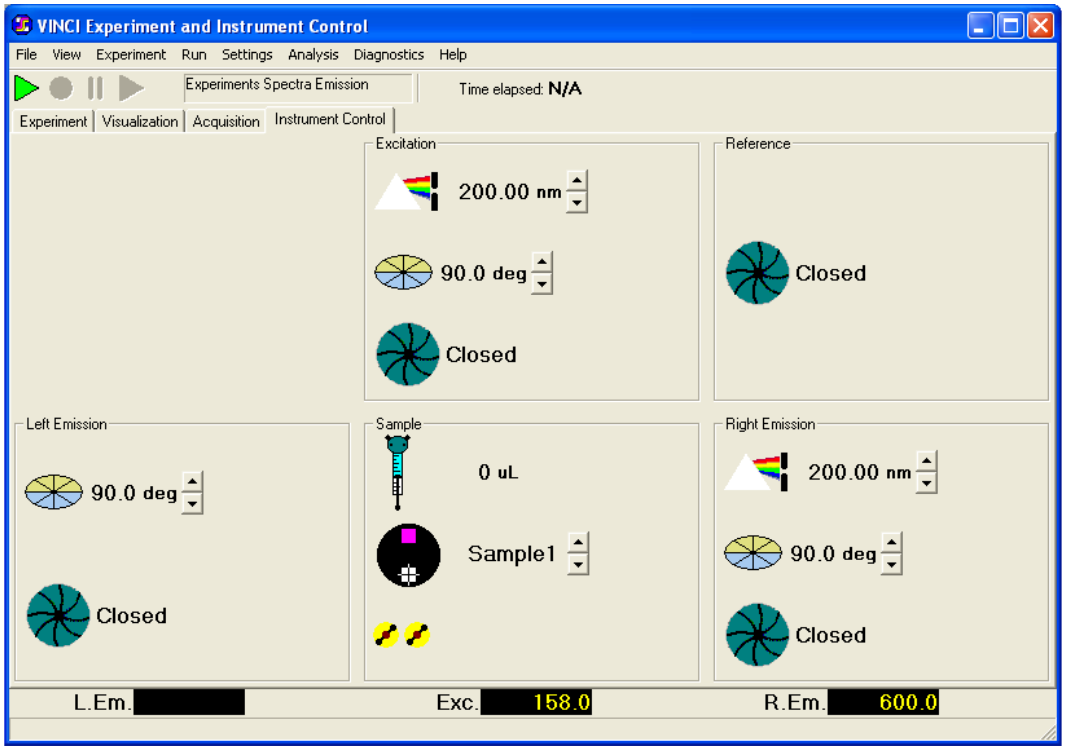
- Acquisition of *n* emission spectra from the same sample in the same wavelength range
- Acquisition of *n* emission spectra at a set time interval *t*
- Acquisition of emission spectra at different temperature values T
- Acquisition of emission spectra using differently polarized excitation light
- Acquisition of emission spectra where the sample is diluted *x* times using a titrator

In all these examples, with the exception of the first one, the measured parameter (fluorescence intensity at a certain wavelength) is acquired by changing other experimental parameters (temperature, plane of polarization of the excitation light, time between data acquisition). Vinci allows the user to program such data acquisition protocols. The following paragraphs will demonstrate how to do this.

10.1 Example

10.1.1 Sequence of Emission Spectra with Dilutions of the Sample Using a Titrator

This experiment requires a computer-controlled titrator. Please refer to the instructions in section 3.4 "Adding Devices to the Instrument" in order to configure the motor parameters of the titrator. Once the titrator is successfully added to the configuration, its icon will be displayed in "Instrument Control".

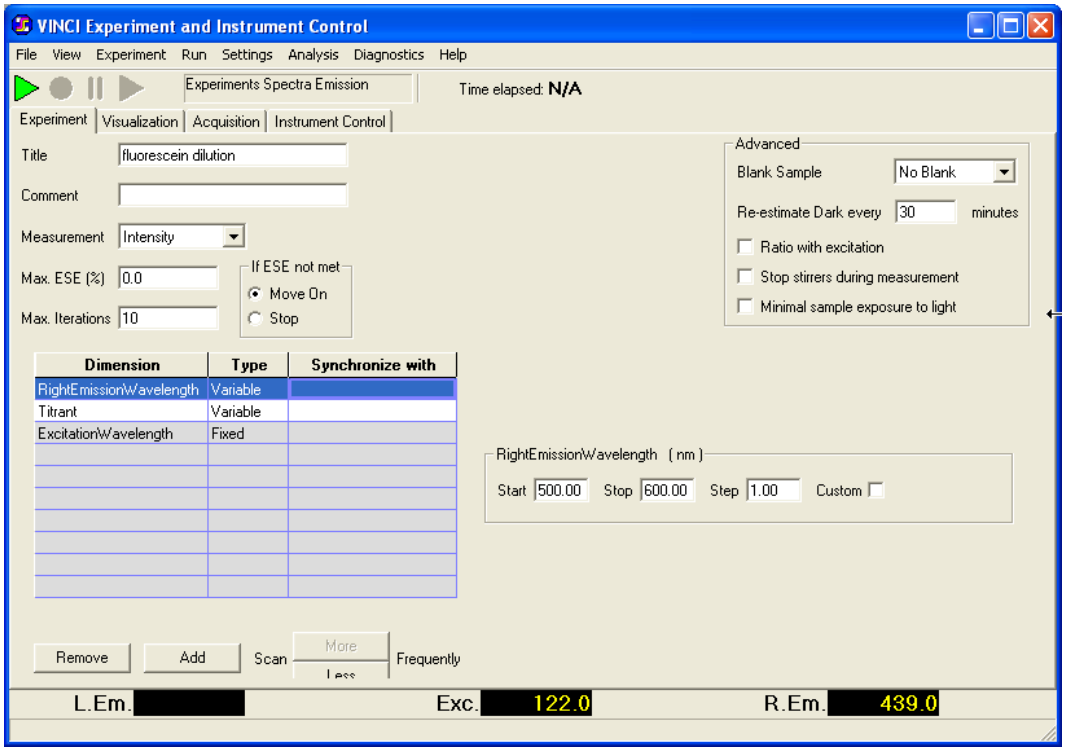


For this experiment, we prepare a cuvette containing a solution of fluorescein; the total volume of the solution is 1.5 ml. We intend to acquire a series of ten emission spectra, with excitation at 480 nm, and scanning the emission monochromator from 500 nm to 600 nm, in 1nm-steps. During the measurement of the 10 spectra, the solution is diluted by adding water. The dilution is made in ten steps by each time adding 100 µl of water.

In the Experiment menu select "Spectra" and then "Emission". Next, instead of starting the data acquisition, select <View>.

In the drop-down window select <Toggle Standard/Power>.

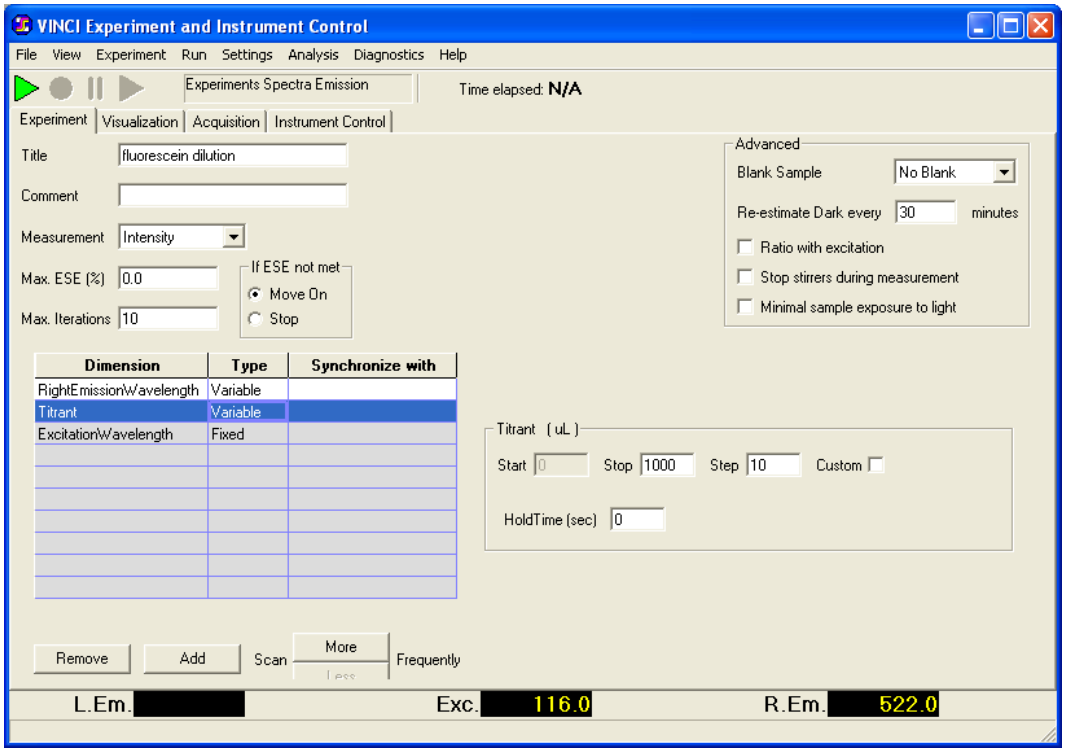
The following screen will appear which will allow to set up the experiment.



Both the Emission Monochromator and the Titrator produce *variable* dimensions. The position of the Excitation Monochromator is a *fixed* dimension.

Once the priority is set, we set the wavelength range for the emission monochromator. We enter "500" in the *Start field* and "600" in the *Stop field* and "1" for the Step-Size.

Now we move the cursor to the titrator. We enter "0" in the *Start* field and "1000" in the *Stop* field; we enter "100" in the *Step* field, indicating the addition of 100 µl per step. The screen will appear as the one in the following page.

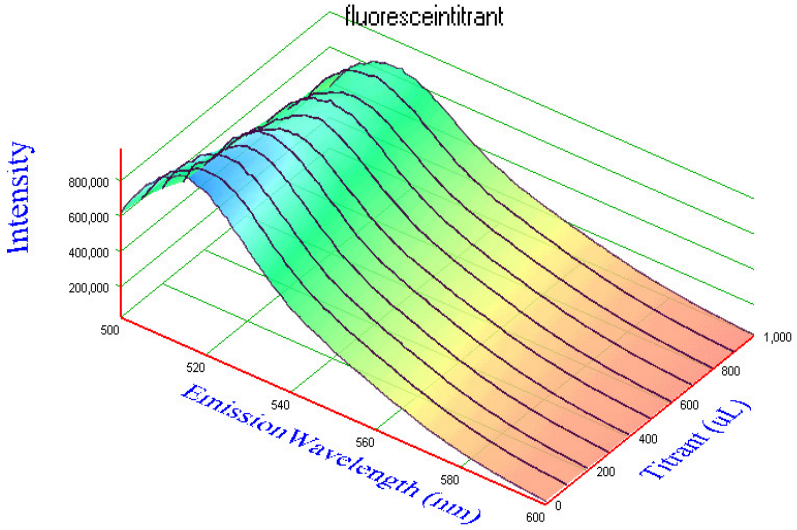


We can decide how we would like to visualize the data; we can select a 2D display (intensity versus wavelength) or a 3D display, where the additional axis (z-axis) reports the volume of titrant added to the cuvette.

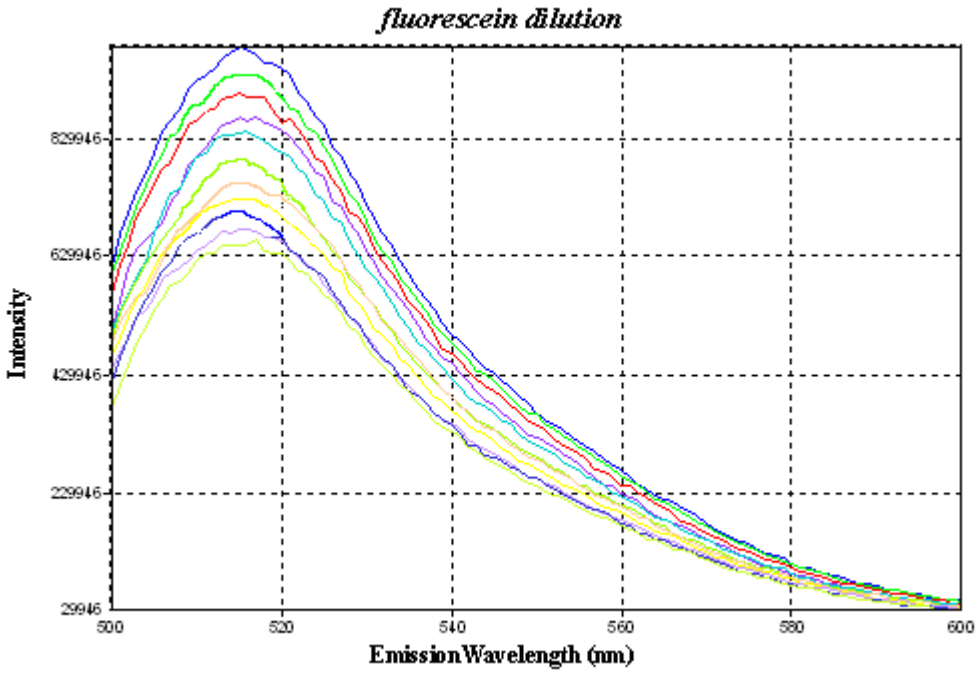
The selection is made by pressing the <View> button and then selecting <Visualization>.

Let us select <3D Surface>. When done, click onto the green arrow to start the data acquisition.

The series of the ten emission spectra will be acquired and displayed as in the following 3D plot.

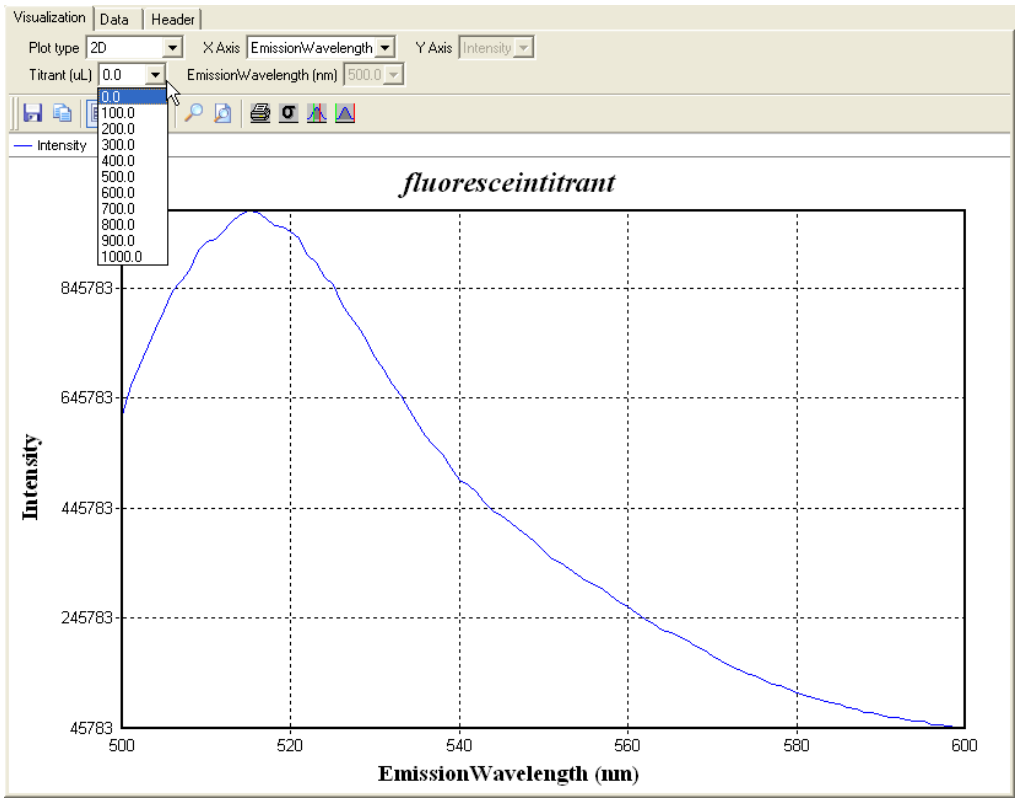


If instead of the <3D> selection in the Visualization menu <2D Series> is selected, the following plot will be displayed:

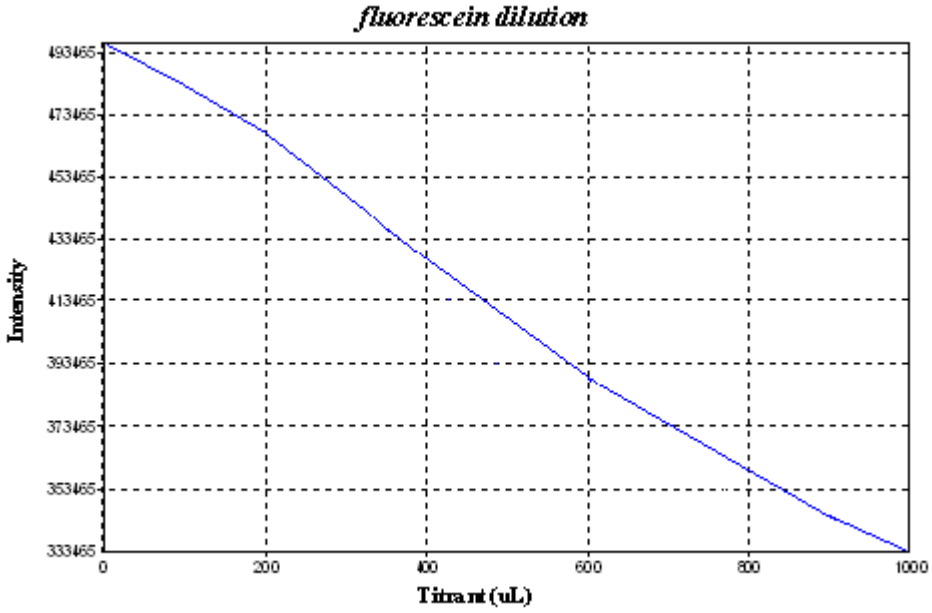


The ten emission spectra are shown and each color represents a spectrum taken after each dilution step.

When the user is interested in a particular spectrum corresponding to a specific dilution of the fluorophore <2D> is selected. The amount of titrant is selected in the drop-down menu:



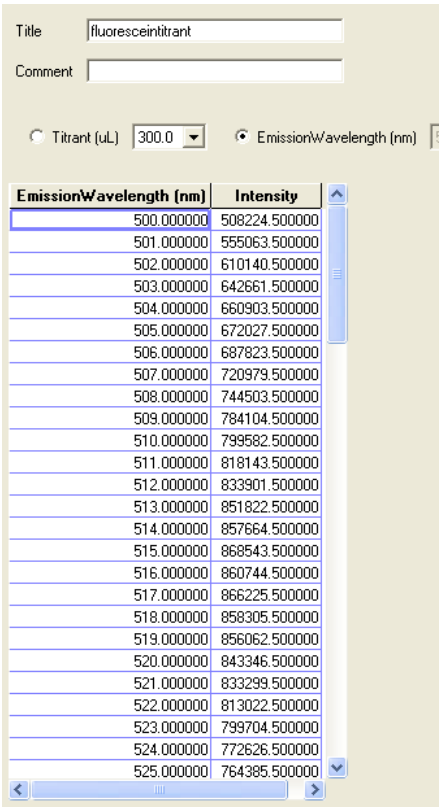
Another useful plot is the plot of the fluorescence intensity versus the amount of added volume to the solution at a fixed emission wavelength.



Data can be visualized by selecting <Data> in the plot window.

In this window, the data corresponding to each record can be visualized by selecting the corresponding amount of titrant added to the solution.

Data are acquired in ASCII file format (see section on data file format) and can be read directly into any spreadsheet program.



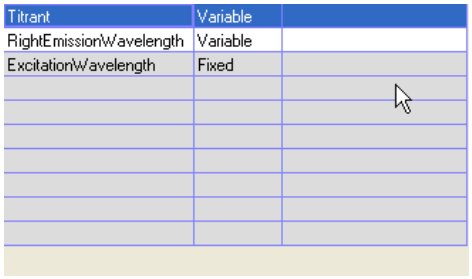
EmissionWavelength (nm)	Intensity
500.000000	508224.500000
501.000000	555063.500000
502.000000	610140.500000
503.000000	642661.500000
504.000000	660903.500000
505.000000	672027.500000
506.000000	687823.500000
507.000000	720979.500000
508.000000	744503.500000
509.000000	784104.500000
510.000000	799582.500000
511.000000	818143.500000
512.000000	833901.500000
513.000000	851822.500000
514.000000	857664.500000
515.000000	868543.500000
516.000000	860744.500000
517.000000	866225.500000
518.000000	858305.500000
519.000000	856062.500000
520.000000	843346.500000
521.000000	833299.500000
522.000000	813022.500000
523.000000	799704.500000
524.000000	772626.500000
525.000000	764385.500000

10.1.2 Establishing the Priority between Devices

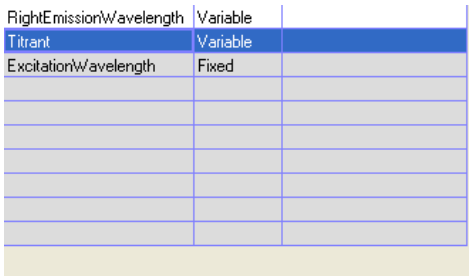
The priority between devices is set by the position in the device table. If a device is static (dimension is *fixed*), it will be placed at the bottom of the table. In the above experiment, the excitation wavelength is fixed therefore its position is lower than that of the right emission monochromator.

Dimensions that can be changed during an experiment are marked *Variable*. Their position in the table determines their priority. Using the buttons positioned below the table can change the position:

In this sequence, the initial volume of added titrant to the solution is “zero”. An emission spectrum is acquired. Then, a 100 µl volume of titrant is added to the solution and a second emission spectrum is acquired. The sequence continues until a total of a 1000 µl volume of titrant is added to the solution.



In this sequence, the position of the Emission Monochromator is set at 300 nm, and 11 data points are acquired, each corresponding to an addition of titrant. Then, the Emission monochromator moves to 301 nm and the sequence is repeated.

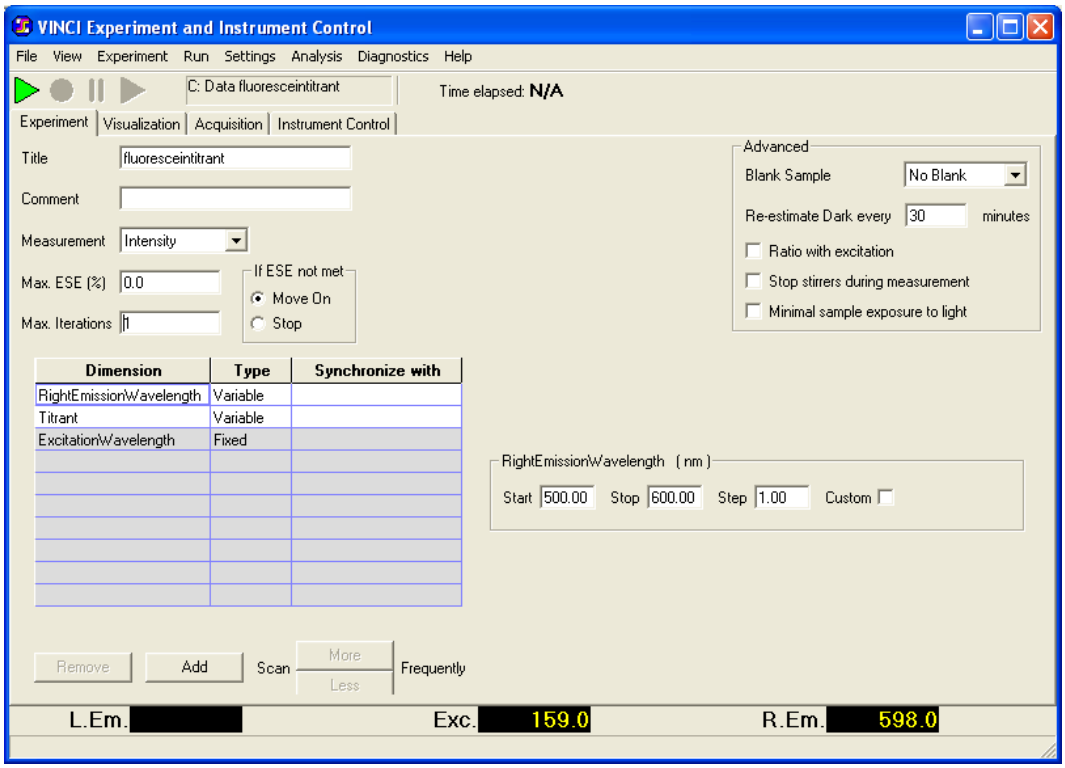


10.2 Re-run an Experiment

Any experiment that has been set up and saved can be rerun under the same conditions at a later time. The record to be rerun is selected in the data files location and then the <re-run> button is checked:

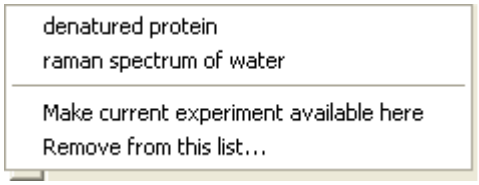
Load the record from the data file. Click on <Experiment> and select <Re-run>.

When choosing re-run for the titration experiment described in the previous section, the following display is shown: Please note that both the emission monochromator and the titrator parameters utilized in the experiment are shown as default values; they can of course be modified by the operator.



10.3 Saving an Experiment for Future Use

The Advanced Application feature allows the user to setup a custom-made acquisition protocol. Once an experiment is defined, it can be saved for future use.



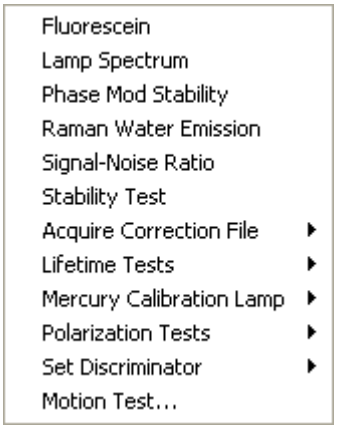
In <Experiment>, select <User Defined> and then click on <Make current experiment available here>. The experiment is added to the list of experiments already stored. The experiment will be saved with the name entered in the title. Whenever it will one needs to reuse it in the future, all that needs to be done is to load it and run it.

Experiments can be deleted from the list by clicking on <Remove from this list>. A table listing the experiments is displayed and the one highlighted will be deleted from the list.

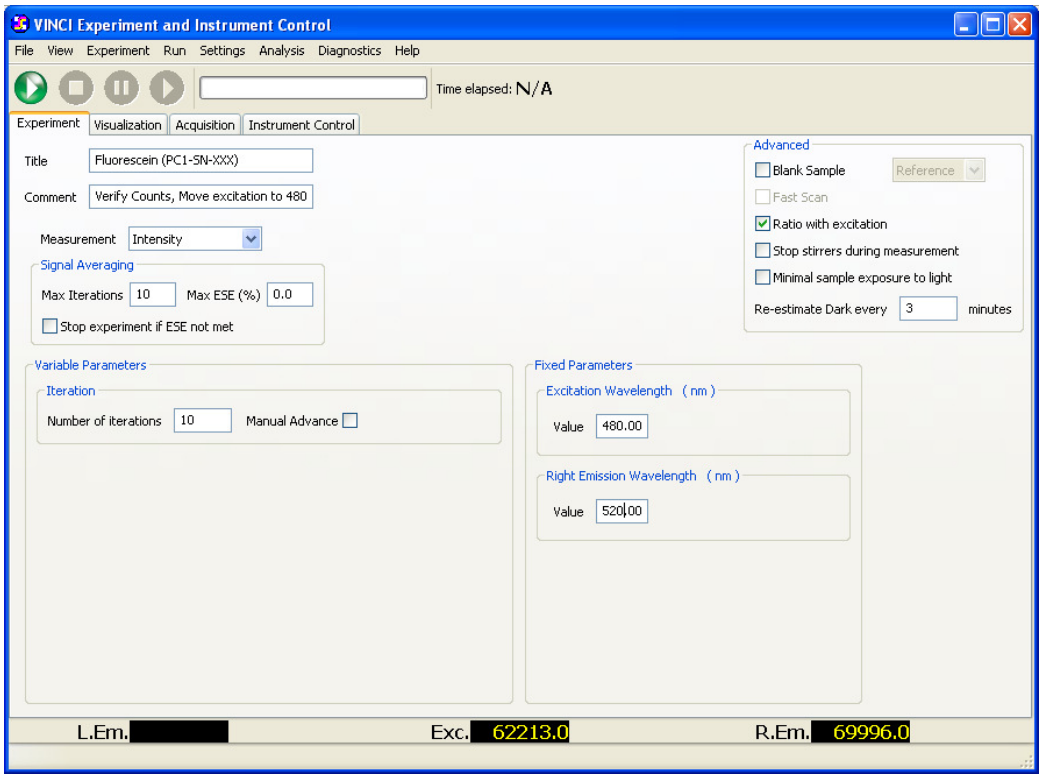
11 Instrument Diagnostics and Performance Validation

Vinci also includes a comprehensive diagnostics tool to ensure proper operation of major system components at start-up (see Vinci Log). In addition several pre-set experiments allow the user to check the performances of the instrument any time during its operation.

Select <Diagnostics> in the main menu bar and the following drop-down window is displayed:

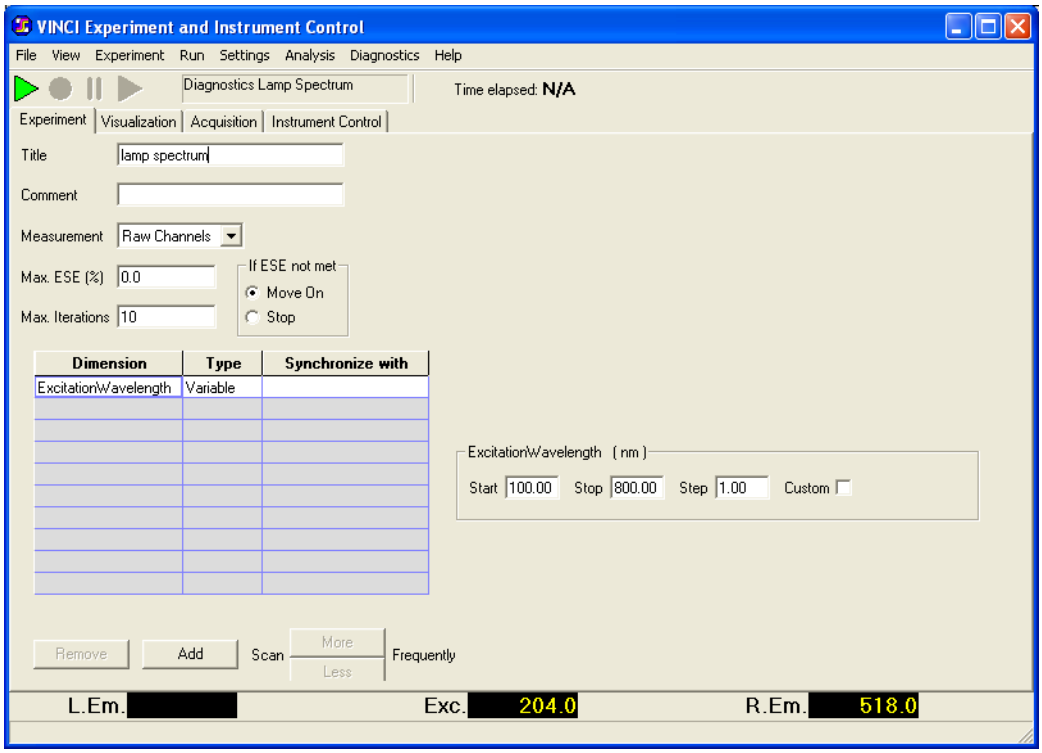


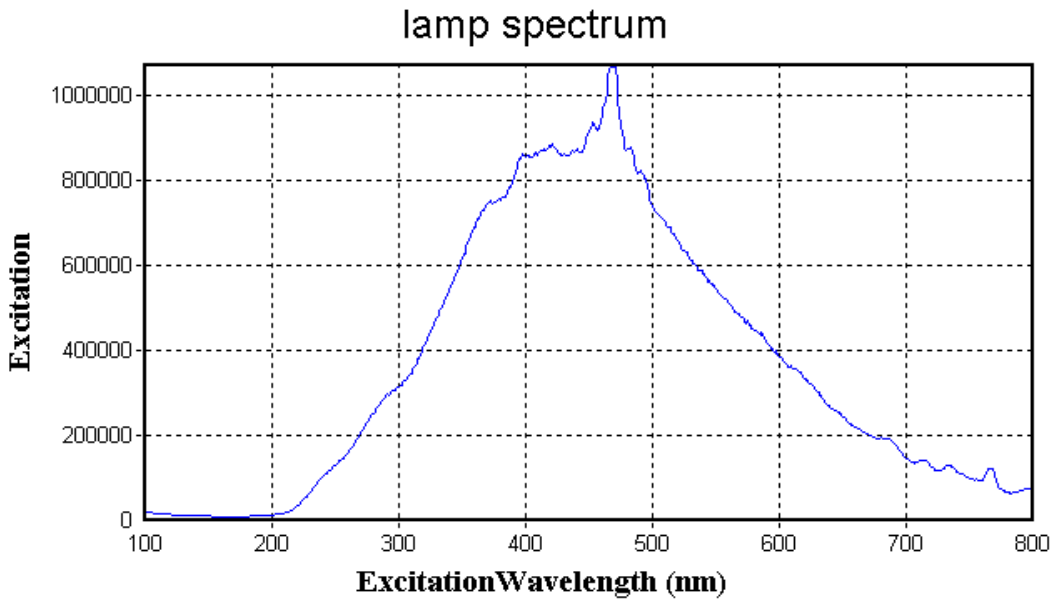
11.1 Fluorescein



11.2 Lamp Spectrum

In this experiment, the excitation monochromator is scanned between the two wavelengths set by the user and the light is detected using the reference photomultiplier tube (PMT). The routine can be utilized to check the calibration of the excitation monochromator using the peak at 468 nm.

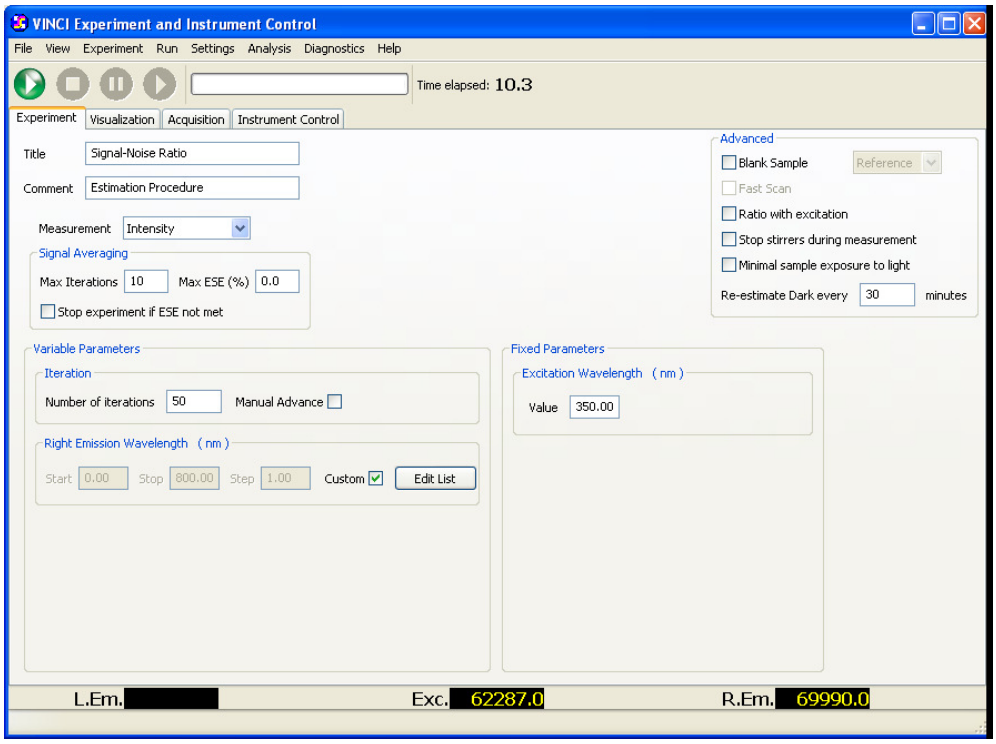


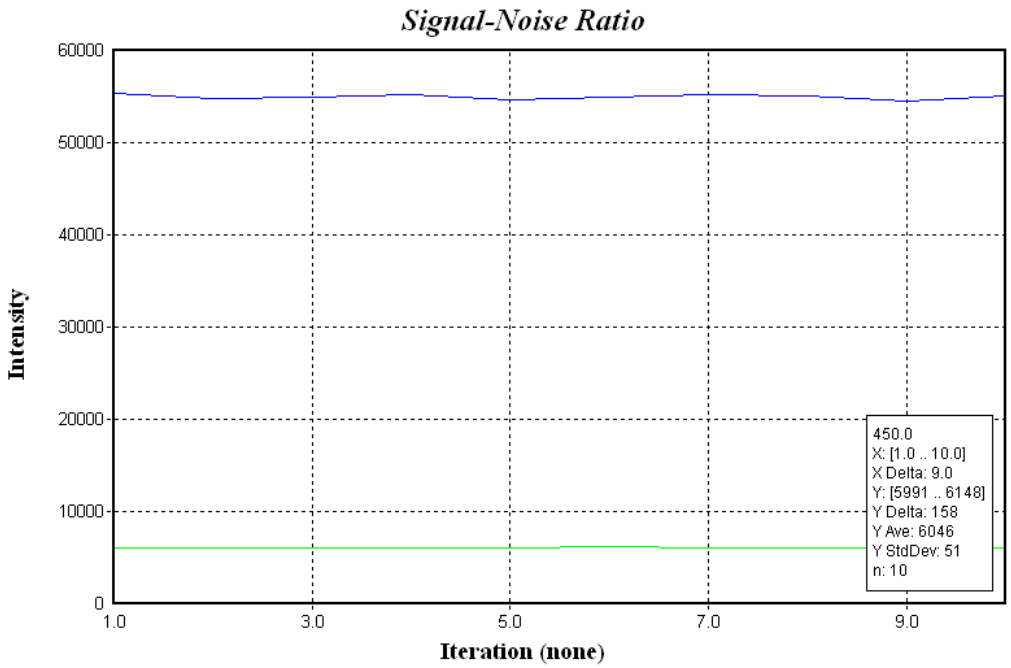


Please note that the recorded spectrum is not the “true” lamp spectrum: the spectrum is a convolution of the lamp spectrum and of the response of the photomultiplier tube in the reference channel of the instrument.

11.3 Signal-Noise Ratio

This test is aimed at checking the signal-to-noise ratio of the instrument. In a typical test, HPLC water is utilized; the excitation wavelength is set at 350 nm and the signal is measured at 397 nm and 450 nm. The 397 nm corresponds to the position of the peak in the Raman spectrum of water and 450 nm is utilized to measure the background and the peak-to-peak value of the noise.

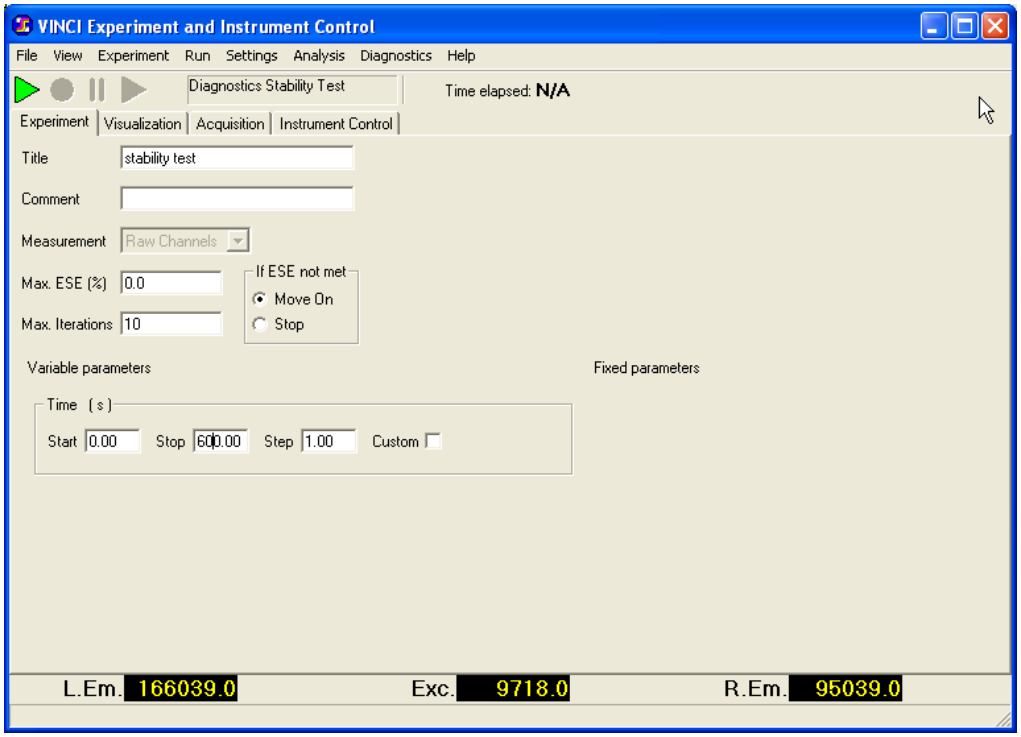


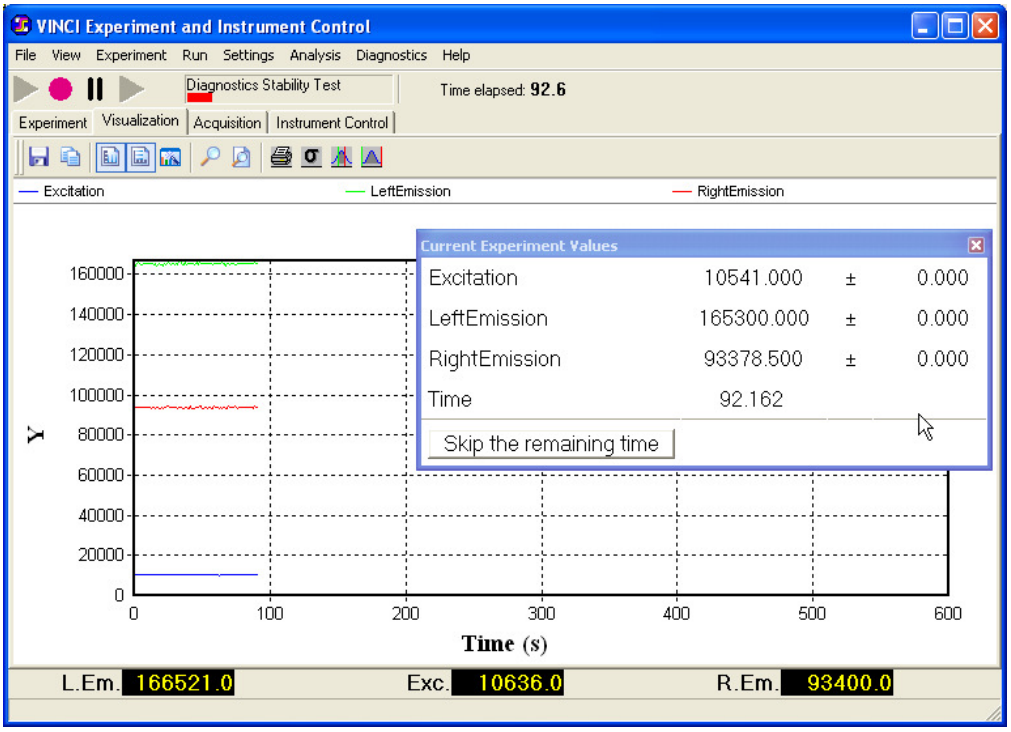


The figure above shows the average signal and noise levels estimated by excitation at 350nm and collecting the emission at 397 and 450 nm.

11.4 Stability Test

The test is aimed at checking the stability of the signal on each of the three light detectors, independently. Two types of stability are checked: the short-term, peak-to-peak ripple of the signal and the drift of the light detectors. For experimental conditions on how to run the test, the user should consult the Test Report provided with the instrument.


VINCI Experiment and Instrument Control
File View Experiment Run Settings Analysis Diagnostics Help
Diagnostics Stability Test
Time elapsed: N/A
Experiment Visualization Acquisition Instrument Control
Title
stability test
Comment
Measurement
Raw Channels
Max. ESE (%)
0.0
Max. Iterations
10
If ESE not met
Move On
Stop
Variable parameters
Fixed parameters
Time (s)
Start 0.00 Stop 600.00 Step 1.00 Custom
L.Em. 166039.0
Exc. 9718.0
R.Em. 95039.0


VINCI Experiment and Instrument Control
File View Experiment Run Settings Analysis Diagnostics Help
Diagnostics Stability Test
Time elapsed: 92.6
Experiment Visualization Acquisition Instrument Control
Excitation
LeftEmission
RightEmission
Current Experiment Values
Excitation 10541.000 ± 0.000
LeftEmission 165300.000 ± 0.000
RightEmission 93378.500 ± 0.000
Time 92.162
Skip the remaining time
160000
140000
120000
100000
80000
60000
40000
20000
0
Y
0
100
200
300
400
500
600
Time (s)
L.Em. 166521.0
Exc. 10636.0
R.Em. 93400.0

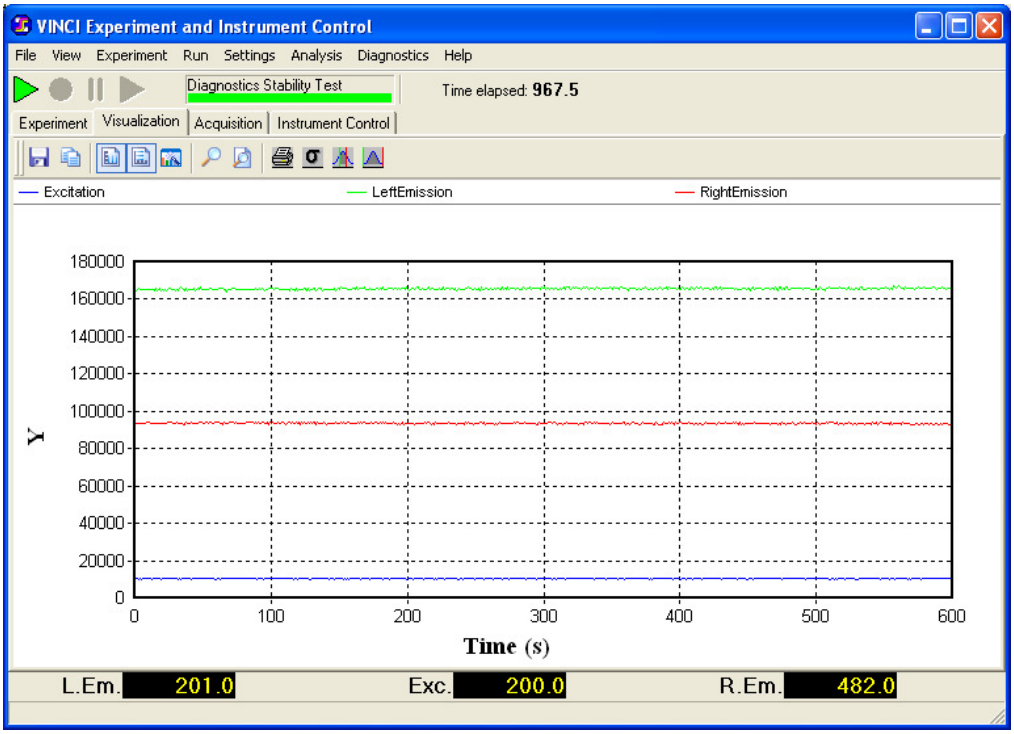
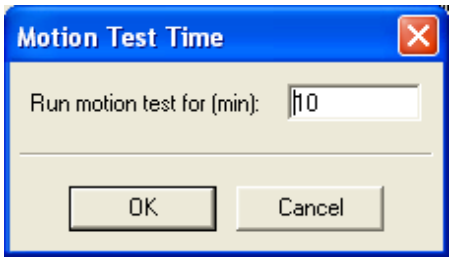


Figure 3. Intensity measured on the emission channels

The above plot shows the stability of three photomultiplier tubes within a time frame of ten minutes.

11.5 Motion Test

These tests are designed to check the functionality of the stepper motors and of the automation of the instrument. Upon selecting this feature, the window on the right is displayed and the user is requested to enter the duration time (in minutes) of the test.



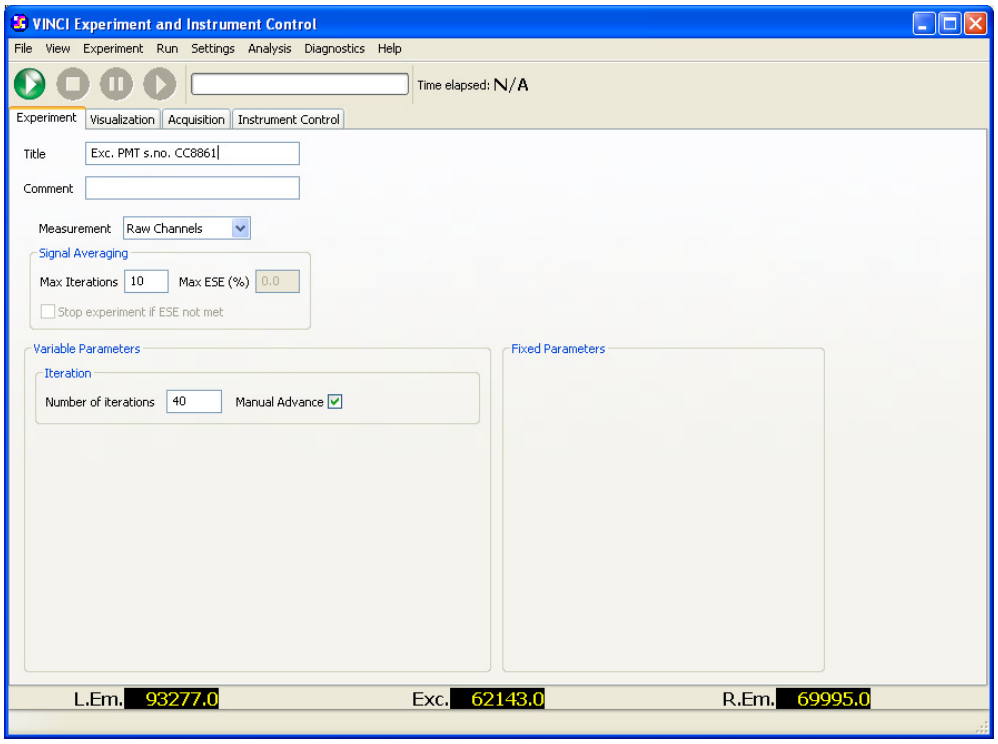
Each stepper motor utilized on the instrument undergoes a long-term *burn-in* test during the assembly of the instrument.

Once the test starts, the stepper motors of the instruments are moved in sequence from number zero to number fourteen. The log view window of the experiment is displayed and any malfunctioning will be reported in the window.

```
Taking RightEmissionFilter to the low limit ...
Taking RightEmissionFilter to -20.0 nm ...
done
Taking RightEmissionPolarizer to vertical position ...
done
Closing RightEmissionShutter ... Done
Taking SampleHolder to first position...
Taking ExcitationFilter to the high limit ...
Taking ExcitationFilter to 800.0 nm ...
done
Taking ExcitationPolarizer to horizontal position...
done
Opening ExcitationShutter ... Done
Taking LeftEmissionFilter to end position ...
Taking LeftEmissionPolarizer to horizontal position...
done
Opening LeftEmissionShutter ... Done
Opening ReferenceShutter ... Done
Taking RightEmissionFilter to the high limit ...
Taking RightEmissionFilter to 800.0 nm ...
done
Taking RightEmissionPolarizer to horizontal position...
done
Opening RightEmissionShutter ... Done
Taking SampleHolder to last position ...
Taking ExcitationFilter to the low limit ...
Taking ExcitationFilter to -20.0 nm ...
done
```

11.6 Setting the Discriminator

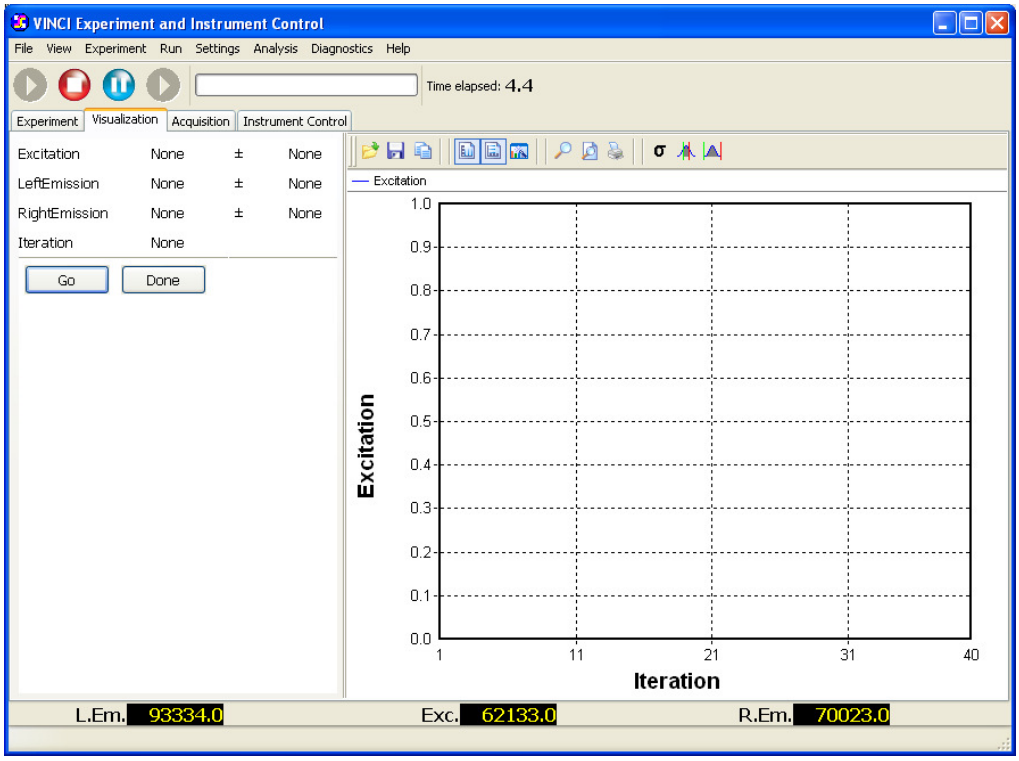
For setting the discriminator select “select discriminator” under Diagnostics menue:



In this screen, enter the following parameters:

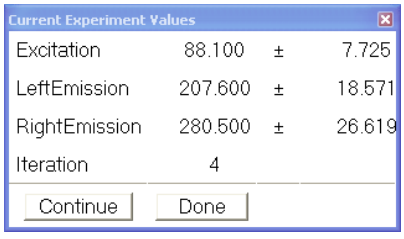
Title	Exc. PMT s.no. Explanation: Enter the serial number of the PMT installed in the Excitation Channel
Measurement Iterations	1 (default value) Explanation: one second acquisition when unit is in seconds
Number of Iterations	40 (default value)

When done, click onto the green arrow to start the data acquisition. Move to "Visualization" and the following window is displayed

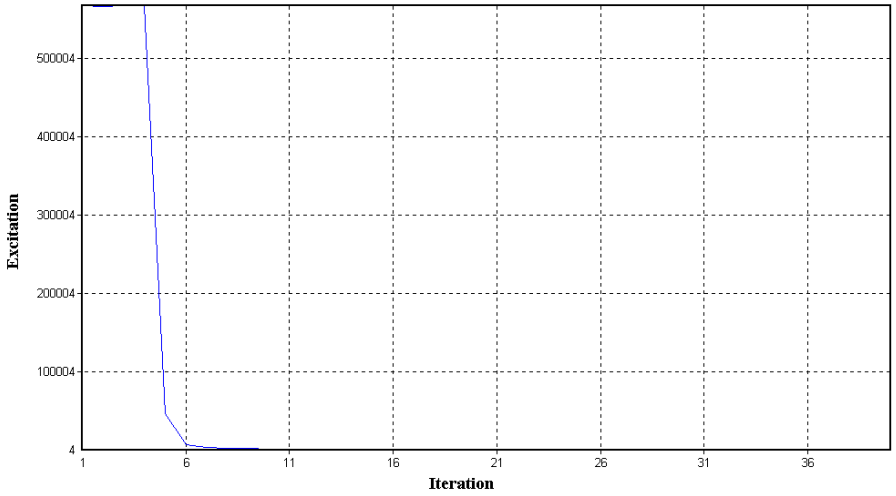


Use the screwdriver tool that was supplied by ISS to set the Discriminator Threshold. Identify the appropriate slot on the right side of the instrument and insert the screwdriver into the slot. The first step is to rotate the screwdriver counterclockwise so that the signal goes to its maximum. Press <Go> to record the first data point then rotate the screwdriver half a turn clockwise and press <Go> again. Then again rotate the screwdriver half a turn clockwise after pressing <Go>. Repeat this procedure until the signal goes to "zero" (the signal is displayed in the bottom row in the screen above).

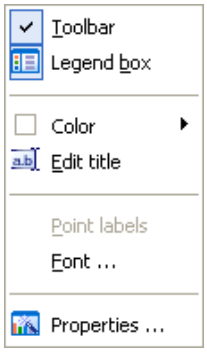
Press <done> in this window when the signal goes to "zero".



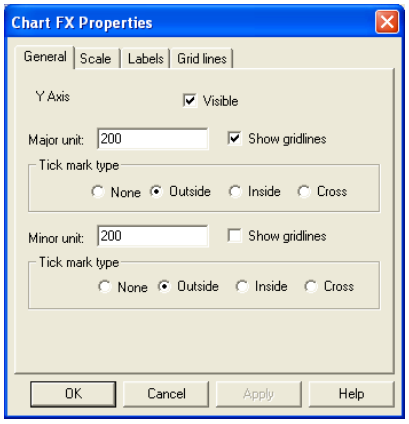
The resulting plot should be similar to what is displayed below:



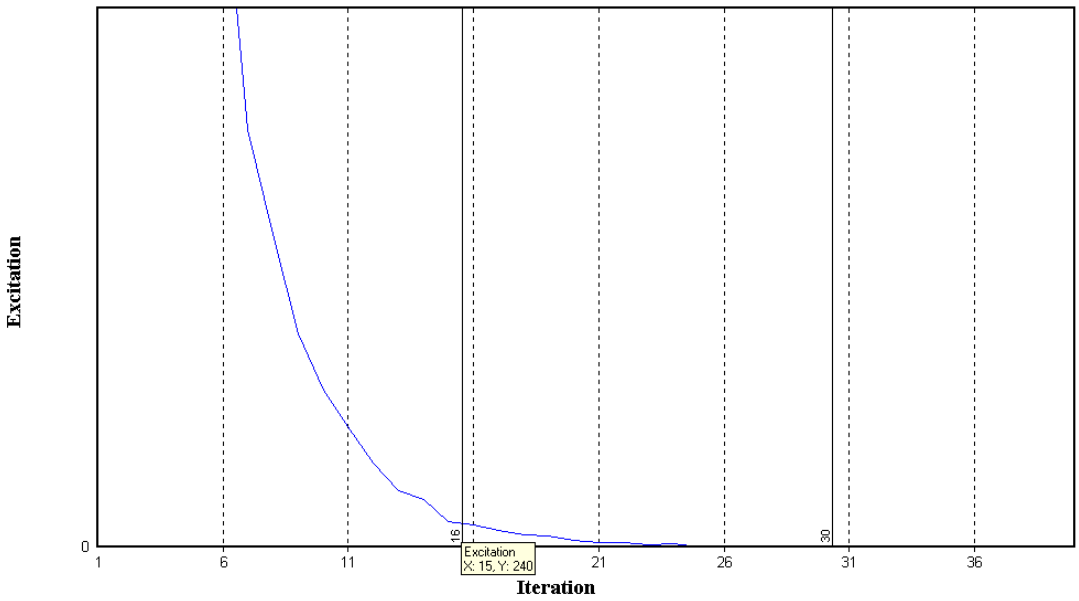
Right-click on the left axis to change the scale. Select <Properties>



And then select <axis>.



Use the cursor to locate the position where the slope of the curve becomes less steep (in the plot above it is at 15 and the value of the signal is 240). Rotate the screwdriver 15 half-turns clockwise until the signal is at that value. The discriminator threshold for the excitation PMT is set.



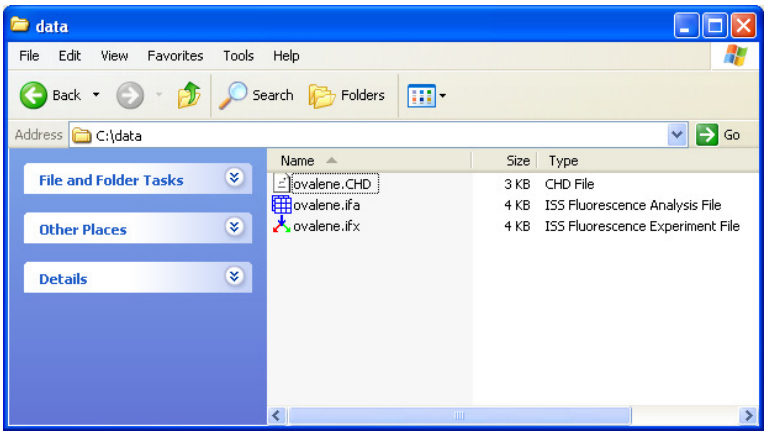
Repeat the same procedure for the Right and Left Emission PMT.

12 Data Files Structure

Two types of data files are stored and handled by Vinci. The files that are saved by Vinci as a result of the acquisition of an experiment have the extension "ifx" and the user never modifies these files. Whenever an operation is performed on an experiment file (smoothing, change of wavelength range, etc.) the new file is stored with the extension "ifa".

File Extension	Description
ifx	Experiment files
ifa	Analysis files

Different icons mark the two types of files as well.



An experiment file contains all the information that allows the experimenter to reload and run the exact same experiment again at a later time. Whenever the file is manipulated and/or some of its attributes are changed (for instance the color of the lines) the new version has to be saved as an analysis file with the "ifa" extension.

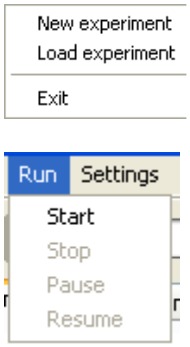
Note: An experiment file cannot not be altered. From an experiment file, several analysis files can be generated.

12.1 Experiment Files

The header of an experiment file contains information on how to rerun the experiment.

Title	ovalene
Comment	
Signature	ISS_Experiment_Ver_1_0
Timestamp	Mon Jan 27 14:46:06 2003
AcquisitionType	Photon Counting
AcquisitionFormat	L
AcquisitionSide	Right
Timebase	2
Measurable	Intensity
MeasurementIterations	1
CloseExcitationShutterAfterEveryMeasurement	0
UseExcitationIfAvailable	0
StopStirrerDuringMeasurement	1
DelayAfterMovingTurret	0.1
DarkIterations	0
DarkDelayAfterClosingShutters	0
DarkUpdateFrequency	1800
DelayAfterOpeningShutters	0.0
UseBlank	0
Visualization	PlotType:2D,X:EmissionWavelength,Y:Intensity,Y2:
EmissionWavelength	type:numeric,unit:nm,from:400.0,to:550.0,step:1.0
ExcitationWavelength	type:numeric,unit:nm,fixed:340.0
Space	EmissionWavelength
Columns	EmissionWavelength,Intensity

To rerun an experiment, the user loads the file and selects <Run> in the experiment section of the Vinci software



12.2 Analysis Files

The header of an analysis file only contains information about the type of file (wavelengths, intensity, etc.).
This file is generated during the analysis of experimental data.

Signature	ISS_Analysis_Ver_1_0
Title	ovalene
Comment	
Visualization	Y:Intensity,X:EmissionWavelength,Z:,Y2:None,PlotType:2D
ExcitationWavelength	type:numeric,unit:nm,fixed:340.0
EmissionWavelength	type:numeric,unit:nm
Space	EmissionWavelength
Columns	EmissionWavelength,Intensity

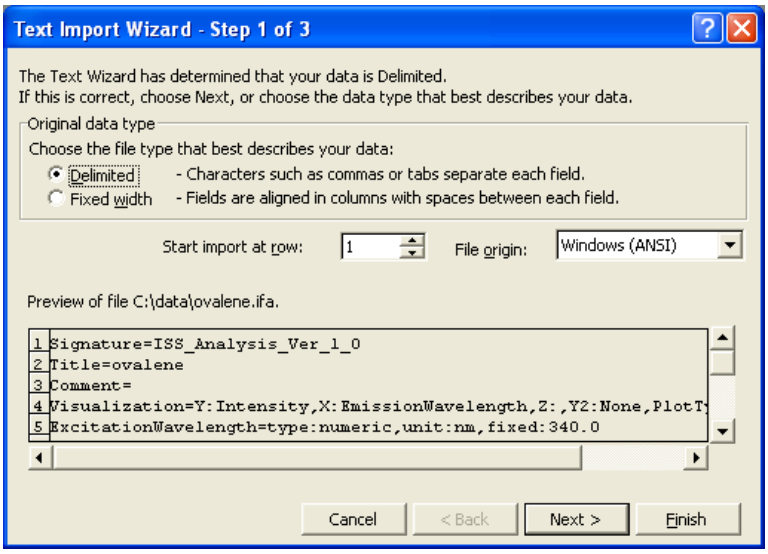
12.3 Compatibility of Vinci Data Files with Other Programs

Vinci uses an ASCII format for data file storage. As such, data files can be opened with popular commercial programs such as Microsoft Notepad, WordPad and Microsoft Word.

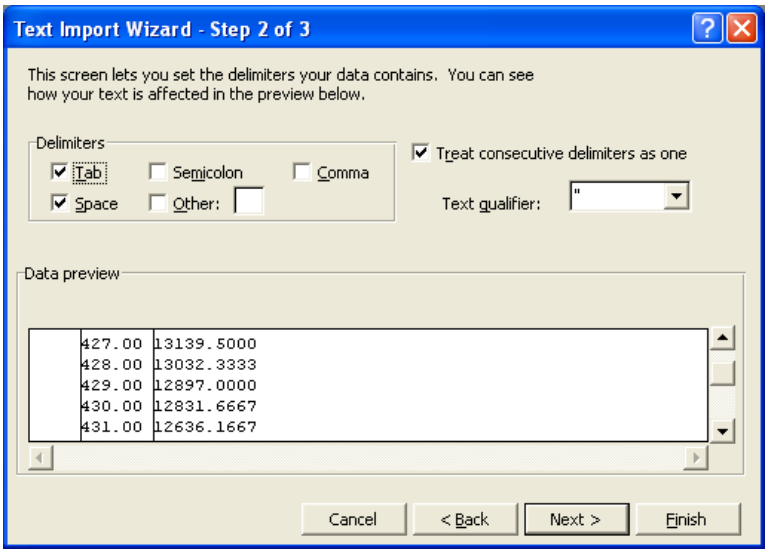
12.4 Open Vinci Data Files in a Spreadsheet

Vinci data files can also be exported and opened directly in a spreadsheet, such as Microsoft Excel.

When the text import wizard starts, Check on <Delimited> and click <Next>.



Select <Tab> and <Space> and click on <Next>.



The data file is imported and can be manipulated with Microsoft Excel.

12.5 "Cat" Data Files Import

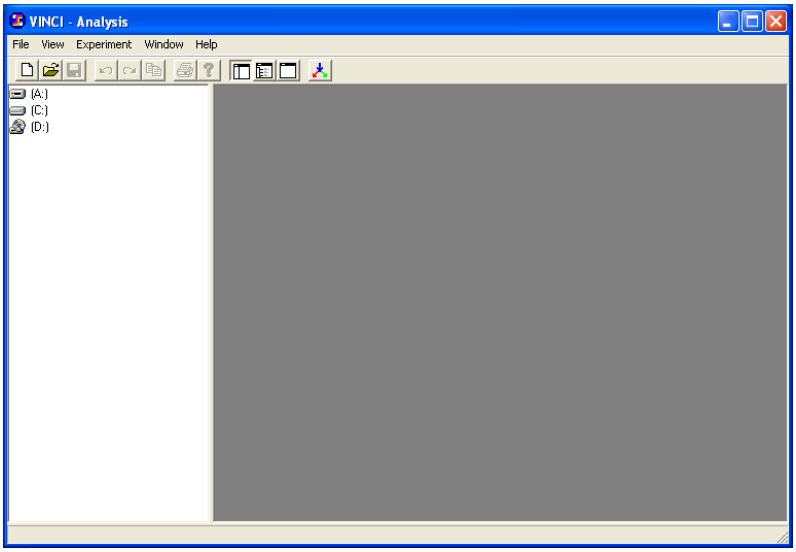
Data acquired in the ISS binary format ("cat" extension) can be imported into Vinci. These files are stored with the "ifa" extension and therefore cannot be utilized to re-run an experiment.

We also have developed a routine to assist users of SLM spectrofluorometers who update the instrument with ISS electronics and Vinci.

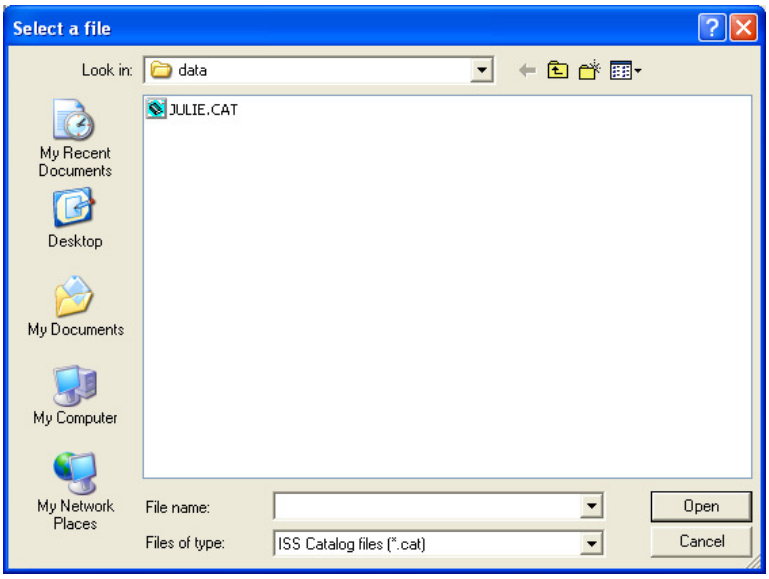
12.5.1 How to Import ISS binary Files into Vinci

ISS “cat” files are stored in binary format and up to 160 records can be stored. First the file to be converted should be copied into the destination directory.

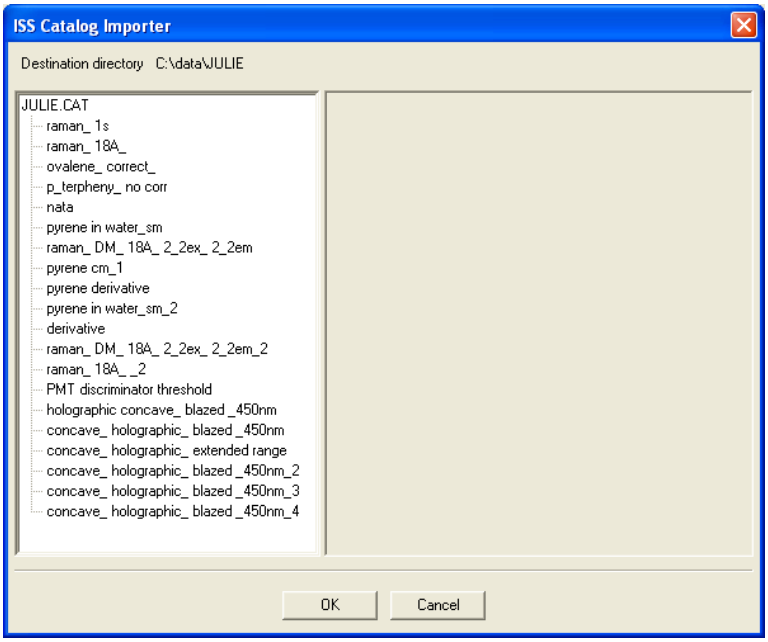
Start Vinci and select <File>.



In the drop-down window, select <Import>. Locate the directory where the files are stored. In the example, we are converting the files “Julie.cat” located in the directory “data” on the hard drive.

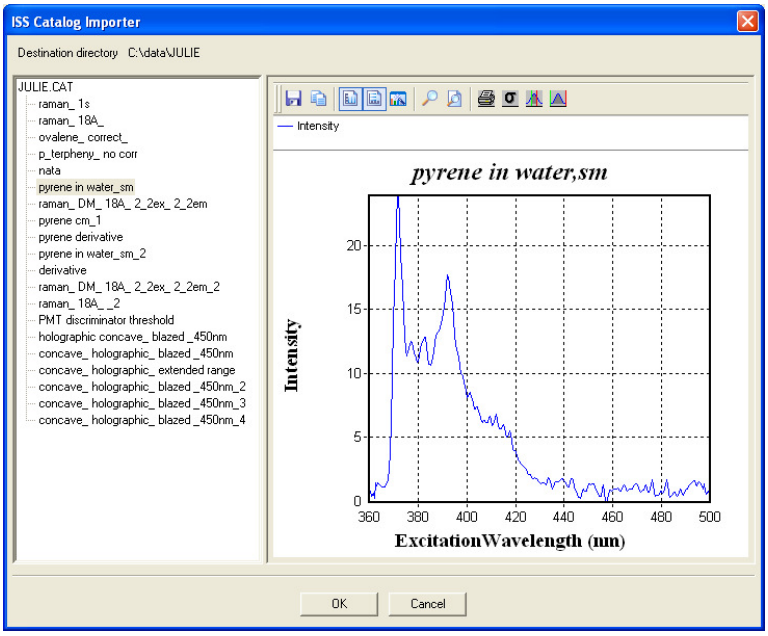


The listing of the files stored in “Julie.cat” is displayed.



By right-clicking on the file each plot can be displayed in Vinci.

Multiple files can be selected by pressing the <Shift> key and highlighting them.



Once the files have been selected, click on the <OK> key to complete the import procedure.

The selected files are imported and stored with the “ifa” extension in the destination directory.

12.5.2 SLM ASCII-format files

Vinci also allows the direct import of ASCII files generated with the original software running on SLM spectrofluorimeters.

Two types of files can be imported: files of spectra that display intensity versus wavelength and files of kinetic measurements that display intensity versus time.

13 Measurements, Standard Deviation and Standard Error

This chapter briefly explains how the reported errors associated with a measurement are calculated. Also, as the acquisition software is built to be adaptive, the chapter explains the way the statistics is used to acquire data with the same standard deviation.

13.1 Acquisition of a Data Set and Calculation of the Standard Deviation

Let us suppose that we measure a quantity X a number of times *n*. We obtain n values of the quantity X that we can write as in the following:

$$\{ X_1,\ldots, X_n \}$$

The average value of the measurement for the quantity X can be calculated using the relation:

$$\bar{X} = \frac{1}{n}\sum_{i=1}^{n} X_i$$

For a large number of measurements, the accumulated uncertainties leading to random noise are distributed according to a Gaussian curve. That is, the following statements apply:

$$\left|X - \bar{X}\right| \le \sigma_X \qquad 68.3\%$$

$$\left|X - \bar{X}\right| \le 2\sigma_X \qquad 95.4\%$$

$$\left|X - \bar{X}\right| \le 3\sigma_X \qquad 99.7\%$$

In other words, once the average $\bar{X}$ and the standard deviation σ_X are established, an additional measurement X_i has 95% probability to fall in the interval $(\bar{X} - 2\sigma_X,\ \ \bar{X} + 2\sigma_X)$.

For an infinite set of data, the standard deviation of the average is:

$$\sigma_X = \sqrt{\frac{1}{n}\ \sum_{i=1}^{n}(X_i - \bar{X})^2}$$

Practically, the number of measurements performed is a finite number. Whatever the distribution of the variable X_i is, the average X approximates a Gaussian distribution as the number of measurements goes to infinity. The convergence is rapid: the average of 6 measurements is already a good approximation to a Gaussian distribution. With a 20 measurements sample, the distribution is practically a Gaussian function. Yet, for a small set of data the standard deviation is best represented by:

$$\sigma_X = \sqrt{\frac{1}{(n-1)} \sum_{i=1}^{n} (X_i - \bar{X})^2}$$

The standard deviation of the average, or standard error, for *n* observations is:

$$ESE = \frac{\sigma_X}{\sqrt{n}} = \sqrt{\frac{1}{n(n-1)} \sum_{i=1}^{n} (X_i - \bar{X})^2}$$

13.2 How Vinci Determines a Measurable Quantity

In Vinci, data are acquired in a time window that can be specified by the experimenter. Two questions have to be answered:

a. How is a measurable quantity determined in Vinci?
b. How is the error associated to a measurement calculated in Vinci?

The shortest data acquisition time window for all of the measurements is 100 ms, the only exception being the measurements grouped in the *Fast Time* dimension, or Fast Kinetics, where 0.1 milliseconds is the time window.

The experimenter can change the time window by changing the number of the <Max Iterations> in the Experiment page of the acquisition. The default value is ten (10); that is measurements are acquired for 10 repeated intervals each with duration of 100 ms; the total data acquisition time is one (1) second. The minimum value is 3, that is, the software needs to repeat the measurement three times for a total of 300 milliseconds.

Let us suppose that the intensity x_i is measured for 0.5 seconds. We have the following five quantities:

$$\{X_1, X_2, X_3, X_4, X_5\}$$

In general, if we were to measure the quantity for a total time T, the number to write in the <Max Iterations> field is n= T/ 0.1, which the total number of time base windows. In this case, the following quantities are collected:

$$\{ X_1, \ldots, X_n \}$$

The average value of the quantity X can be calculated using the relation:

$$\bar{X} = \frac{1}{n} \sum_{i=1}^{n} X_i$$

The estimated standard error (ESE) of the average is:

$$ESE = \frac{\sigma_X}{\sqrt{n}} = \sqrt{\frac{1}{n(n-1)} \sum_{i=1}^{n} (X_i - \bar{X})^2}$$

Note that the value for ESE differs from the value of σ_X. The quantity $\overline{X}$ can be the value of the intensity at a specific wavelength (as in an emission spectrum); or the value of the intensity at a specific time (as in a slow kinetics); or the value of the polarization at either a specific time or wavelength.

Data files include the standard error for any quantity measured in the acquisition.

13.2.1 Data Acquisition with a Set ESE Value: Adaptive Acquisition

In many measurements, the acquisition of a set of data points with the same standard deviation can be very convenient. An example is when we utilize these data points to fit a physical-chemical model using a minimization routine. Vinci allows the experimenter of this type of acquisition using the "adaptive acquisition" module. In each Experiment page, the user can specify the ESE value for a set of measurements.

The specified ESE value is given as percentage of the ESE associated with the measurement set. The acquisition proceeds until the value has been reached; at that point, the acquisition stops and the measurement proceeds to the next step. If the set value for the ESE has not been reached, the measurement proceeds; alternatively, the measurement can be stopped.

In summary:

If ESE=0	Data acquisition is performed for a time specified by the <Max iterations> field.
If ESE(%) ≠ 0	Data acquisition is performed until the specified ESE is reached. Once the ESE is reached, the next data point acquired. If the specified ESE is not reached within the time set in the <Max iterations> field, the program automatically continues to acquire the next data point, unless the <stop> box is checked.

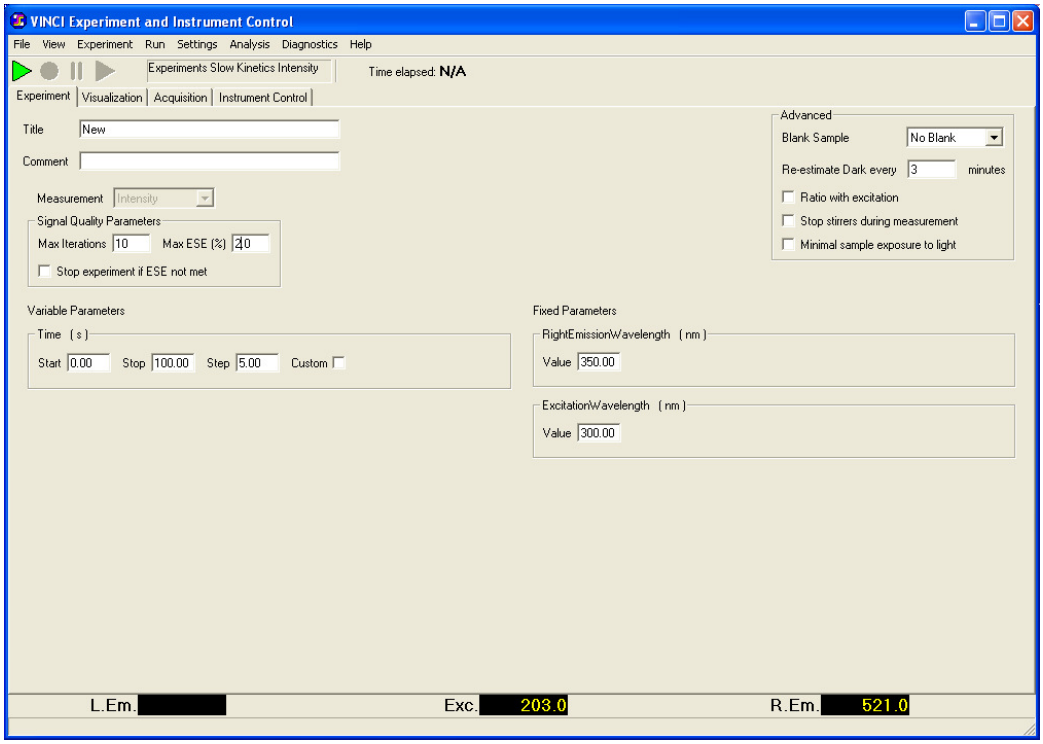
The ESE is expressed in percent rather than in absolute units. The reason for this is due to the amount of the measured fluorescence signal. The signal can go from almost zero counts per seconds to over four (4) million counts per seconds. A percentage is a convenient way to specify the ESE in a measurement.

During data acquisition, the ESE value utilized for the calculations is the value of the signal as read on the monitor channels at the bottom of the screen. This value is stored in the data file when the measured quantity is the quantity stored (for instance fluorescence intensity at selected wavelengths). For more complex quantities, such as fluorescence anisotropy or intensity-ratios, the ESE stored in the data file is determined using error propagation algorithms.

13.2.2 Slow Kinetics

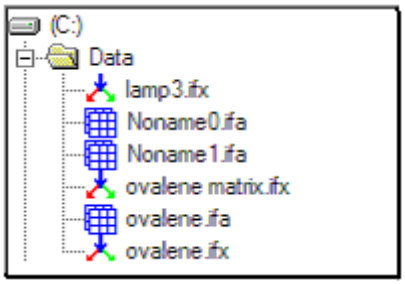
In this experiment, the fluorescence intensity is measured at specific time intervals Δt and at each time interval data are acquired for a time T. Let us suppose to measure the intensity for 1 second at time intervals of 5 seconds for a total time of 100 seconds: a total of 20 data points will be acquired in this measurement. Additionally, we would like to acquire data with an ESE equivalent to 2% of the value of the measured intensity.

The Experiment and Instrument Control screen has to be set as follows:



14 Accessing and Moving Data

14.1 Data Stored on the Computer Running Vinci



The Analysis section is accessed whenever the Vinci software starts. The Vinci browser displays a list of the folders on the computer; for the following, we will assume that the fluorescence data are stored in the *Data* folder.

An alternative way to open data files is to click on the Open Folder icon displayed on the top row of the monitor.

14.2 Data Stored on the Network

The computer running Vinci can be connected to a Local Area Network (LAN). The Vinci browser cannot see a folder stored on a computer of the network.

In order to open data stored on the network, one has to click on the *Open Folder* icon displayed on the top row of the monitor.

Similarly a data file can be stored on the network using the *Save As* utility in the <File> menu.

15 Linear Operators and Data Files

This chapter details how to perform mathematical operations on the fluorescence data acquired. The operations can be done on the set of data or between different sets of data.

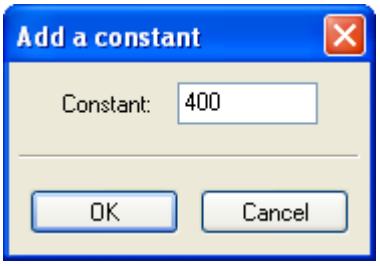
When selecting the <Math> menu on the top row of the monitor, the list of operations is displayed.

In the following we will refer to each of these operations as to an *operator*" applied to the data file. Whenever an operator is applied to a data file, a new data file is generated.

15.1 Add a Constant

Syntax If Y is the set of Y_i data values and k is a constant, the new data file D is generated by $D : Y \rightarrow (Y + k)$

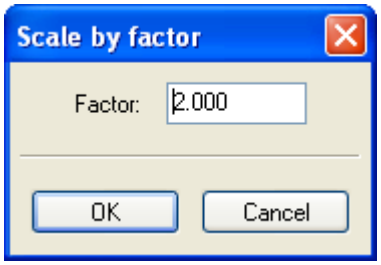
When this operator is selected, the window to the right is displayed; just enter the constant that needs to be added to the Y-values of the entire data file and click on <OK>. The value is a relative number.



15.2 Scale by Factor

Syntax If Y is the set of Y_i data values and k is a constant, the new data file D is generated by: $D : Y \rightarrow (Y * k)$

The <Scale by a Factor> operator multiplies all the Y-values of the data file by the factor entered by the operator.



15.3 Reciprocal

Syntax If Y_i is the set of data values, the new data file *D* is generated by:

$$D : Y \rightarrow \left(\frac{1}{Y} \right)$$

The <Reciprocal> takes the inverse of the Y-values of the data file.

15.4 Normalize

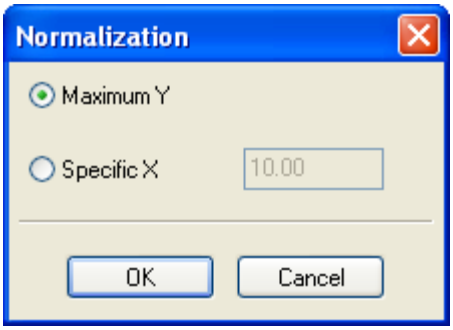
Syntax (normalize at maximum) If Y_i is the set of the Y data values and *k* is the value of the maximum of the Y^i, the new data file *D* is generated by

$$D : Y \rightarrow \left(\frac{Y}{k} \right)$$

Syntax (normalize at point x) If Y_i is the set of Y data values and *K* is the value selected by the operator for the point x, the new data file *D* is generated by

$$D : Y \rightarrow \left(\frac{Y}{k} \right)$$

The Y-values of the data file are normalized either at the maximum, or at a specific x-value selected by the experimenter.



15.5 Derivative

Syntax If Y_i is the set of data values, the new data file *D* is generated by:

$$D : Y \rightarrow \frac{\partial}{\partial x_i} Y_i$$ First derivative

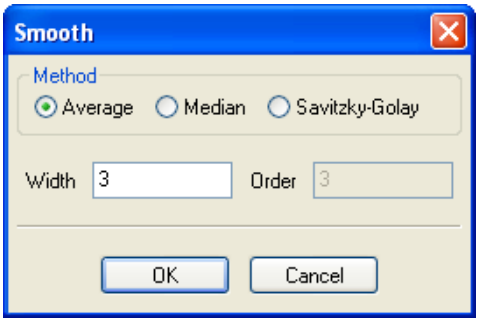
$$D : Y \rightarrow \frac{\partial^2}{\partial^2 x_i} Y_i$$ Second derivative

$$D : Y \rightarrow \frac{\partial^n}{\partial^n x_i} Y_i$$ *N-order* derivative

15.6 Smoothing

Three types of filters are available in Vinci for smoothing of the data; respectively:

a. Average
b. Median
c. Savitzky-Golay



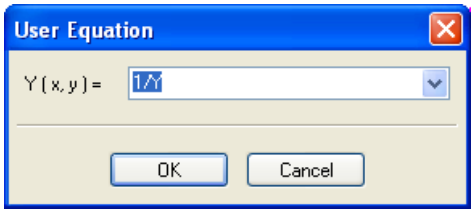
The selection of the filter, along with the smoothing parameters is done using the dialog box.

15.7 Apply Custom Equation

Syntax If Y_i is the set of data values and *E* is the equation, the new data file *D* is generated by

$$D : Y \rightarrow (Y \otimes E)$$

The Y-values of the data file are convoluted with a generic equation entered by the user.



The equation can include trigonometric functions and exponential functions. For instance, the following equations are included in the software, as an example:

$$Y(x, y) = y + \exp x^x$$
$$Y(x, y) = y + \sin x^\pi$$

15.8 Math Between two Data Sets

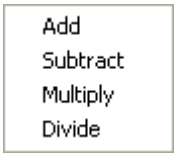
Syntax

Lets assume that $Y_1 = Y_1(x)$ is a spectrum defined for in the interval $\lambda_1 < x < \lambda_2$ and $Y_2 = Y_2(x)$ is a spectrum defined in the interval $\lambda_3 < x < \lambda_4$.
The arithmetic operators applied to the spectra produce data sets D as defined in the following intervals:

Addition and subtraction: the resulting spectrum is defined in an interval that is the union of the two starting intervals. $D_1 : Y_1 \bigcup Y_2$

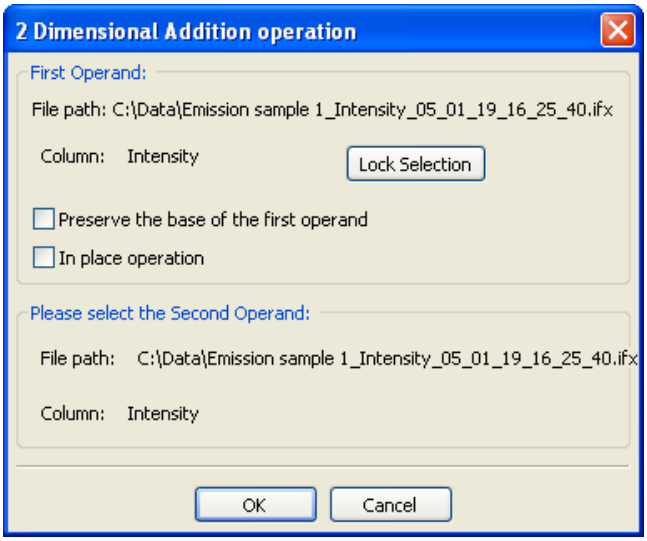
Multiplication and division: the resulting spectrum is defined in an interval that is the intersection of the two starting intervals. $D_1 : Y_1 \bigcap Y_2$

The available arithmetic operators between two spectra are displayed when the <Between two Data> operator is selected:



Open the two data files on which the arithmetic operator needs to be applied and click onto the window of the first operand.

Then select the arithmetic operator; the dialog box to the right is displayed. The file path of the first spectrum is shown. Now click on the window of the second spectrum and the result will be an additional plot displaying the result of the operation.



The interval in which the first operand is defined can be maintained by checking the <Preserve the base of first operand> box.

The operators can be applied to data sets stored in the same file. For instance, a polarization measurements file includes the intensities of fluorescence measured at the four different orientations of the polarizers; an arithmetic operator can be applied to two such columns and the selection of first column can be set by clicking the <Lock Selection> button; any other column can then be selected.

15.9 Mean along Z

Syntax If Y=Y(x_i, z_i) is a 3-dimensional plot, the new data file *D* is generated by:

$$D:(x_i,z_i)\rightarrow\left(x_i,\frac{1}{n}\sum_{1}^{n}z_i\right)$$

In short, the operator applies an average on the second independent variable of the experiment, the variable associated with the z-axis.

As an example, let us suppose to acquire a series of polarization measurements on a sample, each for a 100 seconds duration. At each measurement, adding a set volume of a titrant has changed the sample. A 3D plot can be displayed: the volume of the added titrant is on the x-axis, while the time is on the z-axis. It may be convenient to average the values of each polarization data set; a 2D display will be generated using the <Mean along Z> operator.

15.10 Slice

Syntax If Y=Y(x_i, z_i) is a 3-dimensional plot, defined for in the interval $\lambda_1<x<\lambda_2$ and $\lambda_3<z<\lambda_4$. the operator deletes the data set defined by:

For all x in the interval $\lambda_1<x<\lambda_2$
At $z=z_n$, where z_n is selected by the user.

In a series of spectra, the operator allows the deletion of one entire array of one-dimensional data.

15.11 Delete Data Points

This option allows for deletion of measured data points. Upon checking this option a table of the measured X-values is displayed and the user can choose those values that he wants to delete. Several data points can be deleted at the same time.

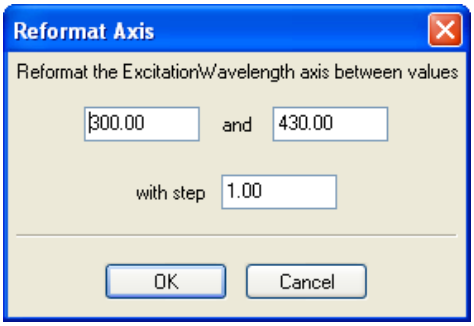
15.12 Reformat Axis

The <Reformat> operator allows the user to apply to each spectrum two operations:

a. Change the wavelength range of the spectrum:
b. Change the step-by-step spacing between the data points of a spectrum.

For instance, a spectrum has been acquired between 400 nm and 600 nm with a 2-nm step-size of the emission monochromator. Using the <Reformat> operator, data points can be extrapolated for a 1-nm-step-size. Or, a new spectrum between 400 and 500 nm with 5nm resolution can be generated.

When the <Reformat> operator is selected the dialog box to the right is displayed. Enter the wavelength range and the desired resolution of the new spectrum.



15.13 Merge Data

This option allows combining two data sets with the same X and Y dimensions.

16 Spectral Operations on Data Files

Several operations can be performed on data files; they are dubbed “spectral” due to their encompassing nature.

The list of operations is displayed when the <Spectral> operation button is pressed. Each of them is explained in the next paragraphs.

16.1 Spectral Moments

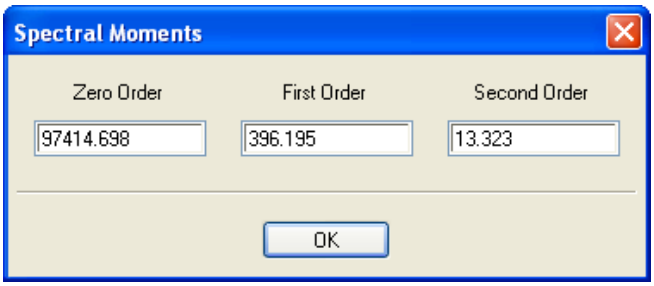
A spectrum is a function of fluorescence intensity versus wavelength. For the standpoint of this paragraph, a spectrum can be the polarization versus wavelength, or simply a kinetics versus time. For each spectrum, the moments, such as the area, the average and the standard deviation of the function can be calculated.

Whenever a spectrum is selected, and the <Spectral Moments> button is pressed, the following numbers are displayed:

zero order ⇔ *area*

first order ⇔ *average*

sec*ond order* ⇔ *s*tan*dard deviation*



16.2 Polarization Spectrum

This option allows to user to generate an excitation polarization spectrum (or anisotropy spectrum) using from the following four separate spectra:

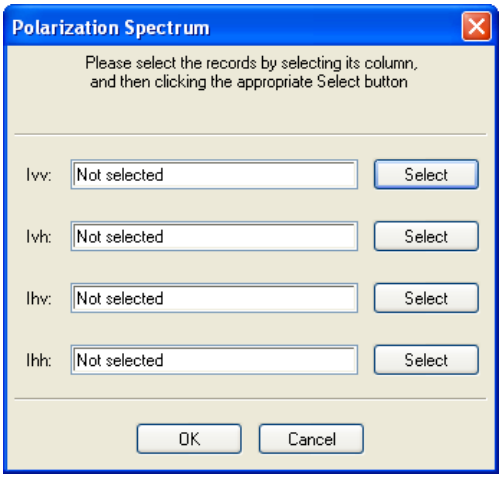
Position of Polarizers	Description
I_{HH}	Excitation polarizer horizontal – emission polarizer horizontal
I_{HV}	Excitation polarizer horizontal – emission polarizer vertical
I_{VH}	Excitation polarizer vertical – emission polarizer horizontal
I_{VV}	Excitation polarizer vertical – emission polarizer vertical

These four spectra are then used to derive the excitation polarization spectrum using the dialog box below.

Polarization $$P = \frac{I_{VH} - gI_{VV}}{I_{VH} + gI_{VV}} \qquad \text{Where } g = \frac{I_{HH}}{I_{HV}}$$

Anisotropy $$r = \frac{I_{VH} - gI_{VV}}{I_{VH} + 2gI_{VV}} \qquad \text{Where } g = \frac{I_{HH}}{I_{HV}}$$

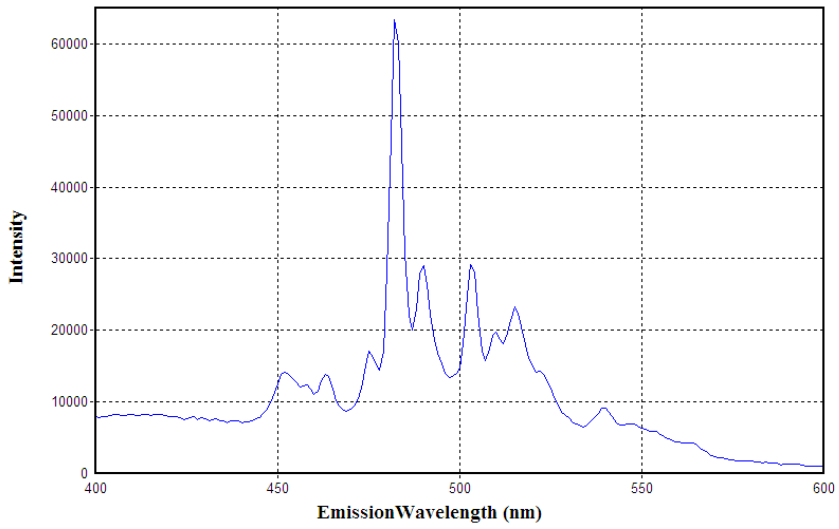
When selecting "Polarization Spectrum", the dialog box to the right is displayed. After uploading the proper spectra click 'OK" and the excitation polarization spectrum is displayed.



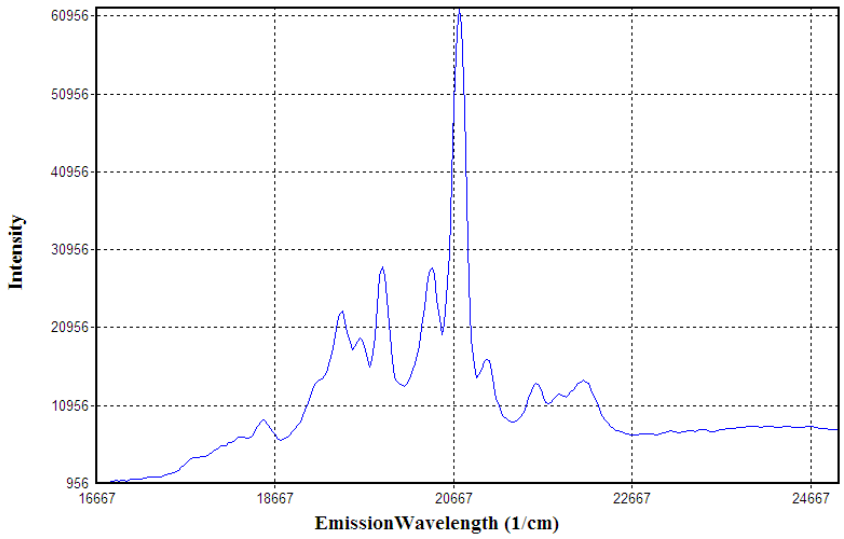
16.3 Convert to Wavenumbers

The feature allows a rapid conversion of a spectrum with the wavelength expressed in nanometers into a spectrum with the wavelength expressed in inverse centimeters (cm^{-1}). The conversion is displayed as soon as the selection is performed. Also the type of spectra can be saved.

Ovalene emission spectrum: intensity versus wavelength in nanometers.

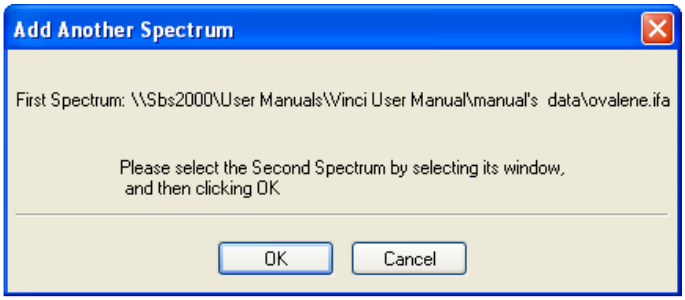


Ovalene emission spectrum: intensity versus wavelength in cm^{-1}.



16.4 Append Spectrum

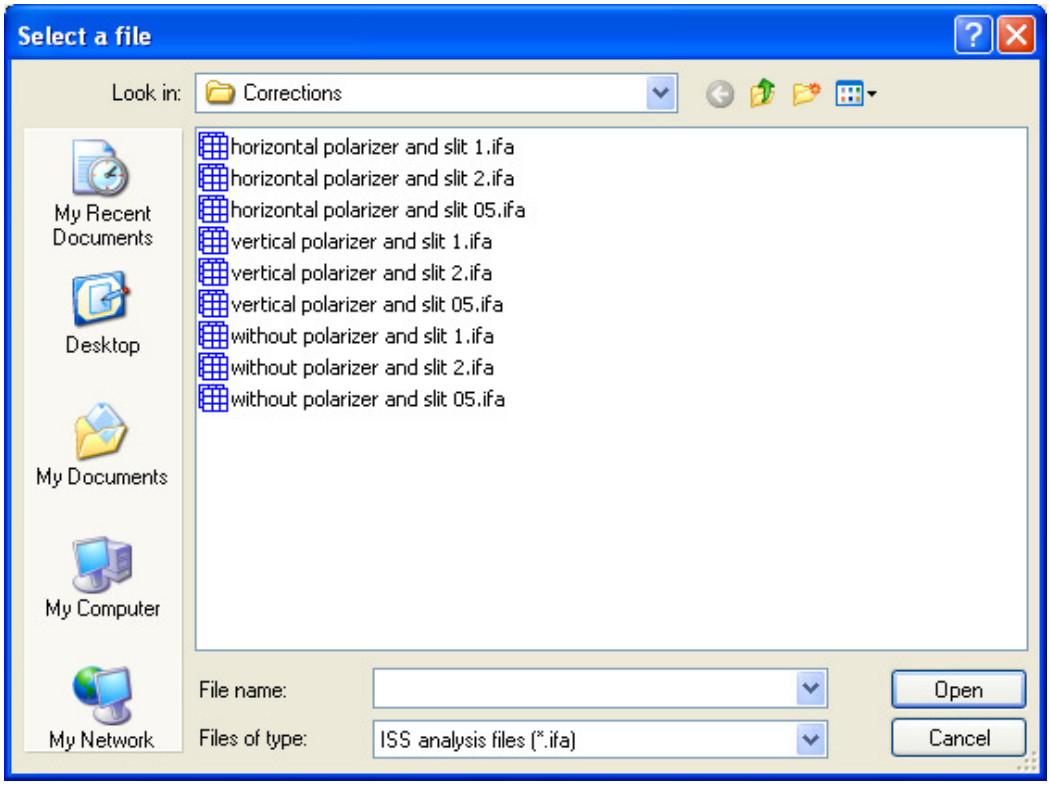
The *Append Spectrum* feature allows one spectrum to be merged with a second spectrum. The resulting spectrum will be defined in the wavelength range that is the union of the two wavelength ranges; its intensity will be the superposition of the respective intensities.



16.5 Correct Spectrum

A technical spectrum, that is a spectrum acquired with no correction, can successively be corrected using this routine and the correction files stored in Vinci.

Once the spectrum is selected, the list of correction files embedded into the software is displayed; the user will select the file corresponding to the experimental conditions utilized in the experiment.



These files apply to ISS instruments equipped with monochromators models H-10 and H-1061 only. If another monochromator is mounted on the instrument, please contact ISS for instructions.

16.6 Determine Absorption Spectrum

The spectral feature is to be used with the ISS accessory Model no. K444; the accessory allows for a rapid determination of the absorption spectrum of a solution. For a comprehensive explanation on how to utilize the accessory, please refer to the respective technical note available through ISS.

For the determination of the absorption spectrum of a solution two separate measurements are taken by scanning the excitation monochromator:

- In the first measurement, a cuvette filled with the appropriate solvent is placed in the cuvette holder in order to determine the intensity I_0 of the light reaching the sample at each wavelength of the selected range; $I_0 = I_0(\lambda)$
- Subsequently, the cuvette containing the solution is placed in the sample holder of the accessory and a new measurement of the intensity $I(\lambda)$ in the same wavelength range is taken. $I = I(\lambda)$

According to Lambert-Beer's law these two intensities are related as follows: $I(\lambda) = I_0\, e^{-k(\lambda)d}$

Where $k(\lambda)$ is the extinction coefficient of the solution and d is the optical path, that is the distance traveled by the light in the substance.

The optical density of the solution in the selected wavelength range is then given by:

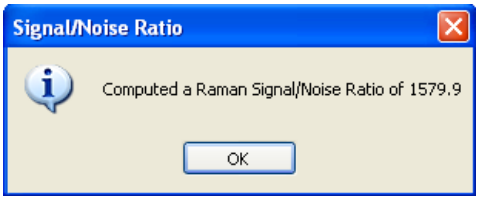
$$OD(\lambda) = k(\lambda)\, d = \ln \frac{I_0(\lambda)}{I(\lambda)}$$

16.7 Raman Signal/Noise Ratio

The Signal-to-Noise (S/N) ratio feature is utilized to automatically calculate the S/N ratio when using the corresponding routine in the Diagnostics section of the Experiment part of Vinci. Two parameters are recorded and stored: the signal at 397 nm and the signal at 450 nm. The root mean square of the latter is taken as the noise. The S/N ratio is calculated as follows:

$$\frac{S}{< N >} = \frac{(\text{average signal at } 397)}{\frac{(\text{peak - to - peak signal at } 450)}{5}}$$

By selecting a file and clicking on the <Raman S/N Ratio>, the value is returned right away.



17 Vinci Analysis

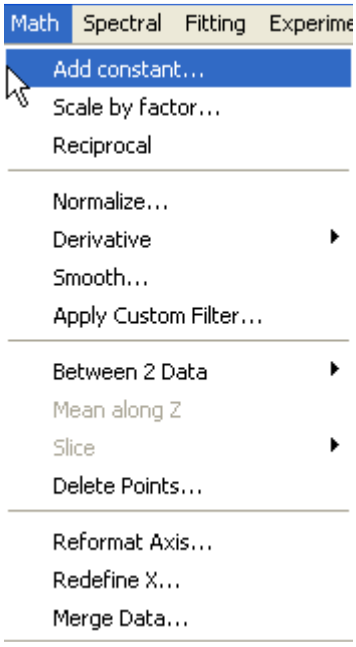
Vinci is a very versatile software program. Vinci's fitting routines allow to retrieve multiple decay times and multiple rotational correlation times, or to separate steady-state spectra of up to three components in a mixture with the phase- and modulation-resolved spectra measurement option. Other operations include smoothing, derivative, calculation of area and arithmetic between files.

The Vinci menu contains the following features:

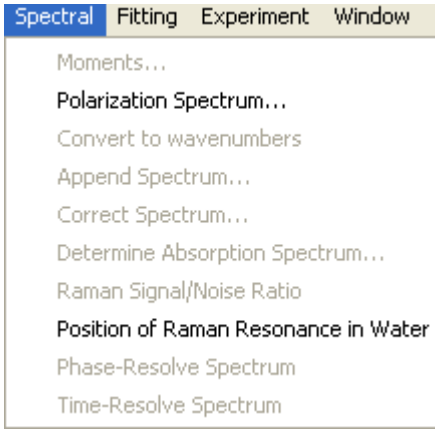
Vinci Analysis Menu List

In this list the most important ones are: **View, Math, Spectral, Fitting and Experiment.**

The View menu offers several options for viewing data:
Auto-scale Y axes allows to auto-scale the y-axes
X and Y Axis Progression allows to change the axis features from linear to log
Reset Plot Style
Overlay … allows to overlay 2 or more data sets
By checking "Browser+Data" both data and browser options will be shown on the screen while checking "Data" only the data plot will be shown



The Math menu includes all features for manipulation of data. We refer you to Chapter 15 for more detailed information on each of these tasks.



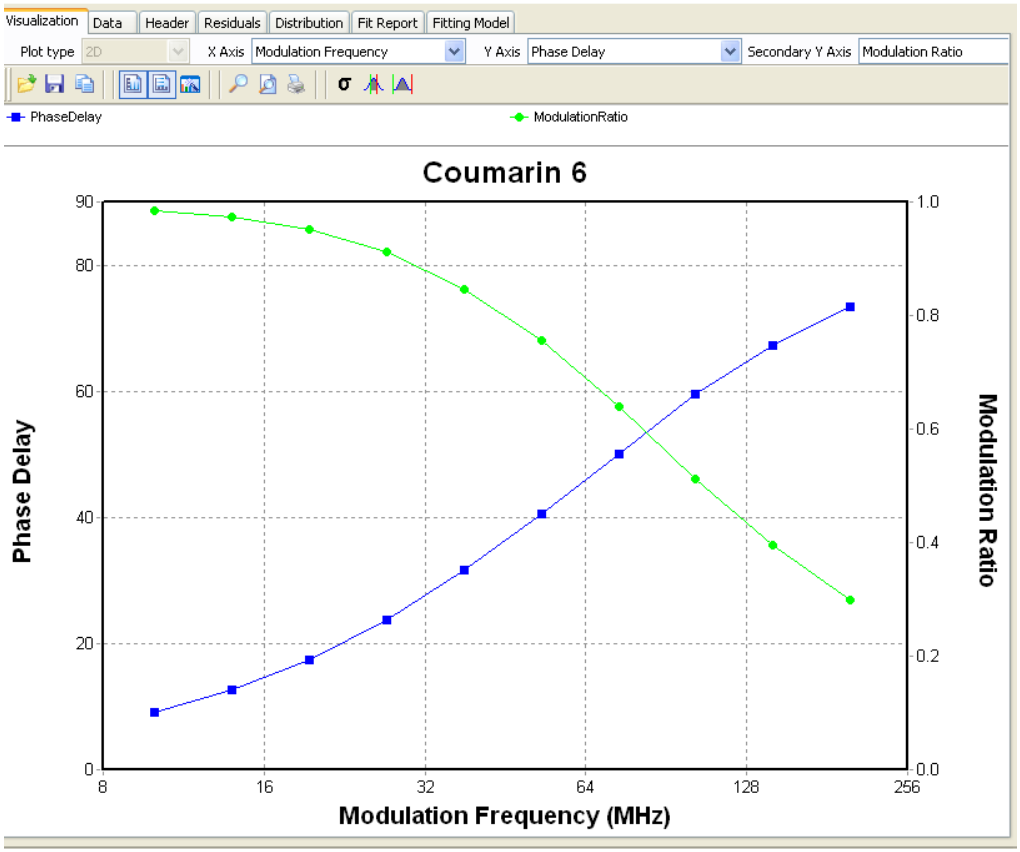
The Spectral menu contains tools for the manipulation of data and spectra. These functions are described in detail under Chapter 16.

17.1 Analysis of Time-Resolved Data in Vinci

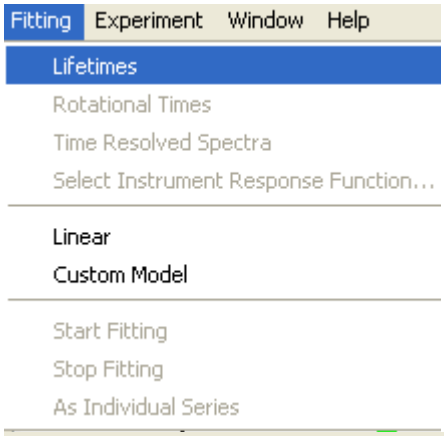
17.1.1 Analysis of Time-Resolved Frequency-Domain Data in Vinci

17.1.1.1 Fluorescence Lifetime Analysis

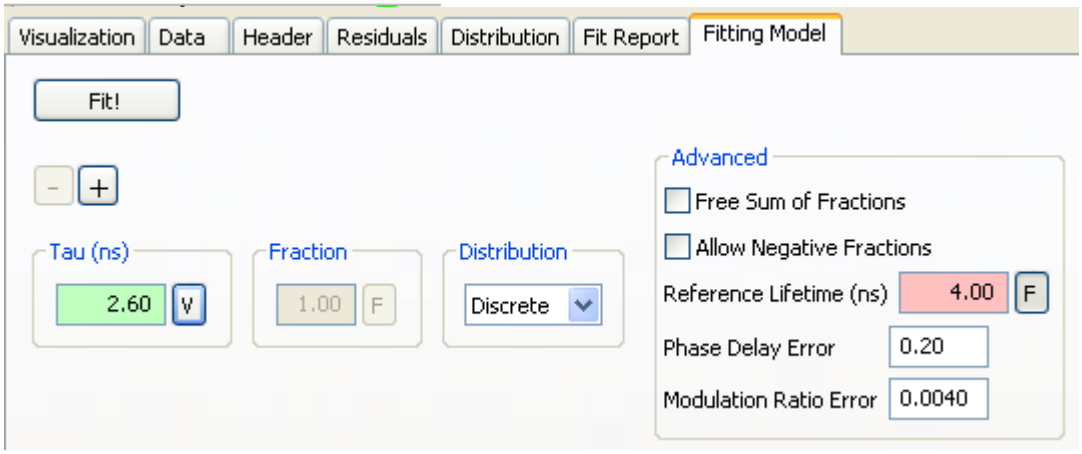
After completing a time-resolved measurement in Vinci-*Instrument and Experiment Control* the software can be programmed to automatically switch to Vinci Analysis and a frequency response curve similarly to the one shown below is displayed on screen:

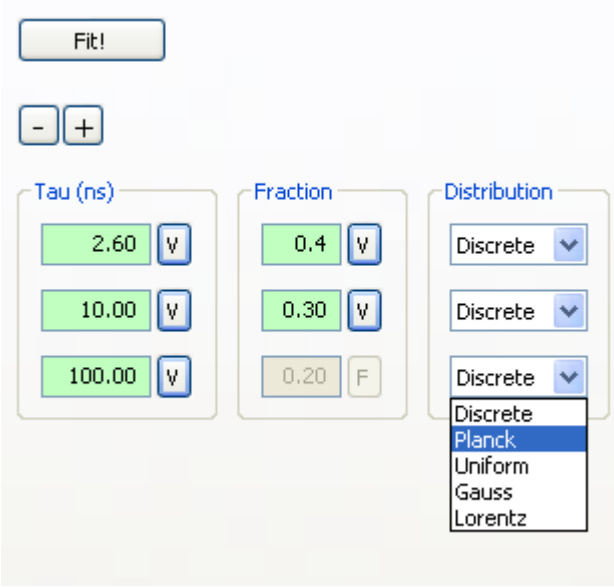


Example of a displayed plot in Vinci Analysis.



To start the fitting process, click on Lifetimes in the Fitting menu and a new page showing the parameters for the fitting process will come up (see below).



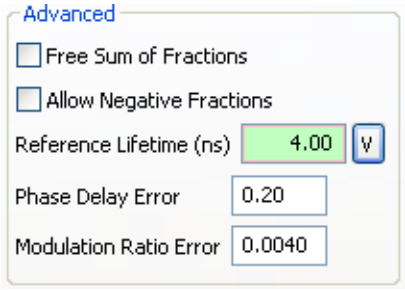


On the right of the lifetime fitting menu page are the most important parameters for fitting of phase-modulation data:

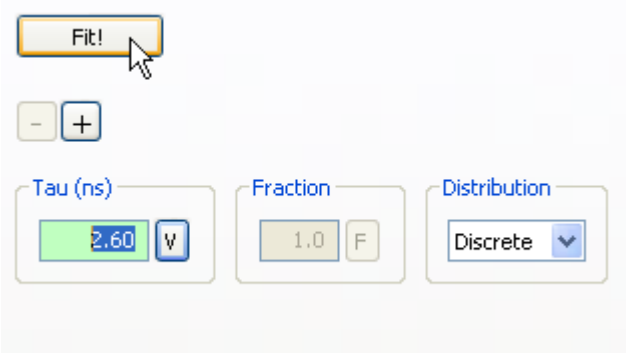
Lifetime (Tau), the fractional contributions (Fraction) and the distributions. Lifetime fits with up to 4 components are possible in Vinci Analysis.

The Fractions of these components add up to 1, thus the freedom for the fractional contributions is the number of lifetime components minus 1.

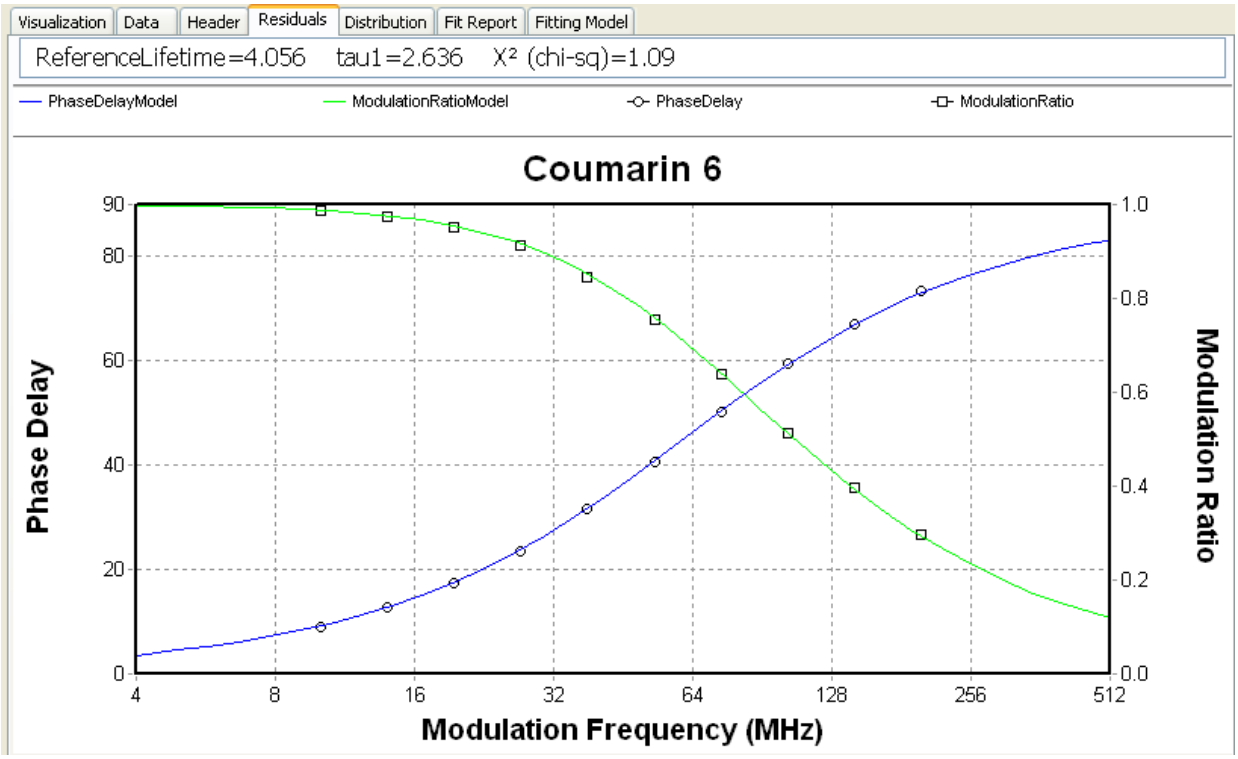
From the Distribution list one can choose between Discrete, Planck, Uniform, Gauss, and Lorentzian distributions.



There are also advanced fitting parameters in Vinci Analysis:
"Free Sum of Fractions" and "Allow Negative Fractions" allow to analyze the data with negative fractions and the sum of them not being fixed to 1.
The reference lifetime is by default a fixed parameter but can be freed in the advanced analysis option. Phase delay errors and modulation ratio errors are set to be 0.2 and 0.004 by default but can be changed by the user in the advanced analysis box.



It is recommended to start the analysis with a one- component fit because the number of the chi-square function will immediately give an idea about the goodness of the fit. If the chi-square is too high, one can add additional components and redo the fitting. The optimal chi square for a fit is 1. Chi-squares below 2 are acceptable.



17.1.1.2 Anisotropy Decay Analysis

In frequency domain there are two parameters, which characterize the anisotropy decay:
Differential phase angle between the perpendicular and parallel components of the emission:

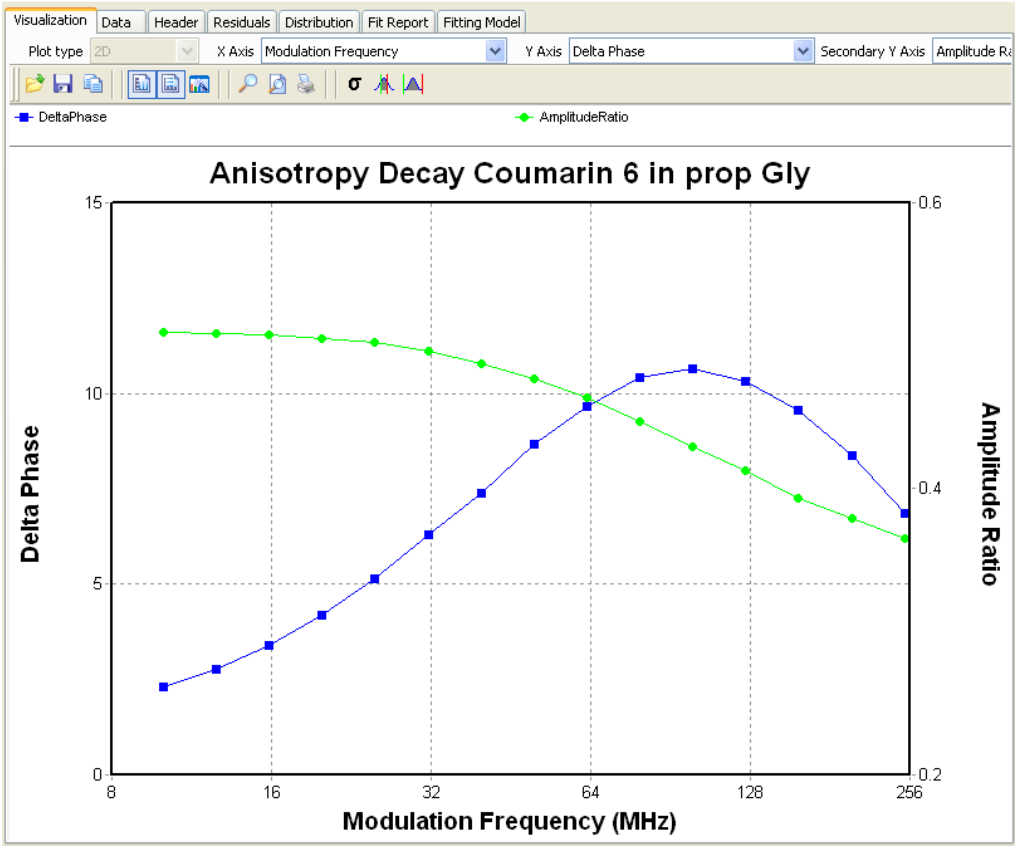
$$\Delta\omega = \phi_{\perp} - \phi_{//}$$

and the amplitude ratio between the parallel and perpendicular amplitudes of the modulation:

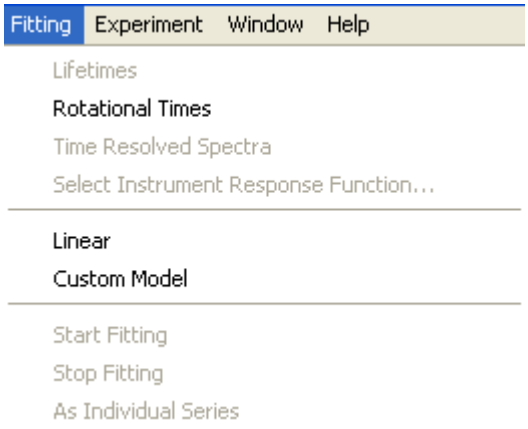
$$\Lambda\omega = \frac{m_{//}}{m_{\perp}}$$

For more information on time-resolved anisotropy measurements we refer you to our technical notes on our website.

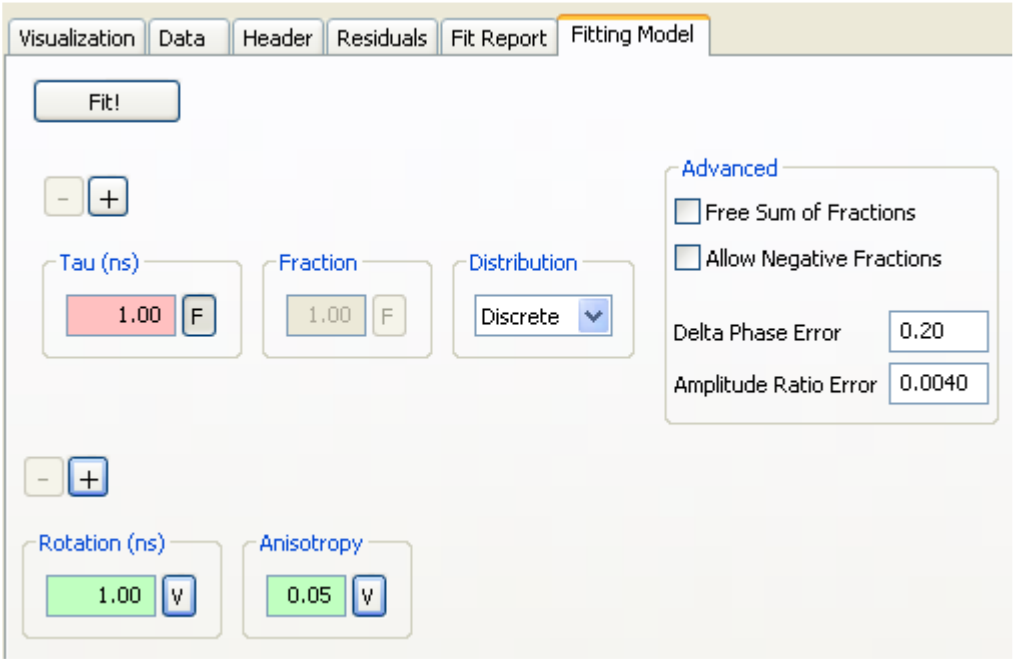
After measurement of an anisotropy decay with Vinci- *Experiment and Instrument Control* the software can be programmed to automatically switch to Vinci Analysis and a frequency response curve similarly to the one shown below is displayed on screen:

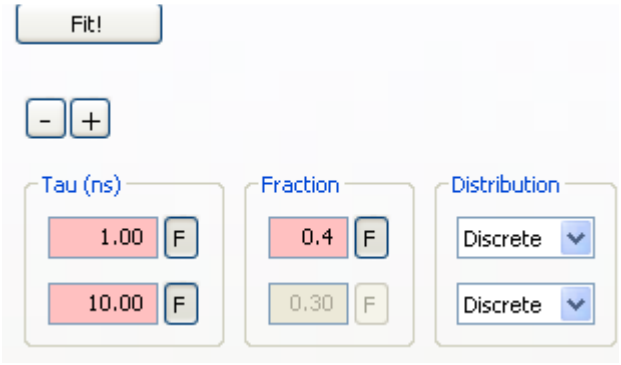


Example of a displayed plot in Vinci Analysis.



To start the fitting process, click on "Rotational Times" in the Fitting menu and following that a new page showing the parameters for the fitting process will appear (see below).



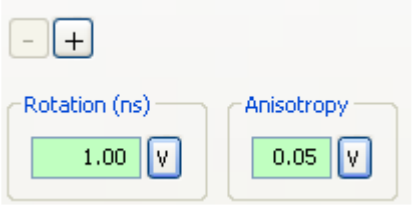


Frequency domain anisotropy decays require the following fitting parameters, which are the same as for lifetime fits:

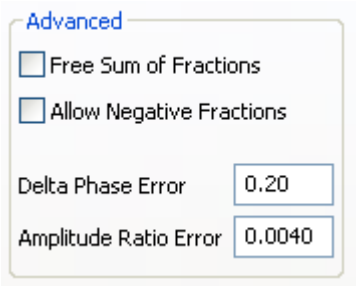
Lifetime (Tau) fits with up to 4 components are possible in Vinci Analysis.

The Fractions of these components should add up to 1, thus the freedom for the fractional contributions is the number of lifetime components minus 1.

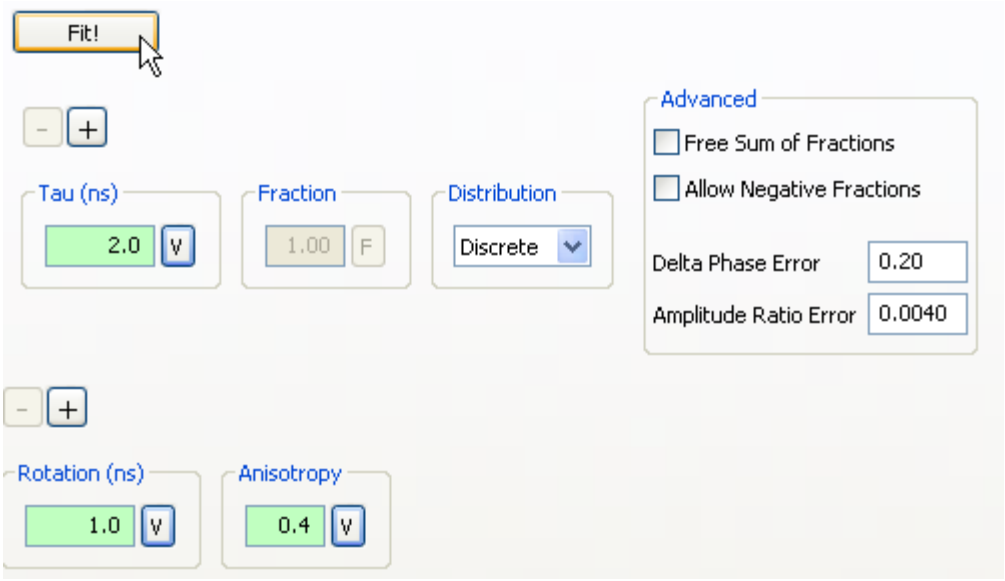
From the Distribution list one can choose between Discrete, Planck, Uniform, Gauss, and Lorentzian distributions.



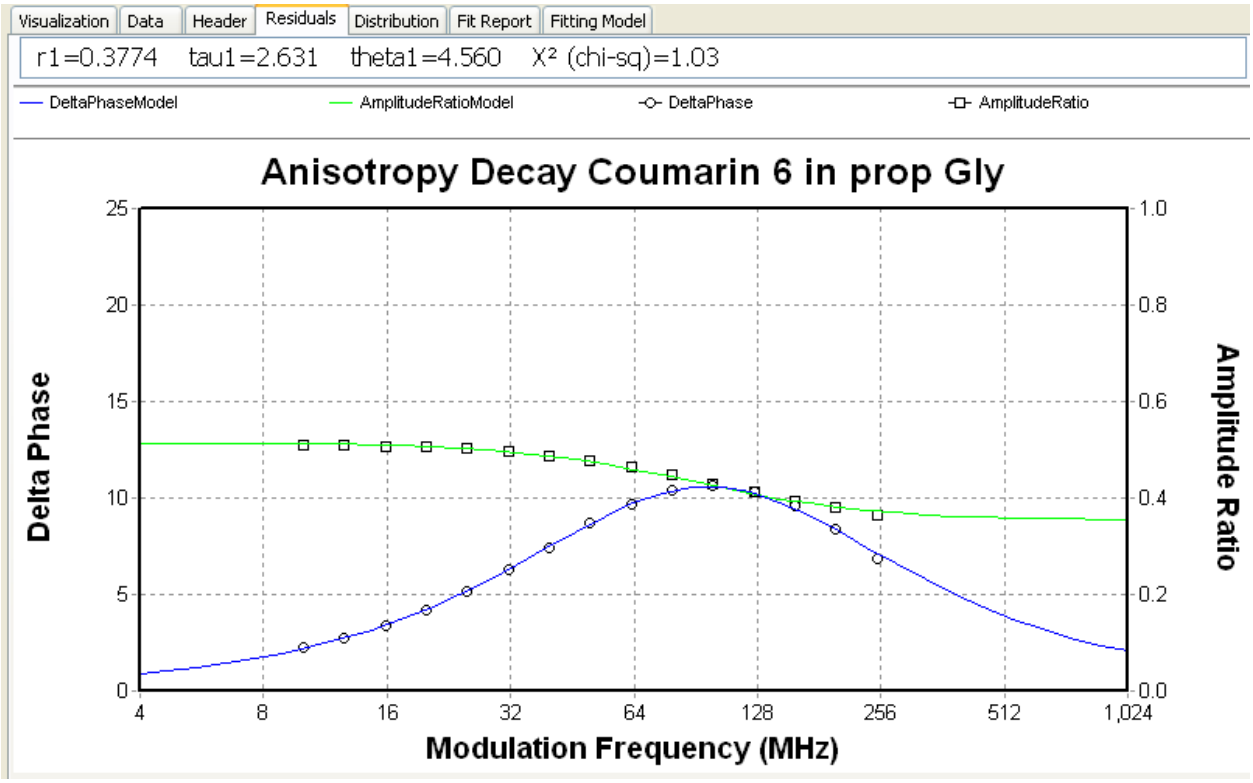
Additional parameters in the anisotropy decay analysis are the rotational correlation time and the limiting anisotropy R_0.



The advanced fitting parameters in Vinci Analysis, "Free Sum of Fractions" and "Allow Negative Fractions" allow analysis of data with negative fractions and the sum of them not being fixed to 1. Delta Phase Errors and Amplitude Ratio Errors are set to be 0.2 and 0.004 by default but can be changed by the user in the advanced analysis box.



It is recommended also for the anisotropy decay analysis to free all parameters and to start the analysis with a one-component fit for both the lifetime and the rotational correlation time because the chi-square function for this fit will immediately give an idea whether there are additional components needed to obtain a better fit. If the chi-square is too high, add another rotational component and redo the fitting. If the lifetime information is known (through a separate lifetime measurement) it should be implemented in the analysis as a fixed parameter. The optimal chi square for a fit is 1.



17.1.2 Definition of Chi-Square X^2 in Vinci Frequency-Domain Analysis

17.1.2.1 Frequency-Domain Lifetime

The chi-square X^2 is given by:

$$X^2 = \frac{1}{\nu}\left\{\sum_{j=1}^{N}\left[\frac{\phi_\omega - \phi_{c\omega}}{\sigma_\phi}\right] + \sum_{j=1}^{N}\left[\frac{M_\omega - M_{c\omega}}{\sigma_M}\right]\right\}$$

Where:

N Total number of frequencies

ν Number of degrees of freedom. Since the number of data points is twice the number of frequencies, $\nu = 2N - p$. p is the number of variables.

σ_ϕ , σ_M Uncertainties used in the phase and modulation values. Unlike in time-domain, σ_ϕ and σ_M cannot be given by a Poisson distribution. The effects of σ_ϕ and σ_M have been studied (Lakowicz JR et al, 1984, Analysis of fluorescence decay kinetics from variable-frequency phase shift and modulation data. *Biophysics J* **46**:463-477. Gratton E. et al, 1984, Resolution of mixtures of fluorophores using

variable-frequency phase shift and modulation data, *Biophysics J* **46**:479-486). It was found that the experimental result is not strongly dependent on σ_ϕ and σ_M. For consistency and ease of day-to-day interpretation, a constant error is used, σ_ϕ = 0.020 and σ_M = 0.004 for the calculation. The use of constant error does not introduce any ambiguity in data analysis since the accepting and rejecting a model is decided by the relative value of X^2. The X^2 for one, two and three exponentials are compared. If X^2 deceases by 50% or more as another component is added, it is better to include another component in the model. The X^2 calculated in this way gives information about the degree of error in the experiment data. If the X^2 is still quite high even for the best fitting option it would indicate that there is a systematic error or poor signal-to-noise ratio.

ϕ_ω Measured frequency-dependent values of phase angle

M_ω Measured frequency-dependent values of demodulation

$\phi_{c\omega}, M_{c\omega}$ Calculated frequency-dependent values of phase angle and demodulation, respectively. The phase angle and modulation can be predicted for any decay law by using sine and cosine transform from intensity *I(t)*:

$$\phi_{cw} = \arctan(N_w / D_w),$$
$$M_{c\omega} = (N_\omega^2 + D_\omega^2)^{1/2}$$

Where:

$$N_\omega = \frac{\int_0^\infty I(t)\sin\omega t dt}{\int_0^\infty I(t)dt}$$

$$D_\omega = \frac{\int_0^\infty I(t)\cos\omega t dt}{\int_0^\infty I(t)dt}$$

ω is the circular modulation frequency (2π times the modulation frequency), the equation above can be transformed to:

$$N_w = \sum_i f_i \frac{\omega\tau_i}{(1+\omega^2\tau_i^2)},$$

$$D_w = \sum_i f_i \frac{1}{(1+\omega^2\tau_i^2)},$$

f_i is the fluorescence fractional contribution:

$$f_i = \frac{\alpha\tau_i}{\sum_i \alpha\tau_i}$$

17.1.2.2 Frequency-Domain Anisotropy Decay

Chi-square X^2 is given by

$$X^2 = \frac{1}{v}\left[\sum_{j=1}^{N}\left(\frac{\Delta_\omega - \Delta_{cw}}{\delta\Delta}\right)^2 + \sum_{j=1}^{N}\left(\frac{\Lambda_\omega - \Lambda_{cw}}{\delta\Lambda}\right)^2\right]$$

Where:

N	Total number of frequencies
ν	Number of degrees of freedom. Since the number of data points is twice the number of frequencies, $\nu = 2N - p$. p is the number of variables.
Δ_ω	Measured differential phase, $\Delta_\omega = \phi_\perp - \phi_{//}$
Λ_ω	Measured modulation ratio, $\Lambda_\omega = M_{//} / M_\perp$
$\delta\Delta, \delta\Lambda$	Uncertainties in the differential phase and modulation ratio
Δ_{cw}	Calculated phase shift

$$\Delta_{c\omega} = \arctan\left(\frac{D_{//}N_\perp - N_{//}D_\perp}{D_{//}N_\perp + D_{//}D_\perp}\right)$$

Λ_{cw}	Calculated modulation ratio

$$\Lambda_{c\omega} = \left(\frac{N_{//}^2 + D_{//}^2}{N_\perp^2 + D_\perp^2}\right)^{1/2}$$

$N_{//}, N_\perp, D_{//}, D_\perp$ are calculated in a similar way as in lifetime with parallel and perpendicular intensity.

$$I_{//}(t) = \frac{1}{3} I(t)\left[1 + 2r(t)\right]$$

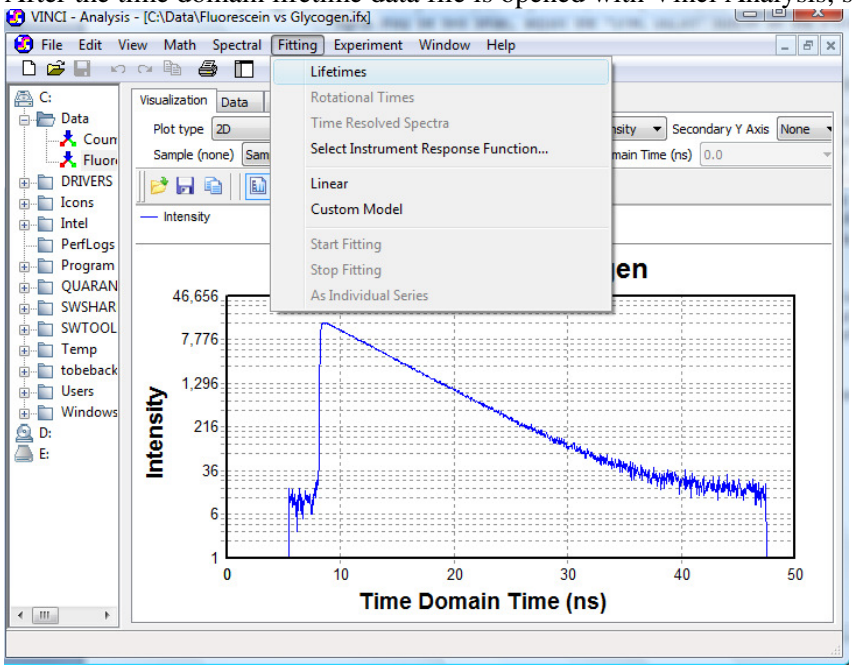
$$I_\perp(t) = \frac{1}{3} I(t)\left[1 - r(t)\right]$$

Where *I(t)* is the intensity and *r(t)* is the time-resolved anisotropy. Please refer to "Principles of Fluorescence Spectroscopy (Joseph R. Lckowicz, Third Edition)" for more information.

17.1.3 Analysis of Time-Resolved Time-Domain Data in Vinci

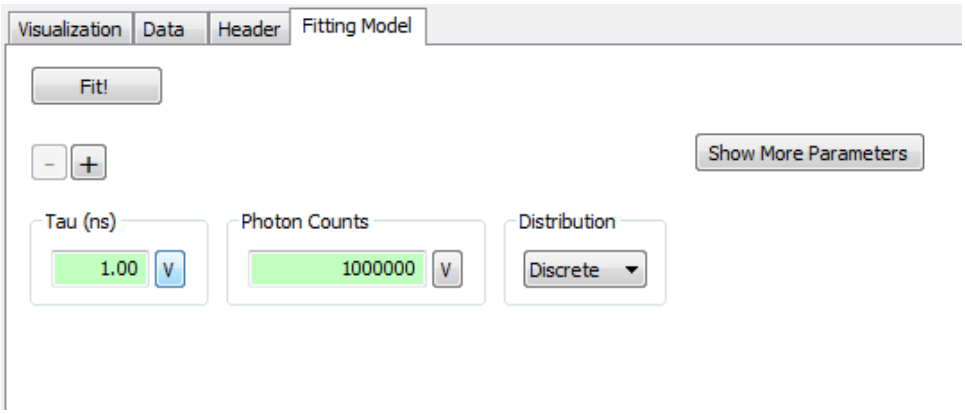
17.1.3.1 Time-Domain Intensity Decay Analysis

After the time domain lifetime data file is opened with Vinci Analysis, select Fitting->Lifetimes.



The "Fitting Model" panel is displayed. Select a proper fitting model.

There are several options to fit the decay curves: one is to increase the number of components in the fit. Vinci allows fitting with up to 4 components. Typically 3 are more than sufficient. Additional components for the intensity decay fit are selected by clicking on the plus sign above the Tau. The other option is to choose from several different lifetime distribution: The default is discrete; other distributions to choose from are Planck, Uniform, Gaussian and Lorentzian.



Go to the "Visualization" window, and move the cursor and select a proper range for the lifetime analysis. Make sure that the IRF is fully included in the analysis.

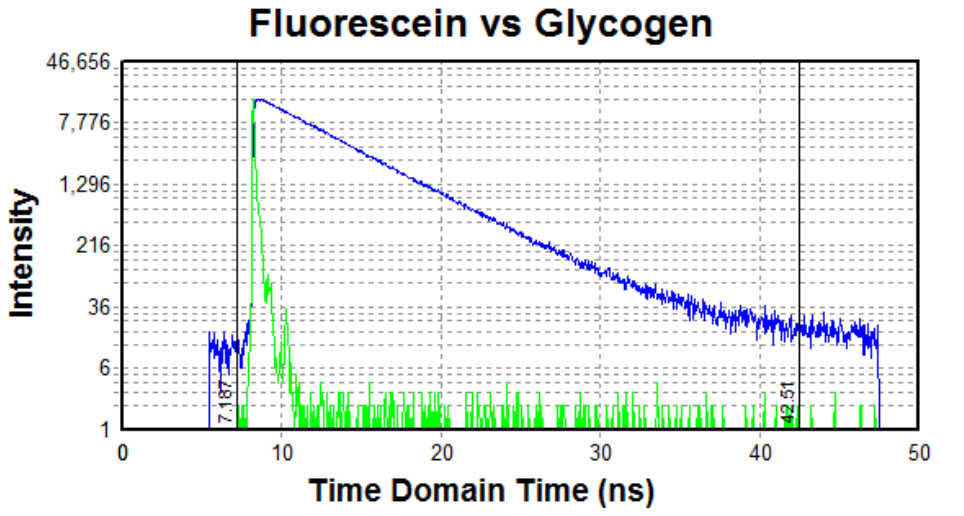
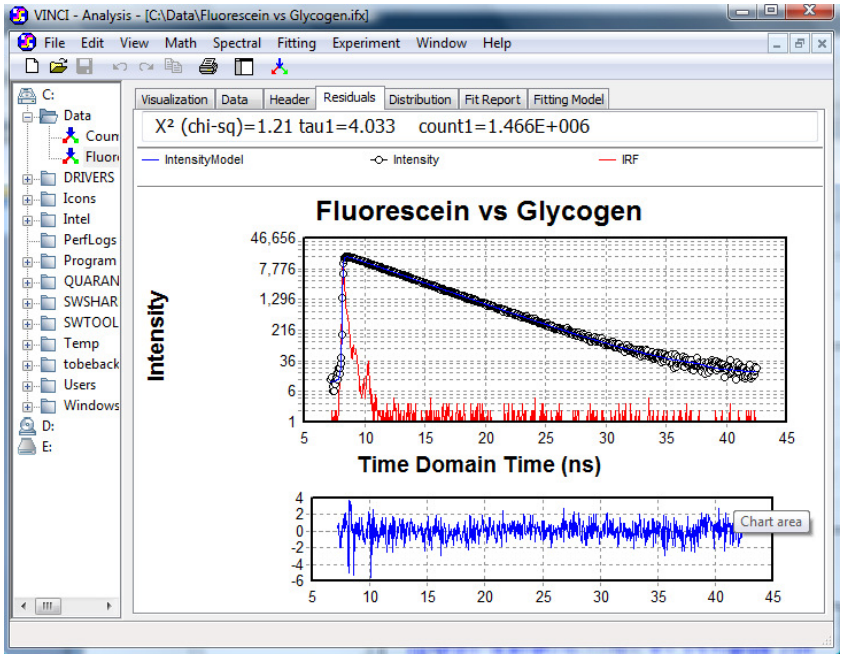


Figure 0-1 Selection of proper fitting range

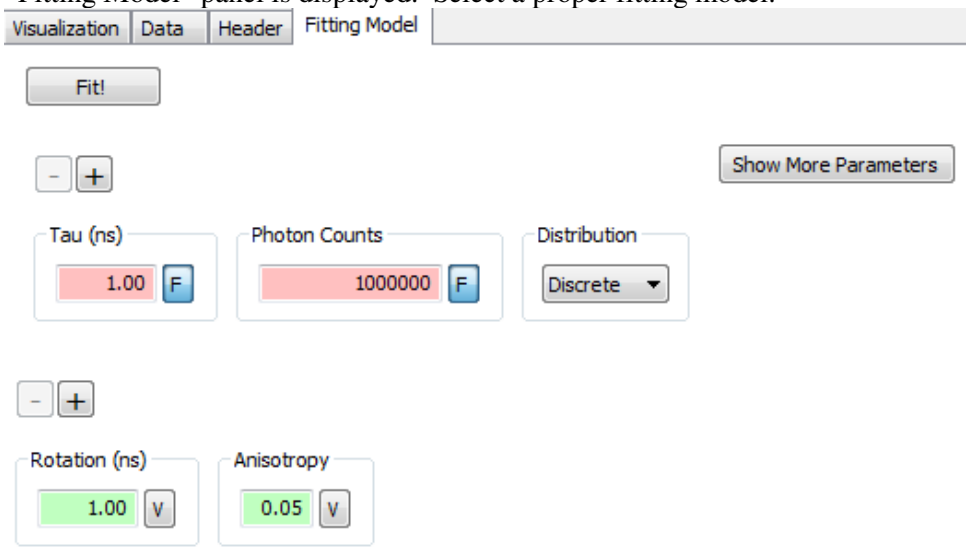
Go to the "Fitting Model" panel and click on the "Fit!" button.

The Residuals window will display the fit result including the lifetime value(s) and chi-square value for the fit.

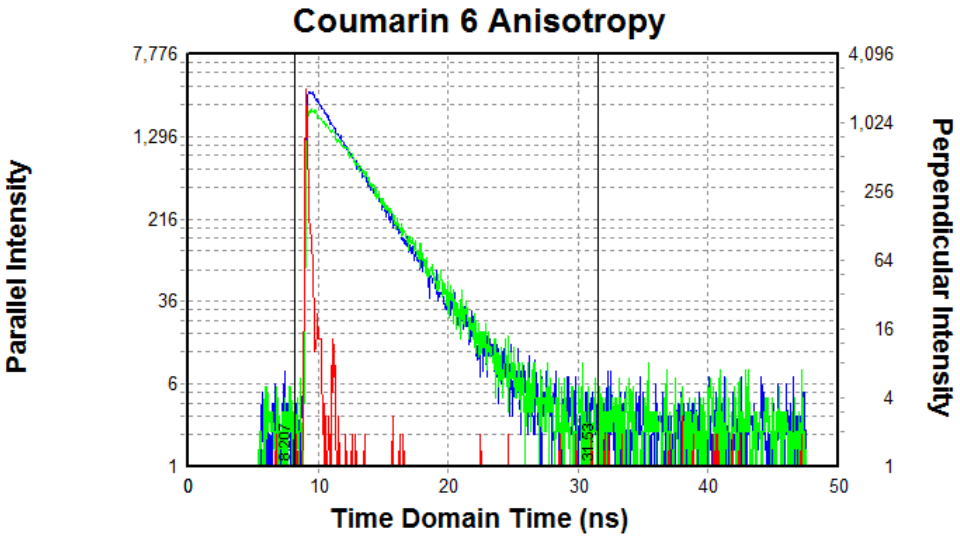


17.1.3.2 Time-Domain Anisotropy Decay Analysis

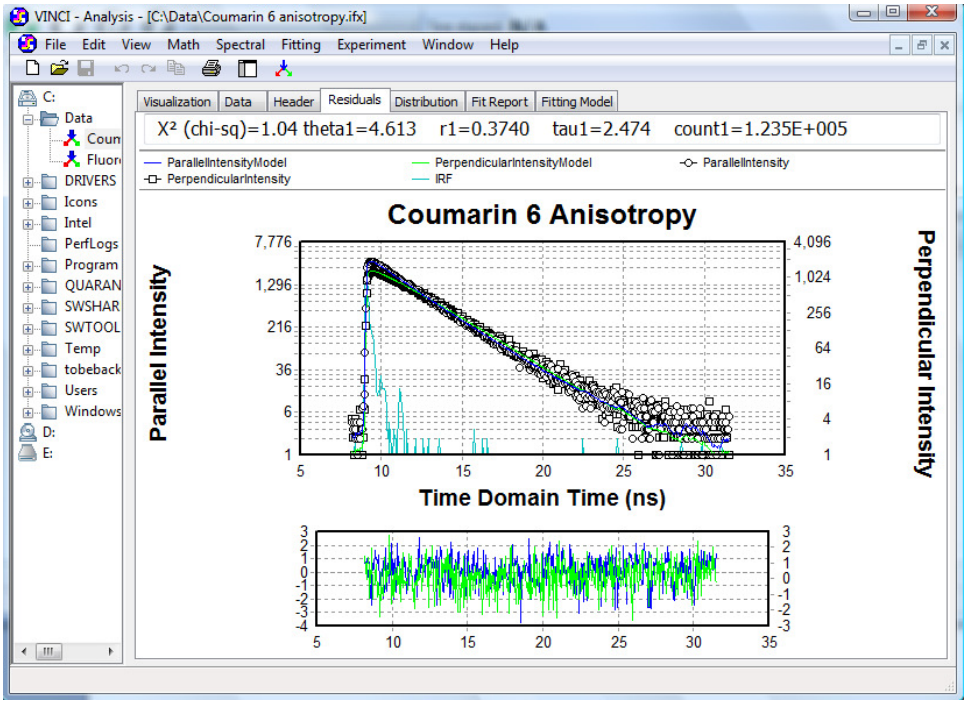
After the time-domain anisotropy dada is opened in Vinci Analysis, select Fitting->Rotational Times, the "Fitting Model" panel is displayed. Select a proper fitting model.



Go to the Visualization window, move the cursor and select a proper range.



Then go back to Fitting Model Panel and click the "Fit!" button. The fitting result will be displayed in Residuals window.



17.1.4 Definition of Chi-Square X^2 in Time-Domain Analysis

17.1.4.1 Time-Domain Intensity Decay

For time-domain intensity decay analyses the Chi-square X^2 is given by:

$$X^2 = \frac{1}{\nu}\sum_{k=1}^{n}\frac{[N(t_k) - N_c(t_k)]^2}{\sigma_k^2} = \frac{1}{\nu}\sum_{k=1}^{n}\frac{[N(t_k) - N_c(t_k)]^2}{N(t_k)}$$

Where:

n number of channels or data points

ν Number of degrees of freedom. $\nu = n - p$. p is the number of variables. Since p is negligible comparing to n in time domain measurement, $\upsilon \cong n$

$N(t_k)$ Measured intensity decay data, number of photons collected at time channel t_k.

σ_k Standard deviation. Known from poisson distribution, $\sigma_k = \sqrt{N(t_k)}$

$N_c(t_k)$ Normalized calculated intensity decay data at time channel t_k using assumed parameter values. For a multi-exponential model, the impulse response function of sample is:

$$I(t) = \sum_{i=1}^{n} \alpha_i \exp(-t/\tau_i),$$

τ_i is the decay times and α_i represent the amplitudes of the components at $t = 0$.

However, it is impossible to just measure the impulse response function of sample. The measured intensity is a convolution of impulse response function from sample and instrument.

17.1.4.2 Time-Domain Anisotropy Decay

For time domain anisotropy decay, Chi-square X^2 is given by:

$$X^2 = \frac{1}{\nu}\left(\sum_{k=1}^{n}\frac{\left[N_{//}(t_k) - N_{//}^c(t_k)\right]^2}{\sigma_{//k}^2} + \sum_{k=1}^{n}\frac{\left[N_{\perp}(t_k) - N_{\perp}^c(t_k)\right]^2}{\sigma_{\perp k}^2}\right)$$

Where:

n number of channels or data points

ν Number of degrees of freedom. $\nu = 2n - p$ p is the number of variables. Since p is negligible comparing to n in time domain measurement, $\upsilon \cong 2n$

$N_{//}(t_k)$ Measured parallel intensity decay data, number of photons collected at time channel t_k.

$N_{\perp}(t_k)$ Measured perpendicular intensity decay data, number of photons collected at time channel t_k.

$\sigma_{//k}$, $\sigma_{\perp k}$ Standard deviation for parallel and perpendicular intensity. Known from Poisson distribution

$$\sigma_{//k} = \sqrt{N_{//}(t_k)}$$

$$\sigma_{\perp k} = \sqrt{N_{\perp}(t_k)}$$

$N_{//}^c(t_k)$ Calculated parallel intensity decay at time channel t_k using assumed parameter values.

$N_{\perp}^c(t_k)$ Calculated perpendicular intensity decay at time channel t_k using assumed parameter values.

The calculated parallel and perpendicular intensity decays are:

$$I_{//}(t) = \frac{1}{3}I(t)\left[1 + 2r(t)\right]$$

$$I_{\perp}(t) = \frac{1}{3}I(t)\left[1 - r(t)\right]$$

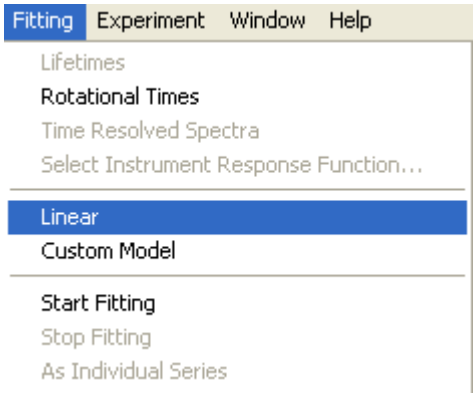
Where *I(t)* is the intensity decay as listed in time domain lifetime section, *r(t)* is the time-resolved anisotropy, general r(t) for a multi-exponential anisotropy decay is:

$$r(t) = r_0 \sum_j g_j \exp(-t/\theta_j) = \sum_j r_{0j} \exp(-t/\theta_j)$$

$r_0 = \sum r_{0j}$ are the limiting anisotropy in the absence of rotational diffusion, the θ_j are the individual correlation times, and g_j are the fractional amplitudes of each correlation time in the anisotropy decay ($\sum g_j = 1$).

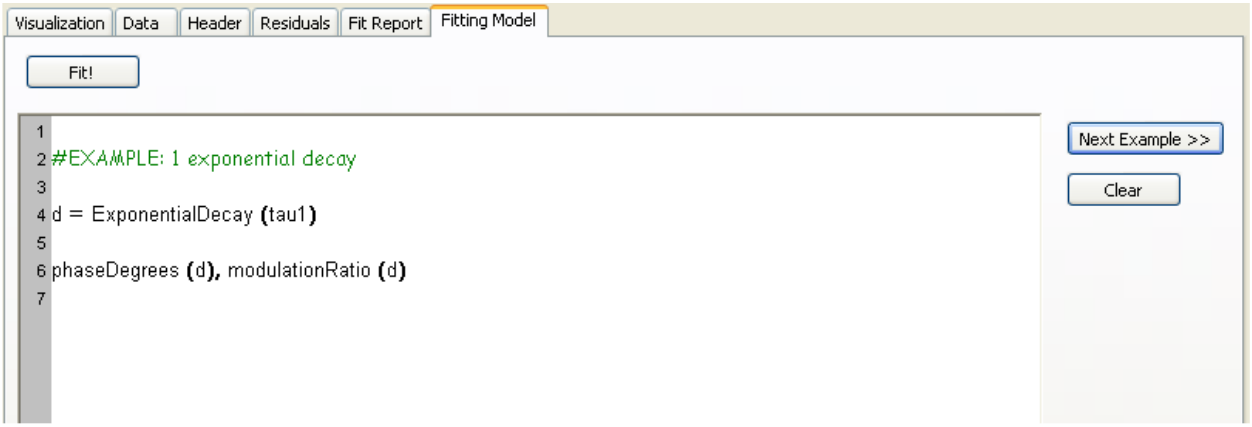
For a system with multiple τ_i and θ_j, interaction between τ_i and θ_j could be quite complicated. Please refer to "Principles of Fluorescence Spectroscopy (Joseph R. Lckowicz, Third Edition)" for more information.

17.2 Analysis using a Linear Regression or Custom Models

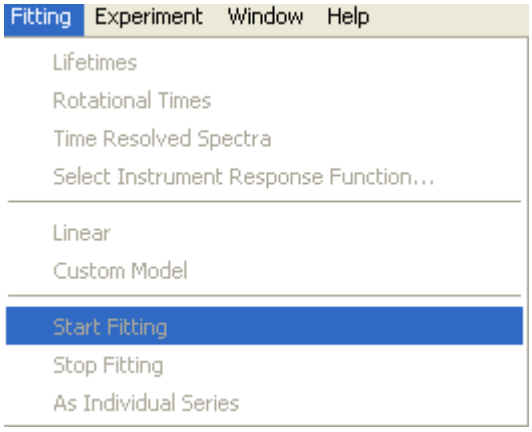


Vinci also includes analysis tools that enable fitting with linear regression or other custom models. The option to built user-defined custom models for the analysis can be accessed by checking on "Custom Model". Several examples of custom models can be selected by checking on: Next Examples.

Example 1: single exponential decay
Example 2: bi-exponential decay
Example 3: anisotropy decay

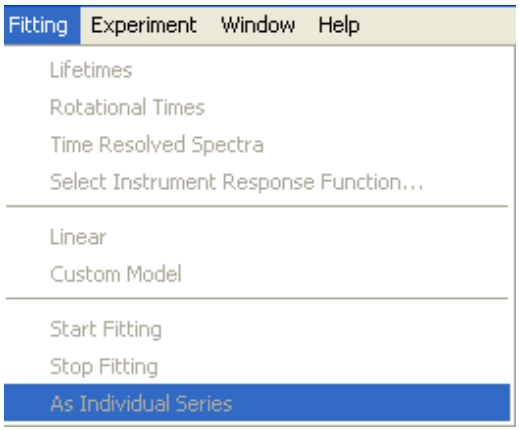


17.3 Starting and Stopping the Analysis Process



The “Start Fitting” and “Stop Fitting” options allow to start and to stop the fitting process.

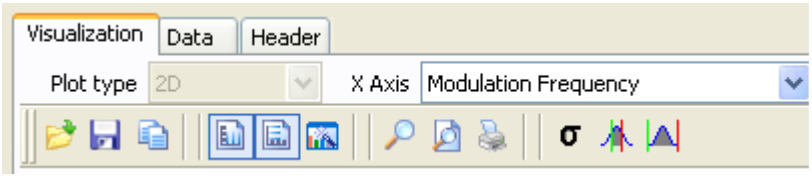
17.4 Individual Fitting of a Record that is Part of a Series of Measurements



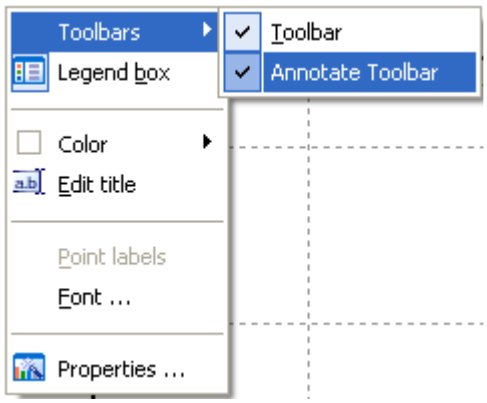
This option is used when a sequence of measurements was obtained and these measurements need to be analyzed separately. Checking “As Individual Series” allows for individual fitting of a record that is part of a series.

18 Generating and Manipulating Plots in Vinci

This chapter is aimed at helping the user of Vinci-*Multidimensional Fluorescence Spectroscopy* to generate plots and data sets that are publishable in ACS journals without the need for having to use external software. Vinci includes 2 sets of toolbars that can be utilized to generate and manipulate these data plots:



The Vinci-Plotting Toolbar contains important functions to manipulate and generate data plots. It also contains functions to choose from different plot styles and to display different measurement parameters.



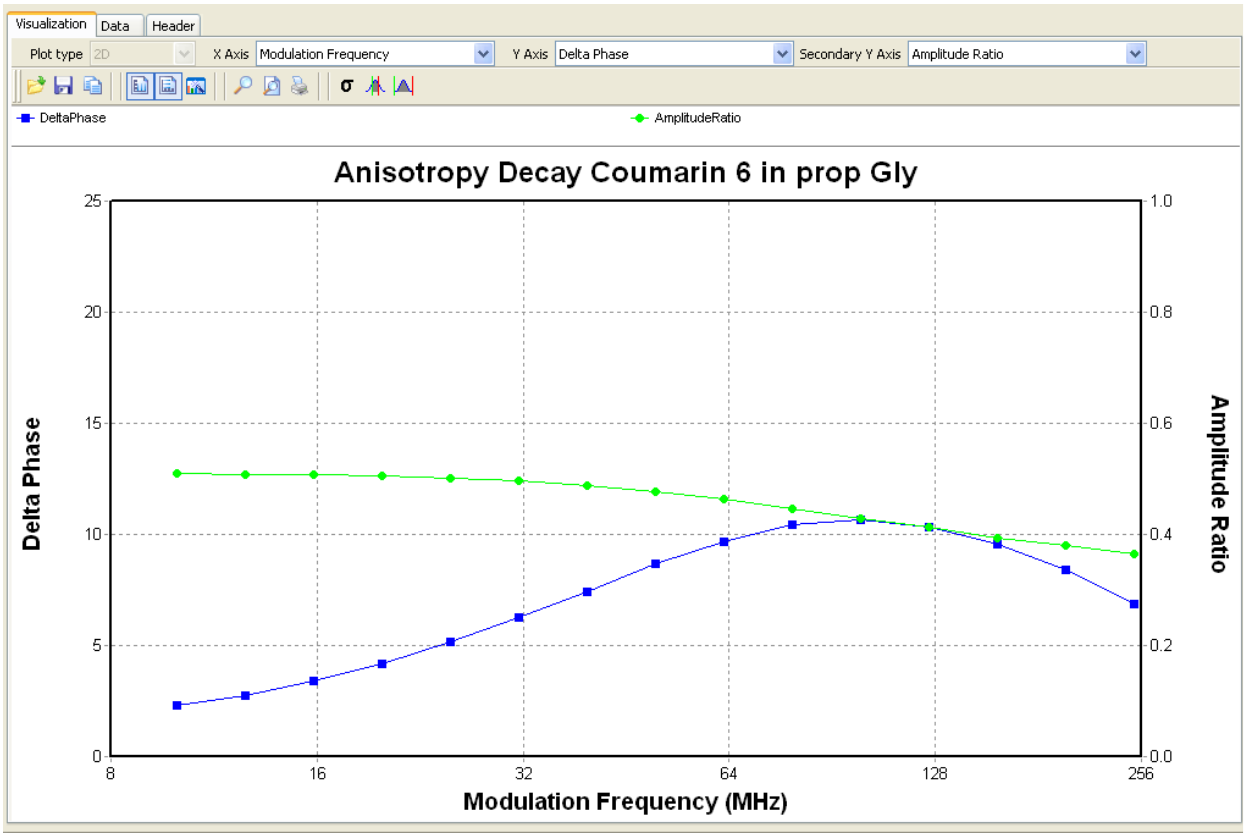
The Vinci annotation toolbar is hidden. Press “F2”, right click mouse button and select “Toolbars” and “Annotate Toolbar” to display it.

Both toolbars can be hidden by right clicking the mouse button and selecting “Toolbars” and then unchecking “Toolbar” or “Annotate Toolbar”.

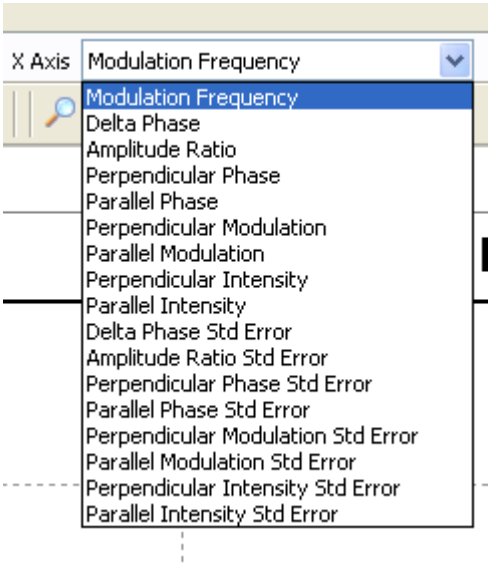
18.1 Vinci Analysis Page Set Up

Vinci Analysis presents the data and data plots in the following order:

18.1.1 Visualization:



Vinci-Visualization showing data in plot format.



Vinci Analysis enables to display a variety of different parameters in graphic format. These parameters can be chosen from the list under the X, Y or a 2nd Y-axis.

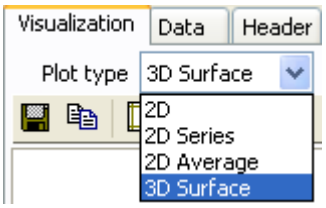
The Visualization page contains several functions (listed on top) that enable to save, copy and manipulate the data plot.

These functions are explained in detail below:

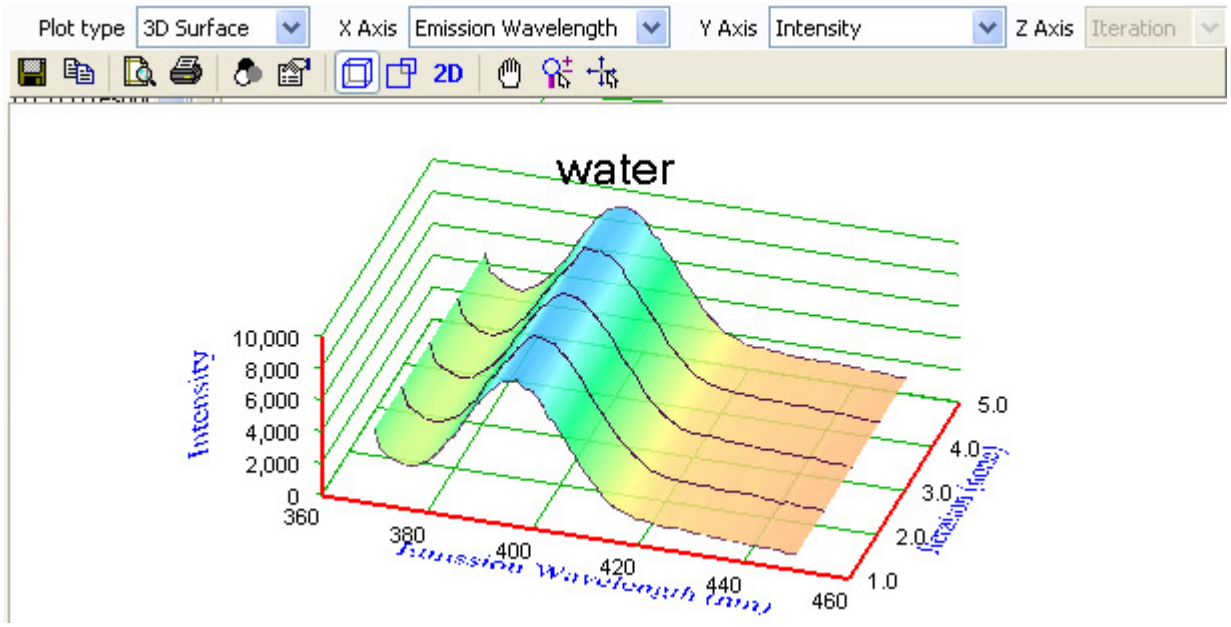
Open Chart — Check this box to open files and folders

Save Chart	Check this box for saving of data and data files
Copy Chart	Check this box to copy plot to clipboard
Vertical and Horizontal Grid	Allows to add/remove horizontal and vertical grid
Plot properties tool	Checking this box opens the plot properties chart that allows to manipulate data plots
Zoom-Tool	
Page Setup Tool	Definition of margins, orientation of plot, colors etc.
Print	Opens up printing setup page
Statistics Tool	Provides an Y average for measured data including the standard deviation
Vertical Markers Selection Tool	Shows vertical markers
Vertical Markers Hide Tool	Hides vertical markers

Different Plot Styles:



Vinci Analysis allows displaying and manipulating multi-dimensional data.



Example of a plot of 5 Iterations of the Water Raman Spectrum in 3-D.

	Save Chart	Check this box for saving of 3D data files
	Copy Chart	Check this box to copy plot to clipboard
	Print Preview	Opens up a print setup and preview window
	Print	Print figure
	Render Greyscale	Display figure in grey scale
	Property Sheet	Open a property sheet window
	Orthogonal Projection	Display figure in orthogonal projection
	Perspective Projection	Display figure in perspective projection
	2D View	Display figure in 2D
	Trackball Mode	Click this button will enable rotate 3D figure by click and drag mouse
	Zoom Mode	Click this button will enable zoom in and out figure by click and drag mouse
	Offset Mode	Click this button will enable move figure in window by click and drag mouse

18.1.2 Data

Data collected during experiments is displayed in the Data window.

18.1.3 Header

Title and other experimental conditions are displayed in the “Header”

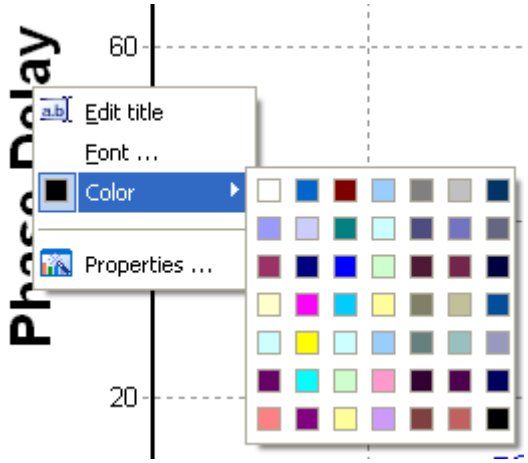
Visualization | Data | Header

Attribute	
Title	Bodipy vs Fluorescein
Comment	
Signature	ISS_Experiment_Ver_1_0
Timestamp	Wed Nov 14 10:05:22 2007
AcquisitionType	Analog
AcquisitionFormat	L
AcquisitionSide	Left
FrequencyDomainAcquisition	yes
CrossCorrelationFrequency	400
Measurable	Phase-Mod
DarkUpdatePeriodInMinutes	30.0
ReferenceLifetime	4.0
ReferenceLifetimeSample	Reference
ExcitationWavelengthBandwidth	8
Visualization	PlotType:2D,X:ModulationFrequency,Y:PhaseDelay,Y2:ModulationRatio
ModulationFrequency	type:numeric,unit:Hz,from:2000000,to:100000000,numberOfFrequencies:15,progre
ExcitationWavelength	type:numeric,unit:nm,fixed:0.0
Sample	type:label,unit:none,fixed:Sample
Space	ModulationFrequency
Columns	ModulationFrequency,PhaseDelay,ModulationRatio,TauPhase,TauModulation,Sample

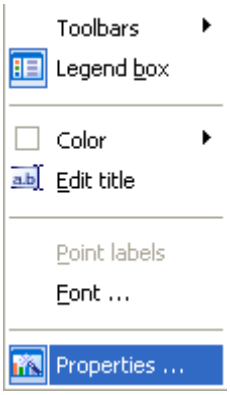
Example of header information of a frequency-domain lifetime measurement

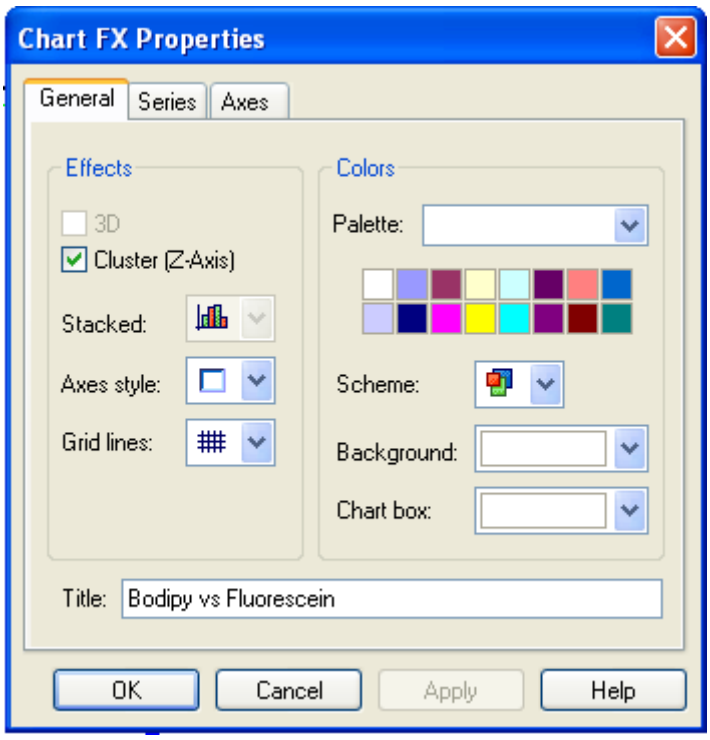
18.2 Manipulating Plots in Vinci

A plot can be manipulated in either the “Visualization” or “Residuals” window.



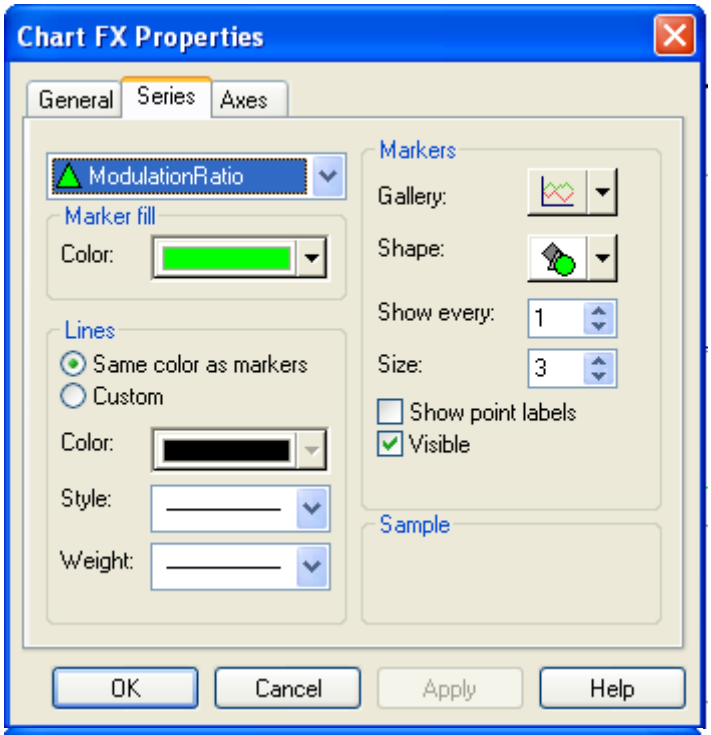
Right clicking on title or label will bring up the “Options” window which allows you to edit, change fonts and color of a title, and/or the properties of axes, series etc.





By right clicking on the plot area, one can choose among the option as shown on the left:
Toolbars will show or hide tool bar
Legend Box will show or hide legend Box
Color option allows the selection of the colors for the chart.
Edit title allows to change the title of chart
Changes of fonts of the title or label are possible by clicking on title or labels.
Clicking on "Properties" will display the Chart FX properties window (see below).
The Chart FX Properties window allows to select and change the features of the plot:

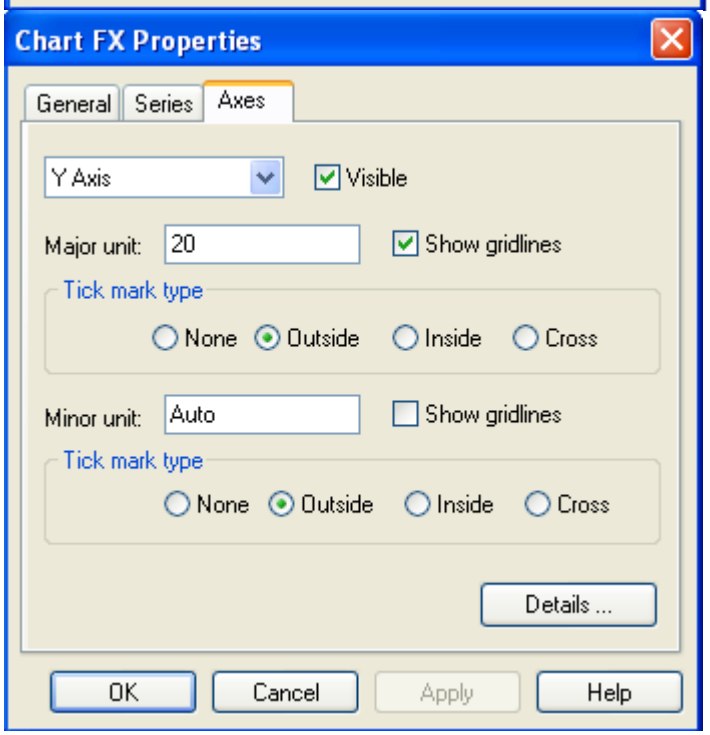
The "General" window gives the option to change the general properties of a chart such as the title, color etc.



The "Series" panel allows to select/change the following:

Color, Style, Weight of lines;

Type, Shape, and Size of markers.



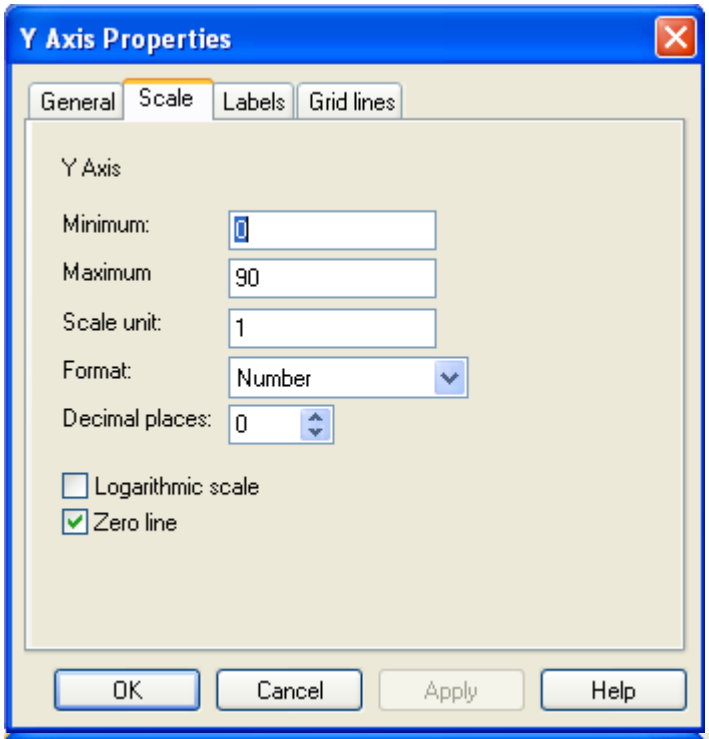
The "Axes" panel allows changing the properties of selected different axes by clicking on the drop-down box. The options available in this window are:

Checking or unchecking "Visible" will display or hide axes;

Checking or unchecking "Show gridlines" for major and minor units will display or hide the gridlines.

Placing tick marks inside or outside of axis;

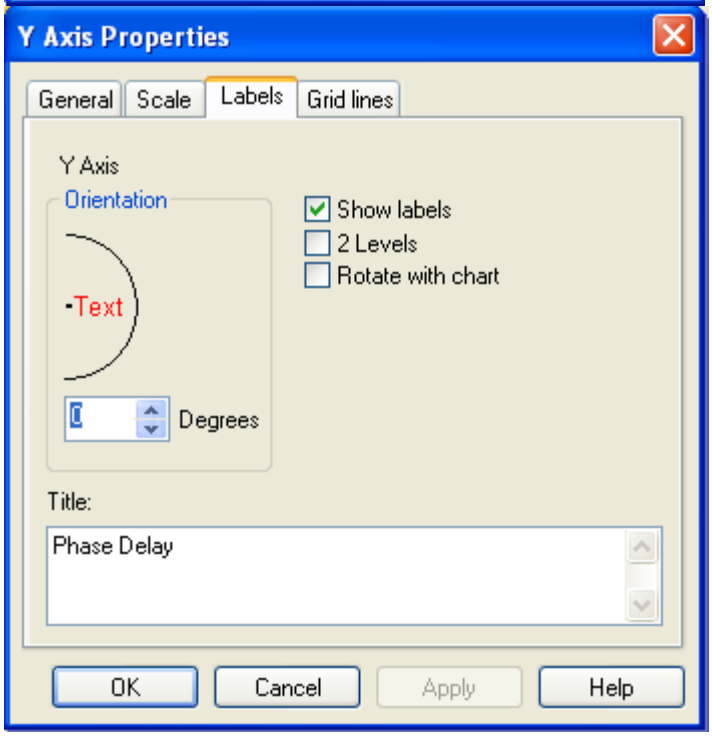
Selecting an axis and clicking on details displays a new window e.g. "Y Axis Properties" that allows changing the properties of that axis (see below).



Options for displaying a particular axis are provided in the "Scale" panel:

Change the minimum and maximum of an axis, the scale unit, the label display format and decimal points;

It also enables to display data in logarithmic scale, and allows for the axis to go through zero.



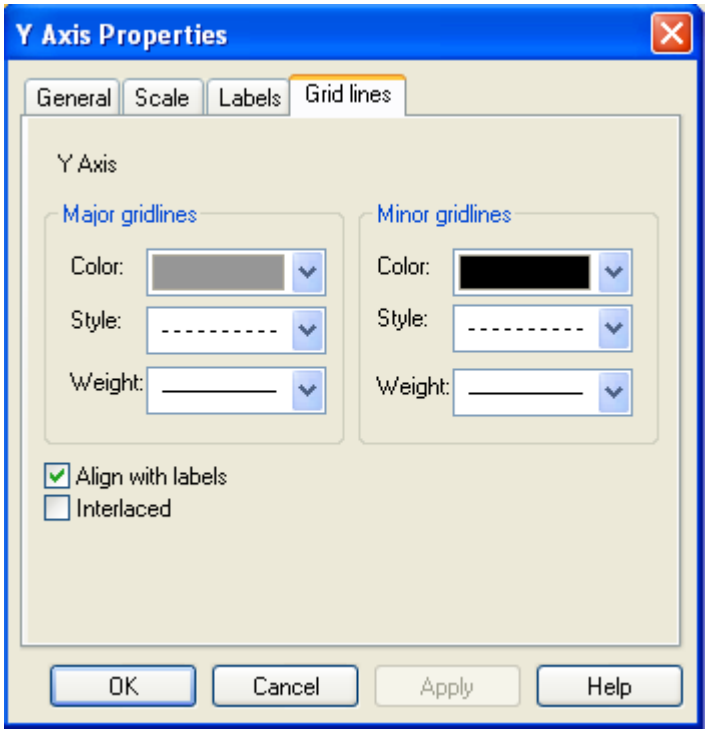
Options for displaying the labels of an axis are available in the "Scale" panel:

Change the orientation of label

Show or hide labels by clicking the check box "Show labels"

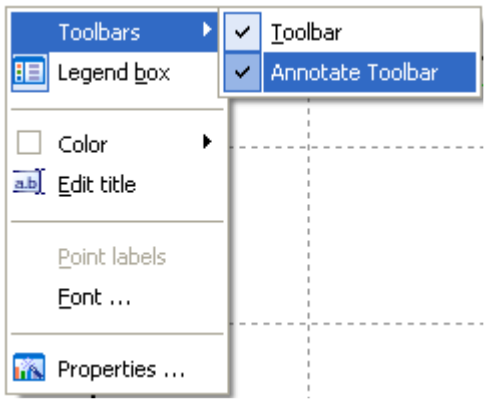
Show label number in "2 levels"

The title of the label can be edited in "Tile" field.



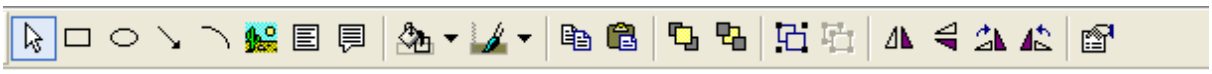
The grid lines of a plot can be formatted with the Grid Lines panel.

Annotate Toolbar



The Vinci Annotate Toolbar is made visible by clicking F2, and subsequent right clicking of the mouse and selecting “Toolbars” and “Annotate Toolbar”.

The Vinci Annotate Toolbar contains all the options for modifying graphs and charts using the following symbols:



Pointer

Rectangle	Draw Rectangle on chart
Circle	Draw Circle on chart
Arrow/Line	Draw arrow on chart
Arc	Draw arc on chart
Picture	Generate a picture object. Right click on object will display a property window. A picture can be select and properties of an object can be modified.
Textbox	Add textbox to chart.
Balloon with text	Add a balloon with text to chart
Background Color	Change the color for background
Foreground (border) Color	Change the color for foreground
Copy	
Paste	
Bring to front	
Sent to back	
Group	
Ungroup	
Flip vertical	
Flip horizontal	
Rotate right	
Rotate left	
Properties	Change the property of an annotate object. This button will be activated when an object is selected. For example, when a text box is selected, clicking on this button will bring up a property window for a textbox.

19 Appendix 1: Units used in Vinci

Quantity	Abbreviation		Conversion
Wavelength	nm	Nanometer	1 nm = 10^{-9} meter
	cm^{-1}	1/centimeter	
Time	s	Second	
	ms	Millisecond	1 millisecond = 10^{-3} seconds
	µs	Microsecond	1 microsecond = 10^{-6} seconds
	ns	Nanosecond	1 nanosecond = 10^{-9} seconds
Pressure	Mpa	Megapascal	10^5 Pa = 10^5 N/m^2 = 1 bar
Concentration	nM	Nanomolar	1 nanomolar = 10^{-9} molar
Volume	mL	Milliliter	1 milliliter = 10^{-3} Liter
	µL	Microliter	1 microliter = 10^{-6} Liter
Temperature	C	Degree Celsius	

Quantity	Abbreviation	Notation
Giga	G	10^9
Mega	M	10^6
Kilo	K	10^3
Milli	m	10^{-3}
Micro	µ	10^{-6}
Nano	n	10^{-9}
Pico	p	10^{-12}
Femto	f	10^{-15}

20 Appendix 2: End User License Agreement

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