# chronosBH

Time- Resolved Fluorometer

**User Manual**

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# Table of Contents

# 1. Safety and Requirements

## 1.1 Laser Safety

### 1.1.1 Warning

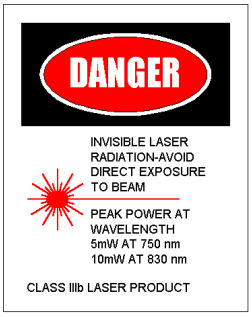
Never attempt to access/control the laser beam without wearing proper goggles certified for the type of laser utilized by the instrument.

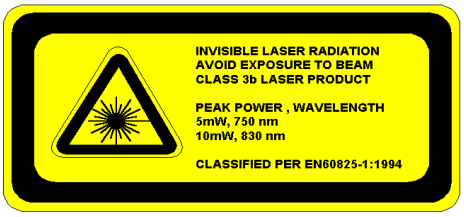
Three types of lasers are used by ChronosBH as excitation source:

- Single-photon laser (Class IIIA and Class IIIB)
- Supercontinuum laser (Class IV)
- Multiphoton laser (Class IV)

### 1.1.2 Warning Labels

The following cautionary labels are attached to the laser diode power supply.

| | |
|---|---|
|  | Refer to accompanying documents. |
|  | Caution, visible and or invisible laser radiation is contained within the labeled enclosure or is emitted as shown by an accompanying arrow or picture. |
|  | Laser safety warning label required in the United States of America. |

| | |
|---|---|
|  | Laser safety warning label required in the European Union. |

## 1.2 Environmental

### 1.2.1 Electrical

It is recommended to connect the instruments' cords to a power strip with surge protection capabilities. This will be utilized for the instrument, computer and the monitor, and lasers, as well as additional devices connected to the instrument.

Sufficient amperage must be provided for the instrument to work properly, see Appendix A for details. The lamp power supply should have its own connection directly to the wall. If possible, it is recommended to connect the lamp power supply to a separate outlet. If the spectrofluorometer is utilized in conjunction with a water bath, please note that water baths need at least 8.0 A.

### 1.2.2 Temperature

The instrument is designed to work in the temperature range from +10 ºC to +35 ºC. Operations of the instrument at environmental temperature different from the ones stated is not recommended. For optimal performance it is recommended that the ambient temperature of the laboratory be between 20-25 ºC and held constant within ± 2 ºC.
For on-operating conditions (transport) the instrument should be kept in the range 5-45 ºC.

### 1.2.3 Humidity

The instrument best operates in an environment with relative humidity (RH) <60% non-condensing.
For on-operating conditions (transport) the instrument should be kept at RH=20-80%.

### 1.2.4 Altitude

The instrument best operates at altitudes 0-1000 meters.

# Part I: Instrument Description

## 2. The ChronosBH platform

ChronosBH is an instrument module that has been designed for research-grade fluorescence applications; it can be used for steady-state fluorescence measurements, frequency-domain lifetime acquisition and time-domain lifetime acquisition (time-correlated single photon counting measurements). Monochromators and polychromators can be coupled directly - or indirectly through fiber optic bundles – to the instrument. The fluorescence signal is sent to the light detectors, either photomultiplier tubes (PMTs), avalanche photodiodes (APD) or microchannel plates detectors (MCP). A diagram of the module is given in Figure 2.1 below.

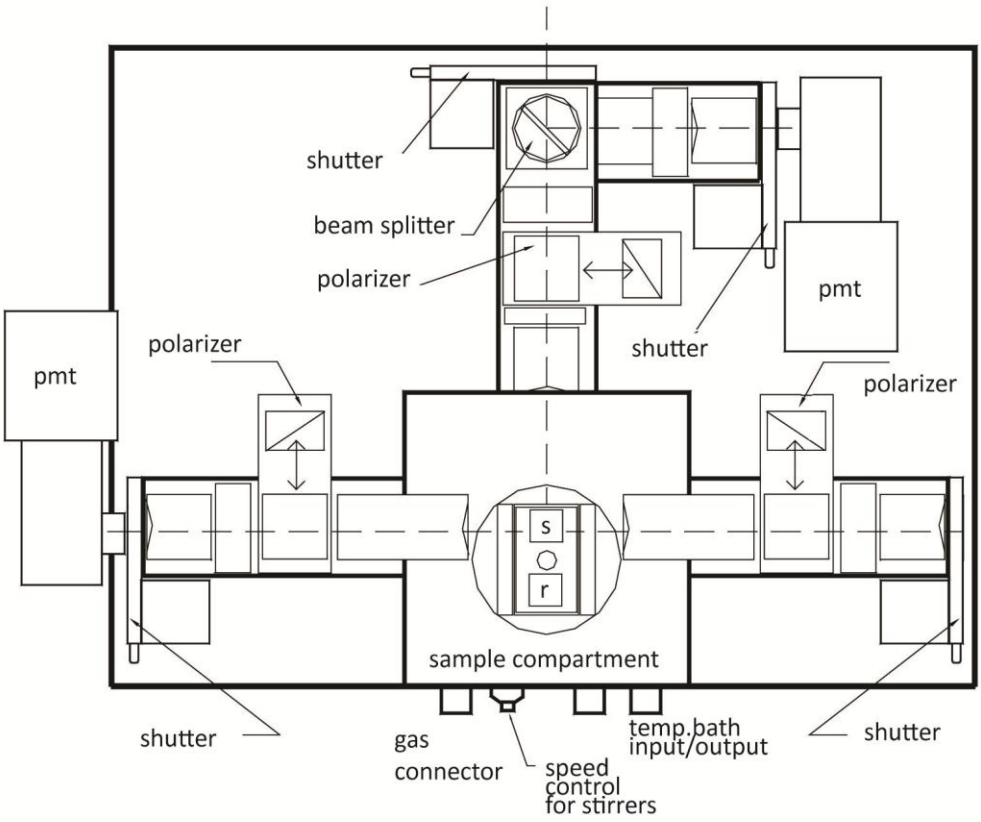



**Figure 2.1** - Chronos top view

Chronos is designed on a T-format geometry: one excitation channel and two identical emission channels positioned on the same axis and at 90 degrees with respect to the optical axis of the excitation channel (T-format).

The excitation channel houses an automatic shutter, a beam-splitter, a filter holder, and an automatic polarizer holder. An additional automatic filterwheel can be positioned in the excitation channel. The light beam enters into the excitation channel from the back aperture and travels down the excitation channel all the way to the sample.

A fraction of the excitation light beam diverted by the beam splitter enters into the reference channel that has a filter holder and an automatic shutter. A PMT detects the light from the reference channel and the signal is used for correction of excitation spectra and for correction when acquiring fluorescence on a long time period.

Each emission channel is equipped with an automated polarizer holder, filter holder, and an automatic shutter. The connections at the ends of the two emission channels are M28-threaded coupling rings, which accommodate the ISS housings for photomultiplier tubes or the monochromators. Computer controlled filterwheels can be placed in the emission channels.

The polarizers holders are controlled by stepper motors which make polarization measurements fully under computer control. A polarizer is permanently attached to each motorized holder; that eliminates the need to insert or remove any polarizer from its holder.

The light detectors are mounted on the emission channels. The signals from the light detectors are diverted to the acquisition card inserted into the computer or to the processing electronics.

## 2.1 Front view of the ChronosBH

The front view of the instrument is shown in Figure 2.2 below. The central area is taken by the sample compartment which can be removed.
The left and right emission channels are symmetrically located on the sides of the instrument; the lid is open by using the handles.


**Figure 2.2** Front view of the ChronosBH.

The instrument is sitting onto four (4) height-adjustable feet. When the instrument is installed onto a laser table, holders are available to fix the feet in plate.

## 2.2 Back Panel of the ChronosBH

Figure 2.3 shows the back panel of the ChronosBH, with the power entry module the connection port to the computer and ports for controlling external devices.


**Figure 2.3** Back panel of the ChronosBH

The back panel, from left to right, features the following components:

| | |
|---|---|
| ***Power entry module*** | The power entry module holds the ON/OFF switch for the instrument and the receptacle for the power cord. |
| ***REMOTE*** | The 25-PIN DSUB connector is used to control the automation devices on the instrument. It connects directly to the PCMC card (inserted in the computer) or to the USB module used to control through the USB port of the computer. |
| ***Voltage output: V1, V2, V3*** | These are 5-PIN DIN connectors used to provide voltage to PMT housings such as the Models K218 and K219 by ISS (see Figure 2.4) |
| ***Motor controls for: 02, 03, 08, 13*** | Each 9-PIM DSUB connector is connected to the respective motors on the control electronics. (see Figure 2.5) |


| Pin Number | Connection |
|---|---|
| 1 | +5 V |
| 2 | Ground |
| 3 | +15 V |
| 4 | -15 V |
| 5 | Analog Voltage |

**Figure 2.4** V1, V2 and V3 connectors pinout.

| Pin Number | Connection |
|---|---|
| 1, 2, 3, 4 | The 4-phases of the signal driving the stepper motor |
| 5 | Limit switch A |
| 6 | Limit switch B |
| 7, 8 | GND |
| 9 | Not connected |

**Figure 2.5** Motors pin out

## 2.3 Right Side Panel

The right side panel includes two BNC jacks that are connected to the PMT installed on the reference channel.


**Figure 2.6** Right Side panel of the ChronosBH

| | |
|---|---|
| ***RF*** | The RF signal input for the frequency-domain option upgrade of the instrument |
| ***OUT*** | Signal output from the PMT |

## 2.4 Reference channel

The reference channel function is to allow for the acquisition of corrected excitation spectra and to stabilize the emission when fluorescence is collected over a long time (slow kinetics).
Upon entering the instrument, a fraction of the light beam is diverted into the reference channel. A filter holder is available where the quantum counter assembly is positioned. An automated shutter is positioned in front of the detector.

## 2.5 Excitation channel

The excitation channel comprises a filter holder, the polarizer holder and the excitation (focusing) lens. An automated shutter is positioned at the entrance of the channel.

## 2.6 Emission channels

Each of the two emission channels includes the collection lens, the polarizer holder, a filter holder and an automated shutter.

## 2.7 Polarizer Units

Each polarizer unit consists of a stepper motor driven polarizer assembly. The assembly is mounted on a brass slide that slips on an aluminum holder. The slide has two stop positions that correspond to POLARIZER-IN and POLARIZER-OUT positions (IN and OUT of the optical path). The axis of the polarization (zero degrees) is given by the set-screw located on the polarizer mount. Vertical position of the polarizer corresponds to the screw being perpendicular to the plane of the instrument. The angular positioning of the polarizers is better than 0.3 degrees.

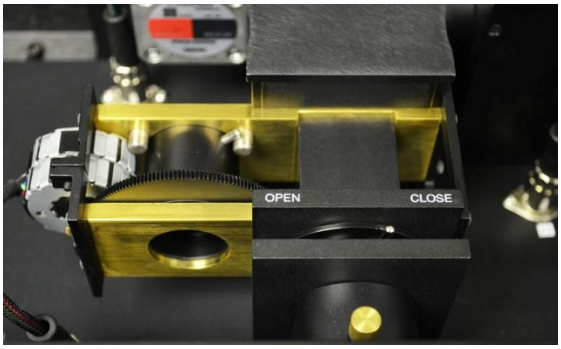



**Figure 2.7** Excitation channel polarizer unit. In the position shown in the picture the polarizer is not inserted in the optical path; for insertion, the entire slide is pushed from the left until the bracket comes to a stop.

The standard polarizers used in ISS equipment are ultraviolet Glan-Thompson prisms, with a length-to-aperture ratio of 2 and transmission in the wavelength range between 214-2300 nm.

## 2.8 Shutter Units

The Chronos is equipped with four automated shutters, one in excitation, one on the reference channel and one on each emission channel, for a total of four shutters. The shutters are controlled using stepper motors. Each shutter has two positions: OPEN and CLOSED; when in the CLOSED position the handle is parallel to the plane of the instrument (Figure 2.8). Each shutter can be moved manually or controlled through the computer.

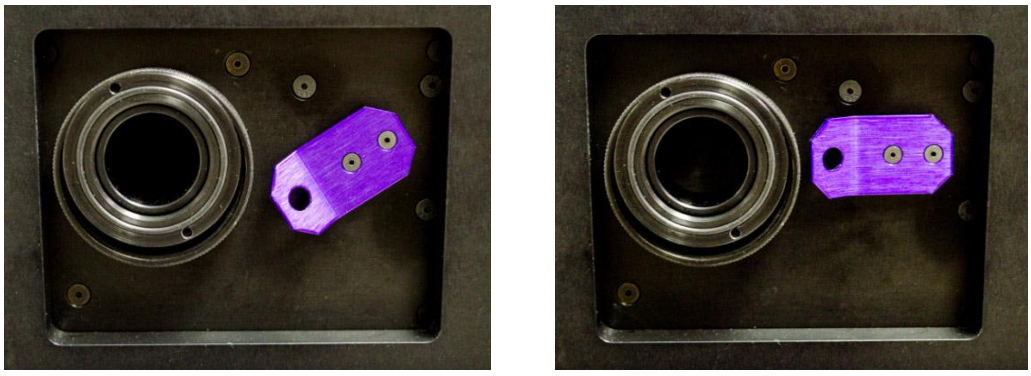


**Figure 2.8** Excitation shutter OPEN (left) and CLOSED (right)

## 2.9 Sample Compartment

The standard sample compartment of ChronosBH is a computer-controlled, two-cuvette rotating chamber. The temperature of the sample compartment may be controlled through an external liquid circulating bath down to -25 ºC. Magnetic stirrers can be accommodated and their speed is controlled by the knob on the front of the compartment. Several sample compartments are available in order to accommodate various applications (see Table 6.1).

The inner dimensions of the sample compartment chamber are 162 x 165.2 mm. The height of the optical axis with respect to the optical base plate is 63.4 mm.
Each sample compartment slides on a bracket fixed onto the instrument. The bracket can be utilized to accommodate a custom sample compartment; the height of the optical axis from the bracket is 97.7 mm.

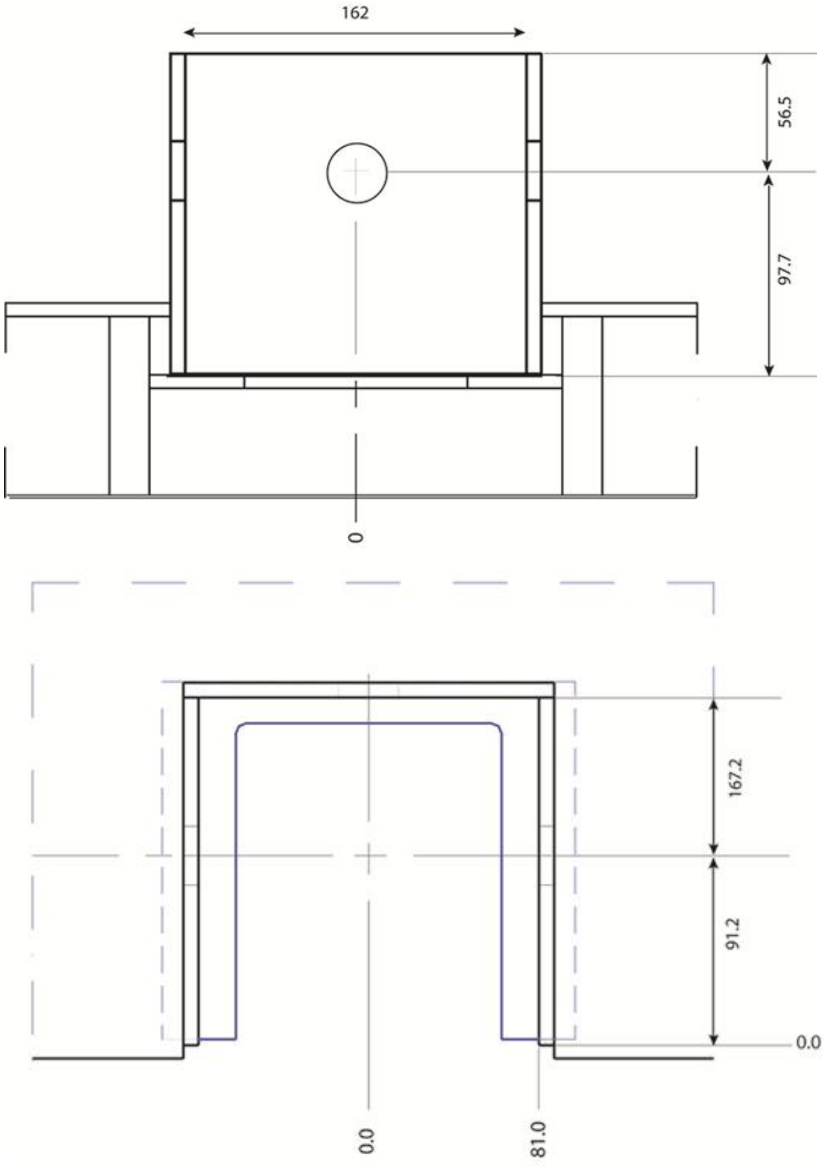



**Figure 2.9** Front view of the sample compartment area (top) and top view (bottom). The dimensions are in cm.

For the 1-, 2- and 3-cuvette holders, the temperature control is achieved through an external bath circulator and the typical temperature range for a standard configuration is -25 ºC to +125 ºC (depending upon the circulator used). A connector for gas flux is provided on the right hand side of the compartment. For 1- and 4-cuvette, a Peltier sample holder is available.

The stirrer speed control knob is located between the connectors for the bath and the gas flux. To increase the stirrer's speed, turn the potentiometer knob clockwise and decrease the speed by rotating it counter-clock-wise. The walls of the sample compartment are made of Delrin for best thermal insulation.

## 2.10 Light Detectors

The standard light detector for the ChronosBH is the fast PMT Model H5773 by Hamamatsu (model PMC-100). It features an instrument response function (IRF) of about 250 ps. Other detectors can be used as well.
For a complete list of detectors available for the ChronosBH see paragraph 6.5 below.

## 2.11 Software

The *Vinci Multidimensional Fluorescence Spectroscopy Analysis* software for Windows7 controls the instrument automation and its interface to computer-controlled external devices (temperature bath, titrator). *Vinci* includes routines for the automatic acquisition of unidimensional data files (excitation and emission spectra; polarization and anisotropy spectra; synchronous luminescence spectra; slow and fast kinetics studies; lifetime data; rotational correlation times) and multidimensional data files (intensity and polarization versus excitation and emission wavelengths, time, temperature, lifetime). Raw data are stored in ASCII format along with the experimental parameters.

The analysis portion of the software includes data manipulation (operations between spectra, smoothing, correction, derivative and integration). The analysis includes fitting routines for the determination of multiple decay times (exponential, non-exponential, lifetime distributions); for the determination of rotational correlation times (isotropic, anisotropic and hindered rotators); for the display of time-resolved spectra and the resolution of spectra using phase and modulation. The sophisticated user can use Vinci to input a custom analysis model: the $X^2$-function is minimized with the custom equation.

The graphical display allows for 2D and 3D plots as well color display of user defined functions with zooming and rotation capabilities, statistical operators, and plot export to popular formats (metafile, bitmap).

## 3. Instrument Configurations

Due to its high level of modularity, ChronosBH can be configured to best match the intended application. Below we display the schematics of some popular configurations.

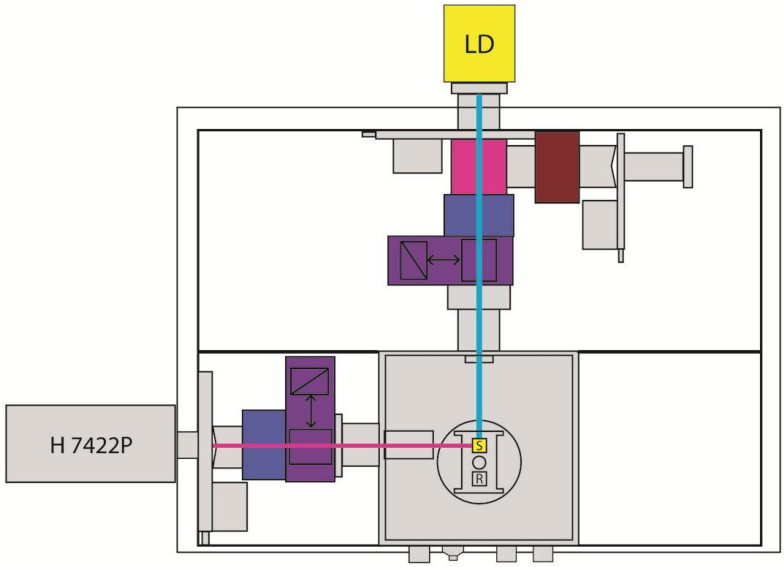



**Figure 3.1** Lifetime spectrometer equipped with laser diode LD in excitation, one emission channel with PMT H7422P.

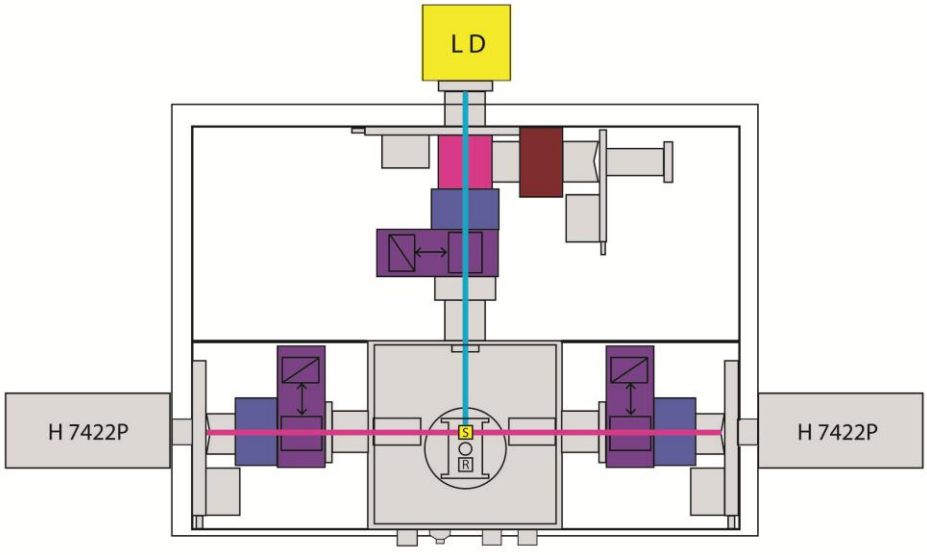



**Figure 3.2** Lifetime spectrometer equipped with laser diode LD in excitation, two emission channels with PMTs H7422P.

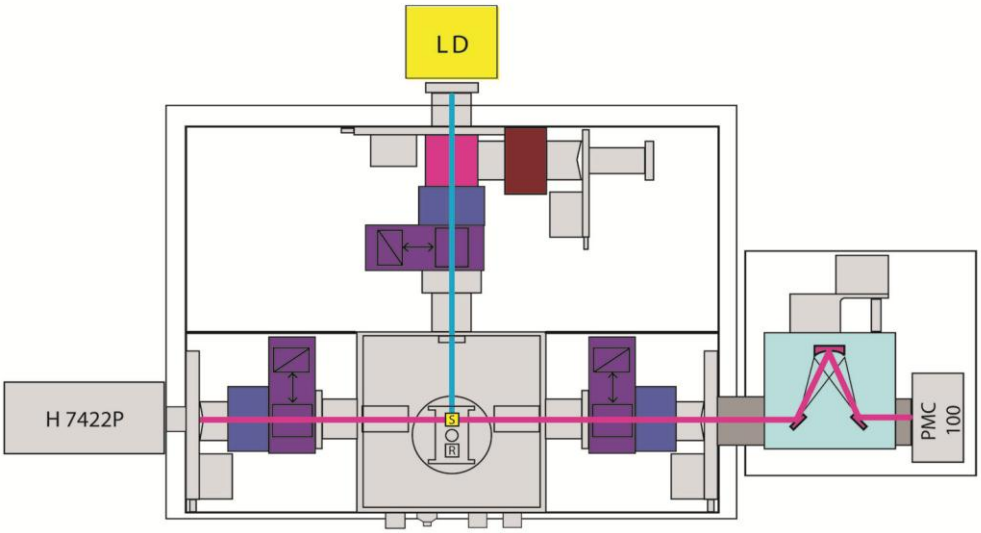



**Figure 3.3** Lifetime spectrometer equipped with laser diode LD in excitation, one filter emission channels with PMT H7422P and a second channel with monochromator with PMT PMC-100.

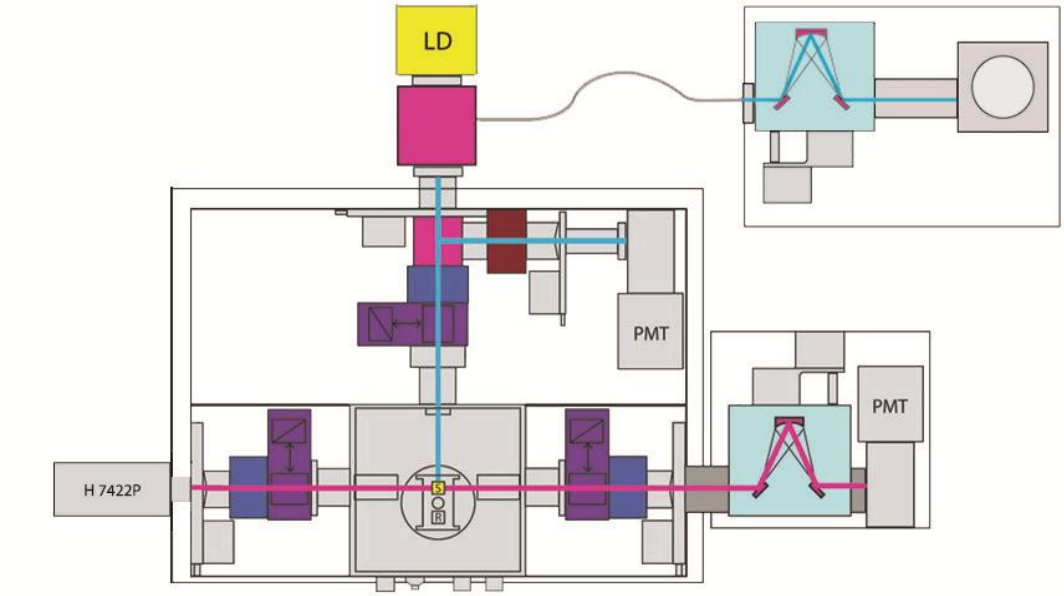



**Figure 3.4** Lifetime spectrometer equipped with laser diode LD and xenon arc lamp in excitation, one filter emission channels with PMT H7422P and a second channel with monochromator with PMT R928 for steady-state measurements.

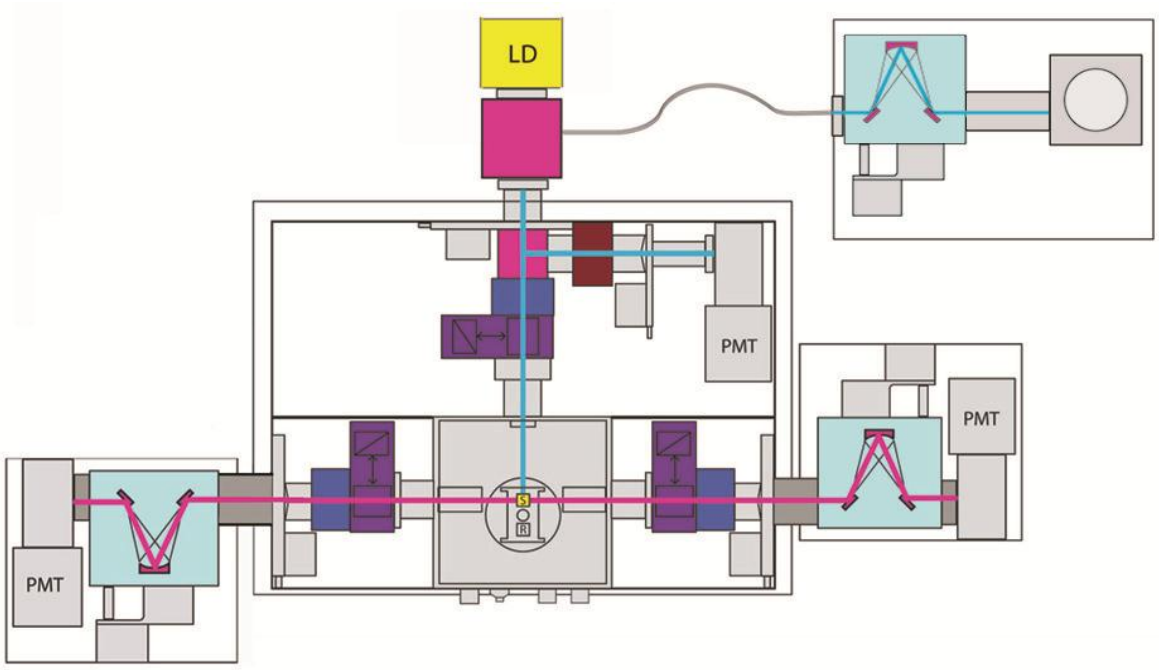



**Figure 3.5** Lifetime spectrometer equipped with laser diode LD and xenon arc lamp in excitation, two monochromators on the emission channels.

# 4. Measurements Acquired with ChronosBH

## 4.1 Basic configuration

The standard instrument configuration for lifetime measurements (Figure 3.1) can be utilized to acquire the following measurements:

| | | |
|---|---|---|
| Time-resolved fluorescence (using the laser or LED) | Determination of multiple decay times | |
| | Rotational correlation times | |
| | Time-resolved spectra | Requires an emission monochromator (Figure 3.3 and above). |
| Steady-state fluorescence (using the laser or LED) | Single point intensity | |
| | Single point polarization | |
| | Slow kinetics in intensity | Manually activated, time resolution about 1 s. |
| | Slow kinetics in polarization | Manually activated, time resolution about 1 s. |
| | Fast kinetics in intensity | Time resolution of 2ms depending upon the type of stopped flow apparatus. |
| | Fast Kinetics in polarization | Time resolution of 2ms depending upon the type of stopped flow apparatus. |
| | Emission spectra | Requires an emission monochromator (Figure 3.3 and above). |

**Table 4.1** Measurements acquired with ChronosBH standard configuration.

## 4.2 Steady-state option with xenon arc lamp

When the instrument is equipped with the steady-state option, a card counting all of the photons is included. In this way, steady-state measurements with high sensitivity are achievable.

| | | |
|---|---|---|
| Time-resolved fluorescence (using the laser or LED) | Determination of multiple decay times | |
| | Rotational correlation times | |
| | Time-resolved spectra | Requires an emission monochromator (Figures 3.4 and 3.5). |
| Steady-state fluorescence (using the lamp) | Single point intensity | |
| | Single point polarization | |
| | Slow kinetics in intensity | Manually activated. |
| | Slow kinetics in polarization | Manually activated. |
| | Fast kinetics in intensity | Time resolution of 2ms depending upon the type of stopped flow apparatus. |
| | Fast Kinetics in polarization | Time resolution of 2ms depending upon the type of stopped flow apparatus. |
| | Excitation and Emission spectra | Requires an emission monochromator (Figures 3.4 and 3.5). |

**Table 4.2** Measurements acquired with ChronosBH standard configuration and xenon arc lamp.

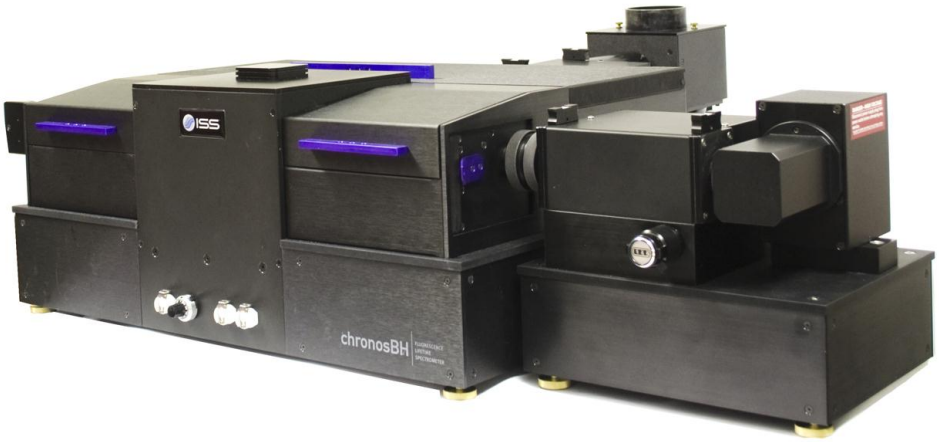



**Figure 4.1** ChronosBH equipped with the xenon arc lamp and monochromator in excitation and monochromator on the right emission channel (configuration of Figure 3.4).

# 5. Computer Requirements

ChronosBH is delivered with a computer. Should the customer decide to supply the computer; a few requirements must be met.

A Pentium P5 series computer (2 GHz or higher) is required, with the following features:

1. Windows7 (32 or 64 bit) or Windows XP operating systems
2. A minimum of 512 MB RAM.
3. At least two USB ports.
4. At least two PCI-bus slots for a ChronosBH without steady state: one for SPC-130 card (or DPC-230 card) and one for DCC-100 card. The DPC-230 card is about 32cm long, a special computer must be ordered for it.

## 5.1 Cards dimensions

The computer has to be able to accommodate two or more cards. Therefore, the space behind the PCI slots has to be sufficient. For the dimensions of each card consult Table below.

| model | description | dimensions (mm) |
|---|---|---|
| SPC-130 | TCSPC card | 225x115x25 |
| DPC-230 | TCSPC card | 312x124x20 |
| MSA-300 | Multi scaler card | 240x110x18 |
| PMS-400A | Multi scaler card | 180x108x15 |
| DCC100 | Control card | 160x110x15 |
| PCMC | Card utilized when the steady-state option is present. This card requires two slots as it has a second bracket | 155x100x15 |

**Table 5.1** Dimensions of cards utilized with the ChronosBH.

# 6. Instrument Optional Accessories

A number of accessories may be mounted on the instrument. For example: fiber optics for remote sensing applications, automated temperature bath, automated titrator, automated stopped-flow accessory for kinetics studies, a TIRF (Total Internal Reflected Fluorescence) detection device. Additionally, diode array detectors can be coupled to the instrument through fiber optics; and a microscope can be coupled to the Chronos for fluorescence microscopy applications.

## 6.1 Sample Compartments

Table 6.1 below lists the available sample holders suitable for several applications. ISS works with any customer to design specialty sample holders.

| Part no. | Description |
|---|---|
| K200 | 1-cuvette, liquid circulator |
| K201 | 2-cuvette, liquid circulator |
| K202 | 3-cuvette, liquid circulator |
| K210 | 1-cuvette, Peltier cooled |
| K212 | 4-cuvette, Peltier cooled |
| K420 | Absorption measurements for the determination of the optical density of a solution using the spectrofluorometer. |
| K421 | Variable-angle front surface sample holder for solid and high-turbidity samples. |
| K424 | Dewar for liquid nitrogen temperatures |
| K425 | Cryostat |
| K426 | Vacuum chamber for studying samples at pressures as low as 1 mTorr. The device can be utilized for the study of both photo-luminescence (PL) and electro-luminescent (EL) phenomena |
| K427 | TIRF flow cell for the study of macromolecules at or near surfaces and interfaces of membranes. |
| HP200 | High pressure cell working up to 3 Kbar with quartz windows and 4 Kbar with sapphire windows. |
| K436 | Fiber optics for in-situ measurements |
| K428 | Microwell plate reader for 96/384 well plates |

**Table 6.1** – Sample compartments available for the Chronos

## 6.2 Light Sources

Several types of light sources can be utilized with the Chronos:

- Pulsed LEDs
- Pulsed laser diodes
- Supercontinuum laser
- Multiphoton laser
- Xenon arc lamp

### 6.2.1 Pulsed LEDs

Pulsed LEDs are packaged by ISS . The typical repetition rate is 40 MHz although slower rep rates can be chosen.

| Part no. | Emission wavelength (nm) | FWHM (nm) |
|---|---|---|
| N804 | 265 | 20 |
| N806 | 280 | 20 |
| N808 | 300 | 20 |
| N812 | 335 | 20 |
| N816 | 345 | 20 |

**Table 6.2** Some LEDs from ISS; other wavelengths are available

### 6.2.2 Pulsed laser diodes

The Chronos uses laser diodes from Hamamatsu, Lasos and Horiba. The repetition rate depends upon the specific driving electronics of the laser diode; while some laser have a limited range of repetition rates, others offer quite a wide range that allows for their use to measure decay times from the millisecond to the picosecond range.

| Part no. | Emission wavelength (nm) | Pulsewidth (ps) | Peak power (mW) |
|---|---|---|---|
| N410 | 375±10 | 80 | 50 |
| N412 | 405±10 | 70 | 100 |
| N414 | 445±10 | 100 | 80 |
| N416 | 473±10 | 130 | 60 |
| N417 | 488±10 | 125 | 80 |
| N421 | 514±10 | 125 | 70 |
| N430 | 635±10 | 100 | 400 |
| N432 | 655±10 | 70 | 50 |
| N434 | 785±10 | 100 | 70 |
| N436 | 850±10 | 70 | 100 |

**Table 6.3** Specifications for the pulsed laser diodes from Hamamatsu.

### 6.2.3 The supercontinuum laser

The supercontinuum laser (or white laser) emits radiation from 420 nm up to 2500 nm. Typical pulsewidth is about 10 ps. The repetition range of this laser goes from 100 KHz to 40 MHz, depending upon the specific options.
The wavelength can be conveniently selected by using the AOTF; alternatively, a monochromator can be utilized. In either case the selection is done though the Vinci software for the lasers made by Fianium (Southampton, United Kingdom).

### 6.2.4 Multiphoton lasers

Multiphoton excitation (2- and 3-photon excitation) is achievable using the Ti:Sapphire laser. The typical emission ranges from 700 nm to 1000 nm; that is, the two-photon excitation is used in the region from 350 nm to 500 nm and the 3-photon excitation in the region from about 240 nm to about 330 nm.
These lasers can be used with a frequency-doubler to obtain single photon excitation in the region from 350 nm

to 500 nm. The high power emitted by these lasers requires attention in controlling it. ISS provides a computer control unit for the control of the power in the range 1 to 200 starting from 2 W.

### 6.2.5 Other lasers

ChronosBH is utilized with several pulsed lasers. The laser has to provide a sync signal for the start of the acquisition. Alternatively, an external beam splitter collects a fraction of the excitation light that is read by a photodiode (reference photodiode); the photodiode provides the sync signal to the acquisition electronics.

### 6.2.6 Synchrotron radiation

ChronosBH can utilize radiation emitted by a synchrotron beam as light source.

### 6.2.7 Continuous wave xenon arc lamp (for steady-state measurements)

ISS uses the compact 300W xenon arc lamp from EG&G Perkin Elmer.

| Lamp Type | LX300F | LX300UV |
|---|---|---|
| Nominal Operating Power (watts) | 300 | 300 |
| Operating Power Range (watts) | 180-320 | 180-320 |
| Nominal Operating Current (amps dc) | 21 | 21 |
| Operating Current Range (amps dc) | 10-22 | 10-22 |
| Nominal Operating Voltage (volts dc) | 14 | 14 |
| Operating Voltage Range (volts dc) | 13-16 | 13-16 |
| Ignition Voltage (kilovolts) | 23 | 23 |
| Beam Geometry – Half Angle at 0/100/1000 hrs. (degrees) | 5/6/7 | 5/6/7 |
| Radiant Beam Characteristics (nominal values) | | |
| Peak Intensity (candelas) | $515 \times 10^3$ | $460 \times 10^3$ |
| Radiant Output (watts) | 50 | 50 |
| UV Output [≤390 nm] | 2.6 | 6.6 |
| Visible Output (lumens) [390 nm ≤ VIS ≤ 770 nm] | 5,000 | 4,500 |
| IR Output (watts) [≥ 770 nm] | 28.8 | 26.8 |
| Reflector Coating [A=Aluminum; S=Silver] | S | A |
| UV Reflective Window Coating | Yes | No |
| Weight (grams/ounces) | 285/10 | 285/10 |

**Table 6.4** Xenon arc lamps specifications

Table 6.4 lists the technical specifications for this lamp. For a comparison of this lamp with the standard (traditional) xenon arc lamps, see ISS Technical Note at (htttp://www.iss.com/resources/research/technical_notes/PC1_xenonlamps.html)

Figure 6.1 displays the spectral output of the lamp. Typically the lamp is used in the range from 230 nm up to about 800 nm for excitation. For using the lamp with longer excitation wavelengths, the monochromator has to mount a different grating.

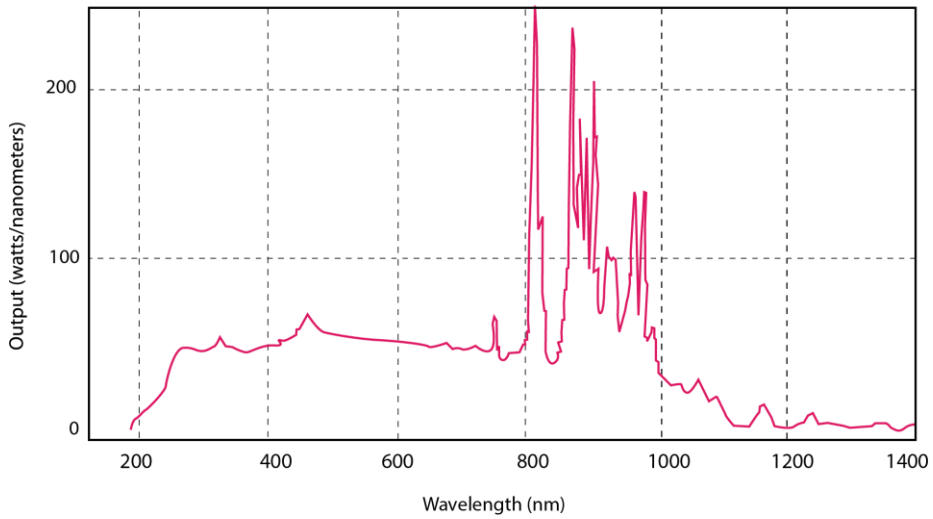



**Figure 6.1** Spectral distribution for the 300W xenon arc lamp.

## 6.3 Monochromators

The monochromators used in ChronosBH mount concave holographic gratings for higher transmission over an extended wavelength range and minimum stray light.

| | excitation | emission |
|---|---|---|
| Focal length (mm) | 100 | 100 |
| Aperture ratio | f/3.5 | f/3.5 |
| Grating size (mm) | 32x32 | 32x32 |
| Blazing wavelength (nm) | 250 | 450 |
| Linear dispersion with 1,200 lines/mm (nm/mm) | 8 | 8 |
| Resolution with 0.1 mm slits, 1200 line/mm (nm) | 1.0 | 1.0 |
| Wavelength accuracy (nm) | ± 1.0 | ± 1.0 |
| Reproducibility | ± 0.25 | ± 0.25 |
| Stray light 9measured at 8 bandpasses outside the 632,8 nm line) | $10^{-5}$ | $10^{-5}$ |

**Table 6.5** H-10 monochromator characteristics.

Gratings can be selected for the working region of the application. Blazing wavelengths include 250 nm, 350nm and 450 nm.

| Wavelength (nm) | Linear dispersion (lines/mm) |
|---|---|
| 190 - 800 | 1,200 |
| 300 – 1200 | 800 |
| 400 – 1600 | 600 |
| 800 - 3200 | 300 |

**Table 6.6** Gratings for the H-10 monochromator.

Figure 6.2 below displays the typical transmission of a 450nm -blazed grating. The transmission drops below 20% for wavelengths shorter than about 300 nm.

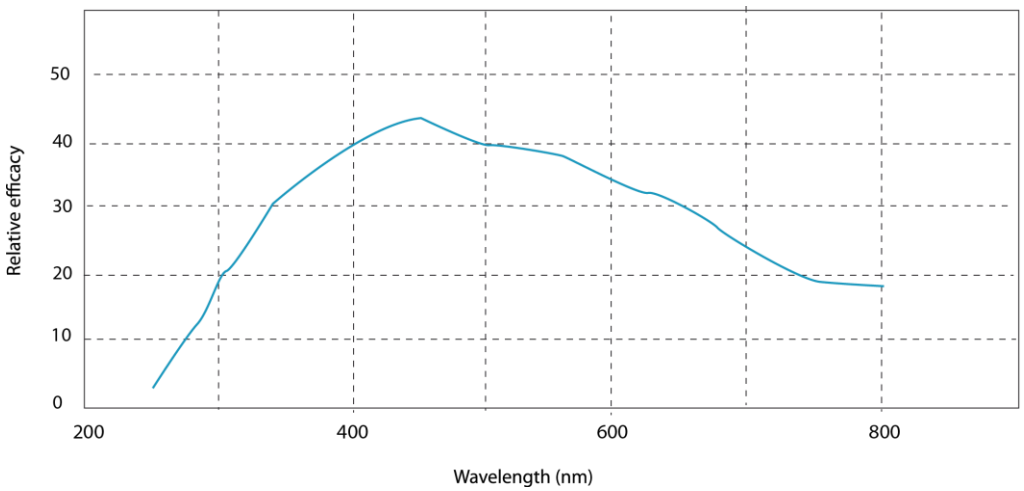



**Figure 6.2** Transmission curve for a holographic, 1200 lines/mm grating, blazed at 450 nm.

## 6.4 Polarizers

The ChronosBH uses calcite polarizers, both in the Glan-Thompson and in the Glan-Taylor configurations, the former being the preferred one due to higher transmission range; yet, the latter is used in excitation when the excitation power exceeds 2 W (as sometimes is the case for multiphoton excitation).

| Polarizer type | Dimensions (mm) | |
|---|---|---|
| Glan-Thompson | 10x10 | 14x14 |
| Glan-Taylor | 10x10 | |

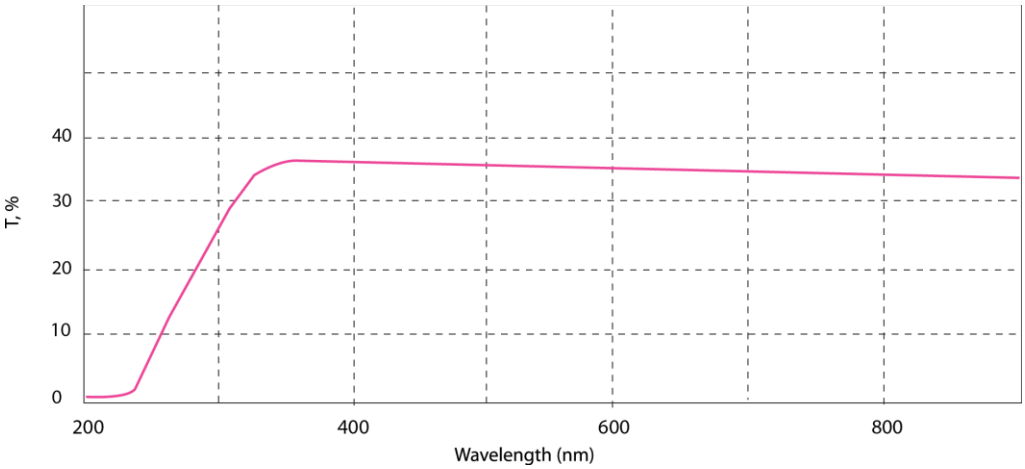



**Figure 6.3** Transmission curve for the 10x10 mm Glan-Thompson polarizer.

## 6.5 Light Detectors

### 6.5.1 Technical Characteristics of light detectors used for TCSPC

Table 6.7 below lists some popular light detectors utilized with the Chronos. All of the detectors are connected to the instrument using a M28-threaded flange.

| Model | Detector type | STT (ps) | SER (ps) | TTS/IRF (ps) | Anode rise time (ps) | Application |
|---|---|---|---|---|---|---|
| H5773 (PMC-100) | | 5400 | 1500 | 140-200 | 780 | lifetimes |
| H7422P-40 | GaAs | 6500 | | 170-220 | 780 | lifetimes |
| R10467U | Hybrid PMT | | 850 | 130 | 400 | lifetimes |
| R3809U-50 | MCP | 550 | 360 | 25-30 | 150 | lifetimes |
| R928 | multialkali | 22000 | 5000 | 1200 | 2200 | Utilized for steady-state measurements |

**Table 6.7** Light detectors for the ChronosBH

It is relevant to have an understanding of the main parameters affecting a PMT:

***SST Signal Transit Time***

The SST is the transit time of the electrons through the PMT. The transit time is taken into account when evaluating the cable length in the signal cable and SYNC cable (see 16.6).

***SER, Single Electron Response***

This is the detector output pulse for a single photoelectron. Due to the random nature of the detector gain, the pulse amplitude varies considerably from pulse to pulse, from 1:5 to 1:10.

The signal can be acquired with a fast oscilloscope. The average peak current of the SER is described by:

$$I_{SER} = \frac{G e}{T_{FWHM}}$$

Where G is the gain of the PMT, $e$ is the electron charge and $T_{FWHM}$ is the full width half maximum of the pulse.

***TTS, Total Transit Time***

Transit Time Spread (or transit time distribution) determines the Instrument Response Function (IRF) of the detector. Usually, when using lasers that feature a pulsewidth of less than 100 ps (single photon laser diodes) or of a few femtoseconds (Ti:Sapphire lasers), the IRF of the instrument is mainly determined by the detector.

***Anode Rise Time***

It is a measure of the time response of the detector.

### 6.5.2 Instrument Response Functions (IRF) of selected detectors

The following IRF have been obtained using a pulsed laser diode (70ps pulsewidth) as light source; the scattering sample was a solution of glycogen in water. Therefore, they are not a “true IRF” as the light travels through the cuvette adding about 45 ps delay; they are meant to provide a comparison between

the various detectors.

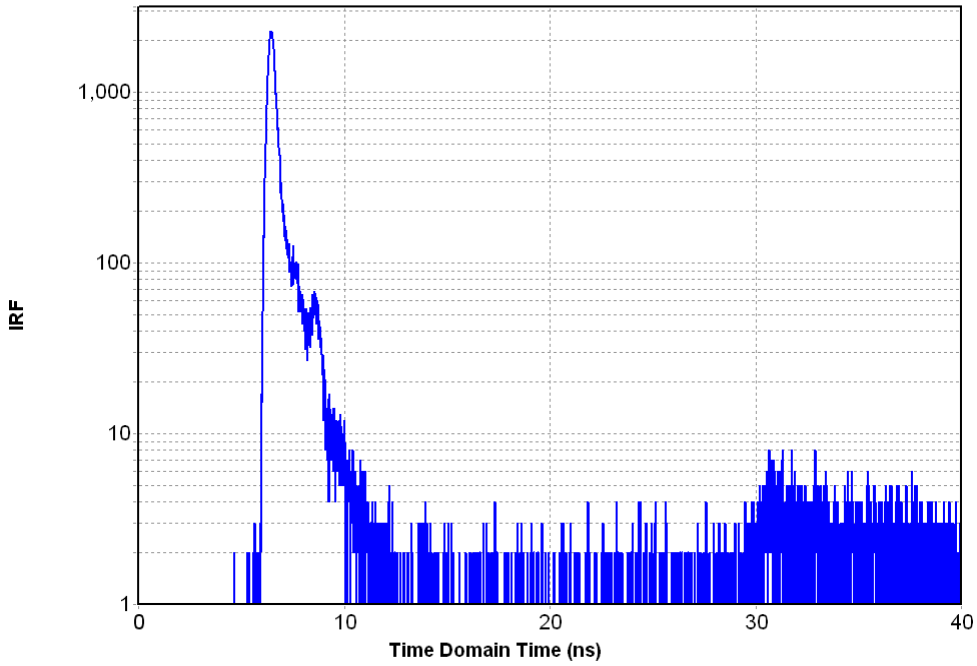



**Figure 6.4** IRF for PMT Model H5773-04 (PMC-100).

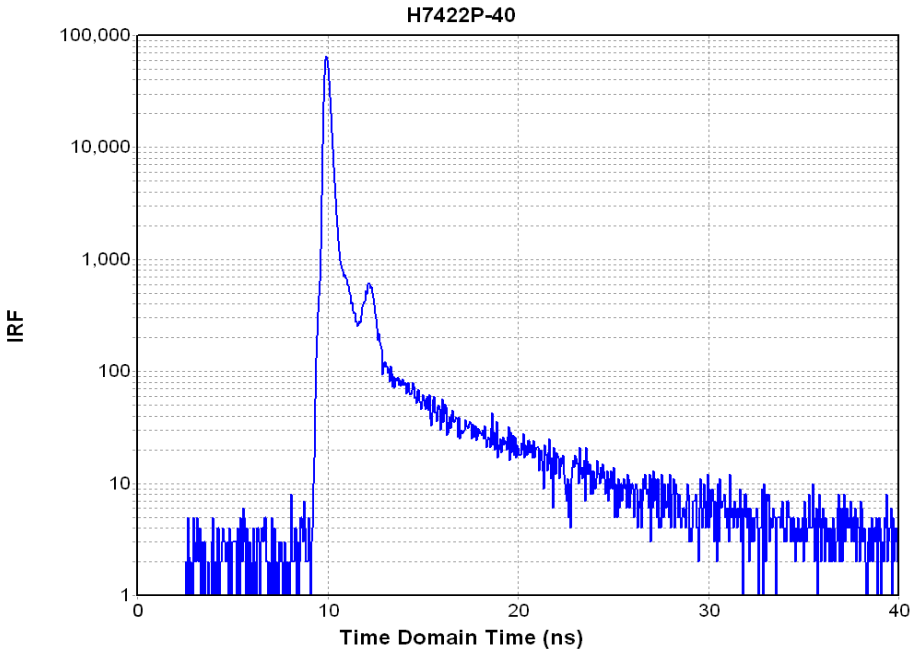



**Figure 6.5** IRF for PMT Model H7422P-40

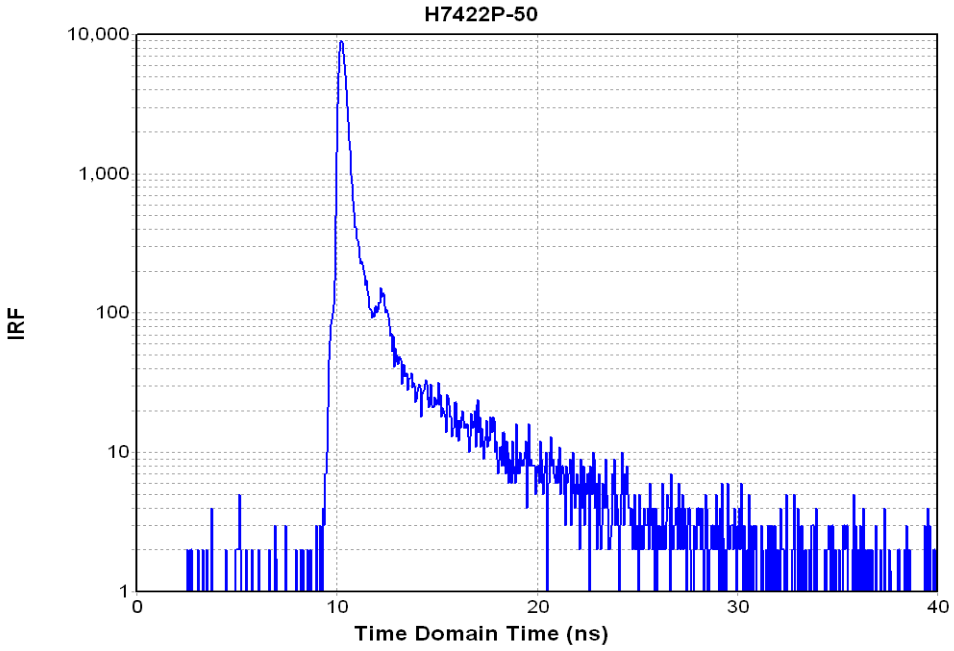



**Figure 6.6** IRF for PMT Model H7422P-50

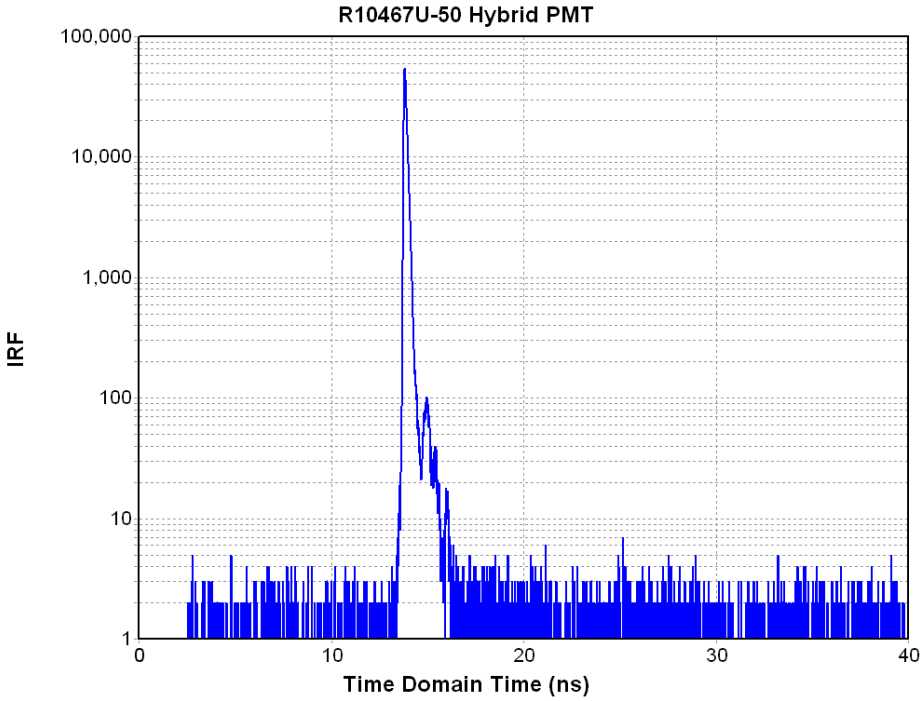



**Figure 6.7** IRF for PMT Model R10467U-50

### 6.5.3 Pictures of selected detectors


**Figure 6.8** ChronosBH equipped with the PMT model PMC-100-4 mounted on the right emission channel.


**Figure 6.9** ChronosBH equipped with the hybrid PMT model H7422P mounted on the right emission channel.


**Figure 6.10** ChronosBH equipped with the hybrid PMT model R10467U mounted on the right emission channel.

**Figure 6.11** ChronosBH equipped with the MCP model R3809U mounted on the left emission channel.

## 6.6 Data Acquisition cards

### 6.6.1 Data Acquisition Cards for TCSPC

Two different time-domain data acquisition cards are used in the ChronosBH: SPC-130 and DPC-230, although other cards can be utilized as well (SPC-150, SPC-730, SPC-830). The SPC-130 card (and the cards of the SPC family) records photon signals in the time-correlated single photon counting (TCSPC) mode.
The DPC-230 card can record photon signals in TCSPC mode with single or multi photon capability. The DPC-230 card uses TDC (Time-to-Digital convertor) technology and delivers a much coarser channel resolution compared to the SPC-130 card; yet, it covers a wider range of decay times.

Table 6.8 lists the most important differences between the SPC-130 and the DPC-230 cards.

| Parameter | SPC-130 | DPC-230 |
|---|---|---|
| **No. of channels** | 1 | 16 – LVTTL inputs<br>4 - CFD |
| **Recording Technology** | TAC-ADC<br>(Time-to-amplitude converter) | TDC<br>(Time to Digital Converter) |
| **Time Resolution** | 813 fs | 165 ps |
| **Channels per curve** | Up to 4096 | |
| **Mode** | TCSPC | TSCPC<br>TCSPC with multi photon capability<br>Multiscaler (lower than 1MHz excitation pulse, total counts less than 1 million) |
| **Measurement Range** | low picosecond to microsecond | 400ps-10ms (dependent on laser) |
| **Application** | Fluorescence decay | Fluorescence decay and luminescence decay |
| **Dimensions** | 225x115x25 mm | 312x124x20 mm |

**Table 6.8** SPC-130 and DPC-230 Specifications

### 6.6.2 Multiscaler cards

These cards are used to measure long decay times (from 1 µs to a few milliseconds), typical of phosphorescence processes.

| parameter | MSA-300 | PMS-400A |
|---|---|---|
| Counter channels | 1 | 2 |
| Time/point | 5 ns to 50 ms | 250 ns to 100000 s |
| Time resolution | 5 ns | |
| Count rate | Up to 100 MHz | 800 MHz |
| No. of points per curve | Up to 512K | |
| dimensions | 240x110x18 | 180x108x15 mm |

**Table 6.9** Multiscaler cards for the ChronosBH.

### 6.6.3 Data Acquisition cards for steady-state applications

| parameter | PCMC |
|---|---|
| Counter channels | 3 independent channels |
| Analog inputs | 4 |
| External Reference CLK Frequency Range | 5KHz-20MHz |
| External Reference CLK Signal | 15-70m$V_{PP}$ |
| dimensions | 155x100x15 |

**Table 6.10** Features of the PCMC card.

## 6.7 Data Control Cards

### 6.7.1 Control of Stepper motors

The stepper motors of the ChronosBH are controlled through the MSM card installed in the instrument. The card receives the commands from the computer either through the parallel port, the PCMC card, or the ISS USB adapter.

### 6.7.2 Control of Light Detectors

The light detectors are controlled through the DCC-100 card (PCI bus) inserted in the computer. This card provides the voltage for controlling the gain of the PMTs, the current to control the Peltier element of the PMTs and can detect, in some system, the overload conditions. Some of the features of this card are listed in Table 6.11 below.

| | |
|---|---|
| Power supply output | Connectors 1, 2, 3 |
| Detectors gain control | Connectors 1, 3 |
| Power supply for thermoelectric coolers | Connector 3 |
| Overload | Connectors 1, 2, 3 |

**Table 6.11** Features of DCC-100 controller card

## 6.8 External devices controlled by the Vinci software

External devices are sometimes integral part of a measurement. Several devices can be coupled to the Chronos; they all are controlled through the Vinci software; their operations are fully integrated with the acquisition software.

### 6.8.1 Stopped-flow apparatus

Several fast stopped-flow devices can be directly interfaced with the Vinci software.

| Model no. | Manufacturer |
|---|---|
| SFA-20 | TgK Scientific |
| SF | OLIS |
| SFM-20, SFM-3001S, and SFM-4001S | Biologic |
| RX-2000 | Applied Photophysics |

**Table 6.12** Stopped-flow devices supported by ISS

### 6.8.2 Titrator

Two models of titrators are available through ISS, the 1- and 2-syringe. Their operations are controlled through the Vinci software for automatic data acquisition.

| Part no. | description |
|---|---|
| K430 | 1-syringe |
| K432 | 2-syringe |

**Table 6.13** Titrators supported by ISS

### 6.8.3 Temperature bath circulators

Several bath circulators are controlled by the Vinci software. They are utilized to set and control the temperature in the sample compartments requiring a liquid circulator unit.

# Part II: Installation of ChronosBH

## 7. Unpacking ChronosBH

Upon receiving the instrument inspect the outside of the crate and boxes for any sign of shipping damage. If damage has occurred, contact the carrier, take a picture and contact ISS immediately for further instructions.

If no obvious damage has occurred, proceed as follows:

- Make sure the crate is situated on a flat surface with the lid facing upward (The lid is marked "THIS SIDE UP" and contains the address labels). The bottom is supported by two "2 x 4" pieces of lumber at the opposite ends.

- Open the crate by removing the screws that fasten the lid to the walls of the crate. You will need a standard Phillips screwdriver. When the lid has been removed, visually inspect the protective wrap and instrument for any noticeable damage. If damage is discovered to components inside the crate contact your carrier, take a picture and contact ISS immediately; otherwise, continue to unpack the instrument.

Please save all packing materials for future relocation or shipping needs.

# 8. Assembling the ChronosBH: Instrument Components

The instrument is installed by ISS technical personnel. If you decide to proceed with the installation, please contact ISS for the authorization. After unpacking the instrument, place it on a sturdy optical table and remove and save the protective wrap. Remove all protective materials from mirrors, lenses, polarizer holders and the sample compartment The optical bench supports excitation and reference channel, sample compartment bay, two emission channels and the reference photomultiplier tube (PMT) and PMT housings. The photomultiplier tubes and the polarizers are shipped in separate boxes.
All optical elements have been aligned at the factory during the instrument testing. No further alignment is required.

## 8.1 Shutters

Locate your tool set. Replace the shipping rubbers keeping the shutter blades fixed during shipment with the four supplied 4/40 x ½ screws contained in a small bag labeled as such. Use a 1/16 inch Allan wrench from the tool set included in the shipment. Remove the shipping rubbers from the excitation, reference and left and right emission shutter. Locate the replacement screws and fasten them to the shutter bodies. When the lever is horizontal the shutters are closed; when the handles are pointing 45 degrees up, the shutters are open. For the moment, close the shutters by hand.

## 8.2 Installation of the Polarizer

The polarizers are shipped in separate boxes. Unpack them above a table or desk and install them according to the instructions described in this manual. The mark on the polarizer holder is aligned with the set screw in the mount.

Identify the packages containing the polarizer. Each polarizer is packed in a separate box. Open each box and remove the holders. Carefully remove the protective red cap, wrapping or parafilm. Do not touch the polarizer's optical surfaces. Please use gloves, do not fingerprint the polarizer surfaces. Clean surfaces when required with a very small amount of ethanol and lens tissue. When working on a laser table, place a piece of bench paper on its surface. When assembly is done on the paper sheet a setscrew cannot disappear in a table surface opening. Prevent the polarizers to roll from the table.

**WARNING:**
THE POLARIZER SLIDES ARE ALREADY MOUNTED IN THEIR HOLDERS AND CALIBRATED FOR PROPER ANGULAR POSITION.

Once you have a polarizer holder in front of you, remove the polarizer slide's packing material. Push the slide to free the access to the round holder.

Remove the set screw on the round holder of the polarizer slide. Insert the polarizer and overlap the mark on the polarizer with the set screw opening and gently fasten the set screw. Overlapping the mark on the polarizer with the opening in its holder requires some giggling of the polarizer and some gently back and forth positioning with a ballpen tip for example. In case mounting is considered too difficult remove the slide's "stop plate" mounted on two brass rails.

The stop plate is located opposite the stepper motor, and is attached to the brass rail by four screws (4-40 x 1/2" socket head cap screws). Slide the polarizer holders between the lens holder mount and the filter holder wall. The correct way to insert them is to place the stepper motors close to the DIN-9 electrical plugs located on the optical bench with the stop-pin visible. Once the polarizer holder is in place, mount the stop plate. Verify manually that the polarizer holder still easily rotates. Otherwise slightly tweak the slide wall distance with your fingers while refasting the stop-plate. Connect the stepper motor to the plug located on the optical bench:

**Note 1:** Each polarizer is mounted in its holder. When the set screw on the holder points upward the principal axis of the Glan-Thompson polarizer indicates the vertical (V), that is zero degrees.

**Note 2:** An increase of a factor of two in detected signal is possible by exchanging the 10x10 mm for 14x14 mm polarizers. They all fit in the same holders.

**Note 3:** For work with laser excitation light sources a Glan-Taylor, air-spaced, polarizer should be placed in the excitation channel. This type polarizer can withstand much higher laser intensities. In case a Glan-Thompson excitation polarizer receives laser damage during a later upgrade from LED or lamp to laser light source carried out by the user.

| Device | Port number |
|---|---|
| Polarizer, excitation | 4 |
| Polarizer, Left emission | 5 |
| Polarizer, Right emission | 6 |
| Shutter, excitation | 1 |
| Shutter, reference | 11 |
| Shutter, Left emission | 10 |
| Shutter, Right emission | 9 |
| Sample Compartment | 0 |
| Stirrers motors | 7 |
| Monochromator, Excitation | 2 |
| Monochromator, Emission | 3 |
| Free port (external) | 8 |
| Free port (external) | 10 |
| DAC1 (used for controlling the gain of PMT housings models K218 and K219) | 14 |
| DAC2 (used for controlling the gain of PMT housings models K218 and K219) | 15 |

**Table 8.1** Port assignment of each automated device

## 8.3 Connection of ChronosBH to the Computer

ChronosBH is controlled by either a PCMC card or a UBS motion control unit.

For the PCMC card, the instrument automation functions are controlled by the parallel port of the ISS-PCMC card via a 25 pin D-Sub connector. Locate the 25 pin cable and connect the REMOTE Port on the rear of the Chronos to the ISS PCMC card in the computer (Note: Don't connect the cable to a 25 pin printer port).

For a USB control box, connect the 25 pin D-Sub cable between Chronos rear REMOTE port to the USB control box PRIMARY port. Next connect the UBS control box to the USB port of computer.

### 8.3.1 Mapping of the devices

ChronosBH is delivered with each device properly connected. Table 8.1 above reports, for reference only, the port number for each automated device of the instrument.

# 9. Installation of Light Sources

Various light sources can be used for the ChronosBH time-domain instrument. This section describes how to connect Hamamatsu Diode Lasers and ISS LEDs. Please contact the ISS service department for information about other types of light sources.

## 9.1 Pulsed LEDs by ISS

The pulsed LED from ISS comes with the following components (Figures 9.1 – 9.3).

**Figure 9.1** Pulsed LED by ISS with built in pulser electronics. The power is applied to the mini-DIN connector; the SMA connector brings the sync signal to the acquisition card.

**Figure 9.2** Coupler ring (left) and Spanner wrench (right)

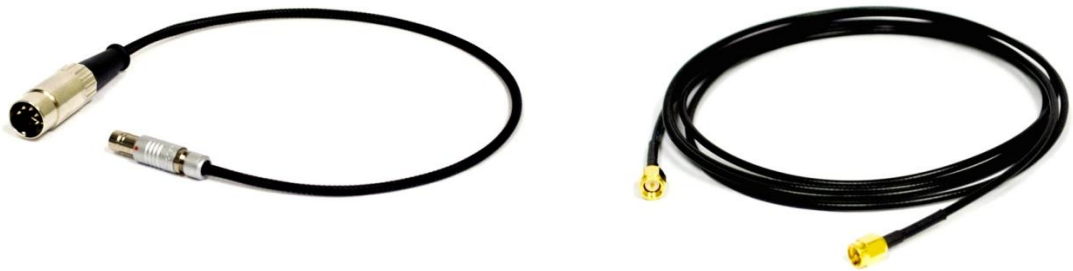


**Figure 9.3** Power cable (left) and sync cable (right)

### 9.1.1 Installation of the ISS LED

The LED is installed on the backside of the ChronosBH.

Locate the area on the back of the instrument.

Attach the coupler ring on the back-side of the ChronosBH and gently tighten with the supplied spanner wrench (Figure 9.2).


Mount the LED onto the flange

Connect the power cable, one end to the LED and the other end to the V1 position on the back lower panel of the Chronos.

Connect the sync cable from the LED to the acquisition card (inserted in the computer).


Connect the LED house “SYNC out” to the “SYNC” on the SPC-130 card via the supplied SMA cable.

**Note:** The light from the pulsed LEDs is much weaker than that from laser diodes. The lens tube is supplied to increase the light intensity. The iris diaphragm behind the sample compartment in the excitation path of the Chronos can also be used to increase the light intensity.

### 9.1.2 Verification and adjustment of LED alignment

Attach power-cord. Turn on Chronos. Open the excitation shutter manually. Open the iris diaphragm fully. Check with a business card in front of the cuvette turret the location and intensity of the LED spot. Pinch the iris diaphragm to its smallest opening. When the LED light is still bright the LED is perfectly aligned. In case the LED spot is invisible or dim adjust open the iris opening somewhat and adjust with a hex driver # 5/64 (included in the accessories kit) the orientation of the LED and maximize the intensity. Insert the hex driver alternatively in either small 1/16 inch opening on the rear of the LED house, see Figure 9.1. When optimized switch off Chronos.

**Note: Rotation of LED housing will change the light intensity.**

## 9.2 Laser diodes by Hamamatsu

The Hamamatsu laser has two components: a controller unit and the laser head module. The laser head module is mounted to a metal block; the block is then attached to an “L” shaped adapter bracket (See Figure 9.4 below).
The L-shaped adapter is attached to the back of the Chronos.

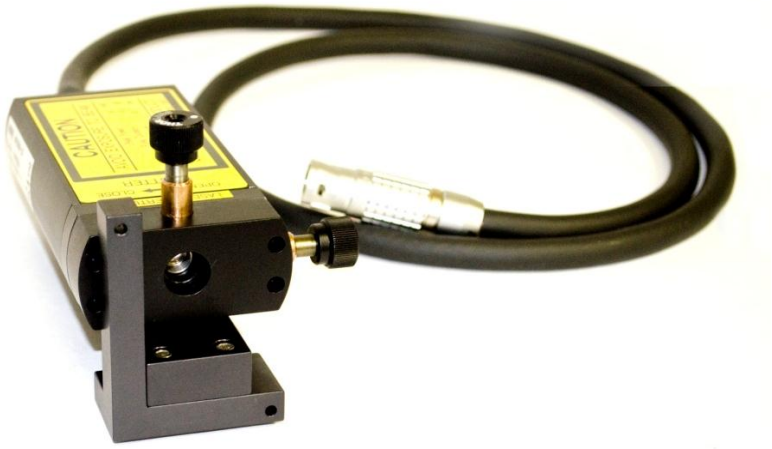
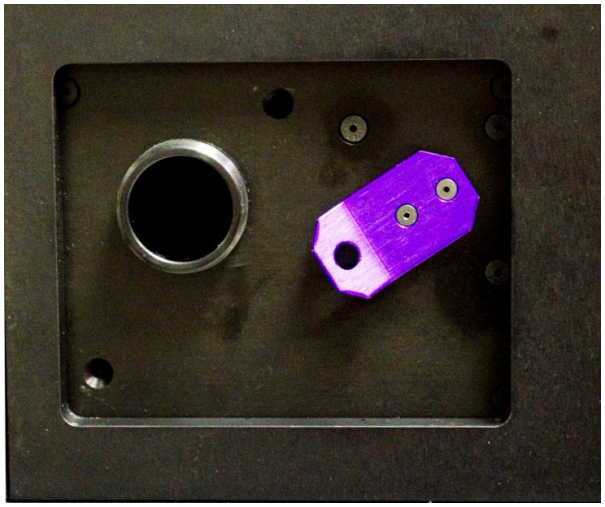


**Figure 9.4** Hamamatsu laser on the mounting bracket.

In order to install it follow the steps outlined below:

Remove the two screws from the back port of the ChronosBH (close to the location of the entrance shutter)



For mounting the laser use the two 4-40 stainless steel screws.

**Figure 9.5** Connection of the laser to the Chronos.

The laser module is connected to the front panel of the Laser Controller via an attached cable to the location marked <HEAD DRIVE> (Figure 9.6).


**Figure 9.6** Front Panel of the Picosecond Light Pulser Unit

We report below a description of the knobs relevant to the use of the unit with the ChronosBH.

| | |
|---|---|
| ***POWER*** | Turn the Key clockwise in order to turn the power ON |
| ***INTERNAL TRIGGER*** | The knobs allow for the selection of the repetition rate of the optical pulses. The rate is the product of the number selected on each knob; for instance:<br>Left knob set at 10M and right knob set at X1/2 give a signal at a rep rate of 5 MHz. |
| ***Sync DELAY*** | This knob allows for the addition of a delay between the optical pulse and the sync signal.<br>When REF is selected, the delay is 10 ns. |
| ***Sync OUT*** | The sync signal is collected from this jack. Note that the type of signal (TTL or NIM) is selected on the rear panel. For the ChronosBH is required the NIM signal (-500mV to -700 mV, 50Ω).<br>The "SYNC Out" from the front panel of the laser controller box is connected to the "SYNC" input of the SPC-130 card in the PC via a SMA cable. |
| ***Power ADJUST*** | The dial has to be set at the maximum in order to generate a single pulse.<br>When the dial is not set at the maximum the pulse shape changes; more than one pulse can be generated. |

The switches on the back panel are set at ISS and the user is not required to change them.


**Figure 9.7** Back Panel of the Picosecond Light Pulser Unit

| | |
|---|---|
| ***REMOTE INTERLOCK*** | It is required to have the optical output. |
| ***SYNC OUT*** | Set the left switch on NIM<br>Set the right switch on PRE (the sync out signal comes before the optical output) |

For a detailed description of the Picosecond Light Pulser, please refer to the Hamamatsu user manual.

**WARNING:**

**Don’t turn the laser on when it’s not connected to instrument. Don’t stare into the laser when it’s on.**

**The Hamamatsu Pulsed laser is a Class 3B Laser product. Please refer to Picosecond Light Pulser PLP-10 Instruction Manual for safety information.**

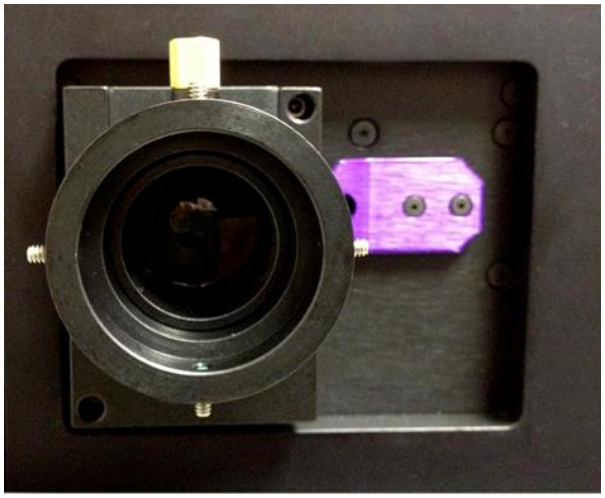
## 9.3 Laser diodes by Horiba

The HORIBA driver controls the laser diodes and some LEDs. It features two components: a controller unit and the laser head module. The laser head module is mounted to the ChronosBH through an adapter bracket that holds a filter holder.


**Figure 9.8** HORIBA laser on the mounting bracket.

In order to install it follow the steps outlined below:

Back of the ChronosBH with the adapter for the laser diode. The adapter holds a filter holder; a 25mm-diameter filter for “cleaning” the emission of the laser diode and LED can be placed in the filter holder.



The DeltaDiode allows for direct control through the knob located on the front panel, or remote control through the USB port (using the software supplied by the manufacturer).


**Figure 9.9** Front Panel of the DeltaDiode Light Pulser Unit

We report below a description of the knobs relevant to the use of the unit with the ChronosBH.

***POWER*** Turn the Key clockwise in order to turn the power ON

***Status Indicators LEDs***

Various LEDs located on the front panel of the unit report the status; specifically:

- Cw or pulsed mode of operations
- External trigger
- Interlock
- Mains power.

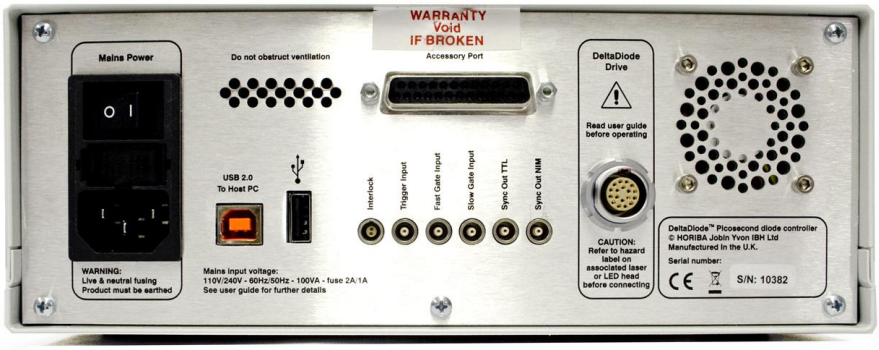



**Figure 9.10** Back Panel of the DeltaDiode Light Pulser Unit

We report below the function of the switches on the back panel relevant for the operations with the ChronosBH.

***Mains power*** ON/OFF switch to the power line.

| | |
|---|---|
| ***USB*** | Connection to the computer |
| ***USB port*** | For connection to other USB devices (downstream) |
| ***INTERLOCK*** | It is required to have the optical output. |
| ***TRIGGER INPUT*** | Optional connection to an external trigger source |
| ***SYNC OUT NIM*** | To connect to the data acquisition card |
| ***DELTADIODE DRIVE*** | Connection to the laser head |

For a detailed description of the DeltaDiode, please refer to the Horiba user manual.

**WARNING:**

**Don't turn the laser on when it's not connected to instrument. Don't stare into the laser when it's on.**

**The HORIBA pulsed laser is a Class 3B Laser product. Please refer to DeltaDiode Light Pulser Instruction Manual for safety information.**

## 9.4 Laser Diodes by B&H/Lasos

The laser diodes by B&H/Lasos are mounted onto a bracket that is used to connect the laser to the ChronosBH.

**Figure 9.11** Laser diode by B&H/Lasos (model BDL-xxx-SMC) with mounting bracket for the ChronosBH.

In order to install it follow the steps outlined below:

Remove the two screws from the back port of the ChronosBH (close to the location of the entrance shutter)

Locate the holding bracket that is shipped with the laser

Mount the holding bracket using the two 4-40x7/8 SHCS screws included in the package.


Once the holding bracket is in place, the laser can be fixed onto it.

For mounting the laser use the two 8-32x 3/8 SHCS stainless steel screws.

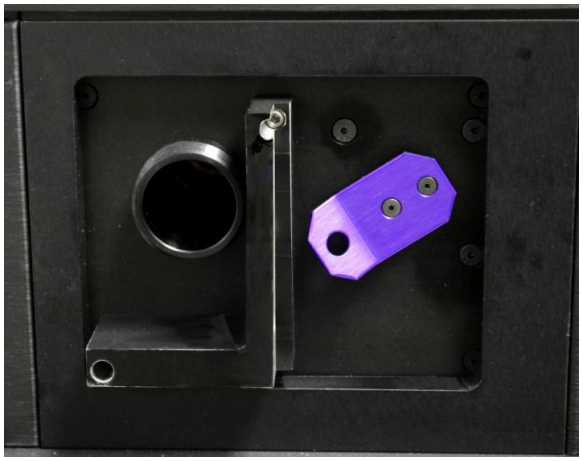



Figure 9.12 shows the controller for the BDL lasers. The controller includes the key switch (mandatory for class 3B lasers) and a switch to select between three repetition rate frequencies (20, 50, 80 MHz) and continuous wave (cw) operation.

The controller includes the pulse generator and driver electronics, the control electronics and an active temperature stabilization of the laser diode.


**Figure 9.12** Laser controller for the B&H/ Lasos lasers.

For a detailed description of the laser, please refer to the B&H user manual.

**WARNING:**

**Don't turn the laser on when it's not connected to instrument. Don't stare into the laser when it's on.**

**The B&H/Lasos laser is a Class 3B Laser product. Please refer to user manual of the manufacturer for safety information.**

## 9.5 Using a multiphoton laser

The multiphoton laser emits in the range from 700 nm to 1000 nm; it allows for excitation using 2- and 3-photons. When used with a frequency doubler it allows for single photon excitation in the region 360-490 nm.

The laser is coupled to the Chronos using a beam steering device and mirrors (if the pulsewidth is important, fast mirrors have to be utilized in the setup). When using this source particular attention has to be put into the rejection of the excess infrared radiation that is present in the environment. It is recommended using filters (such as the BG39 by Schott Glas) in the following positions:

- reference channel (present in steady-state configurations);
- emission channel.

Optics with a NA=0.4 are used in the excitation channel of the Chronos when using this laser.

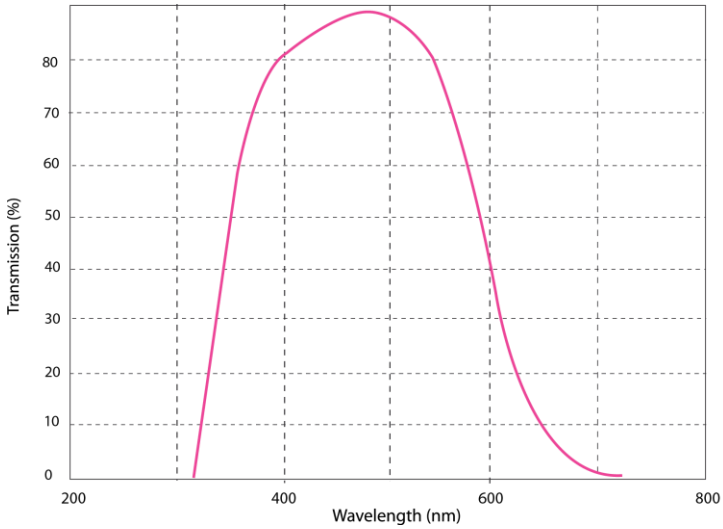



**Figure 9.13** Transmission curve of BG39 filter (courtesy of Schott Glas)

### 9.5.1 Controlling the intensity of the multiphoton laser

Unit A450 allows for the intensity control of the multiphoton laser through the computer. The unit is controlled through the USB port of the computer. The intensity is controlled by using a computer-controlled rotating ½ wave plate in conjunction with a Glan-Taylor polarizer; the rejected portion of the beam is dissipated onto a beam dump.

**Figure 9.14** Unit A452 for conditioning the reference signal from multiphoton lasers.

### 9.5.2 Signal conditioning of the laser trigger

The trigger signal of the laser can be generated by placing a beam splitter in front of the laser that diverts a fraction of the light onto a proper photodiode.

Alternatively, the trigger signal from the internal photodiode can be used. In this case, unit A452 is used to condition the signal using a constant fraction discriminator; this allows for using the internal photodiode over a wide wavelength range.

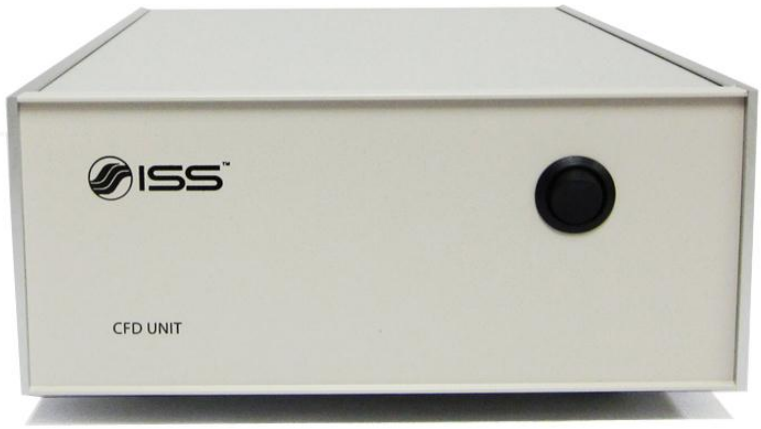



**Figure 9.15** Unit A452 for conditioning the reference signal from multiphoton lasers.

## 9.6 Using the supercontinuum laser

The supercontinuum laser emits in the wavelength range from about 420 nm to 2500 nm (depending upon the specific models); for fluorescence applications a filter allows for the selection of wavelengths in the range up to about 900.


**Figure 9.16** Supercontinuum laser (*courtesy of Fianium, www.fianium.com)*

The selection of a wavelength is done by using an acousto-optical tunable filter (AOTF), computer-controlled. The AOTF system allows for the simultaneous selection of up to eight wavelengths. The laser is controlled through the USB port of the computer; its basic operations and control are included in the Vinci Multidimensional software package driving the ChronosBH. A drawback of the AOTF is the reduced light per unit wavelength available at the output.

The wavelength selection can also be achieved by using a monochromator placed in front of the laser. The monochromator allows for a higher intensity per unit wavelength; when, for instance, using a monochromator with 100 mm focal length, the linear dispersion is about 8 nm/mm. That is a line of 8 nm is obtained by using slits of a 1mm aperture. The wavelength can be selected continuously over the range of operation of the monochromator.

Alternatively, an automated filterwheel allows for the selection of the wavelengths, albeit in this case the wavelengths available are dictated by the filters utilized.

The laser output is pulsed, with pulses of less than 5 ps. The repetition rate is variable from as low as 100 KHz to 60 MHz (the correct specifications have to be provided to the manufacturer). The laser output is unpolarized and it is delivered through a fiber. All these elements make the supercontinuum laser an ideal source for fluorescence applications.

## 9.7 Connecting the Light Sources

### 9.7.1 Sync Signals Unit

Different types of light sources may feature sync signals with different electrical characteristics (see Table below).

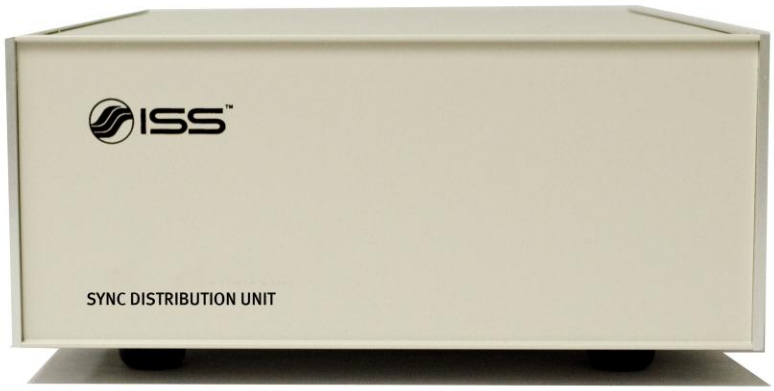
| Light source | Sync signal | Jack |
|---|---|---|
| LED (ISS) | -1 V, 50 Ω | SMA |
| Laser diode (Hamamatsu) | -700 mV, 50 Ω | BNC |
| Laser diode (HORIBA) | -1000 mV, 50 Ω | Lemo |
| Laser diode (LASOS and B&H) | +100 mV to +300 mV, 50 Ω | SMA |
| Supercontinuum laser (Fianium) | +150 mV, 50 Ω | SMA |

**Table 9.1** Characteristics of the sync signals from various light sources.

On the other hand, the TCSPC cards (SPC series) require a negative pulse in the range from -50 mV to -1 V (on 50 Ω) with a minimum pulse width of 400 ps.

The sync signal unit is utilized when different light sources are used for excitation. The unit collects the sync signals from different lasers and delivers the proper signal to the acquisition card. In this way the user does need to change the setup and/or replace cables.

**Fig 9.17** Sync Signal Unit



## 9.8 Aligning the light sources

Any light beam has to enter the excitation channel of the Chronos along the axial direction. Any deviation may compromise the results obtained. In order to check the alignment of the laser, remove the sample compartment and mount the alignment plate (Figure 9.17). The laser beam should hit the center cross of the alignment plate.

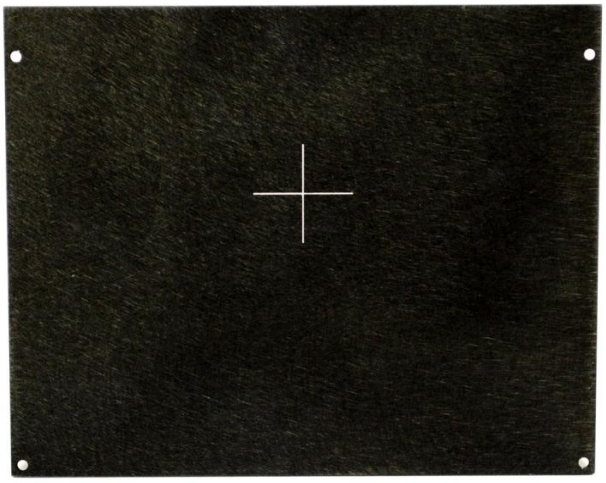
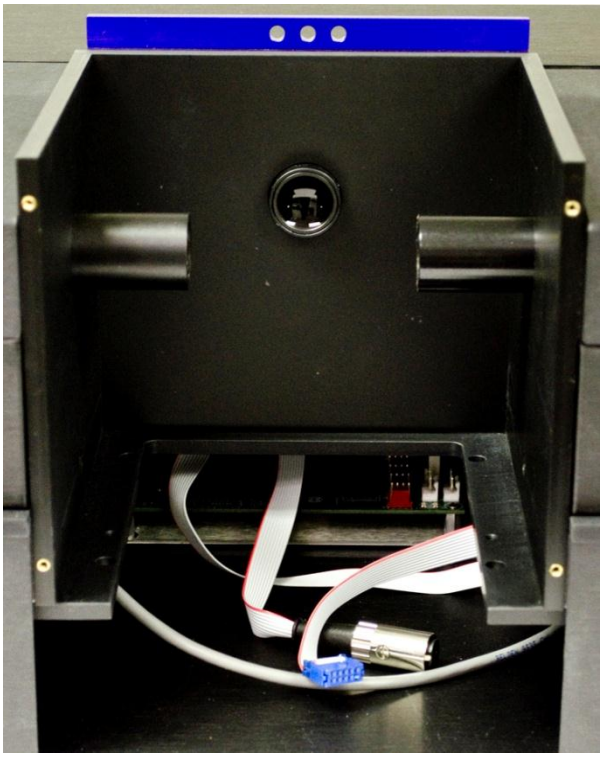


**Figure 9.17** Laser beam alignment plate.

Remove the sample compartment by first removing the four screws (4-40) that keep it attached to the instrument. Disconnect the cables (depending upon the specific sample compartment).
Carefully slide the sample compartment out and detach its cables.



Mount the alignment plate on the ChronosBH whereby the side with the cross faces the laser, and fix the alignment plate with four screws.


Adjust the laser so that the light is centered at the cross with the iris position open and closed.

# 10. Installation and Connection of Light Detectors

Different types of detectors can be used with ChronosBH. The installation of each type of detector is described hereafter; for information on detectors not listed below, please visit the ISS website or contact ISS Customer Support.

## 10.1 The H5773 (PMC-100 Module)

The detector is packaged into the unit PMC-100 module. The unit includes a Peltier element to keep the detector at a constant temperature.

**Note:** Dim the room lights. Detectors are very sensitive to light: direct exposure of the detectors to room light should be avoided as much as possible.

To connect a PMC-100 detector, first remove the cover from the PMC-100. Make sure the O-ring is inside the adapter. Reassemble and screw the detector onto the side of ChronosBH (keep house horizontal and the fan pointing to the front of instrument).
The PMC-100 is connected to connector 3 of the DCC-100 card (inserted in the computer) via a 15 pin sub D cable.

**Note:** Connector 1 and 2 of the DCC-100 card cannot be used since only connector 3 provides thermoelectric, Peltier, detector temperature control.

The output signal is sent to the “CFD” of the SPC-130 card via an SMA cable (see Figure 10.3).

## 10.2 Hamamatsu H7422 PMT Modules

**Note:** Dim the room lights. Detectors are very sensitive to light: direct exposure of the detectors to room light should be avoided as much as possible.

The H7422 PMT comes with an ISS adapter that can be connected onto the emission channel of the ChronosBH. The power for the H7422 PMT is supplied and controlled from connector 3 of the DCC-100 card in the computer via the power supply cable (model C300 DCC H74XL).

The power supply cable includes a cable with an SMA connector, which is connected to the HFAC26-2 preamplifier used for amplification of the signal as well as for indicating the overload protection. The power of the preamplifier is supplied from the DCC-100 card with a C400-12V-1 power cable (Default connected to connector 1 of the DCC-100 card).

The signal output from the H7422 is sent to the input of the preamplifier and then to the “CFD” input of the SPC-130 card. See figure 10.4 for the connection diagram.

## 10.3 R10467 Hybrid detector (HPM-100 Module)

**Note:** Dim the room lights. Detectors are very sensitive to light: direct exposure of the detectors to room light should be avoided as much as possible.

To connect a HPM-100-40 detector, follow the instruction for the HPM-100 detector. The cabling and connection are similar. HMC-100-40 has an adjustable stand that should be used to insure the detector is kept horizontal, and parallel with the Chronos platform.

The HPM-100-40 requires two active power supply voltage that are not used by other detector modules (such as the PMC-100), respectively -5V, +5V and +12V. These voltages are supplied by the DCC-100 board without special cables. The voltages must be turned on by editing the DCC-100.ini file in ISS VINCI software (see paragraph 23.4). The +12V and -5V are kept off by default by both firmware and software.

In *Vinci*, click on <Help> in the Main Menu and select <Open Settings Folder>. Click on the <BH> sub-folder to access the file <dcc100.ini>.
First backup the file *dcc100.ini*, than edit it with notepad. Change, only the settings for +5V, -5V and +12V power for connector 3. Make all these setting “1” (1=on).

Optimizing settings for this hybrid detector is somewhat different than a typical PMT. It is recommended that the CFD level be set to about -30mV for this detector, and the bias voltage (gain) should be adjusted until a plateau of “dark” counts is observed between to gain settings. The dark count to bias voltage curve is very steep outside the narrow plateau. The plateau is typically between 70% and 85% full range. The plateau should be about 5-10% wide in gain units. If an overload shutdown occurs, decrease the gain and clear the overload.


**Figure 10.1** Connection diagram for ChronosBH with Hamamatsu laser and R10467 detector.

## 10.4 R3809U MCP PMT (Microchannel Plate Photomultiplier Tube)

The R3809U MCP PMT has the best time-resolution for time correlated single photon counting (TCSPC) experiments. The R3809U-50 is connected head-on to the side of the ChronosBH via the supplied ISS adapter.

**Note:** Dim the room lights. Detectors are very sensitive to light: direct exposure of the detectors to room light should be avoided as much as possible.

**Note:** Minimize the light exposure of the MCP during the whole process.

The ISS MCP adapter can be separated into two parts as indicated in Figure 10.2 top. The front part can be screwed to the emission side of the ChronosBH. Make sure the O-Ring is and stays properly in place. Check the part that is attached to the ChronosBH and screwed onto ChronosBH as shown in Figure 10.2 (middle).

Take the MCP out of its box. Remove the sticker that covers the light entrance of the MCP, insert it into the ISS adapter and screw the back part of the adapter onto the front part as shown in Figure 10.2 (bottom).

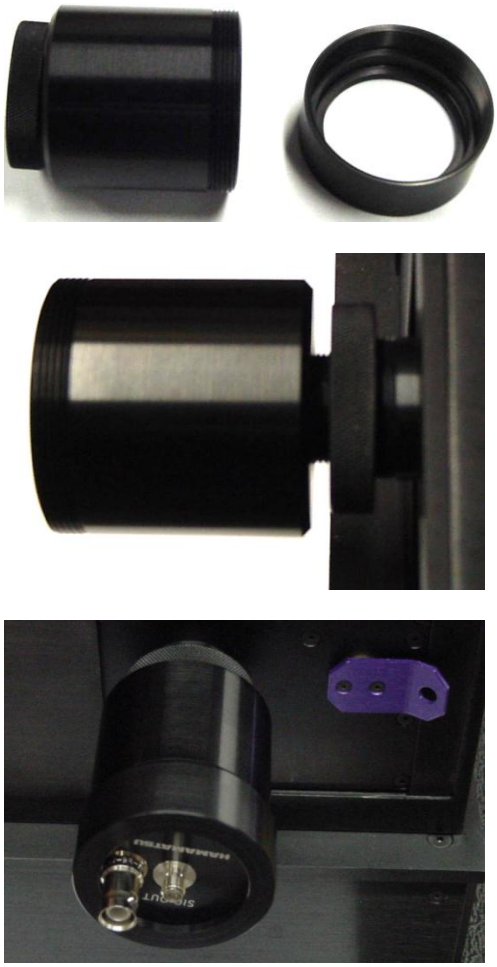


**Figure 10.2** Installation of the R3809U MCP to the ChronosBH.

The cable connection for the detector is shown in Figure 10.6 below. Power for the R3809U-50 is supplied by a FuG HCN14-3500 power supply via a high voltage cable.
Please make sure to not confuse the MCP high voltage cable with BNC cable; the connectors look similar, but they are different in the center with the HV center being longer.

## 10.5 Connecting the Detectors and the Sources: a Summary

Several detectors can be connected to the ChronosBH. The diagrams below display some examples.

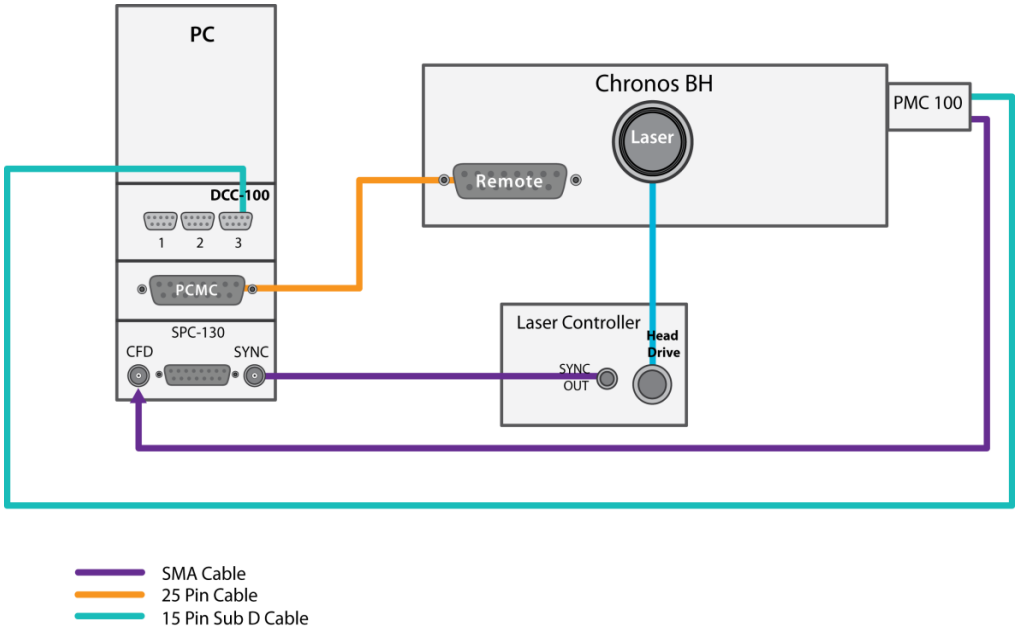



**Figure 10.3** Connection diagram for ChronosBH with Hamamatsu laser and PMC-100 detector

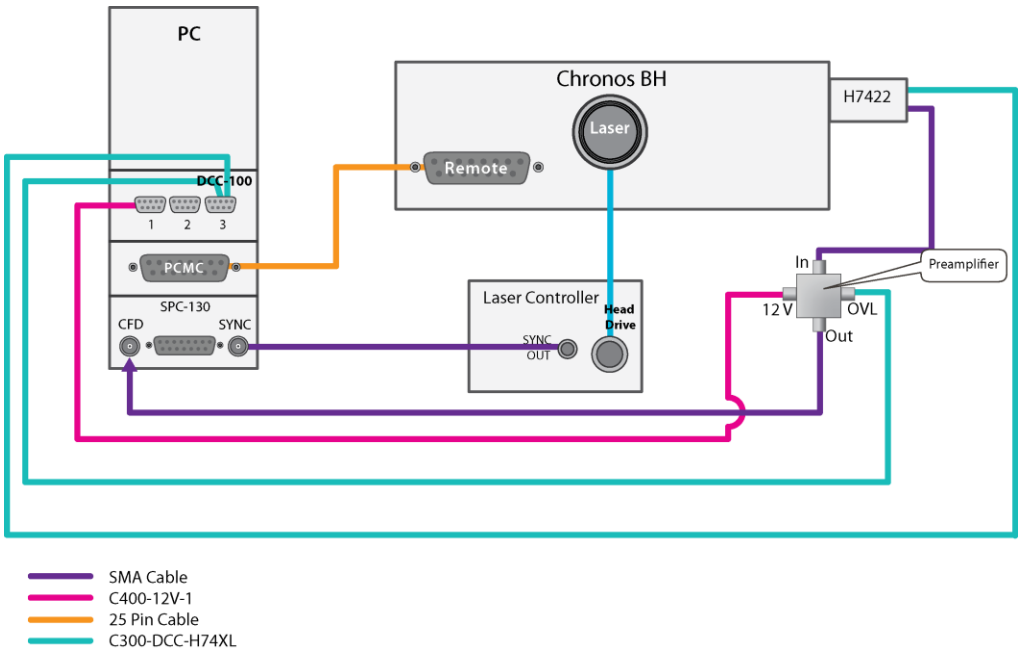



**Figure 10.4** Connection diagram for ChronosBH with Hamamatsu laser and H7422 detector.

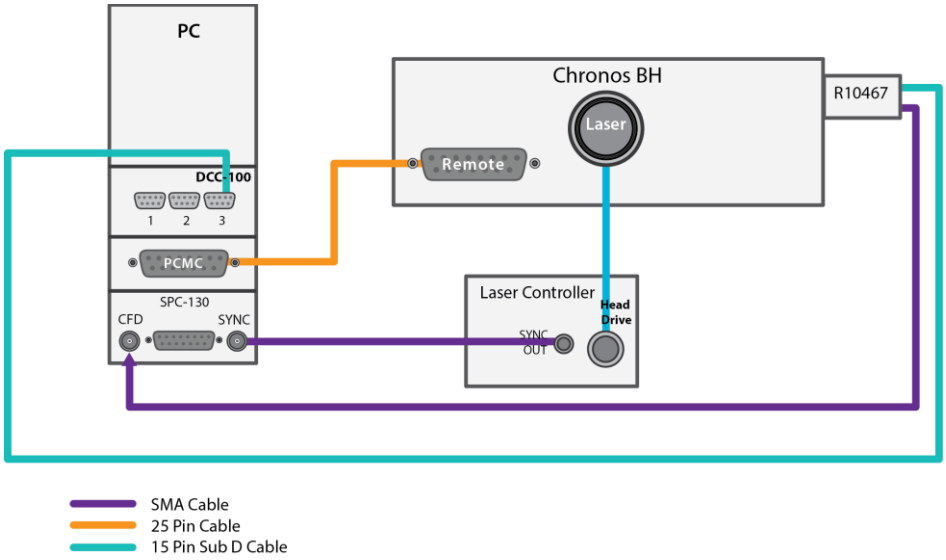



**Figure 10.5** Connection diagram for ChronosBH with Hamamatsu laser and hybrid detector

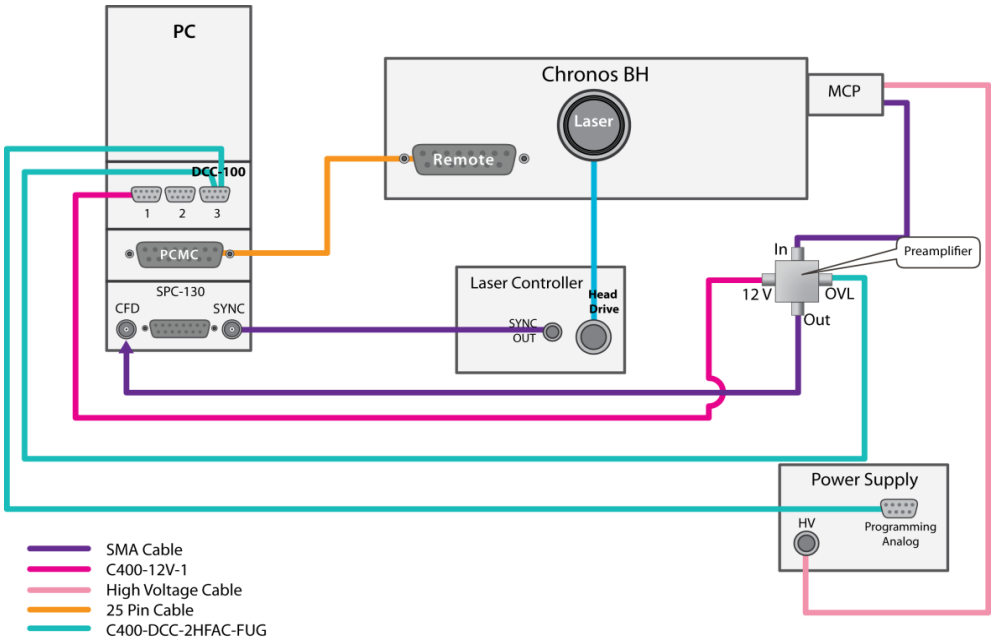



**Figure 10.6** Connection diagram for ChronosBH using the Hamamatsu laser diode and the R3809 MCP detector.

### 10.5.1 Using a router

When two detectors are utilized on the ChronosBH equipped with the SPC- cards, a router is utilized. The SPC-130 card has one input only. The router tags the photons according to the detector they originate from; it assigns an identification label to each photon (detector1 or detector 2). From the router the signal then goes to the single-channel data acquisition card and the photons are recorded according to the originating detector.


**Figure 10.7** Four-channel router

### 10.5.2 Signal distribution unit

The unit is convenient in the following two instances:

1 The signal from a light detector is sent to a two different data acquisition cards (for instance the SPC-130 for fluorescence measurements and the MCA-300 for phosphorescence measurements);

2 The signals from different light detectors are sent to more than one acquisition card (for instance the SPC-130 for fluorescence measurements and the MCA-300 for phosphorescence measurements). In this case, the unit includes the router as well.

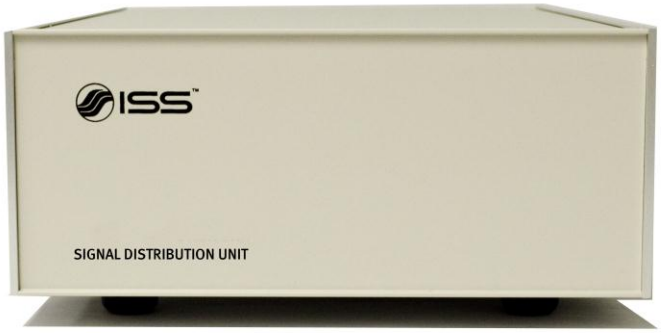



**Figure 10.8** Signal distribution unit front

The Signal Distribution Unit allows for the selection of the acquisition card through the computer; the user does not need to remove and/or replace cables as they are always connected to the instrument.

# 11. Steady-State Measurements with ChronosBH

The acquisition of steady-state fluorescence data requires the use of a proper data acquisition card in order to acquire data with an acceptable S/N ratio in a short time. In fact, when using TCSPC card for steady-state measurements, the S/N ratio of the measurement is poor as most of the fluorescence photons are not collected.

## 11.1 Signal Processing Unit for Steady-state applications

For these applications, the light detector is a R928 by Hamamatsu. The output of the detector is sent to the PX01 Unit where the signal passes through a current-to-voltage converter and through amplifier discriminators. The output (TTL signal) of the pre-amplifier discriminators is directed to the PMC card inserted in the computer for data acquisition.


**Figure 11.1** PX01 unit front panel. The unit accepts the signal from up to 3 detectors; output is a TTL signal diverted to a counter.

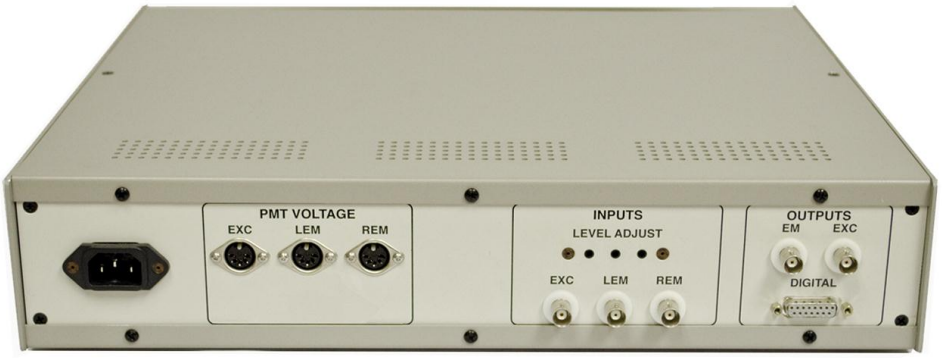



**Figure 11.2** PX01 unit back panel. The unit accepts the signal from up to 3 detectors; output is a TTL signal diverted to a counter.

***PMT VOLTAGE EXC/LEM/REM***

Each DIN connector delivers voltage to the Model K218 and K219 PMT housings. The PMTs are identified as:

EXC – excitation channel
REM, right emission channel
LEM, left emission channel

| | |
|---|---|
| ***INPUTS EXC/LEM/REM*** | The input signal from each PMT.<br>The PMTs are identified as:<br>EXC – excitation channel<br>REM, right emission channel<br>LEM, left emission channel |
| ***LEVEL ADJUST*** | A set screw is accessible in order to adjust the threshold of each PMT, EXC, REM and LEM |
| ***OUTPUTS EXC/EM*** | These are two analog outputs. They are used for frequency-domain applications. |
| ***OUTPUTS DIGITAL*** | The connector carries the digital (TTL) outputs to the ISS PCMC card. |

### 11.1.1 Adjusting the threshold of the discriminators in the PX-01 unit

For setting the discriminators please refer to section 11.6 of the Reference Manual for the Vinci Multidimensional Fluorescence Spectroscopy software.

The PMT output is passed through a pre-amplifier discriminator unit, which allows separation of pulses due to amplitude, which is determined by the threshold level setting. In the ISS spectrofluorometers the unit is installed in the instrument; in other instruments (Chronos) the pre-amplifier discriminators are housed in the PX-01 unit.

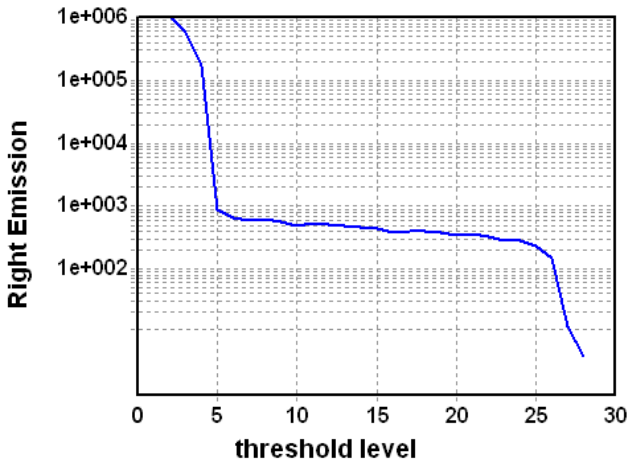



**Figure 11.3** The plot displays the number of counts recorded against the value of the discriminator's threshold for a R928 PMT at 25 ºC.

When the threshold is close to zero, a higher number of counts are recorded. As the threshold is increased, the number of counts decreases and it is almost stable at around 1000 counts per second (c/s). These counts represent the level of the dark current of the PMT.

When the threshold of the discriminator is close to zero, all of the pulses (including the ones arising from noise) pass through the discriminator and are counted by the processing electronics. When the threshold of the discriminator is increased, only pulses that have amplitudes above the set voltage are passed through the discriminator and recorded.

A typical curve of dark current counts versus the discriminator threshold level is displayed in Figure 11.3; for convenience, the threshold level is reported as number of half turns of the potentiometer, which sets

the voltage level on the discriminator. At the zero position of the potentiometer, the threshold level is 263mV and at position 25, the threshold level is equal to about 5 V. At positions of the potentiometer between 5 and 23, the number of dark counts is approximately constant.

We set the threshold at position “6”, which corresponds to about 450 counts/second. There are differences in the level of dark current at room temperature, between different types of PMTs; the dark current varies widely also between PMTs of the same model. PMTs featuring a dark counts level below 100 c/s at room temperature are commercially available. Manufacturers also specify the typical dark current level for a specific PMT.

## 11.2 Light sources for steady-state measurements

### 11.2.1 Using a laser in excitation

Figure 3.3 display the configuration of the ChronosBH with an emission monochromator (the monochromator can be utilized for the acquisition of emission spectra or time-resolved spectra).

### 11.2.2 Using the xenon arc lamp and monochromator in excitation

Figures 3.4 and 3.5 display a ChronosBH equipped with the xenon arc lamp and monochromator in excitation. In this configuration the light is delivered to the instrument via a fiber bundle; in other configurations, the assembly is connected directly to the instrument using a mechanical flange (the laser diode or LED has to be removed first).
The instrument so equipped is capable of excitation and emission spectra. The reference channel is equipped with the reference PMT for the correction of excitation spectra (using the quantum counter) and for stabilization of fluorescence emission measurements when they are acquired on a long time.

## 11.3 Detectors for steady-state measurements

The following detectors are used for steady-state measurements. They are housed either in the room temperature housing Model K218 or the cooled housing Model K219.

| |
|---|
| R928, R928P |
| R3896 |

## 11.4 Room Temperature Housing Model K218

The ISS photomultiplier tube (PMT) room temperature housing Model K218 is designed for both steady-state and time-resolved fluorescence applications. It is routinely utilized on the ISS instrument and yet, it can be utilized as a stand-alone unit. The compact housing features a Hamamatsu E678-11A socket (for Hamamatsu R928 and socket-compatible side-on tubes) with built in voltage divider and the circuitry for injection of the radio frequency signal required for modulation of the signal gain. The housing also includes the high voltage power supply that is set to deliver a maximum of -1,200 Volts. The external panel mounts a switch for selecting between manual and computer-controlled voltage changes, a BNC-jack input for injecting the radio frequency signal and a BNC-jack output for the signal retrieval. The unit requires a +15Volt source for operation.


**Figure 11.4** Room temperature PMT housing unit.

The PMT Housing is composed of two parts:

- PMT Housing
- PMT Screen Body

The Screen Body covers the PMT and protects it from the light of the surrounding environment. An M28 threaded coupling allows for the connection of the unit to the instrumentation.
An ON/OFF switch on the back panel powers the unit. The ON/OFF switch has three positions:

- MAN is used for manual control of the voltage, which is achieved by using the potentiometer;
- REM is used for remote control of the voltage, which is achieved by using a D/A converter embedded in the ISS control electronics;
- OFF turns off the unit.

The voltage is applied to the PMT housing through a 5-pin DIN connector (see Figure 11.5). Table 11.1 reports the pin arrangement for the connector. The PMT housings is powered through one of the connectors V1, V2, V3 (see section 2.2) located on the back panel of the ChronosBH.


**Figure 11.5** The five-pin DIN connector viewed as it sits in Figure 1 on the back of the PMT housing. Please notice the non-sequential numbering of the pin assignments.

| Pin Number | Connection |
|---|---|
| 1 | +5 V |
| 2 | Ground |
| 3 | +15 V |
| 4 | -15 V |
| 5 | Analog Voltage |

**Table 11.1** DIN connector pin assignment

The output of the PMT housing is a current signal, which in ISS instrumentation is carried to a current-to-voltage converter. The signal output is available at the BNC connector labeled OUT.
A second BNC connector, labeled RF, is used for injecting a radio frequency signal that modulates the

gain of the PMT (for frequency-domain applications). Typically, this signal is about 15 Volt PP in amplitude on a 50 Ohm load.

### 11.4.1 Installing the PMT in the K218 housing

**Warnings**

- Only handle the PMT by the plastic portion at the base of the PMT. Avoid touching or handling it by the glass enclosure.
- When installing a PMT into the housing, shut off all light sources and reduce as much light from the room as possible.
- Do not apply voltage to the PMT when it is exposed.

Installing the PMT is done via the following procedure:

1. The PMT screen body is attached to the housing by two screws. Remove the screws.
2. Dim the light intensity in the room to the minimum necessary to see. Remove the PMT from the box and set it into the socket on the housing. You will notice multiple pins on the plastic base. In the center of these pins is a shaft, which has a tab. This tab matches with the notch in the socket.
3. Place the PMT body screen on it and fix it using the two screws.

When handling the PMT only touch the plastic base and avoid getting fingerprints or scratches on the glass over the voltage divider.
Do not power up the PMT unless the body is attached to an optical unit with no light leakage.

### 11.4.2 Operations

The PMT Housing can operate in two ways:

| Operation Mode | Dial Position |
|---|---|
| Photon Counting | With the switch in MAN mode, the dial is set at the Maximum position (10). |
| Analog | With the switch in MAN mode, the dial regulates the signal gain.<br>With the switch in REM mode, the signal gain is regulated through the computer. |

In Photon Counting Mode the number of pulses at the output of the PMT housings is counted in a set time interval. In this mode of operation, the PMT operates at the maximum voltage, that is, with the switch in MAN position the dial is set at the maximum. When in REM position, the voltage is set at the maximum through the computer.

In Analog Mode the average current at the output of the PMT is recorded. The signal intensity varies with the voltage applied to the PMT. The voltage can be varied in manual mode when the switch is set on MAN, or through the computer when the switch is set to REM.

### 11.4.3 Operating the PMT in photon counting mode

In this mode of operations, the gain of the PMT is set at the maximum (-1,200 Volt) using the potentiometer placed on the room temperature housing. The gain is never changed; if the signal is too high, a filter is used to reduce the intensity of the excitation light in the spectrofluorometer.

In photon counting mode the number of photons produced by the PMT in the time unit is counted by the processing electronics and computer. A photon impinging onto the photocathode produces a pulse at the output of the PMT. The pulses are formatted by a discriminator and pre-amplifier prior to being counted.

Typically, not all of the pulses are generated by photons; some pulses are generated by the dark counts of the PMT. Yet, the amplitude of pulses arising from noise is less than the amplitude of pulses generated by photons. This difference allows for the discriminator to separate the two types of pulses; only pulses featuring amplitude greater than the set threshold are counted.

### 11.4.4 Operating the PMT in Analog Mode

When the PMT operates in analog mode, the gain, that is the total voltage applied between the anode and cathode is changed in order to accommodate the signal.
The switch on the PMT control panel is set to <MAN> position. The gain is changed by acting on the potentiometer mounted on the panel of the PMT housing.

In Manual mode, the potentiometer dial regulates the voltage provided by the high power supply, included in the housing, to the PMT. When a +15V is applied, the voltage ranges from -200 up to -1,200V (see Table 11.2).

| Dial position | Voltage applied to the PMT (Volts) |
|---|---|
| 0 | -200 |
| 1 | -300 |
| 2 | -400 |
| 3 | -500 |
| 4 | -600 |
| 5 | -700 |
| 6 | -800 |
| 7 | -900 |
| 8 | -1000 |
| 9 | -1100 |
| 10 | -1200 |

**Table 11.2** Dial position and voltage applied to the PMT

### 11.4.5 Technical Specifications

| Parameter | Property |
|---|---|
| Input Voltage | +15 V (pin 3) |
| Output Voltage | 0 to -1250 V |
| Input Voltage jack | 5-pin DIN connector |
| Input RF signal jack | BNC |
| RF signal input | < 10 Volts |
| Signal output jack | BNC |
| PMT Housing dimensions | 81.0 mm x 105.0 mm x 68.6 mm<br>(3.19” x 4.14” x 2.70”) |
| PMT Cover dimensions | 50.8 mm x 50.8 mm x 88.9 mm<br>(2.00” x 2.00” x 3.50”) |
| Overall dimensions | 114.3 mm x 172.7mm x 81.3 mm<br>(4.5” x 6.8” x 3.2”) |
| PMT Housing Weight | 1.1 kg (2.51 lbs) |
| PMT Connector Thread | M28 x 1 |
| Optical axis (when PMT is placed horizontally) | 63.4 mm |
| Frequency response | DC - 700 MHz |

**Table 11.3** Model K218 PMT Housing Specifications

## 11.5 Cooled PMT Housing Model K219

The ISS Cooled Photomultiplier Tube (CPMT) Housing Model K219 is utilized when the highest signal-to-noise ratio (S/N) is required in a measurement. The noise of a detector is attributable to two types of effects:

- Thermoionic emission, which is due to photoelectrons that detach free from the photocathode surface and are amplified by the dynode chain of the detector.
- Thermal noise due to ionization of gases inside the detector.

By reducing the temperature of the photocathode, thermal noise can be greatly reduced. Therefore, the signal-to-noise (S/N) ratio of the measurement is improved.
The CMPT unit comprises two parts:

- The housing, where the PMT is lodged. It includes a Peltier element utilized to lower the temperature of the photocathode and to keep the temperature stable. The PMT is separated from the outside by a window, which prevents condensation on the PMT when lowering the temperature.
- The controller, connected to the housing by a cable, allows for the user to set the desired temperature. The value of the temperature is displayed on the front panel.

**Figure 11.6** Cooled PMT housing Model K219

**Figure 11.7** Controller front panel.

The compact housing features a Hamamatsu E678-11A socket (for Hamamatsu R928 and socket-compatible side-on tubes) with built in voltage divider and the circuitry for injection of the radio frequency signal required for modulation of the signal gain. The housing also includes the high voltage power supply that is set to deliver a maximum of -1,200 Volts. The external panel mounts a switch for selecting between manual and computer-controlled voltage changes, a BNC-jack input for injecting the radio frequency signal and a BNC-jack output for the signal retrieval. The unit requires a +15Volt source for operation.
The Screen Body covers the PMT and protects it from the light of the surrounding environment. An M28 threaded coupling allows for the connection of the unit to the instrumentation.
An ON/OFF switch on the back panel powers the unit. The ON/OFF switch has three positions: MAN is used for manual control of the voltage, which is achieved by using the potentiometer; REM is used for remote control of the voltage, which is achieved by using a D/A converter embedded in the ISS control electronics; OFF turns off the unit.
The voltage is applied to the PMT housing through a 5-pin DIN connector (see Figure 11.8). Table 11.4 reports the pin arrangement for the connector.


**Figure 11.8** The five-pin DIN connector viewed as it sits in Figure 1 on the back of the PMT housing. Please notice the non-sequential numbering of the pin assignments.

| Pin Number | Connection |
|---|---|
| 1 | +5 V |
| 2 | Ground |
| 3 | +15 V |
| 4 | -15 V |
| 5 | Analog Voltage |

**Table 11.4** DIN connector pin assignment

A power supply is available to power up to three PMTs housings simultaneously.
The output of the PMT housing is a current signal, which in ISS instrumentation is carried to a current-to-voltage converter for analog operation and to a preamplifier discriminator for photon counting operations. The signal output is available at the BNC connector labeled OUT.
A second BNC connector, labeled RF, is used for injecting a radio frequency signal that modulates the gain of the PMT. Typically, this signal is about 15 Volt PP in amplitude on a 50 Ohm load.

### 11.5.1 Installing the PMT in the cooled housing

**Warnings**

- Only handle the PMT by the plastic portion at the base of the PMT. Avoid touching or handling it by the glass enclosure.
- When installing a PMT into the housing, shut off all light sources and reduce as much light from the room as possible.
- Do not apply voltage to the PMT when it is exposed.
- Do not use the Peltier controller when the bath circulator is not running.
- Do not allow the condensation from the housing or bath circulator tubing to drip into the instrument or to come into contact with any instrument electronics.

Installing the PMT is done via the following procedure. Please keep in mind all warnings when performing it.

1. Locate the CPMT housing (part number 90085). On the rear of the CPMT housing you will find the voltage divider and electronics enclosure. It is a black rectangular portion with three connectors (one DIN and two BNC) and the ON/OFF switch located on its back panel. The enclosure is attached to the housing by four screws. Remove the screws and gently pull out the enclosure from the housing.
2. Dim the light intensity in the room to the minimum necessary to see. Remove the PMT from the housing installed on the instrument. If the PMT is included with the CPMT package, remove the PMT from the box.
3. When handling the PMT only touch the plastic base and avoid getting fingerprints or scratches on the glass over the PMT. You will notice multiple pins on the plastic base; in the center of these pins is a shaft, which has a tab. This tab matches with the notch in the receptacle of the CPMT voltage divider.
4. Plug the PMT into the CPMT voltage divider. Insert the enclosure into the housing and fix it with the four screws.

### 11.5.2 Operating the cooled PMT housing

The circulating coolant, which can be tap water, should be at a temperature of no more than +10 °C. As a reference, typical tap water temperature ranges from 6 to 8 °C. The coolant removes the heat generated by the Peltier element located close to the PMT photocathode. Please keep in mind that, when using lower bath temperatures, the coolant viscosity increases; heat flux generated by Peltier elements may not be removed fast enough causing an increase in dark signal.

Turn the controller ON. The controller operates all the way down to –28 °C. The temperature is selected by turning the knob located on the front panel of the controller and is displayed on the front panel.
Turn the knob located on the front panel of the controller to position 6.7, which corresponds to a temperature of about –15 °C.

| | |
|---|---|
| Bath Circulator Setting | 10 °C |
| Cooled PMT Controller Setting | Set the dial reading to position 6.7 |

**Table 11.5** Operational settings for the CPMT

If the bath circulator does not remove the heat produced by the Peltier element, the controller unit will start beeping. In this circumstance, turn the knob located on the front panel of the controller to position “one”. When the beeping sound stops, wait a couple of minutes and then turn the knob to position “three”. Repeat the procedure until the position six is reached. If the beeping starts again, check that the circulator provides enough flow through the housing.
Table 11.5 summarizes the typical operational parameters of the bath circulator and the cooled housing controller.

### 11.5.3 Operations

The PMT Housing Model K219 can operate in two ways:

| Operation Mode | Dial Position |
|---|---|
| Photon Counting | With the switch in MAN mode, the dial is set at the Maximum position (10). |
| Analog | With the switch in MAN mode, the dial regulates the signal gain.<br>With the switch in REM mode, the signal gain is regulated through the computer. |

In Photon Counting Mode the number of pulses at the output of the PMT housings is counted in a set time interval. In this mode of operation, the PMT operates at the maximum voltage, that is, with the switch in MAN position the dial is set at the maximum. When in REM position, the voltage is set at the maximum through the computer.

In Analog Mode the average current at the output of the PMT is recorded. The signal intensity varies with the voltage applied to the PMT. The voltage can be varied in manual mode when the switch is set on MAN, or through the computer when the switch is set to REM. When the PMT housing is used in analog mode, cooling is not required.

### 11.5.4 Operating the PMT in photon counting mode

In this mode of operations, the gain of the PMT is set at the maximum (-1,200 Volt) using the potentiometer placed on the room temperature housing. The gain is never changed; if the signal is too high, a filter is used to reduce the intensity of the excitation light in the spectrofluorometer.
In photon counting mode the number of photons produced by the PMT in the time unit is counted by the processing electronics and computer. A photon impinging onto the photocathode produces a pulse at the output of the PMT. The pulses are formatted by a discriminator and pre-amplifier prior to being counted. Typically, not all of the pulses are generated by photons; some pulses are generated by the dark counts of the PMT. Yet, the amplitude of pulses arising from noise is less than the amplitude of pulses generated by photons. This difference allows for the discriminator to separate the two types of pulses; only pulses featuring amplitude greater than the set threshold are counted.

### 11.5.5 Recording the dark counts of a PMT

A photon impinging onto the photocathode produces a pulse at the output of the PMT. Typically, the amplitude of pulses arising from noise is less than the amplitude of pulses generated by photons.

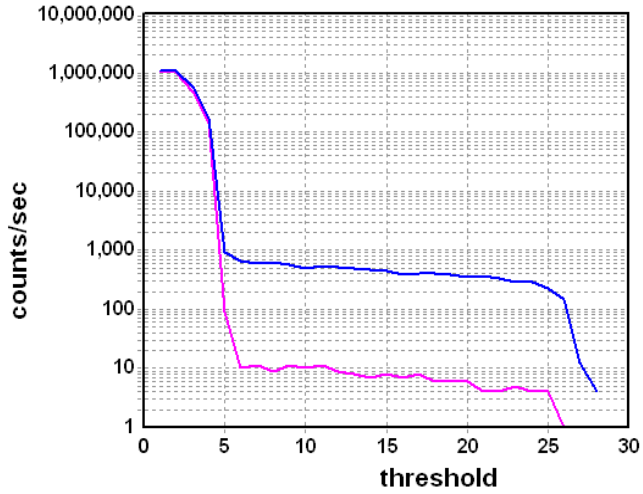



**Figure 11.9** The plot displays the number of counts recorded against the value of the discriminator's threshold for a R928 PMT at 25 ºC (blue line) and at -10 ºC (purple line).

When the threshold is close to zero, a higher number of counts are recorded. As the threshold is increased, the number of counts decreases and it is almost stable at around 1000 counts per second (c/s). These counts represent the level of the dark current of the PMT. When the temperature of the photocathode is lowered at -10 ºC, the dark counts goes to about zero.

This difference allows the instrument electronics to discriminate between the two types of pulses. The PMT output is passed through a pre-amplifier discriminator unit, which allows separation of pulses due to amplitude, which is determined by the threshold level setting. When the threshold of the discriminator is close to zero, all of the pulses (including the ones arising from noise) pass through the discriminator and are counted by the processing electronics. When the threshold of the discriminator is increased, only pulses that have amplitudes above the set voltage are passed through the discriminator and recorded. A typical curve of dark current counts versus the discriminator threshold level is displayed in Figure 11.9 below.

For convenience, the threshold level is reported as number of half turns of the potentiometer, which sets the voltage level on the discriminator. At the zero position of the potentiometer, the threshold level is 263mV and at position 25, the threshold level is equal to about 5 V. At positions of the potentiometer between 5 and 23, the number of dark counts is approximately constant.
There are differences, at room temperature between PMTs of the same model. PMTs featuring a dark counts level of about 100 are commercially available. Manufacturers also specify the typical dark current level for a specific PMT.

### 11.5.6 Operating the PMT in Analog Mode

When the PMT operates in analog mode, the gain, that is the total voltage applied between the anode and cathode is changed in order to accommodate the signal.

### 11.5.7 Manual Control of the Voltage

The switch on the PMT control panel is set to <MAN> position. The gain is changed by acting on the potentiometer mounted on the panel of the PMT housing.
In Manual mode, the potentiometer dial regulates the voltage provided by the high power supply, included in the housing, to the PMT. When a +15V is applied, the voltage ranges from -200 up to -1,200V (see Table 11.2 above).

# 12. Instrument Upgrade to Frequency-Domain Measurements

The ChronosBH can be upgraded to the ChronosFD for performing frequency-domain lifetime measurements. In a typical configuration, one of the emission channels is dedicated to TCSPC acquisition while the other channel is used for frequency-domain measurements. Both configurations are stored in the computer. When the Vinci software is started, the user selects either the configuration for the ChronosFD or the configuration for the ChronosBH.

Contact ISS Service Department for the technical details on this upgrade package.

# Part III: Turning the ChronosBH ON

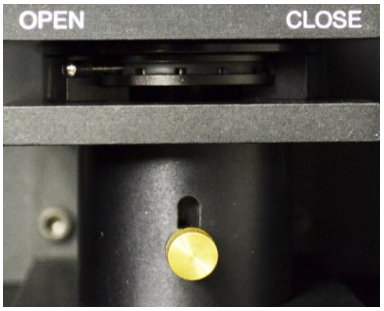
## 13. Instrument Control and Data Acquisition for ChronosBH

All instrument automation are controlled either by the parallel port of the ISS-PCMC card or by a USB controller box via a 25 pin D-Sub cable connected to the port on the back of ChronosBH labeled REMOTE.
The reference signal from the laser and photon signal from the detector are sent to the SPC-130 card or DPC-230 card for TCSPC measurements.

### 13.1 Turning ON ChronosBH

You have already connected all components, the voltage supply and signal cables. Close the instrument covers and all shutters (handles in horizontal position). Detectors are very sensitive to light. Direct exposure of the detectors to room light should be avoided as much as possible.

**Figure 13.1** Iris diaphragm handle; it can be moved at any position between the extremes.




The instrument ON/OFF switch is located on the right rear side of the instrument. Turn the Main Power switch of the instrument ON. Make sure that polarizers are shifted in the light path.
There is a knob in front of the excitation polarizer that controls the iris. Check that the lever of the iris diaphragm behind the turret points is to the right (closed position), Figure 13-1. Close the iris for measurements with a laser. For pulsed LEDs, the IRIS position can be varied to control the light intensity.

### 13.2 Turning ON the Light Source

If the light source is an ISS pulsed LED, it is turned on when the ChronosBH is turned on.

The Hamamatsu pulsed laser is controlled by the laser controller. The turn-on key located at the lower left corner of the laser controller front panel will turn on the laser.

# 14. Starting *Vinci* Software

Your ChronosBH comes with *Vinci Multidimensional Fluorescence Spectroscopy software*. Vinci software is typically located under C:\Program Files\ISS\Vinci. A shortcut icon to Vinci should appear on the desktop after installation.

Click on the <Vinci> icon. Upon starting the software the Analysis page (Figure 14.1) is displayed.

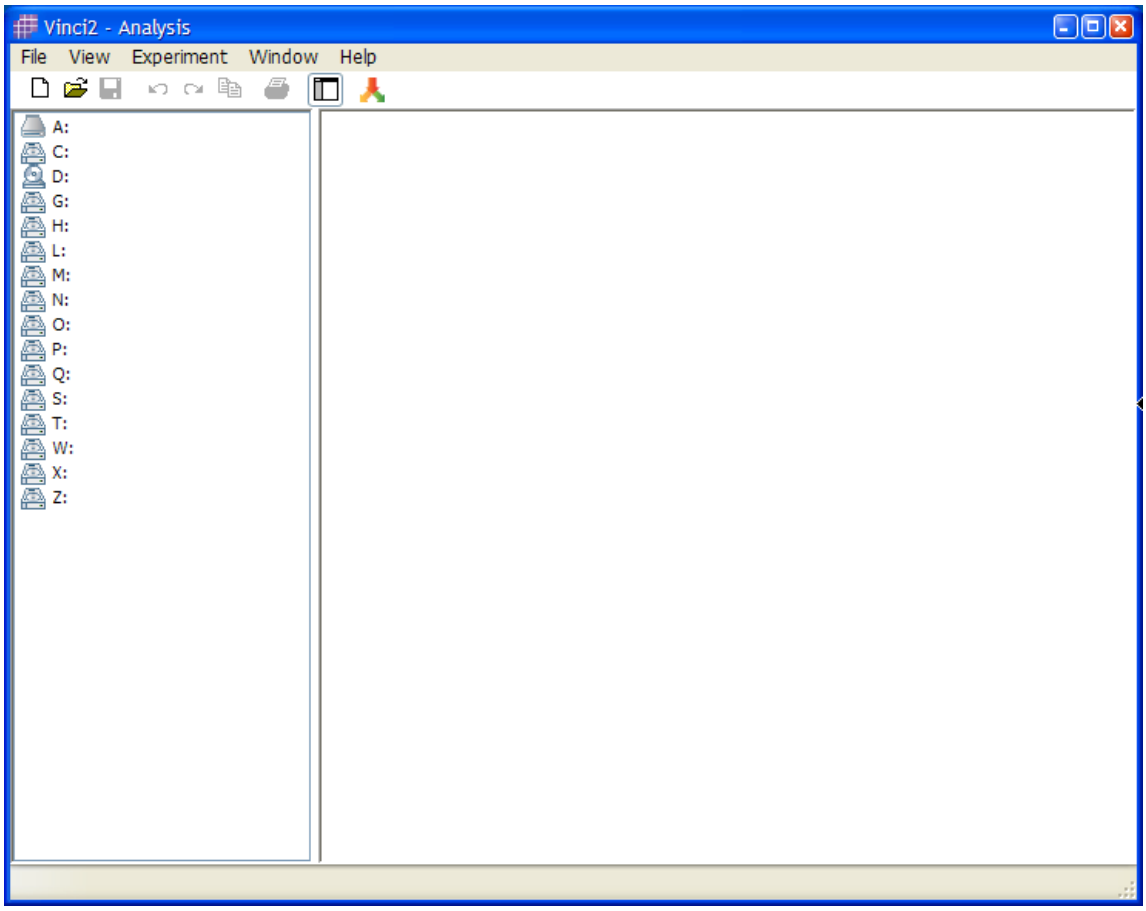
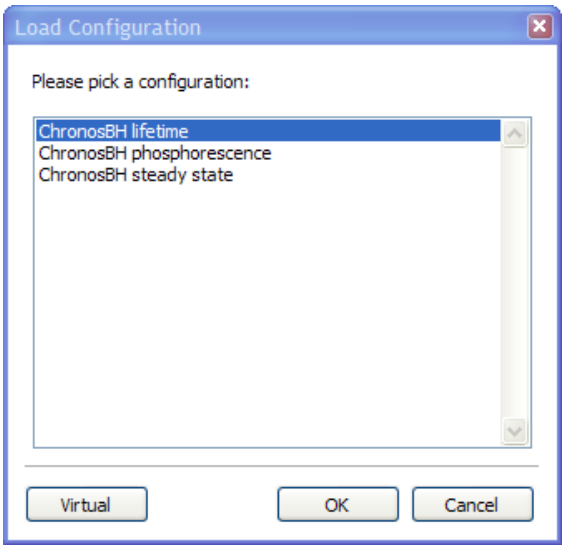



**Figure 14.1** Vinci Analysis window; it is displayed when starting the software.

Select <Experiment> and <Experiment and Instrument Control> from the top menu, which will launch the Experiment and Instrument Control software in a new window.

Alternatively, you can click on the experiment icon 

Select the <ChronosBH lifetime> configuration and press the <OK> button.




You should hear the audible feedback sound of the shutters and motors when the Vinci when the Experiment and Instrument Control portion of the software is loaded.

## 14.1 Instrument Control Page

The Instrument Control Page displays, for each instrument's area (excitation channel, reference channel, right and left emission channels) icons of the various automated devices enabled on the instrument. By clicking on each icon, the device can be moved.
The Figure below displays the Instrument Control page for an instrument equipped with polarizers and shutters in excitation and the two emission channels; the sample compartment is a 2-cuvette holder with stirrers below the cuvettes.

The Instrument Control Page is divided into five areas. Within each area icons show the device activated and controlled by the software. Devices can be added and/or removed using the Instrument Configuration. For details on how to conduct this operation consult the Vinci User Manual.
By clicking on each icon, the user can move the device (for instance, open/close shutters; rotate the sample compartment).

| Instrument area | devices |
|---|---|
| Excitation | Shutter, polarizer, monochromator |
| Reference | Shutter, detector |
| Left Emission | Shutter, polarizer, monochromator, detector |
| Right Emission | Shutter, polarizer, monochromator, detector |
| Sample Compartment | Type of compartment (1-, 2-, 2- 4-cuvettes); stirrers |

An Instrument Configuration reflects the devices installed on an instrument. The user can generate more configurations, depending upon the use. For instance

- ChronosBH lifetime (for acquisition using the SPC-130 card)
- ChronosBH phosphorescence (for acquisition using the Multiscaler)
- ChronosBH stead-state (for acquisition using the PCMC card)

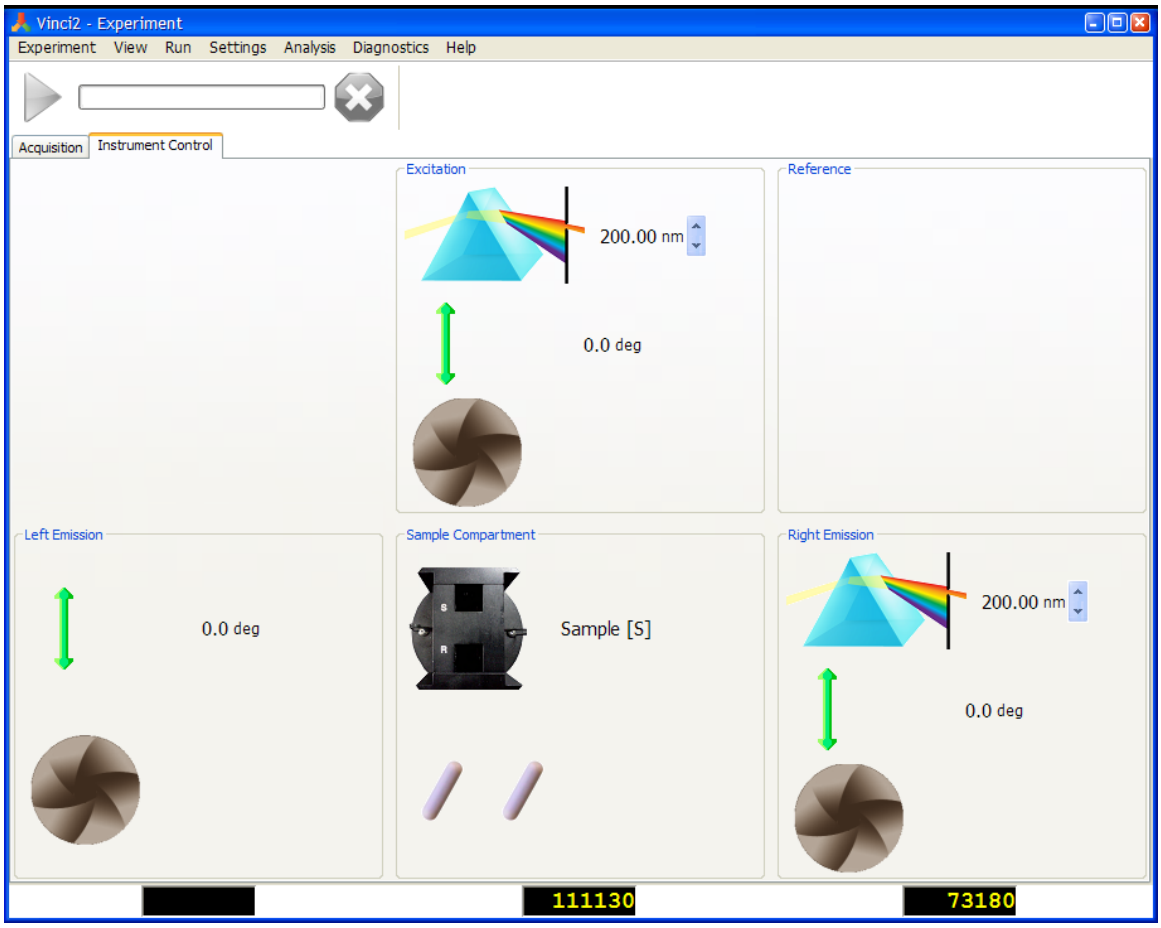
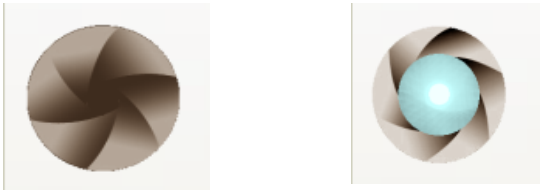



**Figure 14.2** Instrument Control window of a configuration including monochromators in excitation and in the right emission channel.

### 14.1.1 Shutters

The shutter has two positions: <Open> or <Closed>.



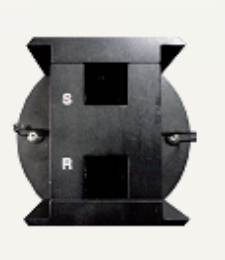
### 14.1.2 Polarizers

When left-clicking on the polarizer icon, it toggles between the (V)ertical and (H)orizontal positions. When right-clicking, the following window:

| | |
|---|---|
| ***Move*** | Allows for the user to move the polarizer at any position ( 0 -90 degrees) |
| ***Calibrate*** | The polarizer is sent to the <Vertical> position (0 degrees) |
| ***Magic Angle (54.7 degrees)*** | Moves to the magic angle position (the icon changes the color to blue) |

### 14.1.3 Cuvette holder

When clicking on the sample holder icon, the device rotates from (S)ample to (R)eference. Position, and viceversa.




Note: the icons are different for the various cuvette holder (1-, 3-, 4-cuvette). Several holders are available (microwell plate, dewar, cryostat, front surface, etc.). For a complete list, see paragraph 6.1 above.

### 14.1.4 Stirrers

The stirrers controls the motors positioned in the sample compartment below the cuvette. A stirrer (magnetic bar) has to be placed into the cuvette for the action to initiate.
When left-clicking on the stirrer icon, the device toggles between rotation and rest. When clicking again, the device stops.

| | |
|---|---|
| ***Move*** | Both devices move. |
| ***Calibrate*** | The motors are stopped. |

### 14.1.5 Monochromators

The monochromators are controlled through this icon.

| | |
|---|---|
| ***Move*** | Entire the desired wavelength position. The monochromator can also be moved step-by-step by clicking on the up/down arrows to the right of the icon. |
| ***Calibrate*** | Enter the position of the monochromator as read on the dial. |
| ***Provide bandwidth factor*** | Enter the bandwidth (in nm) |
| ***Provide slit width*** | Enter the value (in mm) |

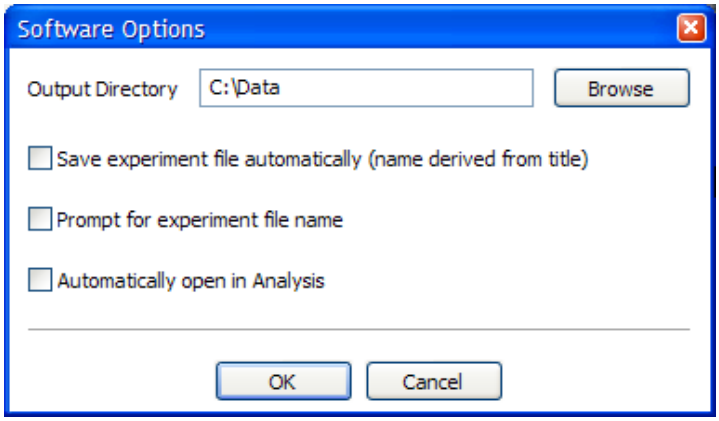
## 14.2 The Acquisition Page

The Acquisition Page shows the relevant information about the acquisition. It also allows, for convenience, to control the polarizers, shutters and rotating sample compartment. In fact, for optimizing the signal, one has to open/close these devices. It is recommended that the user gets familiar with this page. More on the Acquisition Page in the next sections.

## 14.3 General Features of Vinci

### 14.3.1 Saving the data

Three different options are offered in Vinci to save the data. The preferred data saving procedure can be selected by clicking on <Settings> then <Software> and selecting the desired checkbox:




### 14.3.2 Saving the experiment

When an experiment is repeated over and over it can be saved; in this way the user does not need to enter the parameters every time. Select <Experiment> and then <Save Protocol As..>. The next time the user needs to run the experiment, the saved protocol can be loaded.

### 14.3.3 Data Files format

All of the Vinci data files are stored in ASCII format. The Experiment data (data files with the extension <ifx>) are never modified. If a data file is modified, it is stored as a new data file (data files with the extension <ifa>).

### 14.3.4 Start the data acquisition

The data acquisition is started in the <Experiment Page> by clicking on the green arrow on the top:


| | |
|---|---|
| ***To Start*** | Click on the green arrow |
| ***To Pause*** | Click on the bars |
| ***Experiment time*** | It is displayed by the bar utilizing the acquisition parameters selected by the user. The time is also displayed numerically (in seconds). |

During the acquisition, the software switches to the <Visualization Page>, although the user can switch to other pages.

# 15. Lifetime Measurements acquisition using the SPC-130 card

To perform a lifetime and anisotropy measurement with the ChronosBH, follow these steps in a sequence:

| operation |
|---|
| Turn ON ChronosBH |
| Start Vinci Experiment and Instrument Control |
| Turn on Laser |
| Turn on Detector (through the software) |
| Set proper parameters and sample intensity |
| Select an experiment |
| Data collection |
| Data analysis |
| Instrument Turn OFF |

This chapter intends to give a quick starting to the data acquisition.

## 15.1 The Acquisition Page

The page is divided in the following sections.

- Acquisition control area
- Time Domain area
- Instrument control area
- Detectors gain area
- Acquisition parameters area
- Monitor area

### 15.1.1 Acquisition Control Area

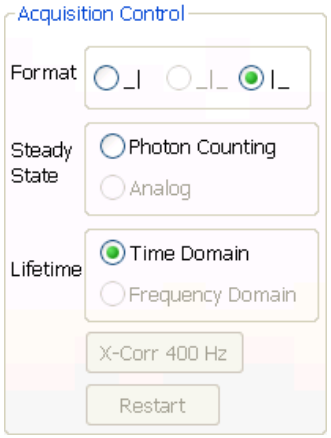
This area includes the parameters to be selected for the measurement acquisition; that is:

***Format:*** Specifies the acquisition channels:
- Left,
- Right ,or
- T-format (both channels)

***Steady-state:*** Photon Counting or Analog

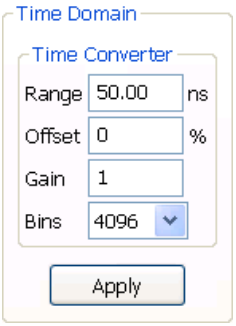
***Lifetime:*** Time Domain (default for ChronosBH) or Frequency Domain
[Vinci places the default according to the acquisition card installed in the computer].

The button <X-Corr> refers to the cross-correlation frequency utilized in the analog frequency domain acquisition.



### 15.1.2 Time Domain area

Time converter. It selects the time scale of the TAC.




***Range:***

This is the acquisition time interval of the TAC; it is the maximum time available for photon detection.

The range can be chosen between 50 ns to 5,000 ns (the default is 50 ns). The value of "Range" can be set to the signal period between laser pulses for normal measurements. If you cannot find your signal in the selected range, increase the range and use a higher gain.

The SPC-130 module works in the reverse start-stop mechanism; a signal from the detector (photon) starts the TAC and a pulse from the source (SYNC) stops the TAC.

***Offset:***

This parameter adds an offset to the TAC range value; by adding an offset, the distribution curve of the photons is shifted in the right direction.

Practically, it shifts the decay curve along the X (time) axis. Decay curves can be also shifted by the delay function of the laser controller and the length of the CFD and SYNC cables. Shorter CFD or long SYNC cables shift the decay curve left. Longer CFD or short SYNC cables shift the decay curve to the right.
The offset of the SPC-130 card is displayed in percentage.

***Gain***

The default is "1". The parameter amplifies the time scale. An example of influence of gain is shown in Table below.

***Bins:***

The number of time channels; it affects the time resolution or signal density.

The default is 4096. Its value can be 256, 1024 and 4096. It defines the time channel width inside the measurement range. For example, if the range is 50 ns and ADC resolution is 1024, each time channel is 48.8 ps.

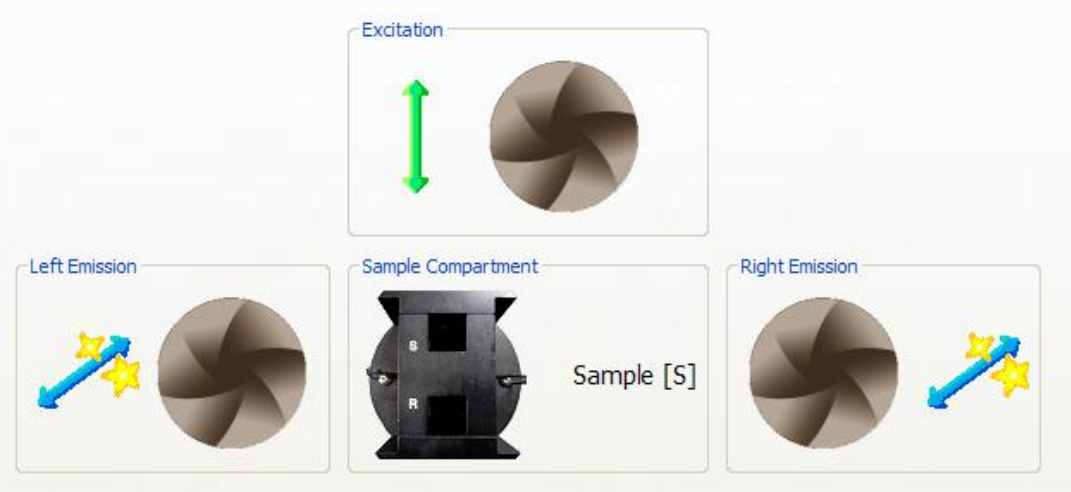
| Range | Gain | ADC Resolution | Time Axis | Time Channel | Signal Density |
|---|---|---|---|---|---|
| 50 ns | 1 | 1024 | 0-50ns | 48.8 ps | 20.5 / ns |
| 50 ns | 2 | 1024 | 0-25ns | 24.4 ps | 41 /ns |

**Table 15.1**

### 15.1.3 Instrument Control area

This area includes the control of some of the devices from the Instrument Control page. The devices accessible here are the ones that are activated to check the signal amplitude.

| | |
|---|---|
| Excitation | Shutter, polarizer, filterwheel or monochromator |
| Reference | Shutter |
| Left Emission | Shutter, polarizer, filterwheel or monochromator |
| Right Emission | Shutter, polarizer, filterwheel or monochromator |
| Sample Compartment | The type of sample compartment |




Refer to 14.1 for indications on how to move the devices.

### 15.1.4 Detectors Gain area

This area allows for the user to activate the detectors by clicking onto the <Outputs ENABLED> button and to set the gain of each detector (installed on the left and right emission channels of the instrument).
The gain of the detector is the voltage applied to the detector; it is expressed as percentage, that is 50% means that half the voltage is applied to the detector.

Once the<Outputs ENABLED> button is checked, the gain on detector A is applied by clicking on the bottom of the slider and dragging the slider up to the desired position; alternatively, one can click on the target area.

The same has to be repeated for the detector B.

If the detector goes in <overload mode> because too much current is drawn on it, the <OVERLOAD> button is colored in red and blinking. The voltage to the PMTs is turned OFF. In order to activate the PMTs again, one has to first click on <OVERLOAD>, then to <Output ENABLE> and drag the slider up.

Click onto the <Outputs ENABLED> to turn OFF the detector. Multiple clicking will toggle detectors between On and OFF.

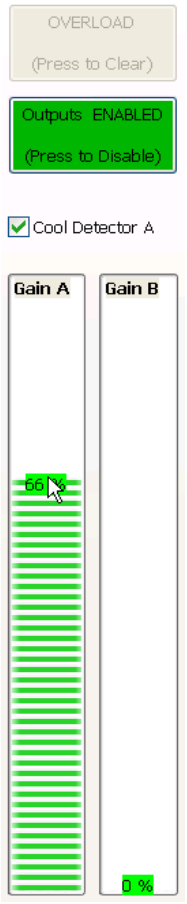



**Figure 15.1** Opening the Detector Voltage Control panel

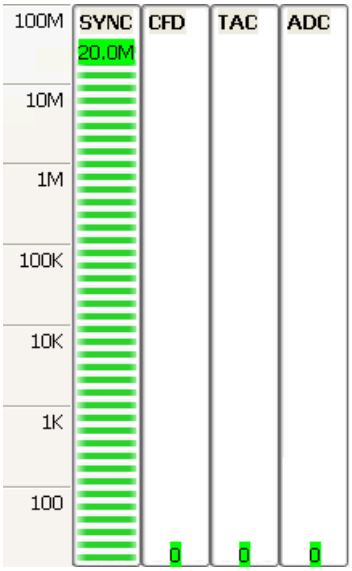
### 15.1.5 Acquisition Parameter area

***Sync:*** The repetition rate of the light source is detected and shown in this column. The symbol <M> stands for MegaHertz.

***CFD:*** The number of photons measured by the Constant Fraction Discriminator

***TAC:*** The number of photons measured by the Time-to-Amplitude Converter

***ADC:*** The number of photons measured by the Analog-to-Digital Converter

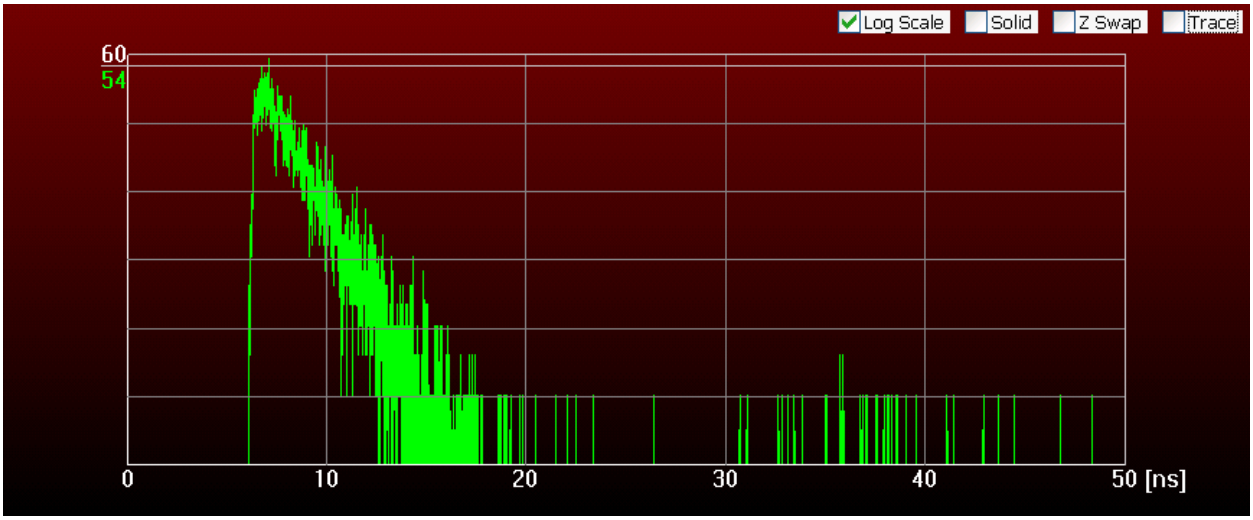



**Note:** The counts shown by the ADC are slightly less than the counts shown by the TAC, which, in turn, are less than the counts shown by the CFD.

### 15.1.6 Monitor area

It shows the histogram of the photons counted by the ADC within the set time scale. In this mode of operation, the trace is updated 10 times a second (that is, the update occurs every 100 ms); this mode of operation is very useful when setting up the experiment and checking for the signal level intensity.

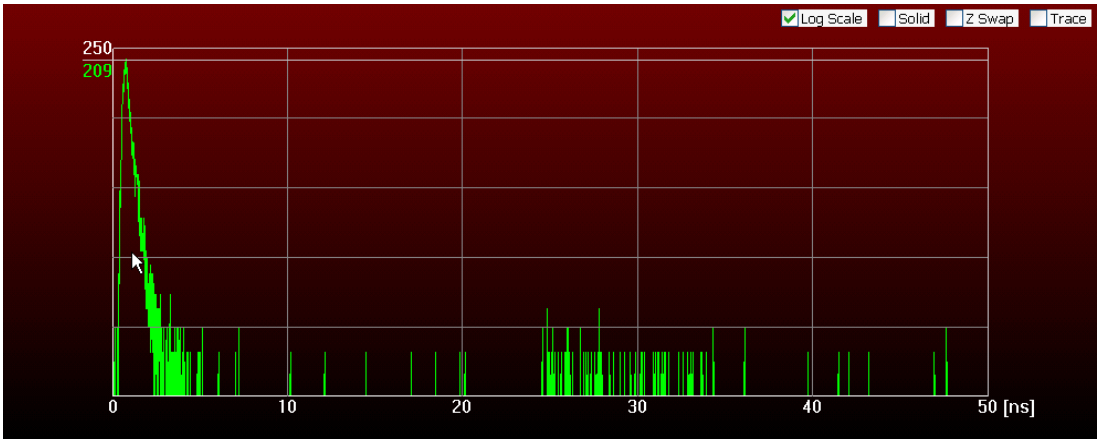
Each point in the histogram is computed from the time between the arrival of a photon and the subsequent pulse (Reverse start-stop; see Chapter 24).



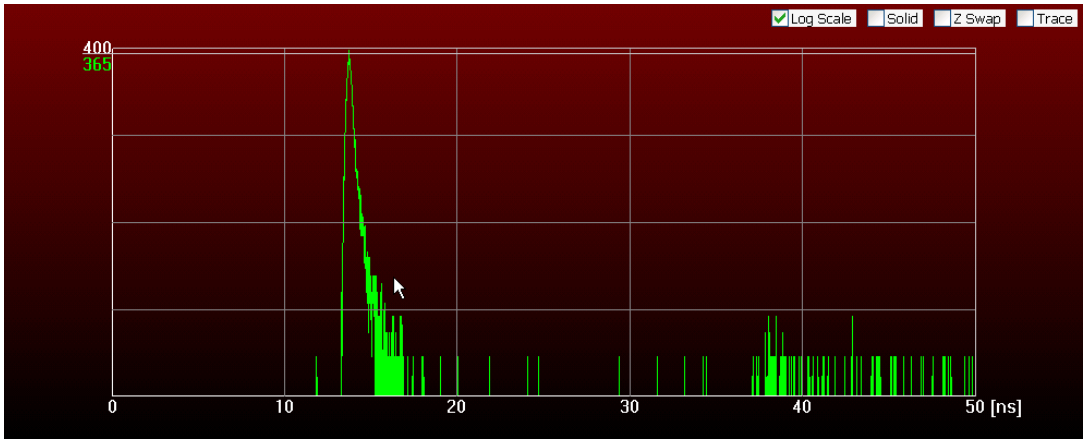
A few parameters can be changed in this area:

| | |
|---|---|
| ***Log scale*** | the scale on the y-axis can be linear or logarithmic |
| ***Solid*** | the area under the histogram is filled |
| ***Z Swap*** | when there is more than one trace (more than one channel), this field decides which channel is going to appear on top. |
| ***Trace*** | this is the oscilloscope mode, the points accumulate in time. |

If the signal has a maximum close to the left edge of the display window, position the mouse cursor on it; press and hold the left button to drag the plot to the right.

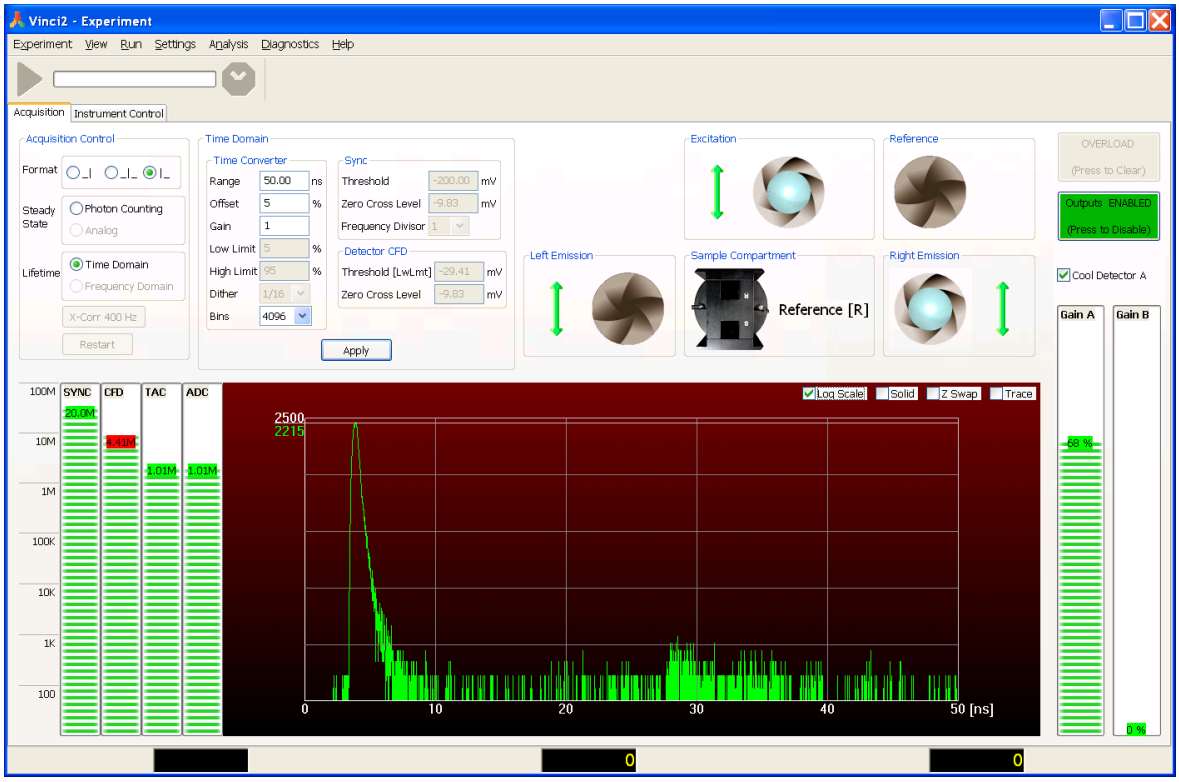



By keeping the mouse button pressed, drag the plot towards the middle of the window. This operation is equivalent to adding an Offset to the Range parameter of the TAC (see Offset in 15.1.2).



## 15.2 Checking the signal

Make sure the lid is closed on the sample compartment. Once the detector is ON, open the excitation and emission shutters by clicking on the respective icons. We suppose a reference solution is placed in the sample compartment (R- position).
A signal will be displayed in the graphical window.




If the number of counts on the CFD column is shown “in red”, lower the detector gain.

### 15.2.1 PMC-100

After the detector is turned on, some residual counts may be seen on the CFD, TAC and ADC. The Peltier controller may take several minutes to cool down the detector. The dark counts will be further reduced after the Peltier is turned on for several minutes.

### 15.2.2 Hamamatsu H7422

Check the preamplifier and fan options, enable the output and gradually increase the PMT Gain to around 80%.

### 15.2.3 R3809U MCP

For an R3809U MCP detector, turn on the high voltage power supply first and set to remote mode before loading the Detector Voltage Control panel. The Current level should be adjusted to about 0.3 on the microchannel plate (MCP) to allow enough current.

Check the Preamplifier choice and click on <Enable Outputs> to enable the Detector Controller. For the protection of MCP, gradually increase the gain to change the voltage output of the power supply. Typical working voltages of the R3809U MCP are between -2.9 and -3.1 kV.

**Note:** The voltage can be read from the front panel of the power supply. The maximum work voltage of the MCP is -3.4 KV; never work above it.

### 15.2.4 Recommended voltages of detectors

Higher gain or voltage of the detector usually yields a shorter IRF and better differential nonlinearity. By increasing the gain of the PMT from a low value, one should see an increase in intensity. At a certain point, the intensity will not further increase even when the gain is increased (this may be not very obvious) but this should be the correct operating point of the detector.

| detector model | suggested operational gain range |
|---|---|
| PMC-100 | 70~90% |
| H7422P | 70-85% |
| R10467U-50 | 75-85% (check on each tube) |
| R3809U MCP | -2.9 - -3.1 KV |

**Note: the maximum work voltage of MCP is -3.4 KV, do not work above it!**

## 15.3 Checking for radio frequency pickup or light leaks

If the counts on the CFD are high (more than a thousand) after the detector is turned ON, there may be electrical noise or light leakage. Make sure there is nothing in the lab irradiating strong signals generated by table centrifuges, neighboring freezers, elevators, building power lines. Lower the CFD low limit by 50-100 mV to reduce the noise.

To identify light leakages, turn the room lights on and off or use a flashlight and see if there is a change in the dark counts of the CFD. If there is light leakage, make sure the detector is tightly attached to the ChronosBH. If all parts are tight and there are still more than 10,000 counts/sec., the O-ring (in the adapter attached to the Chronos) may not be in the correct position or may be worn out. Turn the detector voltage off. Dim the room lights as much as possible. Check to make sure the O-ring is in good condition and in position.

# 16. Optimizing the instrument: Advanced Parameters

It is important selecting the optimum parameters for a successful experiment outcome.

## 16.1 Laser Frequency (repetition rate)

Let us suppose we have to determine a single decay time:

$$I(t) = I_0 \exp^{-t/\tau} \qquad [16.1]$$

| t | $I(t)$ |
|---|---|
| $\tau$ | 0.368 |
| $2\tau$ | 0.135 |
| $3\tau$ | 0.050 |
| $4\tau$ | 0.018 |
| $5\tau$ | 0.007 |

That is, after a time $t = 4\tau$, if the original signal is equal to 10,000 counts, we still have about 180 photons on average.

In general, for a single exponential decay time $\tau$ to be properly resolved, the delay time between two subsequent pulses should be about $4\tau$. That is the max repetition rate of the light source should be at the most:

$$frequency\ rep\ rate \approx 1/4\tau$$

The Hamamatsu Laser Controller delivers pulses from 2 Hz to 100 MHz. The controller is fairly good as it allows for the proper selection of the repetition rate over a wide range of frequencies.

The Lasos/B&H laser diodes have three possibilities, 20 MHz, 50 MHz and 80 MHz. Using 20 MHz, 12.5 ns is the max decay time that can be resolved.

The typical repetition rate of a Ti:Sapphire laser ranges from 76 MHz to 80 MHz. With 80 MHz repetition rate, the longest decay time measurable is about 3 ns.

On the other hand, let us suppose we intend to measure a decay of 10 µs. The max repetition rate of the source should be 25 KHz.

### 16.1.1 Setting the Range and the Gain

The default value (and minimum value of the range) is 50 ns. When using a high repetition rate light source, the scale may be too long. For instance, if the repetition rate is 80 MHz, the excitation pulses are spaced by 12.5 ns in time. In this case, it is useful to use the <Gain=2> or <Gain=4> in order to further reduce the range of the TAC.

Conversely, when using a light source with a 10 MHz repetition rate, the pulses are spaced by 100 ns in time. Select a value for the <Range=100>.

## 16.2 Photon Counting Acquisition Parameters for SPC-130, SPC-730, SPC-830

The general principle of the SPC-130 card is shown in the figure 16.1 below. The detector delivers the photon signal to a Constant Fraction Discriminator (Detector CFD). The Reference Signal from the light source is delivered to another Constant Fraction Discriminator (SYNC CFD).

The outputs of the detector CFD and SYNC CFD are used to start and stop the Time-to-Amplitude Converter (TAC): this technique is called the *reversed START-STOP mechanism* (see Chapter 24 for an introduction to the principles of TCSPC).

The TAC generates a signal that is proportional to the time between the arrival of the photon (Start) and the signal from SYNC CFD (Stop). The TAC signal is sent to a biased amplifier (AMP). The AMP has variable gain and offset. The AMP can selectively amplify a small window inside the whole range of the TAC signal.

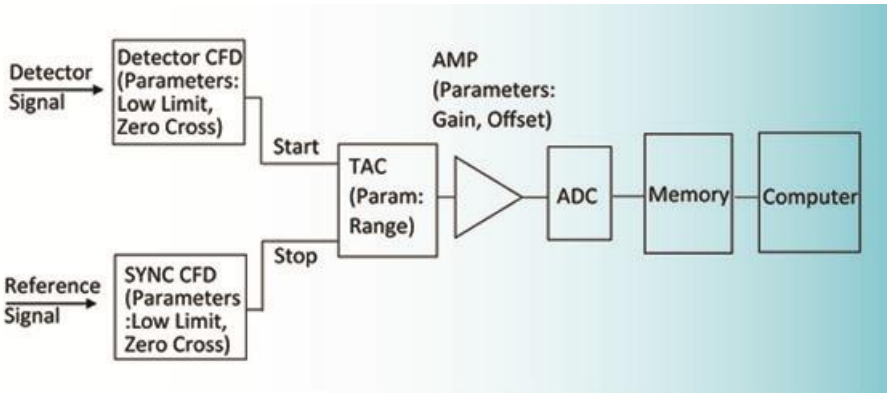
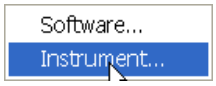



**Figure 16.1** General principle of operation of the SPC-130 card.

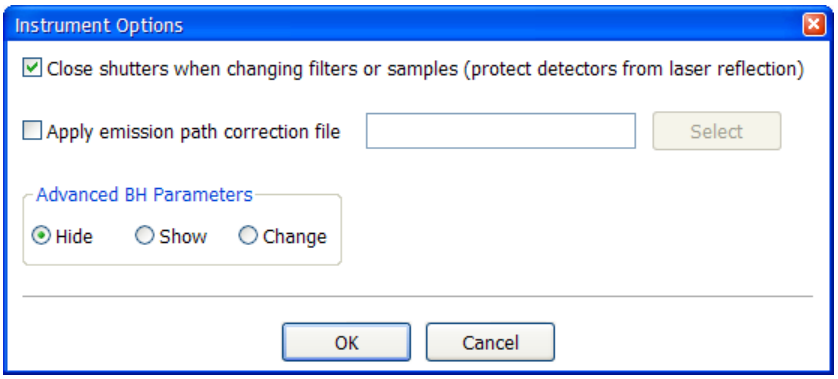
The signal from the AMP is sent to an Analog-to-Digital Converter (ADC). The output signal from the ADC is proportional to the arrival time of detected photon.
Different arrival times are sent to different memory locations (time bins), every photon will incrementally increase the content of the corresponding time bin. The accumulation of photons over the whole measurement period builds up the photon distribution over time.

For the SPC-130, the Range under TAC gain should be set as the time between pulses; this is the observation window within which the decay time is observed and recorded. For example, if the pulse rate is 20 MHz, the range is 50 ns.
Within the observation window set by the <Range>, the user can shift the decay curve along the time-axis, change resolution and amplify the time scale. The <Range>, which determines the observation window, and the position (timing) of the decay curve within the observation window are the two main parameters in a TCSPC experiment. Both can be optimized by a judicial selection of the software parameters (timing, CFD thresholds) as well as the hardware (cable length).

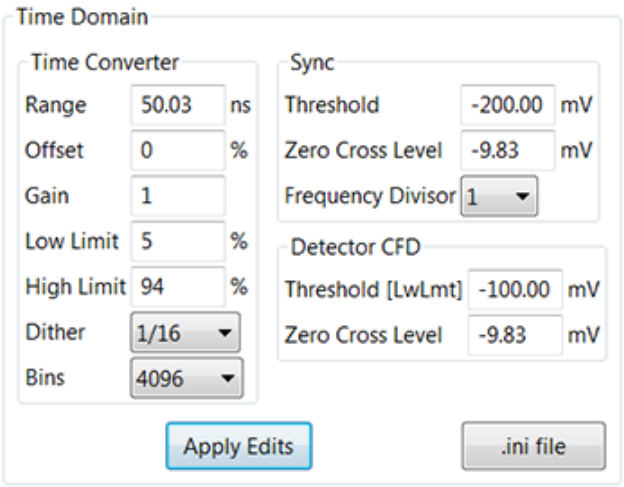
In order to access the software parameters that can be changed, select <Settings> in the Experiment Window and then click on <Instrument>:




The following window is displayed:




When selection <Show> the full list of adjustable parameters is displayed:




This following section gives a brief description of the parameters for photon counting acquisition that can be adjusted in *Vinci*. .

### 16.2.1 Time Converter

The Time Converter parameters act on the TAC and ADC components of the signal acquisition. Low/High Limits parameters act on the range of the TAC; that is, depending upon their value, if the range is set at 50 ns, data below the high limit (short time) and above the low limit (long times) are eliminated. The Dither acts on the TAC/ADC circuitry.

***Range***

This is the acquisition time interval of the TAC; it is the maximum time available for photon detection, or the observation window.

The range can be chosen between 50 ns to 5,000 ns (the default is 50 ns). The value of <Range> can be set to the signal period between laser pulses for normal measurements. If you cannot find your signal in the selected range, increase the range and use a higher gain.

The SPC-130 module works in the reverse start-stop mechanism; a signal from the detector (photon) starts the TAC and a pulse from the source (SYNC) stops the TAC.

| | |
|---|---|
| ***Offset*** | This parameter adds an offset to the TAC range value; by adding an offset, the distribution curve of the photons is shifted in the right direction.<br><br>The Offset can also be added by "drifting" the curve in the Monitor window (see 15.1.6).<br><br>Practically, the Offset removes the earliest times of the TAC; this is equivalent to delaying the starting of the TCA, or to starting the recording of the photons at a longer time. Therefore, it shifts the decay curve along the X (time) axis from left to right.<br><br>Decay curves can be also shifted by the delay function of the laser controller and the length of the CFD and SYNC cables:<br>a. Shorter CFD or long SYNC cables shift the decay curve left.<br>b. Longer CFD or short SYNC cables shift the decay curve to the right.<br><br>The offset of the SPC-130 card is displayed in percentage.<br>[see also See 15.1.2] |
| ***Gain*** | The default is "1". The parameter amplifies the time scale. An example of influence of gain is shown in Table 15.1 above.<br>[see also See 15.1.2] |
| ***Low Limit*** | Suppresses the data collection for data at the end of the range. |
| ***High Limit*** | Suppresses the data collection for data at the beginning of the range. |
| ***Dither*** | It is an error correction technique, proprietary of B&H. The parameter affects the ADC, by improving the uniformity of the channels.<br>The technique enhances the resolution of the ADC by accumulating a large number of samples with a digitally generated noise (the dither signal) added to the input signal. The digital equivalent of the dither signal is later subtracted from the ADC output. |
| ***Bins*** | The number of time channels; it affects the time resolution or signal density.<br><br>The default is 4096. Its value can be 256, 1024 and 4096. It defines the time channel width inside the measurement range. For example, if the range is 50 ns and ADC resolution is 1024, each time channel is 48.8 ps. |

### 16.2.2 Sync Parameters

The sync signal from the light source should have a well-defined amplitude with low jitter. If the synchronization signal has reflections or ringing, multiple triggering can occur. Some of the issue may be solved by the software parameters that help in securing the threshold of the sync signal.

| | |
|---|---|
| ***Threshold*** | Level of the CFD for the sync signal. Pulses with amplitude lower than the value set in this field are not seen. |

***Zero Cross Level*** — It is the level of the zero-cross trigger. Ideally this parameter is set at zero; yet, a low threshold eliminates any potential noise in the SYNC signal, such as ringing or the presence of a low reflection.

***Frequency Divisor*** — Useful to locate the signal. The <Sync Frequency Divisor> determines the number of signal periods that is recorded (Figure 16.2). Values greater than <1> are convenient to locate the signal when using high repetition rate sources.

The ChronosBH is delivered with the proper sync signal (amplitude) characteristics. The only parameter that the user may utilize is the Frequency Divisor (see also 16.5). Let us suppose that the frequency of the SYNC signal is divided by 4; that is, the SYNC signal delivers a STOP signal to the TAC every fourth signal period. The photon distribution curve is built over 4 signal periods. This is useful to locate the signal period when dealing with a large observation window of the TAC-ADC.

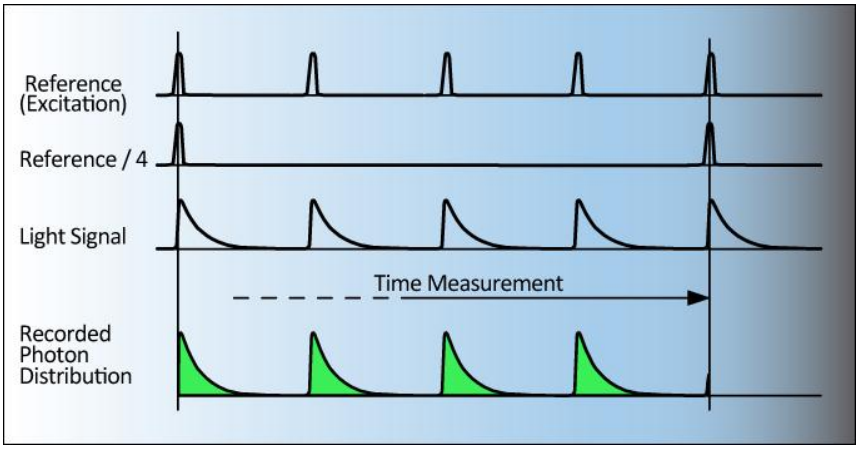



**Figure 16.2** Frequency Divisor

### 16.2.3 Detector CFD

The parameters acts onto the CFD to best discriminate and select the pulses from the light detector.

For a set gain (voltage) of the light detector, when the CFD threshold is decreased the signal as measured by the ADC increases. In fact, pulses with smaller amplitude are counted. At some level of the threshold, the signal (for good detectors) levels off; that is, it reaches a plateau. This is the optimum operational point of the detector.

The optimum CFD threshold level depends upon the detector gain. Generally, a decrease of the CFD threshold and an increase of the detector gain are largely equivalent. However, a higher gain on the detector typically produces a shorter IRF; in fact, both the TTS and SER width of the PMT decrease.

***Threshold [LwLmt]*** — is the detector discriminator threshold level. Pulses with amplitudes smaller than the “Low Limit” are not counted. “Low Limit” can be set between 0 to -500 mV. The input pulse to the CFD is negative, therefore, the lower the number, the higher the threshold. For example, -100 is a lower threshold than -50.

Not only emission photons contribute to pulse amplitudes, the dynode pulses and the electronic noise background all contributes to the pulse amplitudes. The “Low Limit” should be set such that the system will record most of regular photons and suppress most of the noise. A good start value is -50 mV.

is the level of the zero cross trigger. The value can be set between -100 to 100 mV.

***Zero Cross Level***

Theoretically, the “Zero Cross Level” should be set at zero. However, the discriminator could have some offset; therefore the “Zero Cross Level” could be set at a few tens of mV above or below zero. Zero could be a good starting value. Avoid setting “Zero Cross Level” too far away from zero. Try different Zero Cross Levels if double pulses are observed.

## 16.3 Parameters for the DSPC-230 card

In contrast to the SPC-130 card using the TAC/ADC principle, the DPC-230 is able of recording several photons per signal period. For a DPC-230 card, the photon arrival time is still measured with reference to the next arrival reference pulse. However, more than one photon can be recorded in one signal period. The photon signal can be as high as 100% of the repetition rate of the light source.

Both SPC-130 and DPC-230 can operate in TCSPC mode. The DPC-230 card features four CFD channels. Two of them are disabled in the ChronosBH. The detector delivers the photon signal to a Constant Fraction Discriminator (Detector CFD). The Reference Signal from the light source is delivered to another Constant Fraction Discriminator (SYNC CFD). To avoid the timing jitter of the pulse, the CFD triggers at a constant fraction of the pulse (Figure 24.4).

Both the CFD and SYNC signals are recorded with a digital TDC ("Time to Digital Converter") and sent to the FIFO memory and then to the computer to retrieve the photon arrival information.

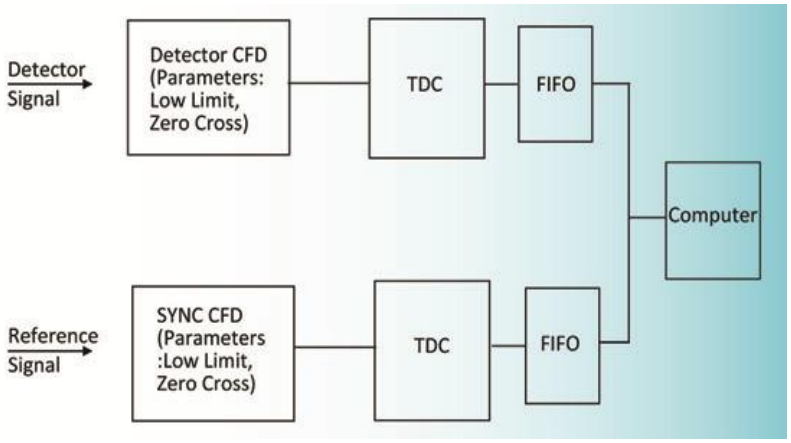



**Figure 16.3** Diagram for the SPC-230 card

### 16.3.1 Time Converter Parameters

The Time-to-Amplitude Converter (TAC) measures the time between the detection of a photon signal and the arrival of the next pulse.

***Range*** is the maximum time for photon detection. The range can be chosen between 42 ns to $10^7$ ns. The start value of "Range" is usually set to the signal period between laser pulses for standard measurements.
If you cannot find your signal in the selected range, increase the range and use a higher "**Frequency Divider**" to locate the signal. To view the whole decay curve, the range should be greater than four times the lifetime.

***Shift*** (or **"Position**" in some software versions) shifts the decay curve along the X (time) axis. The shift of the DPC-230 card is in nanoseconds. A good starting value should be set to around 110% of range, for example, if the range is 42 ns, the shift can be set to 46~50 ns.

| | |
|---|---|
| ***Bins*** | is the time resolution or signal density. The parameter has values of 256, 1024 or 4096. It defines the time channel width inside the measurement range. For example, if the range is 500 ns and ADC resolution is 1024, each time channel is 488 ps. |

For the DPC-230 card, the Range under TAC gain can be set as time between pulses. Shift (or Position) may be adjusted to find the decay curve.

When the card is used in multi-photon mode, the CFD signal can and should be much higher than 1% of repetition rate. It could be as high as close 1 Million counts.

### 16.3.2 Sync Parameters

The SYNC channel constant fraction discriminator (CFD) is triggered by the reference signal obtained from the light source. Reference signal amplitudes are usually more stable than emission signals. If the reference signal amplitudes aren't stable, "Threshold" and "Zero Cross Level" can also be adjusted.

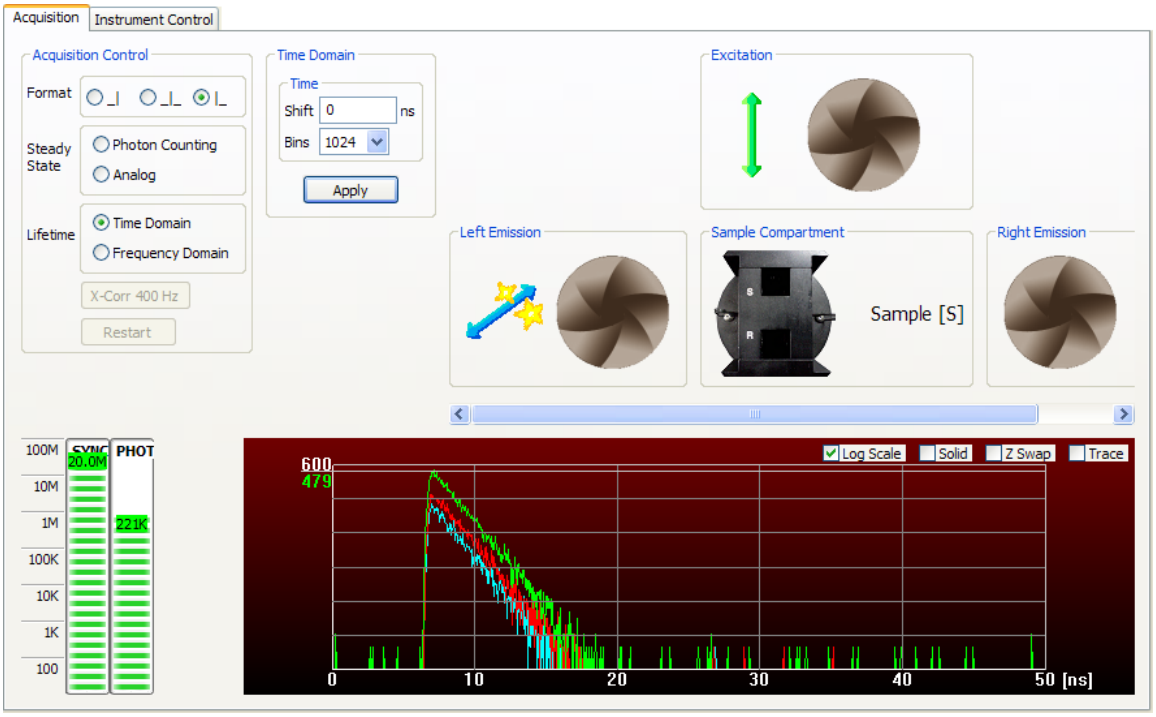
### 16.3.3 Options

| | |
|---|---|
| ***Single Photon*** | By default, the DPC-230 card will record all photons it receives. If the "Single Photon" option is checked, the DPC-230 card will only record one photon per pulse period and ignore all other photons. |
| ***Sync-Divisor*** | " sets the number of signal periods recorded. The "**SYNC Divisor Frequency Divider**" can also be used to calibrate the time scale by comparing the distance between pulses and the repetition rate. Set the "**SYNC Divisor**" to 1 for regular measurements. |
| ***Re-Sync*** | is used to automatically find the decay curve. It is easier for the software to find the decay curve of scattering or short lifetime time compounds. Also, to successfully find the decay curve, the sample or scattering solutions should have proper intensity. |

## 16.4 Parameters for the MSA-300 card

### 16.4.1 The Acquisition Page

The Acquisition Page for this card is slightly different from the page of the SPC-xxx cards.



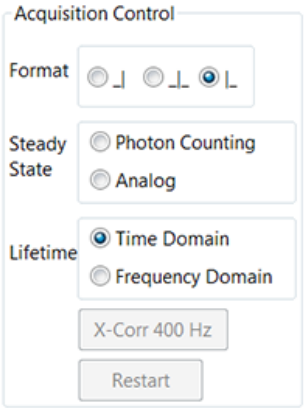

### 16.4.2 Acquisition Control Area

This area includes the parameters to be selected for the measurement acquisition; that is:

***Format:***

Specifies the acquisition channels:

- Left,
- Right ,or
- T-format (both channels)




***Steady-state:***

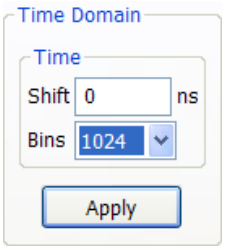
Photon Counting or Analog

***Lifetime:***

Time Domain (default for ChronosBH) or
Frequency Domain
[Vinci places the default according to the acquisition card installed in the computer].

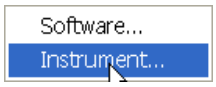
The button <X-Corr> refers to the cross-correlation frequency utilized in the analog frequency domain acquisition.

### 16.4.3 Time Domain area

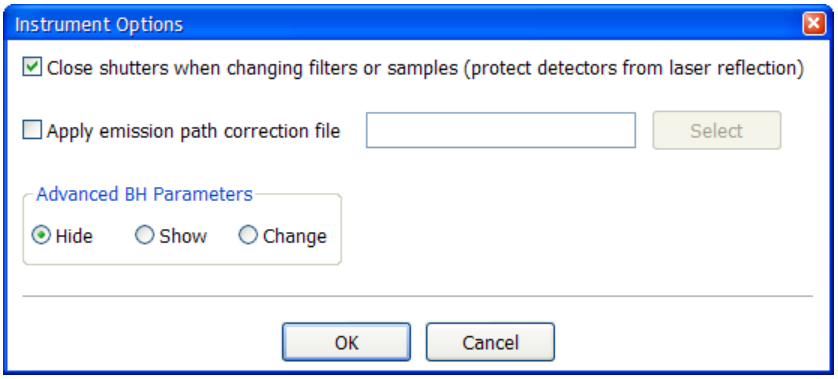



| | |
|---|---|
| ***Offset:*** | This parameter adds an offset to the range value; by adding an offset, the distribution curve of the photons is shifted in the right direction.<br>The Offset can also be added by “drifting” the curve in the Monitor window (see 15.1.6). |
| ***Bins:*** | The number of time intervals set up to acquire the signal. The parameter is either 1024 or 2048. |

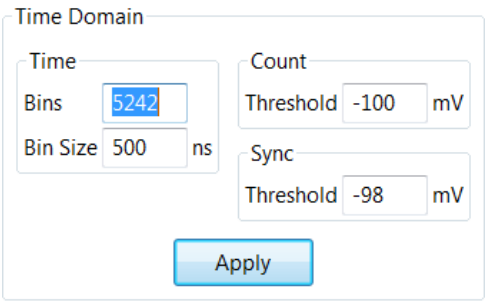
In order to access the software parameters that can be changed, select <Settings> in the Experiment Window and then click on <Instrument>:




The following window is displayed:




The <Advanced Parameters> option is selected, the parameters affecting the signal are displayed

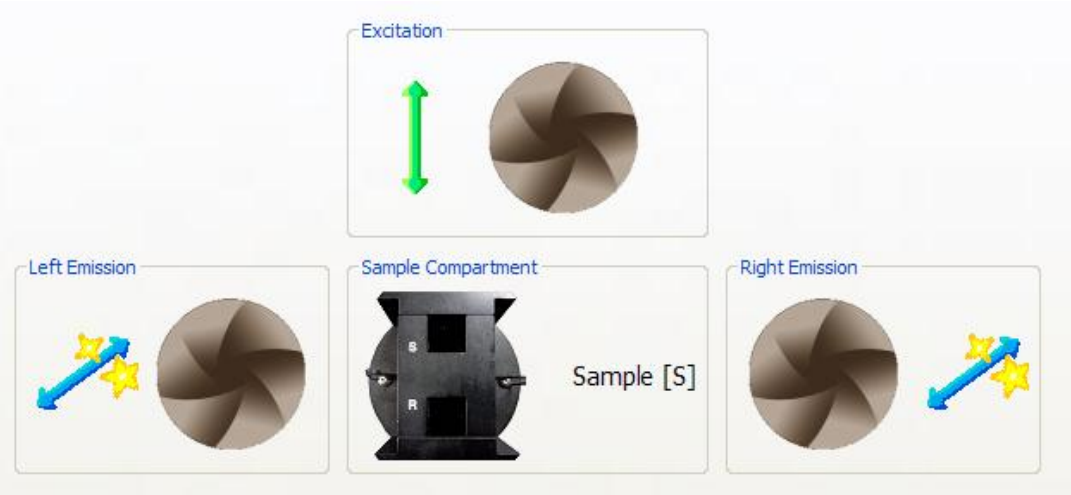



| | |
|---|---|
| ***Threshold Count*** | Value of the threshold for the pulses released from the detector (in mV). |
| ***Threshold Sync*** | Threshold for the sync signal from the source (in mV) |

### 16.4.4 Instrument Control area

This area includes the control of some of the devices from the Instrument Control page. The devices accessible here are the ones that are activated to check the signal amplitude.

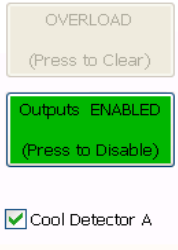
| | |
|---|---|
| Excitation | Shutter, polarizer, filterwheel or monochromator |
| Reference | Shutter |
| Left Emission | Shutter, polarizer, filterwheel or monochromator |
| Right Emission | Shutter, polarizer, filterwheel or monochromator |
| Sample Compartment | The type of sample compartment |




Refer to 14.1 for indications on how to move the devices.

### 16.4.5 Detectors Gain area

This area allows for the user to activate the detectors by clicking onto the <Outputs ENABLED> button and to set the gain of each detector (installed on the left and right emission channels of the instrument).
The gain of the detector is the voltage applied to the detector; it is expressed as percentage, that is 50% means that half the voltage is applied to the detector.




Once the<Outputs ENABLED> button is checked, the gain on detector A is applied by clicking on the bottom of the slider and dragging the slider up to the desired position (see Figure 16.4).

The same has to be repeated for the detector B.

If the detector goes in <overload mode> because too much current is drawn on it, the <OVERLOAD> button is colored in red and blinking. The voltage to the PMTs is turned OFF. In order to activate the PMTs again, one has to first click on <OVERLOAD>, then to <Output ENABLE> and drag the slider up.

Click onto the <Outputs ENABLED> to turn OFF the detector.

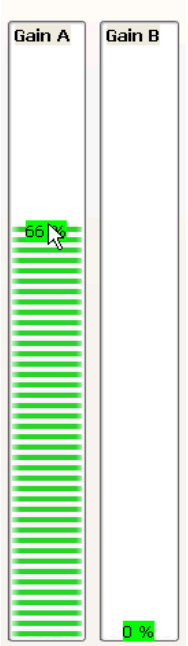
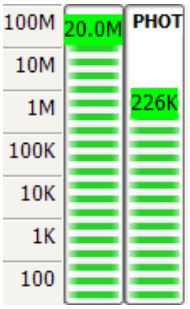



**Figure 16.4** Opening the Detector Voltage Control panel

### 16.4.6 Acquisition Parameter area

***Frequency:*** Repetition rate of the light source.

***PHOT:*** The number of pulses from the detector counted per unit time.



## 16.5 Optimization of Data Acquisition and Sync Parameters

Only a few parameters can be optimized in the ChronosBH, namely the cable length of the signal from the detector and the SYNC signal from the light source and the setting of the proper gain and threshold for the light detector.
The ChronosBH is delivered with the length of the cables from the light source to the SYNC and the detector to the CFD optimized. Yet, it may be necessary some time to modify the cables length if a different light source is utilized or if the repetition rate of the light source is reduced in order to acquire longer decay times.
This chapter explains the parameters that affect the optimization of the instrument. The optimization is achieved by observing the signal in the Monitor Area of the Acquisition Page of the Vinci software (see paragraph 15.2).

### 16.5.1 Recommended Data Acquisition Parameters

As a convenience to the user the Vinci software already sets the default value for detector and SYNC CFD. The customer usually doesn't need to change these parameters.

### 16.5.2 Optimizing the signals (TAC range and TAC gain)

Sometimes, when checking the signal in the Monitor Area, the decay curve is not displayed. This is due to a wrong range (observation window) or offset.

For an instrument with SPC-130 card, you may see that the ADC counts are much smaller than those shown in CFD and TAC, change the <gain> to 1 and increase the <range> to find the signal.
It is also possible that the delay of the synchronized laser signal may be too small: adjust the <SYNC DELAY> button on the front panel of the Hamamatsu laser controller or change the length of CFD and SYNC cables (see below).

### 16.5.3 Checking the counts/sec of the CFD

For TSCPC mode, counts can be as high as 5% of the laser repetition rate (SYNC signal). But it is strongly recommended that the counts be less than 1% of the repetition rate to avoid the "pile up" effect. If counts are too high or too low, attenuate with a neutral density filter or change the sample concentration. Adjust the intensity of the sample and reference solution to be similar.

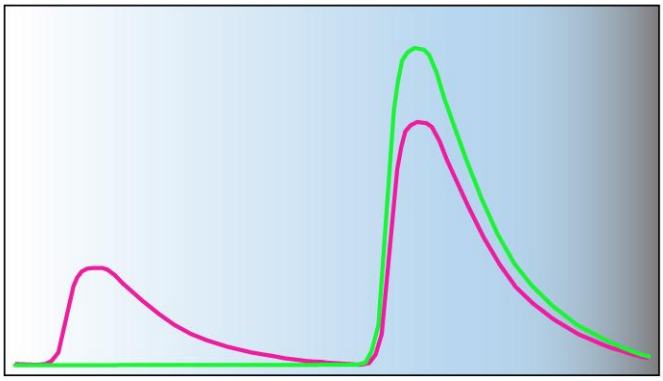


**Figure 16.5** The effect of the ringing of the SYNC signal from the light source can result in multiple triggering.

### 16.5.4 Adjusting the SYNC parameters

The sync signal from the light source has to be stable in amplitude and in phase. If the signal has reflections and/or multiple ringing, multiple triggering can result. Figure 16.6 depicts the correct signal

(green line) and the signal deriving from multiple triggering (purple line). If a similar effect is seen, increase the CDF threshold of the sync signal.

The Sync Frequency Divider determines the number of signal periods that are recorded. Values of the <Frequency Divider> larger than one are utilized to find the signal in a high repetition rate application. They can also be utilized to calibrate the time scale by comparing the displayed pulse distance with the repetition rate of the source.

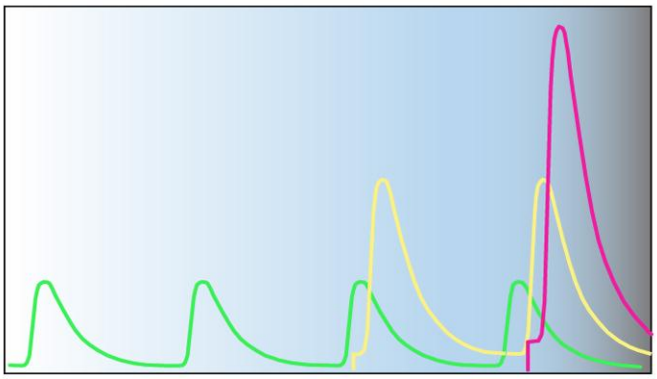


**Figure 16.6** High repetition rate signals recorded with different values of the Sync Frequency Divider, respectively, 1, 2 and 4.

However, data analysis programs, like Vinci, typically analyze one decay signal per signal period. Moreover, recording the signal over several signal periods, decreases the counting efficiency as the average dead time increases and the related time losses. In conclusion, it is recommended to set the Sync Frequency Divider value to one.

## 16.6 Cable length

In general, when optimizing the cable length, one should take into account that a 1 ns shift (the light travels about 30 cm during this time) corresponds to 20 cm of cable length.

### 16.6.1 Adjusting the SYNC and CFD cable length

When selecting the <Sync Frequency Divider = 1> and a reasonable long TAC range, the SPC module records one signal period minus a few nanoseconds required to start and stop the TAC. The location of the recording period within the signal period depends upon the optical path lengths of the light beam, the cable lengths in both the SYNC signal and CFD channels and the delays in the light detector. It may happen that the SPC does not record the correct time interval of the signal.

Figure 16.7 is a schematic of the instrument equipped with an ISS LED. We assume that the sample is a scattering solution (no fluorescence; the photon is scattered with zero time). Let us evaluate the time it takes for a photon emitted by the sample to START the TAC on the SPC-130 card. For the LED, the SYNC pulse is delivered 22 ns before the light pulse. The light pulse reaches the sample after about 1 ns (34 cm is the distance between the source and the cuvette) and it takes another 1 ns to reach the detector (distance is about 26 cm). If the detector is a model H5773, there is a delay of about 6 ns between the time the photon reaches the photocathode and the signal output at the anode. Now, the signal travels to the CFD input of the SPC-130 card; if we use a 2 m long cable, it adds another 10 ns. So far, the total of 40 ns. Because of the dead time of the SPC-130 card, an additional 10 ns are required before the card is ready to accept the STOP signal from the SYNC source. In total, a delay time of about 50 ns after the delivery of the SYNC signal has been accumulated.

The TAC is stopped by the next SINC signal. If the LED is operated with a 10 MHz repetition rate, the

SYNC pulses are separated by 100 ns. We need to have the STOP signal to arrive after the photon signal. A cable of about 10.4 m between the SYNC signal of the LED and the SYNC input on the SPC-130 card is required.

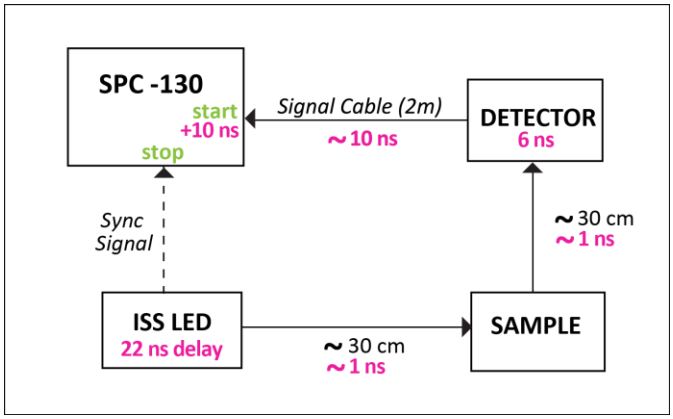



**Figure 16.7** Schematic of the instrument for a representation of the cable length required for adjusting the delay of the signals.

The general effect of changing the cables lengths is illustrated in Figure 16.8 below. The center signal (green line) is the signal as it should be recorded. If the SYNC cable is made longer, or the CFD cable is made shorter, the curve shifts to the left (yellow line). On the other hand, if a shorter SYNC signal cable is used (or a longer CFD cable), the signal shift to the right of the original curve (purple curve). Usually the cable length may require to be adjusted when the repetition rate of the light source is below 20 MHz. A practical indication that the observation window is not synchronized is a relatively high signal on the CFD and TAC and a low ADC rate.

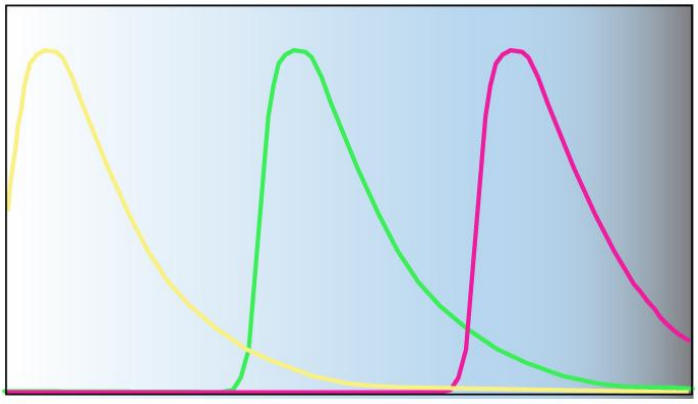


**Figure 16.8** The effect on the signal (green line) of the SYNC cable and signal (CFD) cable length. A longer SYNC cable moves the signal to the left (yellow line); a longer CFD cable moves the signal to the right (purple line)

### 16.6.2 Delay between the sync and light pulse of selected sources

In order to evaluate the cable length of the SYNC signal, it is useful to have an estimate of the delay times between the SYNC signal and the light pulse delivered by a light source. Table 16.1 below lists the delay time for several light sources.

| Light source | Delay (ns) |
|---|---|
| ISS LEDs | 22 |
| Hamamatsu laser diodes | 10 (minimum) |
| Lasos/B&H laser diodes | < 0.5 |

**Table 16.1** Delay between the SYNC signal and the light pulse of selected sources

## 16.7 Adjusting the TAC Offset

The position of the recorded signal can also be changed by changing the TAC Offset.

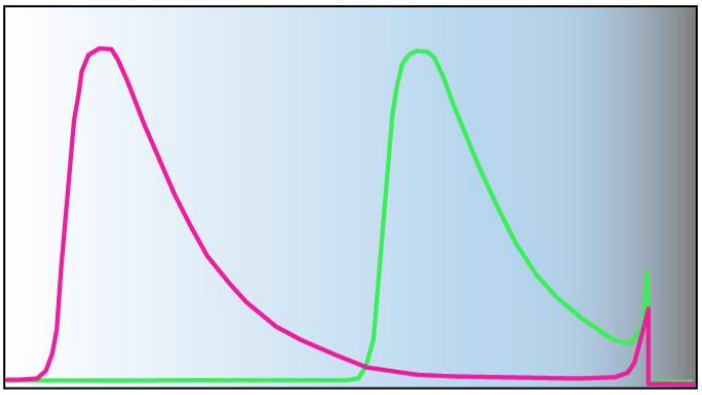


**Figure 16.9** The effect of the TAC offset too small (photons too late).

The initial stage (a few nanoseconds) the TAC is not linear; the Offset is used to bring the observation window out of the non-linearity region. If the TAC Offset is too small, the beginning of the TAC in the next signal period becomes visible (Figure 16.9). The effects appear as a sharp fall of the counts. The effect can be corrected by adjusting the cable length, in this case making it shorter in order to shift the curve right.
A similar effect occurs whenever the photons arrive too early (early photon require a long time window before the STOP signal). The recorded signal drops abruptly to the left. In this case, a longer SYNC cable will shift the curve within the proper observation window.

## 16.8 Optimization strategy of the CFD threshold for PMTs

Figure 16.10 displays the general behavior of the count rate for a PMT versus the applied threshold for different values of the gain, respectively low, medium and high. The figure also indicates the approximate region where the threshold should be set for each case.

In general, one can choose one of the following two strategies in order to find the best value of the threshold for a set gain: (a.) one approach consists in setting the PMT at a reasonable gain and optimize the threshold to find the operational region; (b.) or, select a reasonable CFD threshold and optimize the detector gain.

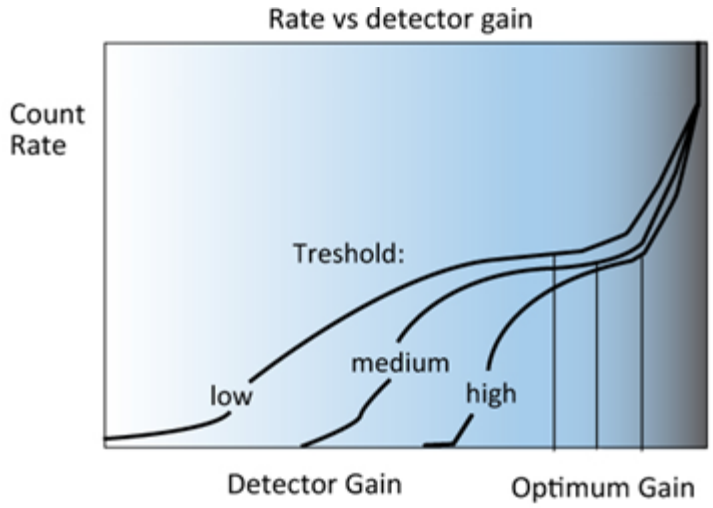



**Figure 16.10** amplitude distribution of the single-photon pulses of a PMT (left) and dependence of the count rate on the gain (right).

We recommend following the second option. In order to find a suitable combination of threshold and gain, we recommend starting with the values given in Table 16.2 below.

| detector | CFD threshold (mV) | DCC gain |
|---|---|---|
| H5773 (PMC-100) | -80 | 80-90 % |
| H7422P-40 | -100 | 70-85 % |
| HPM-100-40 | -30 | 70-90 % |
| R3809U-50 | -80 | 80-85% (-2.9 to 3.0 KV) |

**Table 16.2** Recommended values of the CFD threshold and gain for various detectors.

Figure 16.11 reports the count rate versus the gain for a specific PMT Model H7422P-40 at three different values of the threshold, -30 mV and -50 mV and -100mV, respectively. The optimal region for the gain of this PMT is between gains values of about 78 to 96 with a threshold of -50mV.

### 16.8.1 Pitfalls in the detector CFD adjustment

If the threshold of the CFD is set at values higher than the optimal value, only high amplitude pulses are detected.

High values of the CFD and low gains result in a higher background detected. The background is mainly due to afterpulses.

Too high values of the CFD and low gains can cause another undesirable effect; the recording of multiphoton events within the observation window. Typically, this effect is displayed by a unreasonably narrow IRF; the decay time of fluorophores can be reduced by a factor of two.

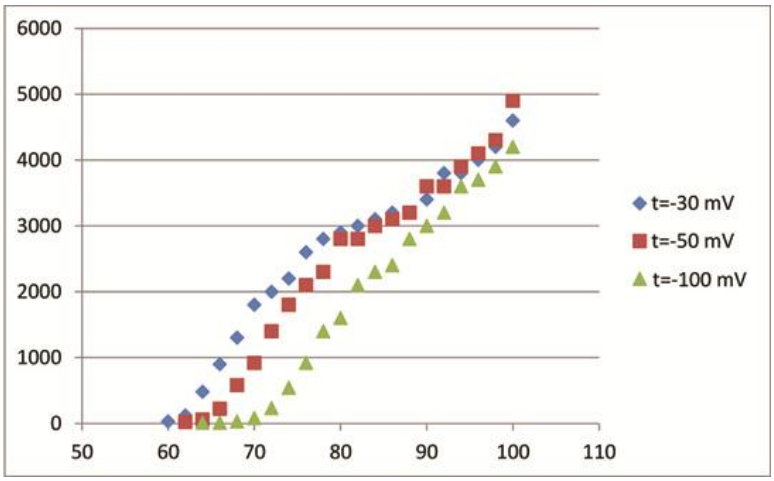
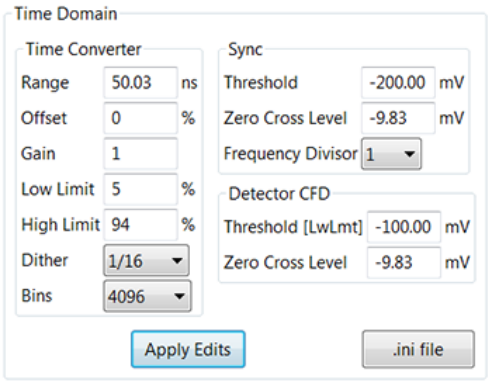



**Figure 16.11** Dependence of the count rate on the gain for the HP7422P detector for three different values of the threshold.

## 16.9 Saving the Parameters

The proper values of the parameters for the SYNC and Detector are set at the company and saved in the <.ini> file. When the user starts the software those values are displayed. If a different detector is utilized, the user can modify the <.ini> file.



# 17. Sample Preparation

## 17.1 Thin Solutions

Fluorescence acquisition theory is built on the assumption that solutions are “optically thin”. That is the optical density of a solution is below 0.1; this number depends upon the concentration of the fluorophore and its excitation coefficient. The optical density can be easily verified by using a spectrophotometer.

Solutions are placed in cuvettes. Standard cuvettes feature a 10x10 mm internal sizes (external sizes are about 12.5x12.5 mm) and fit into any standard sample compartment.
Should the number be higher than 0.1 when using a cuvette with a 10 mm path length, one could use a cuvette with a smaller path length. Cuvettes are available with different path lengths, 1 mm. 2 mm, etc.; the judicial selection of a cuvette is important for the successful outcome of the experiment.

If the required optical density of the sample cannot be reached using a standard cuvette, one should think of acquiring the signal through a front surface accessory.

## 17.2 Turbid solutions

Turbid solution are solution featuring a high optical density (OD); even when using a short optical path cuvette the light is absorbed right away by the layers of the solution close to the excitation path. In addition to having a high absorption coefficient, turbid solutions may exhibit a high scattering coefficient too. Examples of turbid solutions include blood (containing highly absorption hemoglobin and highly scattering cells).
Turbid solutions are studied using the front surface accessory. The fluorescence is collected on the same side of the excitation light. The cuvette containing the turbid solution is usually placed at an angle of 30º with respect to the excitation light.

## 17.3 Solid samples

Solid samples are not transparent to the light beam used for excitation; they scatter the light. Examples of solid samples are crystals, minerals, samples placed on a microslides.
The front surface accessory is utilized for measurements on solid samples. Please refer to the manual for the front surface accessory for proper optimization.

### 17.3.1 Powders

Powders are placed into the proper holder and covered by the glass for keeping the powder into the compartment. The holder is then placed into the cuvette holder of the sample compartment.

### 17.3.2 Microslides

Microslides have a glass substrate; they come in different sizes (20x20 mm, 10x30 mm, etc.). The sample is placed onto the microslide. They are treated as solid samples; the front surface accessory is utilized for measurements on microslides. Insert the microslide into the front surface accessory. Make sure that the area to be investigated is positioned so that the excitation beam illuminates it. Rotate the angle in order to avoid that reflections enter the acquisition channel.

## 17.4 Measuring the IRF (reference solution)

The Instrument Response Function (IRF) is the combination of the excitation light source pulse width, the response time of the light detector and the response time of any optical elements (monochromator, etc.) that may introduce delays to the light beam arriving onto the detector. As an example, when using a light source with a pulse width of the order of 1 ns and a light detector with a TTS (see section 6.5) of the order of 200 ps, the overall IRF is still of the order of 1 ns.

Note that, when using a cuvette with a scattering solution, the light beam is further delayed. In fact, the light travels through a 10 mm solution with a different refraction index and this translates into an additional delay of about 15-20 ps.
The measured IRF is convoluted with the theoretical model describing the fluorescence decay; the result is compared – by using a minimization technique – to the acquired data.

### 17.4.1 Using a scattering solution

Typically, the reference solution is a solution of glycogen (from oyster) in water (see for instance Sigma-Aldrich) at a concentration of about 0.1 mg/ml. Glycogen does not absorb light in the UV/visible spectrum and works as a suitable scatterer. Alternatively, particles of Titanium oxide in water can be utilized. Also Ludox in water (1 small drop to 3 ml water) can be utilized. When in hurry and these compounds are not available, coffee creamer powders works as an excellent scatterer. When preparing the solution, one has to make sure that the optical density (OD) be below 0.2; again, when in doubt, use a spectrophotometer for checking that the requirement is met.

When using a scattering solution as a reference, light at the excitation wavelength is acquired. Scattered light does not have the spatial distribution of the fluorescence light; this can introduce some systematic errors in the measurements as the PMT sees two different spatial distributions of light.
Additionally, the fluorescence is acquired, typically, at wavelengths longer than the excitation wavelength. Some photomultiplier tubes (PMT) may exhibit the *color effect*; that is, their time response is wavelength dependent. This effect is larger for front end PMTs than side-on PMTs. In modern compact PMTs, such as the H5775 or the H7422 series the color effect is much less pronounced.

### 17.4.2 Using a fluorophore

The use of a fluorophore as a reference eliminates the color effect limitation of the light detector and eliminates the differences due to the spatial distribution of light:

1) The sample and reference will emit at similar wavelengths and color effects can be avoided;
2) Moreover, the same filter is used for sample and reference and light paths are the same for sample and reference.

The fluorophore solution has to exhibit a single decay time. Nowadays, tables of fluorophores solutions suitable to be used as a reference are readily available for different wavelength ranges and different decay times ranges (see a compilation at http://www.iss.com/resources/index.html). In the Vinci software acquisition menu, just enter the value of the decay time for the specific fluorophore utilized; the measurement will proceed accordingly.

**Note:** Vinci only works when the lifetime of the reference standard is shorter (by a least a factor of 3) than the sample.

## 17.5 Filters selection

Filters are a very important component of any fluorescence measurement. Filters are used in order to select the wavelength range of the fluorescence measured; they are used to prevent the excitation light from joining the fluorescence light and entering the light detector; they are very important in many instances to purge the excitation light from unwanted components.

Choose proper filters for your sample and for your reference solution. When exciting the sample, a small fraction of the excitation light is scattered and diverted into the detector along with the fluorescence light; a filter will eliminate this component, which otherwise will finish up altering the results. For example, if the sample is Fluorescein, and the reference is a glycogen solution and the excitation wavelength is 470 nm, a 505 or 520 Long Pass (LP) filter can be used to eliminate the scattered light from the sample. Please note that a 520 LP filter passes 50% of the light at the wavelength of 520nm; that is, light at shorter wavelengths passes as well. The filter should be chosen judiciously.

A proper bandpass filter should be used if the sample contains impurities that emit at different wavelengths or to just isolate an emission wavelength range.

No filter is needed for the glycogen solution (scatterer); in this case, the intensity reaching the detector may need to be adjusted (ideally, its value should be close to the fluorescence from the sample). Neutral density filters can be used to attenuate the light intensity of sample or reference.
When a short lifetime fluorophore with matching emission spectrum properties is used as a reference, the filter can be placed in the emission path filter compartment since both the sample and the reference use the sample filter. It's better to use absorptive neutral density filters since reflective neutral density filters may generate extra reflective peaks.

One also has to make absolutely sure that no scattered excitation light can pass through the emission filters selected. An easy way to test the appropriate choice of emission filter is by using a fused silica cuvette with a dilute glycogen solution. With excitation and emission shutters closed a dark signal will be observed. Open both shutters. The detector readings should not change, when the chosen emission filter indeed blocks all scattered excitation light and no phosphorescence signal is created by the absorbed excitation light.

### 17.5.1 Neutral density filters

Neutral density filters are used in the excitation channel to regulate the intensity of the excitation light source.

## 17.6 Using the polarizers

### 17.6.1 Excitation channel

For lifetime measurements, it is convenient to use vertically polarized excitation light. Just slide the excitation channel polarizer in position. The excitation polarizer should be used for both laser diodes and light emitting diodes (LEDs).
When measuring anisotropy, a wave plate can be placed after the polarizer in order the plane of polarization of the excitation light and enable the measurement of the g-factor of the acquisition channel.

### 17.6.2 Emission channel

It is recommended placing polarizers in the light path in order to correct for the depolarization effects. In the emission channel, the polarizer is to be set at 54.7º (magic angle).

# 18. Time-resolved Fluorescence Measurements

## 18.1 Checking the signals on the display

Prepare two solutions, one for the sample (S) and on for the reference (R). Place your sample and reference in the sample compartment cuvette positions labeled S and R.

Once the emission polarizer has been set to the magic angle, open the excitation and emission shutters from the instrument control panel. Switch to the acquisition panel. In the Monitor Area, the real-time decay curve should be similar to the one displayed in Figure 18.1 below.

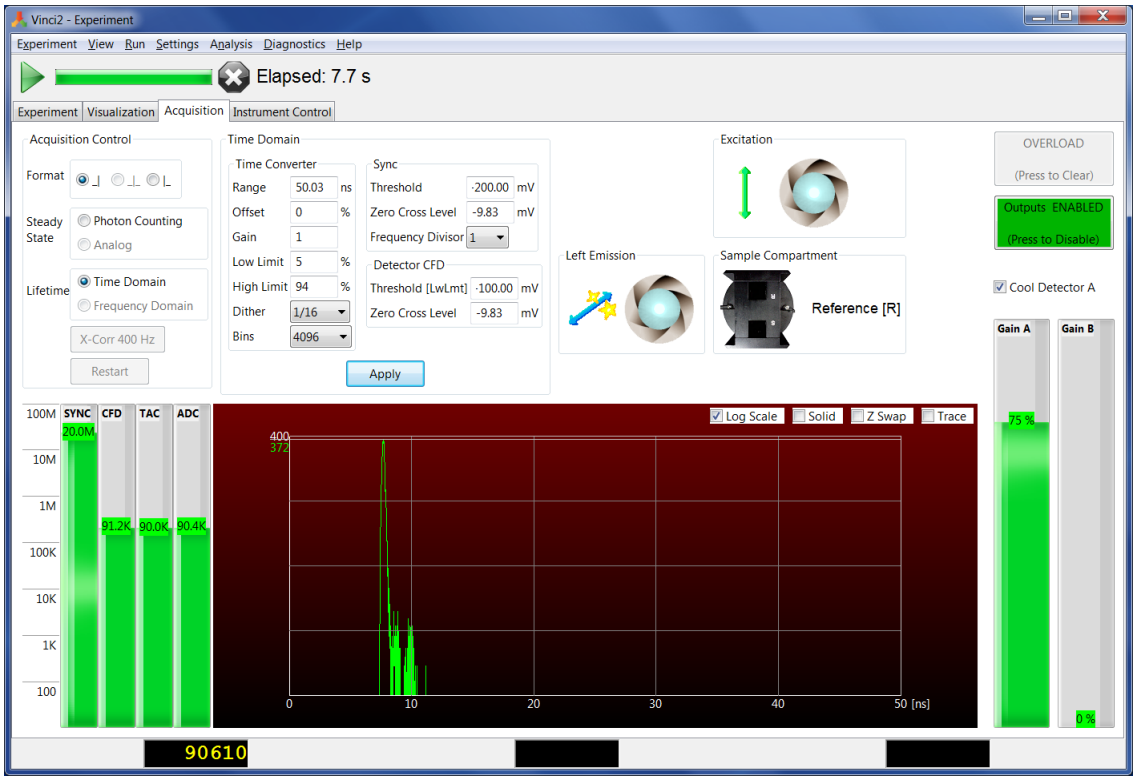
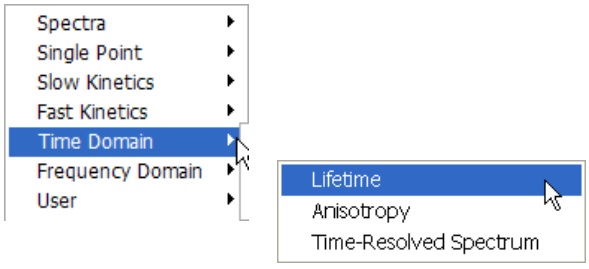



**Figure 18.1** Real time display of the decay curve

## 18.2 Lifetime Measurements

Select <Experiment> and then <Time Domain> and <Lifetime> to bring up lifetime experiment menu.




The lifetime experiment window will be displayed as in Figure 18.2 below.

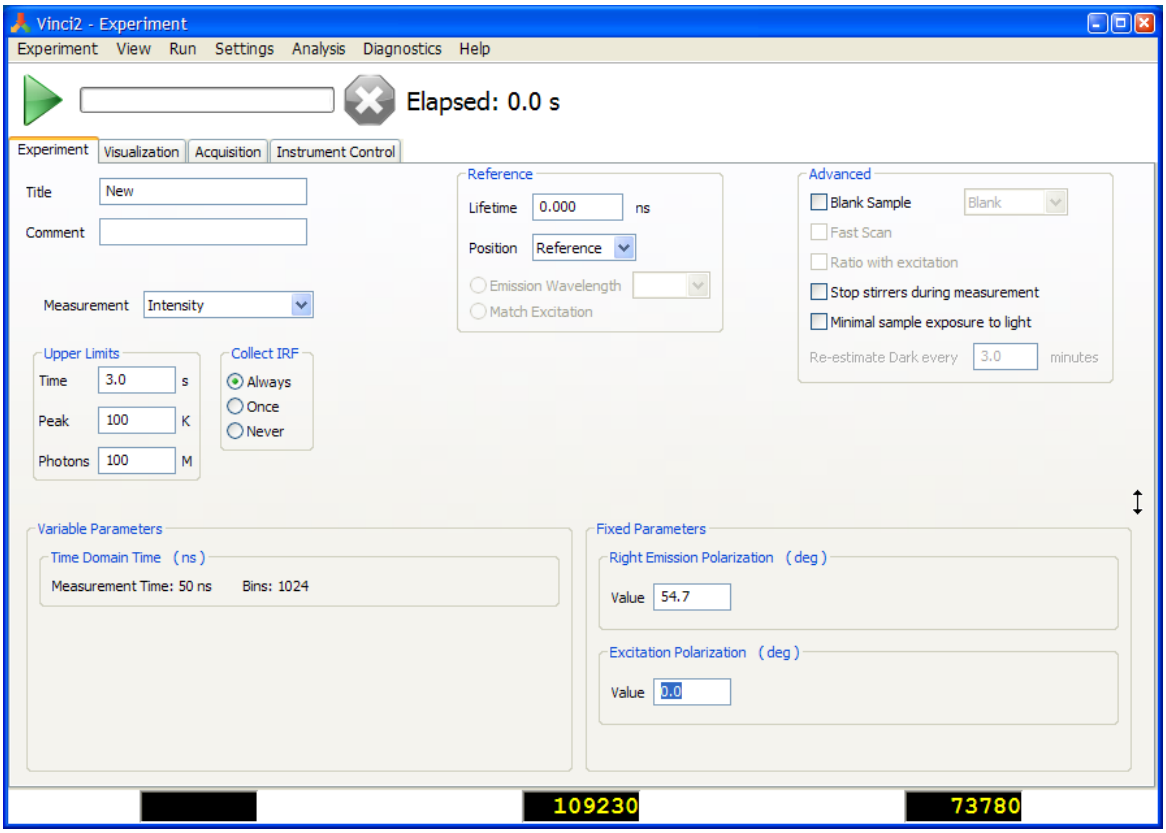



**Figure 18.2** Lifetime Experiment panel

The experiment acquisition window is separated in several functional areas:

- Sample Identification area
- Measurement area
- Reference Solution area
- Advanced Parameters area

### 18.2.1 Sample Identification Area

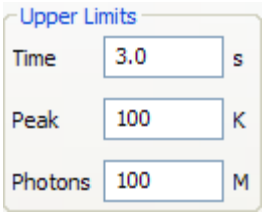
This area includes the parameters to be selected for the measurement acquisition; that is:

***Title:*** Enter an alphanumeric title

***Comment:*** Enter additional identification comments

### 18.2.2 Measurement Area

This area includes the parameters to be selected for the measurement acquisition; that is:




***Upper Limits:***

- Time (s, seconds)
- Peak (K, thousands)
- Photons (M, millions)


***Collect IRF***

Select one of the buttons

- Always
- Once
- Never

***Variable Parameters***

The parameters of the acquisition set in the <Acquisition> page. In the example, we have 50ns for the measurement time (Gain = 1) and 4096 bins

***Fixed Parameters***

Experimental parameters.

In the example:
the Right Emission Polarizer is set at 54.7 degrees
the Excitation Polarizer is set at 0 degrees

### 18.2.3 Reference Solution Area

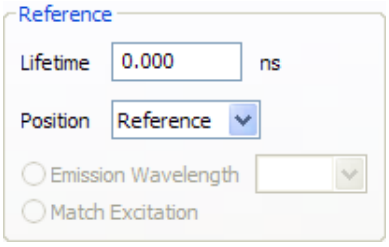
This area includes the parameters for the reference solution:

***Lifetime:***

Specifies the value of the decay time for the reference in nanoseconds

- When using a scatterer, enter <0>
- When using a fluorophore of known decay time, enter the value

***Position:***




Enter the position in the cuvette holder for the reference solution.
In the example, we assume using a 2-cuvette holder and that solution is placed in <Reference>

***Emission wavelength***

If the signal is collected through a monochromator, enter the wavelength position where the reference signal is acquired at (in nanometers)

***Match excitation***

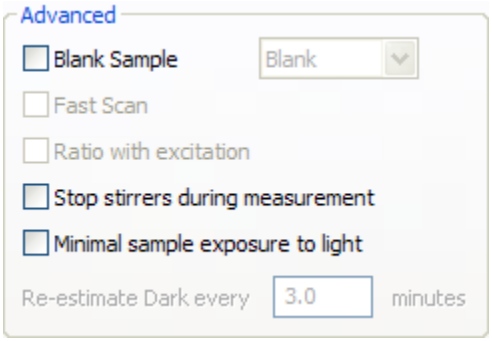
Moves the monochromator to the position of the excitation wavelength. This is useful when acquiring an IRF through the monochromator and using a scatterer as reference.

### 18.2.4 Advanced Parameters Area

This area includes additional control parameters for the measurement. Note that the fields in gray do not apply to the ChronosBH; they only act for the ChronosFD acquisition.

***Blank Sample:***

When acquiring measurements on a fluorophore diluted in a buffer that features fluorescence, it may be convenient to measure the buffer (blank) as well and subtract the results.
The measurement can be automatically acquired if one uses a 3- or 4-cuvette holder.




***Stop Stirrers during measurement:***

The stirrers stop functioning during the acquisition

***Minimal sample exposure to light:***

The shutters are automatically closed when the acquisition is not operating in order to minimize any photobleaching effects.

Start the experiment by clicking the green arrow button.

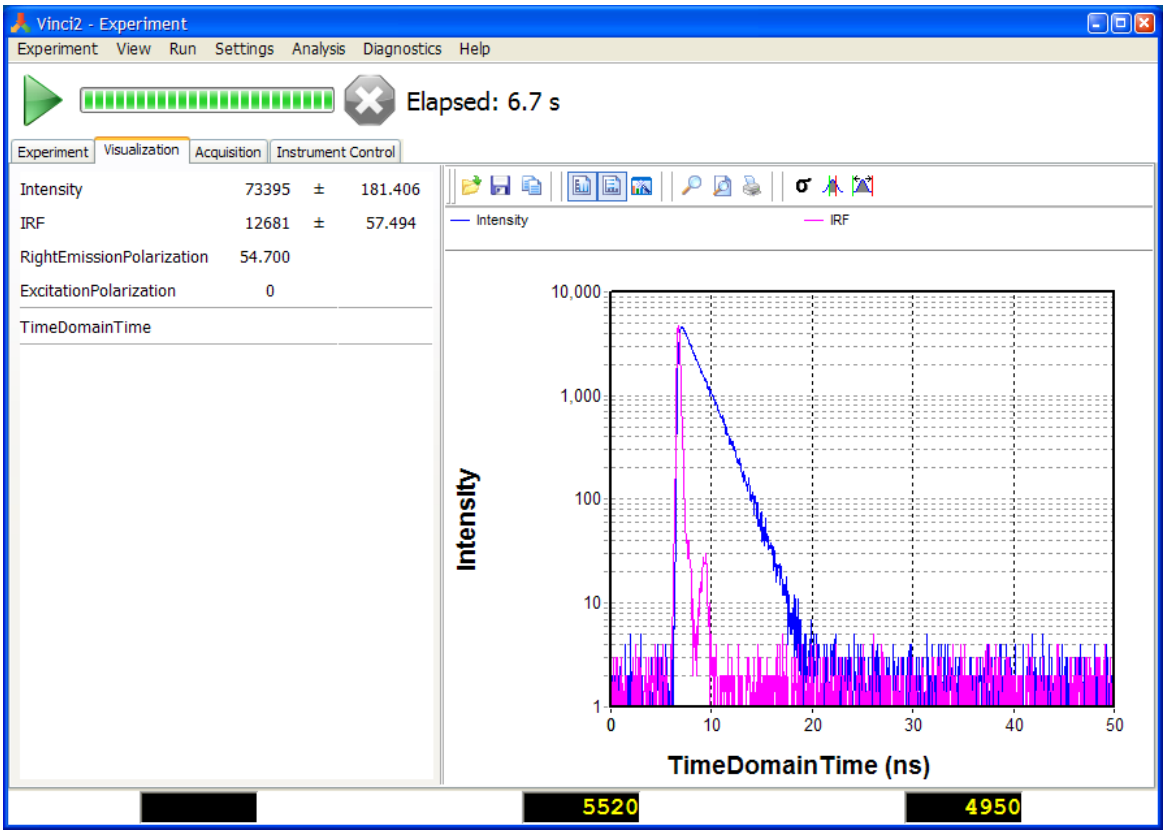



**Figure 18.3** Lifetime acquisition

## 18.3 Anisotropy Decay Measurements

In anisotropy decay measurements, the polarizers are inserted in the optical path both in the excitation and emission channel. Vinci will first measure the sample at vertical-vertical and vertical-horizontal polarizer positions, then measure a scattering solution at the magic angle polarizer position.

In order to perform anisotropy decay measurements place your sample and scattering solution in the sample compartment. Place proper long-pass (LP) or band-pass (BP) emission filters in the emission filter compartment. Check the sample intensity at vertical-vertical polarizer positions and make sure the sample still has a reasonable intensity at vertical-horizontal position. Finally check the reference intensity at the magic angle position.

In case a vertically polarized laser excitation light source is used, a suitable quarter wave plate can be inserted into the excitation path filter holder. This will create a sufficient signal level for the measurement of the G-factor, $G = \frac{I_{HV}}{I_{HH}}$ at the cost of a factor of two in excitation intensity at the sample position.

The G-factor could be measured in advance and entered during the data acquisition.

Select <Experiment> and then <Time Domain> and <Lifetime> to bring up lifetime experiment menu.

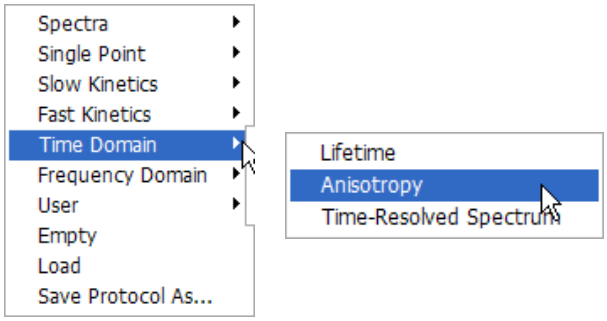



**Figure 18.4** Select <Anisotropy> in “Experiment” menu

Figure 18.5 displays the Experiment Acquisition window.

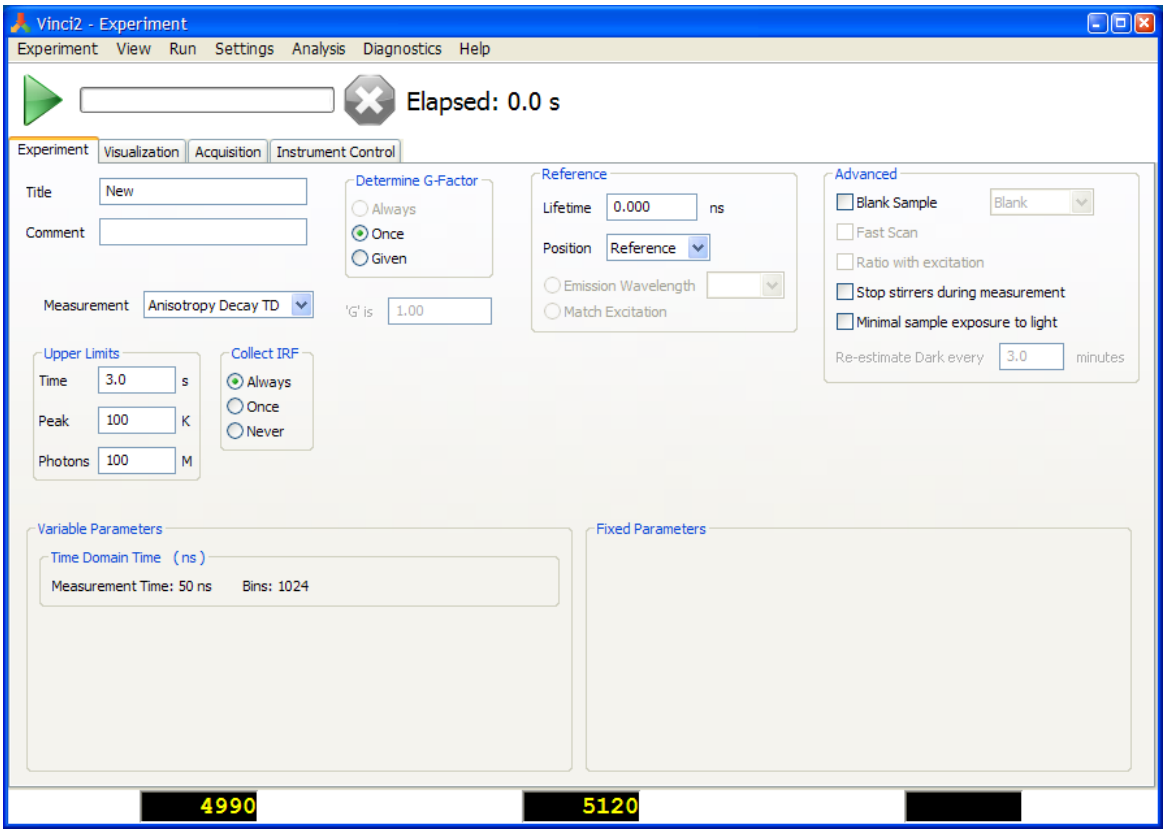



**Figure 18.5** Anisotropy decay experiment window

The experiment acquisition window is separated in several functional areas:

- Sample Identification area
- Measurement area
- G-Factor area
- Reference Solution area
- Advanced Parameters area

### 18.3.1 Sample Identification Area

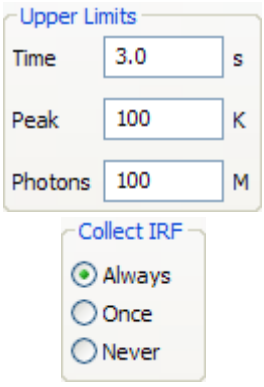
This area includes the parameters to be selected for the measurement acquisition; that is:

***Title:*** Enter an alphanumeric title

***Comment:*** Enter additional identification comments

### 18.3.2 Measurement Area

This area includes the parameters to be selected for the measurement acquisition; that is:




***Upper Limits:***

- Time (s, seconds)
- Peak (K, thousands)
- Photons (M, millions)

***Collect IRF***

Select one of the buttons
- Always
- Once
- Never

***Variable Parameters***

The parameters of the acquisition set in the <Acquisition Page>. In the example, we have 50ns for the measurement time (Gain = 1) and 4096 bins

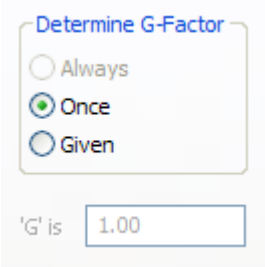
***Fixed Parameters***

Experimental parameters.

In the example:
the Right Emission Polarizer is set at 54.7 degrees
the Excitation Polarizer is set at 0 degrees

### 18.3.3 G-Factor Area

This area includes the parameters for the reference solution:

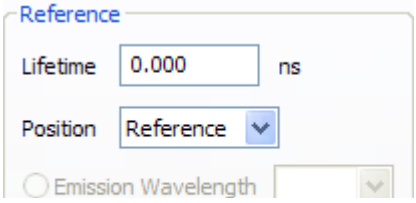



***Determine G-Factor:***

Specifies the
- Always
- Once
- Given

### 18.3.4 Reference Solution Area

This area includes the parameters for the reference solution:




***Lifetime:***

Specifies the value of the decay time for the reference in nanoseconds
- When using a scatterer, enter <0>
- When using a fluorophore of known decay time, enter the value

***Position:***

Enter the position in the cuvette holder for the reference solution.
In the example, we assume using a 2-cuvette holder and the is placed in <Reference>

***Emission wavelength***

If the signal is collected through a monochromator, enter the wavelength position where the reference signal is acquired at (in nanometers)

***Match excitation***

Moves the monochromator to the position of the excitation wavelength. Useful when using a scatterer as reference.

### 18.3.5 Advanced Parameters Area

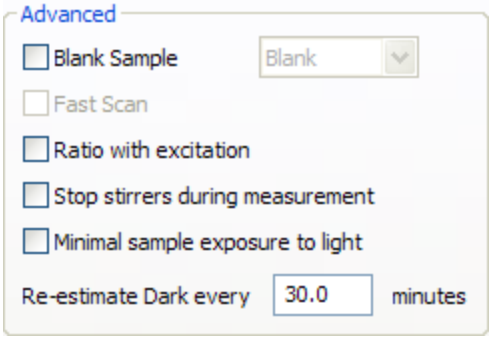
This area includes additional control parameters for the measurement. Note that the fields in gray do not apply to the ChronosBH; they only act for the ChronosFD acquisition.

***Stop Stirrers during measurement:***

The stirrers stop functioning during the acquisition

***Minimal sample exposure to light:***

The shutters are automatically closed when the acquisition is not operating in order to minimize any photobleaching effects.




After you have chosen proper signal levels for sample and reference, go to the experiment panel. Set a proper collection time and start the experiment by pressing the green arrow button. Vinci will collect the decay curves of the sample and reference automatically if you have a two cuvette sample compartment. If you have a one-cuvette sample compartment, Vinci will prompt you to change sample and emission filter after the data collection for the sample is completed. After the measurement is finished the measured intensity decay will be displayed in <Vinci Analysis>.
Start the experiment by clicking the green arrow button. Once the acquisition is completed, data are stored in the data file specified.

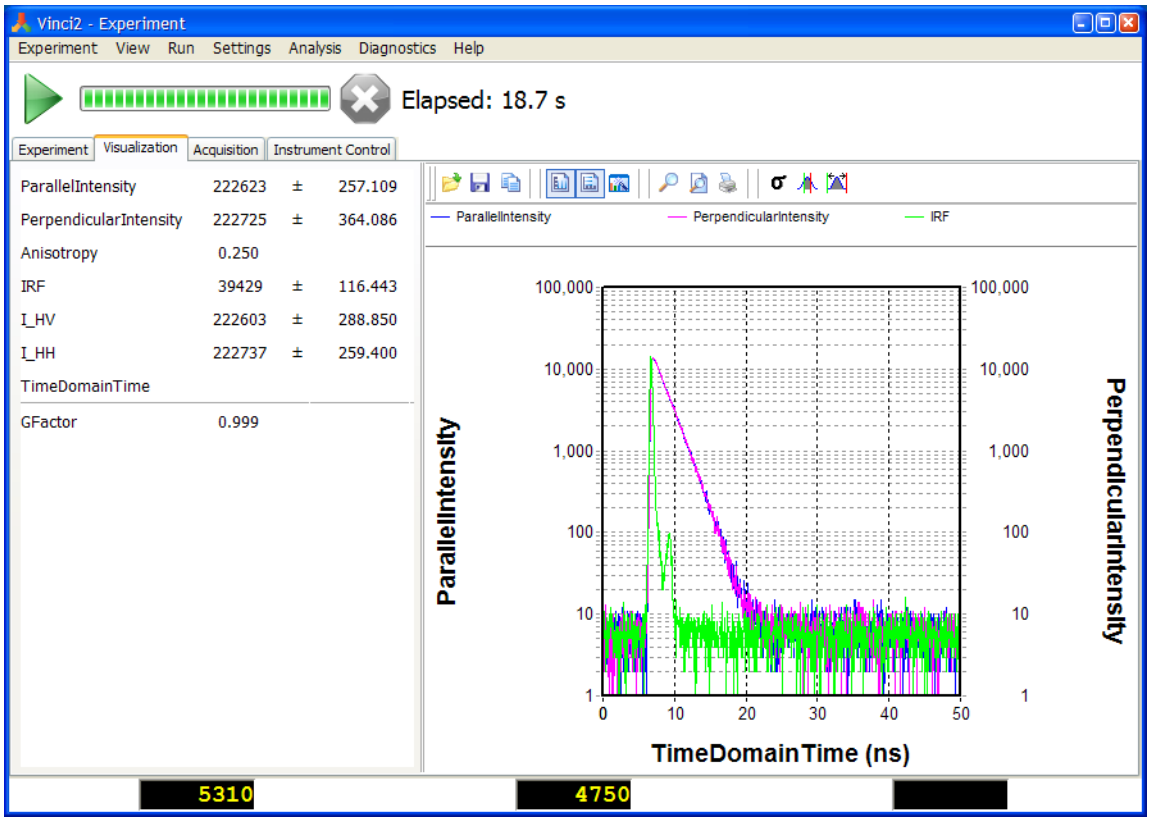



**Figure 18.6** Anisotropy Decay data

## 18.4 Time-resolved Spectra

The time-resolved spectra acquisition routine allows for the recording of decay curves at set wavelengths intervals within a set range. A monochromator is required in the light path of the fluorescence. Alternatively, the measurements can be acquired by using the filterwheel loaded with bandpass filters; in this case the wavelengths are determined by the filters used. In either way, make sure that the Instrument Configuration includes the monochromator or the filterwheel. In the following examples, we will assume that a monochromator is installed in the right emission path of the instrument (see Figure 3.3).

### 18.4.1 The Acquisition page

The Acquisition window when the monochromator is added to the right emission channel looks like the following.

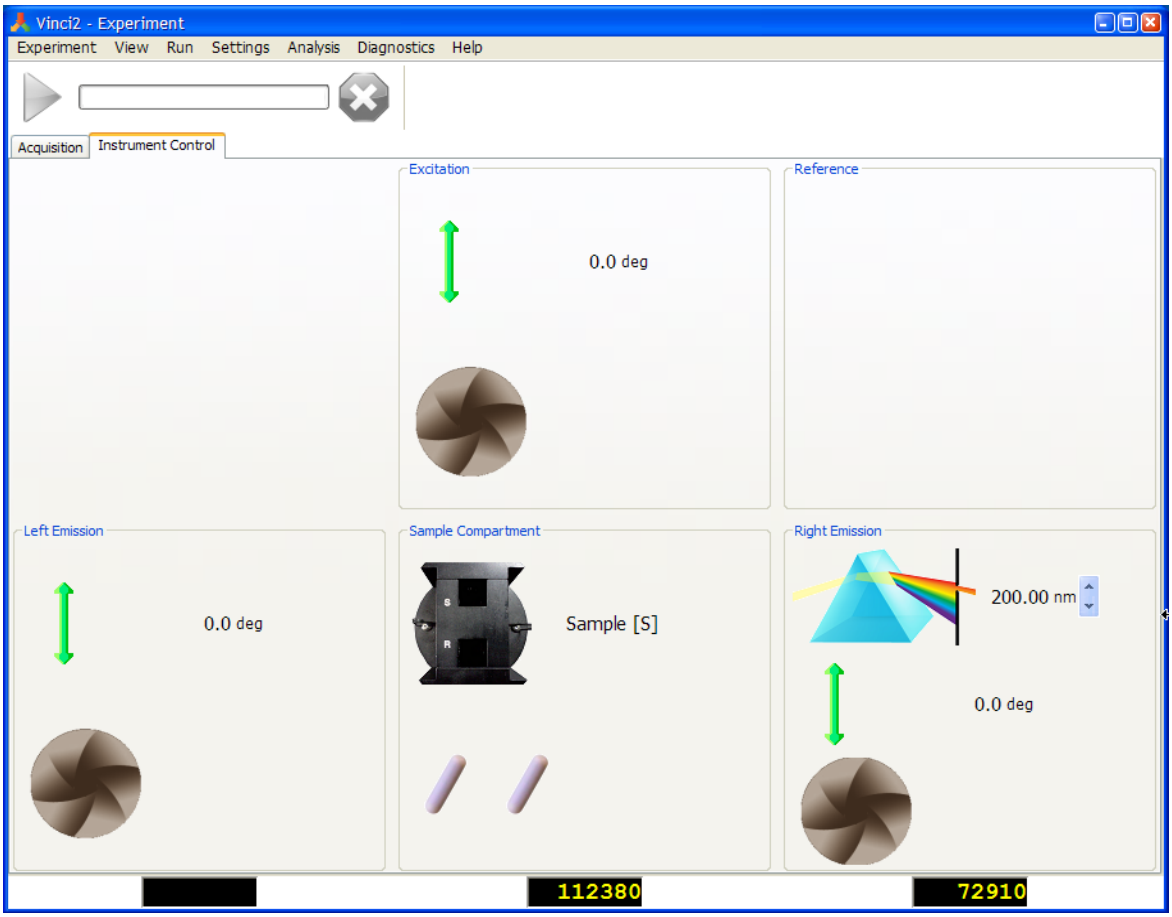



**Figure 18.7** Acquisition page window with control of the monochromator on the right emission channel.

### 18.4.2 Experiment page

In order to perform time-resolved spectra measurements place your sample and scattering solution in the sample compartment. Check the sample intensity at vertical-vertical polarizer positions and make sure the sample still has a reasonable intensity at vertical-horizontal position. Finally check the reference intensity at the magic angle position.

Select <Experiment> and then <Time Domain> and <Time-Resolved Spectrum> to bring up the experiment menu.

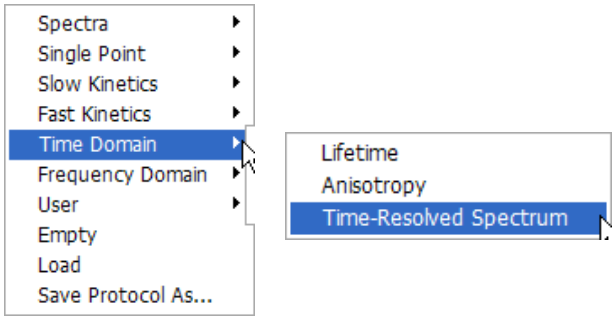



**Figure 18.8** Select <Anisotropy> in “Experiment” menu

The Experiment Acquisition window is displayed:

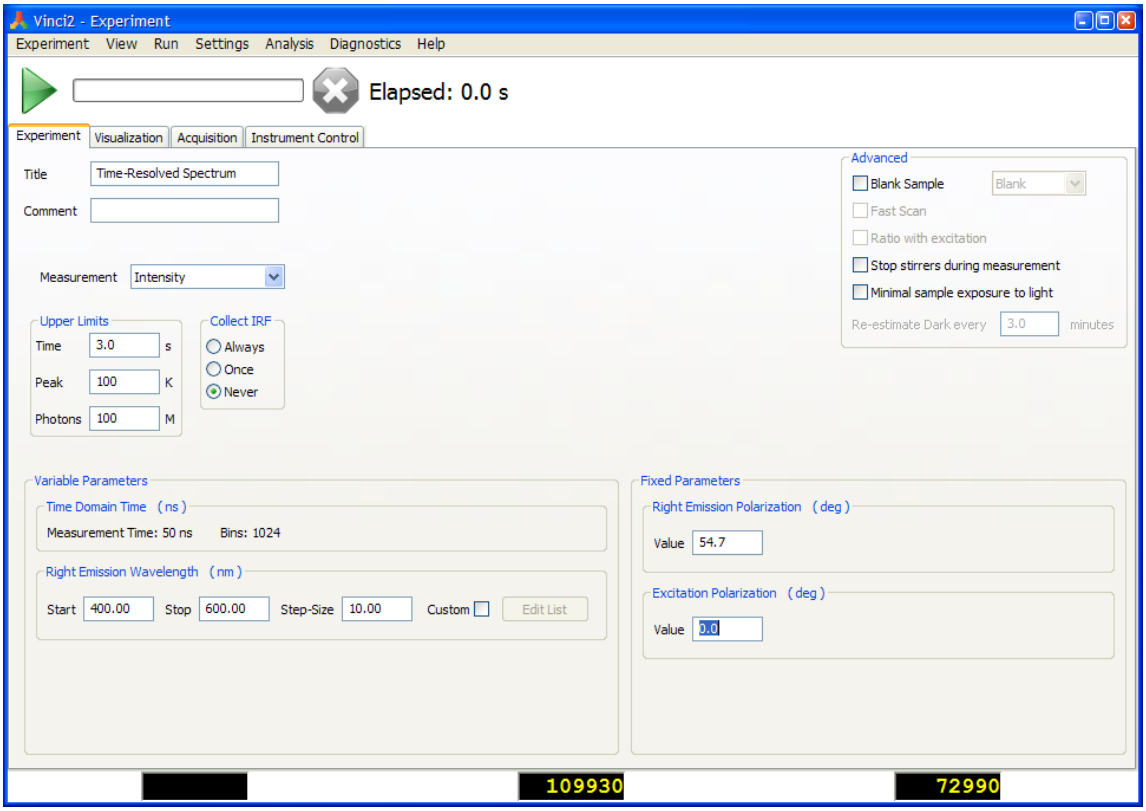



**Figure 18.9** Time-resolved spectrum experiment window

The experiment acquisition window is separated in several functional areas:

- Sample Identification area
- Measurement area
- Advanced Parameters area

### 18.4.3 Sample Identification Area

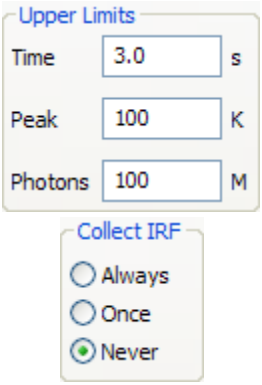
This area includes the parameters to be selected for the measurement acquisition; that is:

***Title:*** Enter an alphanumeric title

***Comment:*** Enter additional identification comments

### 18.4.4 Measurement Area

This area includes the parameters to be selected for the measurement acquisition; that is:




***Upper Limits:***

- Time (s, seconds)
- Peak (K, thousand
- Photons (M, millions)

***Collect IRF***

Select one of the buttons

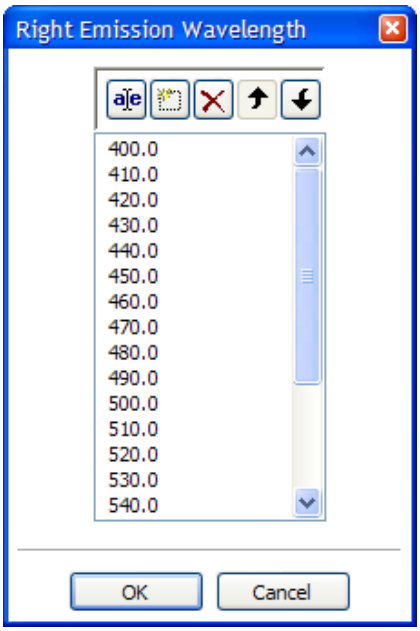
- Always
- Once
- Never

***Variable Parameters***

The parameters of the acquisition set in the <Acquisition Page>.
In the example, we have 50ns for the measurement time (Gain = 1) and 4096 bins

***Right Emission Wavelength (nm)***

Start: enter the value of the starting wavelength for the scan
Stop: enter the value of the ending wavelength for the scan
Step Size: the value of the monochromator's steps between the wavelengths




***Custom***

When clicking on the button, the list of the wavelengths is displayed.

One can delete unwanted wavelengths and add additional wavelengths

***Fixed Parameters***

Experimental parameters.

In the example:
the Right Emission Polarizer is set at 54.7 degrees
the Excitation Polarizer is set at 0 degrees

### 18.4.5 Advanced Parameters Area

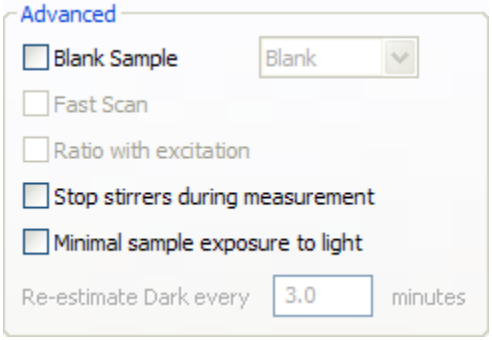
This area includes additional control parameters for the measurement. Note that the fields in gray do not apply to the ChronosBH; they only act for the ChronosFD acquisition.

***Stop Stirrers during measurement:***

The stirrers stop functioning during the acquisition

***Minimal sample exposure to light:***

The shutters are automatically closed when the acquisition is not operating in order to minimize any photobleaching effects.




After you have chosen proper signal levels for sample and reference, go to the experiment panel. Set a proper collection time and start the experiment by pressing the green arrow button. Vinci will collect the decay curves of the sample and reference automatically (depending upon the option selected for the IRF). The IRF can also be acquired in a separate run. Start the experiment by clicking the green arrow button.
After the measurement is finished the measured intensity decay will be displayed in “Vinci Analysis”.

As an example, Figure 18.10 shows time resolved spectra for TNS in glycerol. The excitation is a LED emitting at 300 nm with a repetition rate of 10 MHz. In total, 30 decay times have been collected through the monochromator starting at 380 nm and finishing at 520 nm (although in the plot only seven curves are shown). See the data analysis section for data treatment.

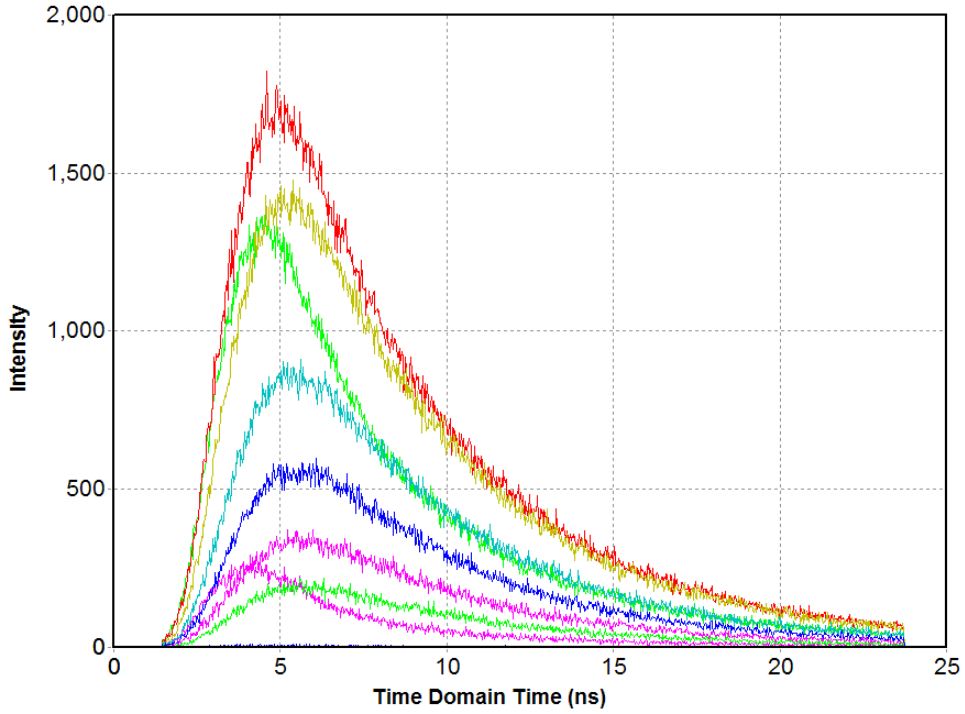



**Figure 18.10** Time-resolved spectrum of TNS in glycerol. Excitation source is a LED emitting at 300 nm with a 10 MHz repetition rate.

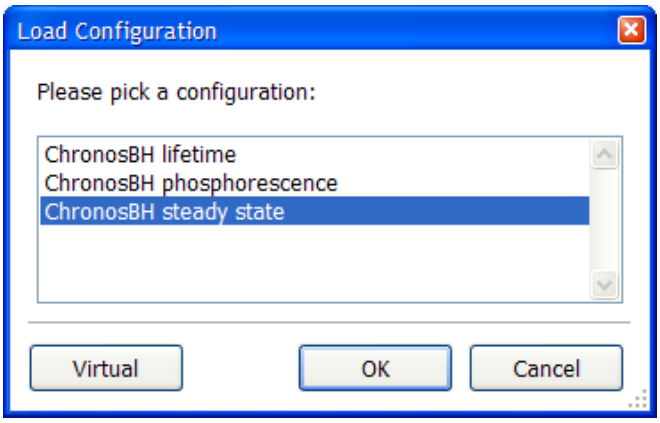
Once the acquisition is completed, data are stored in the data file specified.

# 19. Steady-state Fluorescence Measurements

This section includes an explanation on the most popular measurements in steady-state fluorescence are acquire. Vinci allows more experimental protocols setups; please consult the *Vinci Multidimensional Fluorescence Spectroscopy Analysis* Reference Manual for a complete exposition of these protocols.

## 19.1 Setting up the Instrument Configuration

Before starting to acquire a fluorescence steady-state data make sure you have created a software configuration for the steady-state setup of the instrument. To do so, run the Configuration editor from ISS software package and modify your existing configuration by adding/removing devices (most likely you may want to add the monochromators).
Once the Steady-state Configuration is ready, Save it using a different name, for instance <ChronosBH steady-state>; exit the Configuration Editor and launch the Vinci software. Now, when starting Vinci the new configuration can be selected:

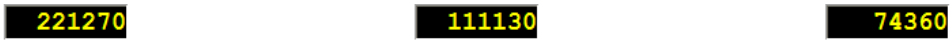



## 19.2 Instrument Control Page

The Instrument Control Page displays, for each instrument's area (excitation channel, reference channel, right and left emission channels) icons of the various automated devices enabled on the instrument. By clicking on each icon, the device can be moved.
The Figure below displays the Instrument Control page for an instrument equipped with polarizers and shutters in excitation and the two emission channels; the sample compartment is a 2-cuvette holder with stirrers below the cuvettes.

## 19.3 Checking the signal using the Instrument Control page

At bottom of the Instrument Control page, the signal from the three channels (reference, left and right emission) is displayed (in counts per second).
The reference signal is displayed in the middle; the signals from the left and right emission channels are respectively the left and right display.




For best performance, the signal (counts per second) should be in the range of 50,000 – 1,000,000. Sometimes, it is not possible to collect a signal level of 50,000 counts/sec; in that case, one has to acquire the signal for a longer time.

The Instrument Control page allows for the user to check the signal from the various channels and to

introduce adjustments if needed, in order to record the best signal level.

The reference channel intensity can be altered by taking one or more of the following actions:

- *insert/remove neutral density filters in the quantum counter holder;*
- *change the slits size in the excitation monochromator;*
- *change the intensity of the xenon arc lamp (by changing the current in the range 16-22 A);*

The emission channels intensity can be altered by taking one or more of the following actions:

- *open/close the iris, located before the sample compartment (Figure 13.1);*
- *insert/remove neutral density filters in the excitation channel in order to reduce the intensity of the light reaching the sample;*
- *change the intensity of the xenon arc lamp (by changing the current in the range 16-22 A);*
- *change the slits size in the excitation monochromator;*
- *insert/remove neutral density filters in the emission filter holder;*
- *change the slits size in the emission monochromator;*
- *change the sample concentration.*

**Note:** The gain setting on PMTs for the photon counting acquisition should always be set to 10 (maximum).

### 19.3.1 Check the excitation intensity at a fixed emission wavelength

Set the emission monochromator to the desired wavelength. To check the excitation intensity, run the excitation monochromator within the set range with the emission shutter in the <open> position. Check the signal on the right display at the bottom of the screen.

Right-click on the emission monochromator icon, select <Move> and enter the desired wavelength.


Repeat the same operation for the excitation monochromator. If the signal is too high, decrease it by using one of the approaches suggested in 19.3 above.

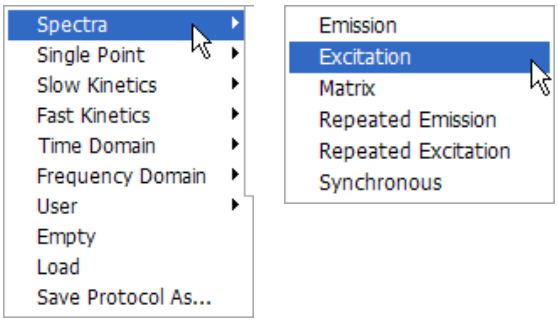
### 19.3.2 Check the emission intensity at fixed excitation wavelength

Set the excitation monochromator to the selected wavelength. To check the emission intensity, run the emission monochromator within the desirable range with the emission shutter open. To do so, set the emission wavelength on the emission monochromator: right-click on the emission monochromator icon, select <Move> and enter the desired wavelength.
Alternatively, one can move the monochromator step-by-step by clicking on the side arrows.

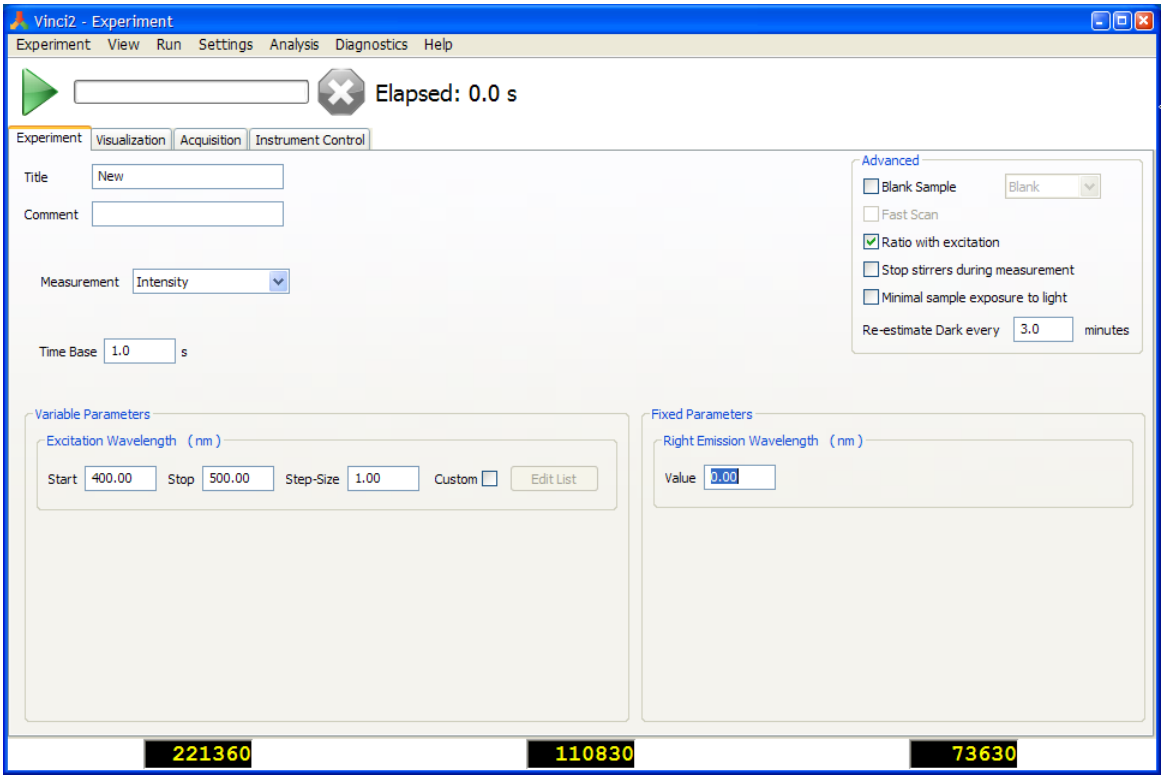
If the signal is too high, decrease it by using one of the approaches suggested in 19.3 above.

## 19.4 Excitation spectra

In the Vinci Experiment window, go to the <Experiment> menu on the top bar menu, select <Spectra> and choose <Excitation>.




The experiment window is displayed:




The <experiment window> is divided into three functional areas:

- Sample identification area
- Measurements area
- Advanced Parameters area

### 19.4.1 Sample Identification Area

This area includes the parameters to be selected for the measurement acquisition; that is:

***Title:*** Enter an alphanumeric title

***Comment:*** Enter additional identification comments

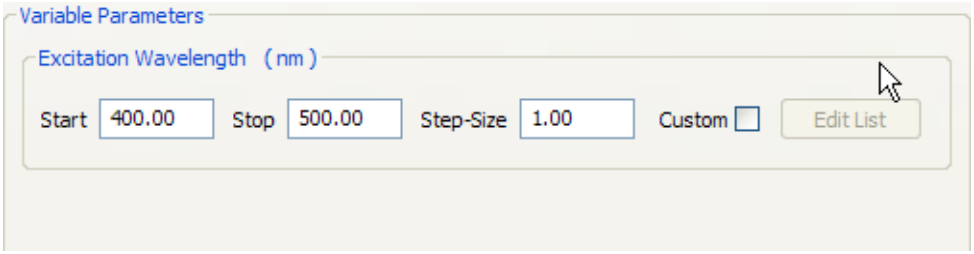
### 19.4.2 Measurement Area

This area includes the parameters to be selected for the measurement acquisition; that is:

***Measurement:*** The type of measurement. The default for this window is <Intensity>

***Time base (s):*** The time base of the data acquisition (the default is 1 sec).

***Variable Parameters***




***Start*** Enter the starting wavelength for the excitation spectrum.

***Stop*** Enter the ending wavelength.

***Step Size*** The step of the monochromator.

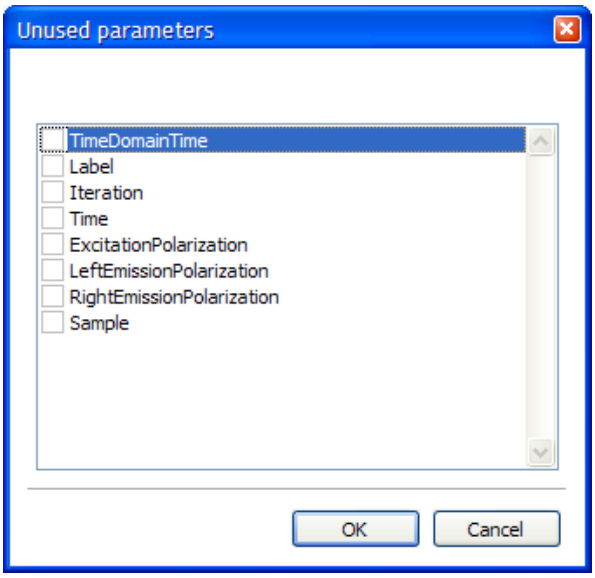
***Custom*** If checked, the wavelength table can be edited; wavelengths can be added and/or removed. For instance, one can decide to acquire a sectrum from 300 to 400 every 5 nm and from 400 to 500 every 10 nm.

Additional variable experimental parameters can be added by right-clicking in the <Variable Parameters> area. When doing so, the <Add> button is displayed.

***Add***

By right-clicking in the Variable Parameters area, additional experimental parameters can be added to make a “multi-dimensional experiment”.

The desired parameter is selected by clicking on the checkbox.




Similarly, the parameters can be removed from the area.

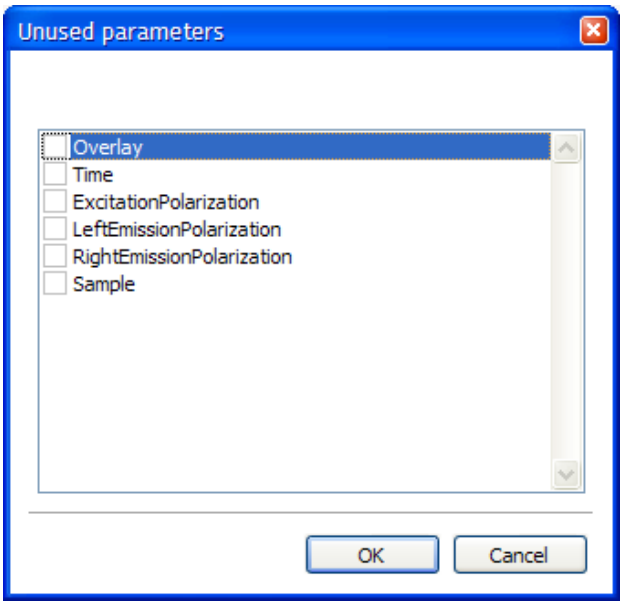
***Fixed Parameters***


***Value*** Enter the wavelength position for the emission monochromator.

Additional variable experimental parameters can be added by right-clicking in the <Fixed Parameters> area. When doing so, the <Add> button is displayed.

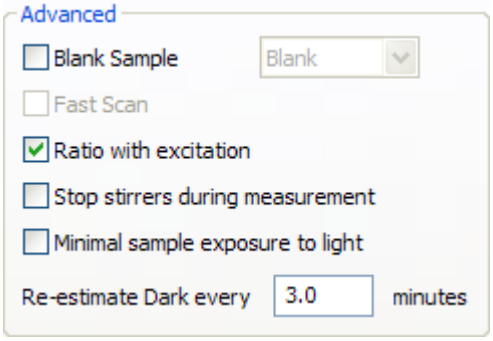
***Add***

By right-clicking in the Fixed Parameters area, additional experimental parameters can be added to make a “multi-dimensional experiment”.




### 19.4.3 Advanced Parameters Area

The parameters in this area are only changed sporadically.




| | |
|---|---|
| ***Blank Sample*** | When checked, a measurement of the blank is acquired (this feature works best with a cuvette holder that holds more than cuvette; when using 1-cuvette holder, the sample has to be replaced manually) |
| ***Ratio with Excitation*** | The box is checked automatically for the acquisition of excitation spectra. The instrument acquires a signal from the reference (excitation) channel in order to automatically acquire corrected excitation spectra. |
| ***Stop Stirrers during measurements*** | When checked, the stirrers are stopped during the measurement |
| ***Minimal exposure to light*** | When checked, the shutters automatically close at the end of the measurement. |
| ***Re-estimate Dark every xx minutes*** | The default is 3 minutes; that is, the dark counts from the detector are automatically acquired (and stored) after the time set in this field. |

### 19.4.4 Start the acquisition

The data acquisition is started by clicking on the green arrow on the top:


| | |
|---|---|
| ***To Start*** | Click on the green arrow |
| ***To Pause*** | Click on the bars |
| ***Experiment time*** | It is displayed by the bar utilizing the acquisition parameters selected by the user  The time is also displayed numerically (in seconds). |

During the acquisition, the software switches to the <Visualization Page>, although the user can switch to other pages.

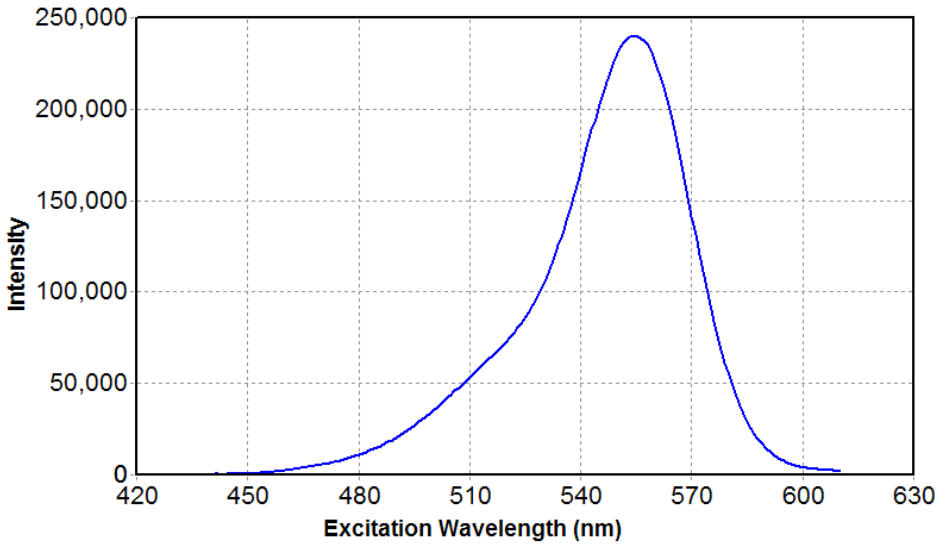
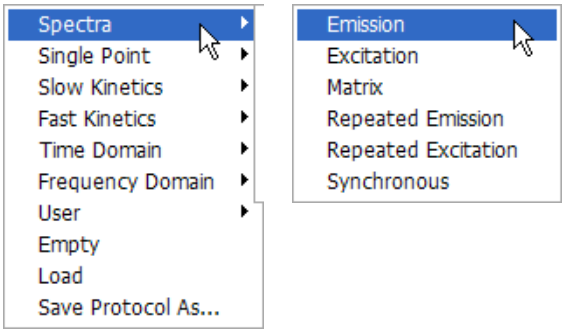


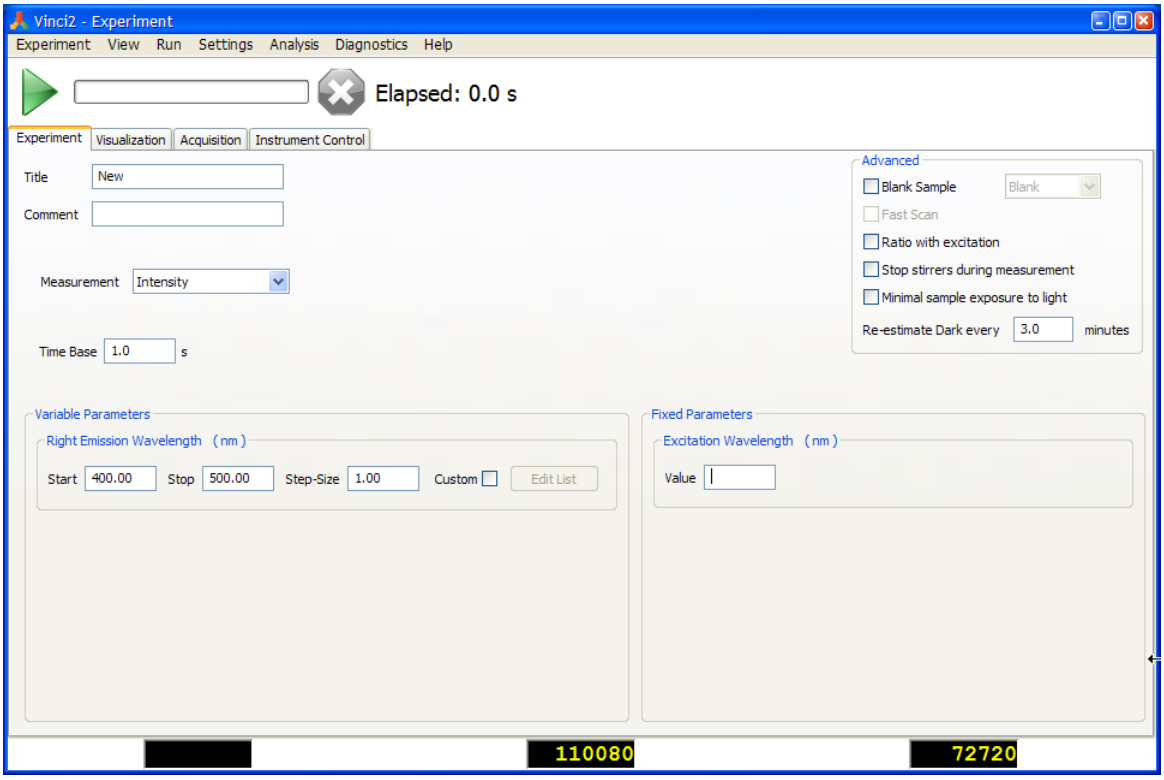

**Figure 19.1** Excitation spectrum of Rhodamine 6G in glycerol

## 19.5 Emission spectra

In the Vinci Experiment window, go to the <Experiment> menu on the top bar menu, select <Spectra> and choose <Emission>.




The experiment window is displayed:




The <experiment window: is divided into three functional areas:

- Sample identification area
- Measurements area
- Advanced Parameters area

### 19.5.1 Sample Identification Area

This area includes the parameters to be selected for the measurement acquisition; that is:

***Title:*** Enter an alphanumeric title

***Comment:*** Enter additional identification comments

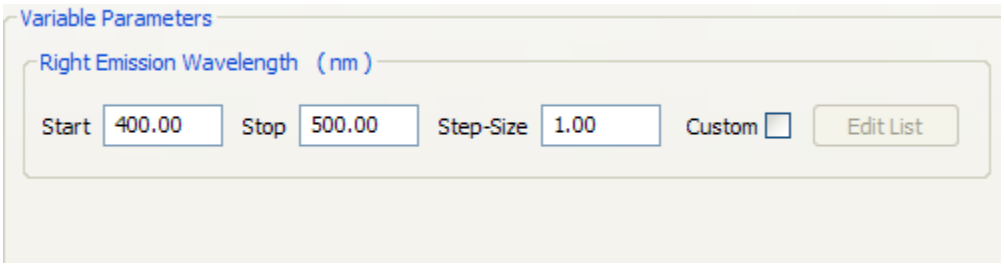
### 19.5.2 Measurement Area

This area includes the parameters to be selected for the measurement acquisition; that is:

***Measurement:*** The type of measurement. The default for this window is <Intensity>

***Time base (s):*** The time base of the data acquisition (the default is 1 sec).

***Variable Parameters***




***Start*** Enter the starting wavelength for the emission spectrum.

***Stop*** Enter the ending wavelength.

***Step Size*** The step of the monochromator.

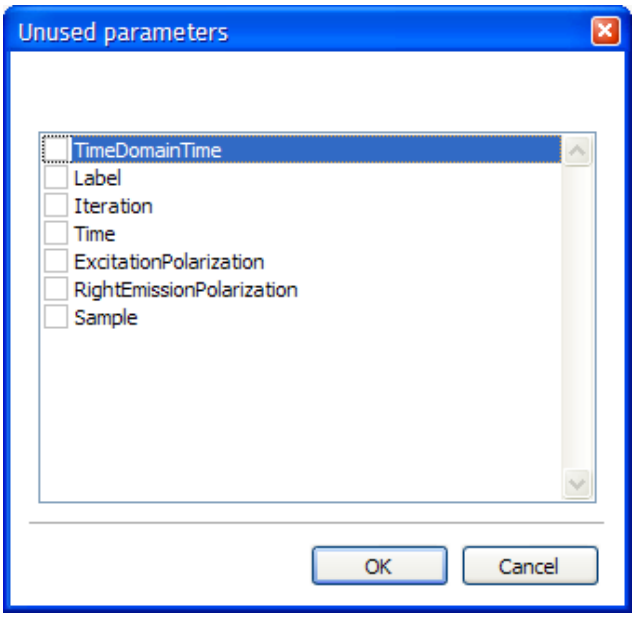
***Custom*** If checked, the wavelength table can be edited; wavelengths can be added and/or removed. For instance, one can decide to acquire a sectrum from 300 to 400 every 5 nm and from 400 to 500 every 10 nm.

Additional variable experimental parameters can be added by right-clicking in the <Variable Parameters> area. When doing so, the <Add> button is displayed.

***Add***

By right-clicking in the Variable Parameters area, additional experimental parameters can be added to make a “multi-dimensional experiment”.

The desired parameter is selected by clicking on the checkbox.




Similarly, the parameters can be removed from the area.

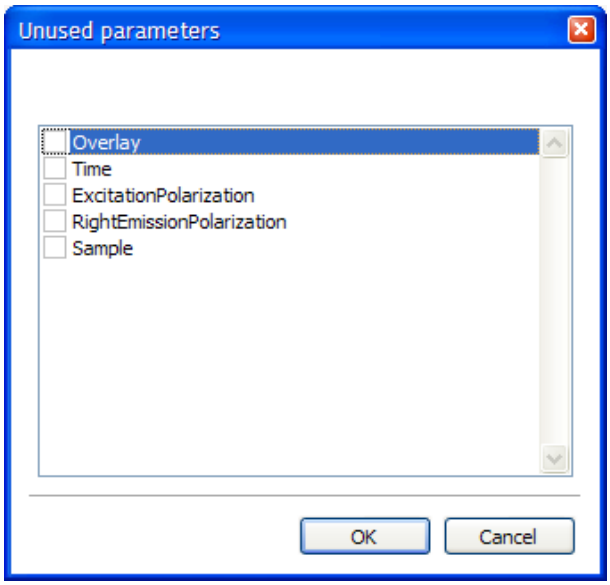
***Fixed Parameters***


***Value*** Enter the wavelength position for the emission monochromator.

Additional variable experimental parameters can be added by right-clicking in the <Fixed Parameters> area. When doing so, the <Add> button is displayed.

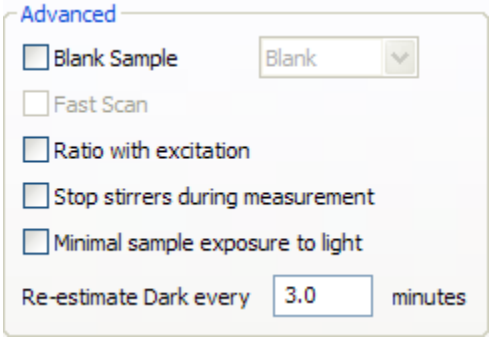
***Add***

By right-clicking in the Fixed Parameters area, additional experimental parameters can be added to make a “multi-dimensional experiment”.




### 19.5.3 Advanced Parameters Area

The parameters in this area are only changed sporadically.




| | |
|---|---|
| ***Blank Sample*** | When checked, a measurement of the blank is acquired (this feature works best with a cuvette holder that holds more than cuvette; when using 1-cuvette holder, the sample has to be replaced manually) |
| ***Ratio with Excitation*** | The box is checked automatically for the acquisition of excitation spectra. The instrument acquires a signal from the reference (excitation) channel in order to automatically acquire corrected excitation spectra. |
| ***Stop Stirrers during measurements*** | When checked, the stirrers are stopped during the measurement |
| ***Minimal exposure to light*** | When checked, the shutters automatically close at the end of the measurement. |
| ***Re-estimate Dark every xx minutes*** | The default is 3 minutes; that is, the dark counts from the detector are automatically acquired (and stored) after the time set in this field. |

### 19.5.4 Start the acquisition

The data acquisition is started by clicking on the green arrow on the top:


| | |
|---|---|
| ***To Start*** | Click on the green arrow |
| ***To Pause*** | Click on the bars |
| ***Experiment time*** | It is displayed by the bar utilizing the acquisition parameters selected by the user. The time is also displayed numerically (in seconds). |

During the acquisition, the software switches to the <Visualization Page>, although the user can switch to other pages.

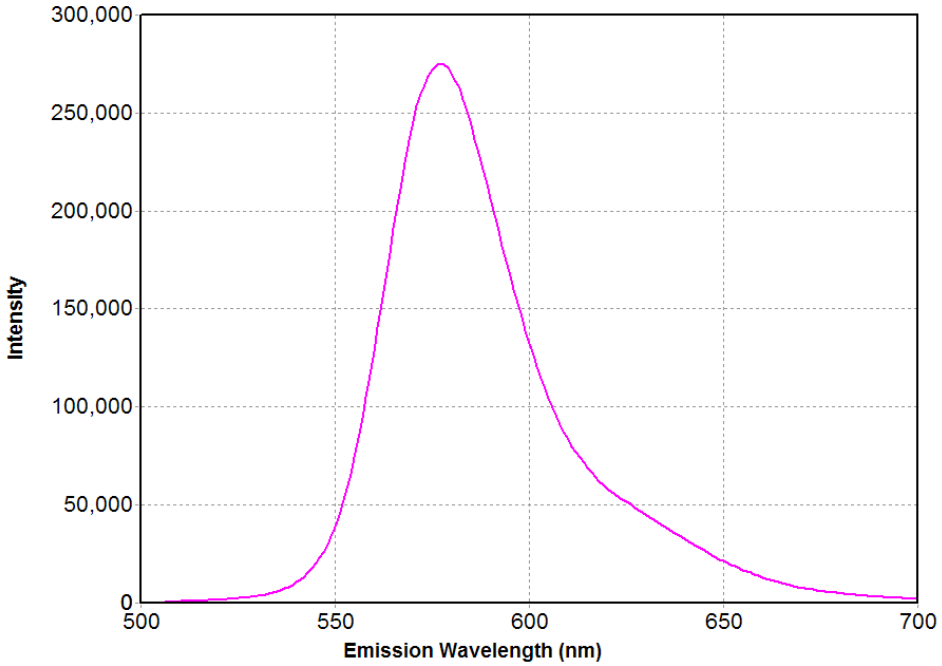
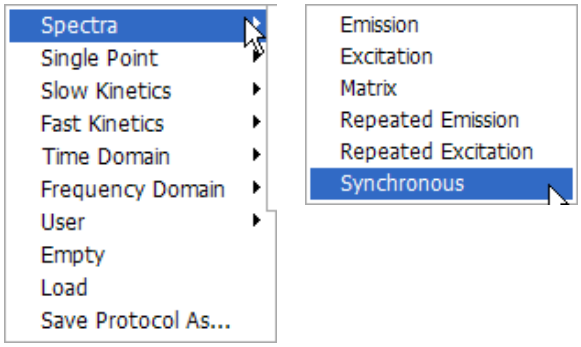


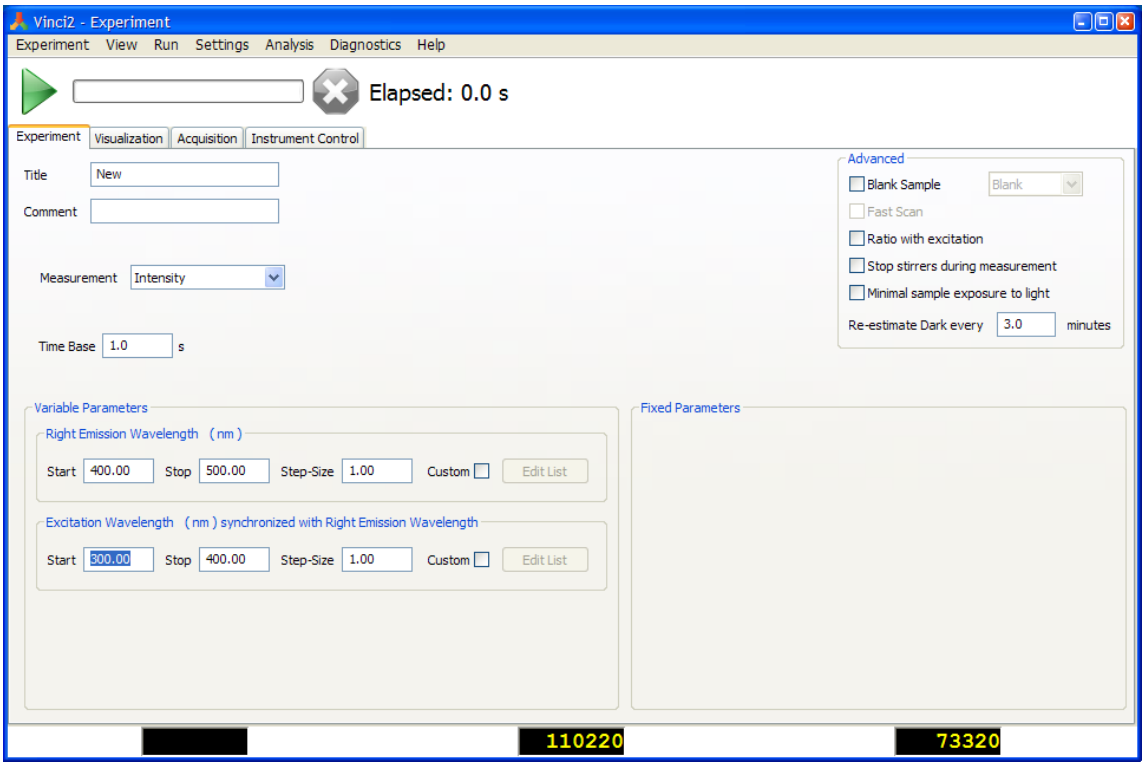

**Figure 19.2** Emission spectrum of Rhodamine 6G in glycerol.

## 19.6 Synchronous Spectra

In the Vinci Experiment window, go to the <Experiment> menu on the top bar menu, select <Spectra> and choose <Emission>.




The experiment window is displayed:




The <experiment window: is divided into three functional areas:

- Sample identification area
- Measurements area
- Advanced Parameters area

### 19.6.1 Sample Identification Area

This area includes the parameters to be selected for the measurement acquisition; that is:

***Title:*** Enter an alphanumeric title

***Comment:*** Enter additional identification comments

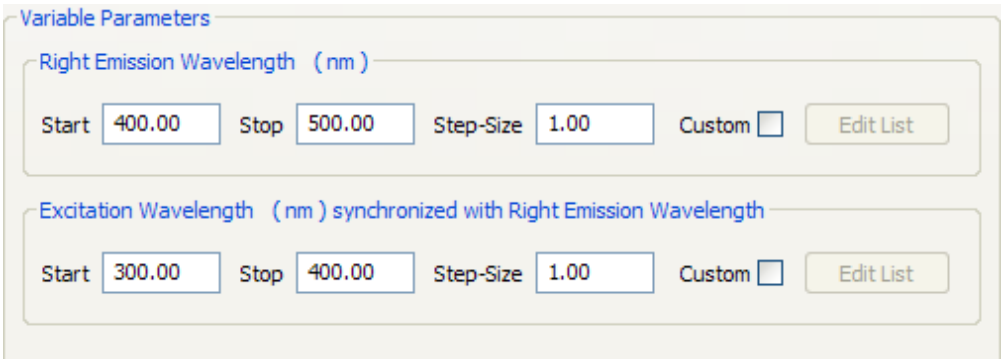
### 19.6.2 Measurement Area

This area includes the parameters to be selected for the measurement acquisition; that is:

***Measurement:*** The type of measurement. The default for this window is <Intensity>

***Time base (s):*** The time base of the data acquisition (the default is 1 sec).

***Variable Parameters***




***Start*** Enter the starting wavelength for the emission monochromator
Enter the starting wavelength for the excitation monochromator

***Stop*** Enter the ending wavelength for the emission monochromator
Enter the ending wavelength for the excitation monochromator

***Step Size*** The step of each monochromator (it should be the same)

***Custom*** If checked, the wavelength table can be edited; wavelengths can be added and/or removed.

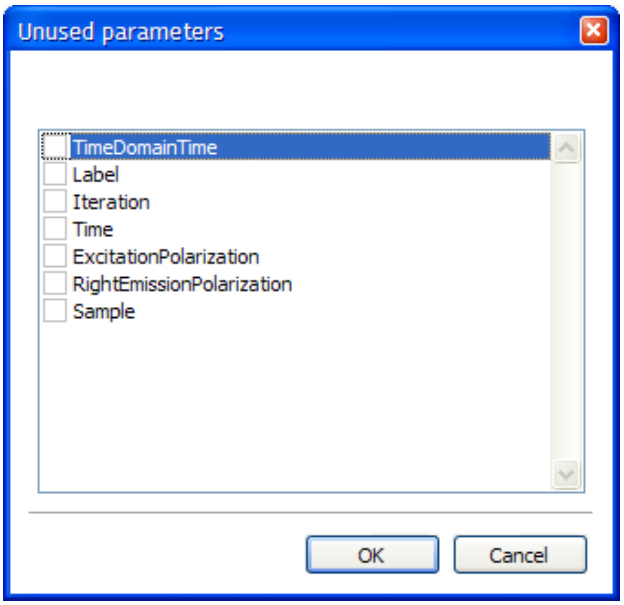
Additional variable experimental parameters can be added by right-clicking in the <Variable Parameters> area. When doing so, the <Add> button is displayed.

***Add***

By right-clicking in the Variable Parameters area, additional experimental parameters can be added to make a “multi-dimensional experiment”.

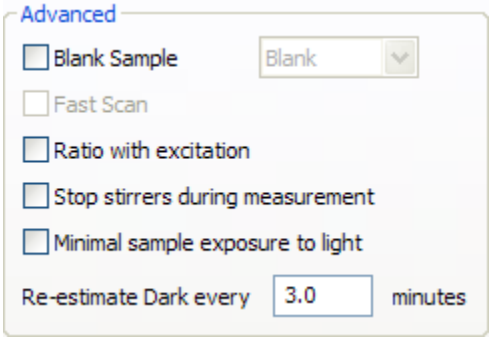
The desired parameter is selected by clicking on the checkbox.

Similarly, the parameters can be removed from the area.




### 19.6.3 Advanced Parameters Area

The parameters in this area are only changed sporadically.




| | |
|---|---|
| ***Blank Sample*** | When checked, a measurement of the blank is acquired (this feature works best with a cuvette holder that holds more than cuvette; when using 1-cuvette holder, the sample has to be replaced manually) |
| ***Ratio with Excitation*** | The box is checked automatically for the acquisition of excitation spectra. The instrument acquires a signal from the reference (excitation) channel in order to automatically acquire corrected excitation spectra. |
| ***Stop Stirrers during measurements*** | When checked, the stirrers are stopped during the measurement |
| ***Minimal exposure to light*** | When checked, the shutters automatically close at the end of the measurement. |
| ***Re-estimate Dark every xx minutes*** | The default is 3 minutes; that is, the dark counts from the detector are automatically acquired (and stored) after the time set in this field. |

### 19.6.4 Start the acquisition

The data acquisition is started by clicking on the green arrow on the top:


| | |
|---|---|
| ***To Start*** | Click on the green arrow |
| ***To Pause*** | Click on the bars |
| ***Experiment time*** | It is displayed by the bar utilizing the acquisition parameters selected by the user. The time is also displayed numerically (in seconds). |

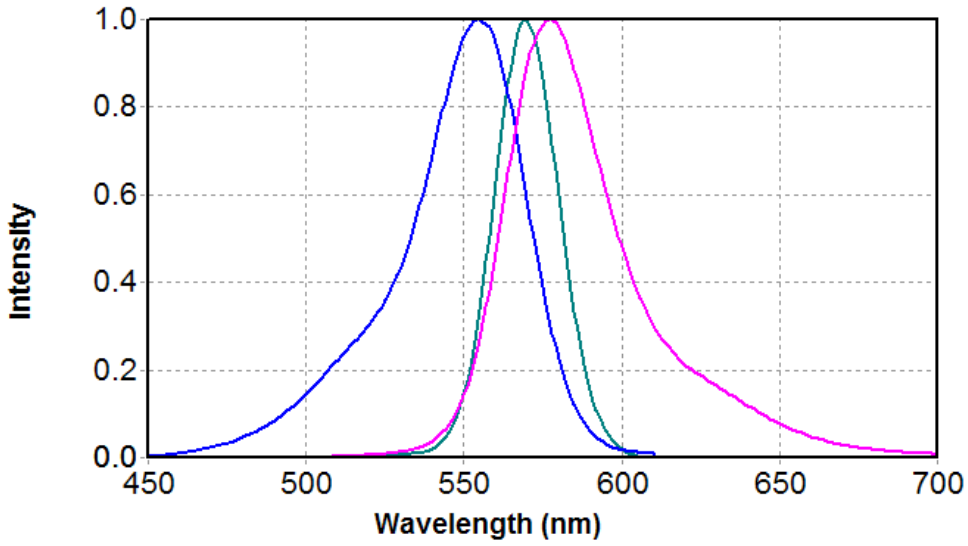



**Figure 19.3** Excitation (blue line), emission (purple line) and synchronous spectra (green line) of Rhodamine B in water. The synchronous spectrum is acquired in the superposition region of the excitation and emission spectra.

## 19.7 Single point (intensity, polarization, ratio)

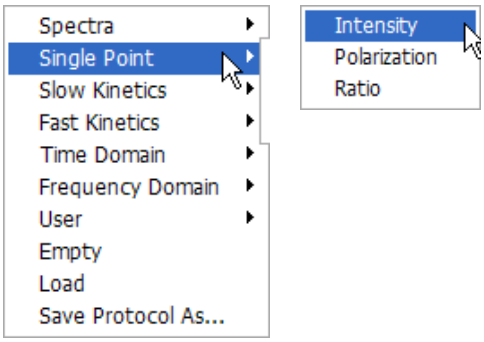
All the <experiment windows> are divided into three functional areas:

- Sample identification area
- Measurements area
- Advanced Parameters area

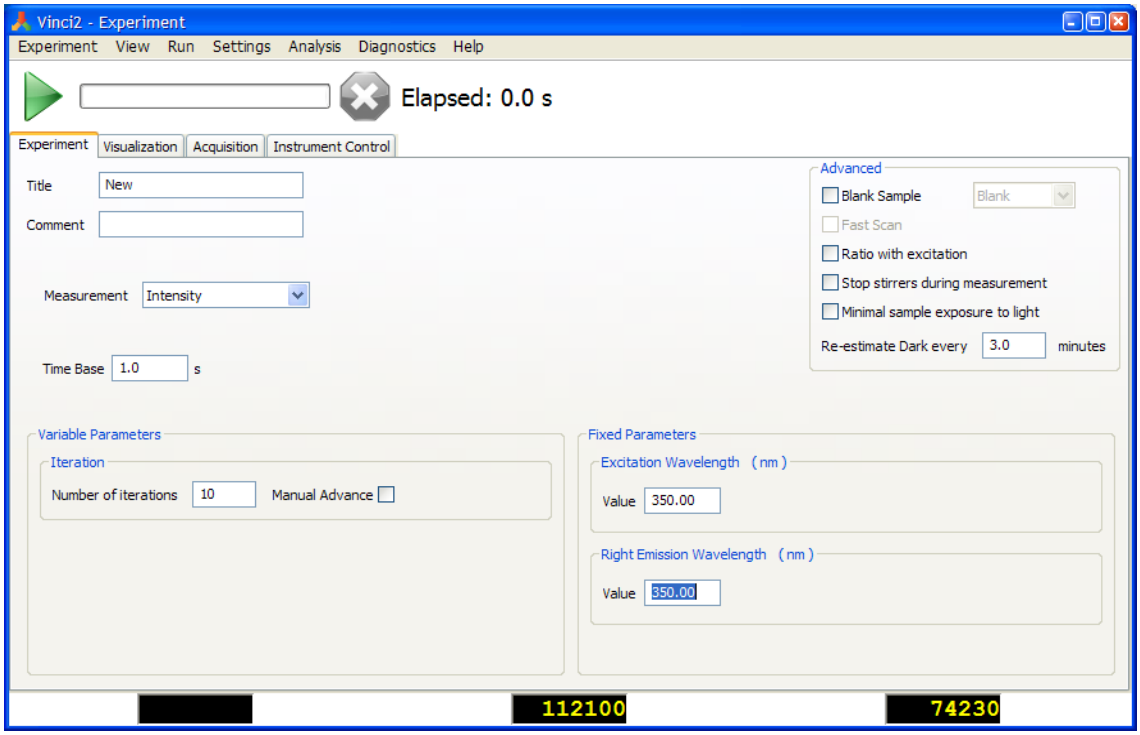
While the Sample Identification area and the Advanced Parameters area are the same for the three measurements, the Measurement Area is specific.

### 19.7.1 Single point intensity

In the Vinci Experiment window, go to the <Experiment> menu on the top bar menu, select <Single Point> and choose <Intensity>.




The experiment window is displayed:

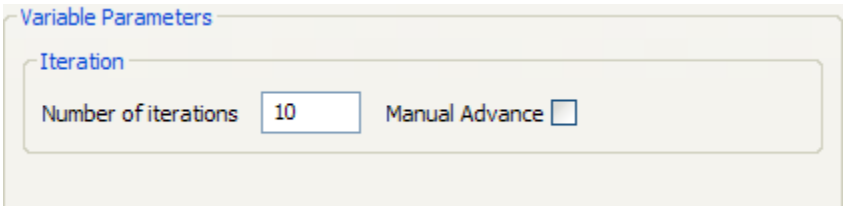



This area includes the parameters to be selected for the measurement acquisition; that is:

***Measurement:*** The type of measurement. The default for this window is <Intensity>

***Time base (s):*** The time base of the data acquisition (the default is 1 sec).

***Variable Parameters***




***Number of Iterations*** Enter the number of times the measurement is repeated

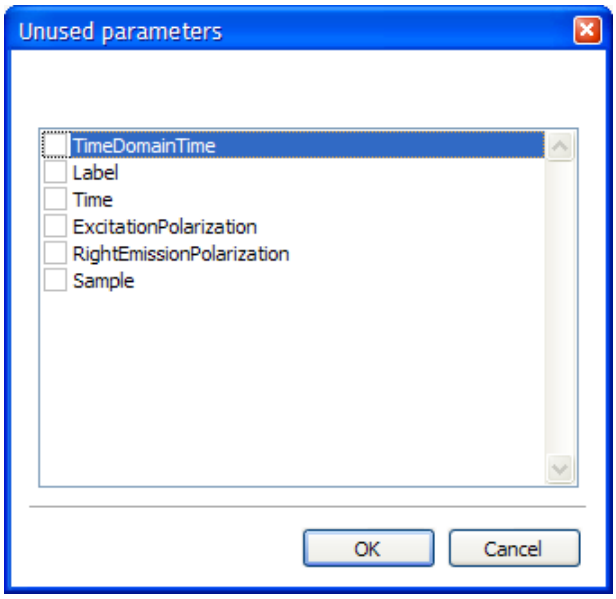
***Manual Advance*** When checked, each measurement (iteration) is started manually

Additional variable experimental parameters can be added by right-clicking in the <Variable Parameters> area. When doing so, the <Add> button is displayed.

***Add***

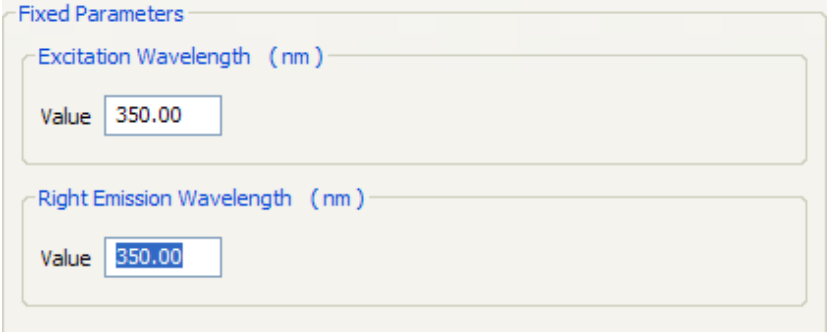
By right-clicking in the Variable Parameters area, additional experimental parameters can be added to make a “multi-dimensional experiment”.

The desired parameter is selected by clicking on the checkbox.




Similarly, the parameters can be removed from the area.

***Fixed Parameters***



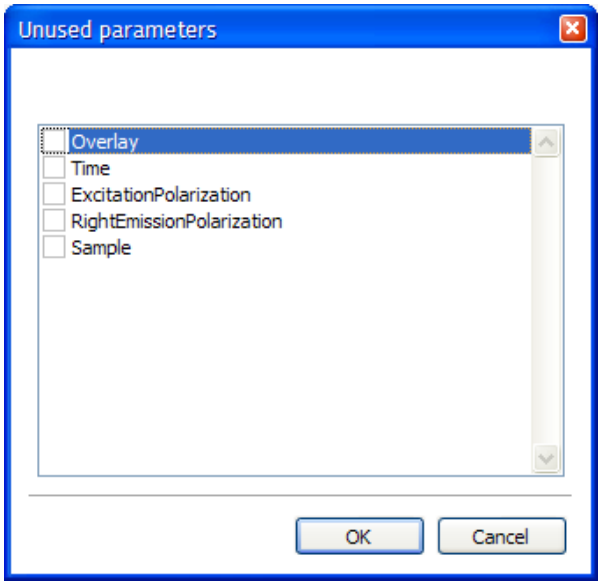

***Value***

Enter the wavelength position for the excitation and emission monochromators.

Additional variable experimental parameters can be added by right-clicking in the <Fixed Parameters> area. When doing so, the <Add> button is displayed.

***Add***

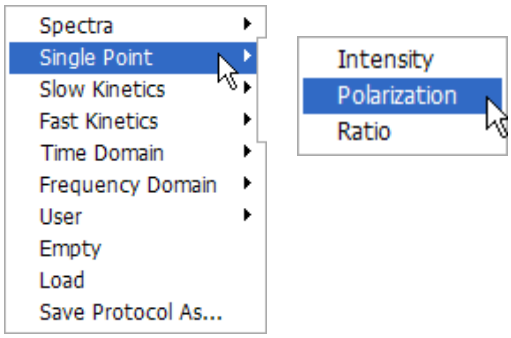
By right-clicking in the Fixed Parameters area, additional experimental parameters can be added to make a “multi-dimensional experiment”.



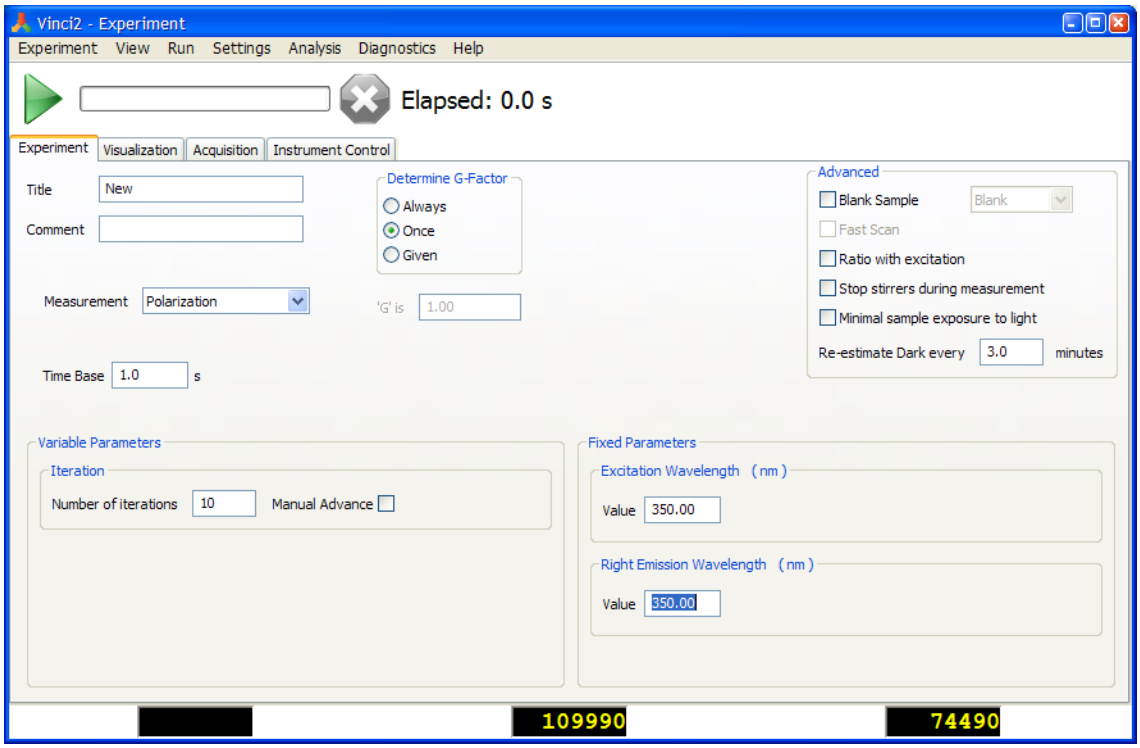

For the <Advanced Parameters> area see 19.7.5 below.

### 19.7.2 Single point polarization

In the Vinci Experiment window, go to the <Experiment> menu on the top bar menu, select <Single Point> and choose <Polarization>.




The experiment window is displayed:




This area includes the parameters to be selected for the measurement acquisition; that is:

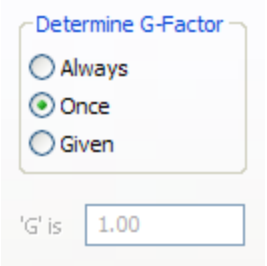
***Measurement:*** The type of measurement. The default for this window is <Polarization>

***Time base (s):*** The time base of the data acquisition (the default is 1 sec).

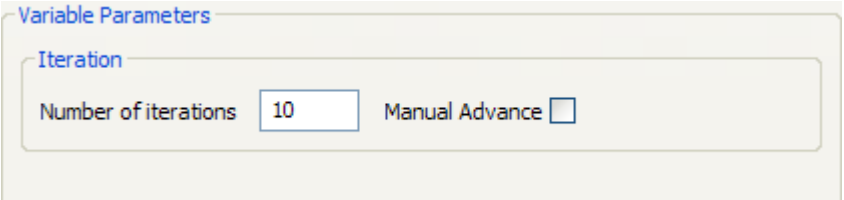
***Determine G-factor***

In this area the user decides how to acquire and/or enter the value for the g-factor. Select one of the three options:

Always
Once
Given




***Variable Parameters***




***Number of Iterations*** Enter the number of times the measurement is repeated

***Manual Advance*** When checked, each measurement (iteration) is started manually

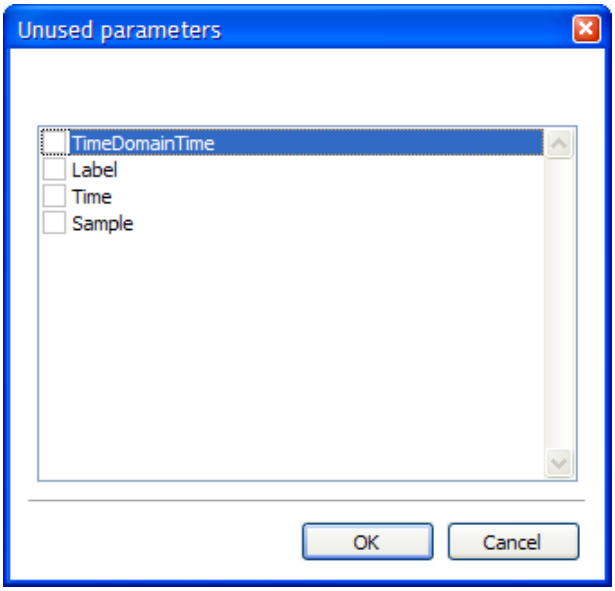
Additional variable experimental parameters can be added by right-clicking in the <Variable Parameters> area. When doing so, the <Add> button is displayed.

***Add***

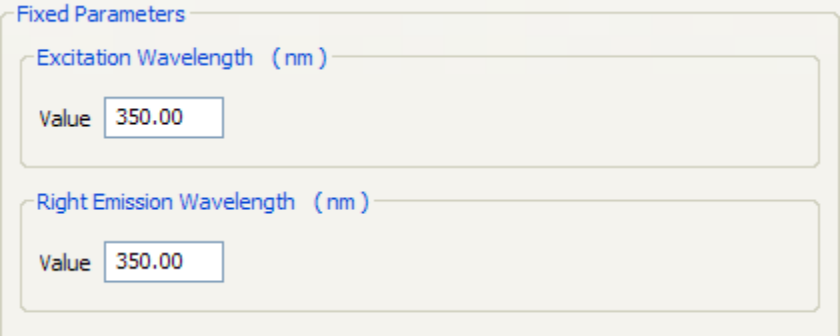
By right-clicking in the Variable Parameters area, additional experimental parameters can be added to make a “multi-dimensional experiment”.

The desired parameter is selected by clicking on the checkbox.

Similarly, the parameters can be removed from the area.



***Fixed Parameters***




Enter the wavelength position for the excitation and emission monochromators.

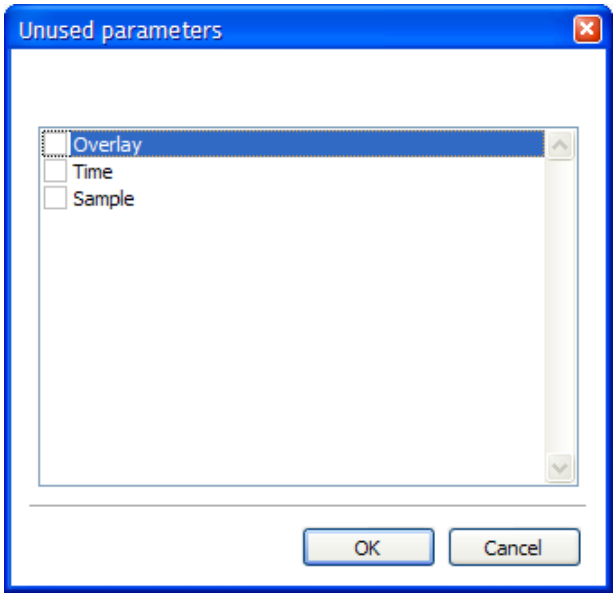
***Value***

If the acquisition is made on the left emission channel, the <Right Emission Wavelength> parameter is not displayed.

Additional variable experimental parameters can be added by right-clicking in the <Fixed Parameters> area. When doing so, the <Add> button is displayed.

***Add***

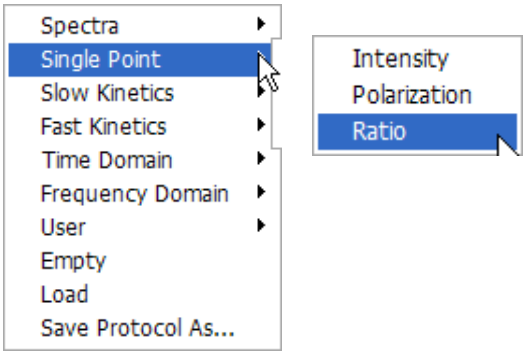
By right-clicking in the Fixed Parameters area, additional experimental parameters can be added to make a “multi-dimensional experiment”.



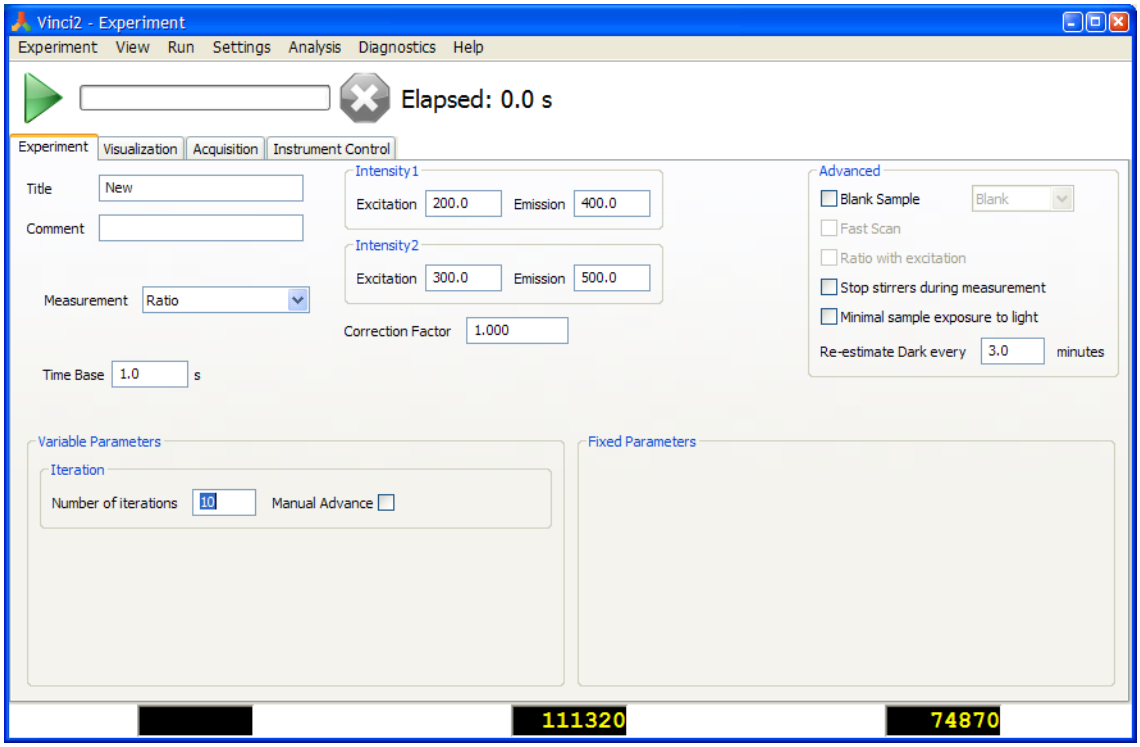

For the <Advanced Parameters> area see 19.7.5 below.

### 19.7.3 Single Point Experiment Ratio

In the Vinci Experiment window, go to the <Experiment> menu on the top bar menu, select <Single Point> and choose <Ratio>.




The experiment window is displayed:



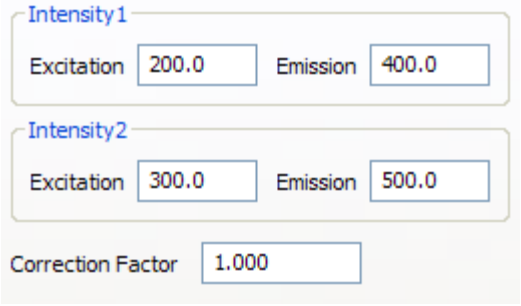

This area includes the parameters to be selected for the measurement acquisition; that is:

***Measurement:*** The type of measurement. The default for this window is <Polarization>

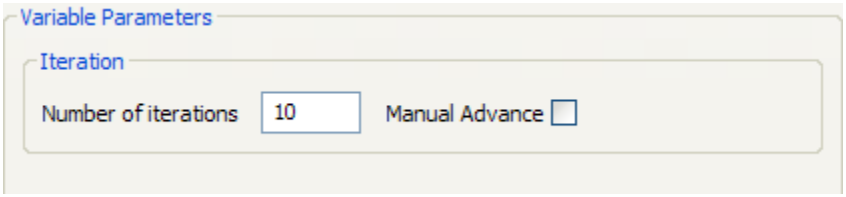
***Time base (s):*** The time base of the data acquisition (the default is 1 sec).

***Intensity1:*** Enter the position of the excitation and emission monochromators, where the intensity has to be measured.

***Intensity2:*** Enter the correction factor for the emission monochromator at that wavelength




***Variable Parameters***




***Number of Iterations*** Enter the number of times the measurement is repeated

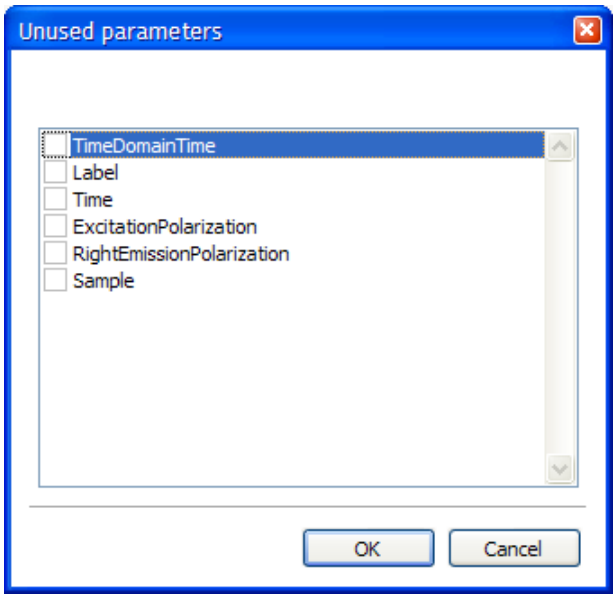
***Manual Advance*** When checked, each measurement (iteration) is started manually

Additional variable experimental parameters can be added by right-clicking in the <Variable Parameters> area. When doing so, the <Add> button is displayed.

***Add***

By right-clicking in the Variable Parameters area, additional experimental parameters can be added to make a “multi-dimensional experiment”.

The desired parameter is selected by clicking on the checkbox.




Similarly, the parameters can be removed from the area. For the <Advanced Parameters> area see 19.7.5 below.

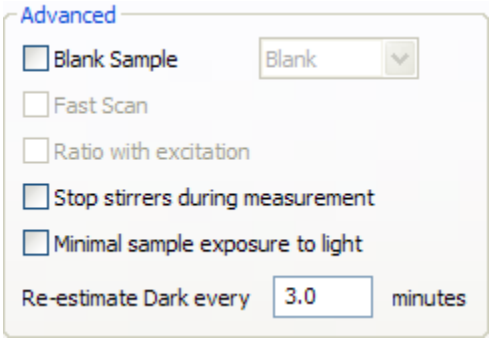
### 19.7.4 Sample Identification Area

This area includes the parameters to be selected for the measurement acquisition; that is:

| | |
|---|---|
| ***Title:*** | Enter an alphanumeric title |
| ***Comment:*** | Enter additional identification comments |

### 19.7.5 Advanced Parameters Area

The parameters in this area are only changed sporadically.




| | |
|---|---|
| ***Blank Sample*** | When checked, a measurement of the blank is acquired (this feature works best with a cuvette holder that holds more than cuvette; when using 1-cuvette holder, the sample has to be replaced manually) |
| ***Ratio with Excitation*** | When checked, the instrument acquires a signal from the reference (excitation) channel in order to automatically correct the emission for any variation of the excitation light intensity. |
| ***Stop Stirrers during measurements*** | When checked, the stirrers are stopped during the measurement |
| ***Minimal exposure to light*** | When checked, the shutters automatically close at the end of the measurement. |
| ***Re-estimate Dark every xx minutes*** | The default is 3 minutes; that is, the dark counts from the detector are automatically acquired (and stored) after the time set in this field. |

## 19.8 Slow kinetics (intensity, polarization, ratio)

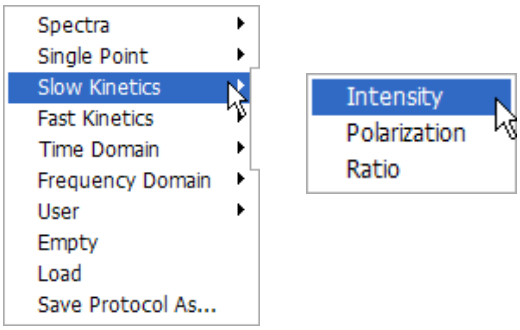
All the <experiment windows> are divided into three functional areas:

- Sample identification area
- Measurements area
- Advanced Parameters area

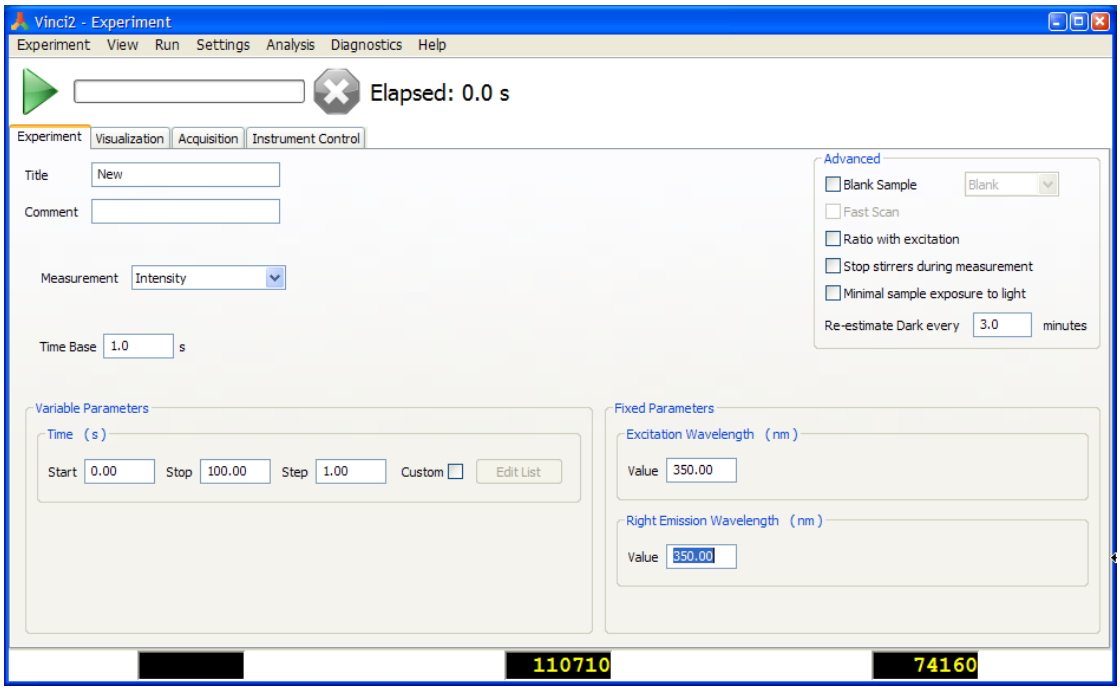
While the Sample Identification area and the Advanced Parameters area are the same for the three measurements, the Measurement Area is specific.

### 19.8.1 Intensity Kinetics

In the Vinci Experiment window, go to the <Experiment> menu on the top bar menu, select <Slow Kinetics> and choose <Intensity>.




The experiment window is displayed:

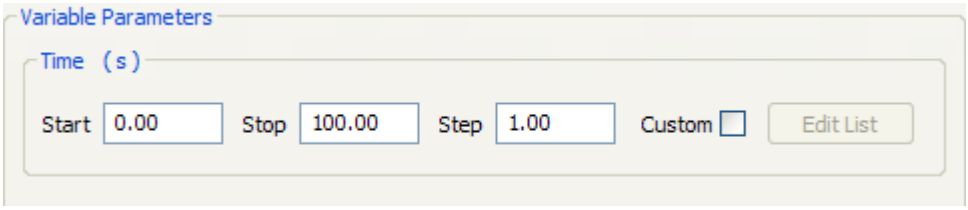



This area includes the parameters to be selected for the measurement acquisition; that is:

***Measurement:*** The type of measurement. The default for this window is <Intensity>

***Time base (s):*** The time base of the data acquisition (the default is 1 sec).

***Variable Parameters***




***Start*** Enter the time starting point for the experiment

***Stop*** Enter the time ending point for the experiment

***Step*** Enter the time interval (in seconds) the measurement is acquired at

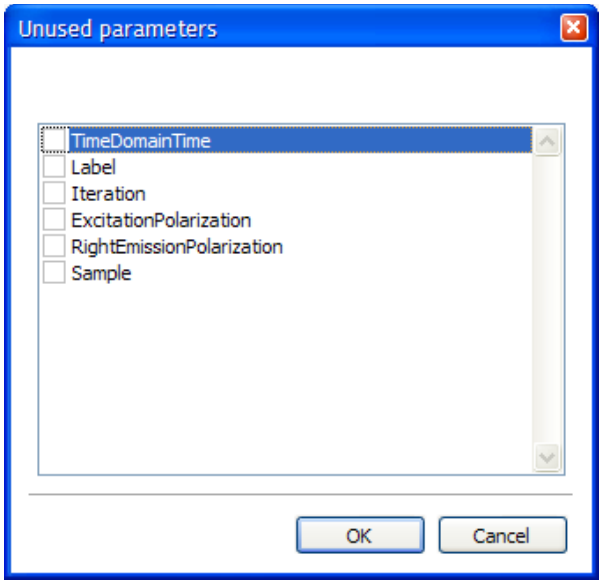
***Custom*** Edit the list of the acquisition time intervals

Additional variable experimental parameters can be added by right-clicking in the <Variable Parameters> area. When doing so, the <Add> button is displayed.

***Add***

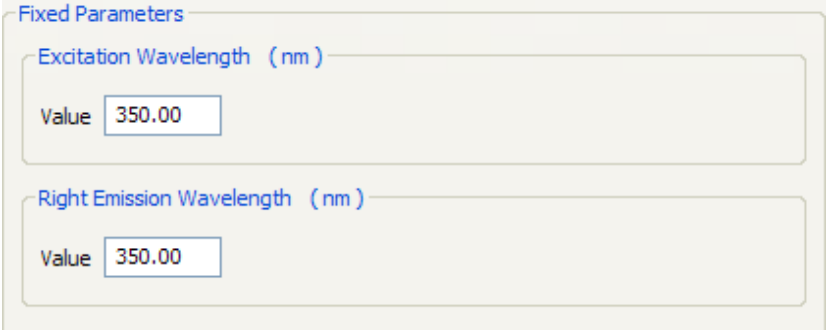
By right-clicking in the Variable Parameters area, additional experimental parameters can be added to make a “multi-dimensional experiment”.

The desired parameter is selected by clicking on the checkbox.




Similarly, the parameters can be removed from the area.

***Fixed Parameters***



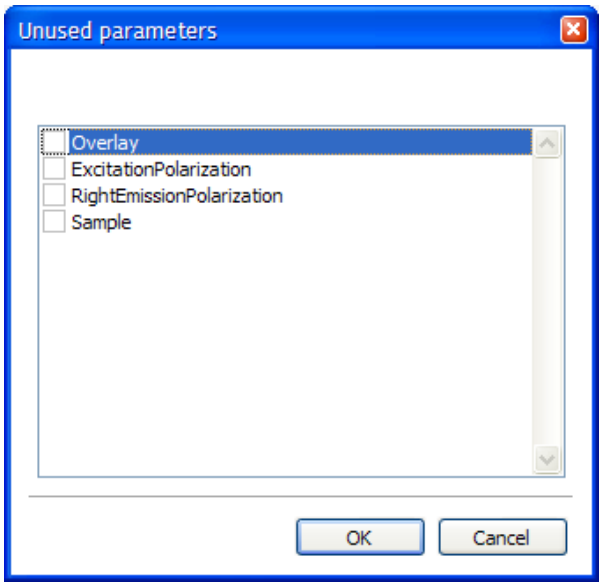

***Value***

Enter the wavelength position for the excitation and emission monochromators.

Additional variable experimental parameters can be added by right-clicking in the <Fixed Parameters> area. When doing so, the <Add> button is displayed.

***Add***

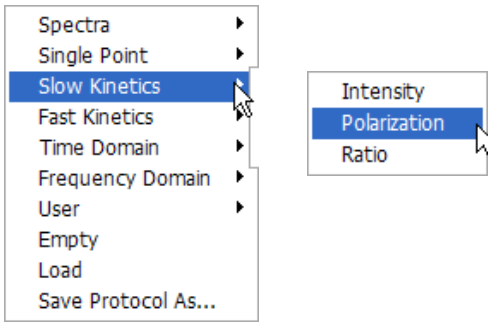
By right-clicking in the Fixed Parameters area, additional experimental parameters can be added to make a “multi-dimensional experiment”.



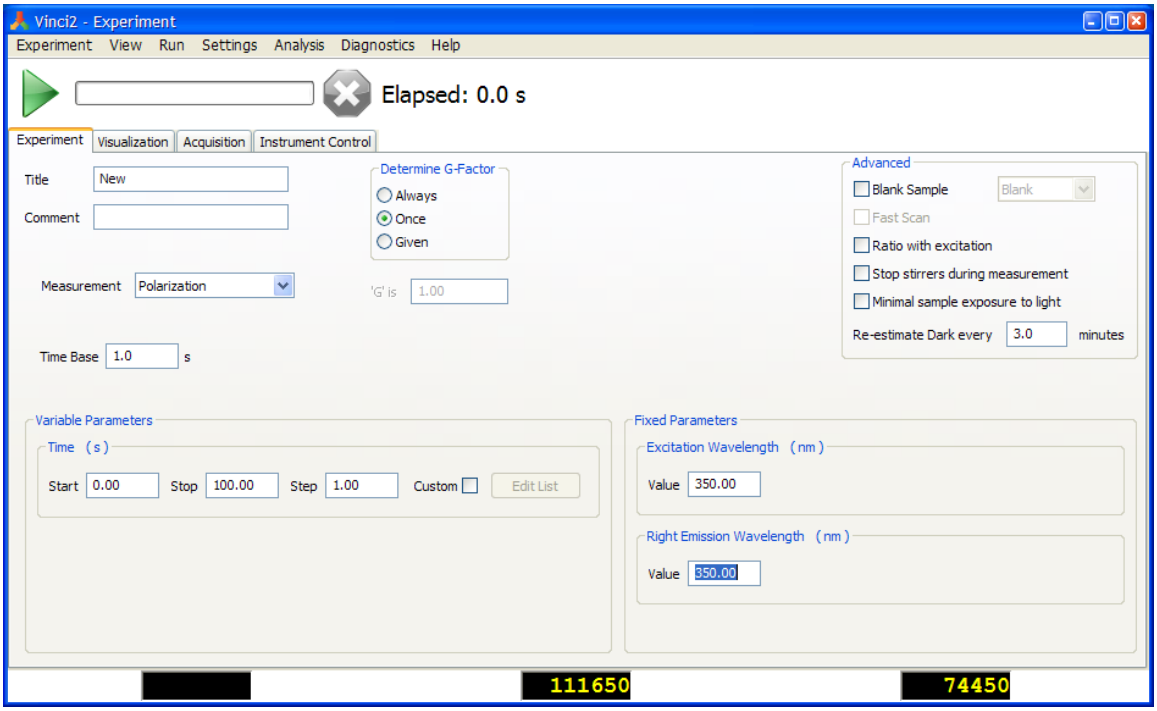

For the <Advanced Parameters> area see 19.8.5 below.

### 19.8.2 Polarization Kinetics

In the Vinci Experiment window, go to the <Experiment> menu on the top bar menu, select <Slow Kinetics> and choose <Polarization>.




The experiment window is displayed:




This area includes the parameters to be selected for the measurement acquisition; that is:

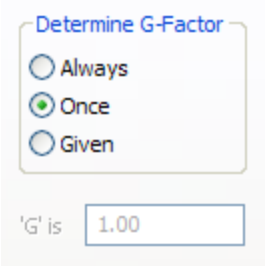
***Measurement:*** The type of measurement. The default for this window is <Polarization>

***Time base (s):*** The time base of the data acquisition (the default is 1 sec).

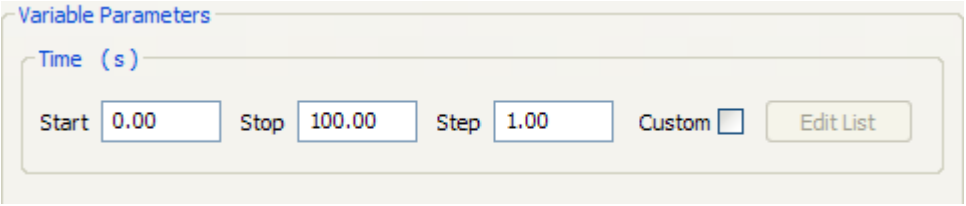
***Determine G-factor***

In this area the user decides how to acquire and/or enter the value for the g-factor. Select one of the three options:

Always
Once
Given




***Variable Parameters***




***Start*** Enter the time starting point for the experiment

***Stop*** Enter the time ending point for the experiment

***Step*** Enter the time interval (in seconds) the measurement is acquired at

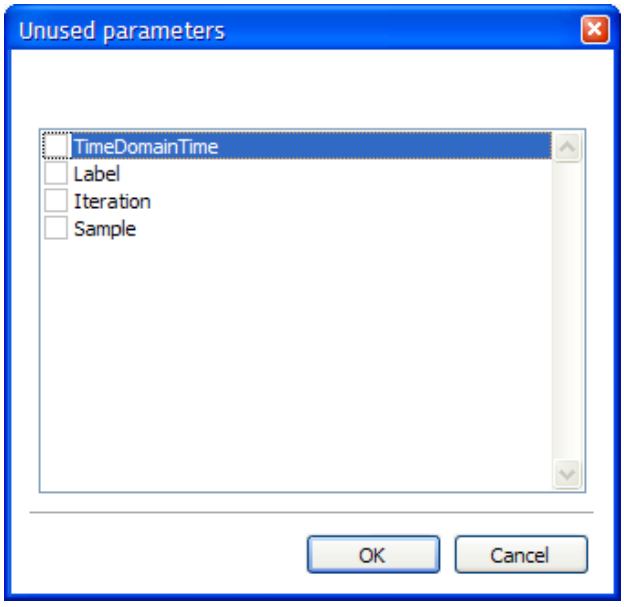
***Custom*** Edit the list of the acquisition time intervals

Additional variable experimental parameters can be added by right-clicking in the <Variable Parameters> area. When doing so, the <Add> button is displayed.

***Add***

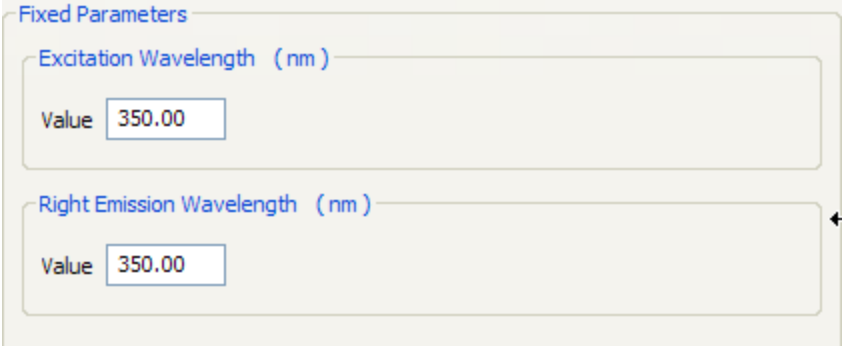
By right-clicking in the Variable Parameters area, additional experimental parameters can be added to make a “multi-dimensional experiment”.

The desired parameter is selected by clicking on the checkbox.




Similarly, the parameters can be removed from the area.

***Fixed Parameters***




Enter the wavelength position for the excitation and emission monochromators.

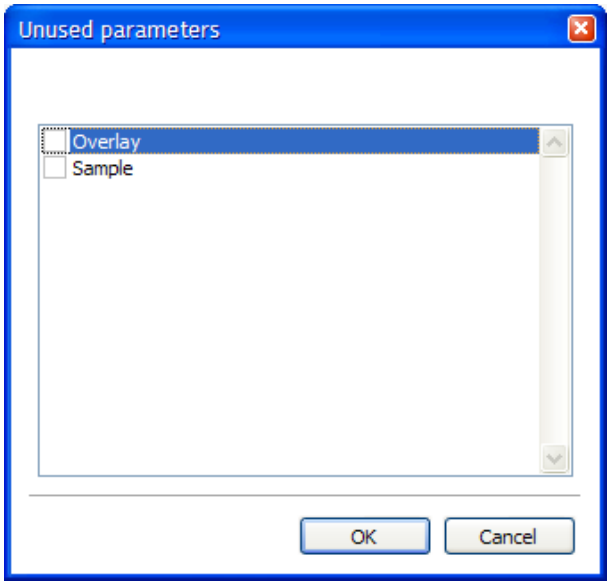
***Value***

If the acquisition is made on the left emission channel, the <Right Emission Wavelength> parameter is not displayed.

Additional variable experimental parameters can be added by right-clicking in the <Fixed Parameters> area. When doing so, the <Add> button is displayed.

***Add***

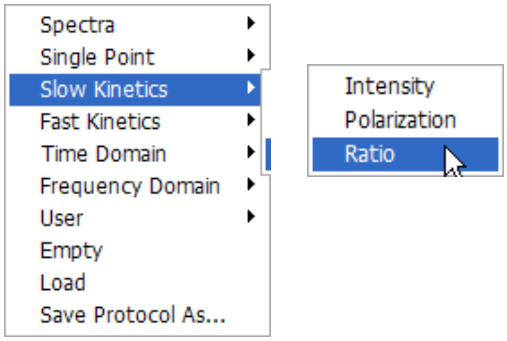
By right-clicking in the Fixed Parameters area, additional experimental parameters can be added to make a “multi-dimensional experiment”.



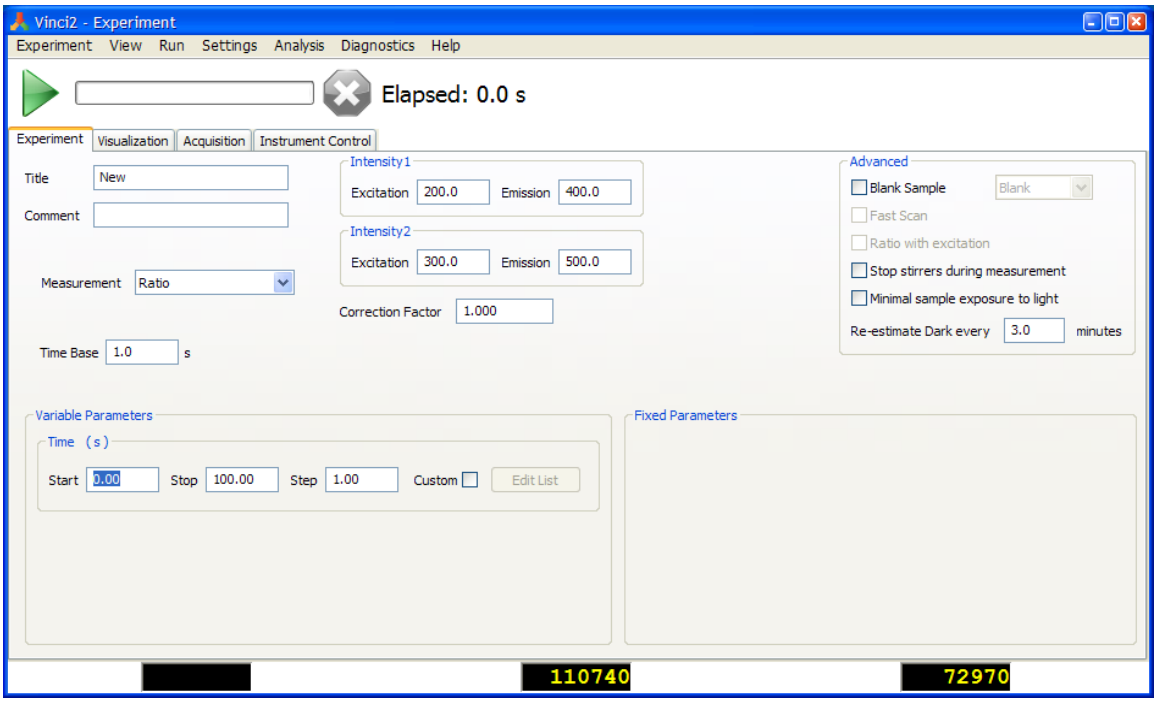

For the <Advanced Parameters> area see 19.8.5 below.

### 19.8.3 Ratio Kinetics

In the Vinci Experiment window, go to the <Experiment> menu on the top bar menu, select <Slow Kinetics> and choose <Ratio>.



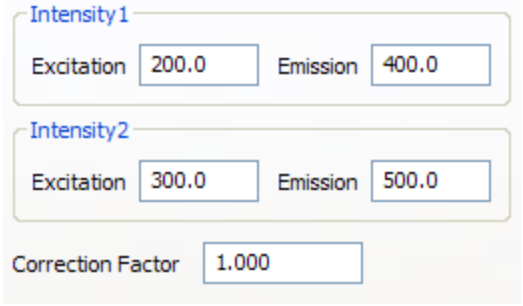

The experiment window is displayed:




This area includes the parameters to be selected for the measurement acquisition; that is:

***Measurement:*** The type of measurement. The default for this window is <Ratio>

***Time base (s):*** The time base of the data acquisition (the default is 1 sec).



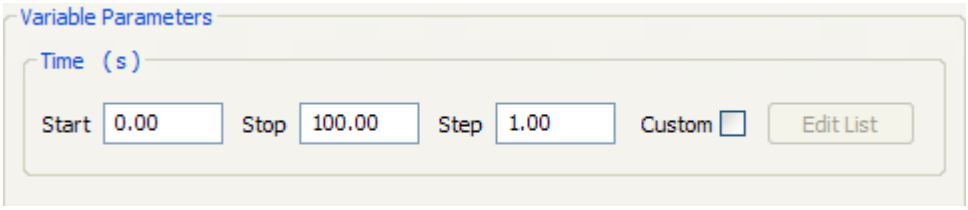

***Intensity1:***

***Intensity2:***

Enter the position of the excitation and emission monochromators, where the intensity has to be measured.

Enter the correction factor for the emission monochromator at that wavelength

***Variable Parameters***




***Start*** Enter the time starting point for the experiment

***Stop*** Enter the time ending point for the experiment

***Step*** Enter the time interval (in seconds) the measurement is acquired at

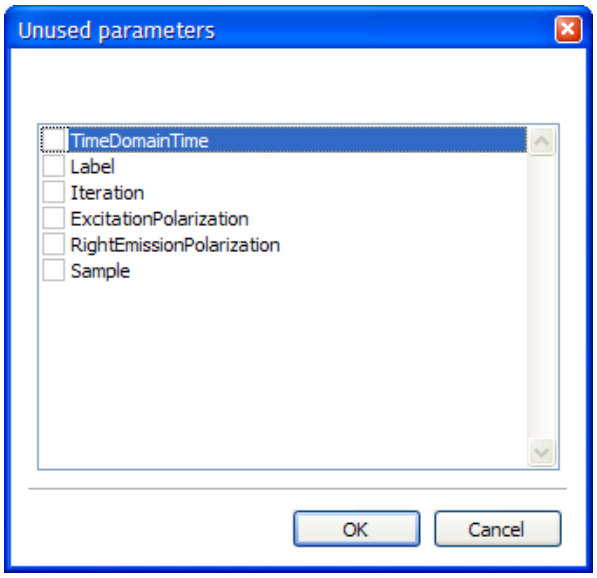
***Custom*** Edit the list of the acquisition time intervals

Additional variable experimental parameters can be added by right-clicking in the <Variable Parameters> area. When doing so, the <Add> button is displayed.

***Add***

By right-clicking in the Variable Parameters area, additional experimental parameters can be added to make a “multi-dimensional experiment”.

The desired parameter is selected by clicking on the checkbox.




Similarly, the parameters can be removed from the area. For the <Advanced Parameters> area see 19.8.5 below.

### 19.8.4 Sample Identification Area

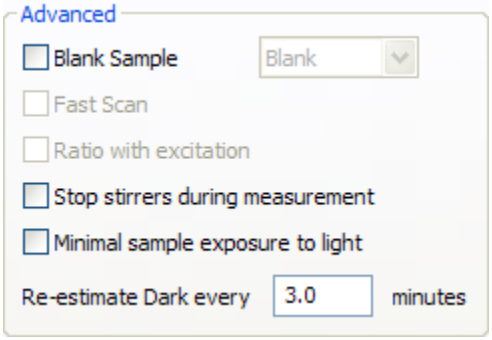
This area includes the parameters to be selected for the measurement acquisition; that is:

***Title:*** Enter an alphanumeric title

***Comment:*** Enter additional identification comments

### 19.8.5 Advanced Parameters Area

The parameters in this area are only changed sporadically.




***Blank Sample*** When checked, a measurement of the blank is acquired (this feature works best with a cuvette holder that holds more than cuvette; when using 1-cuvette holder, the sample has to be replaced manually)

***Ratio with Excitation*** When checked, the instrument acquires a signal from the reference (excitation) channel in order to automatically correct the emission for any variation of the excitation light intensity.

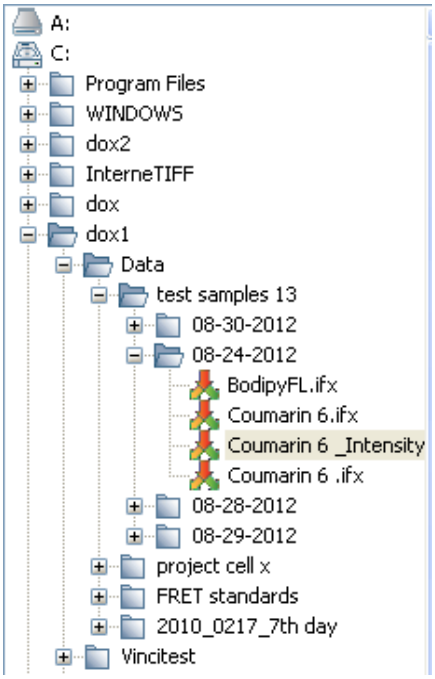
| | |
|---|---|
| ***Stop Stirrers during measurements*** | When checked, the stirrers are stopped during the measurement |
| ***Minimal exposure to light*** | When checked, the shutters automatically close at the end of the measurement. |
| ***Re-estimate Dark every xx minutes*** | The default is 3 minutes; that is, the dark counts from the detector are automatically acquired (and stored) after the time set in this field. |

# 20. Data Analysis

Data analysis is performed by fitting the acquired data to a model using the Levenberg-Marquardt algorithm.

## 20.1 Lifetime Data

Start Vinci and double-click on the data file you intend to analyze.

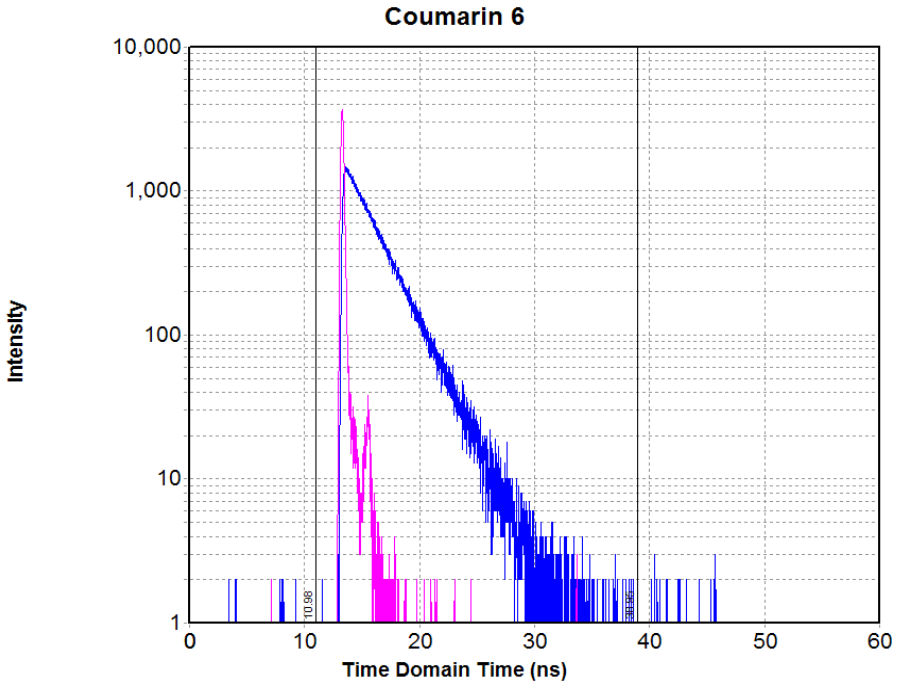



The file is open along with the IRF (if acquired). One can visualize the IRF only by clicking on IRF in <Left Y Axis>


### 20.1.1 Selecting the region to analyze

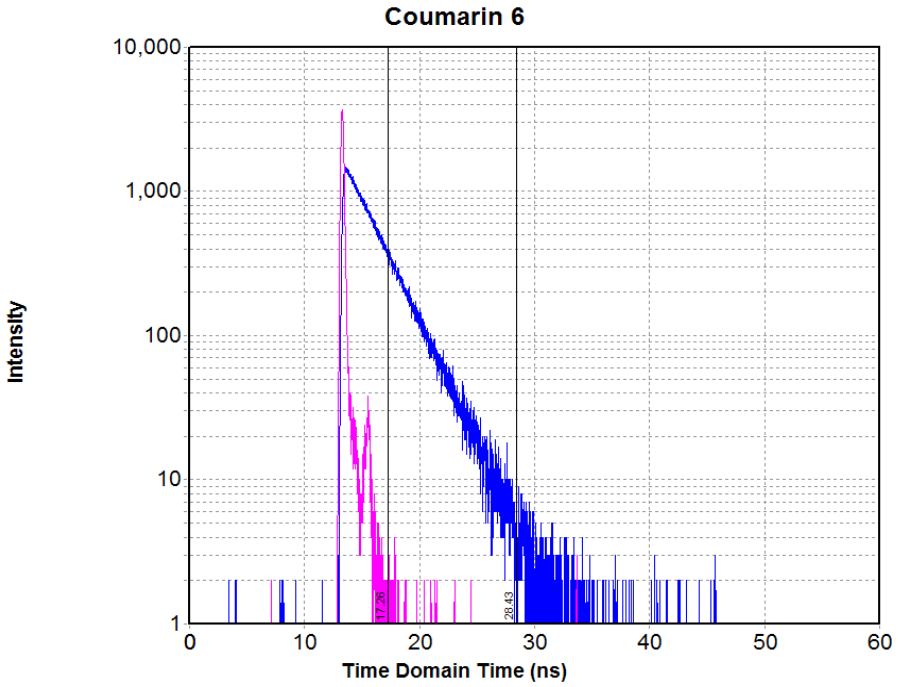
Click on <Show Markers> in order to have the two lines identifying the area to be analyzed on the plot.

Each marker can be moved by left-clicking on it and dragging it to the left or to the right. If the IRF has to be included, the left marker has to be positioned to the left of the plot, prior to the starting of the signal.




### 20.1.2 Tail fitting

Tail fitting allows for analysis of the decay without taking into consideration the IRF. The approach is feasible when the IRF is short comparable to the decay times measured. The example below shows the position of the selection bars for tail fitting: only the data between the bars are utilized in the analysis.



### 20.1.3 Selecting the fitting model

Select <Fitting> on the top row of the software and then <Lifetimes>; the window of Figure 20.1 is displayed.
Parameters can be fixed <F> or variable <V>. If one expects that a parameter be fixed, it is recommended to fix it in order to reduce the number of degree of freedom of the $\chi^2$-function.

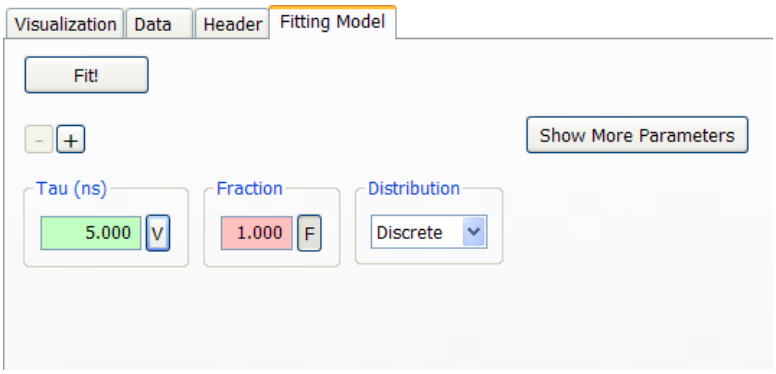



**Figure 20.1** Select lifetime fitting in Vinci Analysis

Select a proper fitting model. As a general approach, follow the sequence outlined below:

- It is a good approach to start with the simplest model, in this case a single exponential decay. The table looks like the one displayed in Figure 20.1 above. The value of the lifetime is variable (it needs to be determined); the default value (5 ns) is a seed value. The fractional distribution is set to <one> and <fixed> as we have in this model one decay only. Click on the <Fit!> button and look at the value of the $\chi^2$-function as well as to the general trend of the residuals.

- If the value of the $\chi^2$-function is not close to one, add a second component by clicking to the <+> button (see Figure 20.2). The page looks like the one displayed in Figure 20.3 below. Now, the two decay times are to be determined (the fields are in green) as well as one of the fractional distribution (the second component is $f_2 = 1 - f_1$). Click on the <Fit!> button and look at the value of the $\chi^2$-function as well as to the general trend of the residuals.

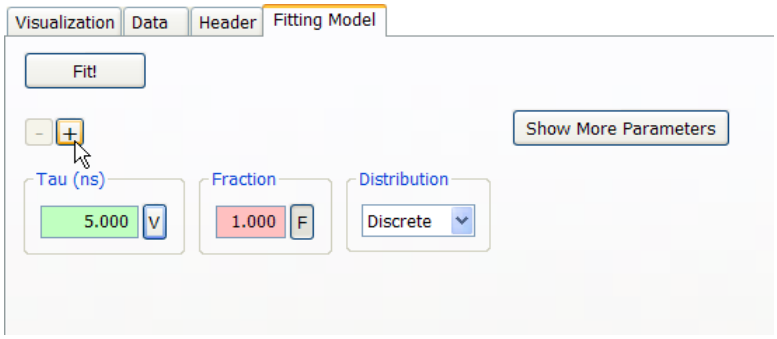



**Figure 20.2** Adding a second component to the analysis.

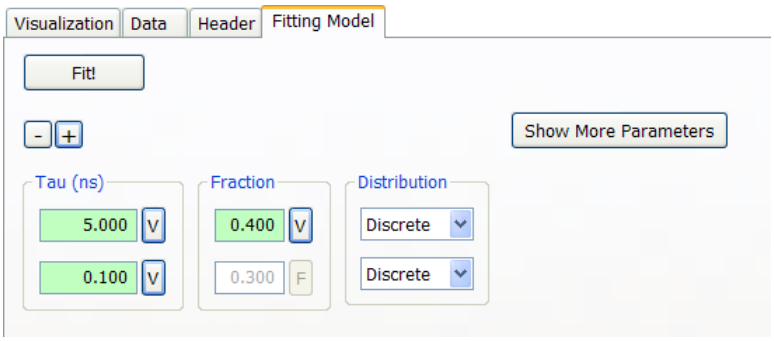



**Figure 20.3** Fitting page for a 2-component decay in Vinci Analysis.

- If the value of the $\chi^2$-function is not close to one, add a third component by clicking to the <+> button again. Follow the same procedure in checking the value of the $\chi^2$-function as well as to the general trend of the residuals.

Vinci allows for a maximum of four (4) decay times. A practical judgment has to be made before concluding that a decay has 3 or more components. Please consult the section 24.6 below for a general outline of the analysis algorithms used in Vinci.

### 20.1.4 Fitting the data with a distribution of decay times

In many instances, the decay times in complex systems are best analyzed assuming a distribution of decay times rather than a sum of exponential (discrete) decays.

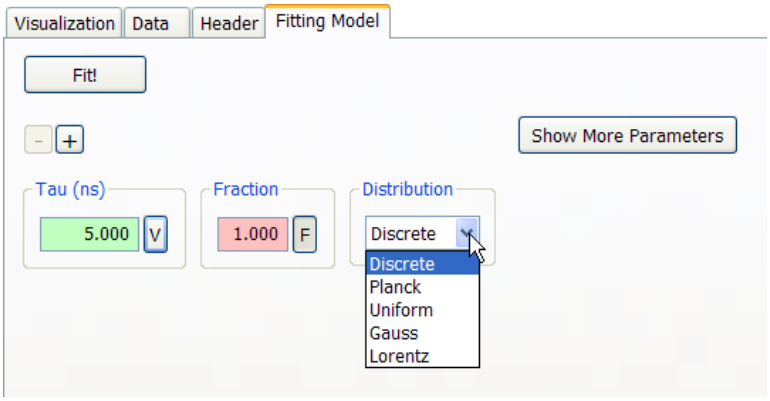



**Figure 20.4** Fitting page for a 2-component decay in Vinci Analysis.

In this case, click on the drop-down window for <distribution> and select the distribution you assume the decay follows. Four different distributions are coded in the analysis software: Planck, Uniform, Gaussian, Lorentzian (see section 24.7.1).
Now, an additional parameter is minimized, the width of the distribution.

### 20.1.5 Fit results

The fitting results for the data set acquired on a solution of Coumarin 6 in ETOH are shown in Figure 20.5 below. Data are fitted using a single exponential decay; the decay time is 2.5 ns.

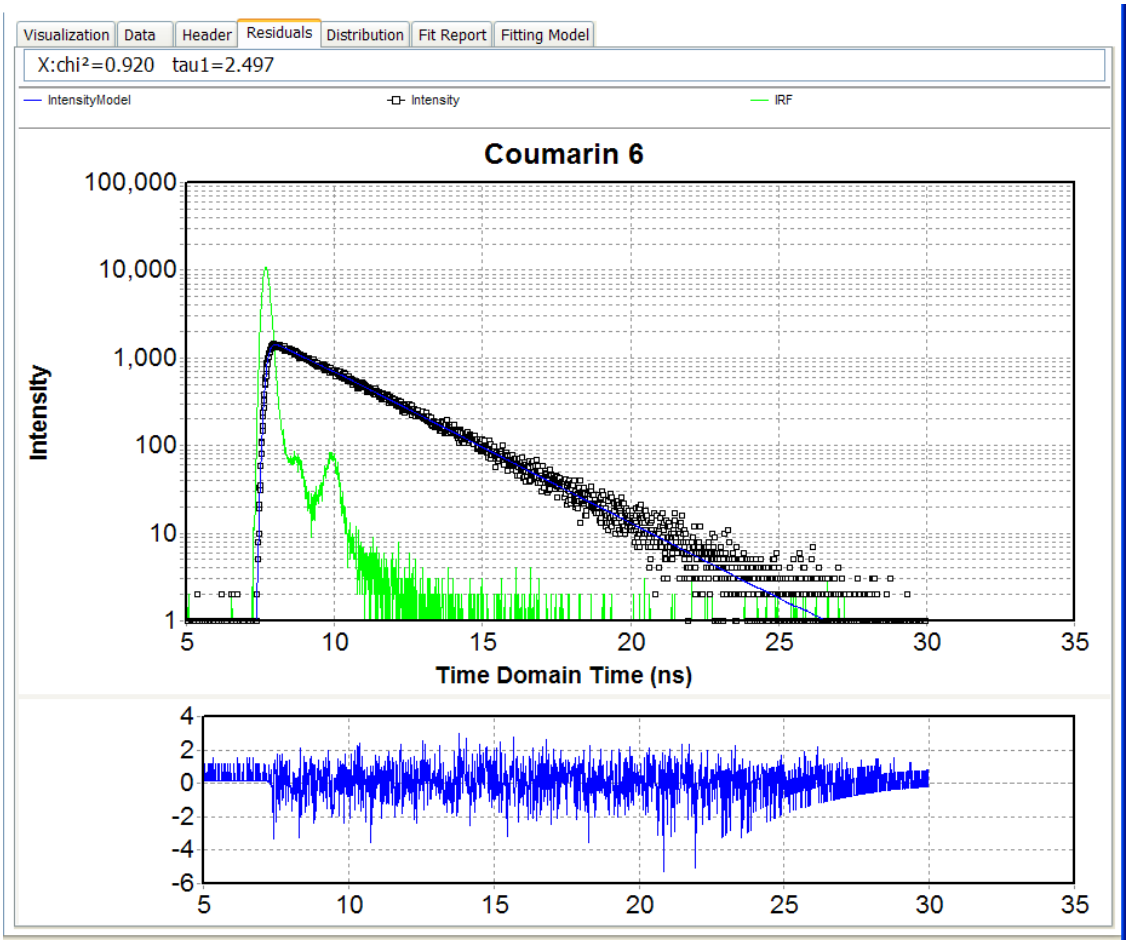



**Figure 20.5** Decay time for Coumarin 6 in ETOH. The data fit to a single exponential decay of 2.5 ns. The bottom plot shows the residuals of the analysis: each point is the difference between the measured points and the fitted curve. If the fit is a good representation of the data, the residuals are distributed around the zero.

### 20.1.6 Fit report

An analysis report is generated by Vinci; click on <Fit Report> to access it. The report can be saved as a pdf-file or a HTML-file for easy electronic transmission. The Fit Report includes the following information:

- Data file title
- Analysis date and time
- Lifetime determined by the fit
- Value of the ratio
- Value of the offset
- Value of the time shift
- Value of the $\chi^2$-function
- Correlation matrix
- Fitted data plot

- Residuals plot
- Lifetime distribution plot
- Decay model plot
- Numerical values of the fit

An example of report for the data showed in Figure 20.5 is reported below.

The first page of the report includes the relevant information about the data file analyzed and the results of the analysis along with the matrix of correlation coefficients.

The second page displays the plot and the plot of the residuals.

The third page displays the distribution of lifetimes (a bar in case of exponential decay) and the theoretical model of the decay associated with the analysis.

The fourth page, and the following pages, includes the numerical values arranged in columnar format.

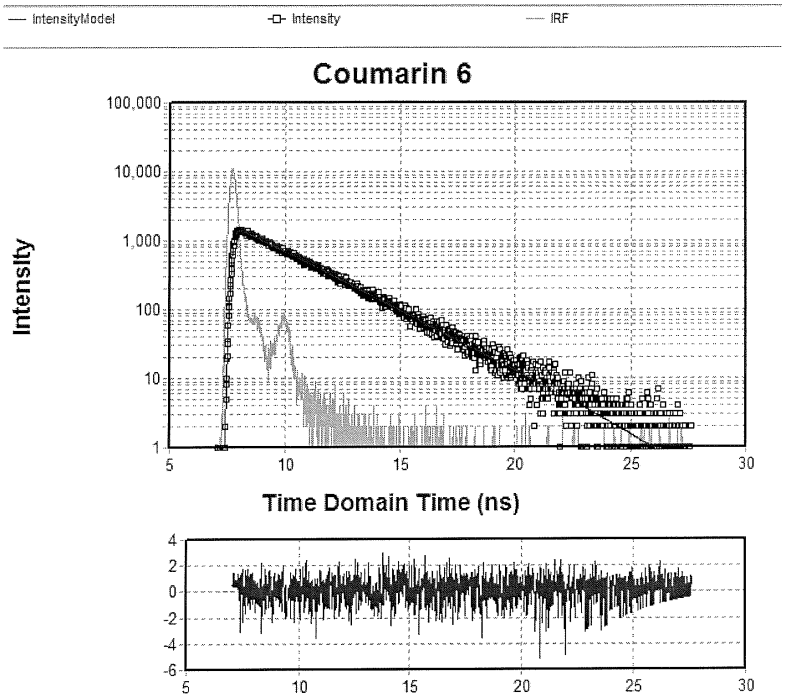
**Vinci Analysis - Fitting Report of**
**C:\dox1\Data\test samples**
**13\08-24-2012\Coumarin 6 ETOH.ifx**
**Nov 14 2012 14:03 PM**

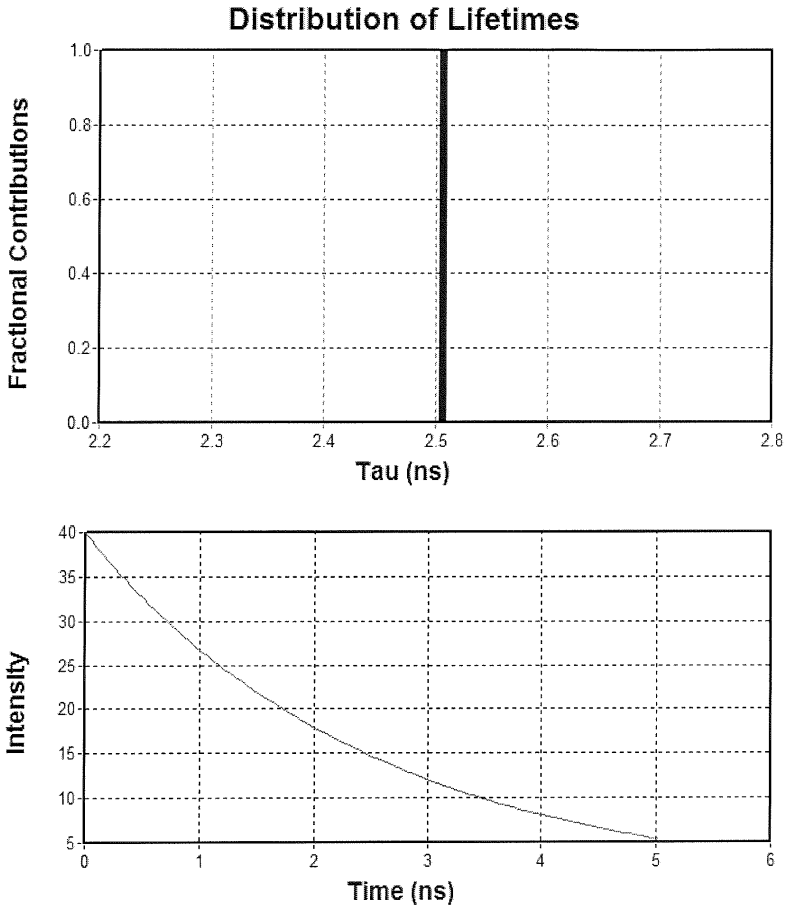
Title: Coumarin 6

| **Lifetime** | [ns] | |
|---|---|---|
| $\tau_1$ | 2.51 | ± 0.005 |
| **ratio** | 1.27 | ± 0.002 |
| **offset** | -0.628 | ± 0.06 |
| **time Shift** | 0.0241 | ± 0.001 |
| $\chi^2$ | 1.03 | |

***Correlation Matrix***

| | $\tau_1$ | **ratio** | **offset** | **time Shift** |
|---|---|---|---|---|
| $\tau_1$ | 1.0 | 0.086 | -0.47 | -0.25 |
| **ratio** | 0.086 | 1.0 | -0.19 | -0.023 |
| **offset** | -0.47 | -0.19 | 1.0 | 0.13 |
| **time Shift** | -0.25 | -0.023 | 0.13 | 1.0 |


IntensityModel
Intensity
IRF
Coumarin 6
Intensity
100,000
10,000
1,000
100
10
1
5
10
15
20
25
30
Time Domain Time (ns)
4
2
0
-2
-4
-6




After the graphical pages, the numerical pages include four columns, respectively:

| | |
|---|---|
| ***Time Domain Time*** | this is the time (associated with each bin) |
| ***Intensity*** | the number of photons in the specific time bin |
| ***Intensity Model*** | the convolution of the IRF with the model selected for the data analysis |
| ***Intensity Residuals*** | the scaled residuals, that is the difference between the convolution result of the model with the IRF and the <Intensity> column, divided by the error |

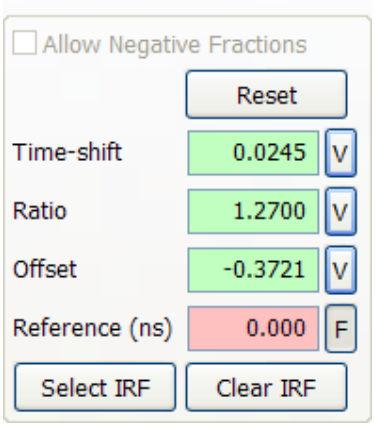
For the example below, about 47 pages have been cut for editorial reasons, the cut being represented by the mark in the middle of the page.

| Time Domain Time | Intensity | Intensity Model | Intensity Residual |
|---|---|---|---|
| 7.01154076867 | 0.0000000 | -0.46875326 | 0.46875326 |
| 7.02375599649 | 0.0000000 | -0.46352050 | 0.46352050 |
| 7.03597122431 | 0.0000000 | -0.46414813 | 0.46414813 |
| 7.04818645213 | 0.0000000 | -0.45893775 | 0.45893775 |
| 7.06040167995 | 0.0000000 | -0.45975950 | 0.45975950 |
| 7.07261690777 | 1.0000000 | -0.46057726 | 1.4605773 |
| 7.08483213559 | 0.0000000 | -0.46121920 | 0.46121920 |
| 7.09704736341 | 0.0000000 | -0.45585119 | 0.45585119 |
| 7.10926259123 | 0.0000000 | -0.45068112 | 0.45068112 |
| 7.12147781905 | 0.0000000 | -0.45154299 | 0.45154299 |
| 7.13369304687 | 0.0000000 | -0.45240068 | 0.45240068 |
| 7.14590827469 | 1.0000000 | -0.45325419 | 1.4532542 |
| 7.15812350251 | 0.0000000 | -0.45410356 | 0.45410356 |
| 7.17033873033 | 0.0000000 | -0.45477696 | 0.45477696 |
| 27.3865407724 | 0.0000000 | 0.43526596 | -0.43526596 |
| 27.3987560002 | 0.0000000 | 0.43009913 | -0.43009913 |
| 27.410971228 | 1.0000000 | 0.42495740 | 0.57504260 |
| 27.4231864559 | 0.0000000 | 0.41984066 | -0.41984066 |
| 27.4354016837 | 0.0000000 | 0.41474878 | -0.41474878 |
| 27.4476169115 | 0.0000000 | 0.40968164 | -0.40968164 |
| 27.4598321393 | 0.0000000 | 0.40463913 | -0.40463913 |
| 27.4720473671 | 0.0000000 | 0.39962112 | -0.39962112 |
| 27.484262595 | 1.0000000 | 0.39462749 | 0.60537251 |
| 27.4964778228 | 0.0000000 | 0.38965813 | -0.38965813 |
| 27.5086930506 | 0.0000000 | 0.38471291 | -0.38471291 |
| 27.5209082784 | 0.0000000 | 0.37979172 | -0.37979172 |
| 27.5331235062 | 2.0000000 | 0.37489445 | 1.1491232 |
| 27.5453387341 | 1.0000000 | 0.37002097 | 0.62997903 |
| 27.5575539619 | 0.0000000 | 0.36517117 | -0.36517117 |
| 27.5697691897 | 0.0000000 | 0.36034494 | -0.36034494 |
| 27.5819844175 | 2.0000000 | 0.35554216 | 1.1628073 |
| 27.5941996453 | 2.0000000 | 0.35076272 | 1.1661869 |

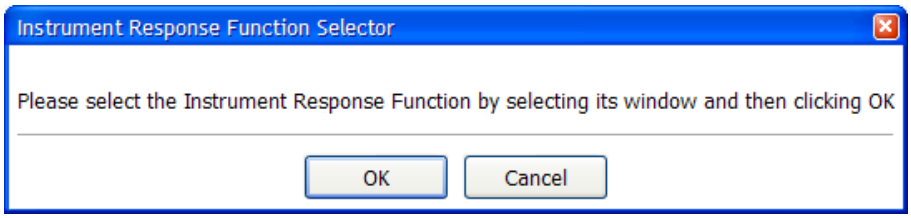
### 20.1.7 Data Analysis using an IRF acquired earlier

Sometimes the IRF is not collected. One can acquire an IRF curve and use it for the rest of the day (assuming the experimental conditions do not change).

When this is the case, click on the <Show More Parameters> button within the <Fitting Model> window:



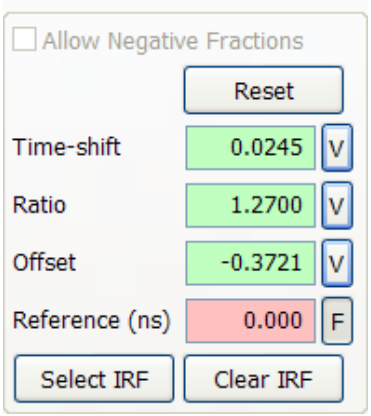

Click on <Select IRF>; the following message is displayed.




Open a plot featuring the IRF that you intend using and click on it. The analysis will be done by convoluting the data to that IRF.

### 20.1.8 Using a fluorophore as a reference solution

ChronosBH allows for using a fluorophore with known decay time in order to determine the IRF. The decay time of the reference solution is entered as a parameter in the <Show More parameters> window.




### 20.1.9 Other parameters

The other parameters that can be chosen are in the order:

| | |
|---|---|
| ***Time shift*** | The time-shift is a parameter that adjust the starting position of the IRF with respect to the starting position of the decay time data |

***Ratio***

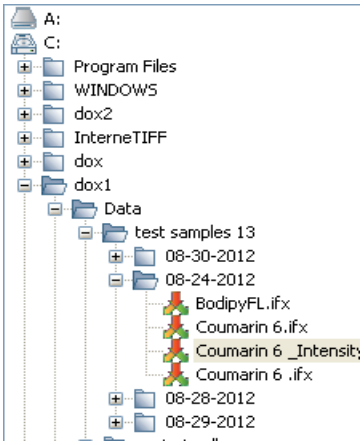
When performing the analysis, the IRF is convoluted with the model M selected by the user to describe the decay rates. The outcome of this operation is used by the software to build the $\chi^2$ function for the data minimization.
The Ratio is a parameter that scales the two sets of data; that is, the result of the convolution of the IRF with the theoretical model M and the set of acquired data.

***Offset***

Whenever the IRF signal is fairly different (number of counts) from the sample data, the offset adjusts for the different background of dark counts.

## 20.2 Anisotropy Decay Data

Start Vinci and double-click on the data file you intend to analyze.




The plot of the data showing the IRF, the parallel and perpendicular components will be displayed.

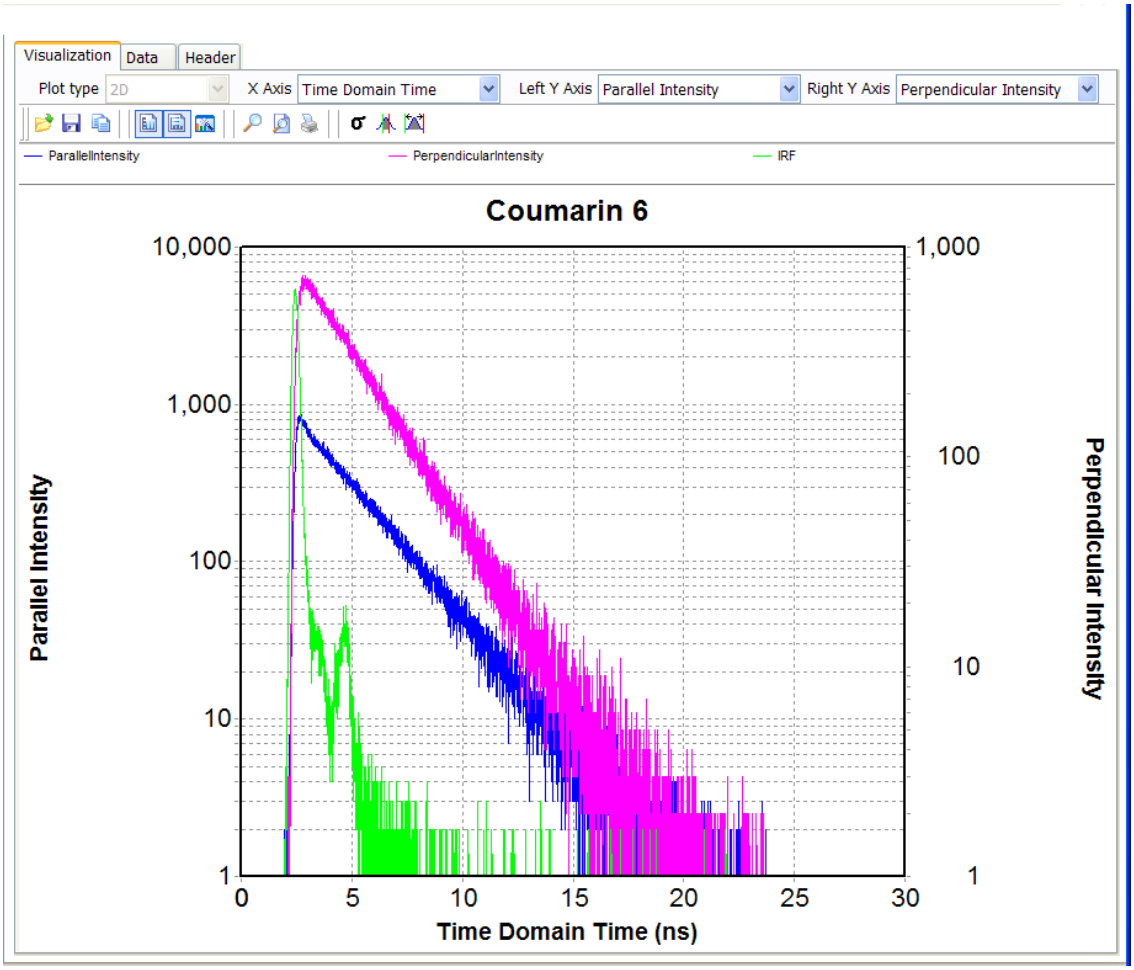



**Figure 20.6** Anisotropy decay data for Coumarin 6 in ETOH. Excitation is a laser diode emitting at 446 nm.

### 20.2.1 Selecting the fitting range

Select <Fitting> and then <Rotational Times>, the “Fitting Model” panel is displayed.

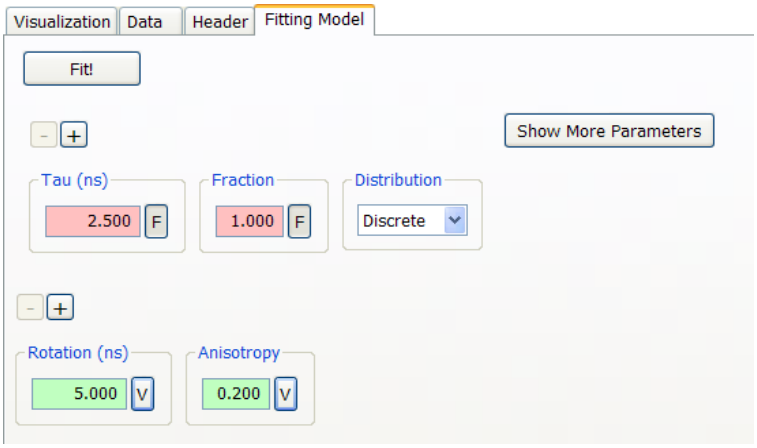



**Figure 20.7** Fitting model page.

Go to the <Visualization> window and click on <Show Markers> in order to have the two lines identifying the area to be analyzed on the plot.

Each marker can be moved by left-clicking on it and dragging it to the left or to the right. Move the cursor and select a proper range as indicated in Figure 20.8 below.

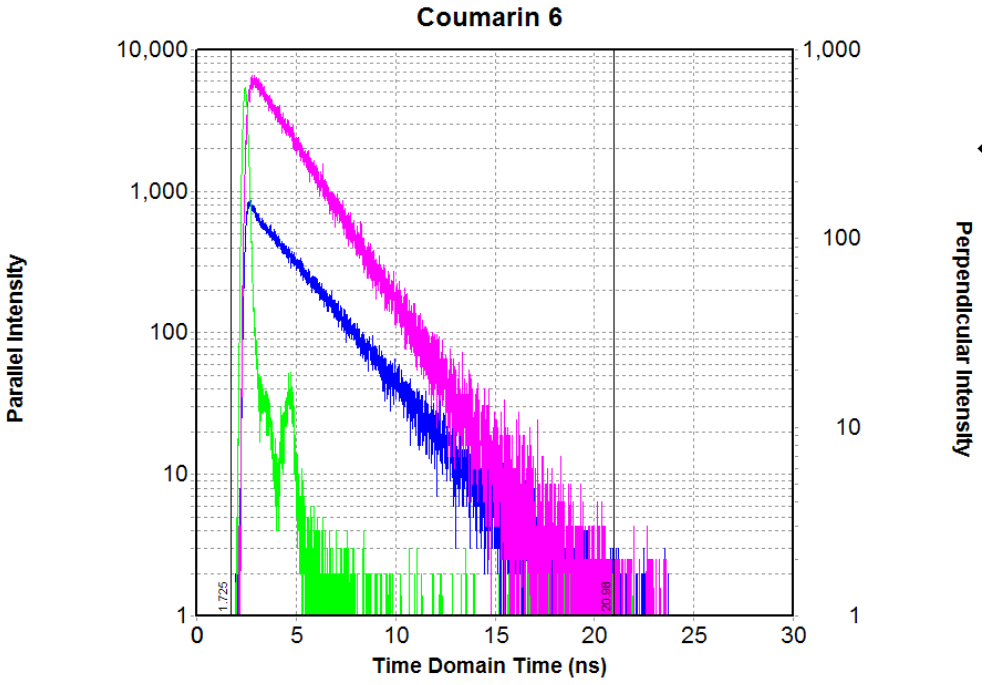



**Figure 20.8** Select a proper fitting range using the selection bars.

### 20.2.2 Selecting the fitting model

When analyzing anisotropy decay data it is convenient to fix the decay time of the fluorophore (see Figure

20.7), although, in principle it is feasible to determine the decay times as well from the equations. Just enter the decay time(s) along with the fractional contributions and set the parameters to <F> for fixed.

Select a proper rotational fitting model (see 24.7.2). Usually the rotational correlation times models are sums of exponentials:

| | |
|---|---|
| Isotropic rotator (spheroid) | Single-exponential |
| Anisotropic rotator (ellipsoid) | Two- or three-exponential decays (one for each axis) |
| Hindered rotator | One exponential decay and a value for $r_\infty$ |

The general approach to the analysis of anisotropy decay data follows guidelines similar to the analysis for the determination of decay times:

- It is a good approach to start with the simplest model, in this case a single rotator (unless it is known that the model is too simple). The table looks like the one displayed in Figure 20.7 above. The value of the lifetime is fixed (as it is determined by a separate experiment). The value of the rotational correlation time is variable (it needs to be determined); the default value (5 ns) is a seed value. The anisotropy value is variable; the default value (0.2 is a seed value).

  Click on the <Fit!> button and look at the value of the $\chi^2$-function as well as to the general trend of the residuals.

- If the value of the $\chi^2$-function is not close to one, add a second rotational component by clicking to the <+> button. The page looks like the one displayed in Figure 20.9 below. Now, the two rotational decay times are to be determined (the fields are in green) as well as their respective anisotropies. Click on the <Fit!> button and look at the value of the $\chi^2$-function as well as to the general trend of the residuals.

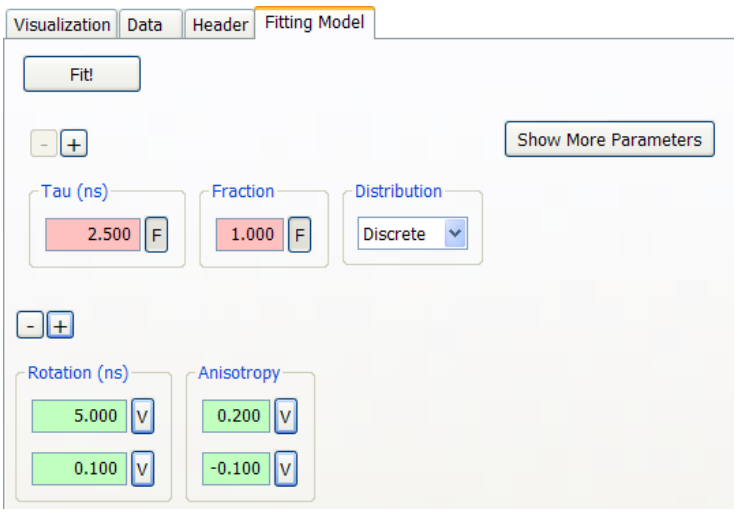



**Figure 20.9** Adding a second component to the analysis.

- If the value of the $\chi^2$-function is not close to one, add a third component by clicking to the <+> button again. Follow the same procedure in checking the value of the $\chi^2$-function as well as to the general trend of the residuals.

Vinci allows for a maximum of four (4) rotational decay times. A practical judgment has to be made before concluding that a decay has 3 or more components. Please consult the section 24.6 below for a general outline of the analysis algorithms used in Vinci.

### 20.2.3 Fitting the data with a distribution of rotational correlation times

In many instances, the rotational correlation times in complex systems are best analyzed assuming a distribution of times rather than a sum of exponential (discrete) decays.

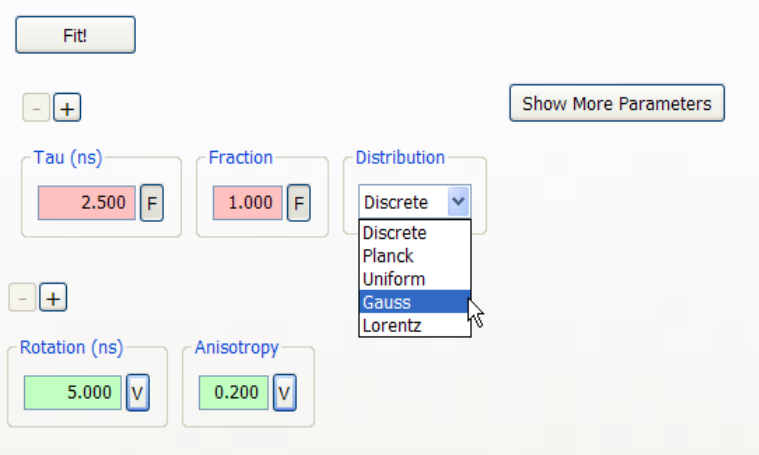



**Figure 20.10** Fitting page using a distribution of rotational correlation times in Vinci Analysis.

In this case, click on the drop-down window for <distribution> and select the distribution you assume the decay follows. Four different distributions are coded in the analysis software: Planck, Uniform, Gaussian, Lorentzian (see section 24.7.1).
Now, an additional parameter is minimized, the width of the distribution.


### 20.2.4 Fit results

For the solution of Coumarin 6 in ETOH it is reasonable to assume that the fluorophore is associated to a single rotational time. Figure 20.11 shows the fit with $r_1 = 0.35$ ns.

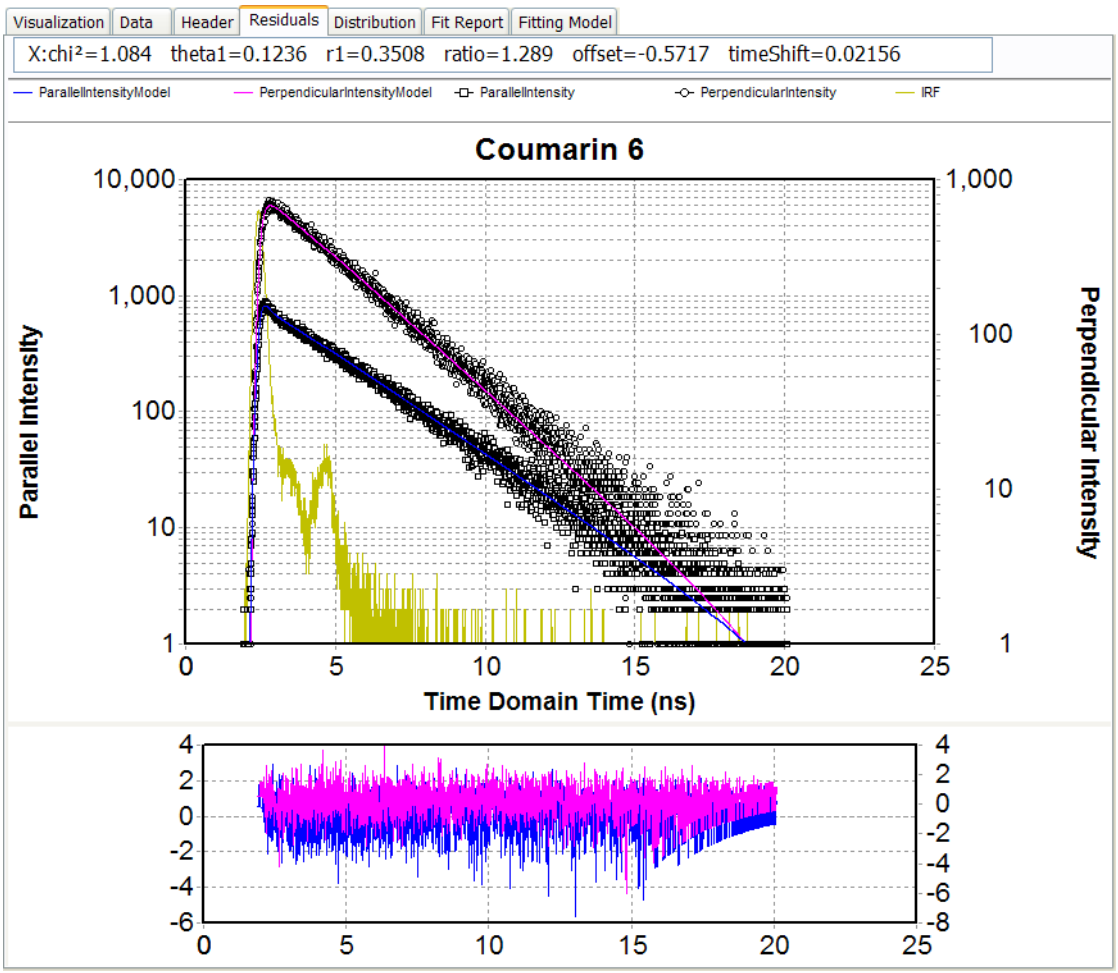



**Figure 20.11** Residuals window displaying fitting result

### 20.2.5 Fit report

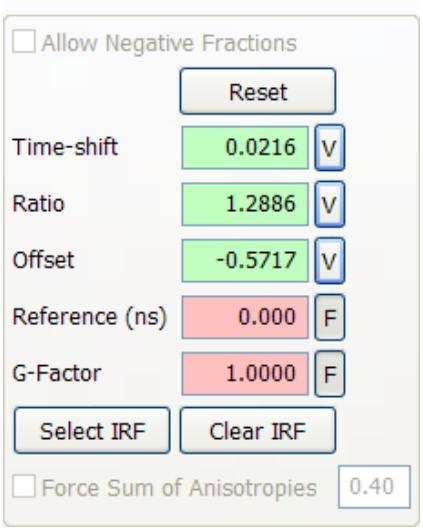
An analysis report is generated by Vinci and can be saved as a pdf-file or a HTML-file for easy electronic transmission. The report includes the following information:

- Data file title
- Analysis date and time
- Lifetime determined by the fit
- Value of the ratio
- Value of the offset
- Value of the time shift
- Value of the $\chi^2$-function
- Correlation matrix
- Fitted data plot
- Residuals plot
- Lifetime distribution plot
- Decay model plot
- Numerical values of the fit

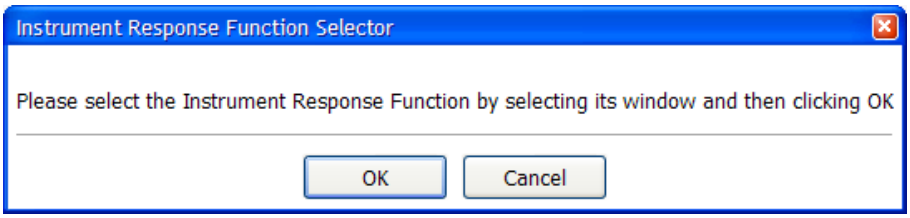
### 20.2.6 Data Analysis using an IRF acquired earlier

Sometimes the IRF is not collected. One can acquire an IRF curve and use it for the rest of the day (assuming the experimental conditions do not change).

When this is the case, click on the <Show More Parameters> button within the <Fitting Model> window:



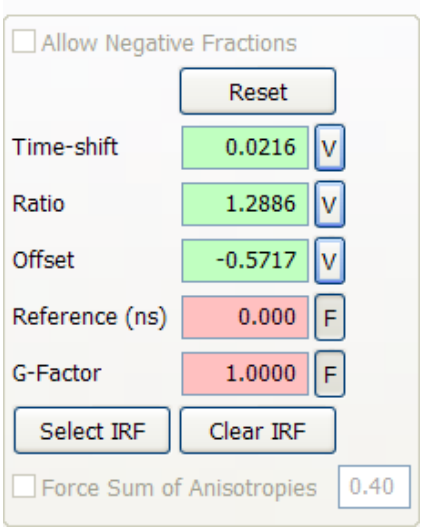

Click on <Select IRF>; the following message is displayed.




Open a plot featuring the IRF that you intend using and click on it. The analysis will be done by convoluting the data to that IRF.

### 20.2.7 Using a fluorophore as a reference solution

ChronosBH allows for using a fluorophore with known decay time in order to determine the IRF. The decay time of the reference solution is entered as a parameter in the <Show More parameters> window.



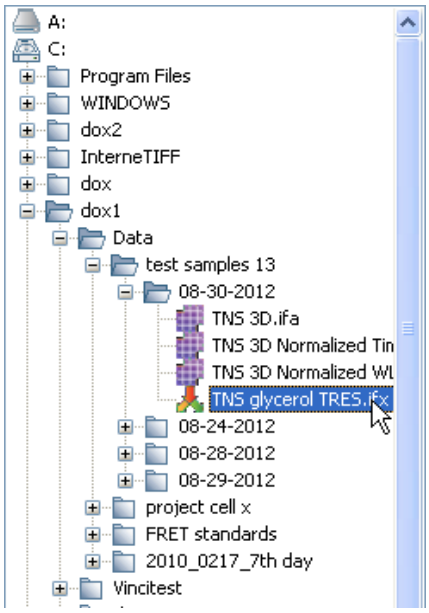
### 20.2.8 Other parameters

The other parameters that can be chosen are in the order:

| | |
|---|---|
| ***Time shift*** | The time-shift is a parameter that adjust the starting position of the IRF with respect to the starting position of the decay time data |
| ***Ratio*** | When performing the analysis, the IRF is convoluted with the model M selected by the user to describe the decay rates. The outcome of this operation is used by the software to build the $\chi^2$ function for the data minimization.<br>The Ratio is a parameter that scales the two sets of data; that is, the result of the convolution of the IRF with the theoretical model M and the set of acquired data. |
| ***Offset*** | Whenever the IRF signal is fairly different (number of counts) from the sample data, the offset adjusts for the different background of dark counts. |
| ***G-Factor*** | The G-factor of the instrument can be determined independently and entered in this field. |

## 20.3 Time-resolved spectra

The data file displayed in Figure 20.12 are retrieved for data analysis. In Vinci Analysis, click on the data file <TNS glycerol TRES>.




The data will be displayed. In the example of Figure 20.12, a total 45 decay curves have been collected between 380 nm and 600 nm (one curve every 5 nm).

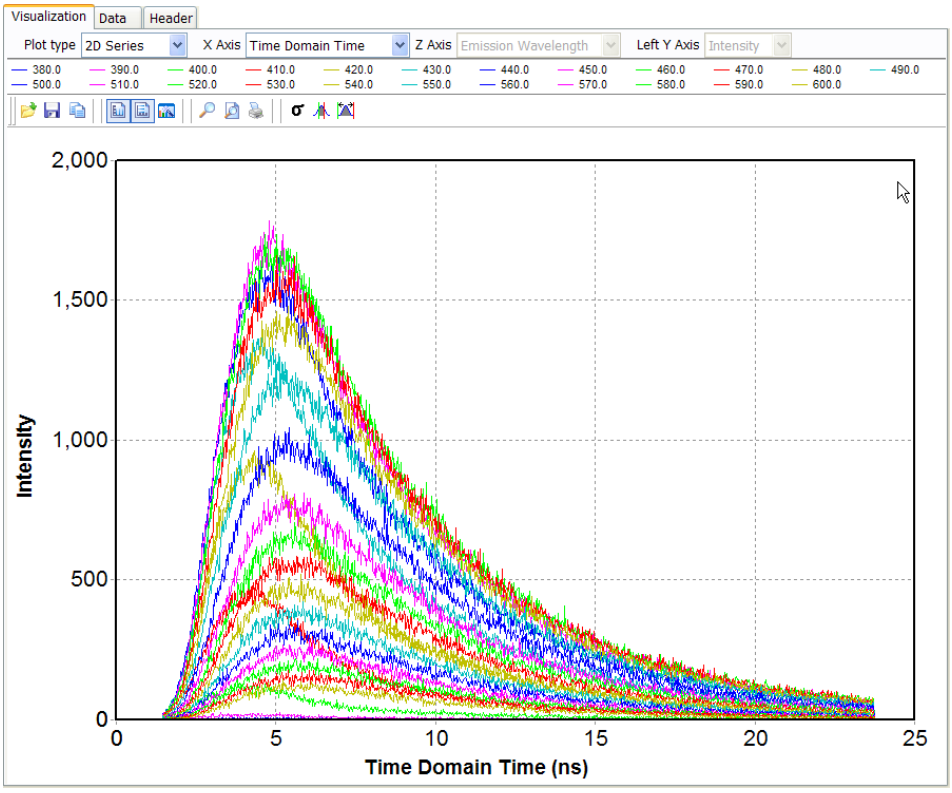
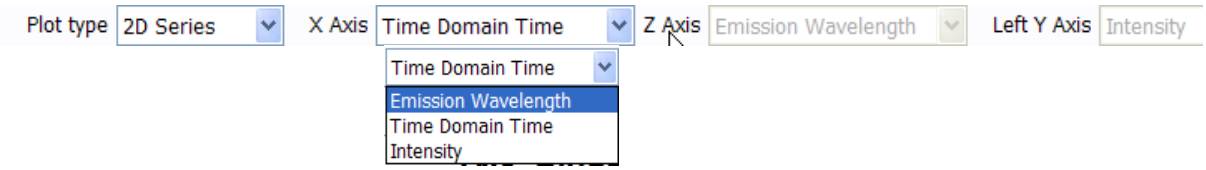



**Figure 20.12** Time-resolved spectra of TNS in glycerol; a total of 22 curves have been collected from 380nm to 600 nm; each curved is spaced by 10 nm. Excitation source is a LED emitting at 300 nm with a 10 MHz repetition rate.

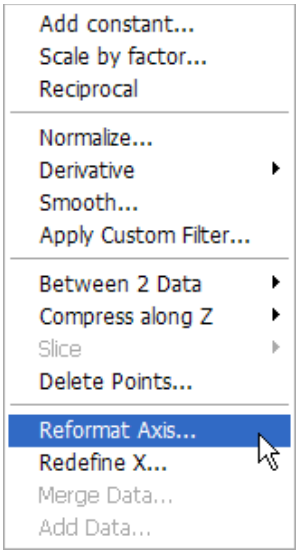
### 20.3.1 Reducing and selecting the data records to be displayed

When the file is too large, one may want to reduce the number of decay times acquired. For instance, if spectra were acquired every 10 nm, one can select a group of them spaced by 20 nm. In order to do so, in the toolbar choose the experimental parameter you want to change by selecting it under the <x Axis> menu. In this case, select <Emission Wavelength>:

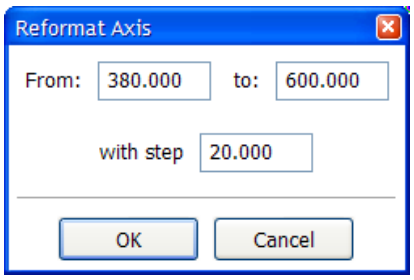



Please note that this operation may take a considerable amount of time when the number of spectra is above ten. Vinci is re-displaying the decay times (intensity versus time) as spectra (intensity versus wavelength).

Once the operation is achieved, click on the <Math> menu in the top toolbar and in the drop-down window select <Reformat Axis>.




In the <Reformat Axis> window, you can choose the spacing range of the data files; for instance, from 5 nm to 20 nm.




Vinci reduces the number of the spectra (in the example from 23 to 12). A new data set is generated and can be saved as a new file (without affecting the original data). In order to visualize the decay times, click on <X Axis> and select <Time Domain Time>. The plot of Figure 20.13 is displayed.

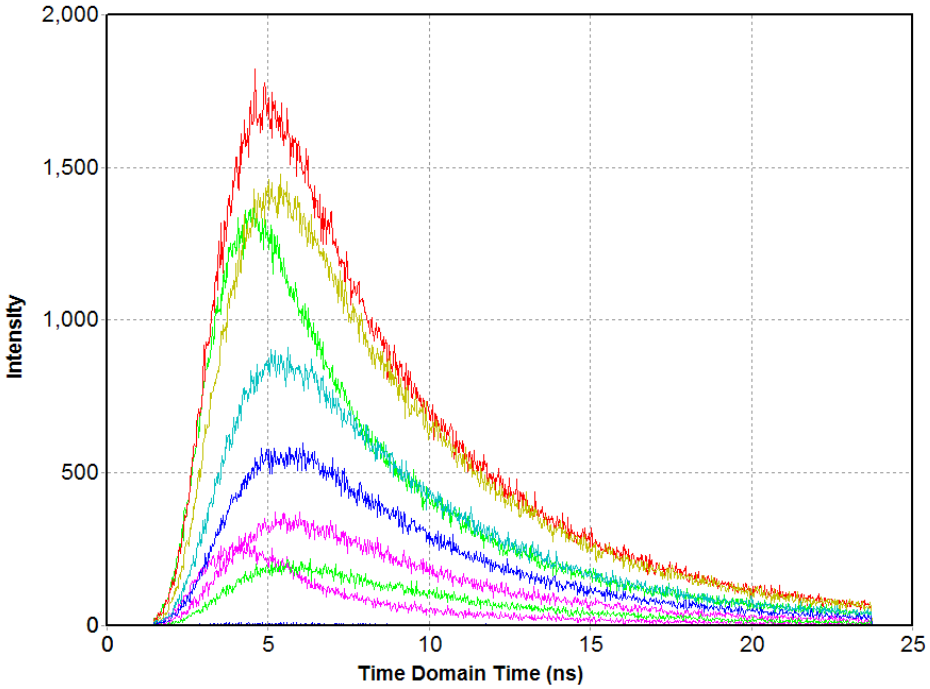
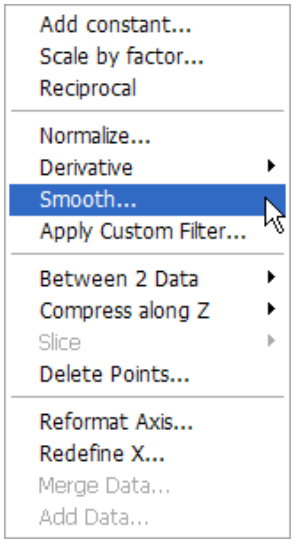



**Figure 20.13** Spectra (intensity versus wavelength); out of the original 23 spectra (each acquired every 10 nm), only 12 have been selected (every 20 nm).

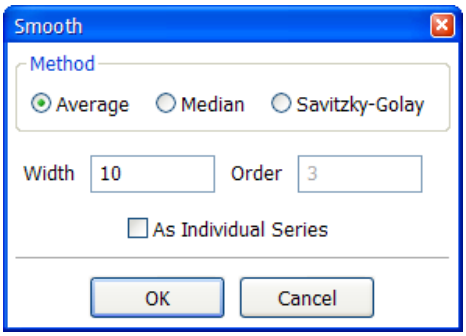
### 20.3.2 Smoothing the data

It is recommended to smooth the data file for a better presentation (alternatively one has to acquire each data file for a longer time).

Click on the <Math> menu in the top menu bar and select the <Smooth> operation.




In the Smooth window, select one of the smoothing algorithms (Average, Median, Savitzky-Golay). Set the smooothing parameters such as, Width and Order. Also, un-click the <As Individual Series> checkbox in order to apply the smoothing operation to the entire data set.
One should keep in mind that for some cases the more smoothing is applied to the original data the more affected the character of the curves that can cause analysis results (e.g. fitting results) of the smoothed data vary from the original data analysis results.




When the smoothing routine is applied to the curves, the plot displayed in Figure 20.14 is obtained.

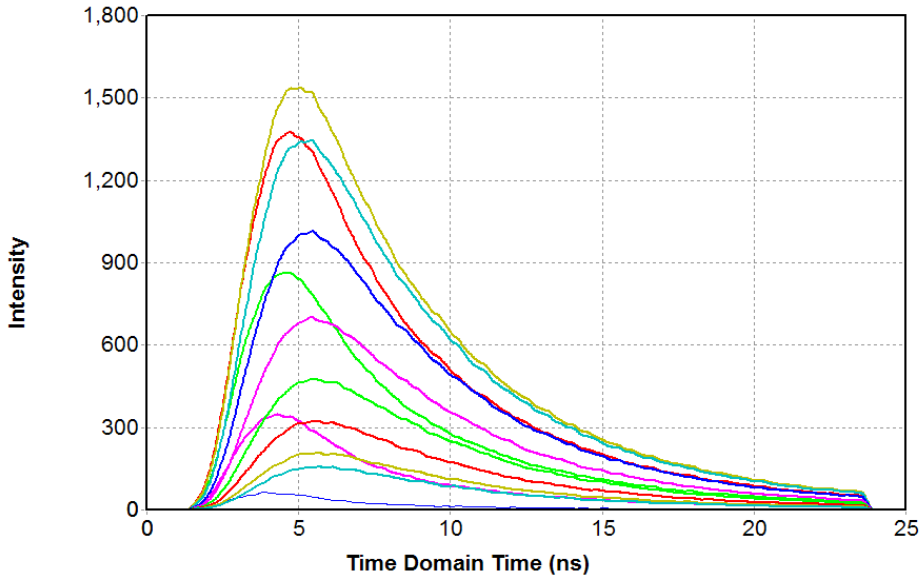
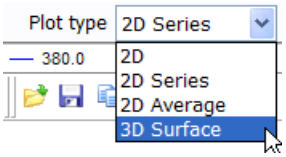



**Figure 20.14** The curves of Figure 20.13 where the smoothing routine has been applied to.

Notice that the smoothing can be rejected by clicking on the <Undo> icon. Alternatively, the new set can be saved as an independent file.

### 20.3.3 Generate the 3D surface

Open the data file and in the Plot Type, select <3D Surface>.




The following plot is displayed:

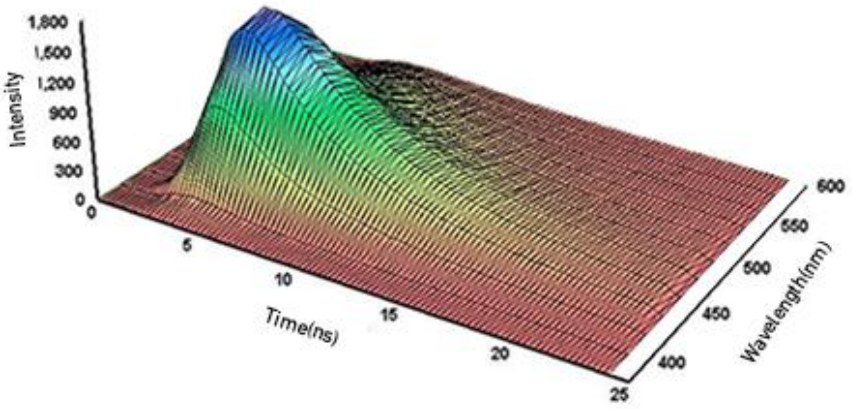


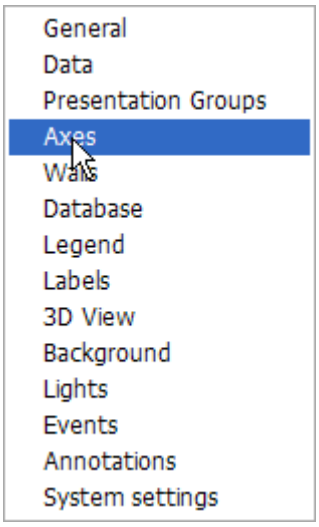

**Figure 20.15** The 3D plot for the curves of Figure 20.14

# 21. Working with Plots in Vinci

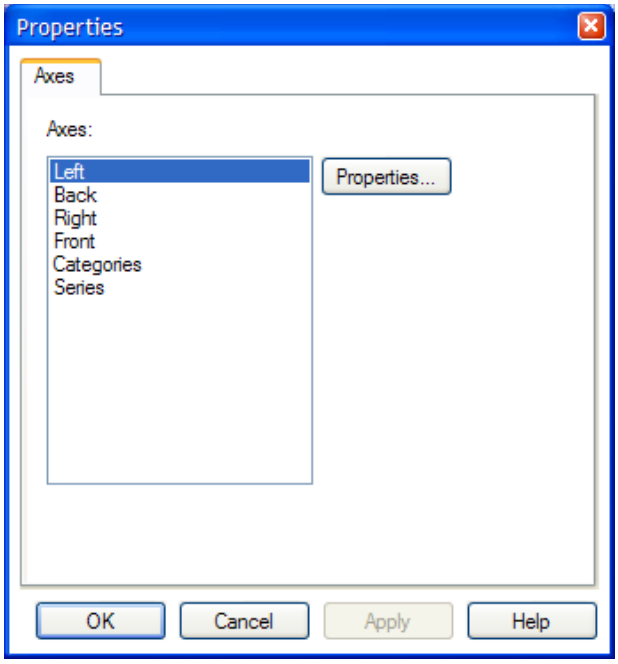
## 21.1 How to edit the 3D plot

Fonts, color, thickness of the axes can be all changed in the 3D plot. Right-click on the plot an select <Control properties>.

In the drop-down window, click on <Axes>.




The following window is displayed:




The XYZ axes of the 3D plot correspond to the following categories:

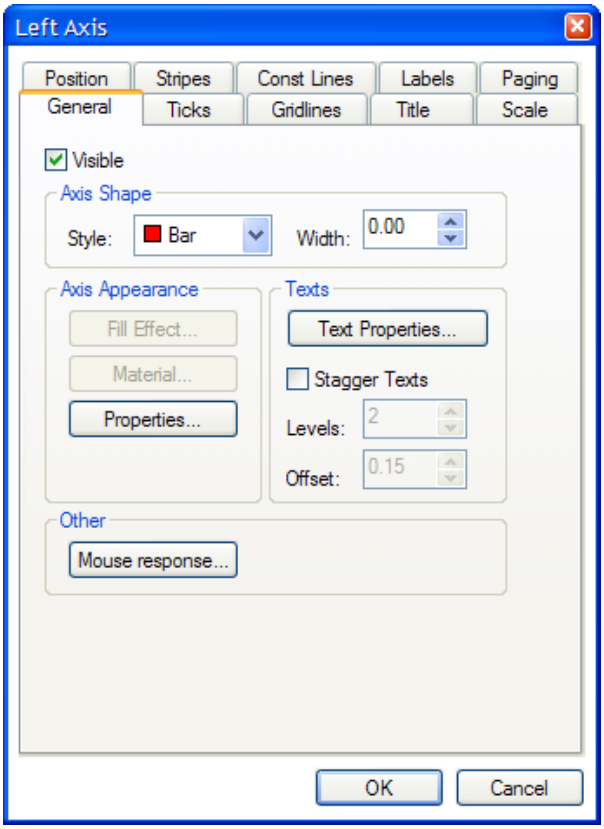
- z-axis:<Left>
- x-axis: <Categories>
- y-axis: <Series>

**Note:** disregard the <Back>, <Right> and <Front> fields.

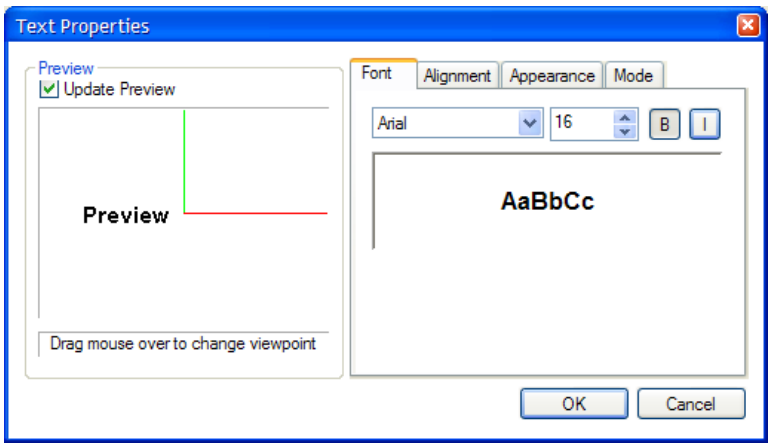
### 21.1.1 Changing the font and color of the units (numbers) on the axis

Let us start with the z-axis.

Select <Left> and click on <Properties>



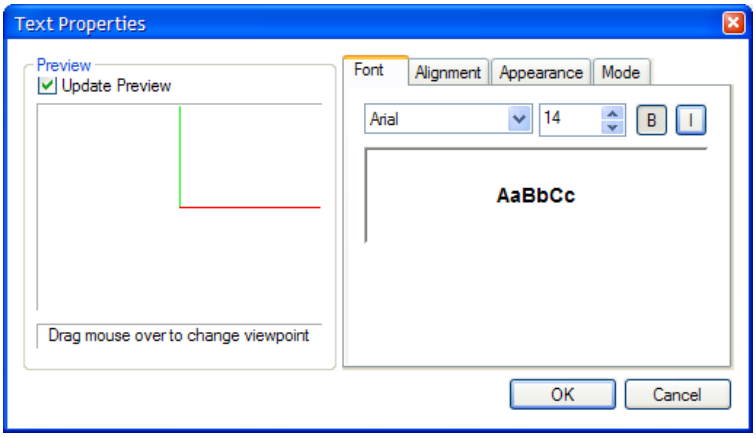

Select <Text Properties>.
Enter font and pitch desired.



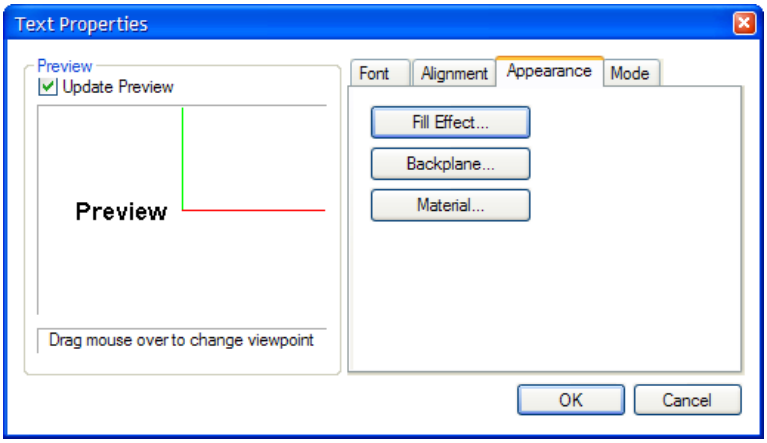

### 21.1.2 Changing the color and the font of the legend of the axis

Click on <Properties> and then click on <Title>.

Select <Font> to change the font and the pitch.

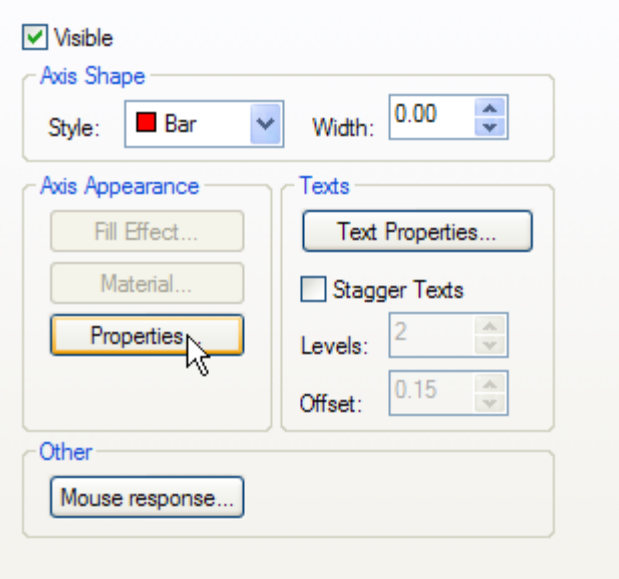



For choosing the color, select <Appearance> and then <Fill Effect>.
In this window the desired color can be selected.

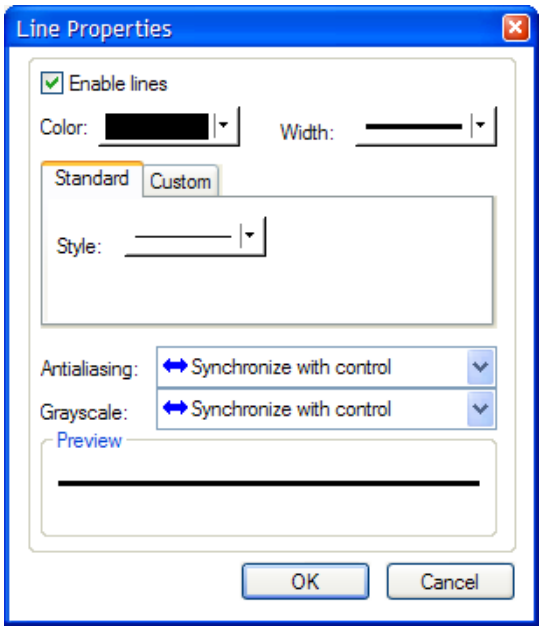



### 21.1.3 Changing the color and thickness of the axis

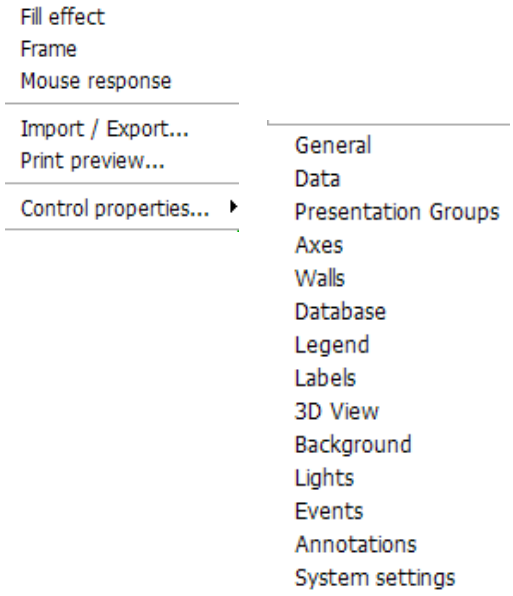
Click on <Properties> in axis appearance, select color of the axis



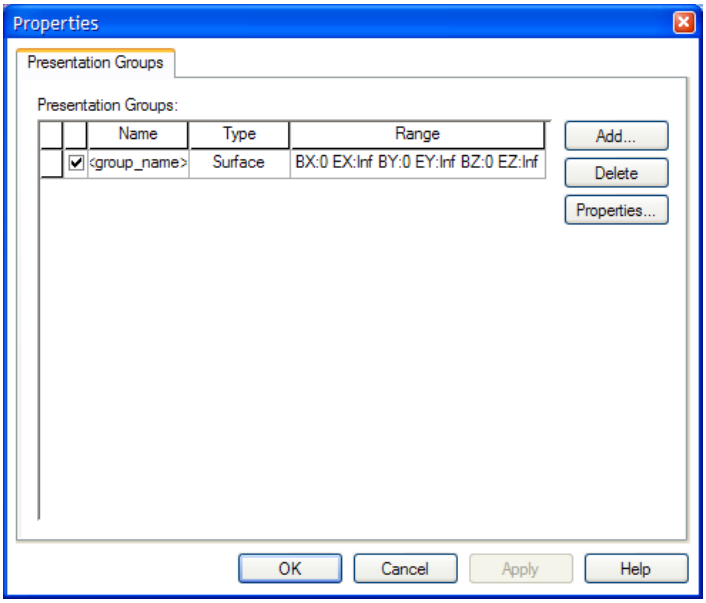
The color and the thickness can be edited in this window.




The look of the 3D plot can be greatly improved. Position the mouse on the plot and right-click on it.




Select <Control Properties> and then <Presentation Groups>; this windows allows for changing the plot styles, filling and textures.




The 3D spectrum can be enlarged/reduced and it can be rotated allowing for the user to see it from different angles. Figure 21.1

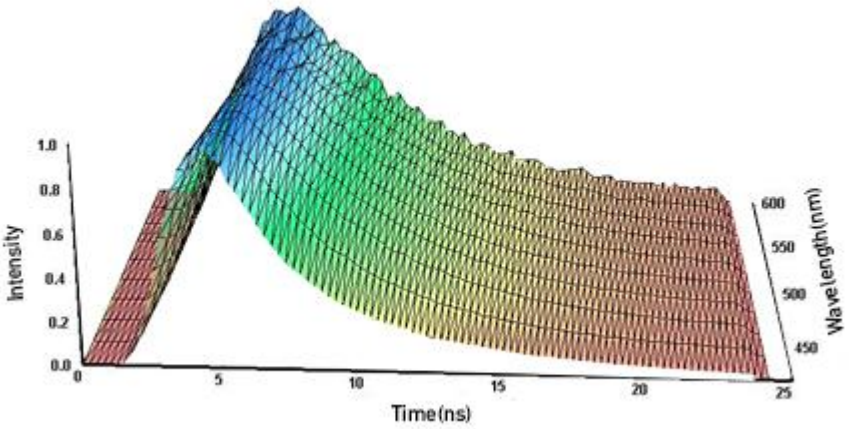
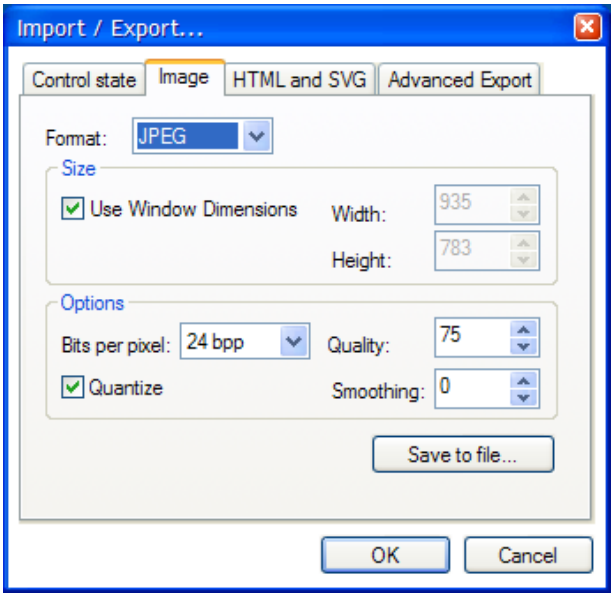



**Figure 21.1** 3D surface of TNS time-resolved spectra. The individual spectra have been normalized.

## 21.2 Saving the 3D plot

The plot can be saved in any of several popular formats (JPEG, PNG, Bitmap, Tiff). Right click on the plot and select <Import/Export>; the following window is displayed:

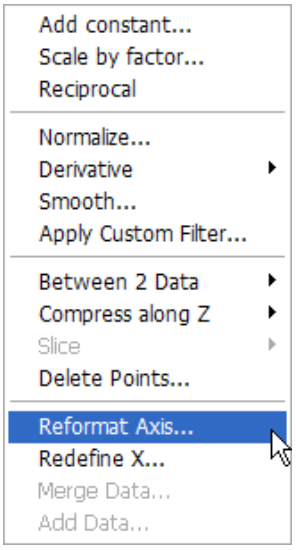
Click on <Save to file>, select the storage location and enter the filename.



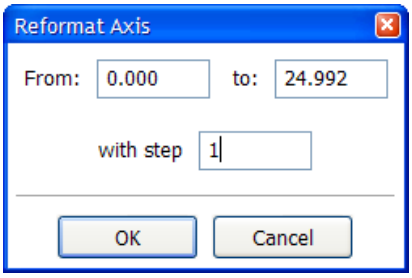

## 21.3 Generate a 2D spectrum showing the shift of the wavelength

It is sometimes interesting, when acquiring time-resolved spectra, to show the evolution of the spectra upon the excitation time.
In order to make the operation faster, it is convenient to select the times after excitation like 1, 2, 3, 4, … n seconds and delete the curves corresponding to any intermediate time. In order to do that, start from the original file containing 12 decay times (Figure 20.13). \

Select <Math> in the main menu and click on <Reformat Axis>




The x-axis reports the time. Select the time from zero to 25 ns in steps of 1 nanosecond.



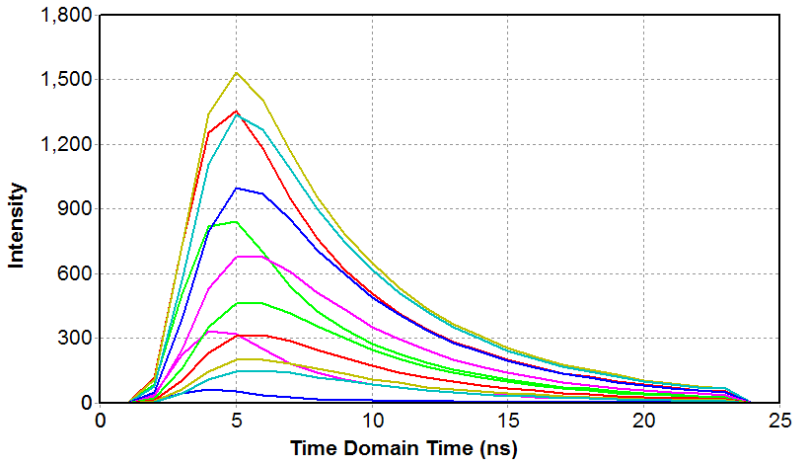
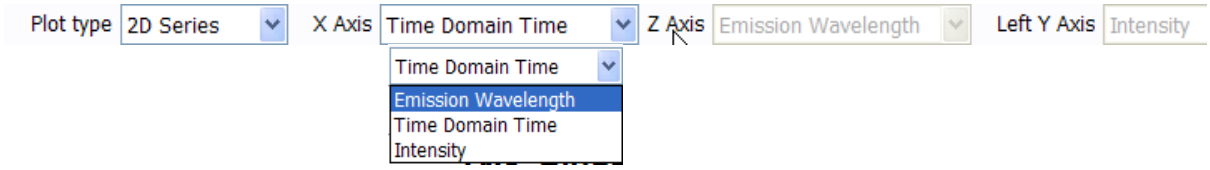



**Figure 21.2** A total number of 12 curves, where the time data are reported every 1 nanosecond.

In the <X Axis>, select <Emission Wavelength>:



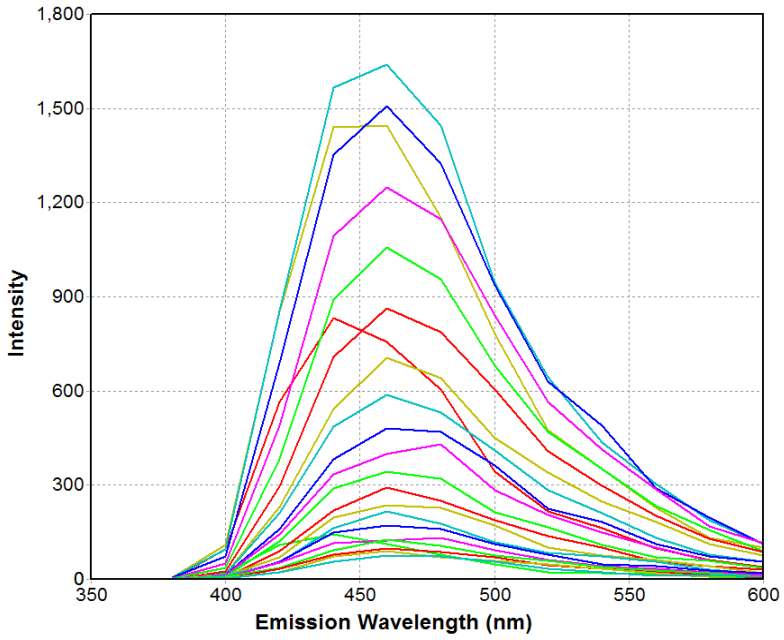
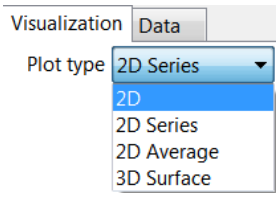




**Figure 21.3** The TNS spectra displayed along the “wavelength” dimension.

In order to show the wavelength shift you need to choose the most representative curves from the series, combine them onto a plot, normalize the curves of the interest and plot them on one graph.

Select <2D> in the Plot Type drop-down window.




To select the curve of interest, click on <Time Domain Time>. The list of the available curve (one every nanosecond) is displayed.

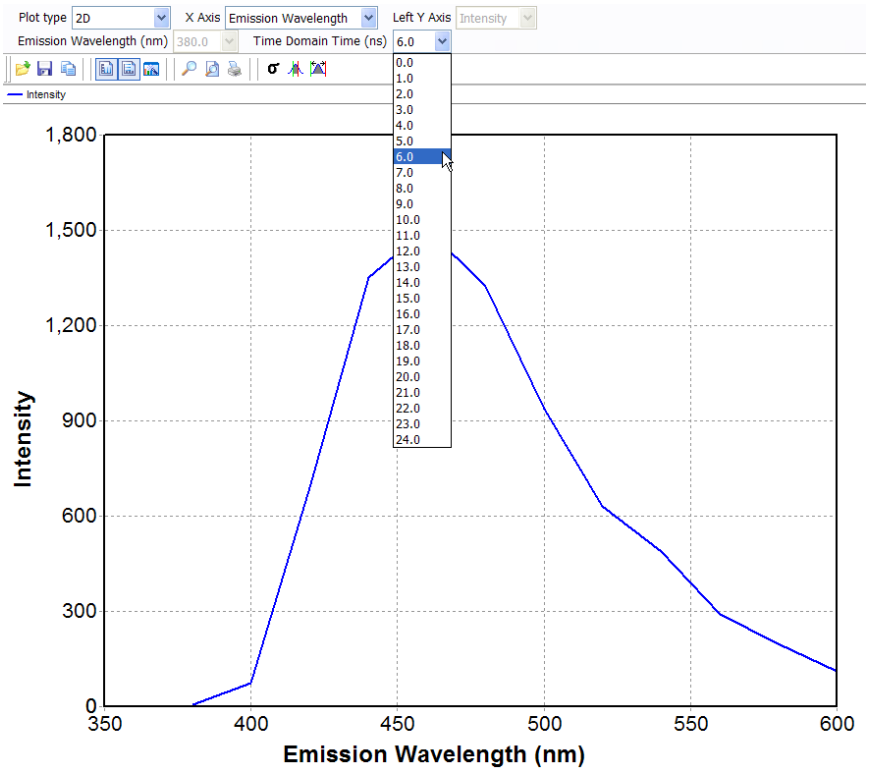


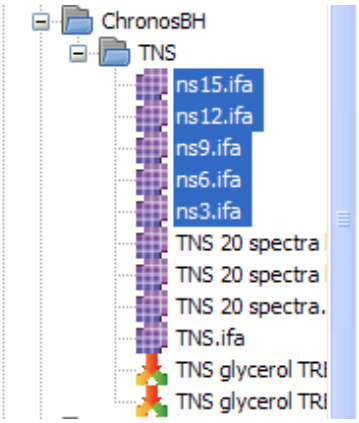

**Figure 21.4** The drop-down list of the curves corresponding to each acquisition time (every 1 nanosecond).

In order to select the relevant curves, click on <Math> and select <Slice>.
Then, select <Extract> in order to extract the selected curve from the 2D Series group. After this step, go to <File>, select <Save as> and save the curve as a new file.

Repeat the extraction for other decay curves (that have pronounced wavelength shifts) and save all of the extracted curves as individual files.

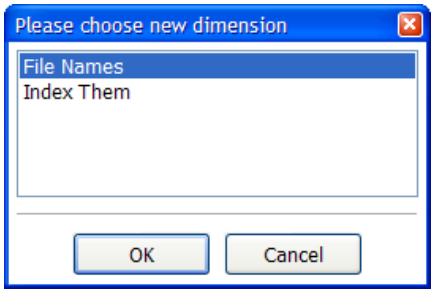
As an example, the folder to the right includes the curves at 3 ns, 6 ns, 9 ns, 12 ns and 15 ns.

Open the data file using the browser and select the files that you intend to combine.




Click on <File> in the main menu and select <Combine>. A new window opens, where you can choose how to name the curves on the future 2D series plot.

Select one of the options and press <OK>.




The selected files are displayed on the same plot.

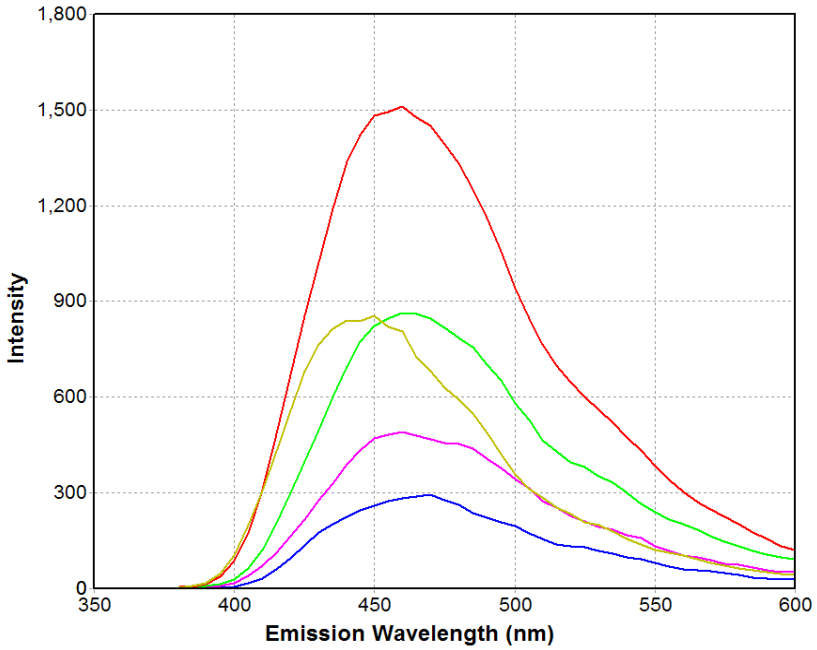
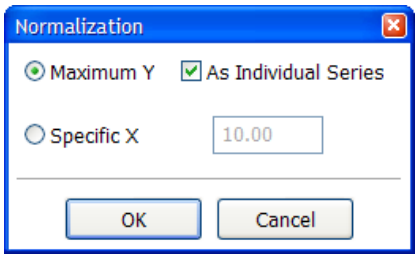



**Figure 21.5** Selected files showing the shift of the maximum as time after the excitation. Each curve, from the left to the right, corresponds to the emission spectrum collected after 3 ns (yellow), 6 ns (red), 9 ns (green), 12 ns (purple) and 15 ns (blue) from the excitation.

The file can be normalized. Select <Math> on the main menu toolbar and then select <Normalize>As a result, a new 2D series graph, containing all the previously extracted files combined in one file will
Go to Math menu on the top bar menu, select <Normalize>.

In the new window, select Maximum Y and As Individual Series and press OK.




The final graph, showing the wavelength shift over decay time, with normalized intensities will be plotted on the screen.

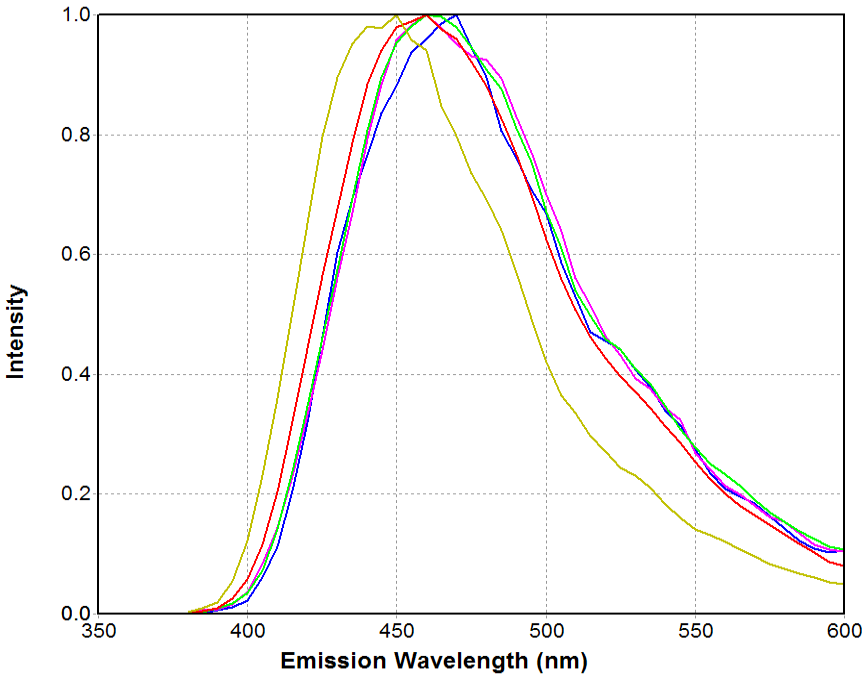



**Figure 21.6** The emission spectra recovered from a series of time-resolved spectra normalized at the maximum. Each curve, from the left to the right, corresponds to the emission spectrum collected after 3, 6, 9, 12, 15 ns from the excitation.

## 21.4 Removing selected curves from a series

Let us consider again the series of curves of Figure 21.2, which are reproduced below for convenience. From the series we want to delete a few curves.

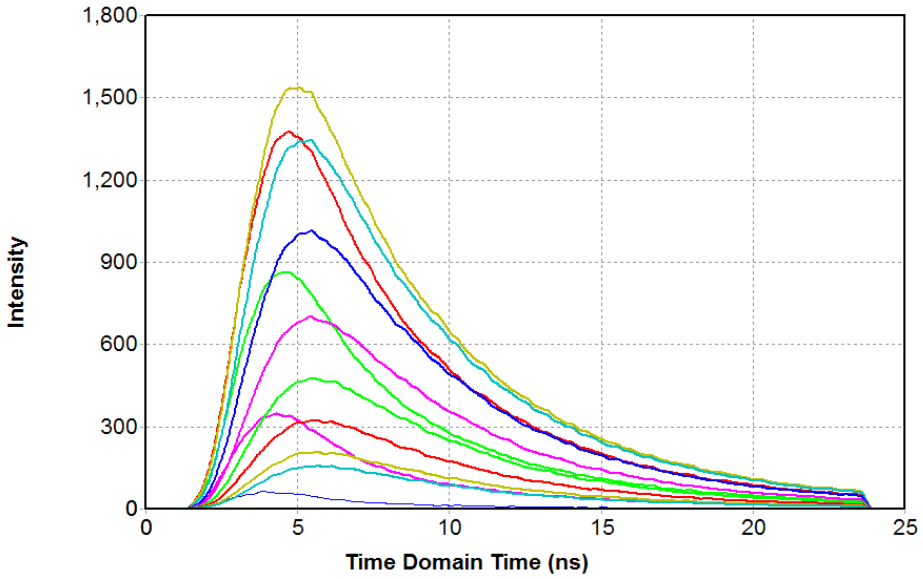
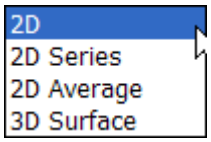


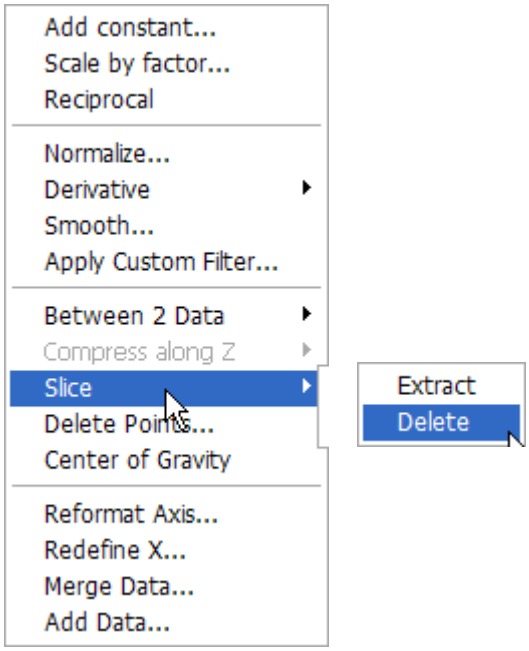

**Figure 21.7** A series of curves.

In the <Plot Type> field, select <2D>.




Now the curves of the series are displayed individually by selecting the <Emission Wavelength> in the field.

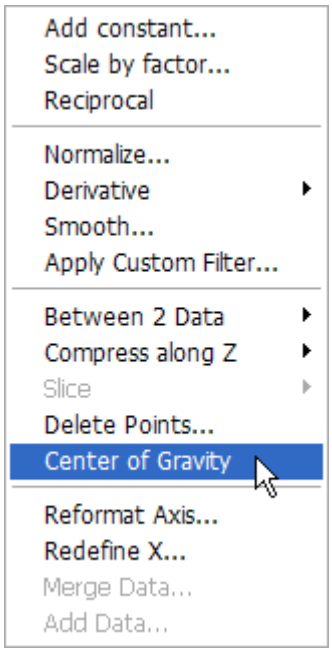
Click on <Math> in the menu bar and select <Slice>: you can either <Delete> the select curve from the series, or Extract> the selected curve from the series.



## 21.5 Displaying the center of gravity

For the spectra displayed in Figure 20.13, the Vinci can calculate the center of gravity. This operation can be done on any group of files.

In the menu bar, select <Math> and then <Center of Gravity>



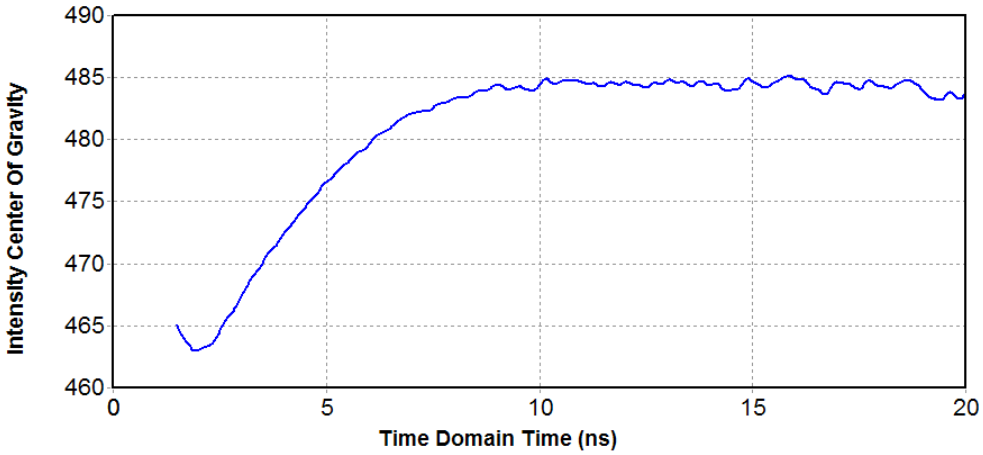




**Figure 21.8** Position of the center of gravity (in nanometers) for the spectra of Fig. 20.13 above.

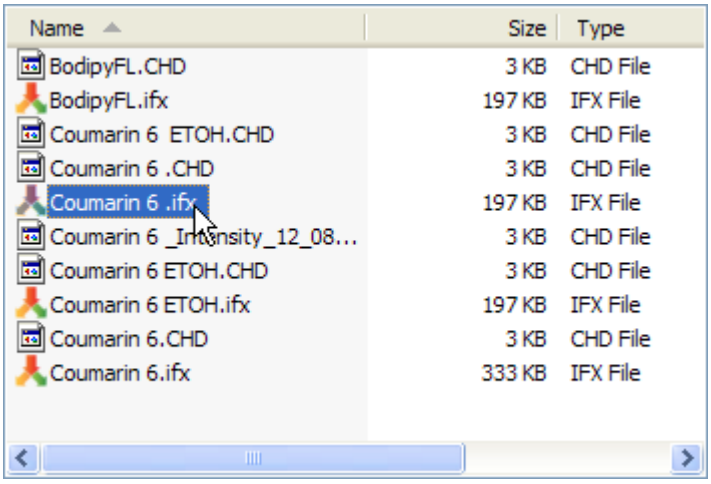
# 22. Managing Data Transfer to other Programs

Data files are recorded in ASCII format for easy transfer to any program. Below we show how the transfer can be achieved from within Vinci.

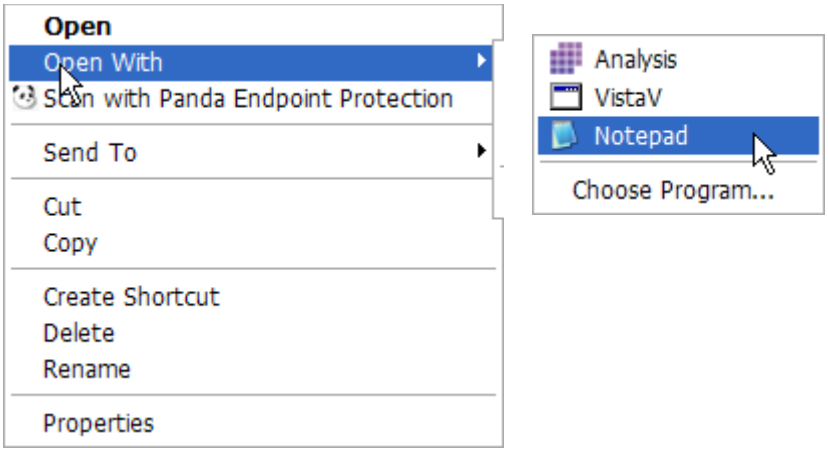
## 22.1 Opening an experiment file (.ifx)

Experiment Files have the extension <.ifx>; they record the data acquired during an experiment. The header of the file contains the information on the experimental parameters utilized for the acquisition. In fact, an experiment can be re-run from any experimental file: it will be using the same experimental conditions. Experiment Files are stored in ASCII format and can be opened by any program.

Open a folder containing the data file you intend opening.
Position the cursor on the file to be opened and right click on it.




Select <Open With> and choose a program (in the example <Notepad>; but it can be any program loaded in the workstation).



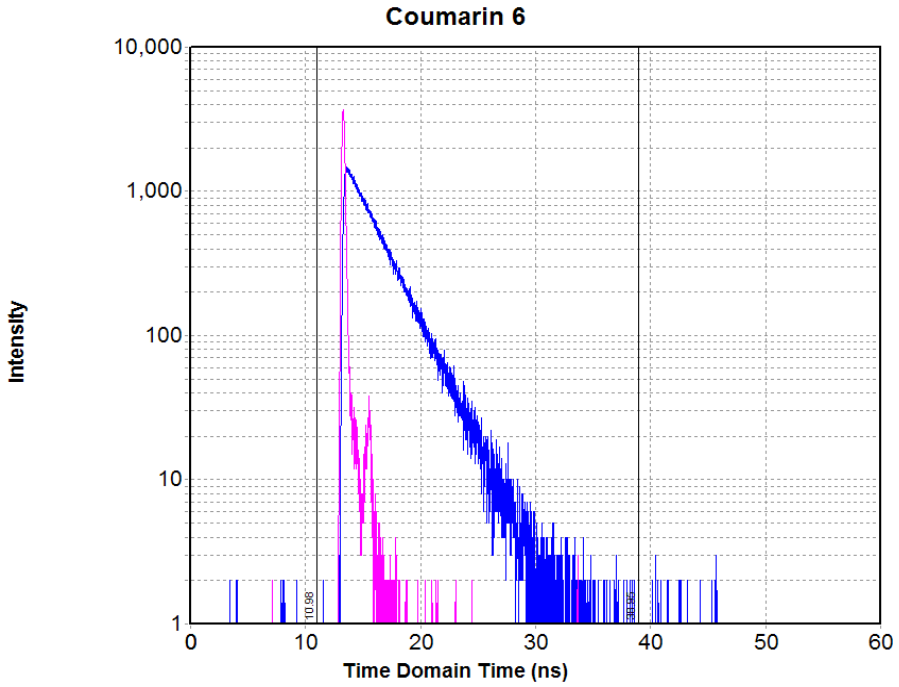

The data file will open displaying the identification information contained in the header as well as the data.

## 22.2 Opening an analysis file (.ifa)

Experiment files (with the extension .ifx) cannot be modified. Any modification to the file can be saved and will be stored with the extension <.ifa>. The experiment cannot be re-run from an analysis file.
An analysis file can be opened using the same procedure utilized for the experiment files.

## 22.3 Transferring data from the Plot page in Vinci

Open in Vinci the data file you intend to transfer.




- When the plot is displayed, left-click on the plot (at any location);
- Click on the <Copy> icon in the menu bar of Vinci. Data are copied in the clipboard.
- Open the destination program you intend to transfer the data to (for instance, Excel) and click <Paste>. The data will be copied into the program.

## 22.4 Transferring data from the Data page in Vinci

Alternatively, one can transfer the data from the data page.

- Select the <Data> tab;
- By holding the cursor (left-click) surf onto the data tables;
- Click on the <Copy> in Vinci menu bar;
- Click <Paste> in the destination program.

## 22.5 Transferring data from the Fit Report page in Vinci

This is a convenient way to transfer data from the Fit Report page. Upon completing the fitting of the data a Report is generated; data can be transferred from the Fitting Report Page to a destination program.

- Open the <Fit Report> page;
- Left-click on the page (at any location point);
- Click on the <Copy> icon;
- Open the destination program you intend to transfer the data to and <Paste>.

### 22.5.1 Transferring the data of a lifetime distribution

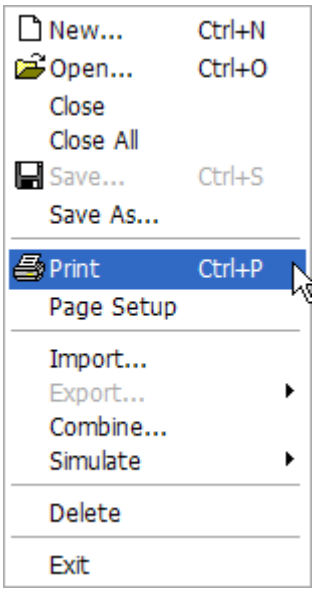
The data generating the lifetime distribution (on page 3 of the Fit Report) can be saved.

- Open the Fit Report and scroll to page 3.
- Left click on the plot of the distribution.
- Click on the <copy> icon in the menu bar
- Open the destination program and <Paste>.

### 22.5.2 Printing and Saving a Fit Report

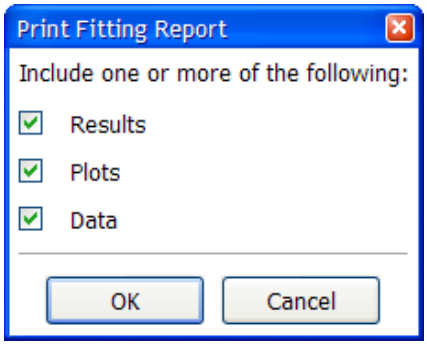
The Fit Report can be printed.

Select <File> in the menu bar and from the list, click on <Print>



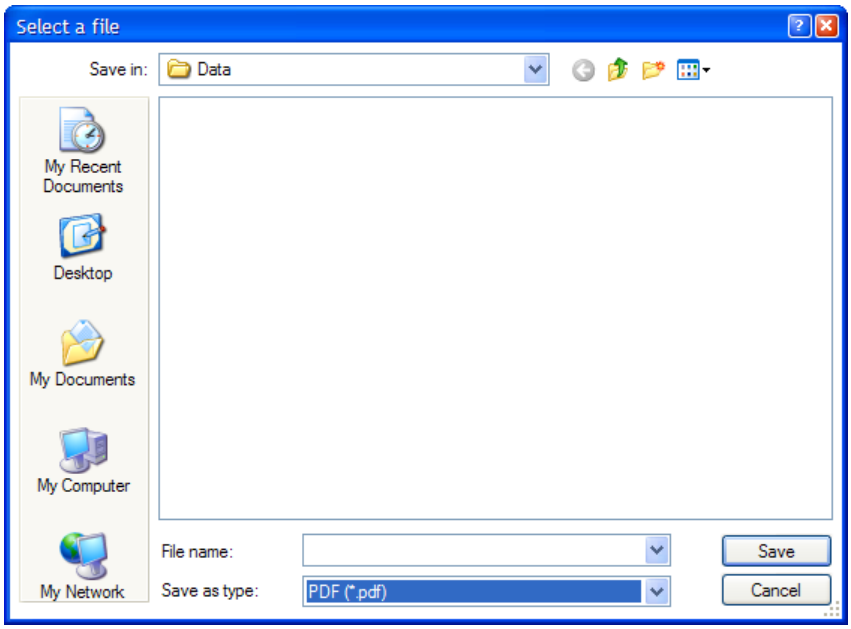

The window allows for the user to select the objects to be printed.

When clicking <OK> the user is prompted to select the printer.




The Fit Report can be saved as document in pdf format or HTML format. Select <File> in the menu bar and from the list click on <Save As>.

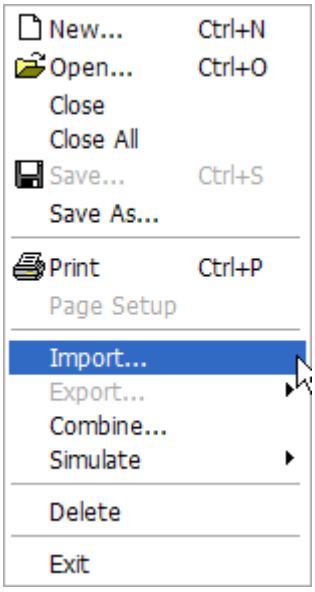
Select in the window the format you desire and give a name to the file.



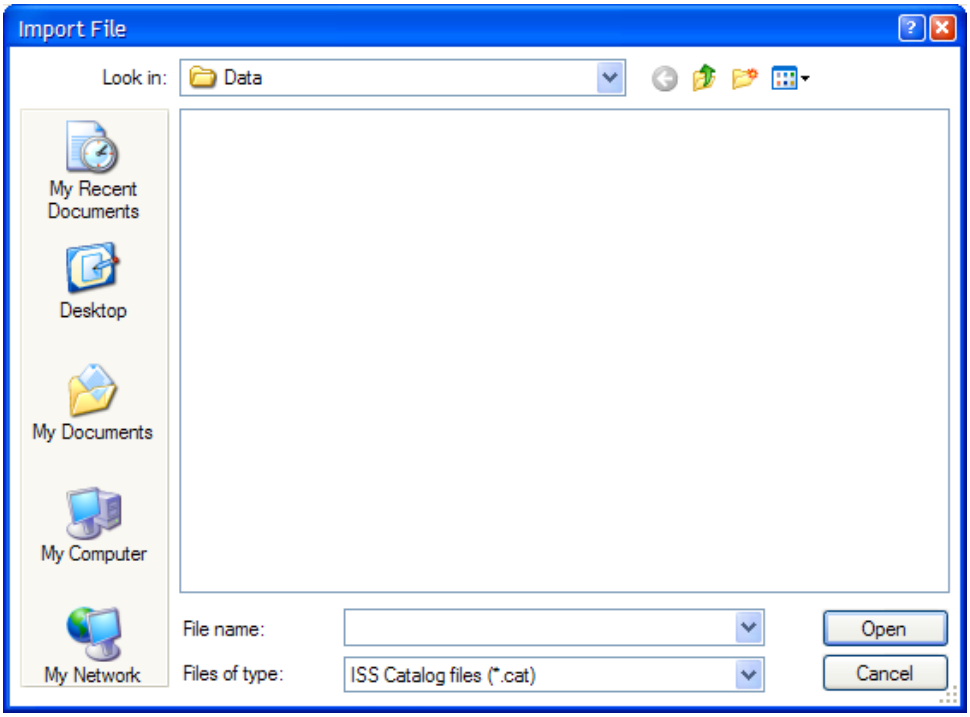
# 23. Importing Data into Vinci

Data can be imported into Vinci from several programs.

Select <File> in the main menu of Vinci and click on <Import>




The following window is displayed.




In the drop-down window, select the format of the file you intend to import. The available data file formats are listed in Table 22.1 below.

| File format | description |
|---|---|
| .cat | Steady-state data file from the binary format used by ISSPC (DOS based software) |
| .lif | Lifetime data file from the binary format used by ISSL (DOS based software) |
| .pol | Anisotropy decay data file from the binary format used by ISSL (DOS based software) |
| .mc2, .sdt | Time domain files by Becker & Hickl |
| .dat | Time domain files by PQ |
| SLM ASCII | ASCII file from SLM Instruments. ASCII files generated by other software packages can be imported as well; the header may require modifications. |

**Table 23.1.** Data file formats that can be imported by Vinci.

Once a data file is imported, it can be saved in Vinci using the <.ifa> extension.

# 24. Turning off the ChronosBH

- If you have a Hamamatsu, Horiba, or Lasos laser, turn off the laser by switching the key in the front panel of controller counterclockwise.
- Close the shutters through the Vinci software.
- In the Voltage Control Panel, reduce the voltage output to the detector to zero by reducing the detector voltage control Gain/HV to zero then disable the output (Note: this step is necessary for an MCP). For the instrument configuration with a MCP detector, turn off the high voltage power supply.
- Close *Vinci - Instrument and Experiment Control* software.
- Switch off ChronosBH. The switch is located in the right of the rear panel.
- Close Vinci Analysis and shut down the computer (Optional).
- Write down name, experiment and number of hours used in the instrument's user logbook.

# 25. Maintenance and Care of the Instrument

The ChronosBH is a rugged instrument and does not require any special care. When kept in the suggested temperature and humidity environmental conditions the instrument will be operational for years. A few recommendations:

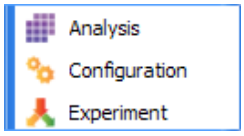
- Lasers and light sources should be turned OFF when the instrument is not in use.
- The ChronosBH should be turned OFF when not in use.
- In order to prevent dust from building on the instrument and/or entering the optics, cover the instrument when it is not in use.

# 26. Adding Hardware to the ChronosBH

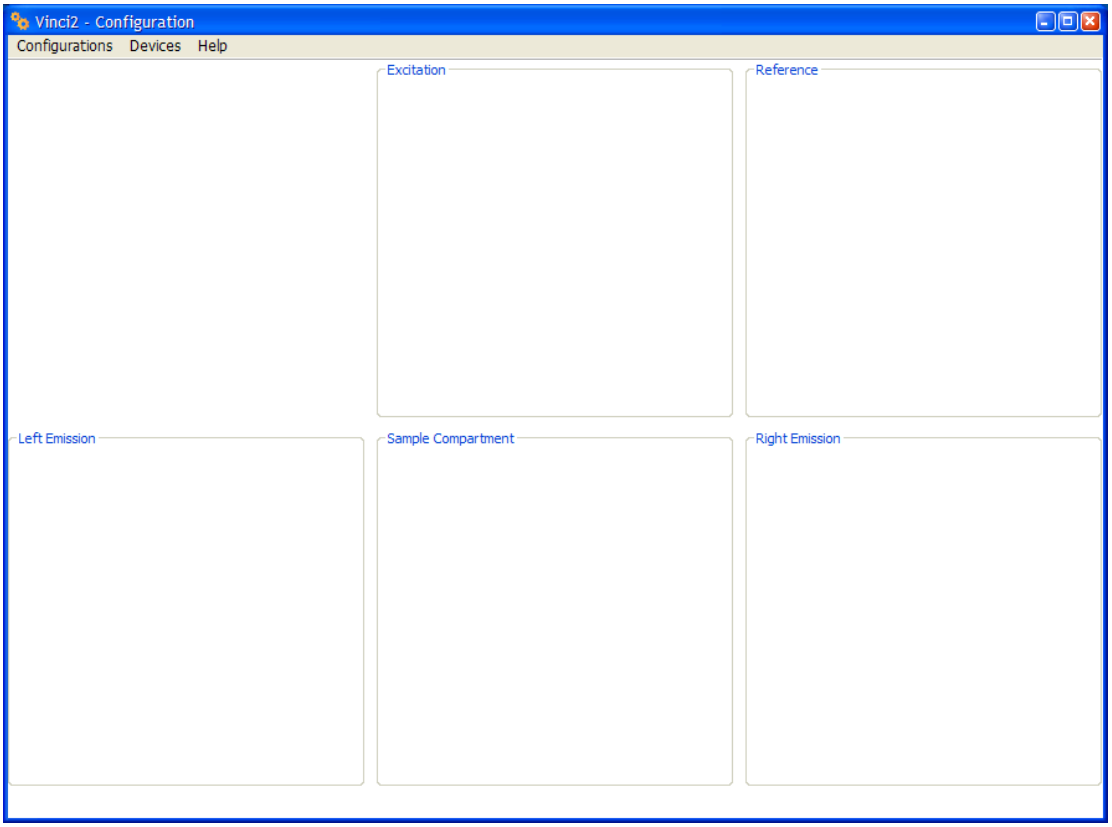
## 26.1 The Configuration Editor

The addition or removal of any hardware to the instrument requires a modification of the Configuration file.
In Windows, select <Start> and then <All Programs>. In <ISS> select <Vinci2> and the following window is displayed.

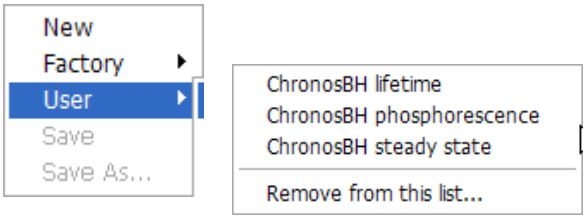
Select <Configuration>.



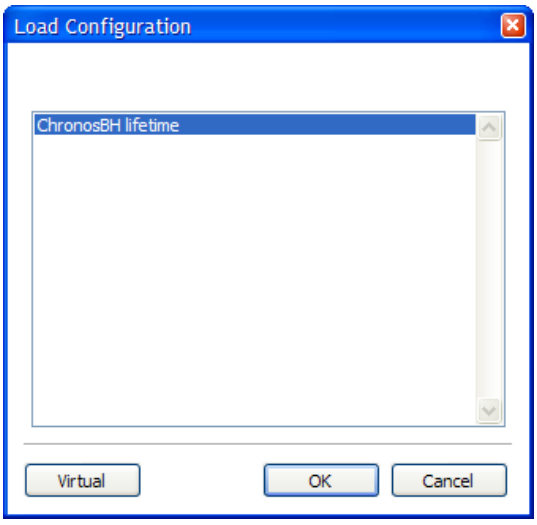

And the following window is displayed.



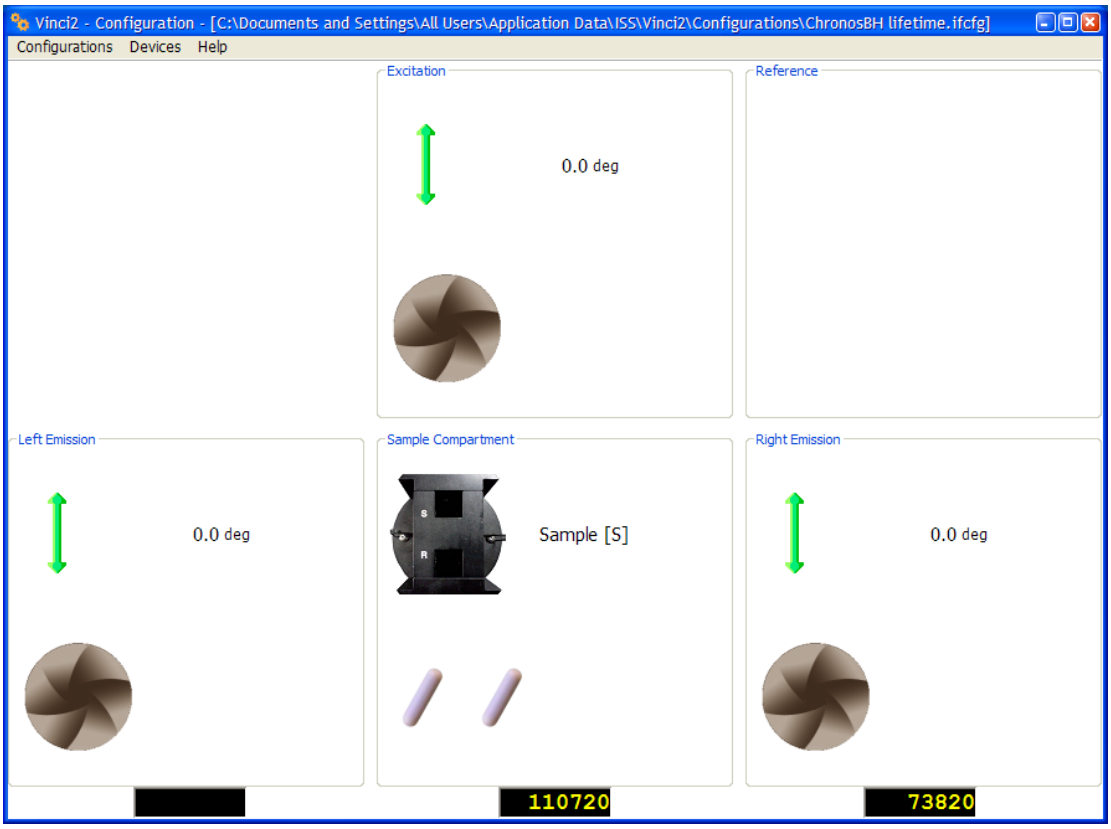

Click on <Configurations>. Select <User> and click on the configuration you would like to modify.




In order to modify an existing configuration, load the configuration, in the example below <ChronosBH lifetime>.

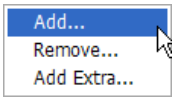



The devices activated in this configuration are displayed in the page below. As an example, we would like to add a monochromator on the right emission channel.

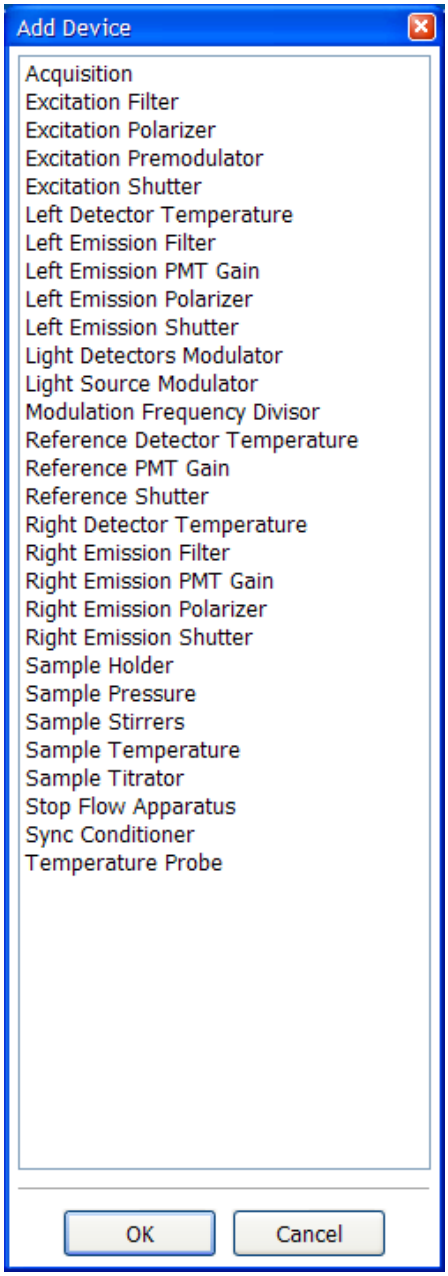



### 26.1.1 Example 1: Adding a monochromator

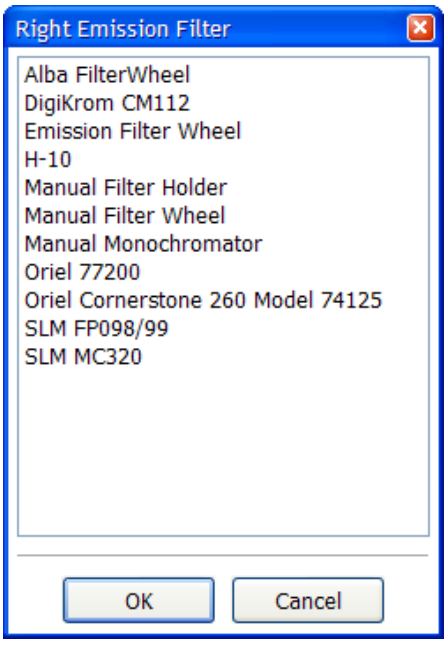
In the Configuration, click on <Devices> and select <Add>.



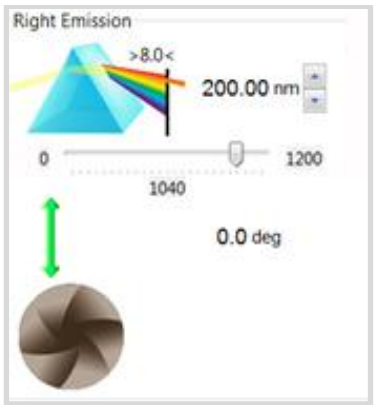
From the list, select <Right Emission Filter>



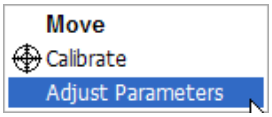

From the specification list, selects your device. For an H-10 monochromator from ISS select <H-10> and click <OK>.



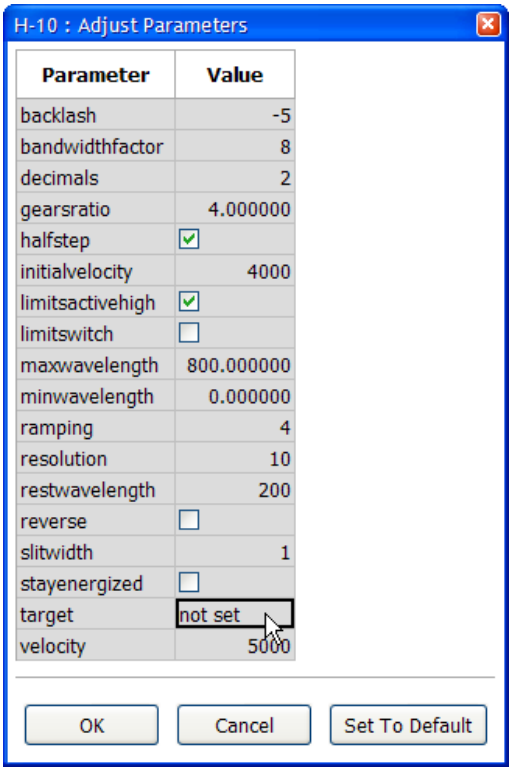
The monochromator icon is displayed in the right emission channel area.




Right-click on the monochromator icon and select <Adjust Parameters>




In the <target> field, enter <m3> and press <OK>. See Table 8.1 for a listing of the devices assignment ports.



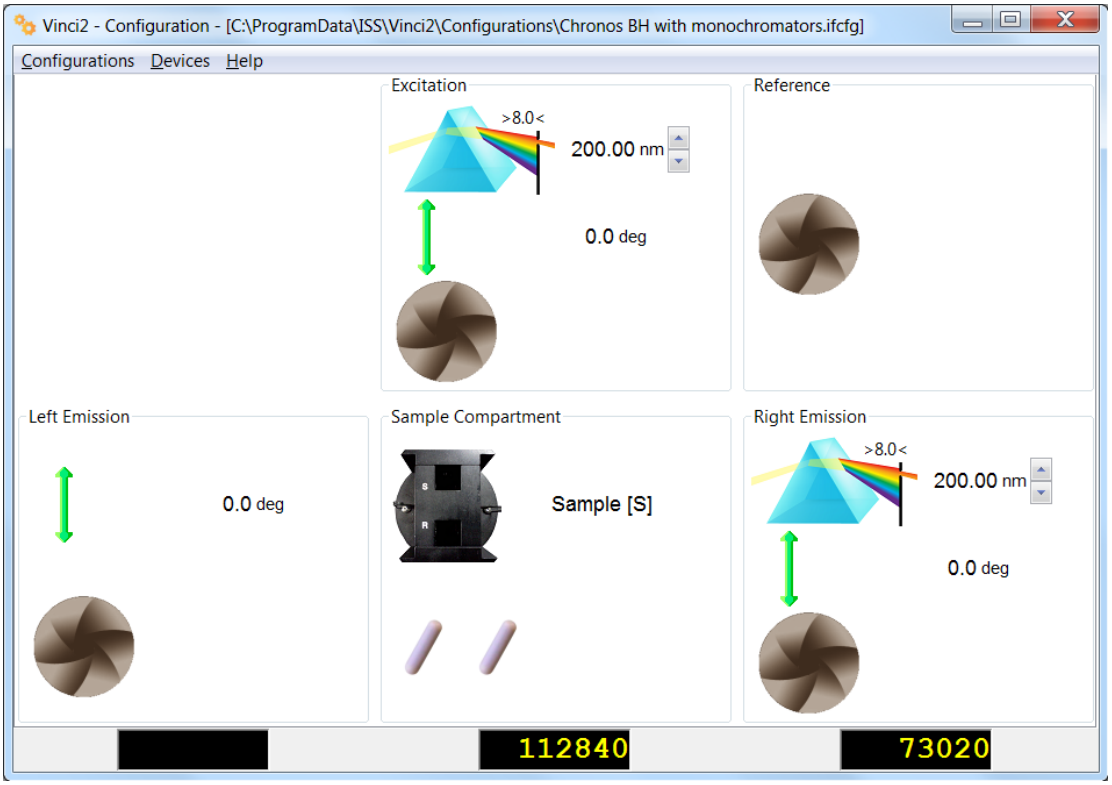
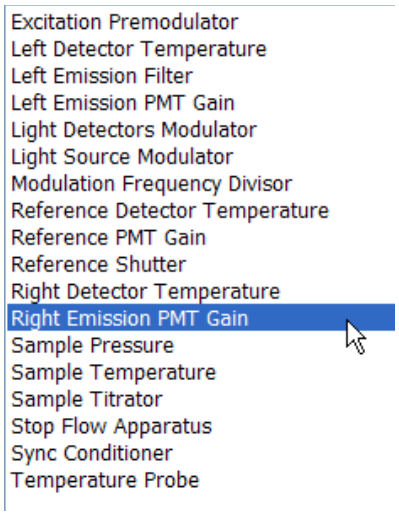



**Figure 26.1** Configuration with monochromators in excitation and right emission channels.

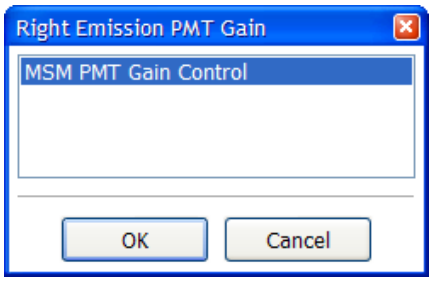
### 26.1.2 Example 2: Computer Voltage Control using the ISS software

The switch on the PMT control panel is set to the <REM> position. To use the Analog Remote High Voltage System for measurements the Instrument Configuration file of Vinci must be modified via the following procedure to engage the high voltage control system. This feature is recommended when using the PMT in analog mode; depending upon the intensity of the signal the gain of the PMT is varied.
Start the <Configuration> of the software.

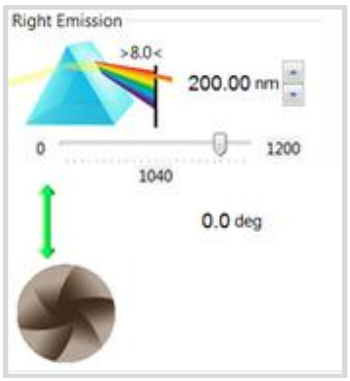
Click on <Devices> and select <Add>.
Select the Right Emission PMT.



Select <MSM PMT Gain Control> and press the <OK> button.

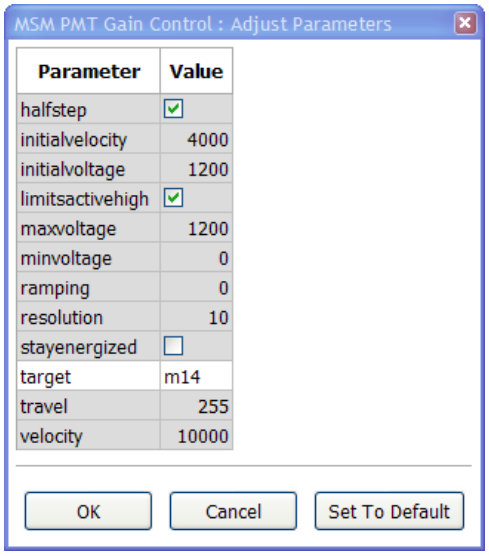



A slide bar will appear in the Right Emission channel area.




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

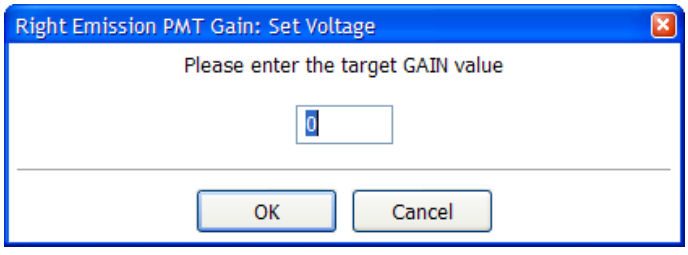
Enter <m14> for the right emission PMT.




The slide bar allows the user to change the gain of the K218 PMT 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.



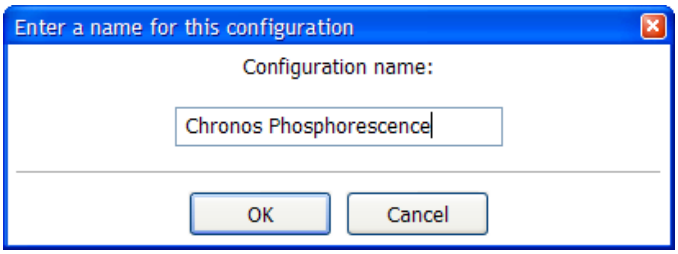
## 26.2 Saving Multiple Instrument Configurations

The Instrument Configuration can be saved with its own identification name. If this Instrument Configuration is used for steady-state fluorescence applications, one could name it Chronos Steady-State.

Similarly, if the instrument is equipped with a Multiscaler card for phosphorescence measurements, one can generate a specific Instrument Configuration and name it Chronos Phosphorescence.

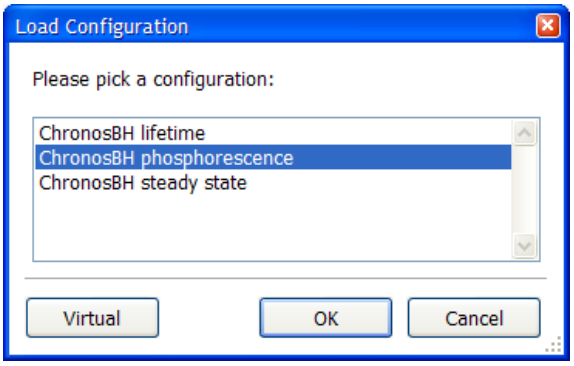
In order to save a configuration, select <Configuration> and then <Save As..>


Enter the name of the new configuration and press <OK>.



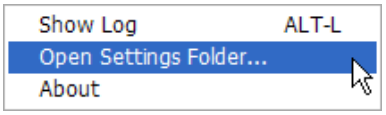

## 26.3 Working with Multiple Configurations

If multiple configurations are saved, when the Experiment is started, the user is prompted to select the proper configuration.
The parameters are loaded and the instrument can be operated.

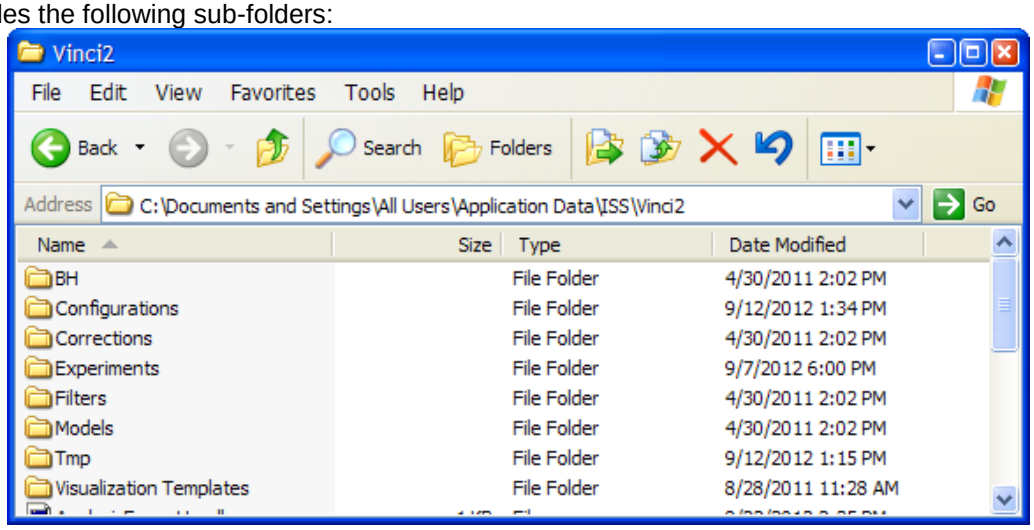



## 26.4 Software location of user-generated files

The Instrument Configuration files as well as additional files generated by the user are stored in the <Documents and Settings> folder.
The file can be accessed from within the Vinci software by pressing <Help> in the main menu of the software and selecting <Open Settings Folder>



The folder includes the following sub-folders:

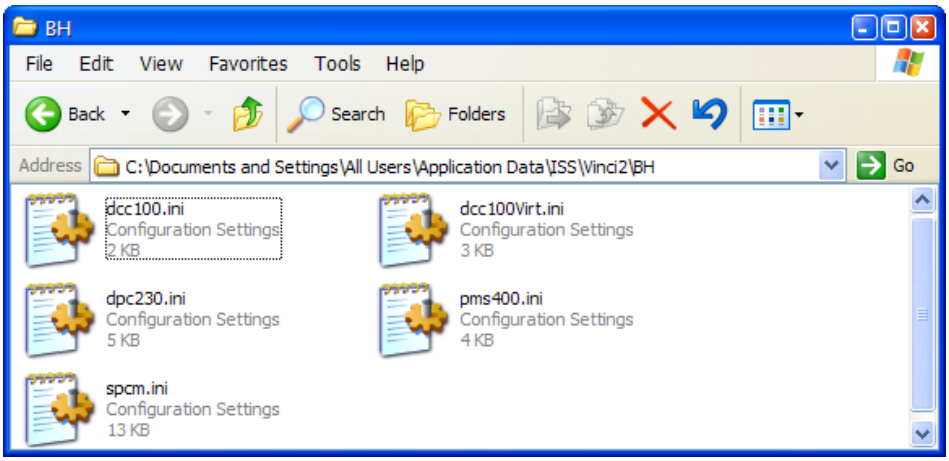



| Sub-folder | Content |
|---|---|
| BH | <ini> files for the acquisition and control cards. |
| Configurations | Instrument configurations files opened by the user. |
| Corrections | Correction factors for the emission spectra. They are collected at the factory and allow the user to correct a technical spectrum or to acquire a “corrected” emission spectrum. |
| Experiments | A User-defined experiment is saved in this folder. |
| Filters | A User-defined processing of data is saved in this folder |
| Models | A User-defined model for fitting is saved in this folder |
| Tmp | Folder where experimental data is stored while the experiment is running. |
| Visualization Templates | Current default choices for plot appearance. |

### 26.4.1 The <ini> files

Some default values for the acquisition cards can be changed in the <ini> files.

Select the <BH> folder and a listing of the <ini> files for the BH data acquisition and control cards is displayed.
The files can be opened using an editor such as <Notepad>.




It is recommended not to change the parameters unless the user is fully familiar with their significance.

# 27. Time Correlated Single Photon Counting

This chapter is intended to give a general introduction to the TCSPC technique; it is not meant to be detailed. The interested reader is referred to the following exhaustive publications by Dr. Becker:

- Wolfgang Becker; *Advanced Time-Correlated Single Photon Counting Techniques*; Springer-Verlag, Berlin/Heidelberg 2005.

- Wolfgang Becker; *The bh TCSPC Handbook*; Becker & Hickl GmbH; Berlin 2010.

## 27.1 Signal from a PMT

The picture displayed in Figure 25.1 depicts the signal delivered by a photomultiplier tube model H7422P-40 on a 1 ns/div time scale (left) and on a 100 ns/div time scale (right). The plot displays several pulses, each with its own amplitude. The amplitude of each pulse (amplitude jitter, or gain noise) is due to the random amplification process in the PMT.

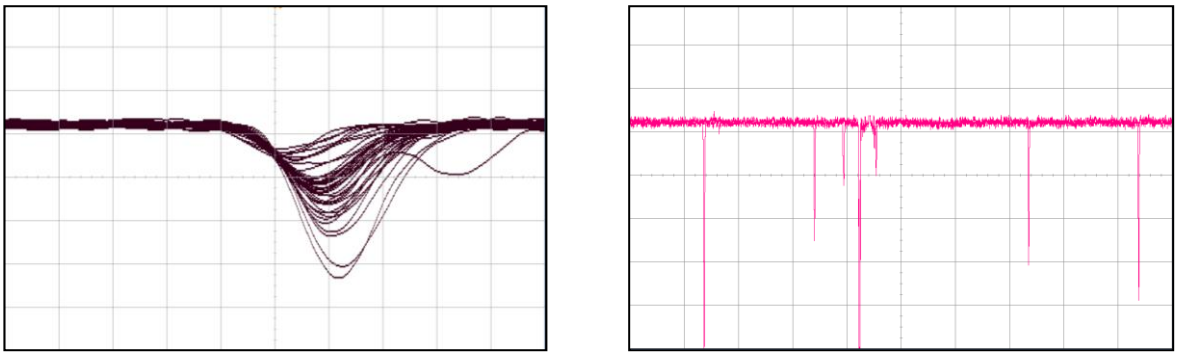


**Figure 27.1** Pulses from a H7422P-40 on a 1ns/div scale (left) and a 100 ns/div scale (right). The number of pulses detected is about 100,000 per second.

## 27.2 Photon Counting Techniques

The goal of Photon counting techniques are based on counting such pulses; they are based upon the assumption that the detector signal is a random sequence of pulses corresponding to the detection of individual photons. The light intensity is a representation of the density of such pulses, not of their amplitude. In general there are three schemes to measure the photons:

- Single photon counting
- Multichannel scaler (multiscaler) cards
- Time-correlated single-photon counting

### 27.2.1 Single Photon Counting

The general scheme for measuring such pulses is displayed in Figure 25.2 below. The pulses from the detector are sent to a pre-amplifier discriminator; the pulses with amplitude above a set threshold pass through and sent to a counter. The advantage of such scheme is that a fairly large number of counts can be counted. The main disadvantages are two: (a.) the discrimination level depends upon the amplitude of the individual counts (amplitude jitter) and this causes a time jitter in the acquisition; and (b.) the time scale is not sufficiently detailed to allow for counting with high precision within short times.

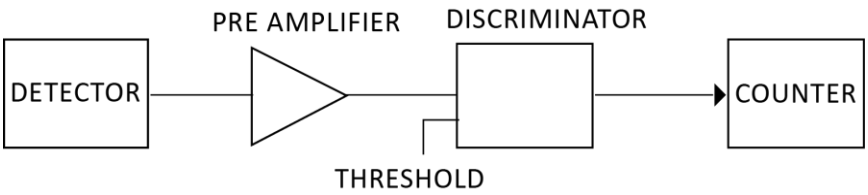



**Figure 27.2** General functional schematic of a photon counting system.

The main advantage of the technique is the extreme large number of photons acquired. The technique is used for steady-state photon counting applications. The PCMC card by ISS (see paragraph 6.6.3) is best utilized for these measurements.

### 27.2.2 Multiscaler cards

A Multichannel Scaler (or Multiscaler) records the input pulses from the light detector into a large number of channels of a fast memory. A synchronization signal from the light source starts the sequential triggering of the fast memory; photons are assigned to specific memory locations. The limitations of this approach are a relatively poor time resolution and count rate. Yet, when recording decay times longer than 1 µs, the multiscaler works fine. Therefore it is routinely utilized for the measurement of long decay times as they typically occur for phosphorescence processes. Multiscalers are utilized for steady-state measurements as well although they are not as suitable as the PCMC card. ISS offers the MSA-300 and PMS-300A cards (see paragraph 6.6.2). These cards can be utilized for the measurement of long decay times as well.

### 27.2.3 TCSPC

In TCSPC, a photon is counted within a set time period with a high precision. The time period is given by the repetition rate of the light source and the precision is given by the acquisition electronics (mainly the TAC and the ADC components). For instance, when using as excitation a rate emitting pulses at 80 MHz, the time period is the distance between two such pulses (12.5 ns). Typically, the repetition rate of some light sources can be set by the user.

## 27.3 Principle of a TCSPC

### 27.3.1 Start-Stop Mechanism

In the tradition TCSPC scheme, the time between an excitation pulse and the detected photon is recorded (start-stop mechanism). The signal from the light source (sync signal) goes through a CFD and “starts” the time; the signal from the light detector goes through a separate CFD and “stops” the time. The TAC provides a voltage output that is proportional to the time interval between the Start and Stop pulses. The signal passes to an ADC and hence to the computer, where it is recorded. When many events are recorded, a histogram is produced; it displays the decay time of the event.

The drawback of such a scheme is that for every sync signal the TAC starts measuring. This is not an issue when the repetition rate of the light source is low. Yet, it is a drawback when the repetition rate is in the MHz range. In fact, in many signal periods, if not most, no photon is detected - in TCSPC the photons stream is equal to 1-2% of the repetition rate of the light source - to stop the TAC, and the TAC has to be reset. The TCA resetting operation introduces dead time during which the electronics cannot operate. Typical dead time ranges 100-130 ns for the B&H cards.

### 27.3.2 Reversed Start-Stop Mechanism

The data acquisition cards by B&H work on the reversed start-stop mechanism (Figure 25.3). That is, the

arrival of a photon triggers the TAC; the stop is determined by the next pulse from the light source. In this scheme the photon arrival time is measured with reference to the next arriving reference pulse and only one photon is recorded in one signal period. Using this scheme, the TAC resets at the rate of the photon arrival, which is a pretty rare event, rather than the time of the excitation pulse.
During most of the time periods set by the excitation light no photon is recorded by the light detector. When a photon is recorded, its time with reference to the excitation pulse is recorded and placed in the memory of the computer. This allows for the generation of the histogram.

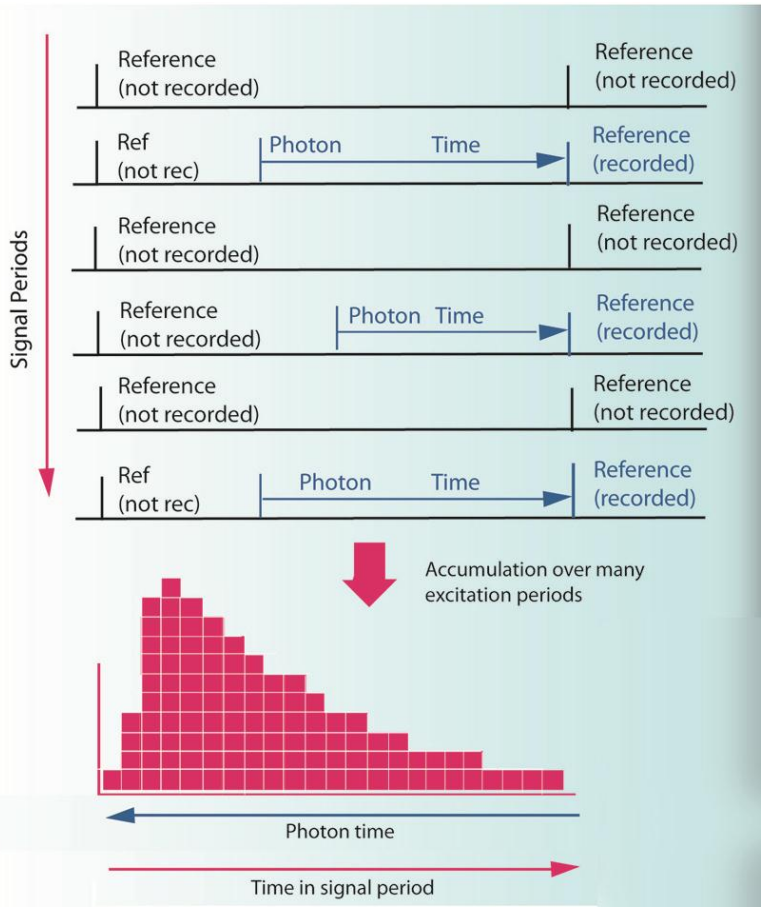



**Figure 27.3** Principle of Start-Stop mechanism for data acquisition.

## 27.4 Components of a TCSPC

### 27.4.1 Constant Fraction Discriminator (CFD)

The CFD eliminates the time jitter associated with the different amplitudes of the pulses delivered by the light detector (Figure 24.4 left). The principle of operation is detailed in Figure 25.4 right: a “copy” of the input pulse is sent to the circuitry and reversed; the difference is generated. The difference triggers at a constant fraction of the pulse amplitude.
In the B&H cards, a CFD conditions the signal coming from the light detector. A second CFD is used to process the sync signal from the light source.

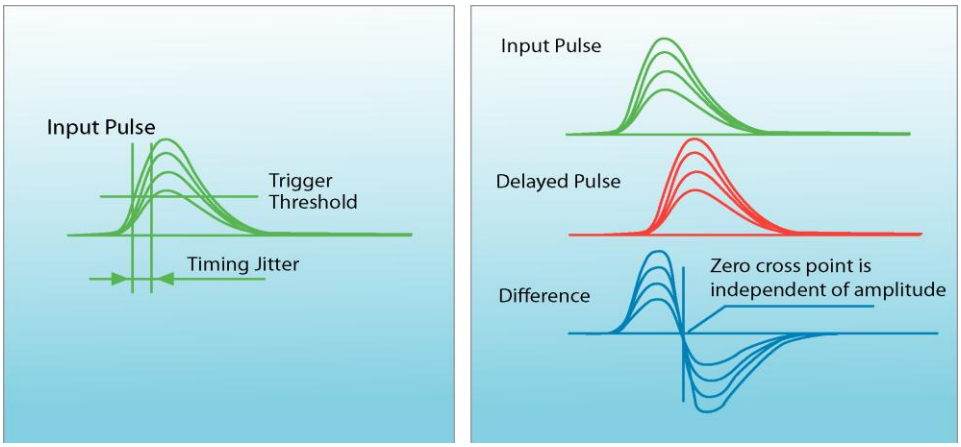



**Figure 27.4** Timing jitter of pulses with various amplitudes and constant fraction triggering.

### 27.4.2 TAC and ADC

The time-to-amplitude (TAC) converter measures the time interval between the detection of a pulse generated by the light detector and the next pulse generated by the sync signal. When the TAC is started by a pulse at the start input, it generates a linear ramp voltage until a stop pulse appears at the stop input.

The analog-to-digital converter (ADC) converts the amplified TAC signal into the address of the memory (MEM). Typically, the TAC signal is resolved into 4096 time channels and the width of the particular channel must be equal to within 1% or better.
The arrival time of each photon is recorded and stored in the corresponding time bin. After the accumulation of many photon arrival times, the photon intensity (number of arrived photons) vs. arrival time curve is built up which results the decay curve of the fluorophore in a lifetime measurement experiment.

## 27.5 Measurements

### 27.5.1 Signal level

If more than one photon is present in any time period, the instrument will detect the first photon only; the histogram is skewed towards the “short time” photons and it is not a representation of the decay time. This is the “pile-up” effect.

In order to avoid pile-up, in a typical TCSPC measurement, the photon signal level is set to about 1% of the repetition rate of the laser which makes it a very rare event for multiple photons to arrive in one signal period.
That is, the number of detected photons should not be higher than 0.01 per signal period; the higher number would cause the instrument to operate in a pile-up effect mode, which distorts the results. Using a light source with a 50 MHz repetition rate (20 ns), the signal should be around 500,000 photons per second.

### 27.5.2 Acquisition Time and Signal-to-Noise ratio

Ideal conditions for a TCSPC single exponential decay time acquisition experiment include:

- using an infinitely short laser pulse for excitation;
- using a repetition rate that is at least four times the inverse of the decay time of the fluorescence;
- using a repetition rate that is not more than 1% of the repetition rate of the SYNC signal;
- using a data acquisition card that features a large number of time channels each with a time width

much smaller that the IRF of the instrument;
- a negligible signal background.

Under ideal conditions, the photons collected from a single exponential decay follow a Poisson distribution and S/N ratio is equal to the square root of the number of photons collected; that is:

$$\frac{S}{N} = \sqrt{N(t_k)} \qquad [27.1]$$

where $N(t_k)$ is the number of photons collected within the time bin $k$.

In order to acquire data that will provide a good statistical response, one should collect at least 5,000 to 10,000 photons. At repetition rates of 20-80 MHz, achievable with the current light sources and data acquisition cards, the acquisition of 10K photons takes about one second.
Of course the situation is quite different when acquiring measurements of long decay times. In that case, one has to use a source with a relatively low repetition rate and the acquisition time of the measurement will become progressively longer.

### 27.5.3 Dead time

The dead time is the time the electronics does not work as it is "busy" counting one photon. In the SPC-xxx modules, the typical dead time is fairly small as it ranges from about 100 ns to 150 ns. During this time the TAC is not operational and the card does not record the arrival of photons.

## 27.6 Data analysis

### 27.6.1 Convolution integral

When the data analysis is performed on a data set, three curves are displayed in the plot, respectively:

- the IRF (green line);
- the experimental data points $N(t_k)$ (dots); and
- the curve that best fits to the data (blue line) determined by the calculated data points $N_C(t_k)$.

The IRF includes the contribution of the light source $L(t_k)$ and of the light detectors $D(t_k)$, as well as other instrumental factors such as the monochromator if present on the optical path of the fluorescence light. The experimental data points curve is the convolution of the decay time function that best describes the decay and of the IRF. Let us suppose that the detector contribution is much narrower than the decay time and that the IRF shape is determined by the light source only:

$$N(t_k) = L(t_k) \otimes I(t_k) \qquad [26.2]$$

Mathematically, the convolution operation can be written as:

$$N(t_k) = \sum_{t=0}^{t=t_k} L(t_k)\, I(t - t_k)\, \Delta t \qquad for\ t > t_k \qquad [26.3]$$

That is, the measured number of counts $N(t_k)$ at time $t = t_k$ is the sum of the responses to an infinitesimal narrow $\delta - function$ occurring up to the time $t = t_k$.

If the interval $\Delta t$ is taken to be very small, the sum can be written as an integral:

$$N(t) = \int_0^t L(t') \, I(t - t') \, dt' \qquad [26.4]$$

The goal is then the determination of the function $I(t)$ that best describes the experimental data.

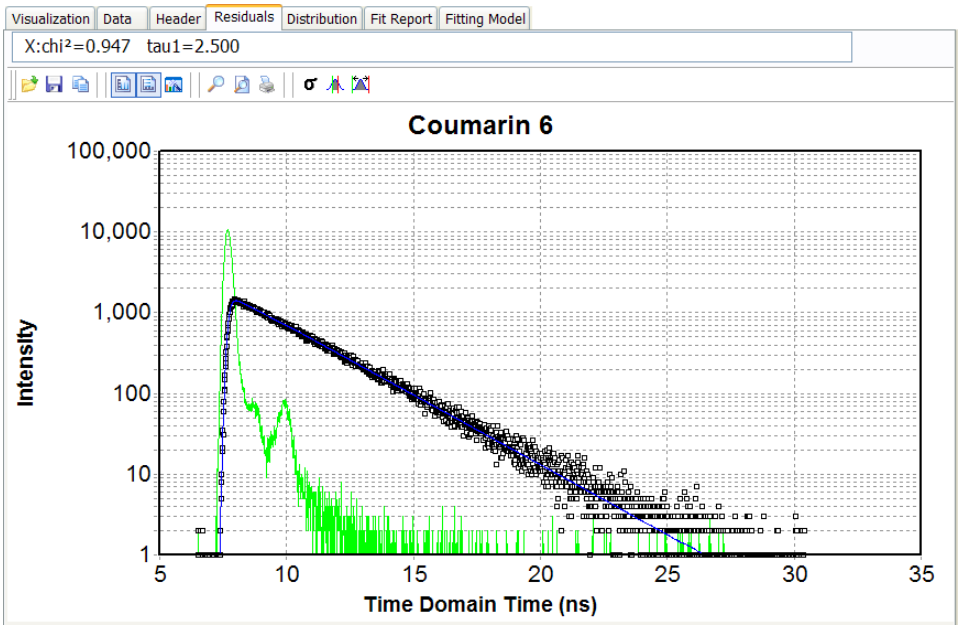



**Figure 27.5** Decay time of Coumarin 6 in ETOH. Three curves are displayed on the plot: the IRF (green light); the experimental data (dots) acquired by the ChronosBH; and the curve that best describes the decay (blue line).

The data analysis proceeds in three distinct steps:

- The user selects a model M for the decay of the fluorescence that, most likely, describes the experiment (for instance, two decay times with one single exponential and one following a Gaussian distribution).
- The software calculates the convolution of the IRF with the selected model for each time bin; the number of photons is $N_C(t_k)$:

$$N_C(t_k) = IRF \otimes M \qquad [26.5]$$

- Finally, the software compares $N_C(t_k)$ with the number of photons acquired during the experiment, $N(t_k)$, in each bin. The decay times $\tau_i$ are the values of the model M that best describes the decay. The "comparison" between the experimental data and the model is done by using the $\chi^2$ function.

### 27.6.2 Mathematical approaches to finding the best data

Several techniques have been suggested for finding the curve that best describes the experimental data. The list includes: the nonlinear least-squares analysis, the method of moments, the maximum entropy method, the Laplace transformation, the Prony's method, the sine transform, the phase plane and the phasor plot. Vinci utilizes the nonlinear least-square analysis. The technique is based upon a set of assumptions on the acquired data:

- The major error is on the dependent variable while the error on the independent variable is negligible;
- The systematic errors are negligible;
- The errors follow a Gaussian distribution;
- The data points are the results of independent measurements;
- The number of data points acquired is sufficient to make the parameters over determined.

The next paragraphs introduce and explain the nonlinear least-square analysis technique and its application in Vinci.

### 27.6.3 The chi-square $\chi^2$ function

Whenever we have a set of *n* measurements $\{x_i, y_i\}$ and we intend to determine a function $y = F(x)$ that describes the data, the nonlinear least-square analysis method consists in the minimization of the $\chi^2$ function, defined as

$$\chi^2 = \sum_{i=1}^{n} \frac{(y_i - F(x_i))^2}{\sigma_i^2} \qquad [26.6]$$

where $\sigma_i$ is the standard deviation on the observed $y_i$ while the error on the $x_i$ is assumed to be negligibly small.

The sum of the square of *n* Gaussian variables is called the $\chi^2$ (chi-square) with *n* degree of freedom. This function, defined in the range from 0 to $+\infty$, is defined by the parameter *n*; its distribution function features a mean value equal to *n*, and a variance equal to *2n*.

Nowadays, in every fitting problem it is common practice to use the reduced $\chi_r^2$, a function normalized as follows:

$$\chi_r^2 = \frac{1}{n-p-1} \sum_{i=1}^{n} \frac{(y_i - F(x_i))^2}{\sigma_i^2} \qquad [26.7]$$

where *n* is the number of experimental points and *p* is the number of parameters to be determined in the operation.

### 27.6.4 Levenberg-Marquardt algorithm

Several techniques have been developed for the minimization of the $\chi^2$ function. Without entering into the merits of each procedure, our preference is for the Levenberg- Marquardt algorithm because of its speed and stability.

Like other numeric minimization algorithms, the Levenberg-Marquardt algorithm is an iterative procedure. To start a minimization, the user has to provide an initial guess for the parameters. In many cases, an uninformed standard guess will work fine; in other cases, the algorithm converges only if the initial guess is already somewhat close to the final solution.

The model that provides a value for the reduced $\chi_r^2$ close to unity is the model that best describes the data collected during the measurement.

### 27.6.5 Judging the goodness of the fit

There are about three parameters that help us in judging if a fit is acceptable.

**A. The value of the reduced chi-square**

It can be proven that for a large number of experimental points $n_i$, the limiting value of this function is unity, that is,

$$\lim_{n\to\infty} \chi_r^2 = 1 \qquad [26.8]$$

Values of $\chi^2 >> 1$ arise from poor measurements, choice of the wrong model function (components) or and underestimate of errors.

Values of $\chi^2 << 1$ are also no good, and probably result from an overestimate of the errors.

The chi-square distribution is a useful and powerful tool (chi-square test) for evaluating the goodness of the fit of an observed distribution to a theoretical one. Goodness of fit means how well a statistical model fits a set of observations. Measures of goodness of fit typically summarize the discrepancy between the observed values and the values expected under the model in question.

This is the way it works: once the number of degrees of freedom *n* is known and the value of $\chi^2$ is calculated, one can consult the tables that provide the percentile values for the $\chi^2$ distribution; such tables are included in many publications on statistical analysis. For instance, let us suppose that we have $n = 100$ and we determine that $\chi^2 = 1.117$; by consulting the tables we find that $P(\chi^2 \le 1.117) = 20\%$; that is, if the measurements are repeated several times, for 20% of the times the value of the $\chi^2$ will be less than 1.117, although for the remaining 80% of the times the value may be higher.

On the other hand, if we find for the same data set a $\chi^2 = 0.993$ by looking at the tables we find that $P(\chi^2 \le 0.993) = 50\%$ , that is, if the measurements are repeated several times, for 50% of the times the value of the $\chi^2$ will be less than 0.993 and for 50% of the times the value of the $\chi^2$ will be higher.

Practically values of the $\chi^2$ in the probability interval between 5% and 95% are considered acceptable.

Values of the $\chi^2$ function too low are to be considered suspicious; in fact, most likely they are due to the presence of systematic errors or to an over-evaluation of the errors. Rather than looking at the absolute value of the $\chi^2$ it is more practical to look at the variations of the function when a different analysis model is considered. In this case, for the change to be considered significant the $\chi^2$ value change should be at least twofold or larger.

**B. The residuals**

The residuals *Ri* are defined as follows:

$$R_i = F(x_i) - y_i \qquad [26.9]$$

Where $F(x_i)$ is the calculated function that best fit the observed data points $y_i$. In many instances it is convenient to utilized the weighted residuals $R_{wi}$, defined as

$$R_{wi} = \frac{F(x_i) - y_i}{\sigma_i} \qquad [26.10]$$

With $\sigma_i$ the standard deviation of the *i*th data point. For a good fit, the residuals should be evenly distributed around the zero.

**C. The covariance matrix**

The variance of a variable is a measure of the dispersion of the values taken by the variable around its mean value. How can this notion be generalized to the case where *n* variables are considered simultaneously, instead of just one? How can the spread of the *n*-dimensional joint probability density be expressed in numbers?

There is no general way of doing that in a practical way, except when the distribution is multinormal. Then, the distribution is entirely characterized by:

a. It's mean vector $\boldsymbol{\mu}$,
b. And the set of pairwise covariances $Cov(X_i, X_j)$, including the variances $Var(X_i)$ (Recall that $Cov(X_i, X_i) = Var(X_i)$).

Whenever the quantities to determine are random variables each with a finite variance and are represented by the vector $\{x_1, \ldots, x_n\}$, then the covariance matrix H is the matrix whose $a_{i,j}$ coefficient is defined as:

$$a_{i,j} = E[(x_i - \mu_i)(x_j - \mu_j)] \qquad [26.11]$$

Where $\mu_i = E(x_i)$ is the expected value of the *i*th entry in the vector x.

$$H = \begin{pmatrix} a_{11} & \cdots & a_{1n} \\ \vdots & \ddots & \vdots \\ a_{m1} & \cdots & a_{mn} \end{pmatrix} \qquad [26.12]$$

The correlation matrix is the covariant matrix normalized by the variance, that is each element is expressed as:

$$r_{i,j} = \frac{a_{ij}}{\sigma_i \sigma_j} = \frac{E[(x_i - \mu_i)(x_j - \mu_j)]}{\sigma_i \sigma_j} \qquad [26.13]$$

$$R = \begin{pmatrix} 1 & \cdots & r_{1n} \\ \vdots & \ddots & \vdots \\ r_{m1} & \cdots & 1 \end{pmatrix} \qquad [26.14]$$

So the diagonal has now "1" in all positions (the value of the correlation coefficient of a variable with itself), and off-diagonal positions (*i*, *j*) are $r(x_i, x_j)$, the correlation coefficient of variables $x_i$ and $x_j$. The matrix is symmetrical.

### 27.6.6 Definition of Chi-Square $\chi^2$ in Vinci Analysis for TCSPC data

The observables to be measured in TCSPC are the number of photons in each acquisition bin $\{N_1, N_2, \ldots N_k\}$.

As we assume that the number of photons acquired in each bin follows a Poisson distribution, the standard deviation is $\sigma_k = \sqrt{N(t_k)}$.

The reduced chi-square $\chi^2$ function is defined as:

$$\chi^2 = \frac{1}{n-p-1}\sum_{k=1}^{n}\frac{\left[N(t_k)-N_c(t_k)\right]^2}{\sigma_k^2} = \frac{1}{n-p-1}\sum_{k=1}^{n}\frac{\left[N(t_k)-N_c(t_k)\right]^2}{N(t_k)} \qquad [26.15]$$

Where $n$ is the number of acquisition channels (or bins), $N(t_k)$ is the number of photons collected in the bin $k$ and $N_c(t_k)$ is the number of photons in the bin $k$ assuming the photon distribution is described by the selected model.
This is the function that is being minimized using the Levenberg- Marquardt algorithm in the Vinci software by ISS.

## 27.7 Analysis models used in Vinci

### 27.7.1 Decay times models

In a multi-components environment containing $i$ fluorescent molecules, the fluorescence is described by the relationship:

$$I(\lambda,t) = I_0 \sum\nolimits_i \alpha_i(\lambda_i)\, \mathrm{e}^{-t/\tau_i} \qquad [26.16]$$

where the coefficients $\alpha_i(\lambda_i)$, called the pre-exponential factors and the decay times $\tau_i$ characterize the fluorescence decay of the $i$ component of the mixture. The pre-exponential coefficients are related to the fractional contributions $f_i$, that is the fraction of the total fluorescence emitted by the $i$-component of the mixture.

$$f_i = \frac{\alpha_i \tau_i}{\sum_i \alpha_i \tau_i} \qquad [26.17]$$

The *Vinci Multidimensional Fluorescence Spectroscopy Analysis* software determines the pre-exponential coefficients $\alpha_i$, the fractional contributions $f_i$ and the decay times $\tau_i$ of up to four components.

Note: a custom model distribution can be entered in Vinci and minimized.

### 27.7.2 A note on the fractional intensity

The fractional intensity is the number of photons associated with each decay. The fractional intensity does not correspond to mole fractions. In order to determine the mole fraction of each component present in the mixture one has to know take into consideration the relationship between quantum yield and lifetime for the particular system.

### 27.7.3 Lifetime distributions

In specific measurement situations the decay time of fluorescence is best described by non-exponential relationships. The fluorescent decay function, $I(t)$, is calculated from the distribution function by utilizing the following equation:

$$I(t) = \int n(\tau)\frac{1}{\tau}e^{-t/\tau}d\tau \qquad [26.17]$$

Where $n(\tau)$ describes distribution.

The Vinci includes the following lifetime distributions models [26.18]:

exponential (discrete) $n(\tau) = N_0\delta(\tau - \tau_0)$

uniform
$$n(\tau) = \begin{cases} 0 & \tau > \tau_0 + w/2 \\ N_0 & \tau_0 - w/2 \le \tau \le \tau_0 + w/2 \\ 0 & \tau < \tau_0 - w/2 \end{cases}$$
with $w = FWHM$

gaussian $n(\tau) = N_0 e^{-\frac{(\tau-\tau_0)^2}{w}}$ with $w = FWHM$

lorentzian $n(\tau) = \frac{N_0}{(1+2(\tau-\tau_0)/w)^2}$ with $w = FWHM$

planck
$$n(\tau) = \begin{cases} 0 & \tau \le 0 \\ \dfrac{N_0}{\left(0.2014052353\dfrac{\tau}{\tau_0}\right)^5 e^{\tau_0/0.2014052353\,\tau} - 1} & \tau > 0 \end{cases}$$
with $k = 0.2014052353 * (\frac{\tau}{\tau_0})$

When using a lifetime distribution, the parameters to be determined are the center decay time $\tau$ and the width $w$ of the distribution; in Vinci the width $w$ of the distribution is expressed as FWHM (full width half maximum).
*Vinci* allows for the assignment of each decay time to either a distribution of choice or to a single exponential decay.

Note: a custom model distribution can be entered in Vinci and minimized.

### 27.7.4 Anisotropy decay models

Following an infinitively short pulse of light, the total fluorescence intensity at time t observed by the ChronosBH is:

$$I(t) = I_{\parallel}(t) + 2I_{\perp}(t) \qquad [26.19]$$

By entering the expression into the definition of anisotropy $r(t)$, one finds the expressions for the parallel and perpendicular components for the emission anisotropy.

$$I_{\parallel}(t) = \frac{I(t)}{3}[1 + 2\,r(t)] \qquad [26.20]$$

$$I_{\perp}(t) = \frac{I(t)}{3}[1 - r(t)] \qquad [26.21]$$

These are the measurements acquired by the ChronosBH. The function $r(t)$ contains the characteristic rotational times of the molecule(s).
When performing the analysis, typically the characteristic decay times $\tau_i$ contained in the function $I(t)$ are fixed (in fact, they can be determined in a separate experiment). Yet, the curve can also be analyzed letting the entire parameters float.

How to model the $r(t)$ functions? When the instantaneous emission is a sum of exponentials, we have:

$$r(t) = r_0 \sum_i \alpha_i \exp(-t/\tau_{ci}) \qquad [26.22]$$

where $\tau_{ci}$ is the rotational correlation time of the $i$ component and $r_0$ is the limiting anisotropy.

A hindered rotator is described by the following expression

$$r(t) = (r_0 - r_\infty)\exp(-t/\tau_c) + r_\infty \qquad [26.23]$$

Finally, the rotational correlation time $\tau_c$ provided by the *Vinci Multidimensional Fluorescence Spectroscopy Analysis* software should not be confused with the rotation diffusion coefficient $D_R$. They are linked by the relation:

$$\tau_c = \frac{1}{6 D_R} \qquad [26.24]$$

# Appendix A: Specifications

| | |
|---|---|
| **Baseplate Dimensions:** | 540 L x 425 W x 235 H |
| **Weight** | 25 Kg (55 Lb) |
| **Polarizer motor:** | 12VDC stepper motor two-phase<br>(7.5 degrees per full step);<br>the motor drives polarizer through a 1 to 10 reduction gear train; full step angle 0.75 degrees. |
| **Input Power requirements** | 115/220 VAC; fuse 250V/2A |
| **Power supply input** | Voltage: Dual range 90-132Vac or 180-264 VAC, user selectable;<br>Frequency range 47-440 Hz single phase. |

# Appendix B: Warranty

**General Conditions**
All ISS manufactured instruments are warranted against defective materials and workmanship for one year from the date of shipment. The instruments must be used for the function they have been designed for, as described in the instruction manual. A Return Material Authorization (RMA) number is required before returning any instrument to the ISS factory for repairs.

Should this product malfunction during the warranty period, ISS will, at its option, repair or replace it at no charge, provided that the products have not been subjected to misuse, abuse, or unauthorized alterations, modifications, and/or repairs.

All expressed and implied warranties for this product include, but are not limited to, the warranties of merchantability and fitness for a particular purpose, are limited in duration to the above one year period. Some states do not allow limitations on how long an implied warranty lasts, so the above limitations may not apply to you.

Under no circumstances will ISS Inc. be liable in any way to the user for damages, including any lost profits, lost savings, or other incidental or consequential damages arising out of the use, or the inability to use, such products.

**Expired Warranty**
ISS will repair instruments with expired warranty at the current part and labor prices. Please contact ISS customer support for more information.

**Non-ISS Parts**
Although ISS Inc. may supply equipment manufactured by other companies, the only warranty that shall apply to such equipment is the warranty offered by the original manufacturer.

**Field Service**
During the one year warranty period ISS Inc. will replace defective parts (parts and labor) free of charge.

**Transportation Damage**
Packages should be carefully examined upon receipt for evidence of damage caused by shipping. If damage is noticed, notify ISS Inc. immediately. Preserve all packages, cartons and documents.

# Analytical Index