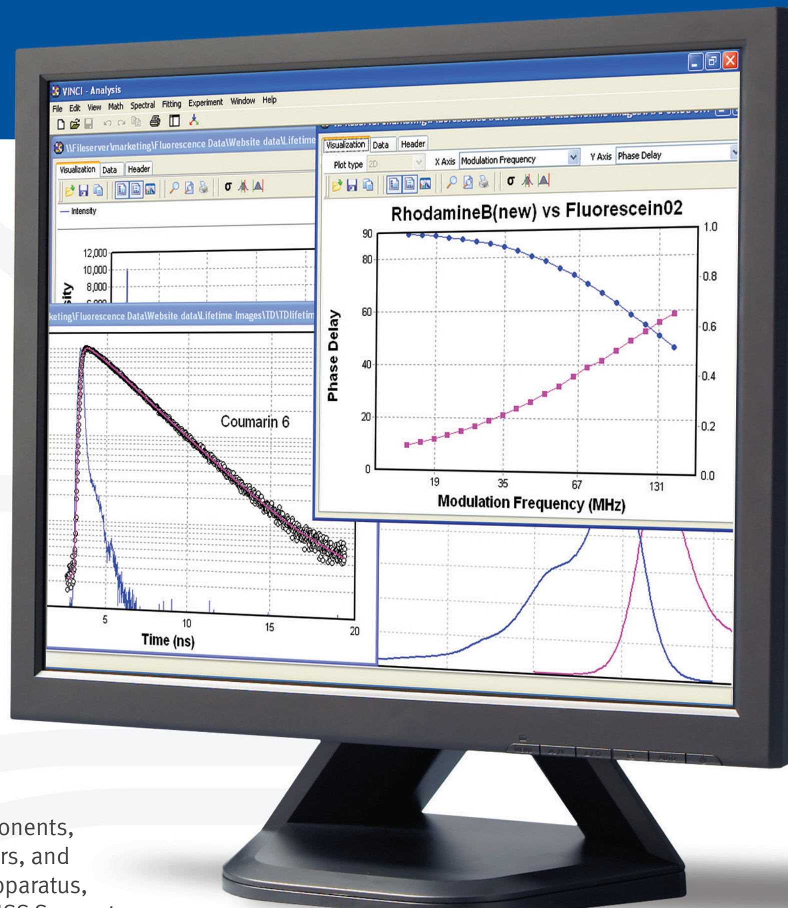
vinci™

MULTIDIMENSIONAL
FLUORESCENCE
SPECTROSCOPY



New
from ISS

Vinci™ now provides you with more flexibility by offering both time- and frequency-domain data analysis.

Vinci – Multidimensional Fluorescence Spectroscopy is a comprehensive software package designed to enhance the capabilities and performance of ISS spectrofluorometers.

Vinci offers unparalleled performance to the end-user through three distinct features:

Instrument Control
Vinci provides full control over all automated instrument components, including shutters, polarizers, sample holders, monochromators, and enables integration of external devices such as stopped-flow apparatus, titrator, and temperature bath. Vinci's remote interface allows ISS Support Personnel to access any instrument in the world via internet and conduct a remote diagnostic check-up.

Data Acquisition
Vinci offers a variety of data acquisition options (spectra, lifetime, kinetics, titrations) through built-in, easy-to-use, routines and allows the user to generate, store and rerun custom-designed data acquisition protocols. Vinci is used for the acquisition of both, time- and frequency-domain data on ISS instruments. Data are stored in ASCII format for convenient access from other software packages.

Data Analysis
Vinci's fast fitting routines allow to retrieve multiple decay times and multiple rotational correlation times, to obtain time-resolved spectra with picosecond resolution and to separate steady-state spectra of up to three components in a mixture using the phase- and modulation-resolved spectra measurement option. Other operations include smoothing, derivative, calculation of area and arithmetic between files. A superb graphical interface generates and displays 2D and 3D plots that can be exported in internet- and presentation-ready formats. Reports can be saved in Word and PDF formats.

Key Features

- Computer-control of all major optical components
- One- and multi-dimensional measurements
- Simulation and analysis of time- and frequency-domain data
- Built-in diagnostic routines to ensure proper instrument operation

Vinci significantly enhances the capabilities and performance of ISS spectrofluorometers

ISS™
Innovations in Fluorescence

Instrument Control

Instrument Configuration and Initialization
The Instrument Configuration Editor of Vinci identifies and enables remote control of all automated devices installed and connected to the instrument. Using this tool, the operator can add or remove devices to or from an existing configuration and save it for future use.

Upon initialization of Vinci for data acquisition, the Instrument Configuration is uploaded, and the software checks the proper operation of each remotely controlled device and alerts the operator in case of any malfunctioning.

Integration of External Devices
Computer-controlled external devices connected to the spectrofluorometer are also identified and synchronized via the Instrument Configuration Editor. External devices may include a titrator, a stopped-flow apparatus, circulators for temperature control, an automated pressure pump, a thermometer and the frequency synthesizers utilized for time-resolved measurements.

Flexibility
Vinci is easily configured for PC1, K2, Chronos, ChronosBH, and Phoenix (SLM upgrade package). K2, Chronos and ChronosBH can be configured to operate with a variety of light sources including laser diodes, light emitting diodes (LEDs), continuous wave lasers, mode-locked lasers and synchrotron radiation.

Instrument Diagnostic
Vinci contains automatic routines designed to ensure proper operation of the spectrofluorometer. Such routines include measurement of the signal-to-noise ratio using the Raman signal of water, recording of the stability of the signal intensity as well as routines for setting the proper discriminator threshold in photon-counting spectrofluorometers. These built-in diagnostic routines together with the remote access option to the instrument from the ISS Support Center help to maintain the optimal performance of the instrument.

Instrument Control Window
All active devices of the spectrofluorometer including all integrated external devices are displayed on the Instrument Control Window of Vinci. From here, the operator, like the pilot of an airplane, takes control of the entire instrument; the operator has direct control over each device either during Experiment Setup or during Data Acquisition. Once the parameters and devices for the measurement are selected, the Data Acquisition session is initiated by selecting the ***Start*** button.

Data Acquisition

Vinci offers several routines for the prompt acquisition of fluorescence data:

- Spectra (excitation, emission, synchronous, excitation and emission matrices)
- Fast and slow kinetics (intensity, polarization and ratiometric)
- Fluorescence decay times
- Rotational correlation times
- Time-resolved spectra

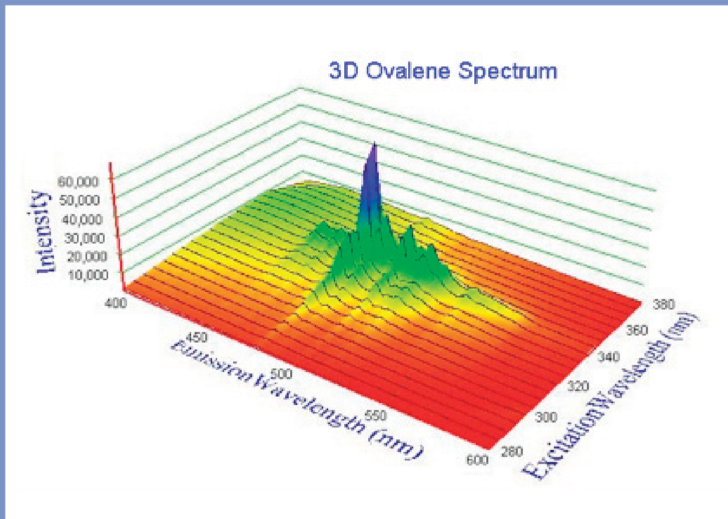


Figure 1

3-Dimensional profile of an **Ovalene** sample

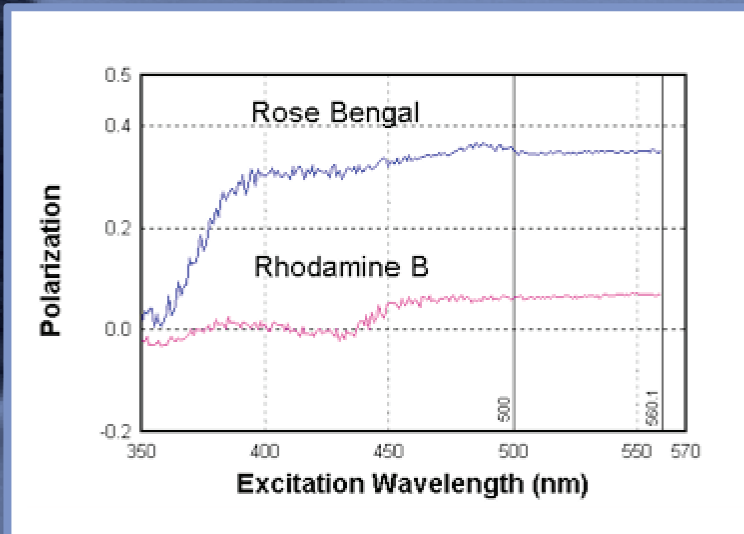


Figure 2

Excitation polarization spectra of **Rhodamine B** and **Rose Bengal** with polarization values of **P = 0.066** and **P = 0.349** for the recommended excitation range for polarization measurements. The data was acquired on PC1™ using a 300W Xenon lamp.

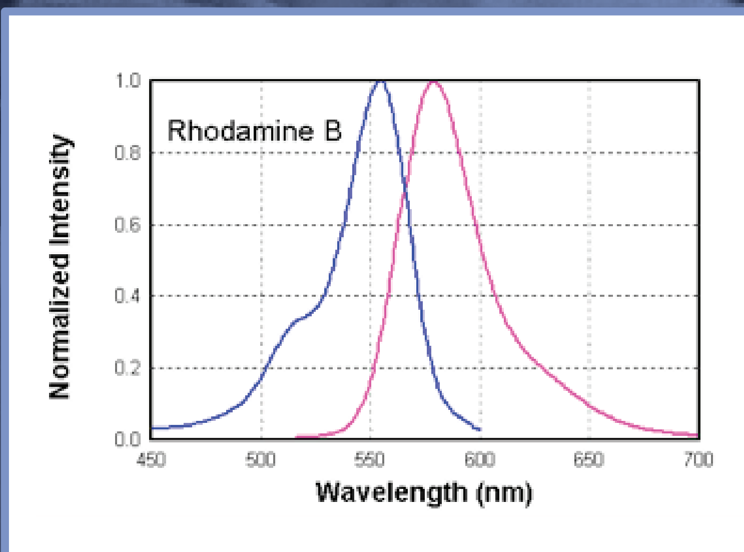


Figure 3
Excitation and emission spectra (λ_{max}(ex)=554 nm, λ_{max}(em)=579 nm) of **Rhodamine B** in water. Data was acquired on PC1™ using a 300W Xenon lamp.

Once the user defines the experiment control parameters and initiates Data Acquisition, the instrument no longer requires the presence of an operator. Multiple data files can be acquired and saved automatically. During Data Acquisition measurement results are displayed graphically and numerically in real-time.

User-designed Protocols
The user can also design a series of measurements with customized experimental conditions of his/her own choice and save this protocol as an experiment file for later use.

Experiment Files with Exact Measurement Conditions
Each experiment file includes not only the raw data but also the experimental conditions for each measurement. Once a saved experiment file is reloaded the measurement can be repeated with the exact same experimental conditions (wavelength range, acquisition time, frequency range, etc.). Experiment files are never modified; once a modification is made, the user is required to save the modified file under a new file name.

One-dimensional and Multi-dimensional Experiments
Acquisition protocols are classified as one- or multi-dimensional experiments. One-dimensional experiments include e.g. the acquisition of emission spectra (intensity versus wavelength); time-traces (intensity versus time) and single-point polarization measurements as well as the measurement of the fluorescence decay times at a set temperature of a sample.

Multidimensional experiments include the acquisition of a series of emission spectra at different temperatures; polarization measurements at different excitation and emission wavelengths, and the acquisition of lifetimes at different pressure, or excitation wavelengths.

Adaptive Data Acquisition
For the acquisition of steady-state and time-resolved data the user has two options for Signal Averaging: Acquisition Time and Standard Deviation.

When Acquisition Time is chosen, different data points may have a different standard deviation. Alternatively, when the Standard Deviation option is used, data acquisition will continue until the chosen standard deviation is reached. The software is adaptive, and automatically optimizes the acquisition time for each data point according to its noise level: data points with a higher noise require additional acquisition time; conversely, data points with a lower noise require a shorter data acquisition time.

Automatic Correction of Instrument Artifacts
ISS instruments also feature automatic correction of instrumental artifacts:

- Dark Count Correction: The light detectors dark current is periodically evaluated by Vinci and automatically subtracted from the acquired data.
- Correction of the intensity output of the lamp: Excitation spectra are automatically corrected for the intensity output of the lamp by using a quantum counter placed in the reference channel of the spectrofluorometer.
- Correction of instruments spectral response: Emission spectra are automatically corrected for the instrument's spectral response during acquisition by applying the correction factors provided with the instrument.

Background Subtraction in Steady-state and Time-Resolved Measurements
Biological samples exhibit background fluorescence, a condition that alters and compromises the quality of the fluorescence signal. Vinci offers an option to automatically correct the background fluorescence of samples both, in steady-state and time-resolved mode.

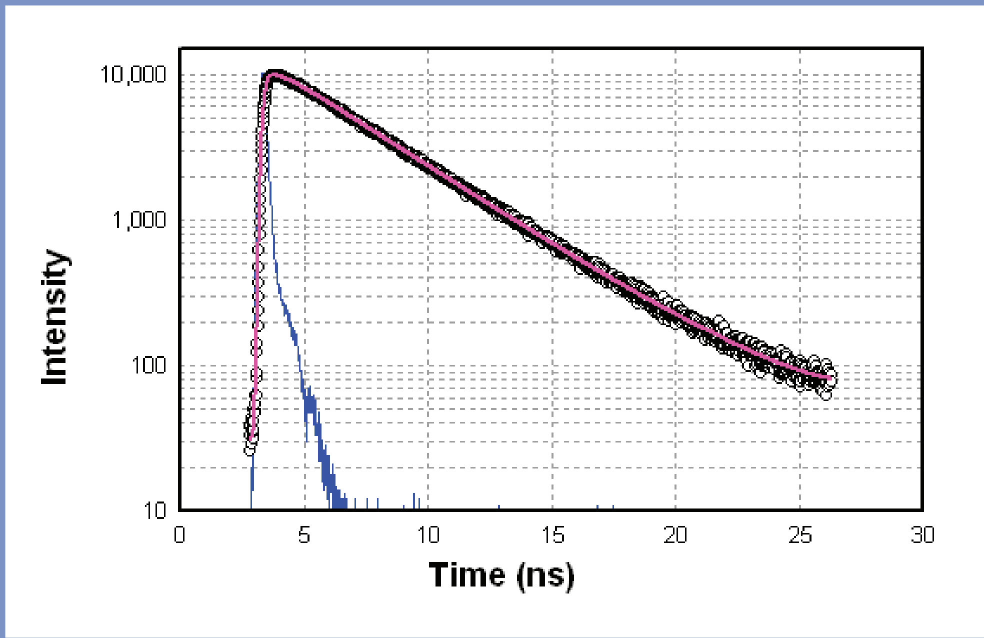


Figure 4

Time-domain intensity decay of Fluorescein in PB 7.4 acquired on ChronosBH™ using a 447-nm pulsed laser diode. The emission was collected through a long pass filter KV 505. The data is best fitted by a single exponential decay time of 4.0 ns (χ^2 = 1.2).

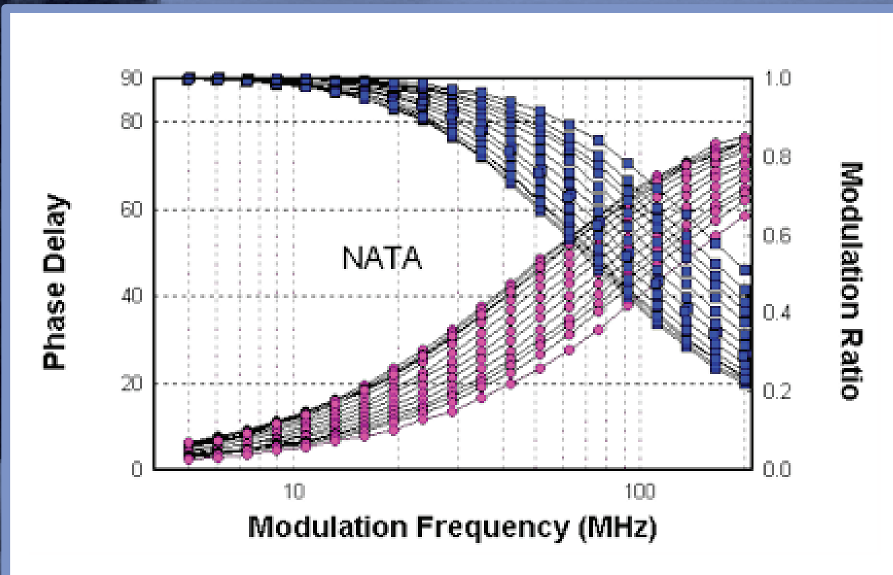


Figure 5

Temperature- dependent frequency responses of **NATA** in water. Temperature range: 5-70°C in 5°C intervals. Excitation source: 280nm (LED). The emission was collected through a long pass filter 320. The lifetime values range from 3.3ns at 5°C, to 1.3ns at 70°C.

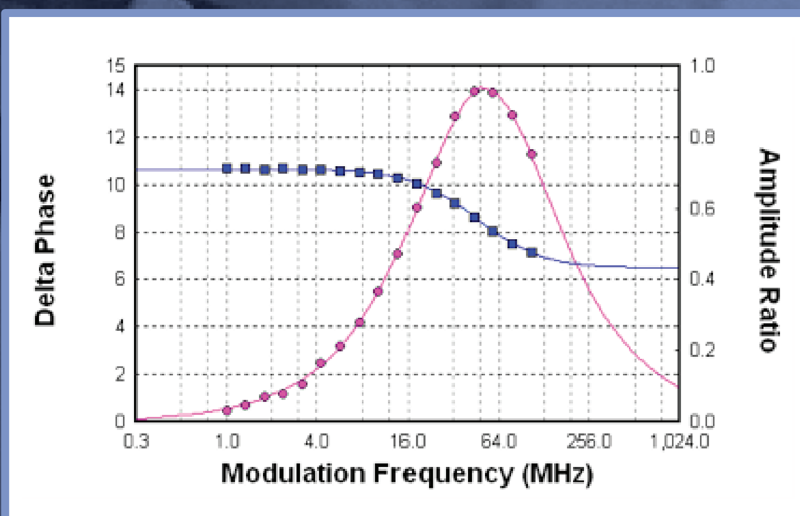


Figure 6

Frequency-domain **anisotropy decays** (Differential Polarized Phase Angle and Amplitude Ratio) of **TNS** in propylene glycol measured on Chronos™ using a **300-nm LED**. The emission was collected using a WG320 long-pass filter. Calculated values for θ = 5.03 ns with R_o = 0.30 and τ = 7.8 ns, T = 27°C.

Data Processing and Analysis

Data File Types

Vinci generates two types of data files: Experiment and Analysis files. Both file types are created in ASCII format and can be identified by their extension and the associated icon.

Experiment files, also called raw data files, include all data and the experimental parameters of a specific measurement (monochromator settings, temperature, sample concentration, polarizer position; or modulation frequency in a lifetime measurement). Experiment files cannot be modified thus ensuring the authenticity of the experimental conditions for future data acquisition.

Analysis files are generated every time a mathematical operator is applied to an Experiment file.

Processing Data Files Using Math Operators

Data files can be combined and superimposed. Several mathematical operators can be applied to a file:

- Normalization of the y-axis at set points of the x-axis
- Reformatting of the x-axis
- Determination of the reciprocal
- Calculation of the area and of the median of a spectrum along with the higher-order moments; smoothing (average, median, Savitzky-Golay)
- Calculation of the nth-order derivatives
- Conversion to wave numbers

Arithmetic operations between two files include subtraction, addition, multiplication and division. Moreover, spectral correction can be applied to technical spectra, and spectra acquired on the same sample can be averaged. The ***Undo*** key in the toolbar allows reversing any mathematical operator applied to a data file.

Fitting Models for Time- and Frequency-Domain Data

Time-resolved fluorescence data analysis is performed by comparing a model (decay time or rotational correlation time) with the acquired data using a Levenberg-Marquardt algorithm as the minimization routine for the χ^2-function. The model with the lowest value for the χ^2-function is chosen for the system. The analysis includes fitting routines for multiple decay times (exponential, non-exponential, lifetime distributions); rotational correlation times (isotropic, anisotropic, and hindered rotators); the display of time-resolved spectra; and phase-modulation resolved spectra.

Fitting Time-resolved Data Using a User-defined Model

Vinci allows for a user to input a custom analysis model and the custom equation is minimized using the experimental data for the χ^2-function.

Simulation of Time-resolved Data

Vinci Analysis also includes a simulation routine that allows the user to simulate time-resolved fluorescence data. Customized frequency response of single, multiple- and non-exponential decays, as well as lifetime distributions can be generated based on user-defined inputs for the decay times and fractional contributions. Similarly, anisotropy decays for isotropic, anisotropic and hindered rotators can be simulated and visualized.

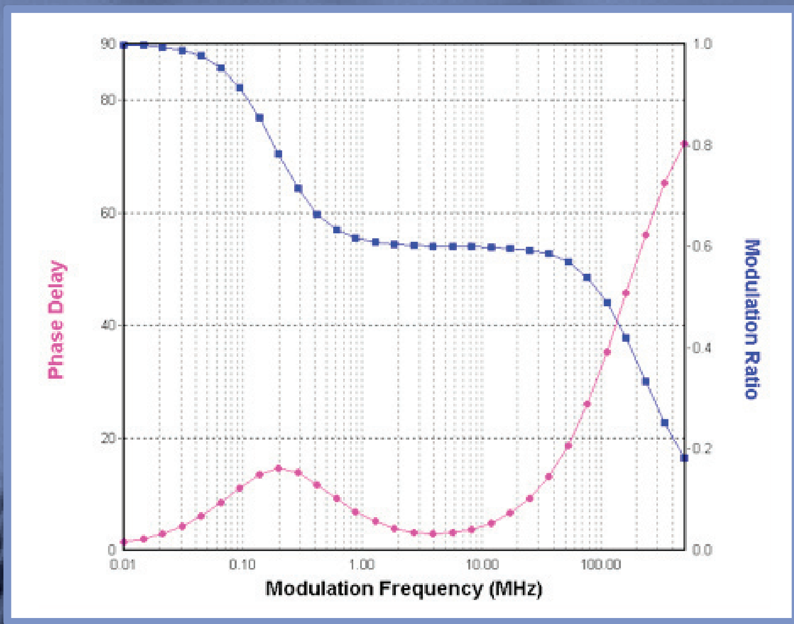
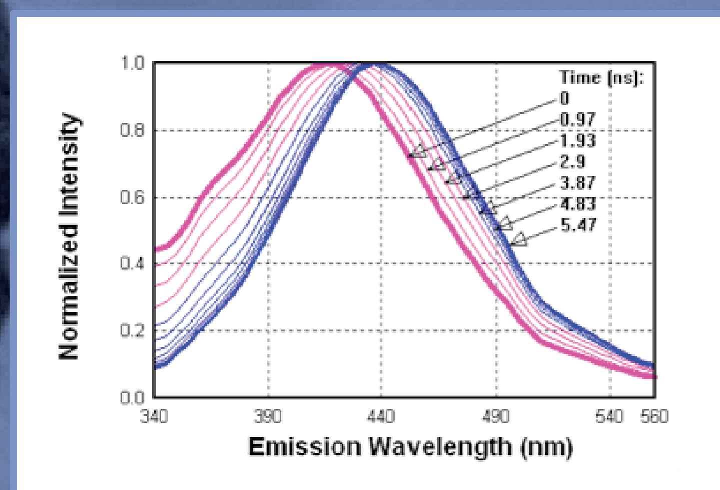


Figure 7

Simulated frequency response (f_1= 0.6, τ_1=1ns, τ_2=1000 ns).

Figure 8

Series of **time-resolved spectra** of **TNS** in glycerol. The excitation source was a 300-nm LED. The emission was scanned from 340 to 560 nm with a 2-nm step size. The frequency was scanned from 5-150 MHz.



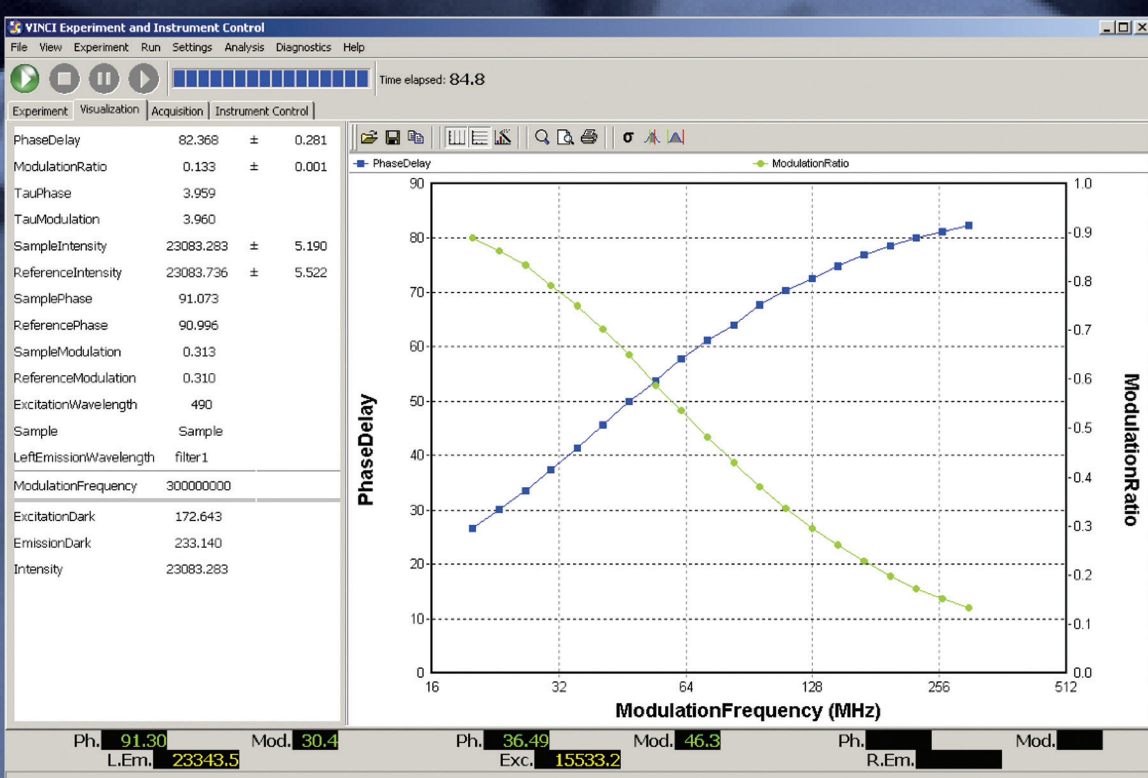


Figure 9

Vinci Data Acquisition Visualization Screen depicting measurement parameters for a single frequency and the resulting phase-modulation curve.

Graphical Interface, Data Transfer and Presentation

Plots
Vinci generates 2D and 3D plots. Areas of 2D plots can be zoomed; hairlines define the position of relevant peaks and the cursor positioned on the plot identifies the XY location. Statistical functions (average and standard deviation) can be applied to the area between the two hairlines.

3D plots can be zoomed and rotated in space. Surface and contour plots can be generated and saved.

Properties of 2D and 3D plots, such as color and thickness of the lines, ticks of the XYZ scales, background and foreground colors and fonts generated in Vinci are user-controlled. The user can save the default values of the plots for future use.

Printing and Export
Plots can be exported to popular internet-formats such as tiff, gif, jpeg; and to publication and presentation formats such as png, bitmap and metafiles. Vinci can also be utilized to generate plots in a publishable format.

Fit Report
All fitting results and plots generated during data analysis are separately saved and presented in the Fit Report. This report, that can be saved either in Word- or pdf-format, includes all selected parameters of the analysis model, as well as the decay times and the respective fractional distributions and pre-exponential factors; further it includes a matrix of the correlation coefficients and two separate numerical tables, one for phase and one for modulation, listing their calculated and measured values, residuals and standard errors for each modulation frequency. The report also includes plots of the frequency responses, the residuals and the lifetime distribution.

Specifications

Instrument Controls

- Automation of ISS instrument components
- Control of external devices
- Temperature bath (Neslab, Fisher)
- Titrator (ISS, Hamilton)
- Stopped-flow apparatus
- (Molecular Kinetics, OLIS, Hi-Tech, Applied Photophysics, Kintek)
- Pressure pump
- Recording from temperature/ pressure sensors
- Storage of all experimental parameters in the experiment data file

Data Acquisition

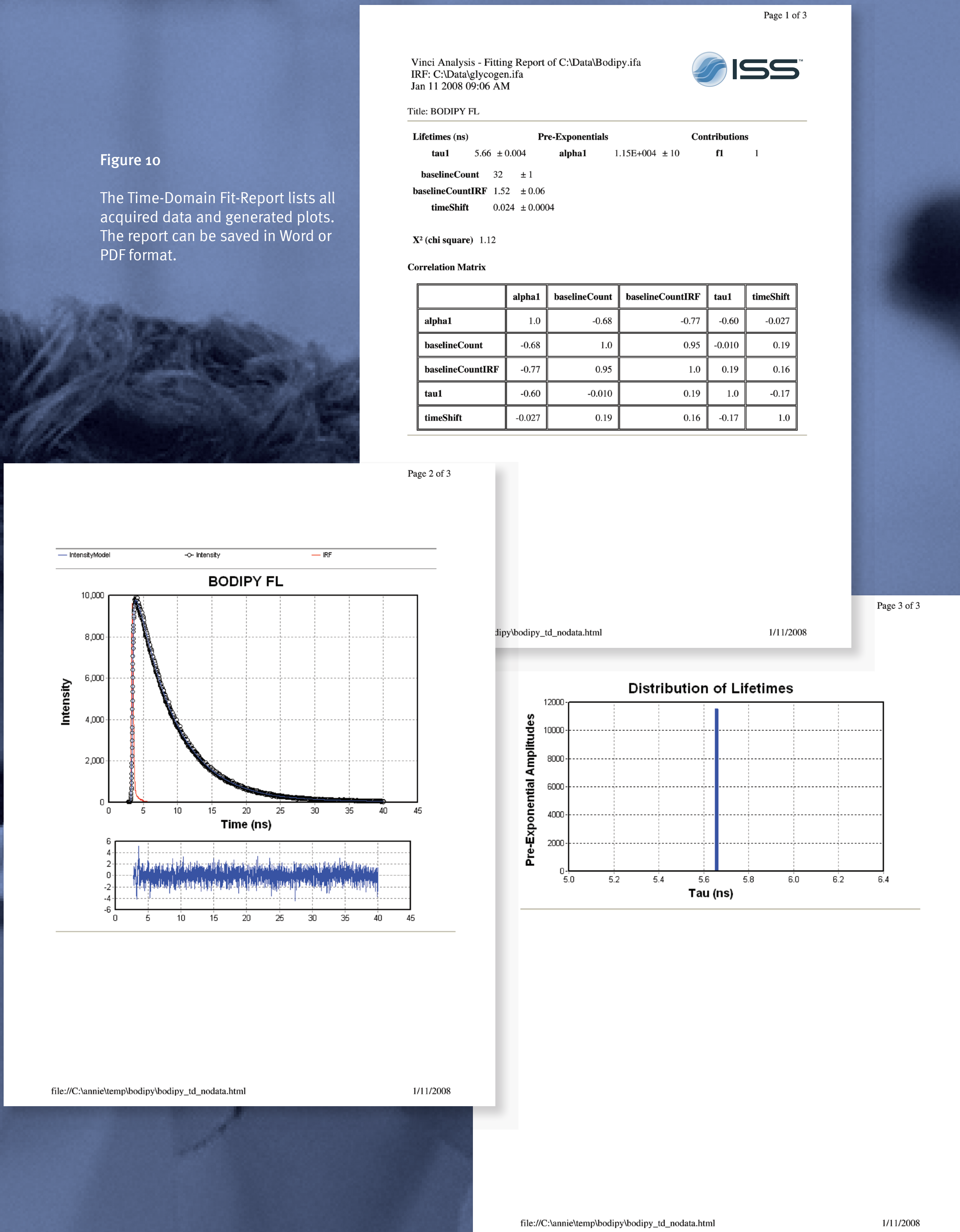
Vinci supports one-dimensional, multi-dimensional and user-defined protocols.

One-dimensional measurements

- Corrected excitation and emission spectra
- Excitation-emission matrices
- Synchronous spectra
- Polarization/Anisotropy
- Kinetics (slow, fast)
- Titrations
- Fluorescence decay times (exponential and non-exponential)
- Anisotropy decays
- Time-resolved spectra
- Phase- and modulation-resolved spectra
- Multi-dimensional measurements

Figure 10

The Time-Domain Fit-Report lists all acquired data and generated plots. The report can be saved in Word or PDF format.



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Vinci Analysis - Fitting Report of C:\Data\Bodipy.ifa
IRF: C:\Data\glycogen.ifa
Jan 11 2008 09:06 AM

Title: BODIPY FL

Lifetimes (ns) **Pre-Exponentials** **Contributions**

tau1 5.66 ± 0.004 **alpha1** 1.15E+004 ± 10 **f1** 1

baselineCount 32 ± 1
baselineCountIRF 1.52 ± 0.06
timeShift 0.024 ± 0.0004

X^2 (chi square) 1.12

Correlation Matrix

	alpha1	baselineCount	baselineCountIRF	tau1	timeShift
alpha1	1.0	-0.68	-0.77	-0.60	-0.027
baselineCount	-0.68	1.0	0.95	-0.010	0.19
baselineCountIRF	-0.77	0.95	1.0	0.19	0.16
tau1	-0.60	-0.010	0.19	1.0	-0.17
timeShift	-0.027	0.19	0.16	-0.17	1.0

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Multi-dimensional Measurements

Vinci also supports multi-dimensional data acquisition. The user can select any of the following parameters:

Fluorescence Intensity Polarization/Anisotropy Ratio Measurements	VS	Excitation (emission) wavelength *and/ or any of the following:* • Emission (excitation) wavelength • Sample concentration • Sample pH • Sample temperature • Sample pressure
Fluorescence Intensity Polarization/Anisotropy Ratio Measurements	VS	Time and any of the following: • Excitation wavelength • Emission wavelength • Sample concentration • Sample pH • Sample temperature • Sample pressure
In Frequency-Domain only: Phase Modulation Differential Polarization Angle or Modulated Anistropy	VS	Modulation Frequency *and any of the following:* • Excitation wavelength • Emission wavelength • Sample concentration • Sample pH • Sample temperature • Sample pressure
In Time-Domain (TCSPC): Fluorescence Intensity Anisotropy	VS	Time *or any of the following:* • Excitation wavelength • Emission wavelength • Sample concentration • Sample pH • Sample temperature • Sample pressure

User-defined Protocols

The user can define protocols for both one-dimensional and multi-dimensional measurements. Protocols can be stored and rerun.

Lifetimes (ns)

tau1	1.36	± 0.022
tau2	8.73	± 0.029

Contributions

f1	0.155	± 0.0021
f2	0.845	FIXED

Pre-Exponentials

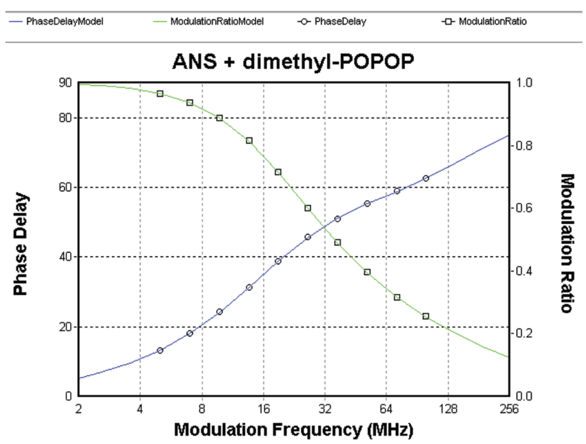
alpha1	0.114
alpha2	0.0968

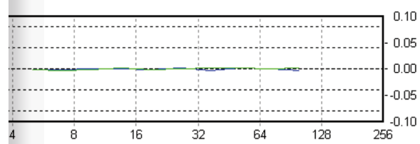
Title: ANS + Dimethyl-POPOP

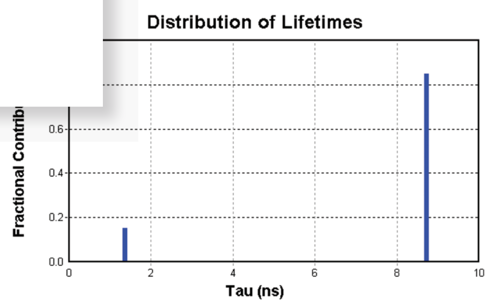
X^2 (chi square) 0.624

Correlation Matrix

	f1	**tau1**	**tau2**
f1	1.00	0.936	0.857
tau1	0.936	1.00	0.771
tau2	0.857	0.771	1.00







ModulationFrequency	**ModulationRatio**	**ModulationRatioModel**	**ModulationRatioResidual**	**ModulationRatioStdError**
5000000.00	0.964984	0.966457	-0.00147254	0.0051342
6974754.00	0.935411	0.937872	-0.00246183	0.0034782
9729439.00	0.888177	0.889347	-0.00116999	0.0051825
13572088.00	0.816662	0.814617	0.00204535	0.0043191
18932395.00	0.714207	0.714276	-6.85041E-005	0.0043012
26409760.00	0.601547	0.599858	0.00168917	0.0033088
36840315.00	0.491664	0.488709	0.00295576	0.003041
51390427.00	0.39548	0.393358	0.00212143	0.0032079
71687116.00	0.316832	0.316476	0.000355504	0.0022994
100000000.00	0.256308	0.253879	0.00242957	0.0023483

ModulationFrequency	**PhaseDelay**	**PhaseDelayModel**	**PhaseDelayResidual**	**PhaseDelayStdError**
5000000.00	13.2659	13.2957	-0.029766	0.060329
6974754.00	18.0919	18.0983	-0.00638342	0.097248
9729439.00	24.211	24.156	0.0550043	0.056871
13572088.00	31.2751	31.2516	0.0234808	0.10308
18932395.00	38.6967	38.7132	-0.0165481	0.087032
26409760.00	45.7199	45.5709	0.148975	0.12009
36840315.00	50.942	51.0876	-0.145656	0.10003
51390427.00	55.3868	55.2618	0.12501	0.11741
71687116.00	58.8841	58.8259	0.0581892	0.11561
100000000.00	62.5373	62.6612	-0.123991	0.14866

Figure 11

The frequency-Domain Fit Report summarizes all fitting results and plots generated during data analysis.

vinci

MULTIDIMENSIONAL FLUORESCENCE SPECTROSCOPY

Data Analysis

Spectral Analysis
- Normalization
- Smoothing (average, median, Savitzky-Golay)
- Derivative (n^{th}-order)
- Integration (area, average and standard deviation)
- Spectral correction
- Spectral moments
- Conversion to wave numbers
- Data interpolation
- User-defined filtering
- Fitting to user-defined model

Spectral Operations
- Addition, subtraction, multiplication, and division
- Scaling factor

Time-resolved Analysis
- Multiple decays times of up to four components for exponential, non-exponential and lifetime distributions
- Rotational correlation times for isotropic, anisotropic and hindered rotators
- User-defined models
- Phase- and modulation-resolved spectra
- Time-resolved spectra

Simulations
- Frequency responses (Phase angle and demodulation versus modulation frequency for up to four components for exponential, non-exponential and lifetime distributions)
- Anisotropy decays (Differential phase and modulation ratio versus modulation frequency for isotropic, anisotropic and hindered rotators).
- Intensity decays (intensity versus time) for mono- and multi-exponential decays

Display
- 2D and 3D with user-defined colors and fonts
- 3D surface rotation and in/out zooming
- Cursor identification of XY spectra coordinates

Data format
- Data files are stored in easy accessible (ASCII) text format.
- Experiment Data files, or raw data files, include all information about the experiment protocol and can be re-loaded to rerun the original experiment.
- Once modified, an Experiment data files cannot be overwritten. Experiment data files, and analysis data files are identified by different extensions and icons.

Fitting
Levenberg-Marquardt algorithm.

Export of Data and Plots
Plots can be saved and exported in gif, tiff, jpeg, png, bitmap and metafiles format. Vinci can also recognize files saved with LFD and CFS formats.

Links to
- Global Analysis by Globals Unlimited
- DATAN by MultiD Analysis AB

Operating System
- Windows2000
- WindowsXP

ISS™

1602 NEWTON DRIVE
CHAMPAIGN, IL 61822, USA
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For more information about Vinci please visit www.iss.com or call 1-217-359-8681