Minimální požadované technické parametry

Název technologie / vybavení: **: Fluorescenčni spektrometr**

Stručný popis technologie / vybavení a stanovení výzkumného účelu a využití:

Pro potřeby projektu FIT musí fluorescenční spektrofotometr umožňit měření spekter, steady state fluorescenci, life-time (metoda TCSPC a frekvenčí doména), polarizace fluorescence, měření vzorků nanočástic a nanovrstev v TIRF cele, měření intracelulární biochemie (např. pH, Ca, K, Mg), měření při definovaných teplotách a s teplotní rampou. Přístroj bude sloužit pro studium struktury a interakce proteinů a peptidů, nanočástic, RNA/DNA konstruktů, které budou připravovány v rámci projektu FIT. Oblast využití bude pro charakterizaci vakcín, vývoj diagnostik a teranostik. Přístroj musí umožňovat relativní a absolutní Další využití je plánováno pro studium elasticity lipidních membrán a formování lipidních raftů, stanovení změny konformace a termální stability proteinů a dalších biopolymerů. Technologie je plánována pro výzkum a vývoj nanočástic na bázi lipoplexů pro cílení léčiv a genetických vakcín ve formě konstruktů nukleových kyselin (siRNA, mRNA, antisens oligonukleotidy, pDNA). Na VUVeL bude fluorescenční spektrofotometr využíván v rámci projektu FIT, CENATOX, Admirevet a dalších navazujících výzkumných projektů. Přístroj bude využíván také v rámci spolupráce s ICRC (společná laboratoř pro nanofarmakologii NanoPharm) při vývoji nových diagnostik a teranostik na bázi cílených nanočástic.

Popis parametru	Zadavatelem požadovaná hodnota	Uchazečem nabízená hodnota	Závaznost
CHROMSPEC spol. s r.o.			
ISS, Inc.			
Chronos DFD			
Fluorescenční spektrometr	Ano	Ano	Podmínka
Preferované technické požadavky Spektrofluorimetr umožňující steady - state měření, měření poločasu života excitovaného stavu (live-time fluorescence) v módu „frequency-domain nebo metodou „time-correlated single photon counting" (TCSPC), preferenčně oběma mody. V případě obou modů měření musí umožňovat přechod mezi výše uvedenými módy pouze softwarovou volbou bez nutnosti fyzické přestavby systému. T - formát přístroje umožňující současně měřit fluorescenci a polarizaci fluorescence včetně Glan - Thomsonových polarizátorů. Xenonová lampa, min. výkon 150W Napájecí zdroj pulzních diod: nastavitelná frekvence od 2 Hz do 100 MHz Další zdroje světla: Pulsed Laser Diode 375 nm Pulsed Light Emitting Diode (LED) 280 nm Fotonásobič - rozsah 185 - 900nm. Rozsah měření doby života excitovaného stavu: niminálně 200 ps – 200 ms	Ano	Ano	Podmínka

Doplňková zařízení Systém musí obsahovat následující příslušenství: Čtyřpoziční automatický výměník kyvet nebo vícepoziční), chlazený Peltierovým článkem. Rozsah teplot: minimálně -20°C až 105°C, dodávka včetně externího chlazení Total Internal Reflection Fluorescence (TIRF) Flow Cell pro studium makromolekul na povrchu a nebo v blízkosti povrchu a na rozhraní membrán. Příslušenství musí být připraveno pro snadné použití a přednastaveno do optické osy fluorimetru. Držák pro měření pevných vzorků a vysoce rozptylujících materiálů	Ano	Ano	Podmínka
Software Součástí dodávky musí být řídící software. Řídící software pro řízení fluorimetru a všech dodaných příslušenství, pro sběr dat, jejich vyhodnocení a prezentaci.	Ano	Ano	Podmínka
Rozměry přístroje (V x Š x H)	Uveďte: rozměry	330x400x540mm	Informativní
Hmotnost přístroje	Uveďte: hmotnost	26 kg	Informativní
Požadavky na napájení: 230 V	Ano	Ano	Podmínka
Další požadavky			
V dodávce budou obsaženy přístroje, příslušenství i spotřební materiál (který je součástí dodávky) ve verzi, která je pro daný typ výrobku aktuální (poslední)	Ano	Ano	Podmínka
Dodání na místo určení, instalace, uvedení do provozu a instruktáž součástí dodávky.	Ano	Ano	Podmínka
Zabezpečení servisu a případných preventivních prohlídek po celou dobu životnosti přístroje	Ano	Ano	Podmínka

DIGITAL FREQUENCY DOMAIN
SPECTROFLUOROMETER

chronos DFD

Chronos DFD - ISS Digital Frequency Domain technology for Lifetime measurements in complex decays in less than 1 second.

ChronosDFD is capable of measuring decay times of fluorescence and rotational correlation times of complex fluorescence mixtures in 1 second with an accuracy of a few picoseconds. The novel instrument opens a number of applications until now hindered by the duration of the acquisition: the capability of measuring fluorescence decay in a short time makes the instrument usable for measuring fast kinetics processes; the decay times of mixtures in a stopped flow apparatus and in chromatography; and lifetimes in samples where the exposure to light has to be minimized in order to avoid photobleaching.

How does it work?

The ChronosDFD uses the unique digital frequency domain technology. The light sources (laser dioded and LEDs) are modulated using square pulses of about 2 nanosecond duration with a repetition rate selected by the user and in the range from 0,05 Hz. to 80 MHz. The frequency signal contains the fundamental frequency at the repetition rate and its harmonics for up to thirty; that is, when the fundamental frequency is, for instance 10 MHz, harmonics at 20,30,40 and all the way up to 300 MHz are generated. The detector photons are separated into phase bins depending upon their individual phase shift and demodulation until a phase histogram is built; from the the histogram the decay times are determined.

Designed for Steady-State & Time -Resolved Applications

Steady-State Measurements

- Intensity measurements at fixed wavelengths
- Polarization (anisotropy) measurements at fixed wavelengths
- Slow and fast kinetics
- Dual wavelength emission-ratiometric measurements

Time-Resolved Measurements

- Frequency responses of single and multi exponential decays
- Anisotropy decays
- Phase and modulation resolved kinetics
- Phase and modulation resolved spectra
- Time resolved spectra
- FRET

ISS™
focus and discover

User friendly Software

ChronosDFD includes *Vinci- Multidimensional Fluorescence Spectroscopy*, a powerful software package that provides several ready-to -use routines for reliable, user friendly acquisition of complex fluorescence data:

- Spectra (excitation, emission, synchronous, time-resolved and polarization)
- Measurements at fixed wavelenghts (intensity and polarization)
- Measurements of kinetics
- Time-resolved measurements (lifetimes and rotational correlation times)

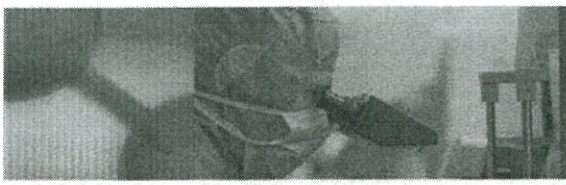
Key Features

- Flexible instrument configuration with a variety of light sources (laser diodes, LEDs, Supercontinuum laser and Ti Sapphire laser)
- Second to picosecond lifetime measurement capabilities using the same hardware
- Complete lifetime scans in one second on routine samples with proprietary Digital Frequency domain technology
- A compact footprint and short optical pathlenght for maximum sensitivity and efficient light coupling into the sample
- T-format and parallel beam optical design for fast and precise polarization measurements
- Full automation of instrument components including: cuvette holder, polarizers, shutters, filter wheel, monochromators and stirrers
- PC-controlled integration of temperature bath, titrator, stopped-flow apparatus & pressure pump
- User selectable data acquisition display: time-domain or frequency-domain

Vinci, the Complete Software Solution for Steady-State and Time-Resolved Applications

A powerful and flexible multidimensional fluorescence spectroscopy software with ready-to-use routines for data acquisition and analysis.

www.iss.com



chronos DFD Specifications

Software Specifications

Instrument Automation
ChronosDFD is the instrument of choice for reliable time-resolved data acquisition using laser diodes and LEDs. All hardware components, including external devices, are automated and PC-controlled.

Instrument Diagnostic and Noise Detection
Vinci includes routines for analyzing the instrument's performance, allowing the user to monitor data acquisition and noise level during the entire measurement. If sample saturation occurs the signal level is adjusted accordingly.

User-friendly Acquisition
A sequence of measurements is acquired through a one-time setup of the experiment file, allowing for the automatic acquisition of multiple data sets.

Personalized Log-on
With its unique system memory design, Vinci allows user-specific access. In multi-user environments each user may perform measurements with a personalized instrument configuration.

Data Analysis

Decay Times
Decay time analysis is performed on multiple data sets using various models including multi-exponential, non-exponential and lifetime distributions.

Rotational Correlation Times
Anisotropy decay data analysis of up to three species using models for isotropic, anisotropic and hindered rotators.

Phase- and Modulation-Resolved Spectra
Vinci also includes routines for the separation of up to three components in phase- and modulation-resolved spectra.

Phasor (polar) plot analysis
A powerful graphical approach to fluorescence decay data analysis used to quantify individual components of a mixture, FRET processes and excited states reactions.

Data Display & Export

- 2D and 3D display with user-defined colors and fonts
- 3D surface rotation and in/out zooming
- 3D display of user-defined functions
- Cursor identification of XY spectra coordinates
- Time-resolved spectra display as 3D and center of gravity plots
- Export to gif, png, jpeg, bitmap and metafile formats
- Data are generated and exported in ASCII format

Instrument Specifications

Light Sources (Internally modulated or pulsed):
Laser diodes: 370, 405, 436, 473, 635,690, 780 and 830 nm

LEDs: (265, 280, 300, 335, 355, 370, 460, 480, 500 and 520 nm)

Optional: Xenon Arc Lamp, Continuous-wave (CW), Super continuum laser and Ti:Sapphire laser

Focusing & Collection Geometry: Parallel beam design for precise polarization measurements

Polarizers: UV grade Glan-Thompson with L/A=2.0

Light Detectors: PMT, hybrid PMT, MCP, APD

Wavelenght Range: From 200 nm to 1700 nm (detector-dependent)

Max Counts Range: Up to 13 million counts/s (hybrid detectors)

Lifetime Measurements Range: 10^{-12} to 10^{-2} sec

OS Requirements: Windows 10

Power Requirements: Universal power input of 110-240 V, 50/60 Hz, 400 VAC

Dimensions: 540 mm (L) x 400 mm (W) x 330 mm (H)

Weight: 26 kg

Information & specifications are subject to change without notice.

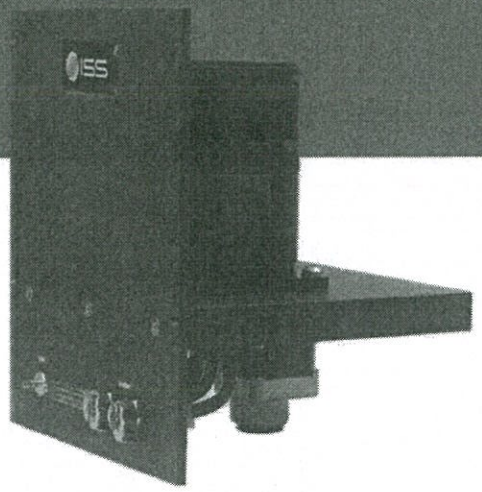
For more information and a complete list of accessories for ChronosDFD, please visit www.iss.com

ISS™
focus and discover

1602 NEWTON DRIVE
CHAMPAIGN, ILLINOIS 61822, USA
217-359-8681

Four-cuvette Peltier

FLUORESCENCE ACCESSORY



Four-cuvette Peltier Sample Compartment

The four-cuvette Peltier-base sample compartment is for use with all of ISS spectrofluorometers and works from -40 °C to +105 °C. The sample compartment comes with temperature controller, magnetic stir bar and tubing.

Specifications

Temperature

- Range of -40 °C to +105 °C*
- Precision of ± 0.02 °C

Optical Port Dimensions

- 12 mm x 10 mm
- Optical axis 8.5 mm

Cuvettes

- 12.5 mm x 12.5 mm O.D. standard

*Operation below the ambient dew point temperature requires dry gas purge. Operation below -10 °C requires dry gas purge and pre-cooled circulating fluid within 25 °C of the desired temperature.

Accessories for Peltier

- Accessory post, a plastic post and support clip that may be inserted in the top of the sample compartment and used to hold tubes, probes and other hardware for special measurements.
- Cuvette adapters for 3x3, 4x4 and 5x5 cuvettes

Key Features

Rapid, precise control over an extended range of temperatures

Highly uniform temperature in each cuvette

Fully automated through ISS Vinci Software package

Calibrated using a NIST-traceable thermometer

Complete package with compartment floor and utilities brought to the front panel

Variable speed magnetic stirring for each cuvette

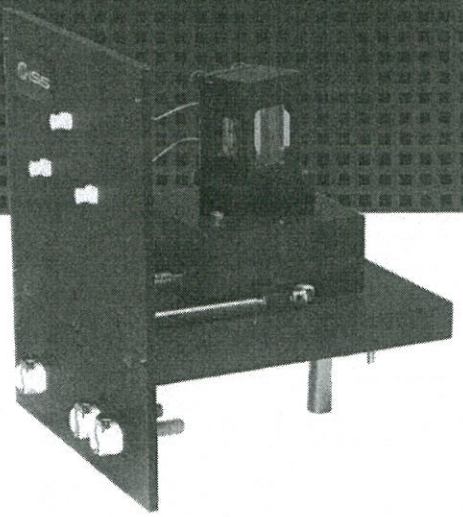
Dry gas purge

Thermometer probe input

ISS™
Innovations in Fluorescence
www.iss.com

FLUORESCENCE ACCESSORY

TIRF Flow Cell



TIRF Flow Cell

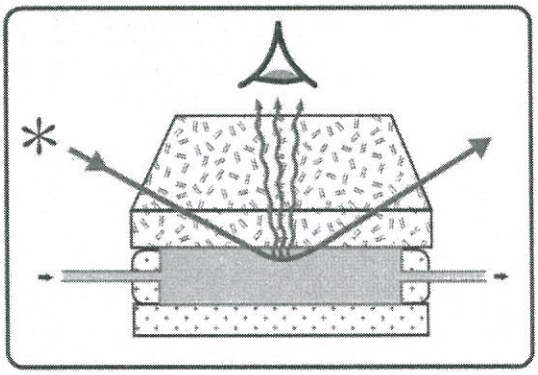


Fig. 2 - Optical scheme of TIRF Flow Cell

Introduction

The specific behavior of macromolecules at or near surfaces, interfaces, and membranes is currently of primary interest in nanotechnology and in the biological sciences. Important applications include: adsorption of blood proteins on biomaterials in thrombogenesis research; the binding to and triggering of living cells by hormones, neurotransmitters, and antigens; cell adhesion to various surfaces; the mechanism of electron transport in mitochondrial and photosynthetic membranes; and also reaction rate enhancement with membrane receptors by nonspecific adsorption and surface diffusion of ligands. Most of the common analytical methods available for investigation of surfaces either lack the extent of surface selectivity required or demand relatively harsh sample handling that severely limits the biological relevance of any results obtained.

However, total internal reflection fluorescence (TIRF) spectroscopy has proven to be a very powerful and versatile technique for the study of surface and/or interfacial behavior of biological molecules and their aggregates [1,2]. TIRF has been successfully applied to numerous studies associated with solute adsorption, orientation, and rotational mobility associated with conformational changes. Surface selectivity is achieved in TIRF by detecting only the evanescent wave excited fluorescence signals which originate within approximately the first 100 nm from the waveguide surface. This exceptionally short optical pathlength allows investigation of surface behavior even in the presence of highly concentrated solutions. In short, TIRF provides in situ, real-time, nondestructive, and highly sensitive detection suitable for studies on expensive biological materials available only in microliter quantities (~10 nl, minimum). The limit of detection is approximately 0.1% of a monolayer in most cases. Additionally, in this case, the optional combination of TIRF with electrochemistry allows control of the physicochemical properties of the surface during a single TIRF experiment. This opportunity can provide new insights into mechanisms of interaction, as well as, facilitate modification of surface properties by an externally applied voltage.

ISS
1602 Newton Drive
Champaign, Illinois 61822
(217) 359-8681
www.iss.com

TIRF cell | ACCESSORY FOR SPECTROFLUORIMETERS

Principles

The principles of TIRF are well documented in the literature [3-5]. In brief, when a beam of light propagating within a medium of refractive index (n_1) encounters an interface with a medium of lower refractive index (n_2), it can undergo total internal reflection for incidence angles (θ_i) greater than the critical angle (θ_c). Although the incident light totally reflects at the interface, a portion of the electromagnetic radiation penetrates the interface into the less dense medium. The intensity of this interfacial field, typically called the "evanescent wave", decays exponentially with distance from the interface.

$$\theta_c = \sin^{-1}(n_2/n_1)$$

The penetration depth (d_p) of the evanescent wave in the less dense medium is a function of incidence angle, refractive index ratio, and incident light wavelength, λ_i, (Eq. 2). The evanescent wave is primarily responsible for the electronic excitation of the fluorophore present in the lower refractive index medium. The penetration depth (pathlength) of the evanescent wave can be conveniently altered by changing incidence angles. The extremely short pathlength of the evanescent wave (on the order of the wavelength of light) excites a very small sample volume and thereby minimizes primary absorption effects. Also, depending on the optical geometry, the emitted fluorescence does not pass through the bulk solution but rather through the waveguide, thus largely avoiding any secondary absorption effects.

$$d_p = \lambda_i / (2\pi n_1[\sin^2\theta_i - (n_2/n_1)^2]^{1/2})$$

Instrumentation

The design of the ISS TIRF flow cell makes the normally difficult TIRF experiment become routine. Figure 1 is a photograph of the TIRF accessory and Figure 2 illustrates the basic optical design. The ISS TIRF flow cell comes prealigned for the ISS spectrofluorometers. along with an easily assembled flow system to study kinetics of various surface interactions. High reproducibility of TIRF measurements is ensured by the exact positioning of optical elements against the excitation beam and emission axis. The ISS TIRF cell differs from similar TIRF cuvettes by simple and fast assembly of the sandwich cell. The transparent gasket which forms the flow chamber (app. 20µl) and transparent back plate facilitate easy visualization of the surface and allows acquisition of microscopic pictures by a long-focus objective.

The standard cell comes equipped with a UV-quartz prism and optically coupled cover slide, as shown in Figure 2. The cover slide provides an easily interchangeable working surface and minimizes wear on the TIRF prism. Cover slides with hydrophilic, hydrophobic, electroconductive thin films, or specific soluble protein docking films can be supplied as options. Additional options include three-electrode electrochemical control by application of an external voltage and temperature control by way of a thermostated block and water bath. The ISS TIRF cell is also available for use with most of the commercially available research-grade spectrofluorometers currently on the market.

Applications

The principle applications of TIRF spectroscopy are:

- protein adsorption: kinetics and isotherms, effect of solvents, competitive adsorption, conformational changes, effect of detergents, surface mobility
- immunoassay systems: antibody-antigen interactions, biosensor & nanotechnology development
- electron transport in mitochondrial and photosynthetic membranes
- cell adhesion to surfaces.

Figure 3 illustrates the adsorption kinetics of immunoglobulin (IgG) at a hydrophilic silica surface. About 70% of IgG is irreversibly adsorbed kinetically and does not desorb back into the flow of pure buffer solution. IgG molecules undergo structural changes after adsorption that result in fluorescent lifetime changes of the fluorescent label, pyrene sulfonyl chloride (not shown). The maximum observed within the first minutes of adsorption can be interpreted as a result of a flattening of the IgG molecules after attachment to the surface [6].

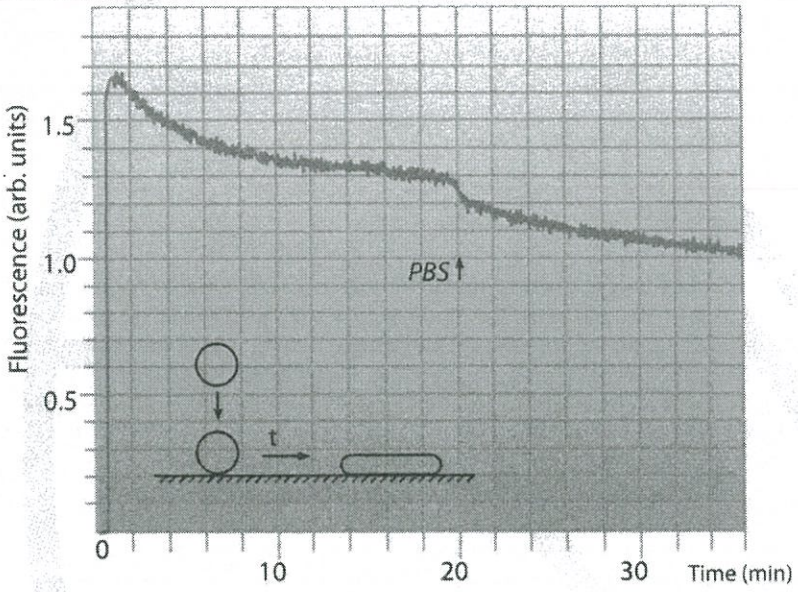


Figure 3.
Immunoglobin (IgG-FITC conjugate) adsorption onto hydrophilic silica from 0.3 mg/ml solution in pH 7.4 phosphate buffer. PBS arrow indicates switching to pure buffer.

An example of multilayer protein absorption is illustrated in Figure 4. Bovine serum albumin (BSA) forms a monolayer when adsorbed from a 50 mM phosphate buffer (pH 6.2 - lower curve) onto a hydrophobic siliconized surface. Ammonium sulphate modifies the protein-protein interactions and allows multilayer adsorption to occur.

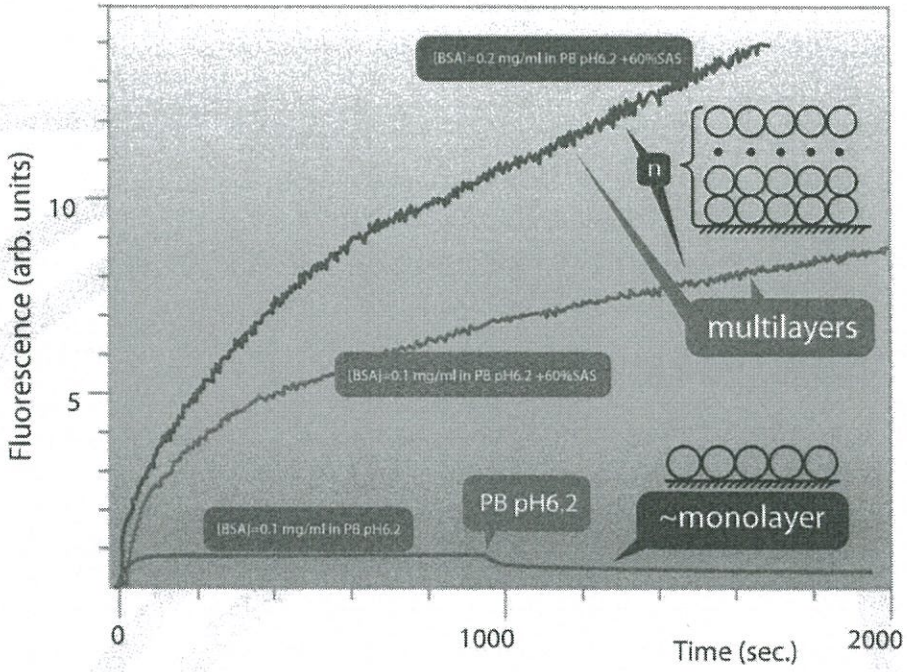


Figure 4. Monolayer bovine serum albumin (BSA-FITC conjugate) adsorption from solution in phosphate buffer pH 6.2 (lower curve) and multilayer adsorption in the presence of ammonium sulfate (60% v/v saturated solution).

Figure 5 illustrates the effect of electrochemical control of human serum albumin (HSA) adsorption at a tin dioxide transparent electrode. Cathodic polarization increases surface affinity for HSA, probably, because of negative polarization which enhances hydrophobicity of the surface. Application of an anodic polarization stimulates desorption of HSA from the tin dioxide surface [7].

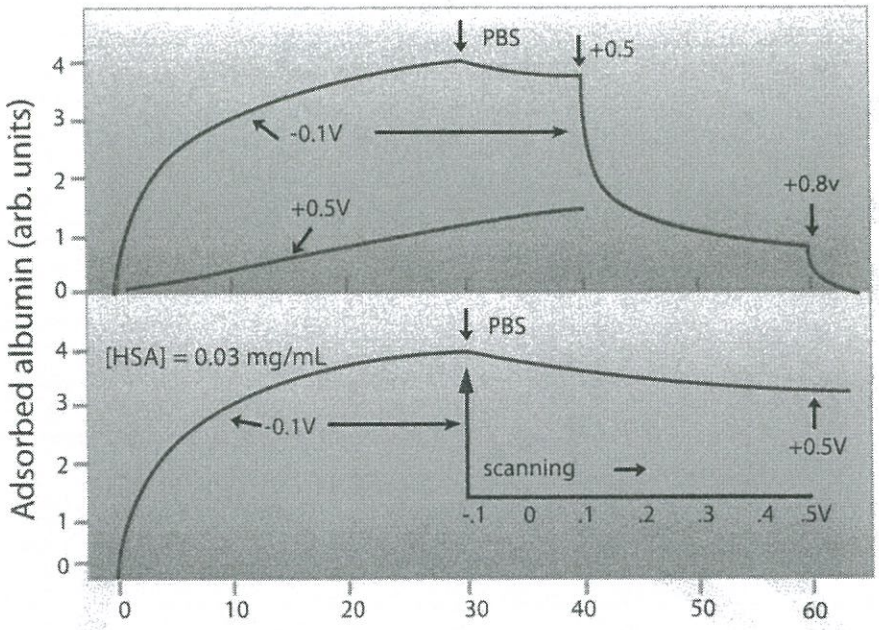


Figure 5. Effect of electrochemical polarization of a tin dioxide surface on the adsorption of human serum albumin (HSA-FITC conjugate) from a 0.03 mg/ml solution in phosphate buffer pH 7.4

Excited state lifetime, rotational correlation time, fluorescence polarization, and quenching experiments often provide important information concerning molecular dynamics, in general, and protein conformation related to adsorption, more specifically. The ISS spectrofluorometers equipped with the TIRF flow cell accessory are quite capable of selectively obtaining this type of information from the surface under study.

Figure 6 illustrates fluorescence lifetime data obtained directly using the K2™ and TIRF cell. Lifetime data for FITC-labelled lysozyme adsorbed onto a quartz surface were obtained. These data provide evidence of at least two types of adsorbed lysozyme characterized by lifetimes of 4.1 and 0.44 nsec, respectively.

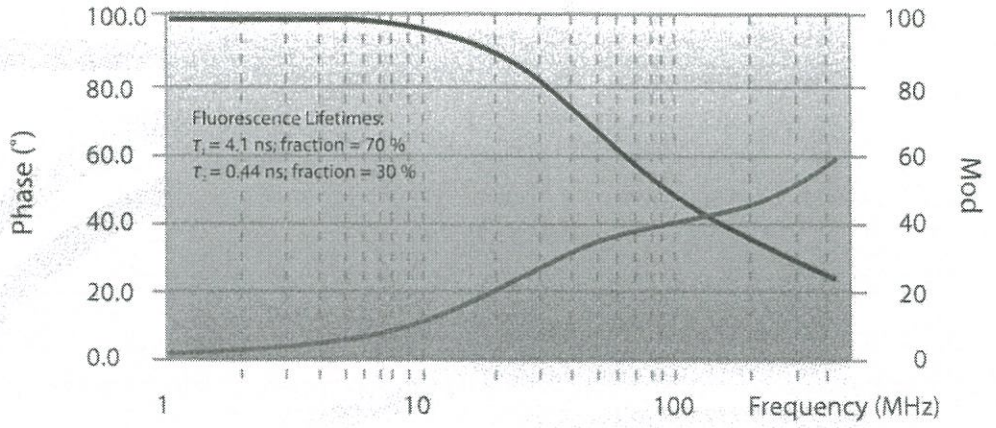


Figure 6. Multifrequency lifetime data obtained with the ISS TIRF cell for lysozyme-FITC conjugate adsorbed onto a quartz surface from a 0.3 mg/ml solution in 50 mM acetate buffer, pH 4.5

CHROMSPEC
SPOL. S R.O.
Plachty 2, 634 00 Brno
Tel: 547246683-4
Fax: 547246685

Položkový rozpočet:

Výzkumný ústav veterinárního lékařství, v. v. i.
Mgr. Jiří Kohoutek, Ph.D.
Hudcova 296/70
621 00 Brno

ChronosDFD: Fluorescenčni spektrofotometr

Pol.	*Objednací číslo a popis*	*Poč.*	*Jednotková cena*	*Celková cena*
1	**C025** **Chronos DFD lifetime spectrometer, two emission channels.** Komlpetní lifetime konfigurace pro digital frequency-domain techniku včetně PC sestavy. T formát přístroje.	1	702 890,00	702 890,00
2	**SW012** **Vinci Multidimensional Fluorescence Spectroscopy Analysis software.**	1	49 860,00	49 860,00
3	**K212** **Four-cuvette sample, Peltier-controlled, sample compartment.** Čtyřpozičvý automatický výměník kyvet. Teplotní rosah –20 °C +105 °C.	1	176 250,00	176 250,00
4	**K446** **Circulating cooling system for Peltier,** Externí chlazení Peltierova termostatu.	1	46 120,00	46 120,00
5	**K421** **Variable-angle,** Držák pro měření pevných vzorků a vysoce rozptylujících materiálů.	1	38 600,00	38 600,00
6	**K427** **Total Internal Reflection Fluorescence (TIRF) Flow Cell** pro studium makromolekul na povrchu a nebo v blízkosti povrchu a na rozhraní membrán. Přednastaven do optické osy přístroje.	1	247 290,00	247 290,00
7	**K401.2** **Set of 2 UV Glan-Thompson Prism Polarizers,** Glan-Thomsonovy polarizátory pro měření polarizace fluorescence.	2	43 350,00	86 700,00
8	**K412.2** **Qty. 2 Automated Filterwheel accommodating four (4),** Držáky pro příslušenství.	1	89 450,00	89 450,00

9	**K218** **Photomultiplier tube (Model R928, by Hamamatsu), multi-alkali photocathode,** Fotonásobič, rozsah. 185-900 nm.	1	90 390,00	90 390,00
10	**N806** **Pulsed Light Emitting Diode (LED) 280 nm.**	1	82 110,00	82 110,00
11	**N110** **Steady-state upgrade option for excitation** Rozšíření pro steady-state aplikace pro excitaci. Včetně Xenonové lampy, výkon 150W.	1	405 390,00	405 390,00
12	**K288** **Steady-state upgrade package for emission** Rozšíření pro steady-state aplikace pro emisi.	1	401 710,00	401 710,00
13	**U510** **Upgrade of ChronosDFD operating in frequency-domain to ChronosBH (TCSPC).** Rozšíření ChronosDFD pro měření time domain (TCSPC).	1	582 660,00	582 660,00
14	**N410** **Pulsed Laser Diode 375 nm**	1	231 050,00	231 050,00
15	**N400** **Picosecond light pulser for laser diode light sources.** Napájecí zdroj pulzních diod: frekvence od 2 Hz do 100 MHz.	1	206 050,00	206 050,00
16	**3/Q/10** **Standardní pravoúhlá kyveta pro fluorimetrii.** Rozsah vlnových délek 170 - 1700 nm.	4	3 345,00	13 380,00
18	**Shipping and handling.** Dodání na místo určení.	1	20 000,00	20 000,00
19	**Installation and training.** Instalace, uvedení do provozu a instruktáž obsluhy.	1	25 000,00	25 000,00

Celkem bez DPH: 3 494 900,00

Sazba DPH: 21 % 733929,00

Celkem v Kč, místo určení, včetně DPH: 4 228 829,00