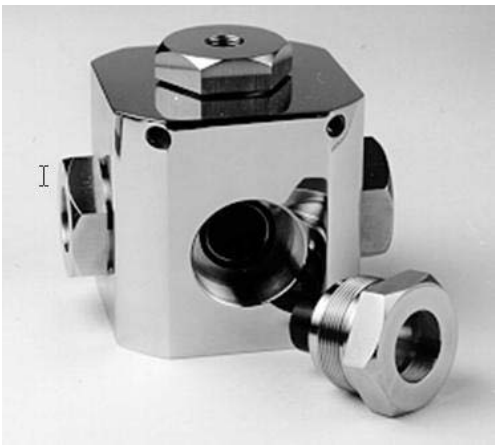
High-Pressure Cell System



The ISS High Pressure Cell System (HPCell) includes the Pressure Cell, the pump, and the software for system control and data acquisition. The system can be interfaced directly to ISS spectrofluorometers or utilized as a stand-alone unit with other fluorometers.

The HPCell has been specifically designed for the fluorescence and absorption studies of samples (solid and solutions in cuvettes) at pressures of up to 4,000 bar (43,500 psi). The cell features three 10mm-diameter quartz windows placed at ninety degrees for the acquisition of either L- or T-format fluorescence measurements. A 4-window cell version is available for the simultaneous acquisition of fluorescence and absorption data.Sapphire windows are also available.

The high aperture 10mm-diameter windows allow for using as an excitation light source either a laser, a light emitting diode (LED) or a collimated beam from a xenon arc lamp. The unusually high numerical aperture (NA = 0.18) for a cell makes it unique for high sensitivity fluorescence studies.

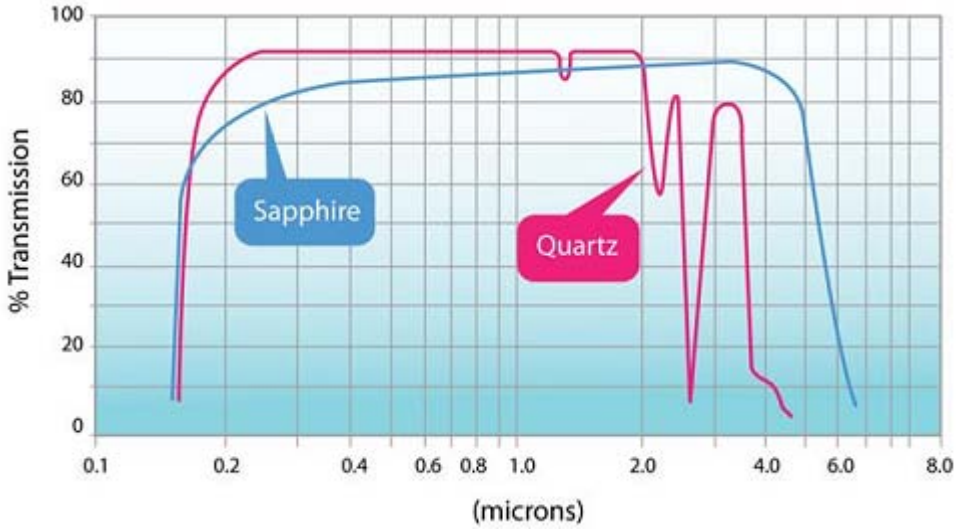
The cell's mounting kits are available for several commercial instruments. If the cell does not fit into an instrument, a kit with fiber optic bundles is available for connection to the instrument.

Windows

Optical windows are made out of quartz [SiO2] or sapphire [single-crystal aluminum oxide Al2O3]. The windows are mounted onto polished plugs and held in place by precise mechanical holders.

The choice of the windows material is dictated by the specific application and measurement to be acquired. Quartz windows withstand pressures up to 3000 bar. The ISS pressure cell windows are constructed of UV grade fused silica. Fused silica is a polycrystalline, isotropic material with no crystal orientation; these windows are suitable for polarization measurements. Sapphire windows withstand pressures up to 4000 bar. Yet, sapphire is a synthetic, hexagonal single crystal anisotropic material, which displays substantially different optical characteristics when measured along different axes; sapphire if birefringent, that is a light beam propagating along the optical axis experiences two different values of the index of refraction. These windows are not suitable for polarization measurements.

The plot below displays the transmission of these materials; both feature excellent transmission down to 200 nm; quartz reaches 2000 nm while sapphire reaches up to 5000 nm.



Temperature Control

The cell is made of a stainless steel alloy which has excellent thermal conductivity. This property facilitates spectroscopic measurements acquired at temperatures other than ambient. The cell includes a built-in circulation path for temperature control through an external liquid circulating bath; temperature control of the sample is achieved by using a bath circulator directly attached to the cell. The cell is designed for operating in the temperature range from -20 °C to +60 °C.

Pressure Generator

Hydrostatic pressure is generated through a manual pump connected to the HPCell by flexible tubing. The pump includes a reservoir for storing the ethanol, and valves for allowing the pressurizing liquid to circulate thorough the circuitry. A pressure gauge indicates the value of the pressure. All of the components are conveniently mounted on a single baseplate which can be located next to the spectrofluorometer.

A computer-controlled pump system is available. The ISS Vinci Multidimensional Software controls the automated pump; a series of measurements at different pressure values can be programmed in the computer and, once started, the acquisition does not require any additional intervention by the operator.

Although water can be utilized as pressure medium, spectroscopic-grade ethanol is the favorite pressurizing medium in order to minimize oxidation processes of the cell body.

Cuvettes for High Pressure Studies

Both circular and square quartz cuvettes are available for the HPCell. The cuvette is placed into a holder, which is positioned into the HPCell. A Teflon stopper on the cuvette separates the internal solution from the pressurizing liquid; the stopper acts as a piston and allows for the transmission of pressure to the solution in the cuvette.

Specifications

Cell

Pressure	. up to 3,000 bar (43,500 psi) with quartz windows . up to 4,000 bar (58,000 psi) with sapphire windows
Temperature	. -40°C to +80°C . Controlled through built-in liquid circulation circuit . Thermal Conductivity . 12 W cm-1 K-1 at 20°C . 16 W cm-1 K-1 at 200°C
Windows	. 10 mm clear aperture . I-Seals for easy use and replacement . Quartz . 19 mm diameter x 8.5 mm thick . Sapphire . 19 mm diameter x 6.4 mm thick
Internal Cell Chamber	. Diameter: 22mm . Height: 50.3mm
Dimensions	. 146mm (W) x 118mm (L) . 39 mm from base to window centers

Pressure Generator

Gauge	. Up to 6,000 bar
Reservoir Volume	. 116 mL
Generator Volume	. 10 mL (manual or automatic pump)
Tubing	. 1/8" diameter . Flexible . Up to 4,150 bar
Thermocouple	. Either Ni-Cr-Ni or Fe-Const

Sample Holder Volumes

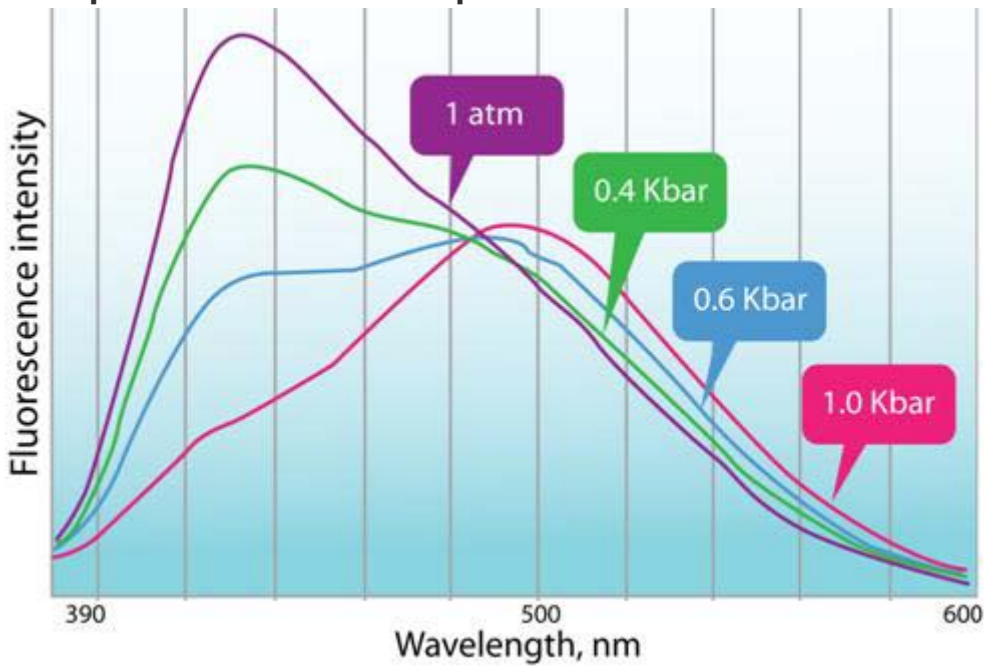
Empty Cell, 3 Quartz Windows	. 15.0 mL
Empty Cell, 4 Sapphire Windows	. 24.5 mL
Holder fitted with 11 mm bottle and cap	. 11.0 mL . Max allowed height of bottle with cap . 40.0 mm
Round cuvette, 11 mm outer diameter	. 0.75 mL
Round cuvette, 9 mm outer diameter	. 0.80 mL
Square cell, 6x6 mm	. 0.30 mL

Hydrostatic Liquid

. Low Fluorescence Background, Spectroscopic-Grade Ethanol

Applications

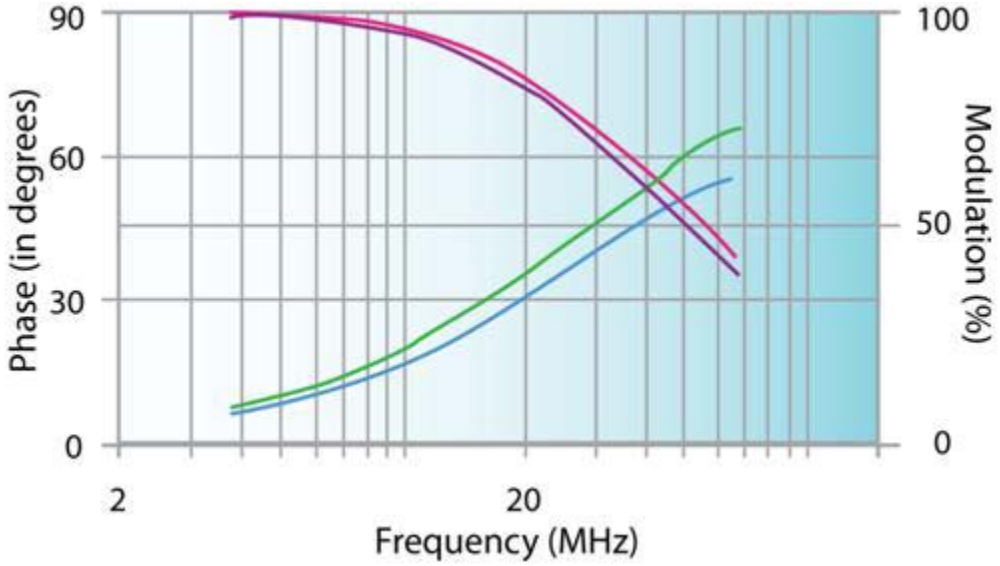
Absorption and Emission Spectra



The effect of pressure on the emission spectrum of Prodan in Dipalmitoyl-phosphatidylcholine (DPPC) multilamellar vesicles at 20 °C in tris buffer (1o mM, pH=8.0) containing 0.8M ethanol. Excitation wavelength was 359 nm.

(Courtesy of Prof. Parkson L.-G. Chong, Temple University, Philadelphia, PA, USA)

Lifetime Measurements



Effect of pressure on fluorescence lifetimes of staphylococcal nuclease. Frequency response profiles at atmospheric and high pressure using the HPCell. Excitation wavelength was 295 nm and emission was monitored at 350 nm.

(Courtesy of Prof. Catherine A. Royer, Centre de Biochimie Structurale, Université Montpellier I, Montpellier, France)

High pressure (2 Kbar) data: blue (phase) and red (modulation) lines
Atmospheric pressure data: green (phase) and purple (modulation) lines

Kinetic Studies

Along with researchers of Temple University (Philadelphia, PA) ISS developed a special cuvette for kinetics studies. The solutions in the cuvette are separated by a membrane that disrupts upon the application of a low voltage signal, which is transported into the special cuvette by wires embedded into the top plug.

ISS provides the suitable baseplates for mounting HPCell into several spectrofluorometers and spectrophotometers.

Whenever HPCell cannot be mounted into an instrument, fiber-optic bundles are utilized to bring excitation light to the cell and then to divert the collected light from the cell to the instrument. Please contact ISS for details on the fiber optics kit.

Selected References for the Use of HPCell in Various Research Areas

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Unusual Properties of Highly Charged Buffers: Large Ionization Volumes and Low Barrier Hydrogen Bonds
Hess, R.A., Reinhardt, L.A.
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Sapphire [Al_2O_3] Window Specifications

Index of Refraction	N_0: 1.768 (c-axis) N_e: 1.760 (c-axis)
Birefringence (N_0 - N_e)	0.008
Density	3.97 gm/cm³
Compressive Strength	300,000 psi
Young Modulus	50-55 x 106 psi
Flexural Strength	~100,000 psi
Tensile Strength	~60,000 psi (at ~25°C)
Poisson's Ratio	0.28-0.33
Hardness	9 mohs 1800 Knoop parallel to c-axis
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	2200 Knoop perpendicular to c-axis
Coefficient of Friction Against Steel	0.15
Porosity	0%
Dielectric Strength	480,000 v/cm
Dielectric Constant (parallel to c-axis)	average 11.5
Dielectric Constant (perpendicular to c-axis)	average 9.3
Volume Resistivity	$>10^{14}$ Ω-cm^3
Melting Point	2,050°C
Coeffecient of Expansion (parallel to c-axis)	5.3 x 10^{-6}
Coeffecient of Expansion (perpendicular to c-axis)	4.5 x 10^{-6}
Conductivity	0.086 cal/gm
Heat Capacity	77.8 joules/deg. Mole