Using Alba with the FemtoFiber laser by Toptica for 2-photon quantitative imaging

Shih-Chu Liao, Yuansheng Sun, Ulas Coskun
ISS, Inc.

Introduction

The advantages of multiphoton excitation for quantitative cell biology are numerous: reduced phototoxicity as excitation occurs in the range from 700 nm to 950 nm (or longer), femtosecond pulses, reduced excitation volume. Yet the cost of standard multiphoton lasers is sometimes out of the range for several research laboratories.

Toptica Photonics (Graefelfing, Germany; Victor, NY) offers a family of ultrafast fiber lasers. Two systems feature dual wavelength output from the single unit. The new Dichro bioMP laser emitting two wavelengths, 780 nm and 1030 nm and the virtual 888nm, is suitable for two-photon laser excitation (see Table I). The FemtoFiber Pro NIR emits 780 nm and 1560 nm (Table I).

Model: Dichro bioMP	Wavelengths [nm]	780	1030
	Laser output power [mW]	>100	>200
	Pulse width [fs]	<100	
	Repetition rate	80 MHz	
	Beam Shape	TEM_{00}, $M^2 < 1.2$	
Model: FemtoFiber pro NIR	Wavelengths [nm]	780	1560
	Laser output power [mW]	>150	>350
	Pulse width [fs]	<100	
	Repetition rate	80 MHz	
	Beam Shape	TEM_{00}, $M^2 < 1.2$	

Table I. Features of FemtoFiber laser family.

The measurements reported in this Note were acquired using the FemtoFiber Pro NIR laser. In fact, several probes are available for the fundamental 780 nm wavelength.

Instrument Setup

The system optical setup is shown below. The laser beam is sent into the excitation head of the Alba (Figure 1) by using a periscope; the intensity of the 2p laser into Alba is controlled using the Intensity Control Unit (ISS part no.

A450) featuring a halfwave plate and a Glan-Thompson laser polarizer; the laser intensity can be tuned continuously from less than 0.1 mW to more than 100 mW. In the Alba, a 720-nm long pass dichroic (D1) is used to separate the 780-nm laser excitation and the emission in the visible range; a 720-nm short pass emission filter is used as an IR blocking (IRB) filter. The Alba is equipped with two Hamamatsu H7422P-40 photomultiplier tubes detectors.

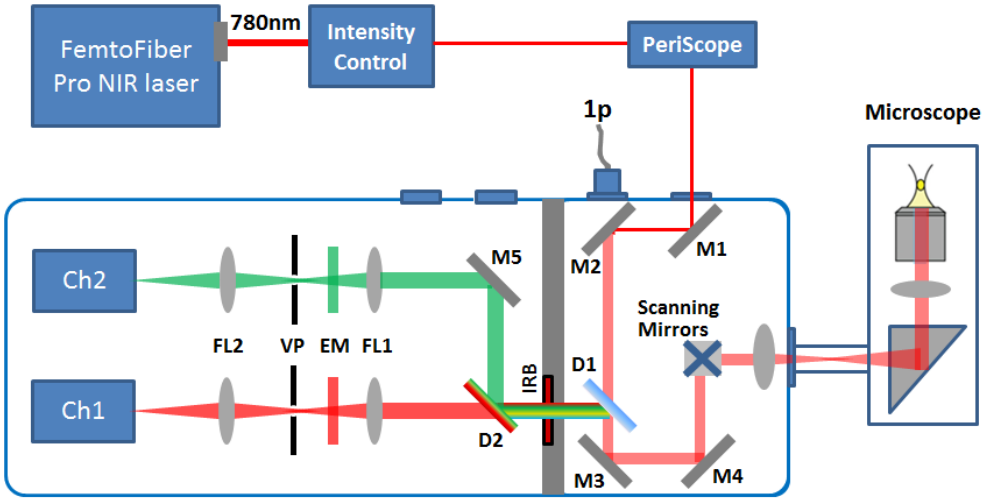

Figure 1. Schematics of the instrument.

FCS Measurements

Fluorescence Correlation Spectroscopy (FCS) measurements were carried out on a solution of Fluorescein (pH 7.4) at different concentrations ranging from 1 nM to 200 nM. The microscope (Model Ti by Nikon) is equipped with a 60X (1.2 NA) water immersion objective. A bandpass filter (525 ± 20 nm) is placed in front of the detector. The Laser power measured before entering the Alba is 20 mW.

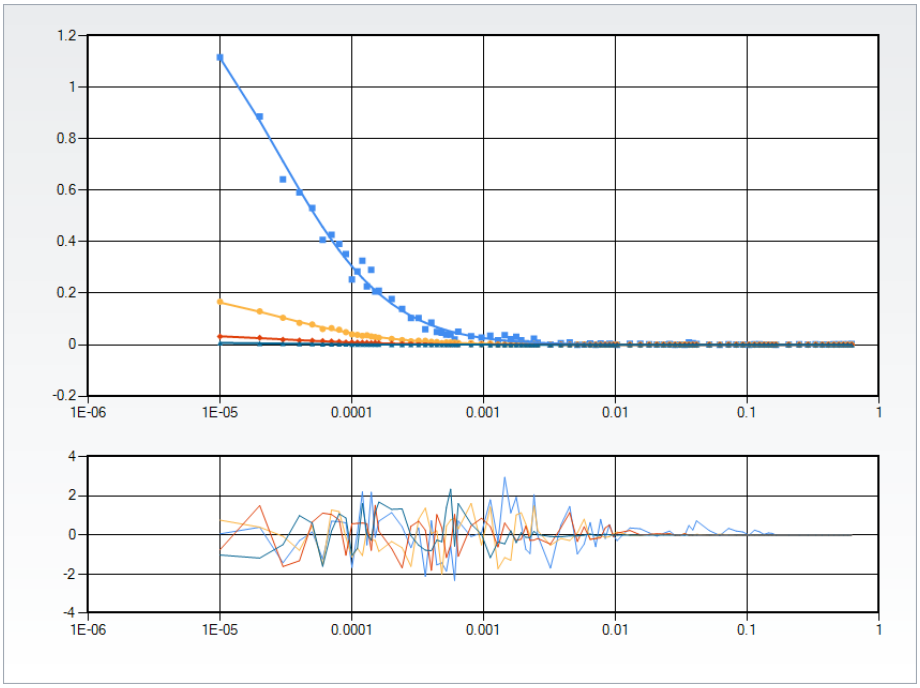

Figure 2. FCS curves for the 1.5 - 200 nM concentration solutions

The auto correlation functions were fitted with the Gaussian Lorentz 1 species model, giving the lateral dimension (w_0) to be 289 nm; while the axial / lateral (z0 / w0) ratio by assuming the 3D Gaussian shape was estimated to be 4.56. The experimental results obtained with the Gaussian Lorentz fit are reported in Table II below. They were determined by fixing the value of the diffusion coefficient to 430 $\mu m^2 s^{-1}$; the concentrations of the solutions were retrieved by the fitting procedure using VistaVision software.

Parameter	1	2	3	4
D, um^2/s	430	430	430	430
C (nM)	1.47	9.97	49.9	232
w0 (µm)	0.289	0.289	0.289	0.289
TauD (µs)	24.2	24.2	24.2	24.2
N, #	0.0247	0.168	0.839	3.89
G	1.12	0.167	0.0319	0.007
G(0)	2.76	0.406	0.0811	0.018
ExcVol (μm^3)	0.028	0.028	0.028	0.028
CPS	1940	15000	68900	3E+05
Chi-Sq	0.857	0.459	0.391	0.409

Table II. Fitting results of the FCS curves for the 1.5 - 200 nM concentration solutions

FLIM Measurements

FLIM measurements were acquired using the Alba equipped with the FastFLIM data acquisition. For calibration, two lifetime standards were used, a solution of Fluorescein in buffer at pH 7.4 and a solution of Coumarin 6 in Ethanol. The lifetime data for the two solutions are displayed on the polar plot in Figure 3: 2.56 ns was obtained for the Coumarin and 4 ns for the Fluorescein.

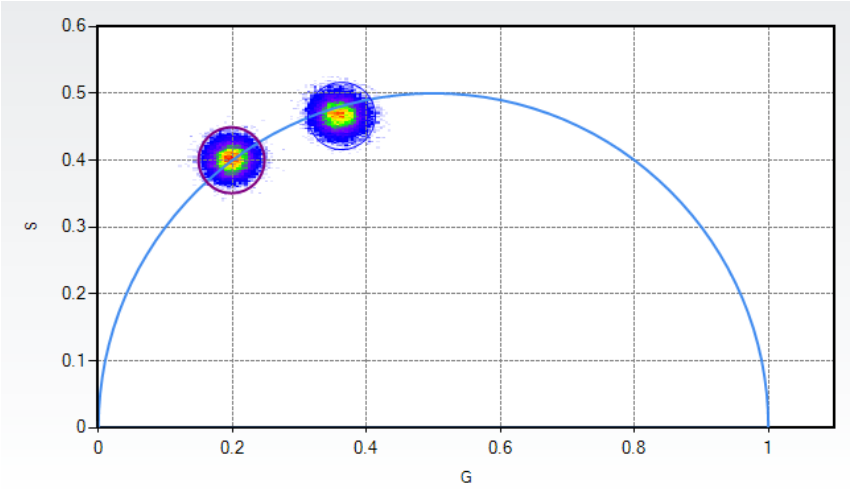

Figure 3. Phasor plot of the fluorescence lifetime data of fluorescein in buffer at pH 7.4 and Coumarin 6 in Ethanol.

FLIM data were acquired on a slide with Lily Convallaria (Figure 4).

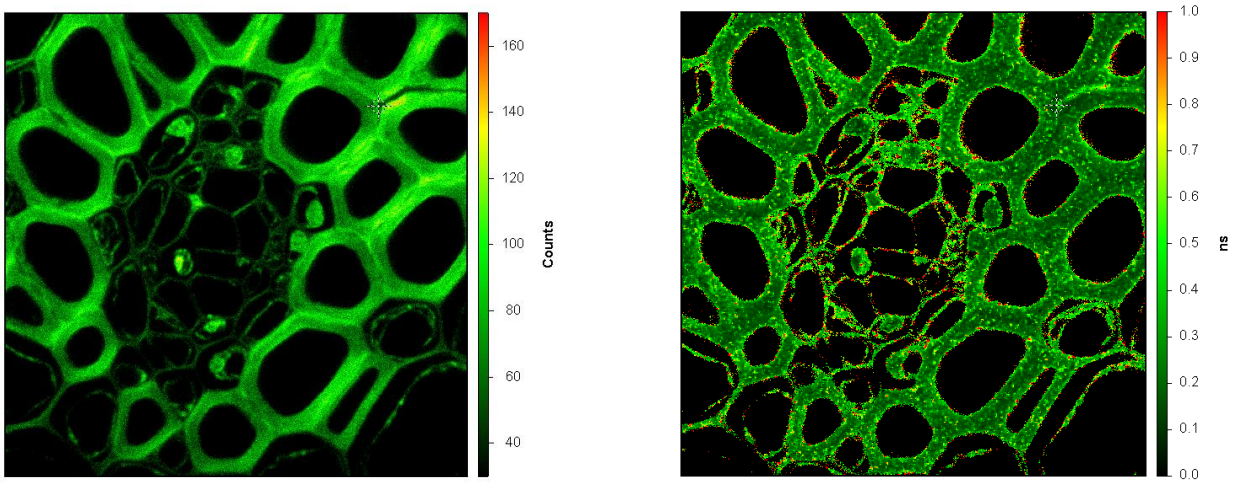

Figure 4. Intensity image (left) and lifetime image (right).
Image size is 1024x1024 (100 µm x 100 µm).

A stack of FLIM measurements (3D FLIM) measurements was acquired using a slide with pollen (Figure 5).

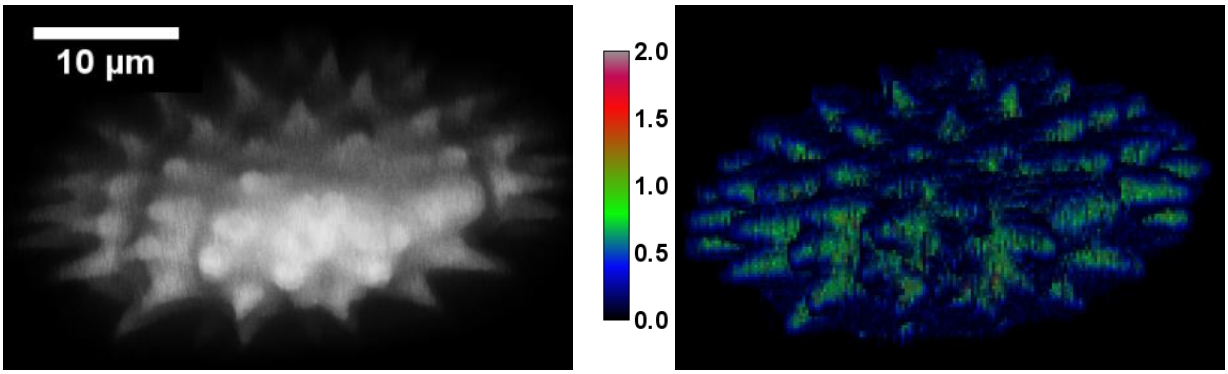

Figure 5. Volume rendering of intensity images (left) and of lifetime images (right),

Stability Test

The FemtoFiber Pro NIR laser unit provides a clock out signal for the laser pulse reference. With a 50Ω termination, the laser clock reference is a clean negative NIM signal with the peak amplitude of -200mV and a FWHM of less than 2ns. The clock out signal is first amplified by a 36-dB preamp and the amplified signal is given to the FastFLIM clock in for synchronization, required by FLIM measurements. A stable laser reference clock is critical for a robust time-lapse FLIM measurement. To test the stability, time-lapse FLIM measurements of fluorescein (pH7.4) were carried out:

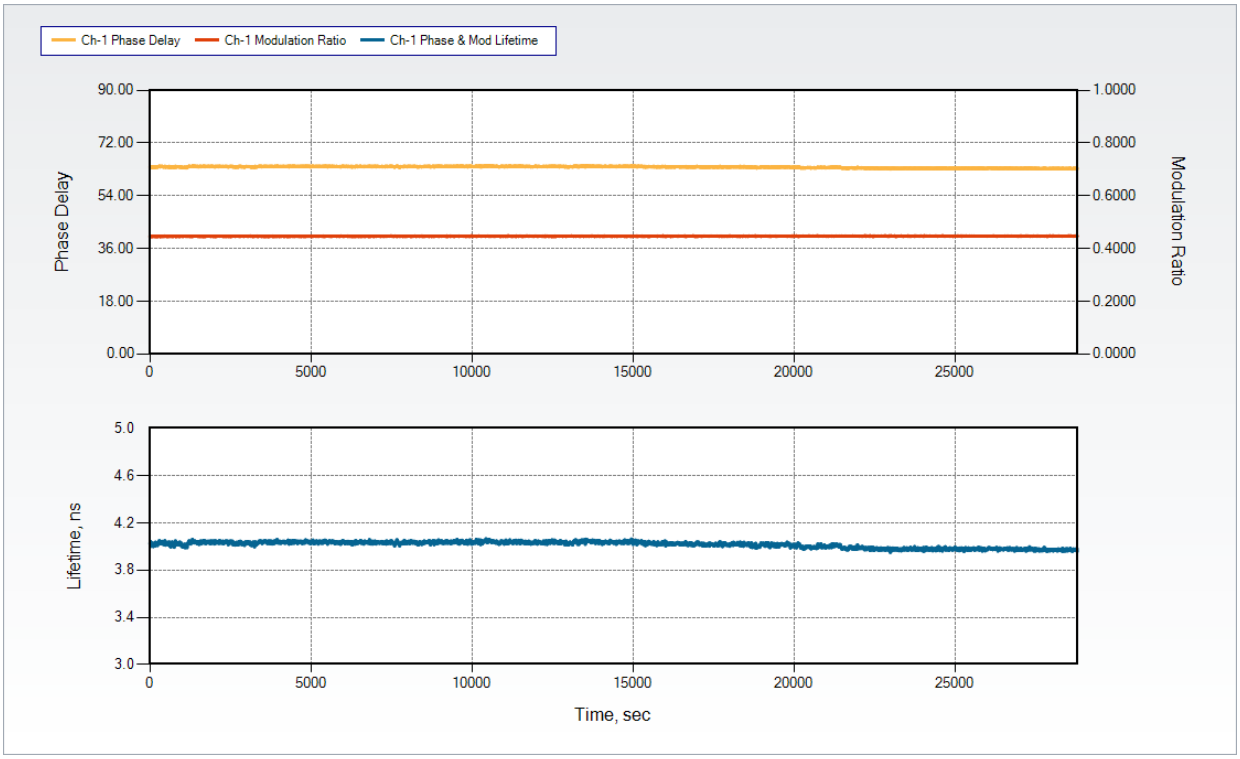

Figure 6. 2760 time points over 8 hours, data accumulation time per time point, 10 seconds: both the laser intensity and the reference clock are quite stable

Conclusions

Alba V combined with the Toptica FemtoFiber Pro laser (780 nm) provides high-sensitivity and time-resolved 2p imaging capability, and is ready for advanced FLIM and FFS (FCS, PCH) measurements.

The results obtained with the FemtoFiber Pro laser are comparable to those obtained with commercial Ti:Sapphire 2p lasers operating at the same wavelength. The reference clock of the FemtoFiber Pro laser is more stable than the clock of the Ti:Sapphire lasers.

For more information please call (217) 359-8681

or visit our website at **www.iss.com**

1602 Newton Drive
Champaign, Illinois 61822 USA
Telephone: (217) 359-8681
Fax: (217) 359-7879
Email: iss@iss.com