Cell-based FLIM Energy-Transfer Measurements Using Alba

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Introduction

This application note describes the use of Alba - Confocal Spectroscopy and Imaging Workstation for the acquisition and analysis of energy transfer images in cells.

Instrumentation

Measurements were performed on Alba - Confocal Spectroscopy and Imaging Workstation. Alba is a dual channel instrument that combines a confocal scanning microscope with fluorescence correlation spectroscopy (FCS) and is optimized for both single-photon and multi-photon excitation.

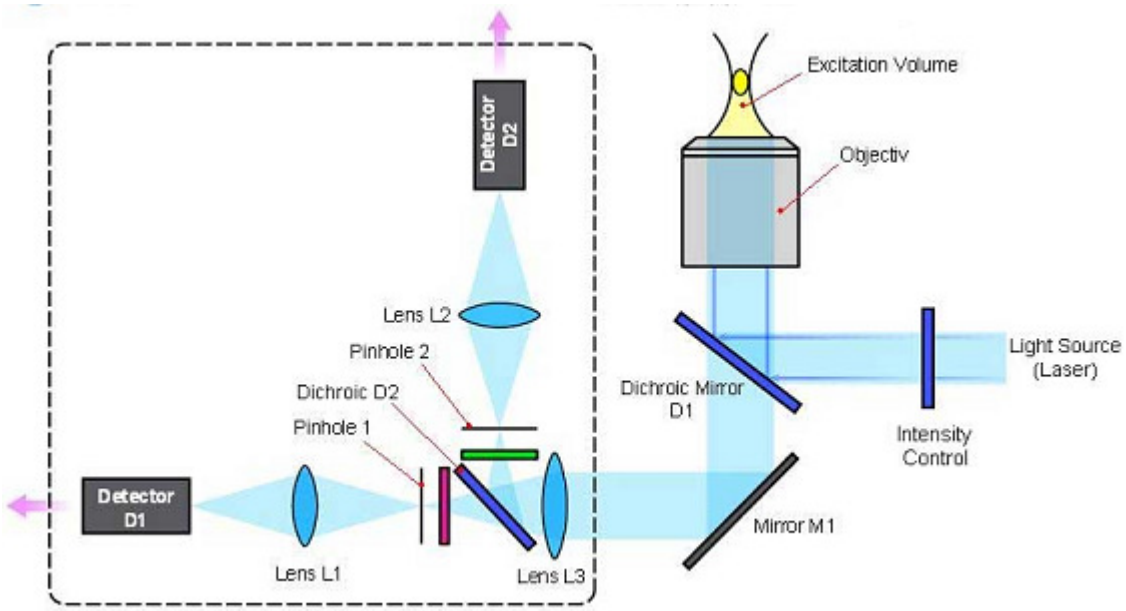

Figure 1. Schematic Drawing of Alba - Confocal Spectroscopy and Imaging Workstation.

Lifetime images are obtained in frequency-domain: the excitation light is modulated at a set frequency in the order of megahertz [MHz] and the gain of the light detector is modulated at the same frequency plus an offset of a few Kilohertz [kHz]. This allows the recording of DC, AC and phase information, thereby enabling the reconstruction of lifetime images. In general each scan includes a measurement of three or four different modulation frequencies in the range between 1-300 MHz.

Alba uses Vista – FCS and Confocal Imaging Microscopy a comprehensive software package for generating high quality confocal spectroscopy data.

Results

The following set-up was used to acquire data on opossum kidney cells that expressed either CFP alone or a tandem CFP/YFP construct: a 4mW, two-photon Ti:Sapphire laser (excitation

wavelength = 850 nm), a 60X water objective with NA=1.2 and a Nikon TE2000E microscope; the acquisition time for each sample was 65 seconds. An 80 MHz modulation frequency was used. The images in channel 1 were collected through a 520 – 560 nm YFP filter and in channel 2 through a 460 – 500 nm CFP filter.

CFP Images

Figure 2 shows images of cells expressing CFP. The upper left image depicts the channel 2 DC intensity image while the upper right image shows the modulation image. The calculated lifetime images from modulation (lower left) and phase (lower right) are also shown.

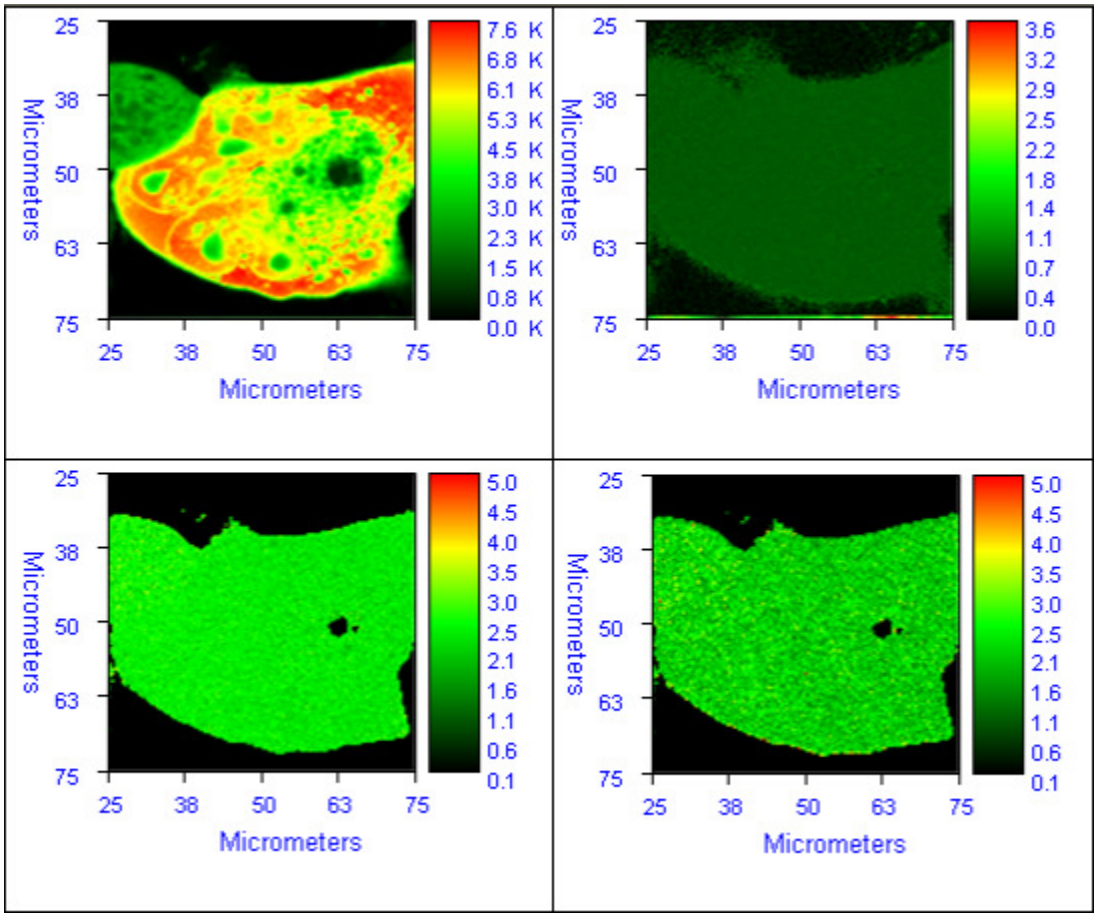

Figure 2. Images of CFP-expressing opossum kidney cells acquired with Alba - Confocal Spectroscopy and Imaging Workstation: Upper left: DC image, upper right: modulation image, lower left: modulation lifetime image, lower right: phase lifetime image.

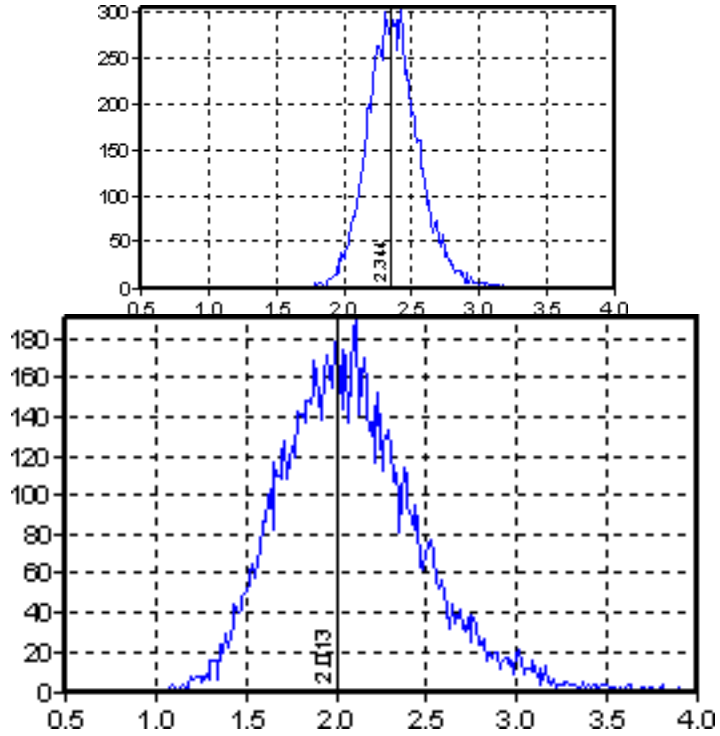

Figure 3. Left: Modulation-lifetime distrubution with a lifetime centered at 2.34 ns; right: phase-lifetime distribution with a lifetime centered at 2.01 ns for the previous images.

CFP/YFP Energy-Transfer Images

Figure 4 shows images of opossum kidney cells expressing the CFP/YFP tandem protein. Compared to the cells expressing only CFP these cells clearly exhibit shorter donor fluorescent lifetimes. This can be seen from the modulation and phase lifetime distributions given in Figure 5 and is indicative of fluorescence resonance energy transfer occurring between the CFP and YFP in the tandem construct.

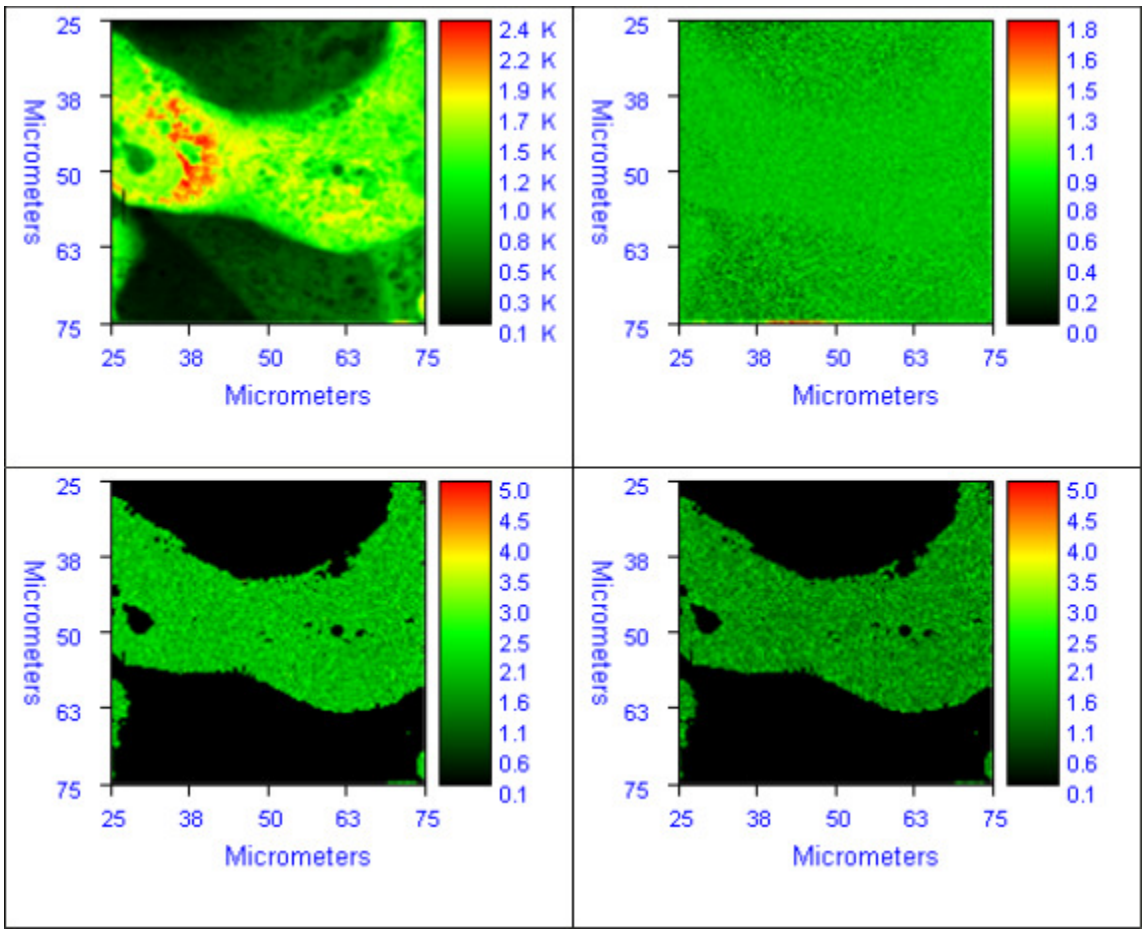

Figure 4. Images of opossum kidney cells expressing a CFP/YFP tandem protein as acquired with Alba - Confocal Spectroscopy and Imaging Workstation: Upper left: DC image, upper right: modulation image, lower left: modulation lifetime image, lower right: phase lifetime image.

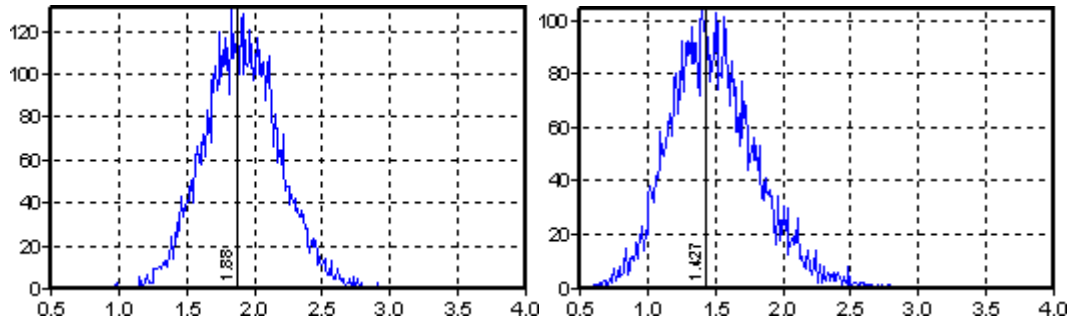

Figure 5. Left: Modulation-lifetime distribution with a lifetime centered at 1.88 ns; right: phase-lifetime distribution with a lifetime centered at 1.43 ns for the previous images.

From the reduction in the CFP modulation lifetime we calculate 28% average energy transfer in the tandem construct. Similarly, 29% average energy transfer is calculated from the reduction in the phase-lifetime. These values are in excellent agreement with values for this tandem construct measured in other systems [1].

Conclusion

These data demonstrate the ability of Alba - Confocal Spectroscopy and Imaging Workstation to acquire FRET images of cells expressed with tandem fluorescent proteins. Image acquisition using Alba is fast and easy and analysis is achieved with Vista – FCS and Confocal Imaging Microscopy.

Acknowledgement

We thank Prof. Moshe Levi, Prof. Nicholas Barry and Dr. Sophia Breusegem in the Departments of Medicine, Physiology and Biophysics, Division of Renal Diseases and Hypertension, University of Colorado at Denver and Health Sciences Center, for their permission to publish these data, which were obtained on their Alba instrument. They in turn would like to thank Dr. Kurt Beam for the CFP/YFP tandem plasmid.

References

1. S. Papadopoulos, V. Leuranguer, R. A. Bannister and K. G. Beam. Mapping Sites of Potential Proximity between the Dihydropyridine Receptor and RyR1 in Muscle Using a Cyan Fluorescent Protein-Yellow Fluorescent Protein Tandem as a Fluorescence Resonance Energy Transfer Probe. J. Biol. Chem. 279, 44046 – 44056 (2004).

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