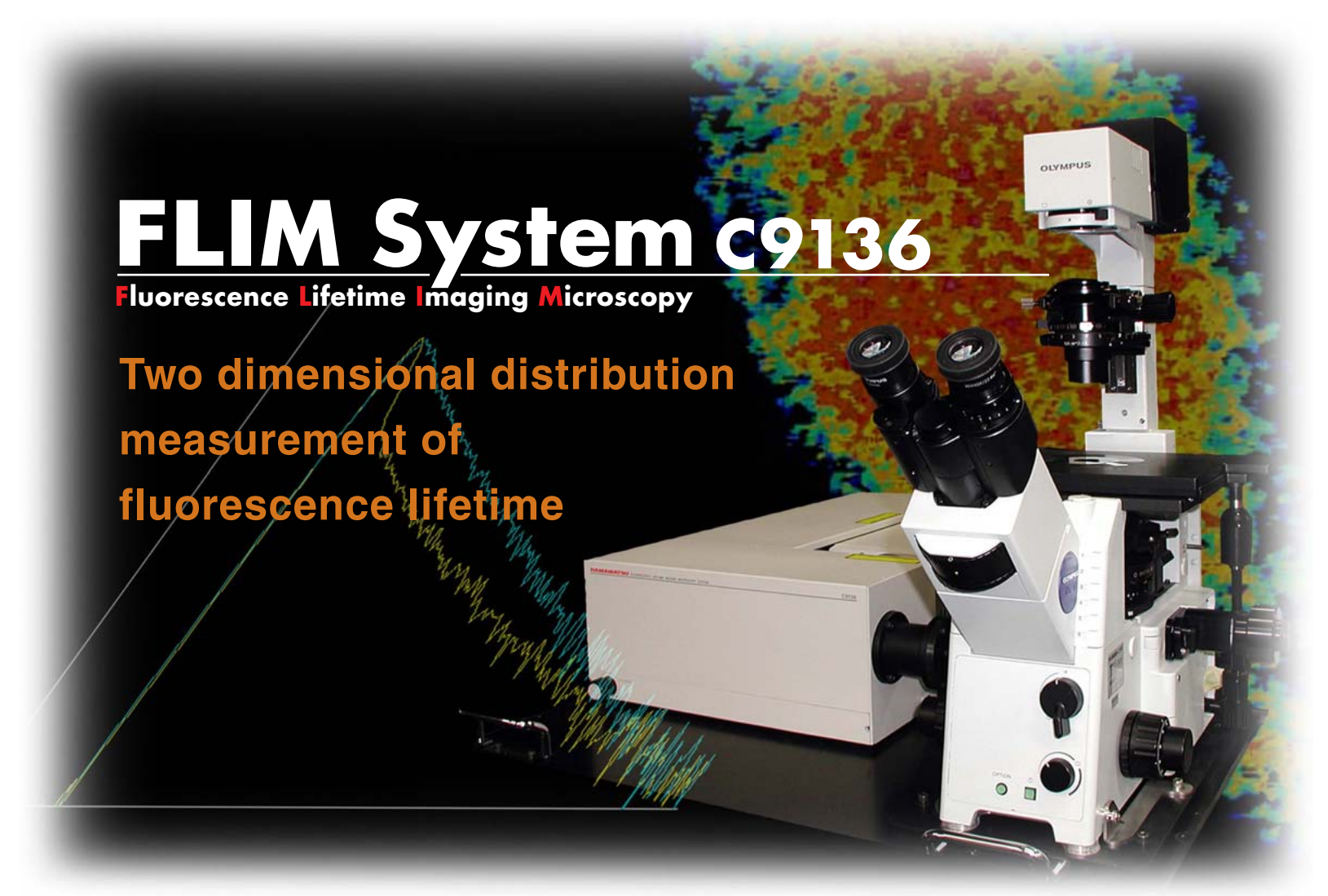
FLIM System C9136

Fluorescence **L**ifetime **I**maging **M**icroscopy

Two dimensional distribution measurement of fluorescence lifetime



What are Fluorescence Lifetime and FLIM?

Fluorescence is the emission of a longer wavelength photon from a molecule as it returns to its ground state energy level after being excited by a short wavelength photon. If a short pulsed light source is used for excitation, a decay of the fluorescence intensity will be observed, as in Figure 1. The exponential decay of the fluorescence intensity over time is characteristic of each fluorescent molecule and is known as τ_F.

Unlike intensity-based measurements of fluorescence, the lifetime (τ_F) is constant even if concentration, path length or illumination is varied in the sample. However, very subtle changes in the local environment of the molecule such as pH, ion concentration, polarity and viscosity produce changes in the lifetime. Molecular non-radiative energy transfers between fluorescent molecules will produce lifetime changes that are easily detected.

FLIM (Fluorescence Lifetime Imaging Microscopy) extends this powerful technique into the microscopic domain. Spatial resolution of intracellular structures is possible in images that provide both temporal and spatial information of changes in the fluorescent lifetime of fluorescently labeled components. Structural, biochemical and photodynamic processes can be observed even in living cells as shown in Figure 2. Even fluorescence resonance energy transfer (FRET) benefits from the high detection and speed of the system. Observation of protein-protein interactions in living cells offers new possibilities in research.

Hamamatsu has combined the space and time dimensions of a streak camera image with the resolution of a microscope to open new horizons in biological imaging.

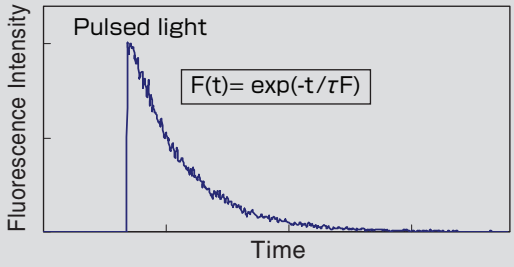


Figure 1:Typical fluorescence decay curve

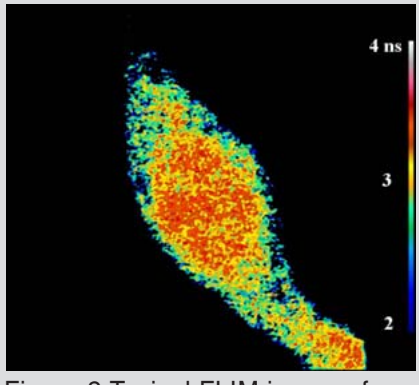


Figure 2:Typical FLIM image of a cell

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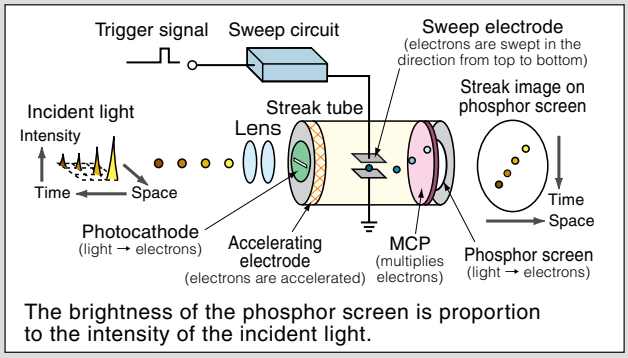
Principle of FLIM System C9136

Operating Principle of Streak Camera

An optical image with intensity information at every pixel is converted to a streak image with spatial information as the horizontal axis and time as the vertical axis. Every point in a streak image provides information representing intensity, location, and time. The CCD detector creates a point-by-point map of this information for evaluation.

In the schematic below, three optical pulses arrive at the slit with varying intensity and which vary slightly in terms of time and space. As the corresponding photoelectrons from photocathode pass between the pair of sweep electrodes, the applied sweep voltage steers the electron paths away from the horizontal direction at different angles depending on their arrival time at the electrodes. The amplified electrons reach the phosphor screen forming an image of three optical pulses arranged in vertical direction according to the time of their arrival at the sweep electrodes. The earliest pulse is arranged in the uppermost position and the latest pulse is in the bottom most portion of the phosphor image. The resulting streak image has space as the x-axis and time as the y-axis.



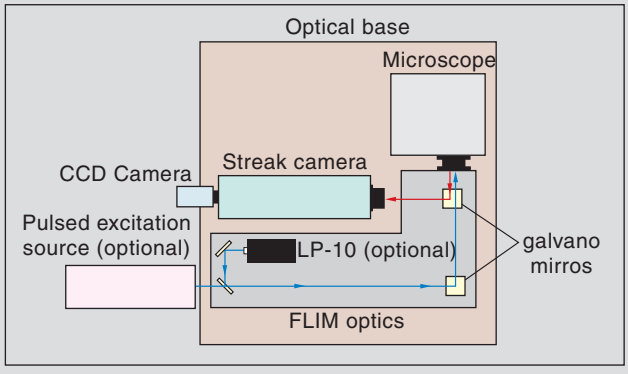
The brightness of the phosphor screen is proportion to the intensity of the incident light.

Configuration of FILM System

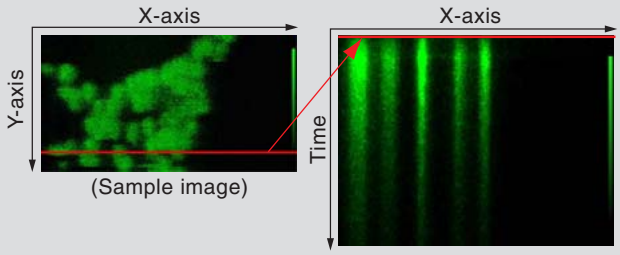
The configuration of C9136 FLIM system is shown below.

A pulsed excitation source is used in the system. Fluorescent substances emit fluorescence after photoexcitation with the pulsed excitation light.

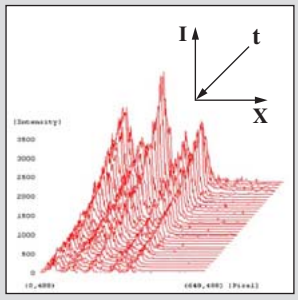
The streak camera captures fluorescence emission through special optics and the lifetime is obtained. By scanning the pulsed excitation light in the two directions spatially with two galvano mirrors in the optics, the 2-dimensional distribution of the fluorescence lifetime is measured.



Streak Image

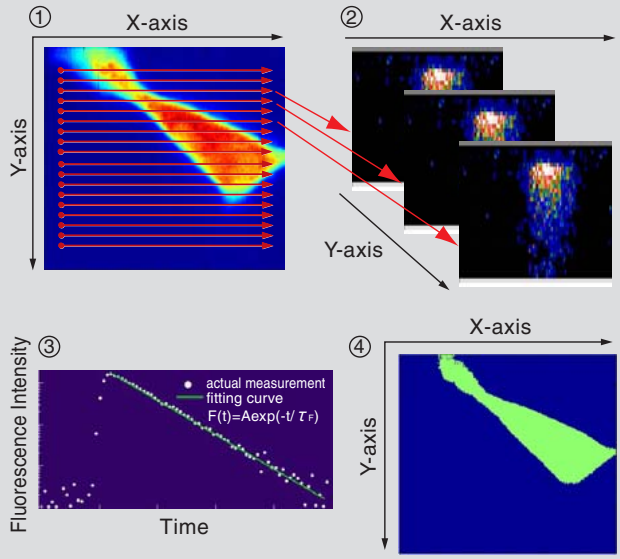


A fluorescence intensity image (left) and a typical streak image (right) of the same sample are shown above. The streak image displays vertically the temporal change in intensity information of those points scanned in the red line of the sample image.



The figure at left shows three-dimentional display of spatial decay of fluorescence intensity as function of time in the streak image. It can be seen that the fluorescence intensity decreases as time passes.

Measurement Principle of FLIM System



① Excitation laser scans along X-axis in sample.*

② Streak camera collects fluorescence emission from individual pixels, and streak image stores three-dimensional information consisting of intensity, space (X-axis), and time. Synchronous Y-axis scanning builds up the streak image stack.

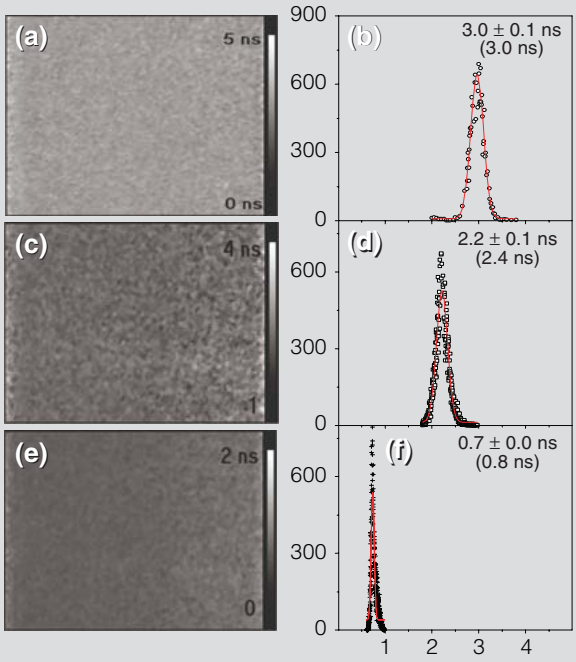
③ Fluorescence decays are calculated and fluorescence lifetimes are obtained at individual pixels on the X- and Y-axis.

④ FLIM image is constructed.

* Slit excitation also available

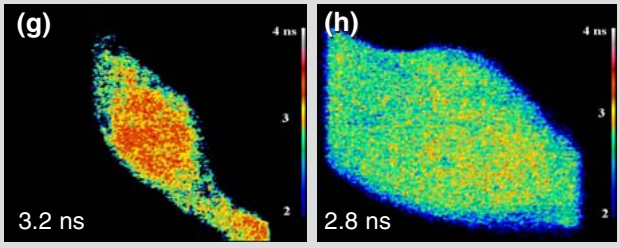
Applications

System Calibration



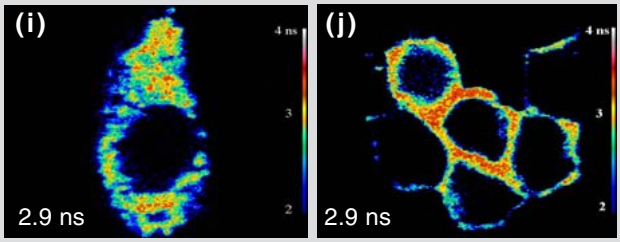
Lifetime images and histograms for standard fluorophore solutions are shown above; (a) and (b): rhodamine 6G in ethanol, (c) and (d): rose bengal in acetone, and (e) and (f): rose bengal in ethanol. Solid lines in the histograms are Gaussian fits to the data. Measured and known (in parenthesis) values of τ F are shown in the histograms.

Lifetime Imaging Cells



Cyto-ECFP **Cyto-BaxECFP**

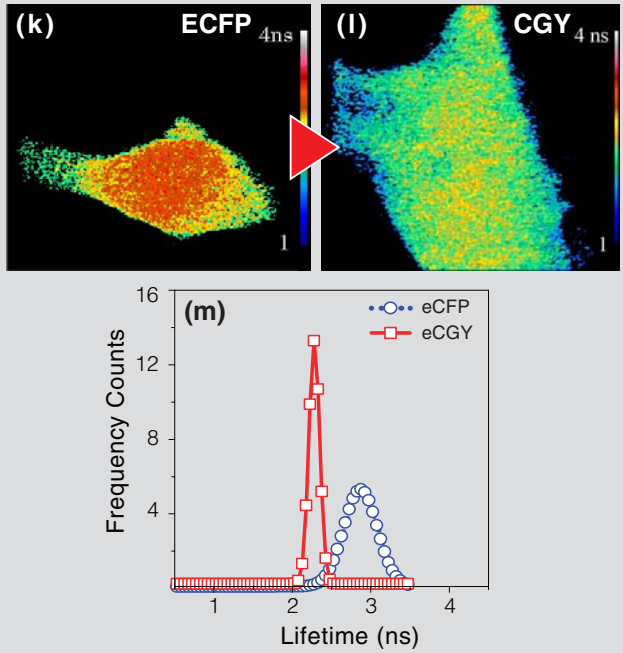
Lifetime images of BHK cells expressing cytosololic ECFP (Cyto- ECFP) and fusion protein Bax-ECFP (Cyto-BaxECFP) are shown in (g) and (h). The mean lifetime values of Cyto-ECFP and Cyto-Bax-ECFP are 3.2 ± 0.3 ns and 2.8 ± 0.3 ns, respectively.



Mito-ECFP **Mem-ECFP**

Lifetime images of cells expressing mitochondrial targeted ECFP (Mito-ECFP) and membrane targeted ECFP (Mem-ECFP) are shown in (i) and (j). Both mean lifetime values of Mito-ECFP and Mem-ECFP are 2.9 ± 0.1 ns.

FRET Imaging in Cells



Lifetime images of BHK cells expressing ECFP, CFP-polyglycine-YFP (CGY), and the histogram of their lifetimes are shown in (k), (l), and (m), respectively. The mean lifetime of ECFP is 2.9 ns, whereas that of CGY is 2.3 ns. The decrease in mean lifetime of CGY compared to that of ECFP is due to FRET process (energy transfer between CFP site and YFP site).

What is FRET?

FRET (Fluorescence Resonance Energy Transfer) is a distance-dependent non-radiative energy transfer interaction between two electronic excited states of fluorescent substances. Excitation energy is transfered from donor to acceptor fluorescent substances. Using this phenomenoma, real-time imaging of protein structural dynamics, protein-protein interactions, enzyme activity and structural change can be observed and quantified.

The efficiency of FRET, ϕ_{FRET} can be calculated from the following expression, where τ_{DA} and τ_{D} are the lifetimes of the donor in the presence and in the absence of acceptor, respectively.

$$\phi_{FRET} = 1 - \tau_{DA} / \tau_{D}$$

Specifications

Effective field of view: 48 μm × 45 μm **
Spatial resolution: 0.2 μm
Effective pixels and lines of full area : 656 pixels on X-axis and 601 lines on Y-axis
Shortest image acquisition time (full area): approx. 3 s ***

Excitation Method
Pulsed excitation source

Temporal Resolution
Less than 20 ps

** With ×60 water immersion objective lens
*** When the data acquisition time for a line on Y-axis is 5 ms.
The image acquisition time
is estimated as (the data acquisition time for a line) x (the number of lines).

References

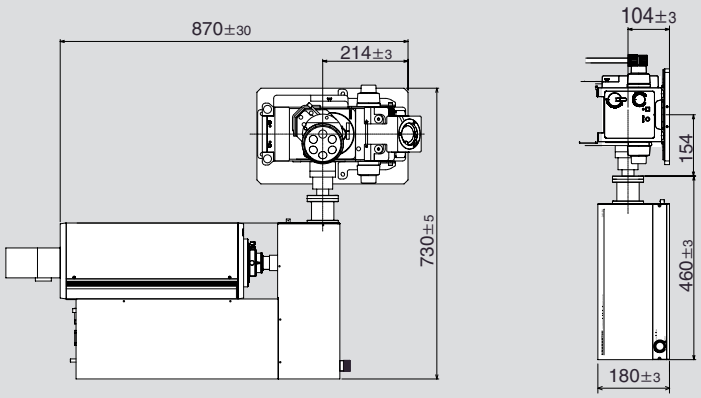
1. Krishnan,R.V.; Saitoh H;Terada H; Centonze, V.E; Herman, B (2003)
Development of Multiphoton Fluorescence Lifetime
Imaging Microscopy (FLIM) system using a streak camera, Rev.Sci.Instrum. 74, 2714-21.

2. Krishnan,R.V.; Masuda A; Centonze, V.E; Herman, B (2003)
Quantitative Imaging of Protein-Protein interactions by
Multiphoton Fluorescence lifetime imaging microscopy using a streak camera. J.Biomed.Optics. 8, 362-367

3. Krishnan,R.V.; Biener E; Zhang J.-H; Heckel R; Herman, B (2003)
Probing subtle fluorescence dynamics in cellular proteins
by streak camera based fluorescence lifetime imaging. Appl. Phys. Lett. 83, 4558-4660.

4. Herman, B; Krishnan, R.V.; Zhang, J.; Masuda, A.; Lopez-Cruzan, M.; Zhang,Y.; Centonze, V, (2003)
FRET and FLIM Microscopy in
the Study of the Role of Oxidative Stress and Apoptosis in Aging. Microscopy and Microanalysis vol.9, suppl.2, 226-227

Dimensional outline (mm)



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