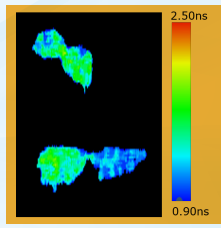
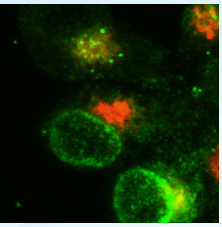
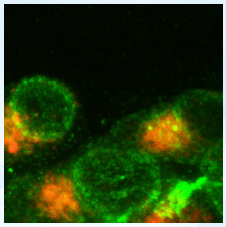
HORIBA
Scientific

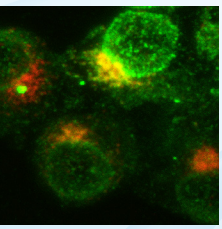
FLIMera™

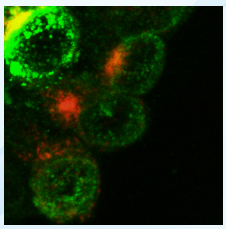
Video Rate FLIM with Pixel-wise TCSPC

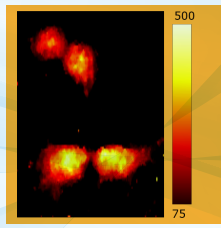












A New Concept in
FLIM Imaging

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FLIMera™

Video rate FLIM with pixel-wise TCSPC

A New Concept in FLIM Imaging

Real-time FLIM camera
from HORIBA
for tracking fluorescence
lifetime dynamics.

FLIMera Camera

The HORIBA FLIMera camera is a new concept in FLIM technology. It is a widefield imaging camera, rather than a confocal point scanning system, with the intrinsic benefit of being able to study FLIM dynamics at video rates with simple camera technology.

Advances in CMOS technology have led to the development of imaging sensors, based on arrays of pixels, with each pixel containing a *single-photon avalanche* photodiode (SPAD) and its associated timing electronics, based on a *time* to *digital converter* (TDC).

This enables rapid (video rate) fluorescence lifetime determination based on the *time-correlated single-photon counting* technique (TCSPC) realized independently in each pixel (Figure 1).

A 192 x 128 pixel image sensor, implemented in 40 nm CMOS technology, is incorporated in a widefield epifluorescence microscope set-up. The sensor has a 13% fill-factor and each 18.4 x 9.2 µm pixel contains a TDC with a resolution <40ps. This enables up to 24,576 simultaneous fluorescence lifetime measurements, each with 4,096 time bins. Through dedicated firmware and the HORIBA EzTime Image software implementation, the fluorescence intensity and the average lifetime, as well as the phasor plot generated from the TCSPC imaging measurement, can be simultaneously displayed in real time at video (>30 fps) rates (Figure 2).

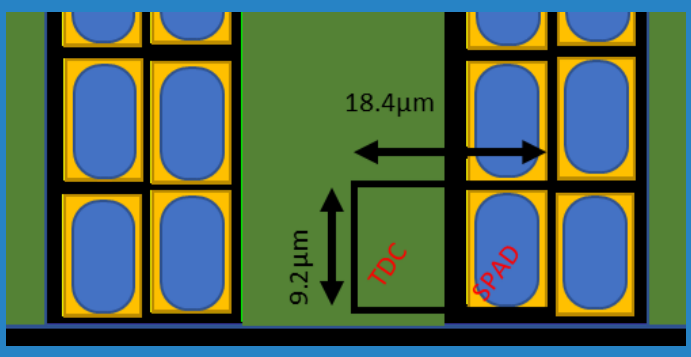

Figure 1: The SPAD and TDC couple

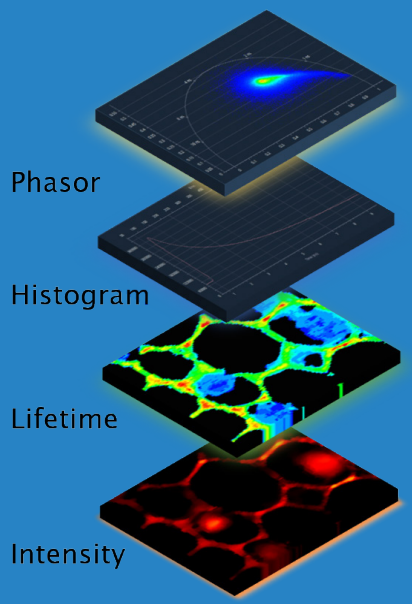

Figure 2: Four simultaneous datasets presented

Modes of Operation

The FLIMera is designed for use with HORIBA's highly intuitive EzTime Image software. This software is used for FLIMera control, data acquisition and analysis. FLIMera provides 4 modes of operation.

• **Manual** – User start and stop of acquisition, providing TCSPC intensity image and FLIM data for lifetime analysis.

• **Timed** – User-defined run time for acquisition, providing TCSPC intensity image and FLIM data for lifetime analysis.

• **Streaming to HDF5 file** – TCSPC data can be streamed, for a user-determined time period, to a HDF5 file. This contains full records for each photon in every pixel, so no data is lost, and can be used to reconstruct frames for full decay analysis.

• **Phasor Plot** – Complementing the histogram representation is the ability to show and select lifetime data in the form of a phasor plot in real time, as seen in Figure 3. The phasor plot requires initial calibration using a lifetime standard.

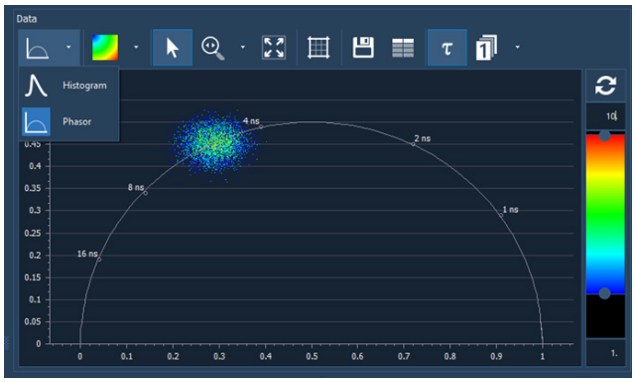

Figure 3: Phasor plot

The video-rate capability is demonstrated using standard samples and FUN-1 labeled yeast cells (Figure 4).

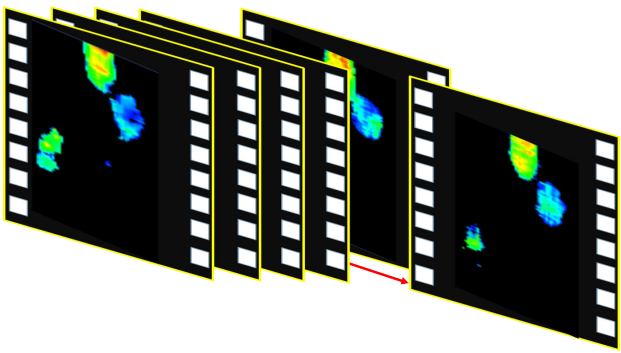

Figure 4: Sequence of images extracted from FUN-1 labeled yeast cell lifetime video https://youtu.be/1Fu7VOCsHuA

A comparison of fluorescence decay data of 9-Aminoanthracene measured in a cuvette system (Figure 5 a) compared to that measured by a single pixel of the FLIMera (Figure 5 b) indicates the per-pixel fidelity of the FLIMera SPAD.

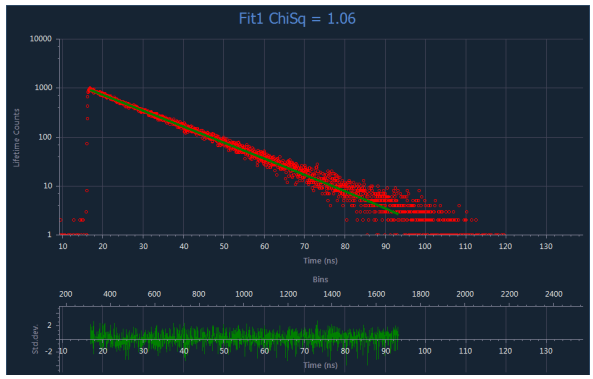

Figure 5 a: Decay from DeltaFLEX cuvette system

Figure 5 b: Decay from just one of 24,576 SPAD pixels demonstrates the very high resolution and TCSPC fidelity of FLIMera

The Science of FLIM

Fluorescence lifetime spectroscopy is an increasingly popular technique for material analysis. This is partly because it is a background-free technique and partly because it offers complementary insights that are not readily accessible through steady-state methods. In addition, fluorescence lifetime imaging microscopy (FLIM) adds spatial information to the measured data set, providing another form of contrast to the image. A FLIM image consists of the fluorescence lifetime at each pixel of the image rather than just the fluorescence intensity, as in steady-state fluorescence. Fluorescence lifetime typically does not depend on concentration, but is highly dependent on the fluorophores' local environment. The spatial information provided by FLIM enables measurement of variables such as FRET, pH, viscosity, binding affinity and temperature, etc.

TCSPC: The Technique

HORIBA's lifetime measurement technology is based on time-correlated single-photon counting (TCSPC) for recording the time-resolved fluorescence decay. This system is capable of lifetime imaging measurements from picoseconds to microseconds, as well as single point phosphorescence measurements.

TCSPC is accomplished by a short pulsed excitation source, typically a laser or LED, flashing at high rates (e.g. 80 MHz) and eliciting a fluorescence flux from the sample of interest. The sophisticated detection electronics register a single fluorescence photon's arrival time and starts the detection cycle again to detect another fluorescence photon during the next excitation flash/event (Figure 6).

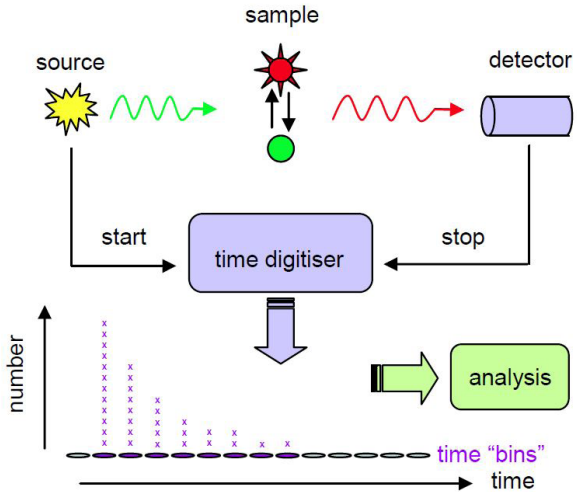

Figure 6: Schematic of the TCSPC measurement

Since only a single photon is registered during each cycle, the histogram of arrival times is used to derive the fluorescence decay curve and the characteristic lifetime value (τ). This process occurs simultaneously for all pixels of FLIMera resulting in a lifetime image.

FLIM and HORIBA

The novelty of the in-pixel detection and timing technology enables a widefield imaging approach, which significantly reduces data acquisition times, enabling the study of dynamic events. FLIMera contributes to HORIBA's long history in the field of fluorescence measurement; recognized by winning the prestigious Institute of Physics Business Innovation Award.

Business Innovation Award **2019**

IOP Institute of Physics

The explosive growth of life science research during the last 25 years focused attention on microscopy and a vast array of related techniques and technologies.

At HORIBA, the attainment of FLIM was accomplished through various configurations.

- Single channel confocal detection with stage scanning
- Unique widefield TCSPC FLIM camera (FLIMera)
- FLIM on a custom micro-spectroscopy platform
- FLIM TCSPC components (for use with stage / galvo scanning)

FLIMera Camera Specifications

Modality	Widefield Time-Domain TCSPC
Sensor	SPAD CMOS with In-pixel TDC
Sensor Wavelength Range	400 to 900 nm
Max Total Count Rate	Up to 294 Mcps theoretical.
Max Pixel Count Rate	12 kcps
Image Array	192 x 128 pixels
Frame Rate (Max.)	30 fps
Fill Factor	13%
Lifetime Range	From 200 ps to 20 ns
Lifetime Resolution	Up to 4096 time bins of 50ps
Connection Type	C-Mount
Electronics Interface Included	HORIBA FLIMera base unit (includes laser synch input TTL)
Software Included	HORIBA EzTime Image
Required Components	Pulsed laser source
Laser Source Options	DeltaDiode Lasers

For more information go to **horiba.com/FLIMera**

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