

LIFETIME



THE FLIM MAGAZINE

The Time Layer

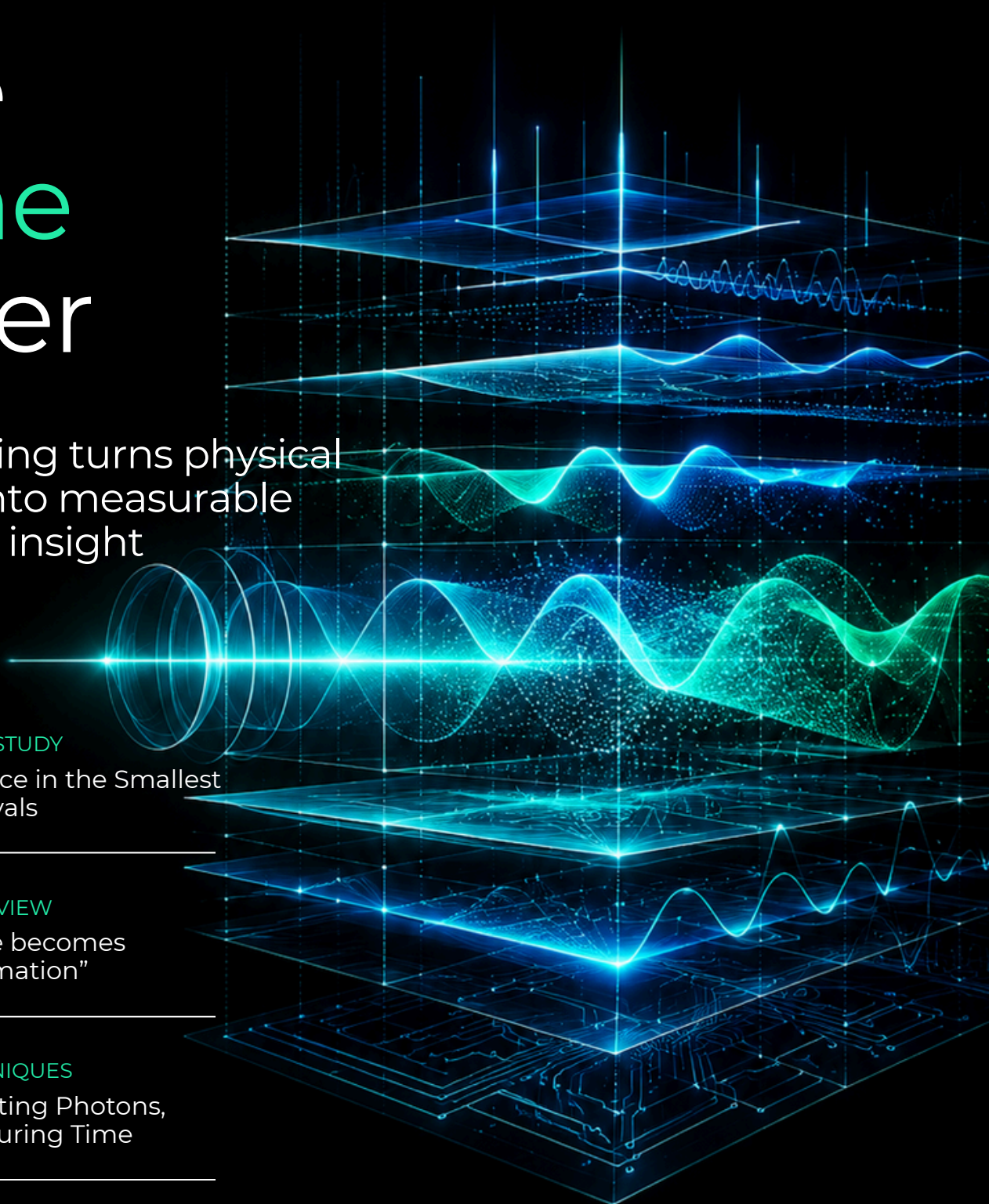
How timing turns physical
signals into measurable
scientific insight

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"Time becomes
information"

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Counting Photons,
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Options, setups, and next steps



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Exploring the science,
technology and applications
of FLIM

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Time-resolved science: photon timing, fast signals, and the instruments that make invisible events measurable.

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NOTE FROM FLIM LABS

Why this issue looks at *timing* - and what fast events let us *see*

This September issue of LIFETIME explores how time-resolved measurements helps science capture signals too fast to see directly.

Some measurements do not become meaningful by looking harder, but by looking in time. In many areas of science, the key signal is not just its intensity or shape, but when it arrives, how long it lasts, and how precisely it can be linked to other events.

This issue begins from that idea. We look at how timing adds a new measurement dimension — one that helps researchers move beyond static images and toward dynamic information. In FLIM, for example, photon arrival times can reveal contrasts that brightness alone may hide.

You will read how techniques such as TCSPC turn single photons into usable decay curves, why timing electronics matter so much for advanced instruments, and how even subtle improvements in signal

handling can shape the quality of a measurement.

At the same time, this issue also looks outward. Time-resolved thinking is not unique to microscopy: it appears across science wherever very fast signals need to be captured, synchronized, and interpreted.

From particle accelerators and synchrotron light to quantum photonics and fluorescence measurements, the same principle returns again and again: a physical event becomes useful knowledge when it is measured in time.

Our goal in these pages is simple: show how advanced timing can become more understandable, more practical, and more connected to the real tools researchers use every day.

“

Time becomes a measurement layer when fast signals are captured clearly enough to mean something

”

CASE STUDY

Science in the smallest intervals

From particle accelerators to FLIM, some of the most powerful measurements in science depend on knowing not only what happened, but when.

A lot of science is built around images. We look at a cell, a material, a signal on a screen, and we try to understand what is there. Shape, position, brightness, structure. Those are the obvious things. But many experiments depend on a quieter question: when did something happen?

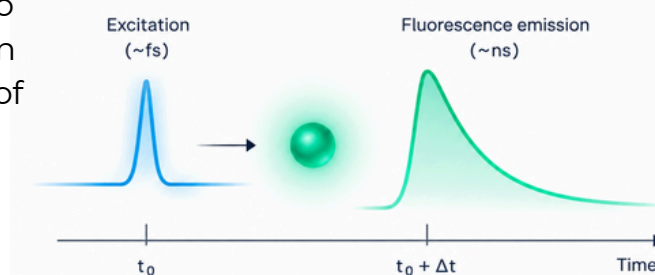
Sometimes that question is simple. Sometimes it is almost absurdly difficult. A particle passes through a detector. A pulse of synchrotron light hits a sample. A fluorescent molecule emits light for only a few billionths of a second. None of these events can be followed with the eye, and in many cases even “fast” cameras are far too slow. The only way to study them is to turn time itself into part of the measurement.

This is familiar territory in particle physics. In an

accelerator, the interesting event is not just that a particle was detected, but where and when it was detected, and how that signal fits with all the others produced inside the machine. Detectors collect fragments of fast interactions and timing helps reconstruct the sequence. Without it, the experiment becomes a crowded room full of voices speaking at once.

Synchrotron facilities work with a different kind of event, but timing is just as present. At a light source such as Elettra - the synchrotron facility in Trieste, Italy - X-ray pulses are used as probes and synchronized with intense laser

sources used as pumps to investigate materials and biological samples. Some experiments ask how a system changes after being



A short excitation pulse triggers nanosecond-scale fluorescence emission.

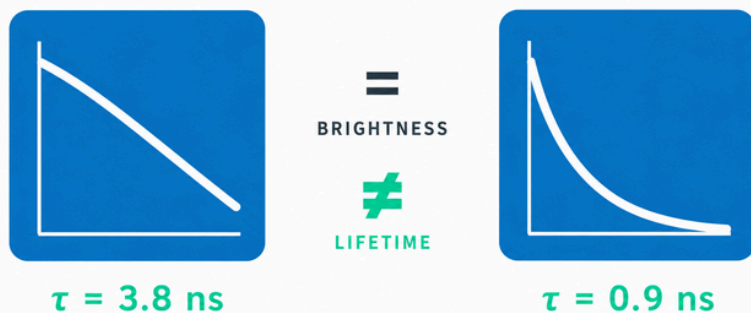
excited, how quickly a process begins, or how a response evolves rather than simply what the sample looks like at one fixed moment. The time axis becomes a way to separate a static picture from a dynamic one.

FLIM brings this logic into microscopy. Fluorescence lifetime imaging does not only ask how much light a sample emits. It asks how long that fluorescence lasts after excitation, usually on the nanosecond scale. That short delay can carry information about molecular environment, interactions, metabolism, autofluorescence, and local conditions inside a sample. Two regions can look equally bright and still behave differently in time. That difference is where lifetime becomes useful. This is why FLIM is not simply another way to colour an image. The image is the visible end of a timing measurement. Behind it there is a chain of pulsed excitation, detection, synchronization, acquisition electronics,

and reconstruction. A photon arrives, its timing is recorded, many events are collected, and only then does the lifetime map begin to take shape. The scale changes from field to field, but the principle stays surprisingly close. Particle accelerators, synchrotrons, and FLIM systems all depend on the ability to organize fast events into something readable. Timing gives structure to signals that would otherwise be too brief, too small, or too mixed together to interpret.

Once time becomes measurable, the questions change. A sample is no longer only bright or dim. A material is not only present or absent. A signal is not just detected. It has a behaviour, a delay, a rhythm, a response.

That is the extra layer. Not a replacement for images or spectra, but a dimension that lets science listen to things that happen too quickly to be seen.



Two regions can appear equally bright (in this example the same blue square) while showing different fluorescence decay times. FLIM captures this temporal difference, allowing lifetime contrast to reveal information that intensity alone may miss.

Inside the *Electronics* of Time

A conversation with Prof. Nicola Lusardi on fast signals, FPGA technology, and the electronics that turn time into measurable scientific information.

Could you briefly introduce yourself and tell us about your background?

My name is Nicola Lusardi, I was born in 1990, and I have been working with electronics for more than 20 years — more precisely, since September 2004, when I began my path by enrolling at the “G. Marconi” technical institute in Piacenza. After finishing high school in 2008, I enrolled in Electronic Engineering at Politecnico di Milano, where I completed my bachelor’s degree, master’s degree — with a thesis on innovative high-resolution time-measurement systems in FPGA devices — and PhD, with a thesis on advanced methods, techniques, and digital architectures for high-speed performance timing of events. I completed my studies in 2017. From 2017 to 2025, I worked as a researcher at Politecnico di Milano, becoming Associate Professor in 2025. My research has always focused on FPGA-based

measurement systems for scientific experiments, with particular attention to timing.

You have worked, and still work, on electronics for measuring very fast signals. What brought you closer to this field?

I started following this path during my master’s thesis in 2013, and over the past 13 years I have consolidated my knowledge and skills day by day, experiment by experiment. I must admit that I came into contact with this field almost by chance, while choosing the topic for my master’s thesis. Once I started exploring it, I became fascinated by the synergy between many different disciplines: digital electronics, because the entire process of measurement, calibration, setting, and user interfacing is completely digital; analog electronics, because the extraction of the raw timestamp — the timing information — is governed by a more analog process, even though it takes place inside a digital device such as an FPGA; mathematics, particularly statistics, which is needed to predict, calculate, and estimate the precision and resolution of the instrument; physics, because every time measurement is used to describe a physical process. There is always a one-to-one conversion between the measurement being made





and the physical process under study.

This issue of LIFETIME is dedicated to time as a measurement dimension in science. From your perspective, why is precise time measurement so important in modern instrumentation?

Essentially, apart from sports competitions, time is not so interesting as a direct measurement, but rather as an indirect one. We measure time because, for the same level of accuracy, it is the simplest and most cost-effective way to understand a phenomenon, even when that phenomenon has little to do with time itself. This is a general concept that extends to many branches of research. To give just a few examples: measuring decay times allows us to analyse matter; measuring particle time of flight allows us to measure space; and measuring the time delay between particles allows us to understand whether two phenomena are correlated, which is fundamental in medical

machines and quantum experiments.

FPGA technology often appears in systems that require speed, synchronization, and real-time processing. What makes FPGAs so useful for these kinds of measurements?

Conceptually, everything you do in a Field-Programmable Gate Array, or FPGA, can certainly be done — even better and with greater design freedom — by designing a dedicated integrated circuit, an Application-Specific Integrated Circuit, or ASIC. The difference lies in development time and budget, which are two fundamental constraints in research. Since research is constantly evolving, it requires state-of-the-art solutions to be reached as quickly as possible and at reduced cost. To day, the major advantage of FPGAs is that they allow development at costs at least 100 times lower, and in timescales about 10 times shorter, compared with an ASIC solution, while still achieving, under compromises (in general more area and power consumption) comparable performance if the design is done well. In addition, FPGAs are programmable. Unlike ASICs, they make it possible to fix bugs immediately and adapt an old setup to new requirements.

When designing electronics for time-resolved measurements, what are the main challenges: signal quality, synchronization, noise, data flow, or something else?

All the aspects you mentioned are important, as are size, area, power consumption, and development time

and cost. Of course, from a technological point of view, it is unlikely that all these constraints can be satisfied at the same time. It is therefore necessary to make compromises, put the specifications in order of priority, and set minimum objectives depending on the application. The difficult part is defining minimum objectives that are both achievable and compatible with the application. I believe this is what makes an experienced designer stand out.

In fields such as FLIM, particle detection, synchrotron experiments, or other time-resolved techniques, what does electronics make possible?

From what I know, electronics is what has made all of this possible. It would be unthinkable to extract information without using electromagnetic mechanisms, and therefore electronics. It is no coincidence that electronics is a subcategory of information technology whose task is to provide the hardware needed for the transfer of information.

What is the difference between building a prototype that works technically and building a system that researchers can use reliably in practice?

Essentially, time: all the time needed to validate, test, solve edge cases, and make the prototype — the proof of concept — user-friendly. When studying a prototype, it may be acceptable to settle for a confidence level of 90–99%. In a final setup, however, the probability of error must be at least in the order of ppm (parts per million), certainly not in

the percentage range.

Looking ahead, where do you think time-measurement electronics and FPGA-based systems are going in scientific instrumentation?

Based on my experience, ten years ago full-FPGA solutions were not particularly favoured. The preference was to work with ASICs that used FPGAs as a “bridge” to interface with the user. Today, I see that full-FPGA solutions are becoming increasingly common. Of course, this trend may change in the future, and predicting it is very complex. I will not go into topics such as AI, open-source ASICs, and so on, which in this context would increase entropy without adding much useful information. The only certain thing is that, ten years from now, things will be different from today, just as today they are different from ten years ago.

IN SHORT

What is FPGA?

Field-programmable gate arrays are reconfigurable chips whose logic can be tailored to a specific task after manufacturing. First introduced in the 1980s, they became widely used in scientific instrumentation because they combine speed, flexibility, and relatively low development costs.

Processor vs FPGA

Processor: *executes instructions sequentially.*

FPGA: *implements custom logic in parallel.*

That is why FPGAs are especially useful for synchronization and real-time measurement systems.

TECHNIQUES



Counting **Photons**, Measuring **Time**

How TCSPC turns individual photon arrival times into fluorescence decay curves and lifetime information.

Time-Correlated Single Photon Counting, or TCSPC, is one of the key techniques behind fluorescence lifetime imaging. Its principle is simple to describe, but extremely powerful: instead of measuring fluorescence only as brightness, TCSPC measures when individual photons arrive after a laser pulse excites the sample.

In a typical experiment, a pulsed laser sends repeated excitation pulses toward a fluorescent sample. After each pulse, the sample emits photons. TCSPC detects these photons one by

one and records the tiny delay between the laser pulse and the photon arrival. Over many repeated cycles, these arrival times are collected and used to build a histogram that represents the fluorescence decay curve.

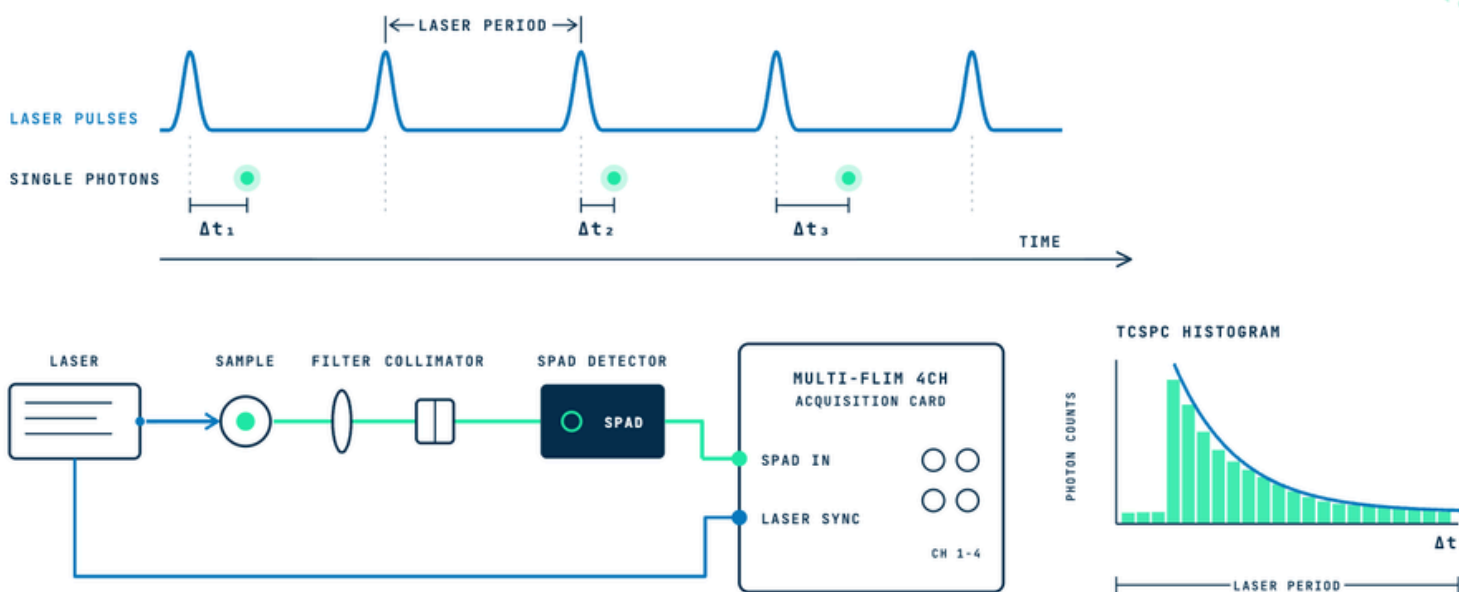
That decay curve describes how quickly fluorescence fades after excitation. From it, researchers can extract the fluorescence lifetime of the fluorophores in the sample. This lifetime can reveal information that intensity alone may miss, including changes in the molecular environment,

interactions between molecules, or shifts in local conditions.

The strength of TCSPC lies in its sensitivity and temporal precision. Because it works by counting individual photons, it can capture very weak signals and reconstruct fast fluorescence dynamics with high accuracy. This makes it especially valuable in FLIM, where nanosecond-

scale timing differences can become meaningful biological or material contrast. For this reason, TCSPC is widely used across biophysics, chemistry, materials science, and microscopy. It turns photon arrival times into quantitative information, helping researchers move from a simple fluorescent signal to a deeper view of how a sample behaves in time.

TCSPC • TIME-CORRELATED SINGLE PHOTON COUNTING



Minimal setup for reconstructing fluorescence intensity decay curves.

Explore FLIM Integration and Validation

From optical devices to solid calibrators,
turn fluorescence into measurable, reliable insight.



Optical Design Integration

Evaluate how FLIM can
extend what your system
can measure



Laser & Microscopy Platforms

Add the time dimension
to existing optical
workflows



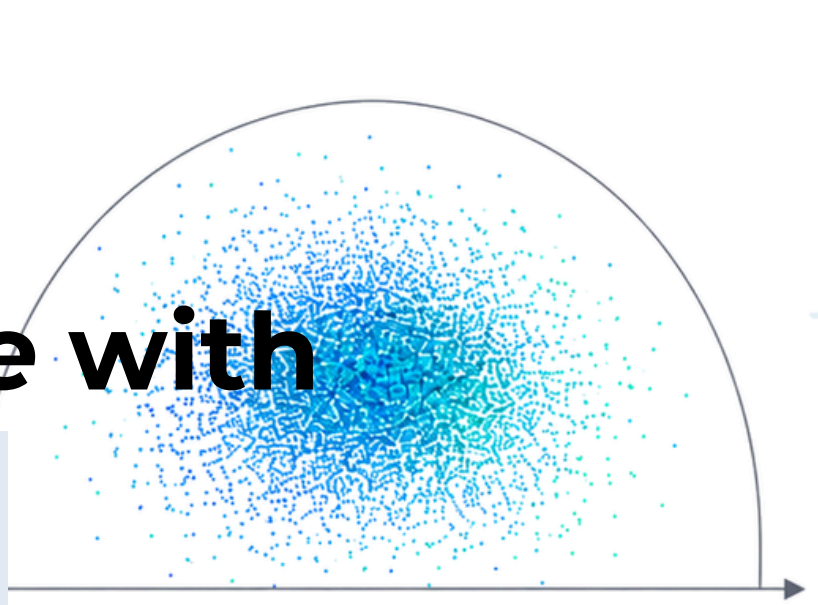
Solid Calibrators & Validation

Build trust through
robust, reproducible
lifetime measurements

→ **Talk to FLIM LABS**

Contact us by sending an email to info@flimlabs.com
or via www.flimlabs.com

Doing More with Less Light



How wavelet-based phasor analysis can help make FLIM data cleaner when photon counts are limited.

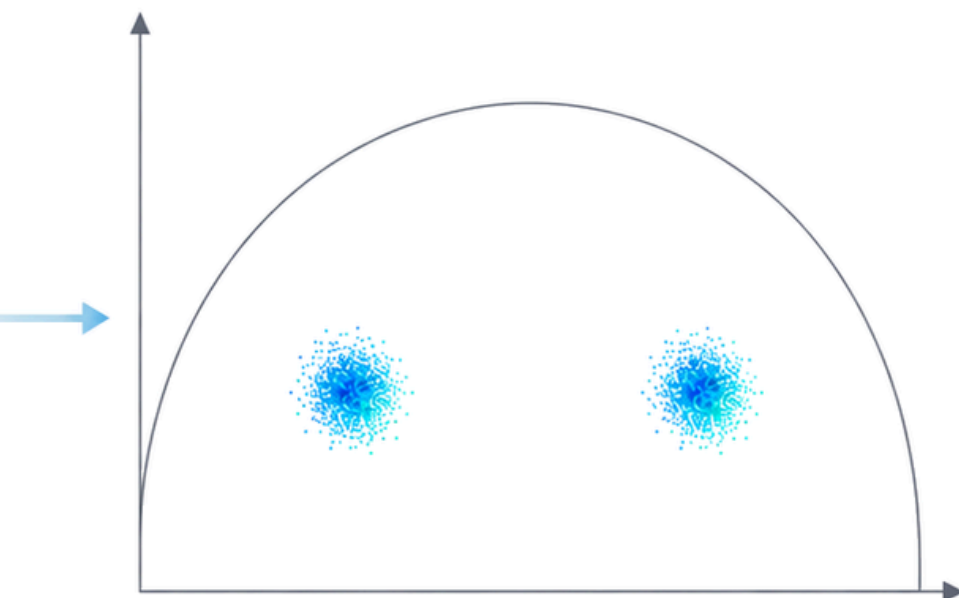
In FLIM, photon counts matter. The more photons a system collects, the more reliable the lifetime estimate usually becomes. But collecting more photons often means longer acquisition times, higher light exposure, or a slower view of fast biological processes.

PawFLIM, or phasor analysis via wavelets, was introduced by Mauro Silberberg and Hernán E. Grecco [1] as a method to address this trade-off. It uses wavelet-based denoising on phasor images, helping reduce bias and uncertainty in FLIM datasets when photon counts are low.

The idea is not simply to smooth an image. Standard smoothing can remove noise, but it can also blur meaningful spatial or molecular differences. pawFLIM takes a more adaptive approach, adjusting the balance between spatial resolution and molecular resolution so that lifetime information can be recovered more reliably.

The method builds on the fact that the measured phasor components are noisy but correlated because they reflect an underlying spatially smooth molecular variable. PawFLIM identifies this correlated information and suppresses the uncorrelated noise using an estimator shown to be optimal under the model; an idea later developed more generally in the Binlets framework [2].

This is especially useful in live-cell and FRET-FLIM experiments, where researchers may need to follow fast dynamics without collecting so many photons that the measurement becomes too slow or too invasive. By improving the quality of phasor-based FLIM data under photon-limited conditions, pawFLIM supports cleaner analysis while preserving more of the information that makes lifetime imaging valuable. In short, pawFLIM helps FLIM do more with less light.



PAPER SPOTLIGHT

IOP Publishing

Methods Appl. Fluoresc. 5 (2017) 024016

<https://doi.org/10.1088/2050-6120/aa72ab>

Methods and Applications in Fluorescence



PAPER

pawFLIM: reducing bias and uncertainty to enable lower photon count in FLIM experiments

RECEIVED
4 November 2016REVISED
21 April 2017ACCEPTED FOR PUBLICATION
12 May 2017PUBLISHED
26 June 2017

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E-mail: hgrecco@df.uba.ar**Keywords:** denoising, Förster resonant energy transfer (FRET), fluorescence lifetime imaging microscopy (FLIM), wavelets, protein-protein interactionSupplementary material for this article is available [online](#)

This paper introduced pawFLIM — phasor analysis via wavelets — as a way to denoise FLIM datasets under low-photon conditions while preserving meaningful spatial and molecular information. By applying a weighted Haar-wavelet algorithm to phasor images, Silberberg and Grecco showed that pawFLIM can reduce bias and uncertainty compared with other methods, especially when photon counts are limited.

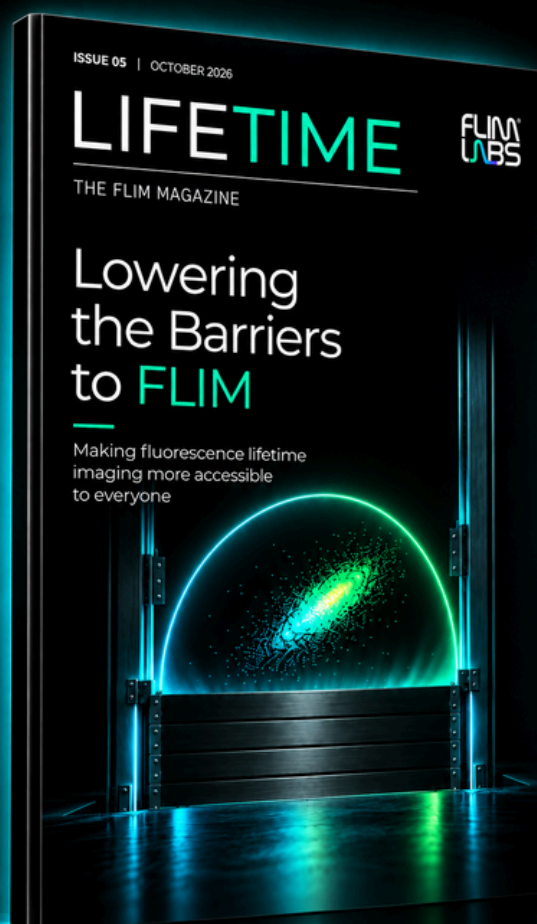
[1] Silberberg, M., & Grecco, H. E. (2017). pawFLIM: reducing bias and uncertainty to enable lower photon count in FLIM experiments. *Methods and applications in fluorescence*, 5(2), 024016.

[2] Silberberg, M., & Grecco, H. E. (2024). Binlets: Data fusion-aware denoising enables accurate and unbiased quantification of multichannel signals. *Information Fusion*, 101, 101999.

NEXT ISSUE

Coming next month: Lowering the Barriers to FLIM

Democratising fluorescence
lifetime imaging



WHAT'S INSIDE

DEMOCRATISING FLIM

How lowering technical and workflow barriers can bring FLIM to more researchers.

CFD INTERVIEW

A conversation on signal discrimination and timing precision in FLIM measurements.

OPENUC2 + FLIM

A look at modular microscopy with the OpenUC2 microscope and the FLIM LABS add-on.

A Modular Path to Quantum Photonics

The Quantum Photonics Bundle is a modular workflow for pulsed excitation, single-photon detection, time tagging, and quantum photonics experiments.



Quantum photonics experiments depend on precise control of light at the single-photon level. The FLIM LABS Quantum Photonics Bundle brings together the key elements needed for this kind of work: a picosecond-pulsed laser source, a SPAD single-photon detector, the TEDIEL TDC bundle, the Felix TimeTagger, and the readout software (TDC-Software).

Together, these components support experiments where timing is central. The laser provides short optical pulses, the SPAD detector captures individual photon events, and the time tagger records their arrival times with high temporal precision. TDC-Software

completes the workflow by giving researchers a dedicated environment for data acquisition and analysis.

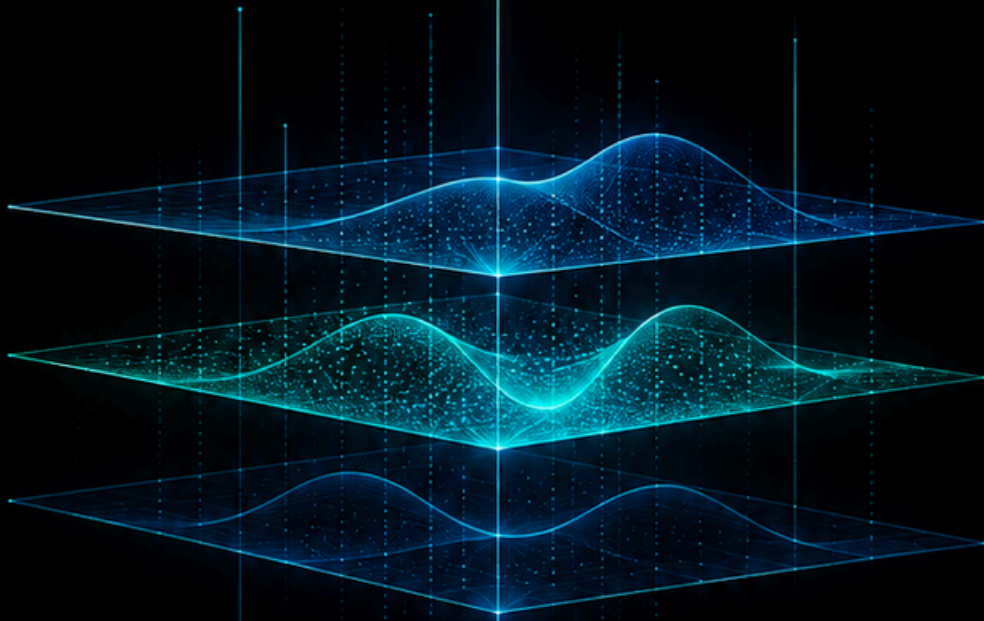
The bundle is designed to reduce the effort needed to assemble a working quantum photonics setup from separate components.

Instead of building the measurement chain piece by piece, researchers can start from a compact, modular system where source, detection, timing, and software are already designed to work together. For quantum photonics, measuring light means measuring events that are extremely small and extremely fast. This bundle is built around that challenge.

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Turn light
into insight



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