TauSense: a fluorescence lifetime-based tool set for everyday imaging

The TauSense technology from Leica Microsystems is a new, straightforward way to generate images using lifetime-based information. Measuring changes in the fluorescence arrival times gives an extra layer of information for understanding the functions of molecules within the cellular environment, increasing image quality, and expanding the number of probes that can be visualized in a specimen. This technology enables the acquisition of lifetime-based information with smaller data size and computational load compared to fluorescence lifetime imaging (FLIM). Moreover, the implementation of different tools (TauConstrast, TauGating, TauScan and TauSeparation) allows exploration of this extra dimension of information at different levels.

Fluorescence microscopy allows researchers to gain unprecedented insights into cellular mechanisms. An intrinsic phenomenon of the fluorescence process is the characteristic time between the excitation of the molecule and the photon emission (Fig. 1a). This time is known as fluorescence lifetime and corresponds to the time that the molecule stays in the excited state ($S_1$) before going back to the ground state ($S_0$). It is typically in the sub-nanosecond-to-nanosecond range. In this last step a photon is generated, as portrayed in the Jablonski-Perrin diagram (Fig. 1a)$^1$. The average fluorescence lifetime is a characteristic value for each fluorophore, but it can vary if there are changes in the fluorophore’s close vicinity (less than 10 nm)$^2$. The changes in the fluorophore microenvironment that translate into changes in fluorescence lifetime make it a powerful analysis tool in life sciences. These changes have been used, for example, in the design of fluorescence-lifetime-based biosensors$^3$.

It is possible to probe lifetime-based changes by measuring the photon arrival times$^4$. A straightforward way of determining arrival times is by measuring the difference between the time when a photon is detected and the time of the excitation laser pulse (Fig. 1a). The zero value of the measurement can be calibrated using the reflection signal from the excitation laser reaching each detector. Having access to the arrival times can reveal information complementary to the fluorescence intensity, which allows characterization of a specimen or a process of interest. As an example (Fig. 1b), lifetime-based information in images of an Arabidopsis thaliana root–hypocotyl junction expressing an actin fluorescent marker$^5$ and labeled with propidium iodide shows three distinct structures—actin network, cell wall and chloroplasts—that are difficult to identify when only the intensity information is available.

Lifetime-based measurements, carried out on a point-scanning microscope, require a pulsed-laser excitation source; fast, low-noise photon counting detectors; and electronics with appropriate time-resolution capabilities. This is the basic concept underlying the TauSense technology for fluorescence imaging: the use and handling of photon arrival times to generate lifetime-based information for

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different applications. The scan head electronics, supported by a field-programmable gate array (FPGA), detect photons and digitize them into a set of gates defined according to the arrival time information in an analogous way to the spectral distribution. This result can limit flexibility for probing additional structures or functions. Lifetime-based information can offer a way to tackle such dilemmas. We applied TauSeparation on the cells expressing LifeAct-GFP and stained with the green mitochondrial marker, previously used for TauScan. With TauSeparation, we obtained a well-defined signal from both the mitochondria and the actin filaments in separated intensity images. In TauScan, the decision on the values of the multicolor separations of the lifetime components is done in the online diagram. Then TauSeparation selects appropriate temporal windows and fits the lifetime-based information in these windows to separate the detected photons. In summary, we present TauSense and show typical applications that benefit from this technology. The TauSense tool set allows one to integrate lifetime-based information and adds freedom for the multiplexing of several probes in everyday fluorescence imaging. TauSense is one of the pillars of the STELLARIS microscopes.

REFERENCES


A different use of the arrival times consists of using them to assign photons to categories, defined according to certain rules and to generate different populations as a result. The classic example of this approach is gating. TauSense offers this possibility with the TauGating tool. TauGating (Fig. 2d) enables the positioning of multiple digital gates up to 101 with precise and flexible time definitions. TauGating delivers images that contain the number of photons intensely detected in one gate (or a set of gates, if several gates are pooled) during the pixel dwell time. A classic application for TauGating is the isolation of a signal of interest from intrinsic or spurious contributions to fluorescence in a specimen. As an example, we imaged zebrafish of the 4xGTIIC:d2GFP line, still containing their native pigments. The fluorescence signal of interest provides a readout of YAP: TAZ-activity and is used here to visualize the striated muscle of the trunk at 45 hpf (Fig. 2e). Using TauGating, we can extract the signal of interest from the contributions of the endogenous pigments. It is worth noting that we generated not only a gated image containing the relevant signal, but also an image with the ‘gated-out’ information to ensure the quality and accuracy of the result.

In addition to providing information based on AAT (TauContrast) and splitting signals into gates (TauGating), TauSense opens the door to a detailed characterization of a specimen in terms of the lifetime-based populations of the detected photons. Previous efforts to harness such information from fluorescence signals have described the use of so-called lifetime distributions to assess the potential lifetime components that constitute a given sample. To this end, we have developed two TauSense tools, TauScan and TauSeparation, to take full advantage of our ability to characterize the distribution of mean lifetime components (Fig. 2f). Using TauScan, it is possible to scan these distributions and separate them into a predetermined number of temporal windows. We can digitally preset gates followed by a multi-exponential components fit to generate an online view (Fig. 2g, top) of the distribution of such lifetime components from the arrival times. The distribution of components enables one to work with the arrival time information in an analogous way to the spectral distribution of a fluorescence signal. Here we obtained the temporal dispersion of the signal, and by positioning the appropriate temporal windows we can split the photon signal according to the arrival time information contained in the photon flux. After such splitting of the signal, the spatial coding of the photons leads to the corresponding images. The image thus obtained results from a TauScan experiment and intensity images containing discrete temporal information (the temporal windows as explained above) across the lifetime component distribution. An example of TauScan describes the lifetime-based distributions in live cells expressing LifeAct-GFP (BioIB GmbH) that were labeled with a green mitochondrial stain (Fig. 2g).

The second tool, TauSeparation, uses the lifetime-based distributions for a different application, namely species separation. Although spectral separation in fluorescence imaging has become routine and a powerful tool for distinguishing multiple fluorophores, there are still instances in which fluorophore choices are limited and the necessary spectral differences are difficult to achieve. This can happen, for example, in experiments with animal models in which GFP or similar fluorescent protein reporters are used to track specific mutations and ensure the correct phenotypes. In this the case, the spectral windows of such labels are unavailable for fluorophores with overlapping emission spectra. This result can limit flexibility for probing additional structures or functions. Lifetime-based information can offer a way to tackle such dilemmas. We applied TauSeparation on the cells expressing LifeAct-GFP and stained with the green mitochondrial marker, previously used for TauScan. With TauSeparation, we obtained a well-defined signal from both the mitochondria and the actin filaments in separated intensity images. In TauScan, the user decides on the values of the multicolor separations of the lifetime components with the aid of the online diagram. Then TauSeparation selects appropriate temporal windows and fits the lifetime-based information in these windows to separate the detected photons. In summary, we present TauScan and show typical applications that benefit from this technology. The TauSense tool set allows one to integrate lifetime-based information and adds freedom for the multiplexing of several probes in everyday fluorescence imaging. TauSense is one of the pillars of the STELLARIS microscopes.