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PrimeView
Fluorescence lifetime imaging microscopy

Fluorescence lifetime imaging microscopy (FLIM) creates spatial maps of fluorescence lifetimes on a pixel-by-pixel basis. FLIM can measure the fluorophore time decay to the ground state, is an intrinsic property of each fluorophore and is independent of its concentration.

Experimentation

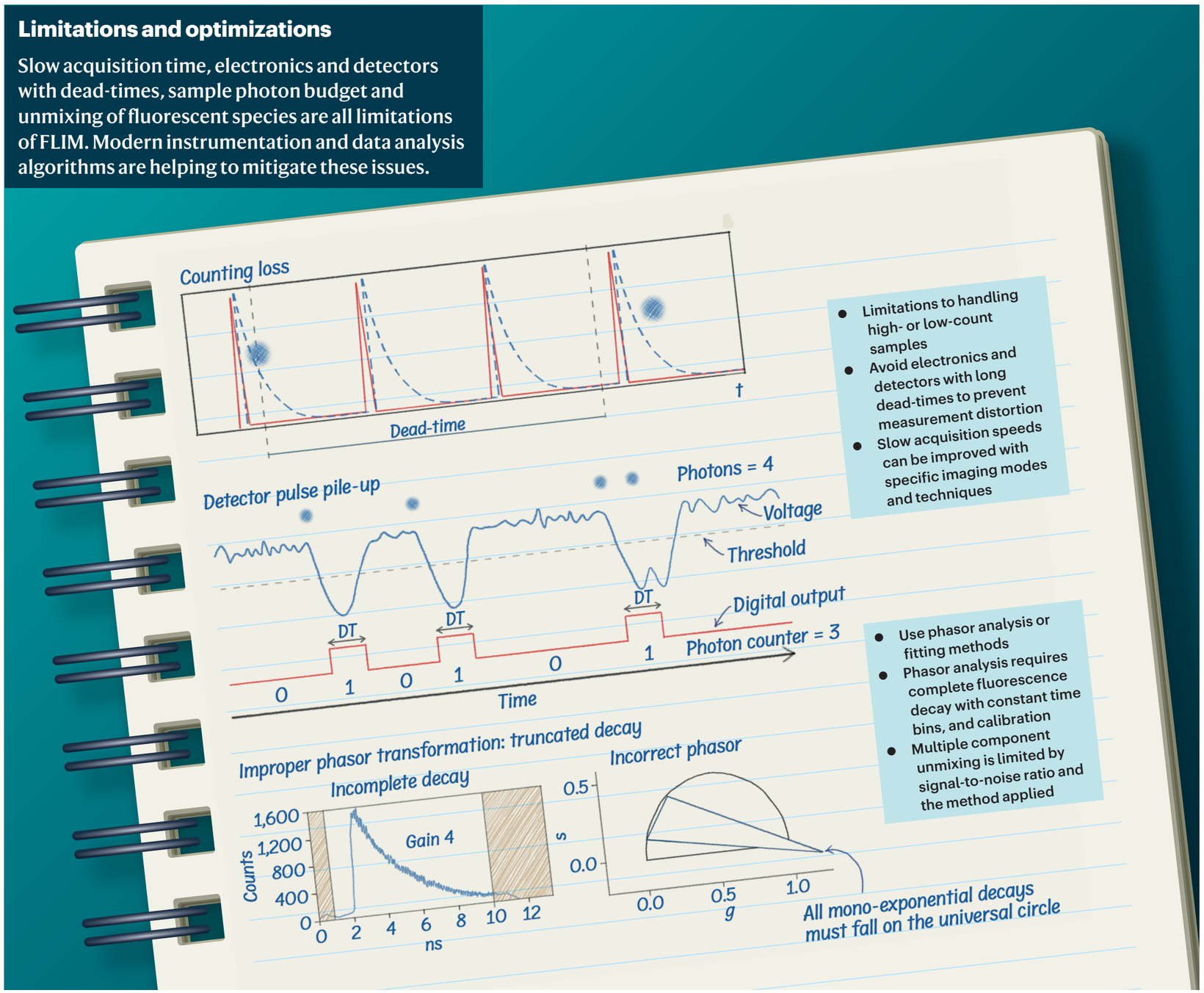
FLIM measures fluorescence lifetime within an image and can be performed using various microscope configurations, including laser scanning confocal, multiphoton, wide-field, light-sheet, stimulated emission depletion and fibre-optic probes. There are two primary approaches to measuring lifetime: time-tagging and phase-modulation shift methods. The phase-modulation shift method employs modulated light sources to collect photon arrivals within a period based on demodulation. Meanwhile, time-tagging methods, the most widely used, precisely measure photon arrival times relative to the laser pulse. FLIM data collection involves accumulating numerous excitation and emission cycles to reconstruct a fluorescence decay histogram for each pixel, enabling extraction of the lifetime values. Selecting the appropriate lasers, detectors and electronics is key for FLIM measurements. Various methods can then characterize the fluorescent molecular species present on a pixel-by-pixel basis. FLIM can be used for a range of samples, including cell cultures, organoids, tissues and animal models. Freshly dissected tissues or fresh frozen tissues are preferred to maintain integrity when measuring metabolism. Potential sources of unwanted fluorescence in samples include cell culture media, matrigel for 3D cultures and coatings on imaging dishes.

Results

FLIM data output consists of fluorescence decay histograms, which can be analysed using phasor plots to determine lifetime values. The phasor approach simplifies calculations by representing signals as complex numbers (phasors) and plotting them in 2D phasor space, enabling simple algebraic operations rather than complex fitting models. Validation steps include calibration with known standards to ensure accuracy and reproducibility.

Limitations and optimizations

Slow acquisition time, electronics and detectors with dead-times, sample photon budget and unmixing of fluorescent species are all limitations of FLIM. Modern instrumentation and data analysis algorithms are helping to mitigate these issues.



Applications

FLIM is widely used in metabolic imaging, particularly for studying nicotinamide adenine dinucleotide autofluorescence to assess cellular metabolism. It enables multiplexing and unmixing of multiple fluorophores, providing detailed insights into complex biological systems. FLIM is also employed in local environment sensing, such as measuring intracellular pH and ion concentrations, and in studying molecular interactions through Förster resonance energy transfer. Additionally, FLIM combined with super-resolution techniques like stimulated emission depletion microscopy enhances spatial resolution beyond the diffraction limit and can be utilized for multi-colour imaging.

Reproducibility and data deposition

Reproducibility in FLIM relies heavily on precise calibration, but the lack of a standardized calibration protocol hinders comparability across sessions and instruments. Reproducibility issues also arise from equipment stability, emphasizing the need for community-driven efforts to establish reporting standards and metadata formats. Repositories for code and data are limited, but initiatives like PhasorPy aim to provide open-source solutions for data analysis.

Outlook

Technological advancements in single photon avalanche diode arrays and machine-learning integration are poised to enhance FLIM's capabilities, enabling faster and more precise measurements. The development of novel fluorescent probes and the integration of FLIM with other imaging modalities will expand its applications in research and clinical settings. As FLIM becomes more accessible, it will have a crucial role in advancing our understanding of cellular dynamics and disease mechanisms.

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