SPLIT-SIM: AN INNOVATIVE APPROACH TO ANALYZE STRUCTURED ILLUMINATION MICROSCOPY (SIM) DATA.

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KEY WORDS: Structured Illumination Microscopy, SPLIT, SPLIT-SIM, Chromatin-DNA.

Structured Illumination Microscopy (SIM) is an important technique among Super-Resolution (SR) Microscopy methods [1]. When compared to other SR techniques, such as Stimulated Emission Depletion Microscopy (STED), SIM has the advantage of performing multicolor channel acquisition using conventional antibodies, live-cell imaging due to its low phototoxicity and performing optical sectioning.
By exploiting a variable pattern illumination, SIM directly encodes sub-diffraction information into the collected data, enabling the reconstruction of super-resolved images.
Here we employ a novel SIM data analysis tool, based on the concept of Separation of Photons by LIfetime Tuning (SPLIT) [2]. In STED-based SPLIT, the sub-diffraction spatial information is encoded into an additional channel of the STED microscope (e.g. lifetime, depletion power) [2][3]. We show that, in SIM, it is possible to use the SPLIT algorithm to analyze information encoded into the images acquired at different illumination patterns. The information contained within the additional channel is visualized and analyzed with a phasor plot, allowing separation of a fractional image component with higher spatial resolution, corresponding to the center of the Point Spread Function (PSF). A particularly important aspect of SPLIT is that the Phasor Plot provides a visual, intuitive and direct evaluation of the acquired data. Coupling SPLIT with SIM allows the formation of super-resolved images avoiding the conventional Fourier reconstruction.
As an application, we use SPLIT-SIM to study oncogene-induced alterations in chromatin high-order folding, because of their relevance in many cellular processes, such as DNA transcription and DNA replication [4]. We exploit SIM advantages to study the spatial distribution of replication and transcription foci in relation to the expression of an oncogene. The relative spatial distributions of nuclear foci, is quantified through object-based co-localization and a recently developed image cross-correlation spectroscopy (ICCS)-based algorithm [5].

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