WHEN HIGH CONTENT SCREENING MEETS SUPER-RESOLUTION MICROSCOPY

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Innovations in High Content Screening (HCS) analysis combined with advanced fluorescence microscopy techniques have enabled image-based screening assays, drastically improving experimental throughput and content richness. In parallel, sub-diffraction limit imaging techniques, commonly referred to as super-resolution microscopy, have revolutionized fluorescence microscopy and cell biology, allowing monitoring protein organization and dynamics with unprecedented spatial resolution. Single Molecule Localization Microscopy (SMLM) approaches such as PALM, *d*STORM or PAINT families, rely on the temporal accumulation of a large number of sparsely distributed single emitters, from which quantitative information such as single molecule localization and dynamics can be extracted. This time-consuming data generation step, in addition to the need for manual intervention to acquire multiple samples, heavily restricts the throughput of traditional SMLM experiments. Here we describe a fully automated quantitative single-molecule-based super-resolution methodology operating in 96 well-plates and using HCS-based analysis software. Our development, called HCS-SMLM, is a quantitative and automated workflow which streamlines the acquisition and data analysis by generating a database online during the super-resolution acquisition, computing localizations and quality parameters (i.e XY coordinates, intensity, sigma …) as well as tracking parameters (i.e trajectories, MSD, diffusion coefficients ...) from thousands of single molecules from living cells. An automated offline data generation step extends our database with single fluorophore photophysics (i.e Ton , Toff, duty cycle, number of blinks …) or structural organization of molecules inside cells (i.e cluster shape descriptors, number of detections per cluster …) using a tessellation approach directly on the single molecule localization data. To be able to effectively navigate through the huge volume of data generated (up to 300 million single molecule detections, 2 billion SMLM localization data and 850 million dynamics data), we have derived the use of Cell Profiler Analyst, a popular freely-available software distributed under an open-source license (data mining approach). We validated our approach with several case studies, such as modification of receptor localization and mobility in response to compounds in living cells (biological application) or the screening of buffer composition on fluorophore photophysics (chemical application).