Grazing Incidence Structured Illumination Microscopy (GI-SIM)

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Current cell biology research is desired to characterize the dynamic interactions between intracellular organelles at high spatiotemporal resolution with low photobleaching/phototoxicity effects, which therefore could continuously resolve the dynamic delicate structures and behaviors of the engaged organelles for the whole interaction process. Structured illumination microscopy (SIM) stands out in the context of super-resolution live imaging, since many fewer raw images and much lower light levels are required. However, high-speed SIM imaging only achieved 11 Hz frame rate at 100 nm resolution under TIRF illumination mode [1, 2], which is still not only too slow, but also too shallow of the penetration depth of evanescent wave to effectively image the ultra-dynamics in live cells. Here, we craft the grazing incidence (GI) illumination mode, which improves ~10 fold deeper penetration depth and ~10 fold higher signal strength than TIRF mode (Figure 1). By integrating GI with SIM, GI-SIM practically achieves the imaging performance of 95 nm resolution, 90 Hz frame rate, hundreds to thousands of time points, and multi-color imaging, which allows us to investigate spatiotemporal coordination among organelles, such as the dependence of ER reshaping dynamics on ER-microtubule contacts (Figure 2), and lysosome translocation with respect to ER-lysosome contacts. Finally, we are pushing GI-SIM to higher resolution level by using of our previously developed patterned activation nonlinear SIM (PA NL-SIM) method [2].

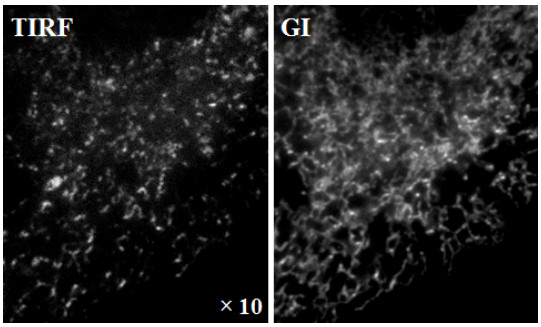

Figure 1. The comparison of imaging depth and signal strength between TIRF and GI mode. The intensity of TIRF image is computationally amplified by 10-fold.

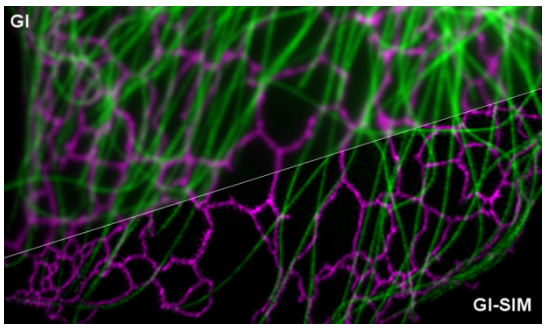

Figure 2. Representative GI-SIM live cell image of ER network reshaping dynamics depending on microtubule cytoskeleton.

[1] P. Kner, *et al.*, "Super-resolution video microscopy of live cells by structured illumination", Nat. Meth. **6**, 339-342 (2009).

[2] D. Li, *et al.*, "Extended-resolution structured illumination imaging of endocytic and cytoskeletal dynamics", Science. **349**, aab3500 (2015).