Structured Illumination Microscopy (SIM) is a powerful technique that enabled resolving biological structures never seen before with a light microscope [1]. In principle it is ideally suited for live-cell imaging, as it requires no special fluorophores or high light intensities to achieve twice diffraction-limited resolution in three dimensions. As it is a wide-field technique, SIM is very light efficient and enables rapid acquisition of large volumes. So far, however, fast implementations of SIM suitable for live cell imaging were restricted to one excitation wavelength. This is unfortunate, as multi-color labeling is one of the main advantages of fluorescence microscopy.

Here, we present rapid three-dimensional imaging of whole cells in two colors with SIM. Using a scientific CMOS camera and fast liquid crystal devices, we achieve volume rates as high as 4s and a lateral resolution of 110nm. We present time lapse movies of mitochondria, clathrin-coated vesicles, and the actin cytoskeleton in HeLa cells and in cultured neurons over tens of time points.

The actin cytoskeleton in a Hela cell as imaged by widefield (left) and 3D SIM (right).
Scale bar 2 microns.