Structured illumination microscopy (SIM) has grown into a family of methods which achieve optical sectioning, resolution beyond the diffraction limit, or a combination of both these effects in optical fluorescence microscopy. SIM techniques rely on illumination of a sample with patterns of light which must be shifted between each acquired image. The patterns are typically created with physical gratings or masks, and the final optically sectioned or high resolution image is obtained computationally after data acquisition. Here we used a high speed ferroelectric liquid crystal microdisplay together with incoherent LED illumination to generate the illumination patterns and a sCMOS camera for widefield image acquisition. The high precision and flexibility of the generated patterns allowed us to use advanced processing techniques relying on the precise knowledge of the display-camera mapping, such as scaled subtraction in the case of optical sectioning SIM [1] and precise determination of spectral parameters (modulation period, direction and phase) in the case of super-resolution SIM. The freedom in choosing the illumination patterns also allows to tune the spatial frequencies and orientations of the patterns. Here we demonstrate the use of multi-frequency one-dimensional patterns to achieve both increased lateral resolution and high contrast optical sectioning with incoherent illumination and two-dimensional data processing in the Fourier domain (see inset in Fig. 1 C). We have also evaluated the impact of incoherent illumination on the SNR (signal to noise ratio) of the recovered high-frequency image components [2].


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Fig. 1: Fixed HT-1080 cells labeled with EdU-Alexa647 (fluorescently tagged nucleotide that incorporates into newly replicated DNA, red) and fluorouridine (synthetic nucleotide which is incorporated into active transcription sites, green), maximum projections of 20 sections, 0.1 µm step. A – widefield, B – optical sectioning SIM, C – super-resolution SIM.