Image restoration of TIRF-SIM

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Combining total internal interference (TIRF) microscopy with structured illumination microscopy (SIM) provides several benefits. TIRF excites only a thin layer of the sample near the glass/water interface, so there is no out of focus light and photobleaching and photodamage to the rest of the sample are minimized. This has been done previously in [1] and [2]. In TIRF-SIM, the maximum spatial frequency of the SIM excitation patterns is set by the excitation wavelength and the index of refraction of the coverslip. By using higher index materials, greater resolution can be obtained. [3] uses special sapphire 1.7 NA lenses with through lens TIRF-SIM to obtain 84 nm resolution in live samples.

We present an image restoration (deconvolution) method for TIRF-SIM This image restoration method is tested with simulated SIM images. In [4] and in the present work we use a finite data, continuum object model. The camera used has only a finite number of pixels but the cell imaged is a continuous object; cameras have pixels, cells do not. The algorithm we will present conceptually produces a dye distribution that is a function on a continuum of 2-dimensional space. We assume the patterns are formed using prism launched total internal reflection with interfering beams from opposite sides of the prism forming the illumination patterns. In our simulations we use 60nm camera pixels and calculate the restored images on a 4nm grid.

We apply our image restoration method to simulated images. With a sapphire prism (index=1.76) and simulated images of two point sources with no noise, we resolve two point sources separated by **22nm**. With more realistic noise levels, we are able to resolve two point sources separated by **44nm** (bright sample) to **52nm** (dim sample). With a glass prism (index=1.52), we resolve two points separated by **52nm** (bright sample) to **64nm** (dim sample). To achieve the same 52nm resolution with the glass prism required images 10 times brighter than with sapphire.

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