Analyzing speckle contrast for HiLo microscopy optimization

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A thorough comprehension of the pathophysiology of disease is based on understanding the molecular mechanisms that regulate cell fate, and in this context, microscopy technology has been one of the most essential tools for biomedical studies during the last centuries. Together with genetic engineering, the applications of laser technology in microscopy became a fundamental part of the ongoing revolution in biotechnology towards finding cures to the most devastating diseases. The constant need of observing rapid intracellular processes demand higher resolution, more sensitive and faster imaging modalities. Hilo is a recently developed microscopy that provides high 3D resolution and is specially suited for fast in-vivo imaging. Briefly, the technique uses a speckle pattern to illuminate the specimen and a set of image processing operations. In this work, we present a detailed analysis and a set of optimization steps to improve this microscopy. We report how the random nature of the speckle illumination produces an underlying roughness that reduces the quality of the image, and explain that when thick samples are considered the out of focus background induces fluctuations in the images. For both cases, we provide a method to minimize these effects and use our results as a guide for implementation and optimization of this powerful technique.