Cell motility plays a critical role in numerous physiological processes such as embryonic development and tissue morphogenesis as well as in pathological processes such as wound healing, tumor invasion and inflammation. Migration of the cell involves remodeling of the actin cytoskeleton and formation/disassembly of focal adhesion sites. Variety of microscopy techniques, including wide field, TIRF, FRET etc. is used to analyze spatio-temporal dynamics of focal adhesions. Interference reflection microscopy was previously used to investigate focal adhesion sites as well as endo-and exocytosis [1]. Compared to other microscopy techniques, interference reflection microscopy enables visualization of focal adhesion sites without overexpression of fluorescent proteins, which might alter focal adhesion dynamics. However the interference patterns of the reflecting membranes sometimes are hard to interpret due to disturbance of changing reflective index of the membrane.

Several attempts have been made to increase the contrast in interference reflection microscopy including use of the crossed polarizer in conjunction with a quarter wave plate an annular aperture [1], which helps to get rid of reflections from the central part of the objective lens and the slide. Here we present an easy method to limit misleading interference patterns and enhance the contrast of the adhesion sites using modifications of the existing setup and illuminating the sample in oblique fashion. Compared to the total reflection where a new wave front is formed [2], presented technique decreases interfering beams and clearly improves established approach of using interference reflection microscopy in total reflection mode.

Figure 1: Visualization of focal adhesions in NIH3T3 fibroblasts using “optimized” oblique reflection microscopy (ORM)” A. Fluorescent image of the cell expressing mCherry-Paxillin to localize focal adhesions. B. Interference reflection microscopy. C. ORM picture. One can see a clear improvement of the contrast and a great overlap of dark spots with those of paxillin positive spots. Please note dark spots seem to be slightly larger than paxillin positive spots.

References: