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Total Internal Reflection Digital Holographic Microscope

Cell-substrate interactions including attachment, spreading, morphology changes, and migration require a complex series of events to occur in a regulated and integrated manner. Cell migration, for example, plays an important role in numerous physiological and pathological processes, such as morphogenesis, wound healing, and tumor metastasis. To date, the primary tools for imaging and studying these processes have been total internal reflection fluorescence microscopy (TIRFM) and interference reflection microscopy (IRM). TIRFM is used for imaging protein dynamics in calcium channels, neurotransmitters, focal adhesion, and other cellular membrane processes. However, information on the morphology of the cellular membrane surface is largely absent in TIRFM. The IRM technique allows estimation of interface thickness profile, however, noise reflected from the cell body beneath interferes and allows only a qualitative interpretation of the surface profile.

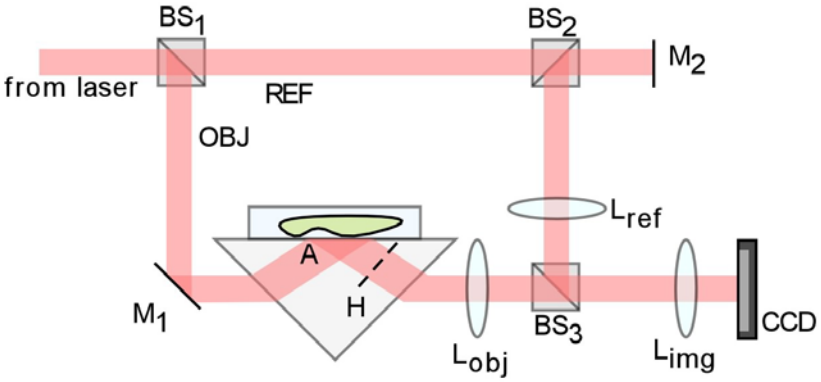
Given these limitations, it is readily apparent that a technique to generate accurate, quantitative surface profile images of live cellular membranes is needed and will greatly help us better understand the important process of cellular motion.

Total Internal Reflection Holographic Microscopy (TIRHM) is a technique developed recently at the University of South Florida which is capable of overcoming these limitations. By applying techniques of digital holography to TIR, researchers are able to generate quantitatively precise images of the cell-substrate interface.

Advantages:

- Image cell-substrate interface with nanometer precision
- High signal to noise ratio allows precise quantitative measurement of surface features
- Observe ligand-receptor interaction for purposes of drug discovery

Precision imaging of cell surface morphology



TIRHM apparatus. BS's: beam splitters; M's: mirrors; L's: lenses; A: object plane; H: hologram plane. Inset: Simplified, equivalent optical path between object plane and ccd plane.

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