# Quantitative DIC Microscopy: Improving Versatility for Live-Cell Imaging

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Our ongoing investigations of DIC microscopy have shown it is possible to *measure* phase variations by using calibration standards and correlating them to actual thickness or refractive index variations within an object. In biological applications, this can provide an alternative modality to fluorescence for imaging and tracking live-cell dynamics, with the advantage that lower intensity illumination can be used and the need for introducing possibly toxic fluorophore dyes can be eliminated.

In this presentation, we will first briefly review our progress to date, including our use of phase-shifting techniques to produce accurate (quantitative) images of the object phase gradients while, at the same time, removing any absorption (amplitude) information which can corrupt the results. We will then describe our progress in calibrating the phase-shifting DIC system in order to convert image intensity into a measure of optical path length (phase) through cell components, as well as present a comparison of algorithms used to reconstruct the phase from images acquired from multiple angles of shear.

Finally, we will describe new techniques we are investigating to improve the speed of image acquisition, including using wavefront coding approaches for increasing the depth of field of the high numerical aperture DIC microscope objectives. Possible ways to combine quantitative DIC imaging with fluorescence microscopy to produce even greater versatility for biologists will be discussed.

## References

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