Height Measurement of Cells Using DIC Microscopy

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Development of fluorescent probes and proteins increased the use of light microscopy notably by enabling the visualization of subcellular components, location and dynamics of biomolecules. However, it is not always feasible to label the cells as it may be phototoxic or perturb the function. On the contrary, label-free microscopy techniques enable the work with live cells without perturbation and the evaluation of morphological differences which in turn can offer useful information for the high-throughput assays. In this work we use one of the most popular label-free techniques, Differential Interference Contrast (DIC) microscopy to estimate the height of cells.

DIC images show detailed information about the optical path length (OPL) differences in the sample. DIC images are visually similar to a gradient image. The mathematical description of the nonlinear image formation model is a recent research topic [1]. In their earlier work [2], the authors proposed a DIC reconstruction algorithm to retrieve an image where the values are proportional to the OPL (or implicitly the phase) of the sample. Although the reconstructed images are capable of describing cellular morphology and to a certain extent turn DIC to a quantitative technique, the actual OPL has to be computed from the input DIC image and the microscope calibration properties. The retrieved phase has already been compared to synthetic data or objects with known properties [3]. Here we propose a computational method to measure the approximate height of cells after microscope calibration. The method starts with calibrating the microscope by setting the Koehler illumination and ensuring that the output image utilizes the full dynamic range of the camera. Then the DIC image of a calibration object with known dimensions and refractive index has to be reconstructed. This allows the mapping of a unit change of image intensity to OPL change assuming a linear formation model. The calculated ratio can be used to determine the height of further samples where the refractive index of the surrounding medium is known. The described method converts DIC to a quantitative technique. The method's precision is tested on a special calibration sample.

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References

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