

# Holographic Technique Aids Live-Cell Imaging

Digital holographic microscopy generates a three-dimensional image of a sample by measuring the phase difference between light transmitted from the sample and light from a reference beam. However, an image captured with this method conjoins information on both the size and shape of a cell and on its refractive index, which is related to the cell's contents.

Now scientists from École Polytechnique Fédérale de Lausanne, Centre Hospitalier Universitaire Vaudois and Lyncée Tec SA, all in Lausanne, Switzerland, have developed an analytical technique to decouple the two types of information, providing separate measurements of changes in the morphology and the

refractive index of living cells placed under stress.

In a study of hypertonic neuronal cells in mice, the investigators put the cells into a standard perfusion medium and acquired images using a digital holographic microscope made by Lyncée Tec. The system uses a 658-nm laser diode, from which the beam is split into two paths: one for reference and one to illuminate the cells.

The light transmitted through the cells is gathered by a 63 $\times$ , NA = 0.8 objective lens that forms an object wave that interferes with the reference beam. The resulting hologram is captured by a CCD camera from Basler Vision Technologies of Ahrensburg, Germany.

The scientists then perfused the cells with a solution of equal molarity but with a higher refractive index and acquired a second set of holographic images. Analysis of the images before and after reperfusion enabled them to derive the mean refractive indices of the cells to an accuracy of 0.0003. Spatial averaging enabled them to account for cellular movements that occurred during the reperfusion process.

They said that separating data regarding the changes in refractive index that occur along with changes in the cellular content will be helpful in interpreting the light signals from highly scattering biological tissues. □

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