Abstract

"Label-free" quantitative measurements of optical volume changes in beating rat cardiac myocytes are presented before and during treatment with IPHC (isoproterenol hydrochloride). This newly developed microscope provides instantaneous measurements of dynamic motions of live cells via a pixelated wire grid polarizer mask in front of the camera sensor. Videos of cell optical thickness topography were generated from quantitative phase data and processed to obtain relative optical volume. Live cells were prepared and grown on #1 coverslips or coated slides. Cells were placed into a Bioptechs FCS3 perfusion chamber and kept at 37°C. These results show dramatically increased rate and strength of contractions with application of IPHC.

Real-Time Quantitative Optical Volume Measurement of Dynamic Cellular Motion

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Applications

- Morphological Studies
- Mechanistic Studies
- Tissue Dynamics
- Quantify & Track Cellular Motion
- Process Monitoring
- Quantify Cellular Changes with Treatment

Enabling Technology

Pixelated Phase Mask

Wire grid polarizer array bonded to detector array pixels

SEM of mask

Brightfield = A + H + C + D

Phase = A - φ - B - 2π / φ - 2π

Interference = A

Optical Thickness = Phase / (2π)

Like a color camera that sees in phase (or polarization), phase shifts are obtained to simultaneously determine brightfield, phase contrast, dark field and DIC images. Fast data acquisition using short exposure times with a pixelated phase mask enables measurement of moving samples without blurring or scanning.

Optical Layout

Microscope schematic for epi-illumination with a Linnik interference objective. Transparent samples in liquid are imaged under a coverslip on a reflective surface. This system measures relative integrated optical thickness (OT) or optical path difference (OPD). OT is related to both physical thickness and index of refraction variations.

Comparison of Optical Volumes

Optical volumes of 2 areas of above culture sampled at 15 fps. Notice variation in strength of contraction in different areas of the culture (upper right). (lower left) Histogram of optical thickness values.

Relative optical volume over time series of 200 datasets. The same area of cells (above) is compared before and after pushing IPHC. Note changes in both strength of contractions and speed of contractions. These are indicative of changes in the force of the contractions.

Microscope Specifications

- Objectives: 20X (NA 0.45) & 50X (NA 0.8)
- 1X or 2.25X FOV lenses
- Source wavelength: 511 or 660 nm
- Fast data acquisition – no scanning
- Real-time processing – 15 fps
- Vibration insensitive