MEASUREMENT OF TRANSIENT CHEMICAL CONCENTRATION IN INDIVIDUAL CELLS USING OPTICAL LOW-COHERENCE REFLECTOMETRY

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BACKGROUND AND OBJECTIVE

The subcellular structure of biological cells effects spatial variation of local index of refraction. Differential interference contrast (DIC) microscopy provides a qualitative measure of thin specimen phase retardation and refractive index. A novel imaging device, a differential phase optical low-coherence reflectometer (OLCR), produces a quantitative spatiotemporal refractive index map of specimens with phase resolution of 5nm [1]. One application of this device is measurement of transient chemical concentration across cell membranes.

MATERIAL AND METHODS

Unlike a conventional single-channel Michelson interferometer that is corrupted with environmental phase noise, the dual-channel OLCR measures the phase difference between two channels. Two orthogonal polarization modes of light are spatially separated using Wollaston prisms. One beam acts as a reference while the other beam passes through the cellular sample and surrounding medium. A priori knowledge of the physical thickness of the sample chamber and initial index of refraction of the surrounding medium permits spatiotemporal calculation of the axial composite index of refraction of the cell. This device produces a quantitative refractive index map with phase resolution of 5nm through the specimen and lateral resolution of 5?m. All measurements are performed across the full thickness of the specimen, and thus there is no depth resolution. Chemical concentration changes are assessed through changes in the intrinsic refractive index.

RESULTS

Preliminary studies using the differential phase OLCR indicate normal human epithelial cell composite refractive index (source wavelength=1.31?m) is on average 1.334 through the cytoplasm and 1.335 through the center of the nucleus when the index of the surrounding water is 1.333. After subjecting the cell to anhydrous glycerol (n=1.47), the cell rapidly dehydrated and the index of the cell constituents more closely matched the environment. Observation of transient chemical transport has been observed but not quantified at this time.

CONCLUSION

Quantitative subcellular variation in refractive index measured with OLCR corresponds with images recorded with differential interference contrast (DIC) microscopy. Differential phase OLCR has the capacity to make quantitative measurements of spatiotemporal subcellular refractive index variation within and surrounding individual epithelial cells. This information can be used to assess chemical concentration gradients.

Calculation of cell membrane transport parameters, such as the water and solute permeability, are often performed by fitting measurable experimental data (such as cell size) to theoretical models [2]. One source of error in the estimation of these parameters is due to the difference between the actual and assumed extracellular concentration at the start of the experiment. A common assumption for the initial condition of the model is that the cell experiences a step change in environmental concentration. Accurate measurement of the spatiotemporal concentration within and surrounding the cell will improve estimation of the cell membrane transport parameters. The validity in the assumption of a concentration step change can be evaluated for different circumstances such as choice of agent and sample geometry.

Other potential uses for the OLCR include the study of subcellular dynamics in living biological tissue where detection of motion with amplitude less than an optical wavelength is necessary [3]. Also, besides concentration, changes in many other physical variables, such as density, temperature, and stress may be assessed through changes in the intrinsic refractive index. The OLCR can be used to investigate and measure changes in these properties as well.

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