Phase resolved retardation measurements of isolated cardiomyocytes

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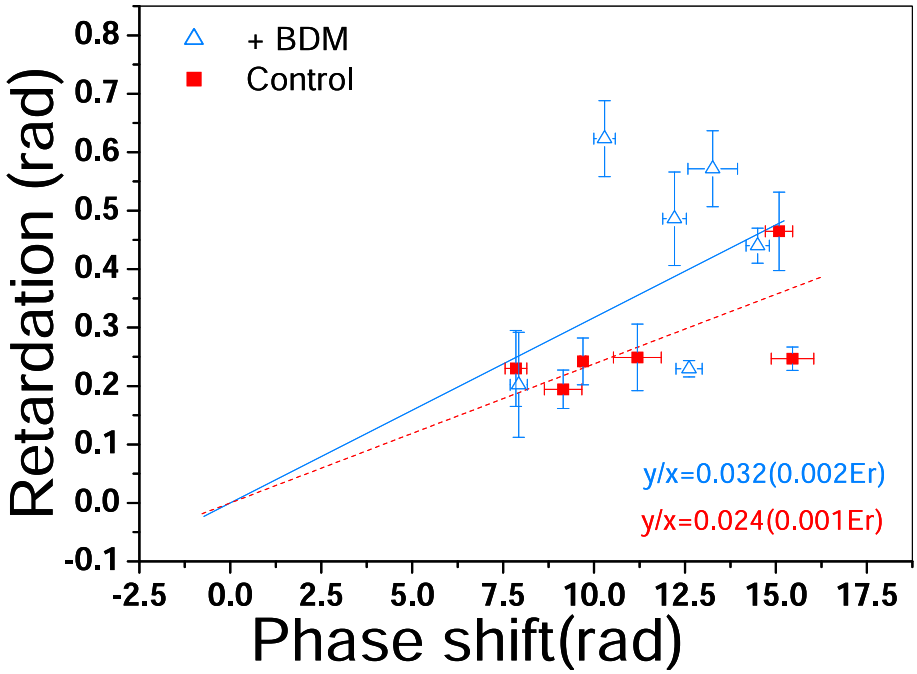
Quantification of the physical and optical properties of the fine structural information of transparent or translucent unstained anisotropic viable cellular specimens is challenging. The isolated cardiomyocyte is a particularly good example of such an anisotropic specimen, where the optical properties are different according to direction of measurement. Phase and retardation measurement are two optical quantities that provide information regarding structural properties. The phase contains information about cell thickness and refractive index. The retardation contains information about the cell thickness and the birefringence, which is a result of more than one index of refraction. Birefringence is highly dependent on biological, physiological and environmental conditions and is responsible of the orderly local arrangement of the thick (myosin) and thin (actin) filaments in the myofibrils of the cardiomyocyte. Phase measurement, performed using Quantitative Phase Microscopy (QPM), was used in conjunction with retardation measurement, performed using Brace-Köhler (BK) compensator.

Freshly isolated ventricular cardiac myocytes were obtained from male Sprague-Dawley rats by a standard enzymatic digestion procedure. Myocytes were suspended in two different buffer solutions (pH 7.4): one containing 2,3-butanedione monoxime (BDM), and the other containing 1mM Ca^{2+}.

Cells of appropriate dimensions (~28 × 125 µm) and shape exhibiting clear cross striations were analysed within 1.5hrs post isolation. Phase images were computed from a set of through-focus bright field images obtained using an *Olympus BX51* polarised microscope (40×/0.70 P, ∞/0.17 Uplan FL objective). Phase calculations were performed using QPm software (QPm^{Tm} V2.1 IATIA, Ltd.). Retardation was determined using BK method by observing the cells oriented at 45 ± 1° under linearly crossed polarisers mode while quantifying the azimuth orientation of a specialized compensator wave-plate of known retardation.



A positive correlation was observed between the retardation and the phase of the cardiomyocytes as illustrated by the linear regression curves fitted for cells placed in BDM (solid line) and control (dash line) buffers (see Figure). Moreover, cells placed in BDM 'relaxing' solution exhibited higher retardation magnitude than cells in control, 1mM Ca^{2+}, solution, despite the lower phase values. Thus myocyte birefringence as evaluated by retardation is affected by a pharmacologic treatment which alters the interaction between cytoskeletal elements. By contrast, phase is less affected by this treatment. For cells in either buffer, the retardation magnitude increased proportionally with increasing the thickness (i.e. phase) as expected.