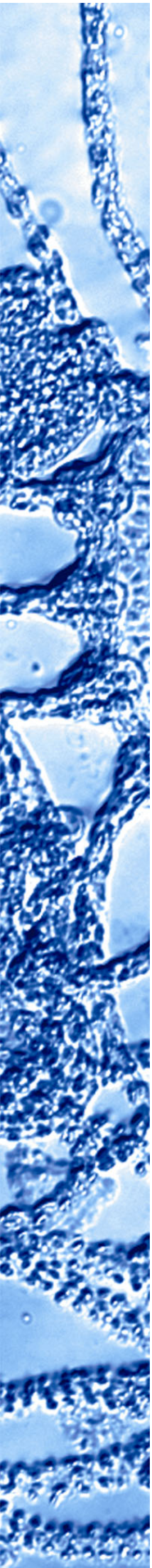

Identification of striations within cardiac myocytes using QPm

Introduction

When viewing a specimen microscopically, structural information can be gleaned from observing how the illuminating light is changed both in amplitude and in phase. Cellular material is frequently quite transparent, lacking the more visible amplitude modulating components but dense with subtle phase components. Although optical phase microscopy can be very useful, as it highlights changes in refractive index and thickness of the cell has changed, it involves expensive specialist optics. This study has demonstrated a novel way of visualising cells and cellular structures without the expensive optical components by utilizing Quantitative Phase Microscopy (QPm).

Method

Enzymatically isolated cardiac myocytes were mounted unstained, on a poly (D-lysine)-coated glass microscope slide for examination. The bright field images were collected using a Zeiss inverted Axiovert 100 M microscope fitted with a Zeiss LD Acroplan x40 0.6 NA air objective. Images were digitally acquired using a 12 bit black and white 1300 x 1030 resolution Roper Photometrics CoolSnap FX CCD camera. Images were enhanced with colour palettes and the colour scale was arbitrary. Optical images were obtained using DIC and standard phase contrast optics with Objectives of LD Achroplan 40x/0.6 NA with fixed polarizer and analyser, DIC prism and slider inserted; LD Achroplan 40x phase.

Quantitative Phase Microscopy (QPm)

The process of constructing a quantitative phase image entails taking a series of three bright field images, consisting of an in-focus bright field image, and a pair of slightly (still within the depth of field) defocused images either side of this. Based on these three brightfield images, Iatia's QPm program calculated and generated a phase map of the specimen. This map can be further processed to generate analogue digital images to emulate other phase contrast modalities .

Results & Discussion

The myocytes are roughly 'rod-shaped' with irregular notches. In brightfield the alignment of repeating sarcomeric structures of contractile myofilaments are clearly seen as periodic striations (Figure A) and constitute the dominant visual component. As our studies show, these cardiac myocytes are of relative uniform refractive index; hence the areas of phase differential may be interpreted quantitatively as variation in cell 'thickness' or depth in the 'z' plane. Thus, the striated appearance reflects an amplitude component of the image that can be dramatically eliminated by constructing the phase map. In other situations where the cell dimensions can be established independently, a phase map can be used to quantitatively assess subcellular variation in refractive index.

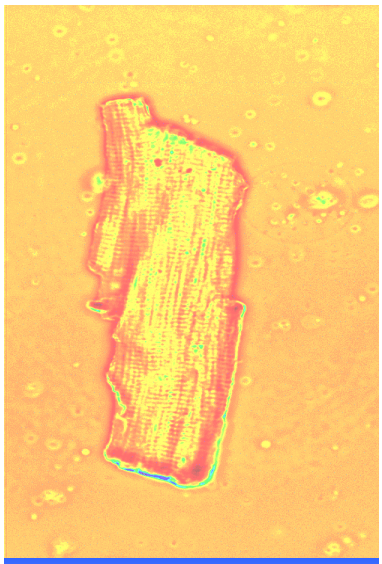

Figure A: Bright field

The periodic cell striations are suppressed and the cell depth contours become evident

With both the phase and amplitude data in hand, it is possible to emulate other contrast image modes using QPm. Comparisons of DIC (Figure B and Figure C) and Zernike phase images (Figure D and Figure E) were generated using conventional methods, and compared with the QPm digitally obtained images. The computed phase image is particularly well rendered for this cell type. Compared with the optical phase image, the 'halo' edge effect is suppressed and the striation pattern more crisp. Similarly the computed DIC image accentuated the surface contour appearance and provides an authentic representation of 'true' optical DIC. In this mode the cell topography is emphasized, and a hint of the three-dimensional aspect emerges- the signature of DIC microscopy.

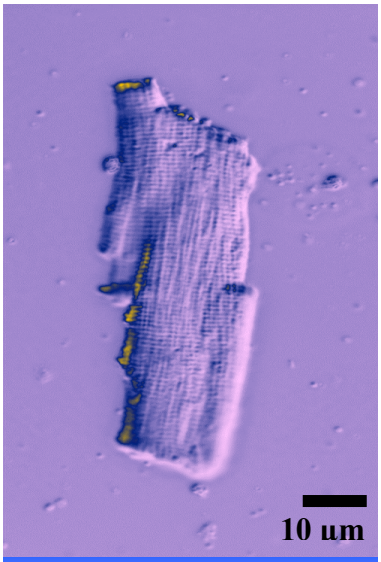

Figure B: Optical DIC

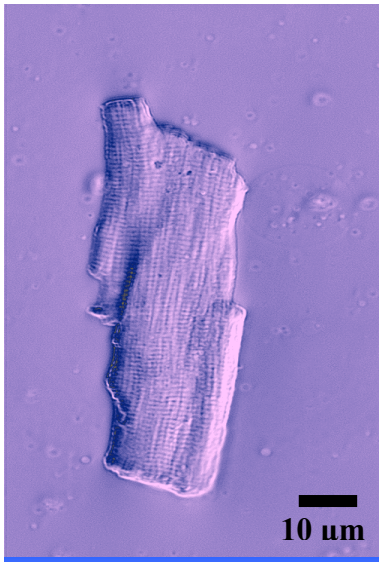

Figure C: QPm DIC

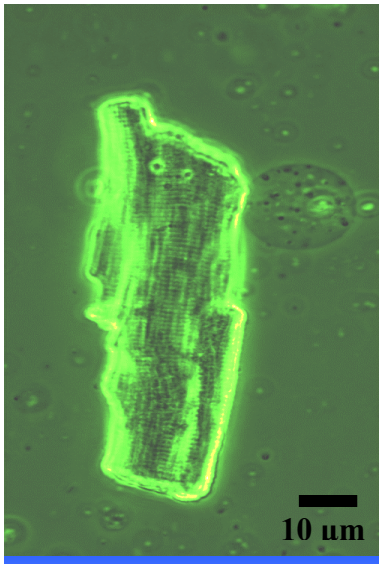

Figure D: Optical Zernike Phase

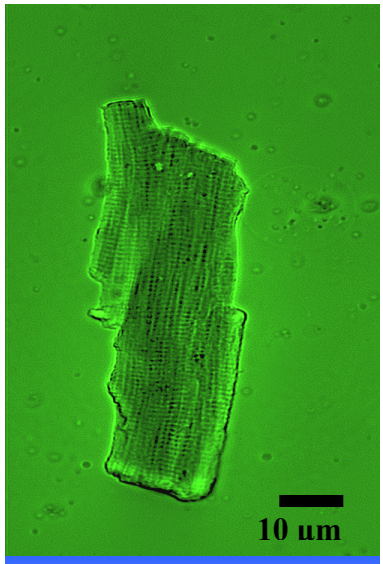

Figure E: QPm Zernike Phase

Conclusion

The development of a convenient and quantitative method of phase microscopy opens up new possibilities for the investigation of cellular structure. QPm provides a cost effective solution for bioscientists, since a standard bright field microscope with basic optical components can be used to obtain quantitative phase images. QPm also provides microscopists with a novel view on cellular architecture that may be readily incorporated into their research.

Reference

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**The work present here was carried out by Lea MD Delbridge, Akram A Kabbara at Department of Physiology; Catherine Bellair and Keith A Nugent at Department of Physics, Melbourne University; Brendan E Allman and Labrini Nassis at Imaging Division, IATIA Limited, Box Hill, Australia. Associated publication "Quantitative Phase Imaging-a new way to 'see' cells" Today's LifeScience January 2002 Issue.*