Meniscus correction on Livecyte to enable quantitative phase imaging (QPI) in multi-well plates

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- Livecyte utilises an adaptive algorithm to correct for the dynamic evolution of the fluid meniscus
- Quantitative phase imaging (QPI) is demonstrated in high density multi-well plates

Introduction

The meniscus of the media in high density well-plates causes deformation of the transmitted light field in optical microscopes. This can result in a drastic reduction in image quality for both conventional modalities (phase contrast, bright field etc.) and novel emerging modalities (quantitative phase imaging (QPI)). Corrective measures must adapt to changes in the surface tension, varying media volumes and dynamic evolution of the meniscus due to evaporation.

Livecyte contains an adaptive algorithm that completely removes aberrations associated with a fluid meniscus, regardless of changes in the form or strength of the meniscus. The algorithm actively senses the deformation induced in the transmitted light and as such does not require calibration nor is it sensitive to changes over time.

Methods

Size standard PMMA (plastic) micro-spheres, with diameter 19.3 ±0.3 μm, are used as a test sample. When mounted in refractive index matched liquids, they are a robust approximation to live cells undergoing mitosis. Furthermore, image quality can easily be assessed since the samples are fixed and have known physical properties. The micro-spheres are mounted on the base of 6, 12, 24 and 96 well-plates and imaged over time. Multiple combinations of media volume, humidity and surface tension are explored.

Results

This note presents an example of typical image analyses that are used to validate the performance of the meniscus correction algorithm. Images are presented, and the measured physical properties of the micro-spheres are compared to the known parameters.

Fig. 1 displays an example image of micro-spheres acquired in a 96 well-plate.

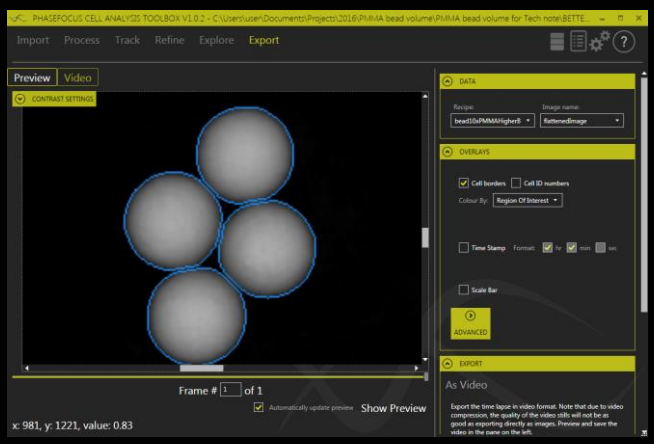

Fig.1: Example segmented phase image of micro-spheres showing high contrast imaging from Livecyte CAT software.

Fig. 2 shows an equivalent QPI image of the micro-beads in a 96 well-plate (1.3 mm by 1.3 mm unstitched field of view). The images are processed in the Livecyte CAT software suite, where the physical properties are calculated and compared to the known values.

Fig.2: QPI image of micro-spheres across 1.7 mm² field of view

Fig. 3 displays a statistical comparison between the measured and known physical properties of the micro-sphere population shown in Fig. 2. Error bars indicate 2σ. All calculated properties match the known parameters, and

the statistical variation of the population of spheres agrees with the manufacturers specification.

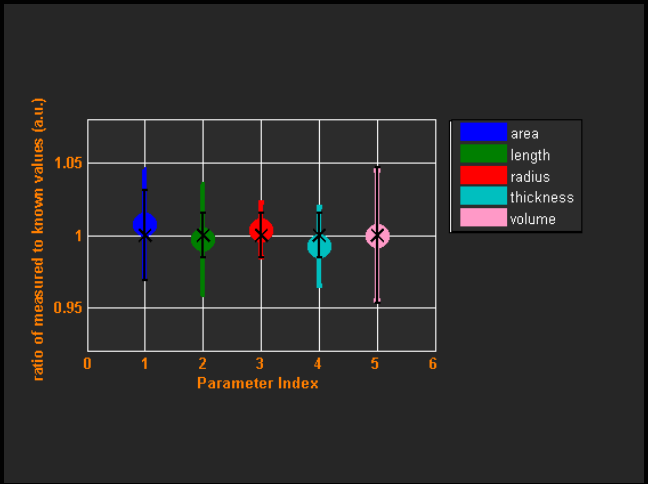

Fig.3: Example compassion between measured and known properties. Manufacturers measurement +-1 standard deviation (overlaid in black).

A further set of experiments monitor the properties of the spheres over time under a variety of conditions. Initially, the well was empty of any media. After 2 hours of imaging, 150µl of media was added to the well. Following this, images were acquired every 30 mins for 24 hours. The lid of the 96 well-plate was then removed to accelerate evaporation. The entire media evaporated over the following 24-hour period. Thus, the meniscus correction algorithm is tested for media volumes of 0-150µl over time. Furthermore, images were acquired of multiple different areas within a 2.3mm diameter square, concentric with the centre of the well.

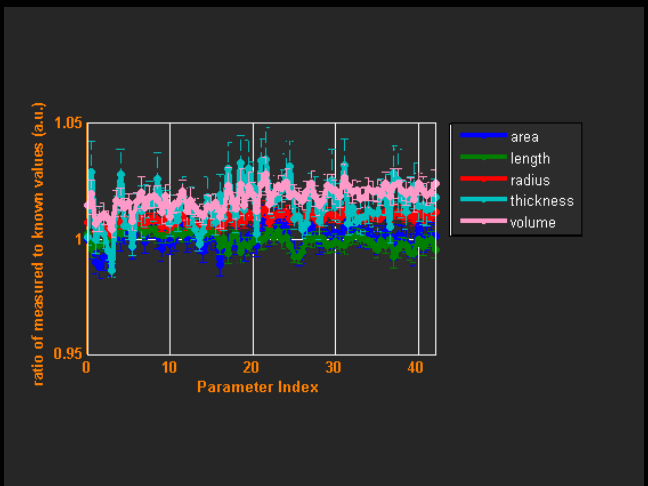

Fig. 4: Example compassion between measured and known properties over time-lapse series

Fig. 4 displays an example of how the physical properties of the micro-spheres are static over the course of this time lapse series and as such unaffected by the evolution of the meniscus properties.

Fig. 5 illustrates an example of a large FOV unstitched QPI image of live cells collected from a 96 well-plate.

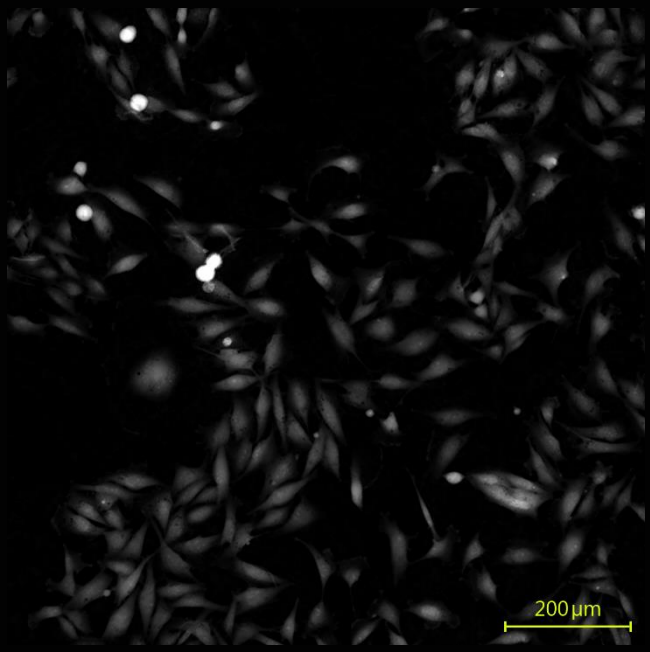

Fig. 5: Example QPI image of live cells in a 96 well plate

Conclusions

The results demonstrate that the adaptive algorithm utilised in the Livecyte is able to correct for the dynamic evolution of the fluid meniscus in experimental conditions across a range of multi well plates. The accuracy and sensitivity of pixel values in the phase images remains unchanged compared to equivalent *dry* measurements. Furthermore, the measured properties of the beads such as area, length, thickness, volume and width, precisely match the known values.

The highly accurate and sensitive nature of the QPI imaging technique allows for subtle changes in phenotypic behaviour to be monitored over time and compared across many wells. This ensures that the highly-refined nature of data that the Livecyte system offers, can be extended across multi-well plates. Thus, Livecyte can be a very informative and efficient tool for running assays that require large number of inter population and/or dose response comparisons.

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For more information on the benefits of the Livecyte system, to access application notes and for additional product information, please visit:
www.phasefocus.com/livecyte

A sample of time-lapse videos can be found at:
www.youtube.com/phasefocuslimited

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