Automated determination of Mitotic Time on Livecyte | Application Note

Automated detection and comparison of Mitotic Time
Compare Mitotic Time without use of fluorescent labels for multiple treatments

- Automatically identify entry to and exit from mitosis, track mitotic cells, and measure time in mitosis without any addition of fluorescent labels

- Quantitative Phase Imaging (QPI) uniquely allows tracking through mitosis using comparison of mass between parent and daughter cells

- Investigate mitotic time and mitotic arrest for different drug treatments on a multi-well plate format

Introduction

Measurement of mitotic time is important to numerous fields of cell biology and has uses in the development of anti-mitotic cancer drugs. Typically, manual methods are employed to identify mitotic events, track through cell division and record mitotic time. Such methods are subject to user bias and are time-intensive to obtain a minimal number of data points to ensure statistical significant comparison between different drugs/treatments. The Livecyte overcomes these issues by combining Quantitative Phase Imaging (QPI) with downstream automated analysis software. Uniquely, QPI allows for more accurate automated approach since the increased cell thickness and hence brightness of the image allows for easy thresholding of mitotic events. Rules within the cell identification recipe remove user-to-user bias in identification of mitotic entry or exit. Moreover, the relationship between the mass of the parent and daughter cells can be used to more faithfully track through cell division and distinguish between mitosis and other fates such as arrest or cell death. Here we show how Livecyte time course image data is automatically processed through Livecyte Cell Analyses Toolbox (CAT) and a Mitotic Time Application Module to produce responses such as changes in cumulative mitotic exit.

Methods

HeLa cells were seeded at 1×10^4 cells/well into 12 wells of a 24 well plate and incubated overnight to allow for adhesion. Prior to imaging the plate, media was removed and replaced with 500µL/well of 6 treatment concentrations of Nocodazole in 10% FBS-DMEM, in duplicate, ranging from 0 (control) to 100nM. Images (500µm x 500µm) were acquired in each of the 12 wells every 5 minutes for 24 hours. These images were automatically processed in Livecyte Cell Analysis Toolbox (CAT). In CAT, the user identified candidates for mitotic events by setting up a segmentation recipe based on individual cell thickness information. These candidates are then analysed with Phasefocus Mitotic Time Application Module which uses a suite of tracking and morphological filters. The filters employed exploit the relationship between daughter and parent mass, and daughter-daughter morphology to calculate mitotic time and cumulative mitotic exit. Figure 1 displays a single frame where mitotic cells are automatically identified by their morphological phenotypes and whether the cell has overgone division and produce 2 or more daughters.
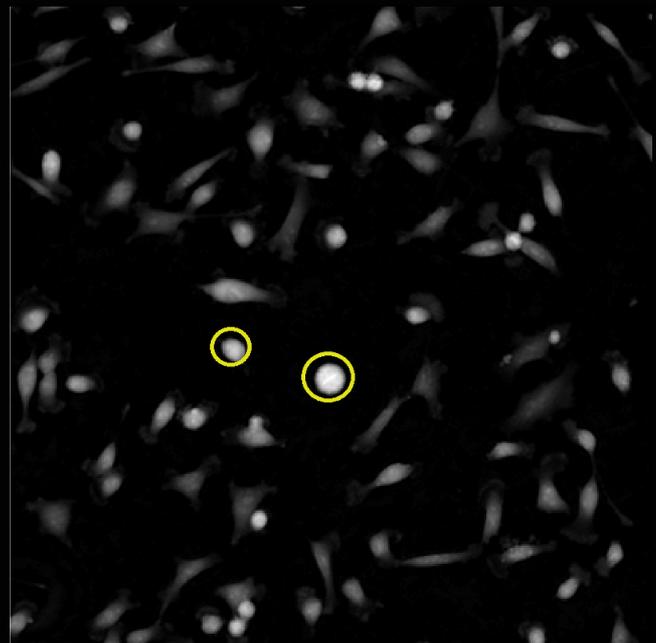

Figure 1 – QPI frame from time lapse movie: Automatically detected mitotic events highlighted with a yellow circle.

Results

Figure 2 displays the series of metrics automatically produced by the Livecyte system and Mitotic Time Application Module. The different coloured outputs identify the varying Nocodazole concentration (matched to the video output illustrated in Figure 3).
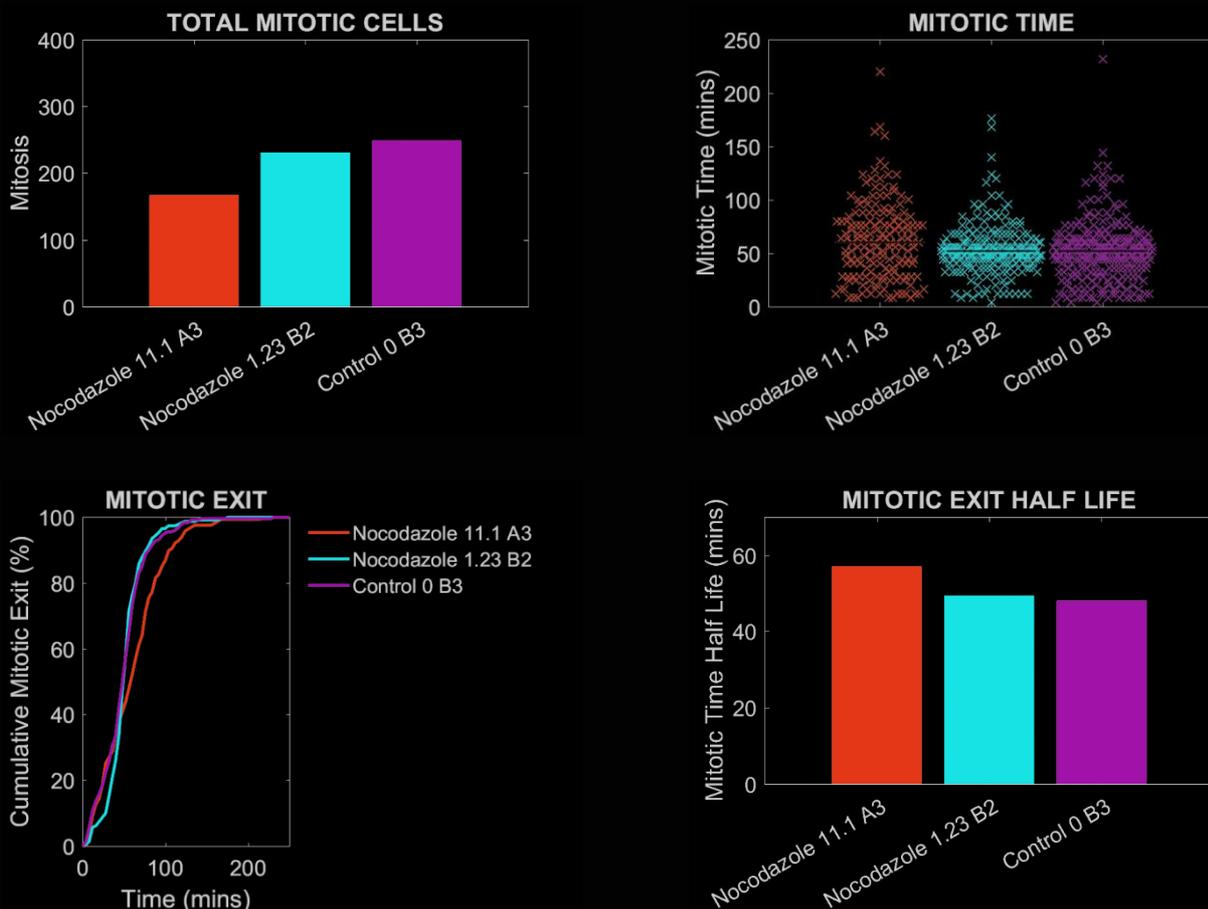
Figure 2 – Metrics outputted from the Mitotic Time Application Module. The above metrics are chosen to provide the user with a complete description of the cell behaviour.

The total number of mitotic cells detected decreases with increasing concentration of Nocodazole (Figure 2; Total Mitotic Cells). Beeswarm plots of the mitotic time for each cell identified as going through mitosis shows that the higher concentration of Nocodazole lengthens the time a cell spends in mitosis; this is also reflected by the cumulative plot of mitotic exit and the corresponding half-life value. Together the graphs illustrate that addition of Nocodazole lengthens the mitotic time (the time from mitotic entry to exit) of Hela cells, which causes fewer mitotic events to be detected as they take longer to transit the cell cycle in the imaging timeframe measured.

The Mitotic Time Application Module also facilitates comparison with ground truth datasets, which can be manually defined by the user in MTrackJ (ImageJ). This allows you to check that the automated analysis produces similar patterns of results to that detected manually. By completing this task for the data presented above we find that the automatically detected mitotic exit half-life follows a similar pattern to that of the GT. Notice that although the relative pattern of increasing mitotic time half-life with increasing Nocodazole concentration is maintained, the ground truth data is systematically higher. In effect, the software module detects a cell as entering mitosis two-three frames after the user notes the same cell as being in mitosis. Another user may detect a slighter shorter time than the software. The software, however has defined values that identify when a cell should be counted as 'in-mitosis', thus removing any user-to-user bias in calculation of mitotic time.
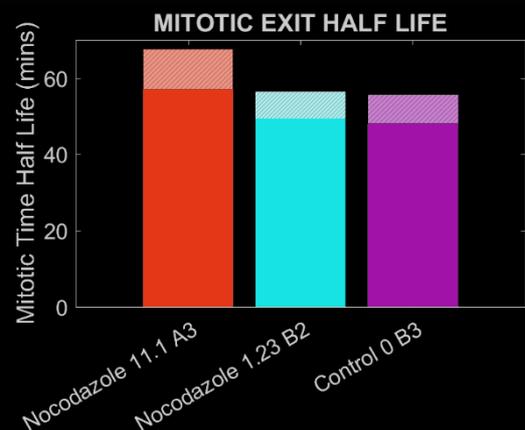

Figure 3 – Mitotic Exit Half Life comparison with Ground Truth The ground truth values for each treatment can be seen overlaid on the values automatically calculated from the Livecyte system.

Conclusion

In this application note it was demonstrated that the Livecyte system can be used to automatically determine mitotic time. The ability to perform the experiment on high format plates allows the effect of different treatments to be investigated. This assay would be of particular importance in the development of anti-mitotic cancer drugs.

The accuracy of the Mitotic Time Application Module was validated with a manual approach. The multi-parametric data produced is combined by the Mitotic Time Application Module to provide the user with a complete description of the cell behaviour and provides a solid foundation to robustly compare the effect of many different treatments.
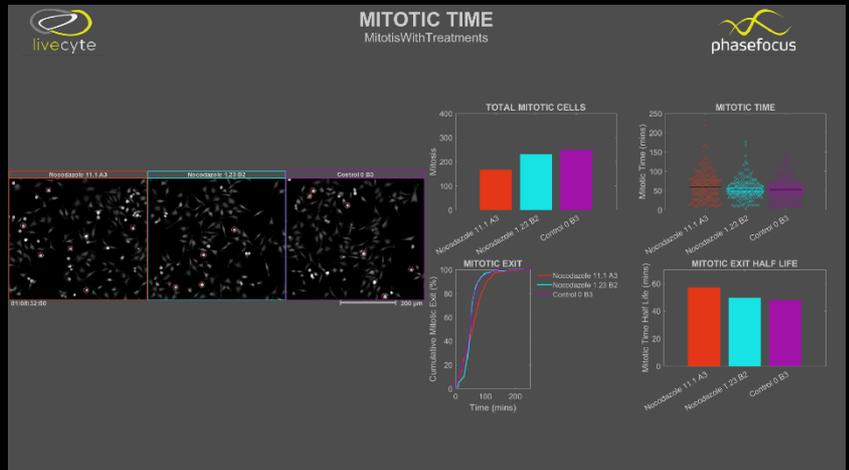

Figure 4— Output screen from the Mitotic Time Application Module. The Mitotic Time Application Module outputs QPI videos, in a multi-well format, colour coded to relate to the multi-parametric data also presented.

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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