Beyond Confluence -
Using novel, label-free intrinsic biomarkers generated from QPI images to gain earlier and deeper insights into microplate cell cultures

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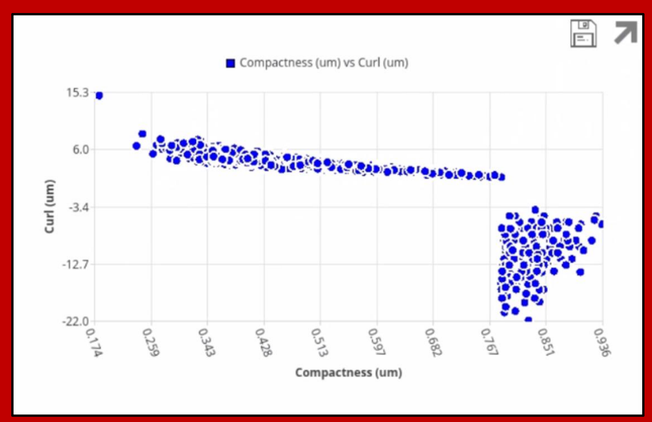
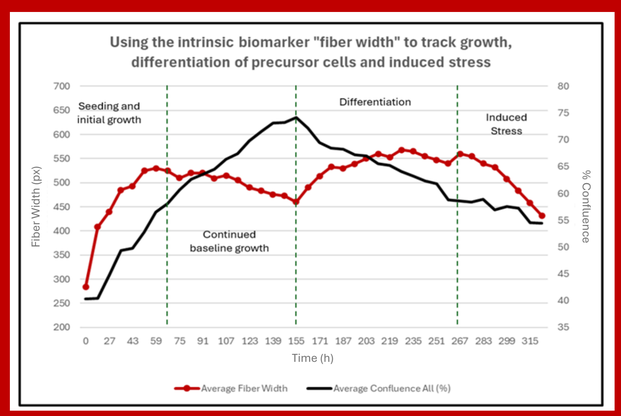


Creation of a label-free intrinsic biomarker to gain deeper insights into cell population dynamics



Unattended label-free imaging of 96 well plates at 8-hour intervals to track growth, differentiation of precursor cells and induced stress by using the intrinsic biomarker "colony fiber width"

1. Introduction

Despite recent accuracy improvements afforded by the development of AI-driven algorithms that adapt to different cell morphologies, confluence cannot give insights into cell health and is prone to errors when 3-dimensional colony and clump formations are present.

In this poster we describe a new three-dimensional, label-free imaging method that goes beyond just confluence to deliver real-time insights into the culture dynamics at the cellular, colony, well and microplate level throughout the entire culture.

We present 3 examples: 1) The isolation of two distinct cell populations in HeLa cell culture 2) The identification of different colony morphologies among iPSC culture and 3) Utilization of an intrinsic biomarker for label-free determination of proliferation, differentiation, and stress in an iPSC culture.

2. Methods

1) Hela Cell Culture
Cells were plated and allowed to adhere. Plates were imaged 6-hour intervals beginning approximately 6 h after plating.
2) iPSC Colonies
Colonies were plated and allowed to establish prior to imaging.
3) iPSC Cell Culture, Differentiation, and Stress
Cells were plated and imaged every 8 hours for 12 days. At this point a stress agent was introduced and the culture was monitored for another 2 days.
Imaging and Feature Quantification
Images were captured utilizing either the CellAssist benchtop imager or CellAssist 50 automated incubator and imager. 3-Dimensional, brightfield, whole well images were captured at each time point and processed in real-time to generate 2-D QPI images using the Thrive IQ software. For each well conventional outputs such as confluence or cell count are automatically generated (fig.1). In addition, we generate 28 different, quantifiable morphological 2D cell features such as area, perimeter, eccentricity, convexity and 3D QPI features such as texture, kurtosis and brightness.
The entire process, from automatic plate removal from the incubator through imaging, analysis and return to the incubator takes less than 10 minutes per plate per timepoint.

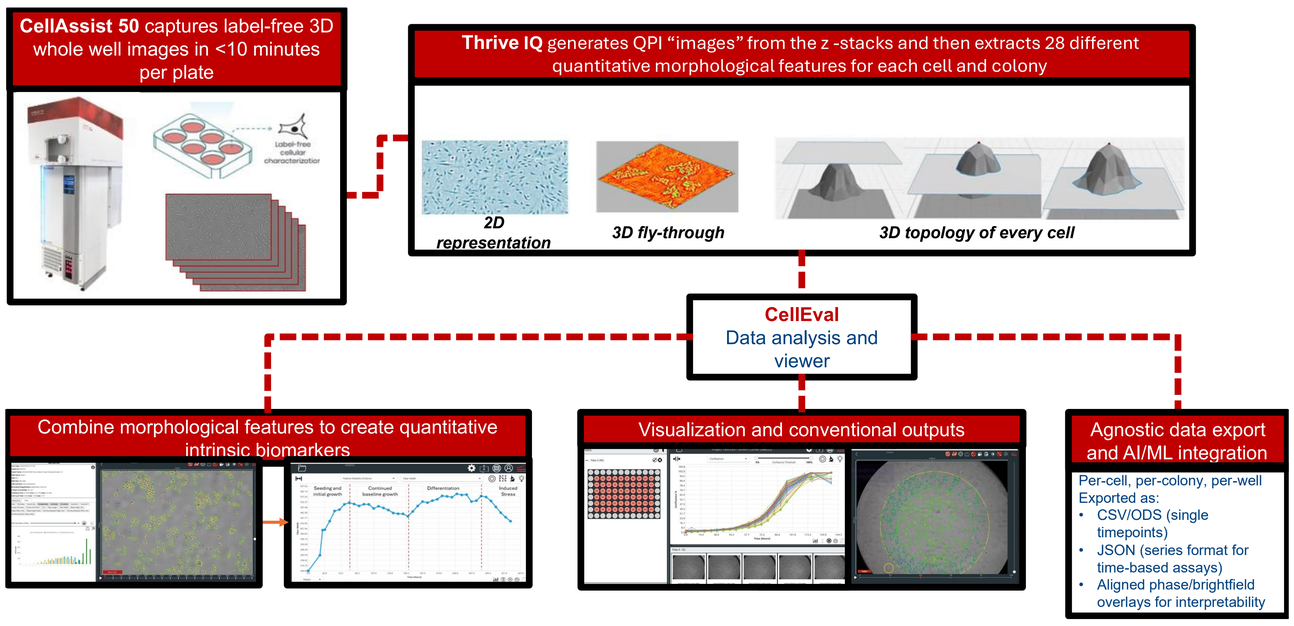


Fig. 1 Overview of image capture and data processing workflow

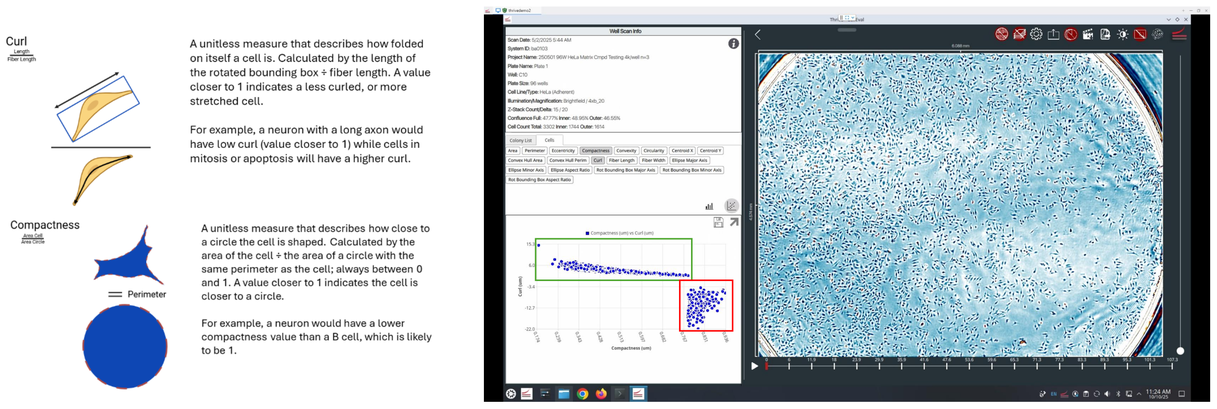


Fig. 2 Quantifiable insights into cell population dynamics using "Curl" and "Compactness"

3. Results

1) HeLa Cell Culture (fig.2)
We combined the morphological features "*curl*" and "*compactness*" .
The well time series review screen shows a color QPI image, and a scatter plot showing the two discreet cell populations, along with the associated QPI image.
We combined "*curl*" and "*compactness*" to create an intrinsic biomarker that is used to probe cell state. Cells with low to intermediate compactness, and relatively high curl (*green box*) are alive, adherent cells, likely in interphase cell growth. Cells with high compactness and low curl (red box) would be a combination of cells that are undergoing mitosis or have rounded up and are dying. By measuring these features over time, it is possible to obtain real-time insights into cell health.
2) iPSC Colonies (*fig*.3)
The 3D morphologies of various iPSC colonies were visualized using a 3D rendering of the QPI image. Distinct cell and colony properties are evident, for example, cell density which is reflected in the brightness measure where brighter cells are denser. The brightness ratio describes the distribution of dense cells within a colony.
3) iPSC Culture, Differentiation, and Stress *(fig.4, 5)*
The confluence plot, (% of the well bottom covered by cells), plot shows 4 phases; (i) lag, (ii) growth, (iii) stationary and then (iv) a linear decrease following differentiation and stress.
We also generated a colony level intrinsic biomarker, "fiber width (*FW*)" and plotted this over time. This plot also revealed 4 different phases, (i) initial growth where *FW* increases sharply, (ii) baseline growth where *FW* decreases slowly, (iii) differentiation where there is an inflexion in *FW* followed by a plateau and then (iv) stress where there is a sharp decrease in *FW*.

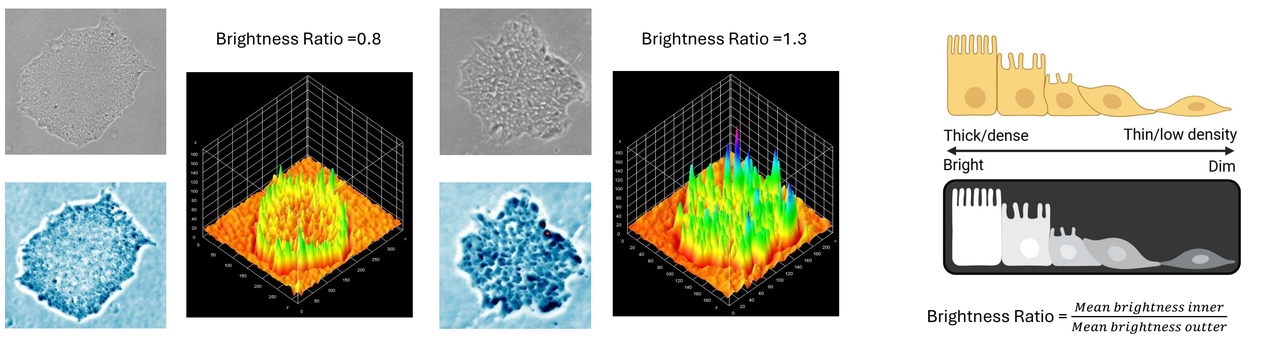


Fig. 3 3D Brightness ratio from QPI image distinguishes between multiple iPSC colony morphologies

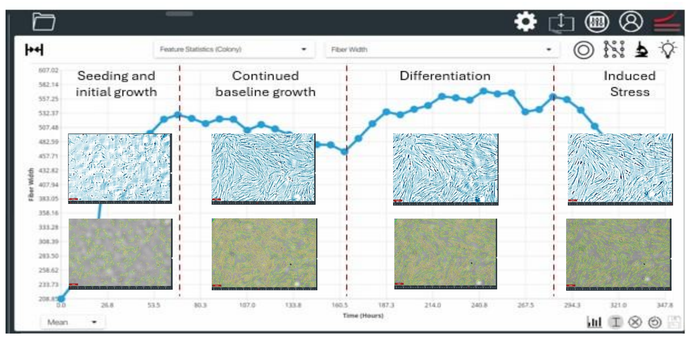


Fig. 4 Use of the morphological feature colony fibre width as an intrinsic bio marker to growth, differentiation and stress response

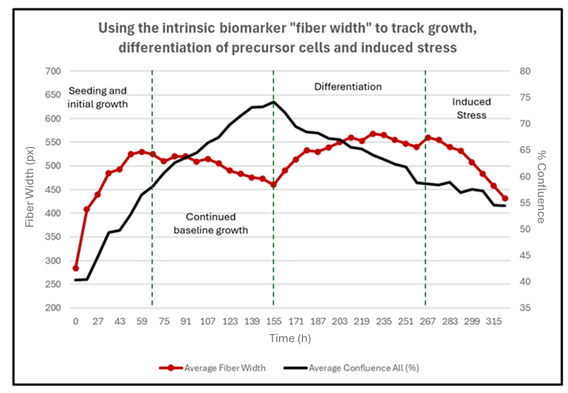


Fig. 5 Comparison of confluence and colony fiber width over time

4. Discussion

In this work we have explored the potential of label-free, intrinsic biomarkers to reveal deeper insights into microplate cultures. While confluence remains an important feature of cells in culture, it provided minimal information regarding cellular health or transitional states. Researchers have long relied on fluorescent microscopy to provide either accurate cell masks and segmentation or probe gene and protein expression. However, fluorescent microscopy creates additional steps and is invasive to cells.

The CellAssist and CellAssist 50 employ quantitative phase imaging to establish highly-accurate cell contours and derive 3-dimensional information from 2-dimensional brightfield images. In combination with whole-well images and rapid image processing, this provides a unique opportunity to measure cellular features over time and at scale.

3D and 2D morphological features may me investigated individually or together as intrinsic biomarkers. These features are provided in standard CSV and JSON format, empowering users to utilize data science and machine learning tools that can predict target cell states.

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