Label-free Cell Viability Assay Using Phase Imaging with Computational Specificity (PICS)

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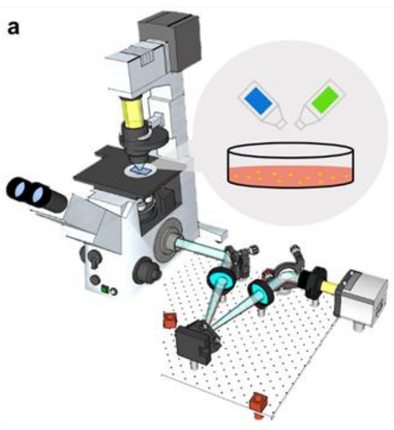
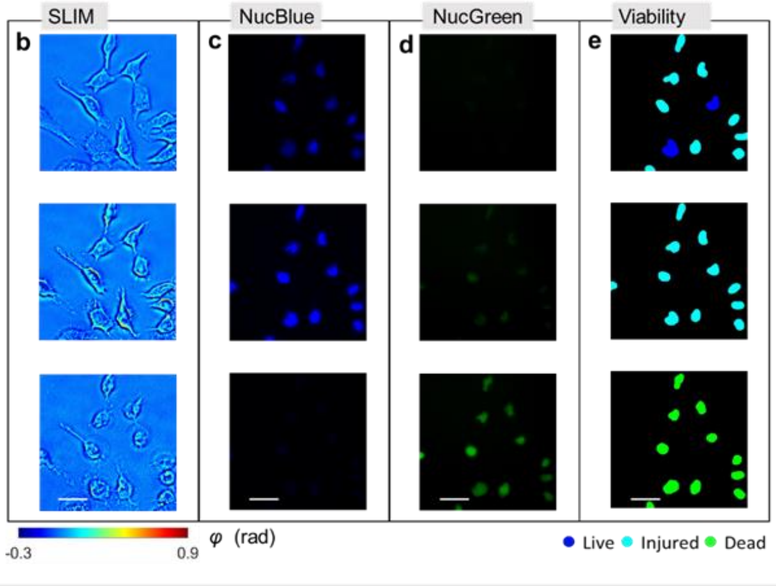
Abstract

Existing approaches to evaluate cell viability involve cell staining with chemical reagents. However, this step of exogenous staining makes these methods undesirable for rapid, nondestructive and long term investigation. Here, we present instantaneous viability assessment of unlabeled cells using phase imaging with computation specificity (PICS). This new concept utilizes deep learning techniques to compute viability markers associated with the specimen measured by quantitative phase imaging. Demonstrated on HeLa and CHO cells culture, the proposed method reports approximately 95% accuracy in identifying injured and dead cells. Further comparison of cell morphology with labeled HeLa cells suggests that potential adverse effect on cell dynamics introduced by the viability reagents can be avoided using the label-free investigation method, which would be valuable for a broad range of biomedical applications.

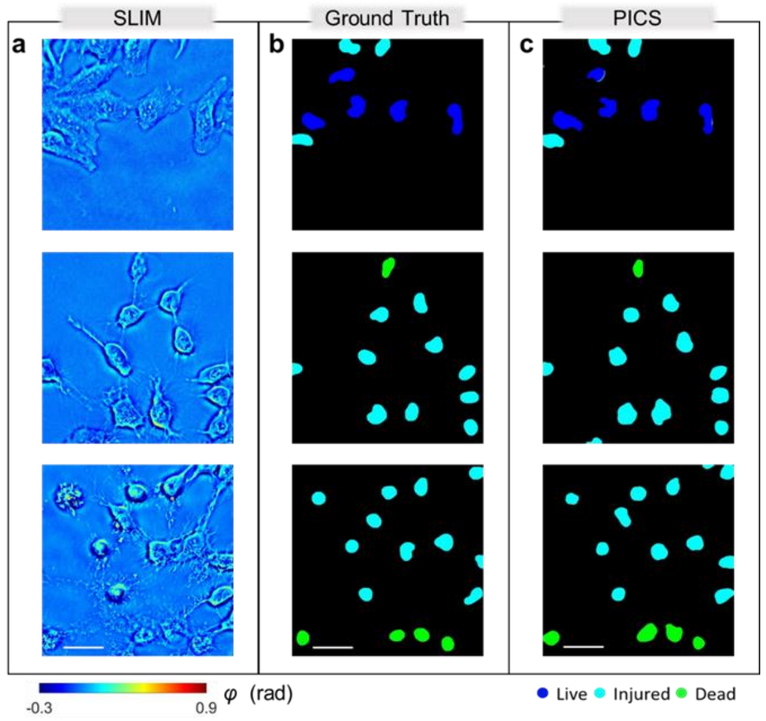
Image Acquisition



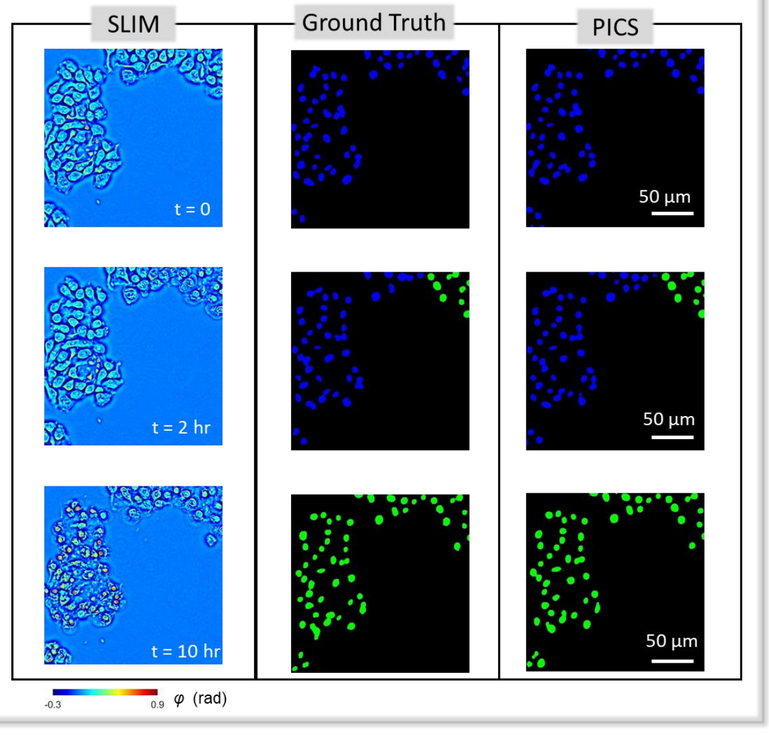
- Live cells are mixed with fluorescent viability reagents, and then measured by QPI and fluorescence microscopy.
- Cell death was introduced by either incubating culture in room temperature or mixing the culture with chemical apoptosis reagents.
- Semantic segmentation maps are generated to label the viable state of individual cells, based on fluorescent signals.



Training on HeLa and CHO cells



- A U-Net based EfficientNet is applied, where the input are QPI images, and output are corresponding viability maps.
- Transfer learning strategy was adapted here to reduce the size of training data. An Adam optimizer and focal loss was used to update parameters.
- Approximately one/two thousand training pairs, and the entire training and validation took nearly 10 hours.



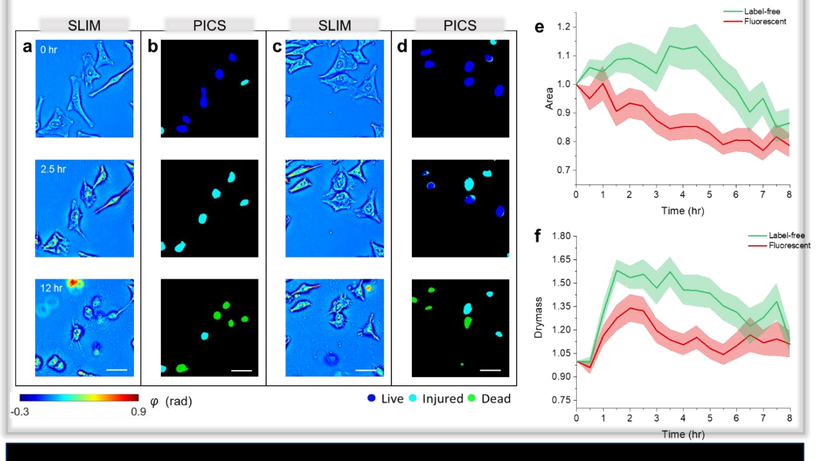
Evaluation

HeLa Cells	Live	Intermediate	Dead
Precision	66.3%	98.6%	91.3%
Recall	82.2%	95.5%	97.6%
F1	73.4%	97.0%	94.3%

CHO Cells	Live	Dead	Mean
Precision	94.6%	96.8%	96.6%
Recall	90.1%	98.3%	94.2%
F1	92.3%	97.5%	94.9%

CHO Cells	High Confluence	Middle	Low
F1	95.1%	94.2%	95.5%

- An object-wise evaluation metric is applied to neglect non-biological related instances.
- Approximately 95% confidence in identify intermediate or dead HeLa caused by necrosis, and CHO cells caused by apoptosis.
- For CHO cells, the deep learning performance seems not affected by levels of cellular confluence.
- Label-free method allows unbiased tracking of cell shape and drymass changes along cell death, as shown below.



Reference and Funding

1. Hu, Chenfei, et al. "Label-free cell viability assay using phase imaging with computational specificity." bioRxiv (2020).
2. Hu, Chenfei, and Gabriel Popescu. "Quantitative Phase Imaging: Principles and Applications." Label-Free Super-Resolution Microscopy. Springer, Cham, 2019. 1-24.

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EBICS Emergent Behaviors of Integrated Cellular Systems
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