

**Title:** Deep Learning Unlocks Label-Free Viability Assessment of Cancer Spheroids in Microfluidics

**Authors and affiliations:**

Chun-Cheng Chiang<sup>a,b,^</sup>, Rajiv Anne<sup>a,c,^</sup>, Pooja Chawla<sup>c</sup>, Rachel M. Shaw<sup>c</sup>, Sarah He<sup>a,d</sup>, Edwin C. Rock<sup>c</sup>, Mengli Zhou<sup>a,b,e</sup>, Jinxiong Cheng<sup>a, c</sup>, Yi-Nan Gong<sup>a,f</sup>, Yu-Chih Chen<sup>a,b,c,g,\*</sup>

a UPMC Hillman Cancer Center, University of Pittsburgh, 5115 Centre Ave, Pittsburgh, PA 15232, USA;

b Department of Computational and Systems Biology, University of Pittsburgh, 3420 Forbes Avenue, Pittsburgh, PA 15260, USA;

c Department of Bioengineering, Swanson School of Engineering, University of Pittsburgh, 3700 O'Hara Street, Pittsburgh, PA 15260, USA;

d Carnegie Mellon University, Department of Biological Sciences, 5000 Forbes Avenue, Pittsburgh, PA, 15213

e Xiangya Hospital, Central South University, Changsha, Hunan, 410008, China;

f Department of Immunology, University of Pittsburgh School of Medicine, 3420 Forbes Avenue, Pittsburgh, PA, 15260 USA

g CMU-Pitt Ph.D. Program in Computational Biology, University of Pittsburgh, 3420 Forbes Avenue, Pittsburgh, PA 15260, USA

<sup>^</sup>equal contributions

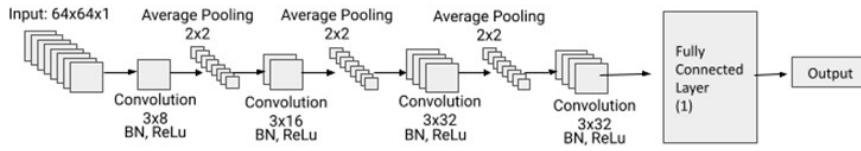
\*Corresponding authors

Yu-Chih Chen

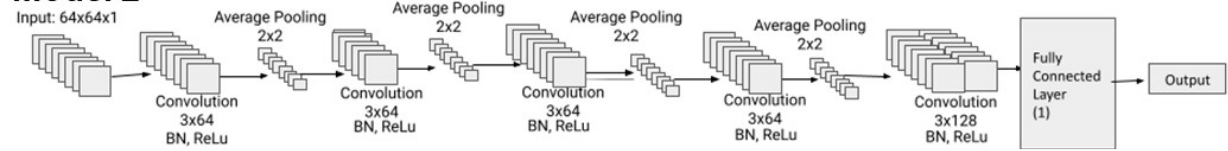
5115 Centre Ave, Pittsburgh, PA 15232, USA

Tel: 412-623-7701; E-mail: chen25@upmc.edu

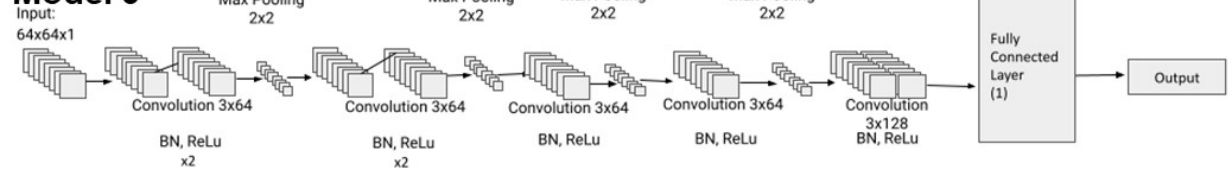
## Model 1



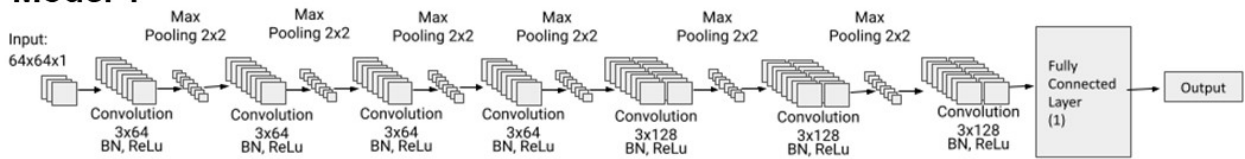
## Model 2



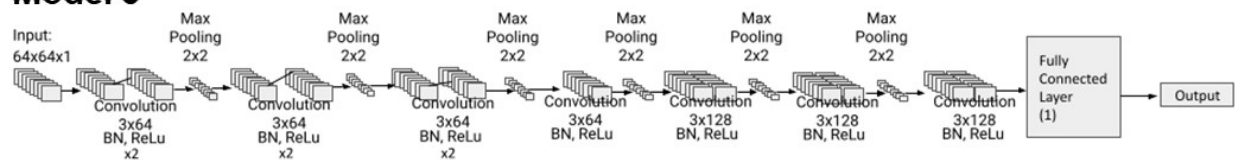
## Model 3



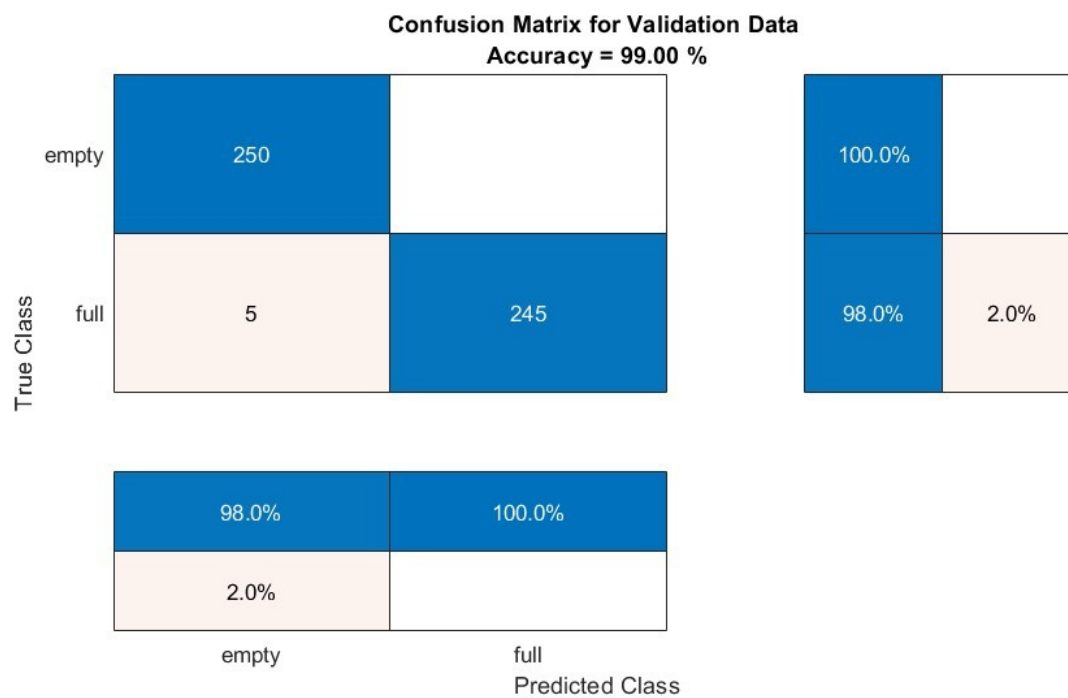
## Model 4



## Model 5



**Figure S1.** Convolutional Neural Network (CNN) models used for label-free prediction of cell status.



**Figure S2.** Classification of chambers with and without spheroids to exclude empty chambers.

**Table S1. The list of compounds.**

| <b>Source</b> | <b>Item #</b> | <b>Compounds Name</b>  | <b>Target</b> |
|---------------|---------------|------------------------|---------------|
| Cayman        | 11421         | Actinomycin D          | Chemo         |
| Cayman        | 10009929      | Vorinostat (SAHA)      | HDAC          |
| Cayman        | 17130         | Romidepsin             | HDAC          |
| Cayman        | 15007         | Doxorubicin            | Chemo         |
| Cayman        | 9003096       | Gemcitabine            | Chemo         |
| Cayman        | 23732         | Ixabepilone            | Chemo         |
| Cayman        | 10461         | Paclitaxel             | Chemo         |
| Cayman        | 21262         | Vinorelbine (tartrate) | Chemo         |
| Cayman        | 10008822      | Bortezomib             | Proteasome    |
| Cayman        | 16139         | Pyronaridine           | antimalarial  |
| Cayman        | 19420         | CC-115                 | mTor/DNA-PK   |
| Cayman        | 20455         | ML162                  | GPX4          |