CNN-based analysis of organoid growth

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Abstract

High-throughput analysis of imaging data is critical for analysing large data typical of modern biological investigations. Here we investigate a convolutional neural network-based approach to analyse the growth of organoids based off of imaging data.

1. Introduction

Predicting tumor growth rate is a first step in determining treatment options for cancer patients. Fast growing tumors necessarily require more aggressive treatment. It would be beneficial if patients could avoid aggressive treatments when possible.

Alexandra Sockell of the Fordyce and Curtis labs in the Bioengineering and Genetics departments, respectively, of Stanford University has developed a microfluidic device to isolate single cells of a tumor and allow them to grow into organoids within the microwell. Organoids are three dimensional stem cell-like cultures that organize into a "mini-organ" [6], and can be used to study cancer in a more natural environment than traditional cell lines [2]. The objective of her research is to study the mechanisms of tumor growth by subjecting a large number of individual cells to a wide range of treatments and conditions and track their condition by imaging. She has taken 14 days of imaging over 8 conditions. For each day, there are approximate 38,000 well images across all conditions. The number of cells per well is approximately Poisson, with most of the wells not containing any cells, 25% have one cell, and smaller portion have more than 1. We believe that the large number of images should provide a sufficient amount of data and information content to apply deep convolution neural network approach. Our hypothesis is that the state of the cells in the early days should be related to their final state. Therefore our objective to determine whether the early stages of the organoid can predict the growth rate and final state of the organoid.

Previous approaches for high-throughput analysis of organoid imaging data did not look at single-cell microwell level data. Instead they typically relied upon a large number of cells to quantify cell proliferation or death [4], used cell counting assays to calculate growth [7], or used single cell tracking to calculate cell motility [3]. To our knowledge, no deep learning approaches have been proposed to analyse organoid imaging data, despite the large success in deep learning to analysing imaging data across a broad spectrum of applications. However, we have found successful convolutional neural network (CNN) approaches in related bio-

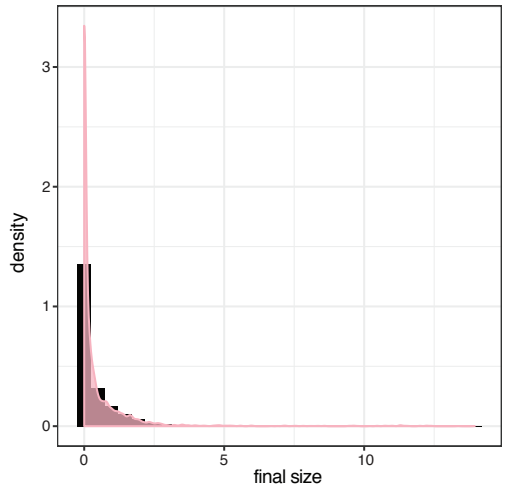

Figure 1. Distribution of normalized final sizes. There is a large peak at zero corresponding to empty wells or cells that died.

logical analysis such as high content screening [8], but this is not similar enough for us to compare against.

The purpose of this project is to provide a proof of principle for CNN-based high-throughput analysis of organoid imaging data.

2. Data

The data is composed of 193×193 greyscale images of 4800 microwells for each of 6 wells, imaged across 14 days. So we have $6 \cdot 4800 = 28{,}800$ wells with 14 images per well. The images were obtained via computational stitching [5] of multiple larger images of well. A small number of images appear distorted. We filtered the images by the estimated area of their interior, computed by the hyst2 function in openCV [1], to try to reduce such artifacts. If the estimated area was larger than possible for any of the 14 days, then we removed all images of that well from the data. After filtering we kept 8,763 microwells. Example images are shown in figure 3.

One difficulty with this project is that we did not obtain all the data at the start. We began with one experiment (4800 microwells), and then obtained data from more experiments as they were processed. We found that some models built on the first experiment did not generalize well, and a major objective is to ensure that our model can is generalizable across biological conditions.

3. Methods

3.1. Proof of principle model

To start we approached the regression problem straightforward. We found difficulties with overfitting using only day 1 on the images from a single well. We therefore decided to first focus on the classification problem, to predict from the early days whether the final day is empty or not, using whether the computed hyst2 area is zero or not as zero and one, respectively. As a proof of principle, we attempted to overtrain a deep convolutional neural network consisting of three convolutional layers, with kernel sizes of 2, 3, and 3, respectively, and channels of 64, 32, 16, respectively. Each convolutional layers was followed by a batch normalization layer, a ReLU layer, and then a max pooling layer with a kernel size of 2. The convolutional layer were followed by a square fully connected layer, followed by a ReLU layer, a fully connected layer with output size of one, and finally a sigmoid for output. We applied this network to all images from a single well, and attempted overfitting on day 1, day 2, and day 13, using cross-entropy loss. Day 13 was included as a sanity check, since the hyst2 area was computed using the day 13 images. The training loss for days 2 and 13 quickly went to zero, while the loss for day 1 did not go below the loss for random guessing (Figure 2). We therefore excluded days 1 and 0 from further consideration because they do not appear to be informative towards our objective. It may be that day is informative when combined with other days, but not by itself.

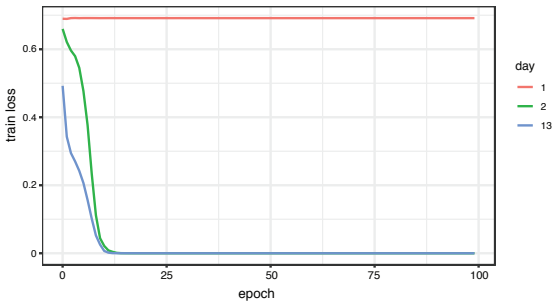

Figure 2. Cross-entropy loss for the training set using days 1, 2, and 13 to predict whether hyst2 area is zero at day 13.

3.2. Deep CNN for multi-day input

After the initial proof of principle we built a classification CNN to predict whether the microwell was empty at the end of the time point (indicating cell death), using hyst2 area as a proxy for whether a cell is empty or not. If the hyst2 area at day 13 is zero then we say that microwell is empty and if the hyst2 area is greater than zero then we say the microwell is non-empty. This CNN had five convolutional layers, all with batch normalization, ReLU, and max pooling following, with channels sizes of 32, 64, 128, 256, and 256 and kernel sizes of 5, 3, 3, 3, 3 (Figure 3). We used cross-entropy as the loss function and the Adam optimizer with learning rate $10^{-}4$. To facilitate generalization of our model we used Dropout regularization with $p = 0.5$. To take into account the temporal nature of our data we used multiple day images as input. We treated each separate image as an input channel (since each image is a single greyscale channel). We initially used days 2 and day 8 images as input, and later used days 2, 5, and 8 as input.

3.3. Pretrained model

We also applied pre-trained models, using transfer learning to train only the final output layer, keeping the other layers constant. We found success with the ResNet 18 model, but not as good as our previously discussed model.

3.4. Data augmentation

Because of the paucity of experiments, we explored data augmentation approaches. Because the primary information is in the center of the image, we hypothesized that random cropping may be a suitable data augmentation strategy. Additionally, the information content of the images should be rotation invariant, so therefore we also explored rotations for data augmentation.

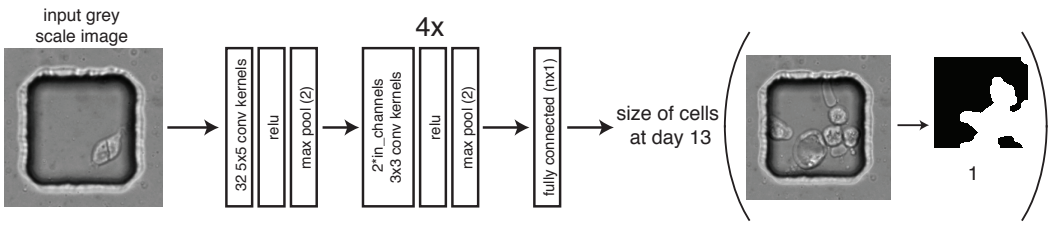

Figure 3. Example workflow of our CNN algorithm. The input is a 193×193 greyscale image of the cell at day one. We pass this through a convolutional neural network to whether the final well is empty, using the hyst2 area as a proxy.

4. Experiments

For classification, first we used one simple 3-conv,1-fully connected layer without any regularization to train 100 images. We obtained overfitting, as the training loss went to 0 and the training accuracy went to 1. However, the validation accuracy remained constant, equal to random guessing. We then added more convolutional layers and more parameters in each of the convolution network, obtaining the model described in section 3.2, and training with more data, 4510 microwells. We obtained 0.19 as the best validation loss , 0.8 as the best training accuracy and 0.6 as the best validation accuracy across all epochs (figure 5). If we run for more epochs, then the training loss goes to zero and the training accuracy goes to one. However, the validation accuracy does not improve. This supports the decision of early stopping. To improve generalization we then added dropout. This improved the validation accuracy slightly, to 0.65. However, the best improvement is when we added more microwells to the training. Then the validation accuracy improved to 0.8. The test accuracy for this model is 0.77 (figure 4).

Then I run for more epoches, the loss almost go to 0 and training accuracy go up to 1 but validation accuracy improves a little bit to 0.65. It was overffiting. So I added dropout and more maxpooling, the validation accuracy went up to 0.68. But it was still overfitting, so I added the training set to 6514. The minum loss is 0.11 and best validation is 0.8 and training accuracy is 0.915. But by changed different models, adding fully connected layer or using pretrained, but validation accuracy did not improve. By adding data augmentation, the training accuracy go down, but validation accuracy did not go up. We have also tried learning rate 1e-2,1e-3,3e-4,1e-4, the best is 1e-4.

For regression, we use pretrained ResNet18 with Adam Optimizer for 3000 training data, the loss go down to 0.01 with training mean square error as 0.03 and validation mean square error as 0.55 which is overfitting. The I added more dataset to 7k and get the validation mean square error down to 0.23 for 4 epoch. The truth and prediction size correlation is 0.85.

5. Experiments

We run the classification model to predict whether the final day well has cell. The best accuracy is 0.8. The we run regression to predict the size for the wells which has cell. The minumum mean square error is 0.22.

For the future work, we could use GAN to generate final day well images so that we do not need to get the size label since it is not very accurate. Besides the final day image has more information about the cancel than just the cell size.

References

[1] G. Bradski. The OpenCV Library. *Dr. Dobb's Journal of Software Tools*, 2000.

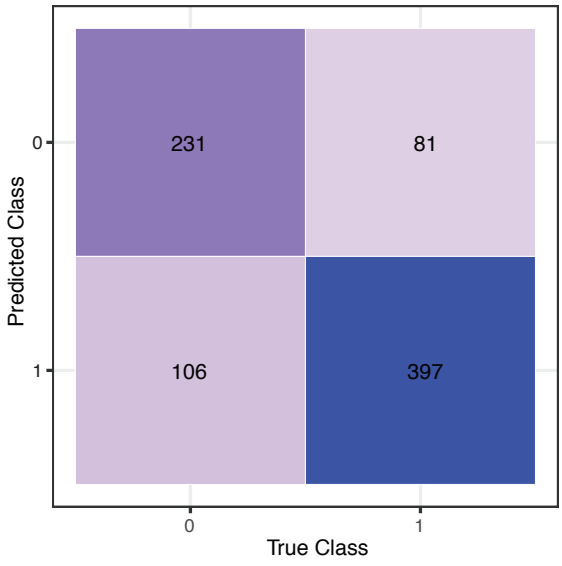

Figure 4. Confusion matrix for the classification problem on the 815 microwell test set.

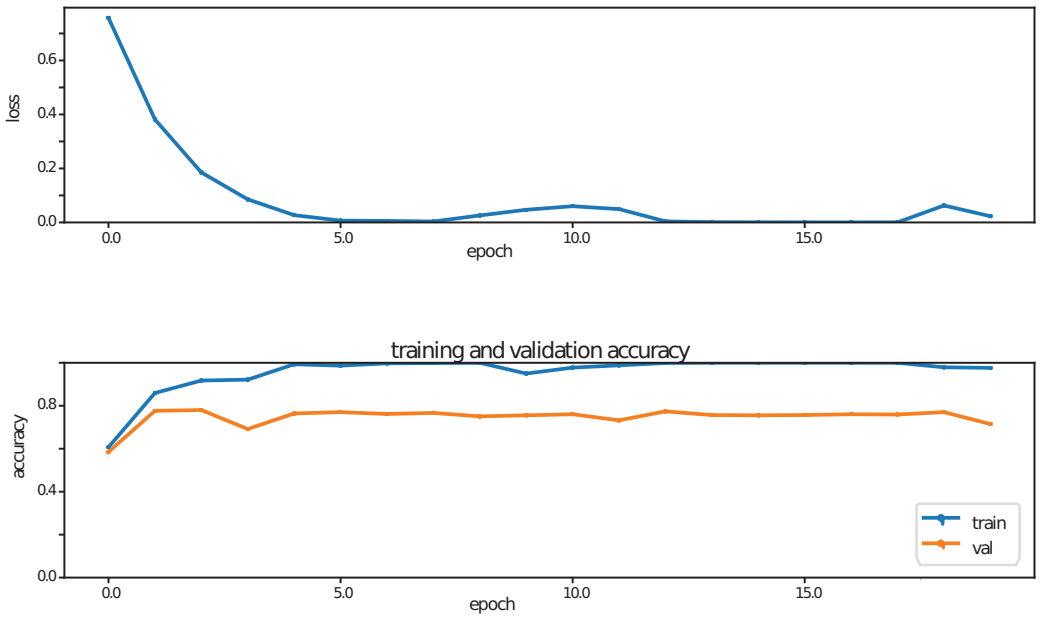

Figure 5. The training cross-entropy loss (top) and training and validation accuracy (bottom) for the classification problem.

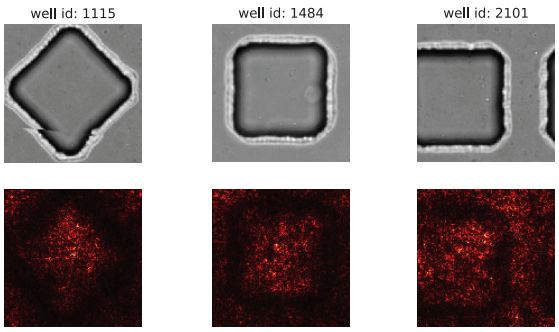

Figure 6. Example images and their corresponding saliency maps.

[2] J. Drost and H. Clevers. Organoids in cancer research. *Nature Reviews Cancer*, page 1, 2018.

[3] D. T. Geum, B. J. Kim, A. E. Chang, M. S. Hall, and M. Wu. Epidermal growth factor promotes a mesenchymal over an amoeboid motility of MDA-MB-231 cells embedded within a 3D collagen matrix. *The European Physical Journal Plus*, 131(1):8, 2016.

[4] J. Jabs, F. M. Zickgraf, J. Park, S. Wagner, X. Jiang, K. Jechow, K. Kleinheinz, U. H. Toprak, M. A. Schneider, M. Meister, et al. Screening drug effects in patient-derived cancer cells links organoid responses to genome alterations. *Molecular systems biology*, 13(11):955, 2017.

[5] S. Preibisch, S. Saalfeld, and P. Tomancak. Globally optimal stitching of tiled 3D microscopic image acquisitions. *Bioinformatics*, 25(11):1463–1465, 2009.

[6] A. C. Rios and H. Clevers. Imaging organoids: a bright future ahead. *Nature methods*, 15(1):24, 2018.

[7] T. A. Sebrell, B. Sidar, R. Bruns, R. A. Wilkinson, B. Wiedenheft, P. J. Taylor, B. A. Perrino, L. C. Samuelson, J. N. Wilking, and D. Bimczok. Live imaging analysis of human gastric epithelial spheroids reveals spontaneous rupture, rotation and fusion events. *Cell and tissue research*, 371(2):293–307, 2018.

[8] J. Simm, G. Klambauer, A. Arany, M. Steijaert, J. K. Wegner, E. Gustin, V. Chupakhin, Y. T. Chong, J. Vialard, P. Buijnsters, et al. Repurposing high-throughput image assays enables biological activity prediction for drug discovery. *Cell chemical biology*, 25(5):611–618, 2018.