Using Generative Adversarial Networks to create multi-channel images of cells undergoing macropinocytosis

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Motivation

The process of imaging proteins inside a cell makes it experimentally impossible to record more than 2 or 3 channels at the same time. As such there is a lack of training data for models that require information about these multiple proteins, for example in modelling the process of macropinocytosis. Due to this, it would be beneficial to have a method for generating realistic data containing these multiple channels from an input cell which has only a single protein imaged. In computational terms, this is learning a complex mapping from a single channel image to a multi-channel image.

Background - Biology

The simple elliptical geometries of dividing yeast fission cells provide a sufficient proof of concept for investigating functional relationships between proteins. A dataset, [1], provides a red reference marker and 41 green channel 'polarity factors'. Each pair of red-green proteins show a relationship with propagating actin structures capable of forcing the deformation of the cell membrane, leading to cell division.

In Dictyostelium cells the process macropinocytosis, or cell drinking, pose a greater challenge due to higher complexity geometries. The engulfment of large amount of fluids by non-selective actin-driven protrusions allow uptake of particles and amoeboids into the cytoskeleton. Like the yeast fission cells, a number of proteins are involved in the protruding membrane for macropinocytosis and their spatio-temporal configuration is of interest. The red reference marker PIP3, one of the key signalling molecules in macropinocytosis, is coupled with proteins marked in green:

- *MyoB*, a motor protein which is able to link the membrane to proteins and the actin nucleators that initiate actin polymerisation and subsequently deforming the membrane.
- *Coronin*, a protein that binds to F-actin and is a suspected influence on aged actin degradation.
- *LifeAct*, a marker for F-actin

Method - Generative Adversarial Networks

General adversarial networks (GANs) implement an adversarial game between two (convolutional) neural networks, a discriminator and a generator, where the discriminator learns to distinguish between real and fake images while the generator tries to fool the discriminator with fake images [2]. This game between the generator and discriminator is shown in figure 1.

In this project, conditional GANs (cGANs) are used as the data is conditioned on the protein marker labels. This is represented by a minmax approach with input data $\mathbf{x}$, random noise $\mathbf{z}$ and labels $\mathbf{y}$, where $D(x)$ classifies the images and $G(z)$ is the generated image.

$$\min_G \max_D \left(\mathop{\mathbb{E}}_{x \sim P(x)} [\log(D(x \mid y))] + \mathop{\mathbb{E}}_{z \sim P(z)} [\log(1 - D(G(z \mid y)))] \right).$$

The pix2pix method was chosen as it uses a cGAN for image-to-image translation with evidence of high-quality images generated. In pix2pix, the generator is a U-net which is specifically good at capturing small scale detail and the discriminator is PatchGAN which outputs an $N \times N$ array and classifies patches of the generated image as real or fake.

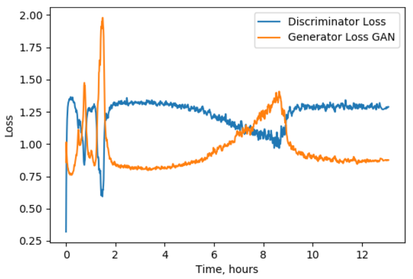


Figure 1: Comparison of Generator and Discriminator L1 losses during training

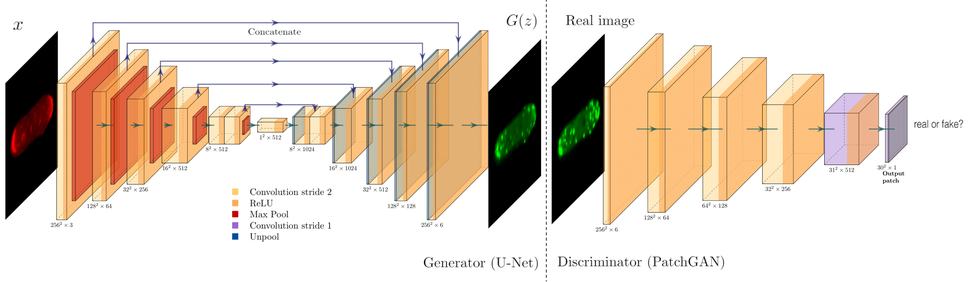


Figure 2: Architecture for the Generator and Discriminator in pix2pix.

Proof of Concept - Yeast Cells

Our first step was to create a simple proof of concept, showing that the relationship between two proteins in a simple cell (imaged into different channels in an image) can be learned through a GAN. To train this proof of concept we used the yeast cell dataset [1], which whilst not being the correct type of cell, still has a correlation between the two proteins.

Computationally, this mapping can be expressed as a single channel image-to-image mapping, similar to that of the original paper, which learnt a mapping between a cell mask and a distribution of a single protein around the cell (a one-channel/grayscale image). We applied pix2pix to a section of the dataset containing 3,247 two-channel images with a marker for *Bgs4* (red) and *Act1* (green). The generated green channel are then combined with the input red as shown in Figure 3.

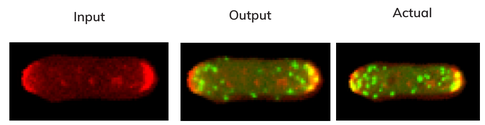
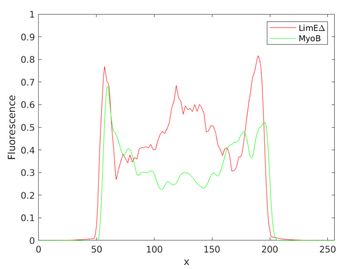


Figure 3: An example run of a trained GAN on a sample yeast cell

Generating Fake Input Data



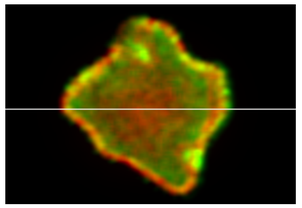


Figure 5: (above) Distribution of red and green fluorescent markers for LimEΔ and MyoB respectively, (below) mock cell showing these markers

To move from yeast cells to Dictyostelium cells, we have been attempting to create fake input data which matches closely the patterns we've been observing in the real data. We've identifyed techniques which appear to be consistent qualitatively in both two dimensions (i.e. cell slices) and in three dimensions (a set of layers of cell slices).

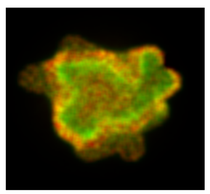


Figure 4: Filters applied to 2D generated mask to mimic behaviour of LimEΔ and Coronin

Two dimensions - Mock cell data was produced mimicking similar cellular protein relationships to those in related papers, but at a lower level of complexity. Arbitrary binary masks were textured with filters to produce mock cell data for Coronin and MyoB as green markers from [3] with the red marker for LimEΔ.

Three dimensions - Noise was used to generate the cell mask due to a similarity between cell structure and terrain generation. Terrain generation uses Perlin noise to create underlying patterns which match mountain ranges, islands, etc. Combining varying frequencies of Perlin noise allowed for cell shapes that remained 'blob-like', but also allowed for protrusions present on cells undergoing macropinocytosis. The 2D filters mentioned above can then be applied to the mask on a layer-by-layer basis.

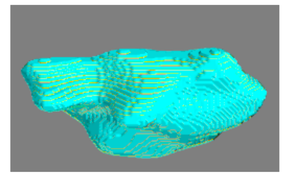


Figure 6: Mask generated by addition of varying frequency perlin noise.

Next Steps and Future Work

- Extend fake data generation to include an extra channel, e.g. crowns are formed at the edge of the red channel through Actin polymerisation.
- Using Poisson distributed random noise to texture the 3D surface of cells.
- Using the time-series of an imaged cell undergoing macropinocytosis, attempting to train a GAN to predict the green channel from the red.
- Extend the CNN used as the generator in the GAN to use a convolution in a third dimension

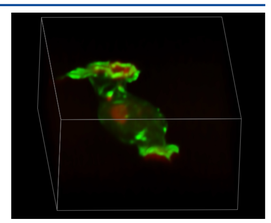


Figure 7: 2-channel cell visualised in 3 dimensions

References

[1] Dodgson, James, et al. "Reconstructing regulatory pathways by systematically mapping protein localization interdependency networks." bioRxiv (2017): 116749.

[2] Goodfellow, Ian J., et al. "Generative adversarial networks." Proc. 27th Int. Conf. Neural Information Processing Systems. 2014.

[3] Bretschneider, Till, et al. "The three-dimensional dynamics of actin waves, a model of cytoskeletal self-organization." Biophysical journal 96.7 (2009): 2888-2900.