Convolutional Neural Networks for Spot Detection

Applied to Fluorescent Microscopy Images

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Abstract

Spot detection is an important task in many fields such as Biology, Astronomy, and Physics. Unfortunately, the task of counting spots in images can take experts many laborious hours to do by hand. Recent studies (cite) have shown that Machine Learning has potential to automate this task. However, It remains a problem to acquire pixel-level annotations needed as targets for any type of supervised learning. Here, we have written a dot simulator to emulate fluorescent spots on background noise – giving us unlimited access to annotated data, and thus allowing users to completely side-step the need for human annotated data sets. Our results exemplify that this method offers a competitive F_1 score on empirical, fluorescent microscopy images when compared to other supervised machine learning methods.

Fluorescent Confocal Immunolabeling

Utilizes fluorescently tagged antibodies to target specific areas within cells, tissue, and organs. This is a very common method in the field of Biology because of how versatile it is. The versatility comes from being able to design both primary and secondary antibodies.

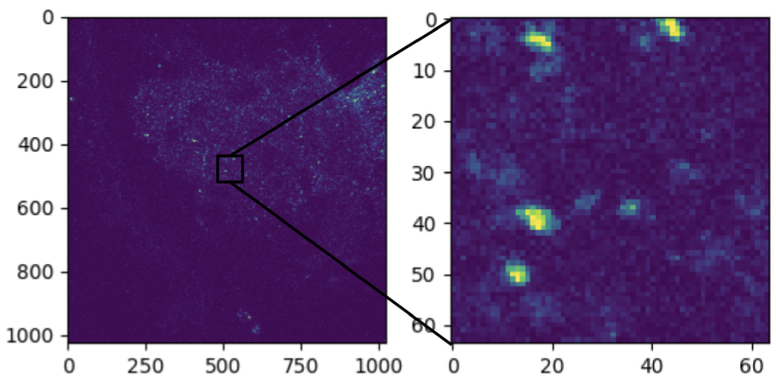

Figure 1:*Left* shows a 1024 × 1024 pixel empirical image of only the z channel. For this channel anitibodies were designed to target the synaptotagmin protein that is present during synapse communication. *Right* shows a 64 × 64 pixel zoomed section that feed into our model for prediction.

Convolutional Neural Networks

Convolutional neural networks(CNN) is commonly used for image classification and recognition. It takes in an input image and divides it into small sections. With the small sections, it is able to detect patterns and output to a class.

Impact

Using our spot detector, the efficiency at which we are able to annotate these images increases by multiple orders of magnitude. Within the context of Neuroscience, a tool like this would be very beneficial for understanding and measuring synapse formation and communication as an observable phenotypic trait. We could then compare this trait between individuals to learn more about synapse formation and common neurological diseases that are affected by this.

Methods

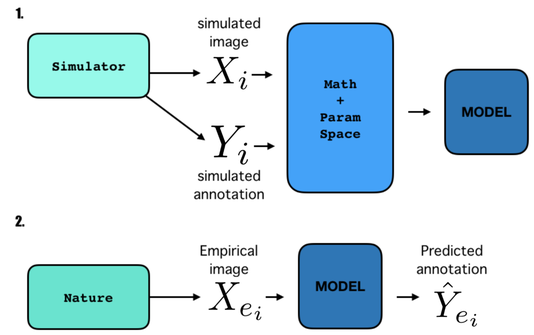

Figure 2:Part 1 (*top*) describes the simulation and training procedure - Each simulation is a pair of both the noisy image, x_i and its respective ground truth binary annotation y_i. These data set are then used to fit our convnet to produce a predictive model. Part 2 (*bottom*) shows an empirical image (in our case a confocal fluoresce image) being fed into the same model to produce a prediction for synapse location.

Simulation

In our simulations, we modeled fluorescent clusters in confocal images with a 2-dimensional Gaussian "bump" scaled by the radius for any given spot. Concretely, given (x_f, y_f) describing the focal point for a bump, the activation of any pixel (x_p, y_p) – within a dot of radius r and distance to focal point $d = \sqrt{(x_f - x_p)^2 + (y_f - y_p)^2}$ – is described as such:

$$A(r, d) = e^{\frac{\ln 10}{r^2} d^2} + \mathcal{N}(\mu = 0,\, \sigma^2) \tag{1}$$

Where σ is provided by the user as a parameter to our simulator. Both the number of dots in any given image, and the radius of any *one* dot are pulled from a binomial distribution, where n, and p, are parameters provided by the user. Once the number of dots are chosen, the simulator uniformly places focal points in the given dimensions for an $M \times N$ pixel image. Finally, Background noise for any pixel not in the radius of a dot is pulled from a Gaussian distribution.

Our Model

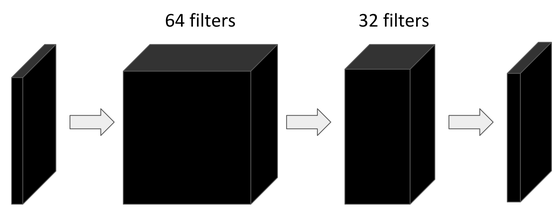

Figure 3:With no down-sampling or pooling, we have three convolutional layers with and 64, 32, and 1 filters, respectively. Each layer has a 3X3 kernel, the first two with leakyReLU activation and the final probability map with a sigmoid activation. Finally, we use binary crossentropy loss function and adam optimizer

Results

This section focuses on the results and the validation of the algorithm. We chose F_1 score as our metric for validation because it balances between precision and recall, which is explained in the box below.

F_1 Score

F_1 score is one of the most common metrics when assessing a machine learning algorithm. It is defined as the harmonic mean of precision and recall, as shown below. In other words, it measures the exactness and completeness of the algorithm. The range of F_1 score is from 0 to 1, where 1 is the best score and 0 is the worst.

$$F_1 = \frac{2}{\frac{1}{\text{precision}} + \frac{1}{\text{recall}}} \tag{2}$$

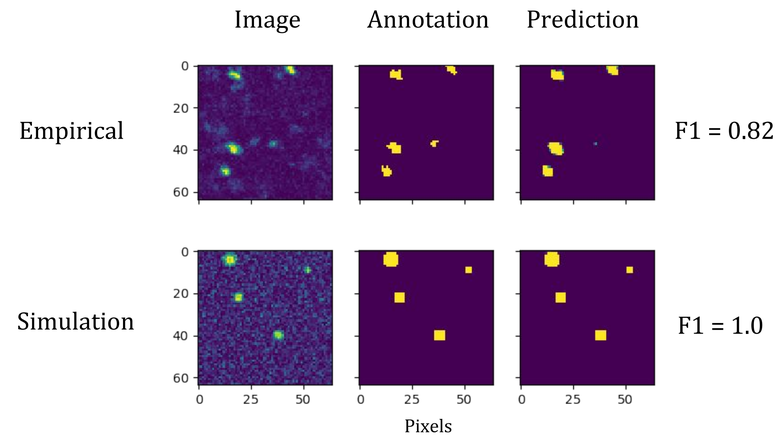

Figure 4:A panel of results for an empirical image and a simulation image including a 64 × 64 section of the original image, a true annotation and a machine prediction. The first row is the empirical image. The bright dots in this row represent the synapses. It is clear that the prediction captures the bright dots and excludes the noisy background. As a result, the F_1 for empirical image is 0.86. The second row is the simulation which has a F_1 score of 1.0. This means that our algorithm picks out all of the bright spots and no background noise.

Software	Precision	Recall	F_1 Score
GoogleNet	0.833	0.751	0.784
AlexNet	0.842	0.703	0.758
detectSpot	0.836	0.740	0.782
SimuSpot	**0.762**	**0.895**	**0.824**

Table 1:A table comparing our method, **simuspot**, to other spot detection results. The results from all other methods were trained and tested on different simulated data - we are simply reporting and comparing results to a study that can be found in Mabaso et. al. 2018.

Future Directions

The future directions of this tool are to continue to improve the simulator with more parameters such that it is able to more accurately represent the more difficult channels, Synapsin and Gephyrin. With more customization our tool will be able to be applied to many other problems and types of fluorescent images.

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