PPSD: Probability Principled Synapse Detection

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***Abstract*—Synapse detection and characterization is an indispensable component of today's brain research. Recent biotechnical progress makes the synapse data widely available to many labs and have never been easier to generate. Awkwardly, the progress in analytical approaches lags far behind. From our experience of real studies we found the performance of existing algorithms are far from satisfactory, with either high false positives or high false negatives, due to the large noise in bioimage data, imperfection in the probe selectivity and heterogeneity in the signal intensity. We realized that the existing algorithms lack rigorous probability foundation to battle with the stochastic nature of synapse data. In this paper, we report a new method named Probability Principled Synapse Detection (PPSD) to reliably detect synapses through a build-up of a series of accurate probability models and strategies, ranging from estimating and stabilizing noise variance, designing test scores using order-statistics theory, and devising an iterative and adaptive thresholding strategy to locate candidate regions. We tested the proposed method on extensive simulation and real studies and the results are very encouraging with significantly higher sensitivity and specificity than peer methods.**

***Index Terms*— synapse detection, iterative and adaptive thresholding, order statistics, variance-stabilizing transformation.**

I. Introduction

Synapse is the critical structure in the nervous system that enables the communication and interaction between neurons. Thorough and accurate characterization of synapse is vital to understanding how normal brain works and how diseased brain goes wrong, as cognitive function hinges on proper wiring and connection in the neuronal circuits through synapse [1, 2]. Recent advances in imaging technique and probe design make it realistic to image the synapses at high resolution and large scale. With the increased capacity of data generation, the bottleneck shifts to the analysis and extraction of information from the data, in which synapse detection is one of the major tasks.

In this paper, we limit our scope to the light microscopy based synapse detection and the electron microscopy will not be discussed. There are a few synapses detection algorithms designed for light microscopy data. Among them, SynD [3] is the most widely used one in the community, to our best knowledge. However, in addition to the requirement of the neurite mask, SynD uses a single threshold and the deconvolution approach will produce a lot of false signals when the image is noisy or the staining is not highly selective. Feng [9] proposed a method to separate synaptic punctas that are clustered together based on the shape and brightness property of synapse using a Bayesian Gaussian mixture model. Again, this method uses a single threshold to segment the image and the foreground part is used to find synapses. However, our experience is that very often there is no single threshold fit to all synapses. In contrast, both SynPAnal [4] and Fish [5] used multiple thresholds to segment the synapse. Yet, none of the algorithms provided rigorous statistical quantification to assess the detected synapses and the whole procedures were rather ad hoc. It is hard for users to determine which region should be regarded as true candidate synapses, when the detected regions should be separated, or when a smaller part should be extracted from a larger one.

The overarching theme that is lacking in the existing algorithms is the adoption of probability principle in an integrated way. We argue that a good approach for synapse detection should be based on accurate probability models in all steps. The adoption of probability principle is particularly pressing for this task, considering the fact that light microscopy data often has low signal-to-noise ratio due to the limits of biological probes or immuno-histochemical staining [8].

Here we developed a new synapse detection algorithm, Probability Principled Synapse Detection (PPSD), by building accurate probability models. PPSD has two unique features.

First, in order to simultaneously detect thousands of synapses whose signal intensities and contrasts may be considerably different, an iterative and adaptive thresholding scheme was designed. Importantly, different from the multiple-threshold algorithms SynPAnal [4] and Fish [5], which select as synapse the region passing the highest threshold, PPSD finds the region with the strongest statistical evidence (smallest p-value under the null hypothesis that it is pure noise). Indeed, a region with multiple moderately bright pixels is more likely to be a synapse than a region with a single brighter pixel.

Second, we propose to use order statistics as a key component of algorithm to determine the statistical significance of each candidate region. The conventional t-test between a group of candidate pixels and their neighbors is not correct since the thresholding operation has already changed the null hypothesis. A candidate region always has higher average values than its neighbors by definition.

Besides, since the order-statistics based approach requires a homogeneous variance of each individual pixels and estimate of the variance, we estimate and stabilize the variance using a Poisson Gaussian model [7].

This paper is organized as follows. In Section II we describe our detection algorithm. In Section III we present the simulation

results and the results from a real-world co-culture data set. After making discussions in Section IV, we summarize the paper in Section V.

II. Methods

The flow chart of PPSD is shown in Fig.1. We first transform the image by variance-stabilizing transformation and then noise variance is estimated. Multiple scans at thresholds from low to high are then performed. For each threshold the significance of each region is computed by order statistics. Each time one best candidate is selected. After that, the algorithm post processes the data with some synapse filtering rules like size and intensity.

A. Transformation and noise variance estimation

Statistical tests require the knowledge of noise variance. This information can be acquired either locally or globally. The local approach picks the samples near the ROI and compute the variance based on those samples while the global method uses the whole image. For local t-test, the variance is considered in computing the test statistics. For order statistics based significance tests, the variance is explicitly needed. A local variance estimates can be unreliable due to the limited sample size near the putative synapses and a global estimate is more desirable.

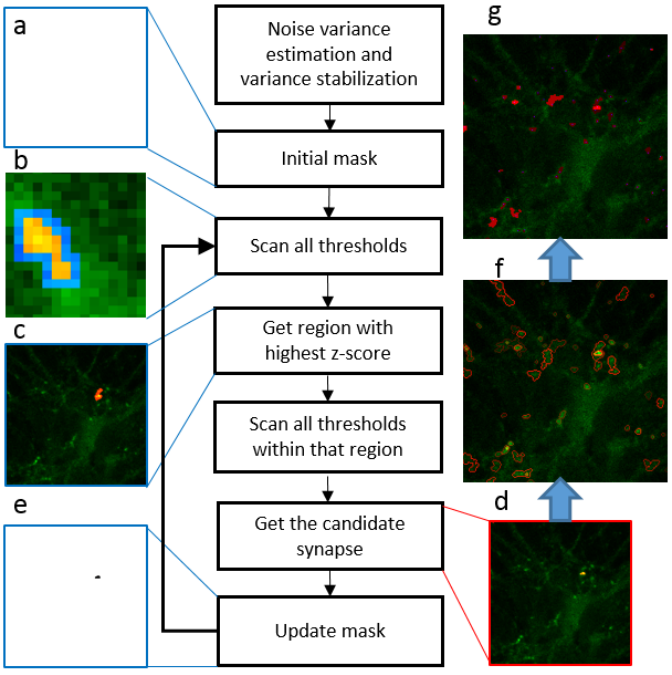

Figure 1. Flow chart of PPSD. (a) The original mask uses all pixels. (b) For each region from each threshold, a z-score is computed from the region (yellow) and its neighbors (blue). (c) For all the regions, find the one with the highest z-score. (d) Within the region with the highest z-score, we search across thresholds for potential sub regions. If succeed, the sub region will be a candidate synapse. (e) A new mask excluding all candidate synapses previously found are computed. (f) All candidate regions are gathered and filtered by rule 1: size and intensity. (g) The remaining regions are filtered by rule 2.

We start by estimating the mean dependent noise variance [7]. Each pixel is modelled as,

$$\mathrm{var}(y_{i,j}) = ax_{i,j} + b. \tag{1}$$

Here (i, j) is the pixel coordinate and $\mathrm{var}(y)$ is the pixel noise variance. x is the true underlying signal intensity, which is not observed. This item models the Poisson type noise and b models the additive Gaussian noise. The model is fit using single image and the resulting a and b are used in the generalized Anscombe transform [7] to stabilize the noise.

B. Order statistics based significance test

In our adaptive thresholding algorithm, for each threshold we will select a set of isolated regions and each of the regions has some pixels. These regions are potential synapses that need to be determined by statistical tests. An individual test is based on the difference of a single region and its neighbor pixels. A larger difference implies a larger possibility that this region is a synapse. For each region, a group of neighbor pixels are selected. Assume there are M pixels $\{x_1, \dots, x_M\}$ in the region and N pixels $\{x_{M+1}, \dots, x_{M+N}\}$ in the neighbor. Due to the thresholding operation, all the M pixels have higher intensities than the N neighbors. Even there is no synapse, some positive difference exists between the means of the two groups, which is undesirable. Thus we define the test statistic as

$$L = \frac{x_1 + \cdots + x_M}{M} - \frac{x_{M+1} + \cdots + x_{M+N}}{N}, \tag{2}$$

where $x_1 \geq \cdots \geq x_{M+1} \geq \cdots \geq x_{M+N}$. Without synapse, L will be positive, and the exact value is determined by the noise variance, the sample size and the ratio of M to N. The mean and variance of L is calculated using the theory of asymptotic order statistics. Let $n = M + N$, we rewrite L as in [6]:

$$L = \frac{1}{n}\sum_{i=1}^{n} J\left(\frac{i}{n+1}\right)x_i. \tag{3}$$

So for $1 \leq i \leq M$, $J\left(\frac{i}{n+1}\right) = \frac{n}{M}$, and for $M+1 \leq i \leq M+N$, $J\left(\frac{i}{n+1}\right) = \frac{n}{N}$. This summation need to be approximated by integration. Let $u = i/(n+1)$, we define

$$\mu(J, F) = \int_0^1 J(u)F^{-1}(u)du, \tag{4}$$

and

$$\sigma^2(J, F) = \iint_{0<u_1<u_2<1} \frac{2J(u_1)J(u_2)u_1(1-u_2)}{f(F^{-1}(u_1))f(F^{-1}(u_2))}du_1du_2. \tag{5}$$

Then we have $E(L) = \mu(J, F)/\sqrt{n}$ and $var(L) = \sigma^2(J, F)/n$, when $n \to \infty$ [6]. Here f is normal probability density function with zero mean and variance estimated as above. F^{-1} is the corresponding inverse normal cumulative distribution function. The integration is computed by summation using all the n samples. The above computation is valid when the sample size is large enough, which may not be the case since one synapse may only contain about 10 or less pixels, depending on the image resolution. Here we apply two corrections for the small sample size. First we notice for the double integration in $\sigma^2(J, F)$, the integration space is a triangle defined by $0 < u_1 < u_2 < 1$. Since we are using discrete samples, the boundary points will noticeably impact the integration results. So half of

the boundary points are incorporated in the integration and the other half are not.

The integration over J is based on a uniform grid, which correspond to the x values. However, the boundary points x_1 and x_n strongly deviate from this uniform assumption and the results will be affected when the sample size is small. We hope the integration can mimic the summation. Therefore we compute the distribution of the largest (or smallest) sample and use the mean to get new grid. This mean value d is computed by

$$d = 1 - F\big(E(x_1)\big) = 1 - F(n\int_0^1 F^{-1}(t)t^{n-1}dt). \qquad (6)$$

Here t should be densely sampled from 0 to 1. Then we get a new grid $[d, \dots, d + (i-1)\frac{1-2d}{n-1}, \dots, 1-d]$.

C. Iterative Detection and post processing

Our iterative synapse detection scheme is driven by the statistical significance of each region, which tries to find the region that has the best contrast with its neighboring pixels. After stabilizing the variance and estimating the global noise, we scan from a lower threshold to a higher one with a suitable step size. For each threshold, some regions with enough pixels will be selected and a z-score is computed based on each of these regions and its neighboring pixels using order statistics, as discussed above. Across all thresholds the region with the highest z-score is chosen. Then an inner loop is applied on this selected region and the neighbors are restricted to reside within that region. If something significant is found within that region, the significant subset is regarded as candidate synapse(s). Otherwise the region itself is treated as a candidate. Then we remove the candidate(s) and repeat the thresholding steps. The statistical significance is based on the Bonferroni correction using all the regions within all the thresholds at this iteration. This is conservative since across different layers the overlapped regions may be highly correlated.

Several rules based on the prior knowledge of the size and relative positions of synapses are applied to the candidates found. First a synapse should not be too large. Otherwise it is likely to be areas with elevated background intensity, such as in soma or dendrites. Second if some region is surrounded by other regions and the surrounding one has smaller values, the surrounding ones should be discarded. The remaining candidates are reported as synapses.

III. Results

A. Simulation with pure noise

In order to compare local t-test to PPSD in terms of false positives, we simulate an image of size 1024×1024 and add additive Gaussian noise with zeros mean and unit variance. The threshold scans from 1.2 to 1.8 with step size 0.2. Ideally no synapse should be identified in this image with pure noise. Here we assume the noise variance level is already known. We count within all layers the total number of candidates given by the threshold and those pass the Bonferroni-corrected significance level of 0.05. Totally we get 2574 candidates. PPSD have only 12 candidates pass the significance threshold while t-test will generate 1310 false positives. So t-test is not capable of dealing with false positives in thresholding based methods (Table I). We also observed that the control of the false positives for our method is conservative, which is likely due to the correlation of scores between overlapped regions as discussed above. Then we repeat the experiment with a poorly estimated noise variance of 0.5. Then both tests generates many false positives. This confirms the importance of noise variance estimation in evaluating the significance level.

TABLE I. False positive rate

Noise variance	T-test	Order Statistics
1, True noise variance	0.5433	0.00466
0.5, a poor estimates	0.5392	0.424

B. Simulation with simulated dendrites and synapses

To study the performance of detecting synapses, we designed a realistic simulation. First we select dendrites based on a real Tuj1 labelled neuron image. Each dendrite is assigned a brightness level randomly to make it close to real image. Then for each dendrite, we randomly put synapses onto it. The number of synapses on each dendrite is proportional to the length of it. The synapse intensity is determined by the dendrite it located. So the synapses on the darker dendrites will also look darker. For simplicity, all synapses here are squares with 25 pixels each. The results are shown in Table II.

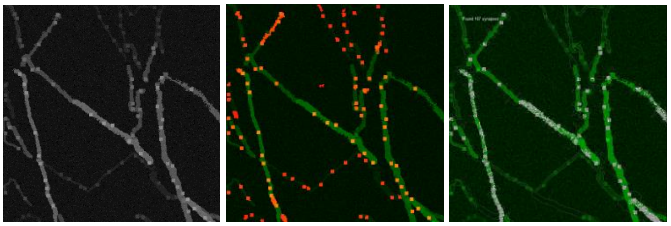

Figure 2. Left: simulated synapses. Middle: detection results by PPSD. Most synapses are found and we have only several false positives Right: results from SynD with thr=1. Many synapses are missed.

We first compare the performance of traditional single threshold based detection algorithm and PPSD algorithm. For single threshold method, no statistical test is applied. The precision and recall is computed for each threshold and the threshold corresponding to the best F1 score is given. The F1 score is the harmonic mean of precision and recall.

PPSD can achieve much better precision and recall performance than the single threshold based methods, which sacrifice either precision or recall. The detected synapses are shown in Fig.2. Although when multiple thresholds are used, t-test produces a good recall by selecting more synapses, however, the precision is very low. The single threshold method SynD can detect similar number of synapses as t-test, but the de-convolution approach reports a much large number of synapses, which lead to lower precision.

C. Neuron astrocyte co-culture data

We apply PPSD to a neuron astrocyte co-culture image. The image includes three channels, the blue channel is for nucleus staining with DAP1, the green channel is for synapse labeling by synapsin I and the red channel is for neurite labeling with

Tuj1. Here we only use the green channel to detect the synapse, although we can further use the Tuj1 channel to filter out the noise parts far away from the neurites.

TABLE II. PRECISION AND RECALL

Method	Precision	Recall	F1 score
PPSD	0.91	1	0.95
Multi threshold and t test	0.47	0.94	0.63
Single threshold, no significance test	0.89	0.48	0.62
SynD Thr=1	0.33	0.54	0.41
SynD Thr=1.5	0.52	0.34	0.41

Under default settings, 1003 synapses are detected, which are labelled in red in Fig.3 (left and middle). The synapse centers are chosen as the pixel with the largest intensity in each synapse, which are labelled as blue dots. Most synapses are detected even without the help of neurite mask and very few false findings exist. We also apply SynD, which identifies a lot of synapses in the noise regions, as shown in Fig.3 (right). Though there are several other tools [4, 5, 7] that can be used to detect synapses and we have not compared them with our method yet, we need to emphasize that none of them is able to provide a statistical evidence of the detected synapse and the selection of synapse is based on an ad hoc approach.

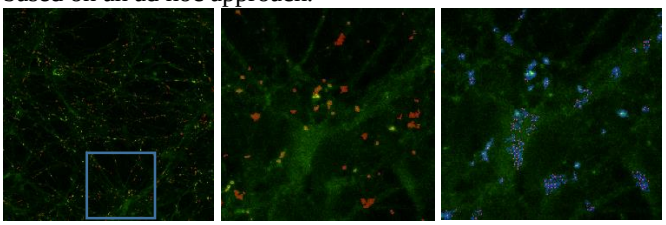

Figure 3. Left: detection results on the synapsin I channel of the co-cultured image. Middle: the blue square in the left. Minimum size of synapse is set to four pixels. The significance threshold is 0.05 after Bonferroni correction. Candidates whose size is larger than three times the typical synapse size (1 μm^2) or with too small means intensities (30 here) are discarded. Right: SynD on the same area. Many synapses are detected on the noise parts. Some dark parts are not detected due to the single threshold.

IV. DISCUSSION

The statistical measure in PPSD is the difference between the synapse and its neighboring pixels, instead of the probability of being a synapse, although they are tightly connected. First, since some properties of synapse like shape and position are not modelled in our statistical framework (although they are considered in the post processing step), the proposed framework may not be able to find the probability of being a synapse. Second, the p-value here should not be simply understood as the probability of an area of being a synapse. To achieve this goal, we need to define an alternative hypothesis, which is out of the scope of this paper.

PPSD can deal with different noise levels since it is fully probabilistic driven. When an image has a large estimated noise level , the null hypothesis will be more difficult to reject, which leads to less significant p-value for each area. The larger the noise, the less synapse will be selected for a given threshold. With noisier images, more false negatives are inevitable in order to control the number of false findings, especially given the conservative p-value reported by PPSD. For extremely noisy image, even no areas can pass the significance threshold.

Some imaging effects can be included in PPSD by linking them to noise variance. The quality of illuminance is related to the noise level since less than adequate illuminating time will lead to lower signal to noise ratio. Besides, the requirement for micro contrast is also directly related to the noise level. If the micro contrast around a synapse is small comparing with the noise variance, that synapse will have a less significant p-value. Simulations for various conditions and more data sets are being performed and are not shown here due to space limitation.

However, when the antibody is not specific, PPSD cannot tell which one is synapse and which is not. This kind of conditions need to be handled case by case. Besides, when the synapses in the cluster do not have clear boundary, which is quite common, the algorithm in [9] can be used as a post processing step to split the cluster to individual synapses.

V. SUMMARY

In this paper, we proposed PPSD method to detect synapse in a rigorous and probability-principled way. Simulation shows that our approach is both more sensitive and specific than existing methods. The results on real images show that our method can detect most synapses without being affected by noisy regions.

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