

A Hypothesis Testing Approach For Fluorescent Blob Identification

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Abstract—Template matching is a common approach for identifying fluorescent objects within a biological image. But how to decide a threshold value for the purpose of justifying the goodness of matching score is a rather difficult task. In this paper, we propose a framework that dynamically chooses appropriate threshold values for correct object identification at a non-arbitrary statistical power based on the local measure of signal and noise. We validate the feasibility of our proposed framework by presenting simulation experiments conducted with both synthetic and live-cell data sets. The experimental results suggest that our auto-thresholding algorithm and local signal to noise ratio estimation can provide solid means for effective spot identity in place of an ad hoc threshold fitting value or minimization method.

Keywords-fluorescent blobs identification; cellular image analysis; auto thresholding; template matching

I. INTRODUCTION AND BACKGROUND

Object identification and tracking in live-cell microscopy experiments provides critical quantitative information for biologists [2]. Correct identification of an object within an image ultimately depends upon how well the intensity pattern captured in the image matches a representative object model. Template matching [1] is a technique in digital image processing for identifying sub-regions of a search image that are similar to a particular target image (template). This method requires a threshold value to serve as a standard for determining whether an image region is more likely to be the known pattern than it is to be noise or a different pattern. Additionally, the detection accuracy changes significantly with varying the threshold value. For the automated matching procedure to work efficiently, one should be able to choose a cutoff threshold of the appropriate parameter for the purpose of justifying the goodness of the matching score and also be able to have a non-arbitrary degree of confidence that this cutoff value will produce good results. Unfortunately, how to decide a threshold value in a systematic way is a rather difficult task. In most cases, the threshold value is determined empirically and will be kept as a constant over the course of template matching operation from regions to regions within images. In fact, the threshold value should be changed dynamically based on the imaging conditions and the “one value fits all” model does not –cannot– scale well with the heterogeneity of the images.

In this work, we employ a version of the model-based pattern matching approaches previously developed for blob identification in time-lapse microscopy experiments [2], [3], [7], [8]. These approaches have in common a model generated for testing against positions in the image. The relative goodness of fit for the model and potential object in the image is used as the primary criteria for object identification. We hypothesize that the criteria for determining threshold values for correct object identification will be inherently limited by the local, and less so by global, signal to noise properties of the image. Therein, we have created methods for determining the threshold values for correct object identification at a non-arbitrary confidence level based on both the local goodness of fit score and a local measure of signal and noise.

II. PROPOSED FRAMEWORK

A. Template Matching

Template matching is a method that makes use a correlation algorithm to locate small parts within an image that have high identify with a target image (a.k.a. template). To do the matching, we shift the target image to every location in the search image and then perform an operation (described below) between the template and search pixels that are overlaid. The output value of the operation will be favorable only when the template and the corresponding sub-region in the image are similar.

Sum of the absolute differences (*SAD*) and cross correlation (*CC*) are two popular techniques for estimating the degree of correlation between a template and a sub-region within a search image [2], [3], [7], [8]. Briefly, *SAD* is defined as the sum of the absolute value of the difference between each pixel in the template and the corresponding pixel in the testing region of a search image, whereas *CC* is defined as the sum of the dot product between each pixel in the template and the corresponding pixel in the testing region of a search image.

B. Local Signal to Noise Ratio Estimation

The signal to noise ratio (*S/N*) of an image is a measure that compares the intensity of the desired signals to the variation in the background intensity. In practice, the *S/N*

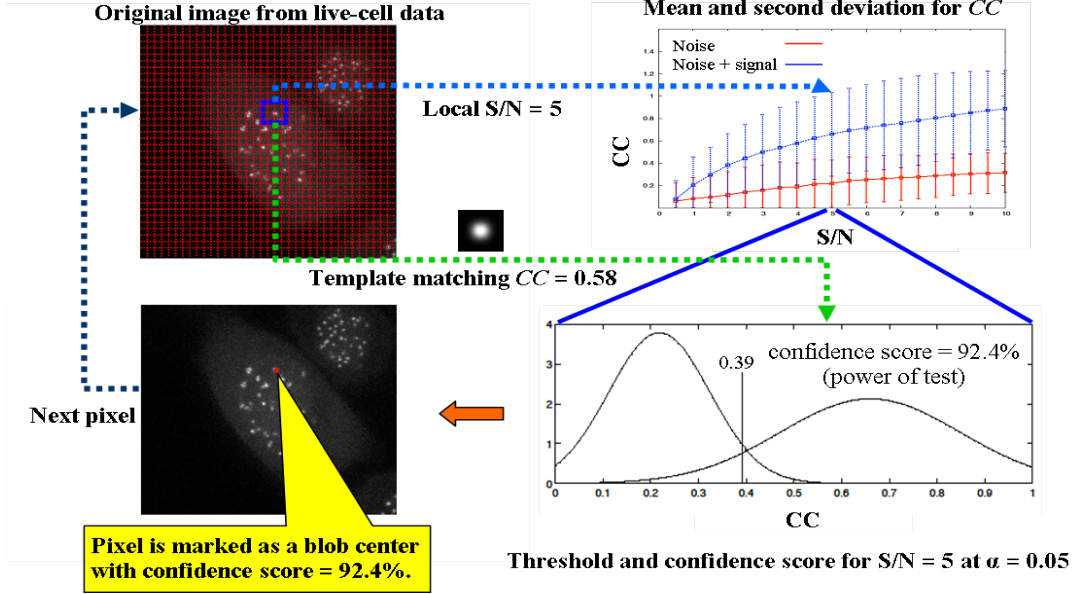


Figure 1. Illustration of the blob identification process at $\alpha = 0.05$. Prior to blob identification, a mapping of S/N vs. CC cutoff value and a mapping of S/N vs. confidence score are generated from images with and without a ghost model image as described in Sec. II-C.

can have different definitions depending on how signal and noise are characterized [5]. For example, S/N is commonly defined as the ratio of the mean value μ of signal strength to the standard deviation σ of noise strength. A higher S/N indicates there will likely be a distinction between the foreground and background pixels. Ideally, S/N determines the absolute detection limit and governs the precision of locating the center of an object. We propose that S/N should be a significant factor in setting an appropriate threshold for correct object identification.

However, for images with more than one uncorrelated source (noise), it is difficult to construct their exact signal or noise models. To cope with this problem, we adopt an approach initially proposed by [6] to compute the S/N of a sub-region within an image:

$$\frac{S}{N} = \frac{(C_{on} - n \cdot X_{back})}{\sqrt{\frac{(C_{on} - n \cdot X_{back})}{G} + n \cdot \sigma_{back}^2 + \frac{(n \cdot \sigma_{back}^2)}{p}}} \quad (1)$$

where C_{on} is the intensity of the signal, X_{back} is the mean intensity of the background, n is the number of pixels within the measuring region, σ_{back} is the standard deviation of the background, G is the gain factor of the system, and p is the number of the background pixels. Note that, this S/N estimation is for a local position within the image and is not for the entire image. We reason that under the conditions typically used for biological time-lapse imaging of fluorescent blobs, knowing the S/N of the entire image will not directly improve our ability to identify blobs, because the search scope is limited by the dimension of the template.

C. Auto Thresholding With Confidence Score

The first step of our auto-thresholding algorithm is to setup a sequence of different S/N values such as ranging from 0.5 to 10. This range should be larger than the maximum local S/N existing in the search images in order to cover all the possible situations. For each S/N , we collect two sets of images in the same size as the template, one set containing only noise and one set containing both noise and signal. Next, we apply template matching on these two image sets and collect the output fitting value of each image. To this end, for each S/N , we have two overlapping fitting result distributions one from fitting to noise and the other from fitting to signal plus noise. We compute their critical value and power of a statistical test based on two assumptions: (1) the type I error (α) equal to 0.05 (one tail on right hand side); (2) the fitting output distribution from the images without signal is the null hypothesis. Finally, for each S/N , the associated critical value will be used as the threshold value and the associated power of the test value will be used as the confidence score ψ for identifying a blob.

The idea is that the power of the test is the probability of correctly rejecting the null hypothesis. For our case, it is the probability of correctly assigning a position in an image as a blob center. Note that, to keep it simple, we set $\alpha = 0.05$ without using any optimization approach such as Neyman-Pearson criterion [4], but this is not a general requirement for the proposed algorithm.

Given a S/N to fitting threshold mapping table W^c , a S/N to confidence score mapping table W^o , a fitting function, and a template, we can now identify the potential

blob centers within images. The identification process begins with the template fitting and local S/N computation (Eq. 1) for each pixel in the search images. If the template fitting value of a pixel is not smaller than the threshold value in W^c given its local S/N , this pixel will be marked as a blob center with a confidence score value obtained from W^o . In contrast, if the template fitting value of a pixel is smaller than the threshold value in W^c given its local S/N , it will be marked as background. Fig. 1 shows an example of identifying a potential blob center from live-cell data by using CC as the matching approach. In this example, one pixel in the image has local $S/N = 5$ and $CC = 0.58$. Based on the W^c and W^o tables, the CC cutoff value is 0.39 and ψ is 92.4% while $S/N = 5$. Therefore, this pixel will be marked as a blob center with $\psi = 92.4\%$.

III. EXPERIMENT WITH SYNTHETIC IMAGES

A. Experimental setup

In this experiment, we want to verify how effective the S/N estimation (Eq. 1) can capture the local image condition as well as to evaluate how far apart the fit metric for the target is (on average) above the number it gives us for noise alone over a range of S/N levels. We construct two sets of synthetic data to simulate images for noise and noise+signal with S/N ranging from 0.5 to 10 in 0.5 increment. For each data set, we create 1000 $7X7$ pixel images at each S/N level. Each noise image contains background intensity = 500 with Gaussian noise $N_e = 10$, and auto-fluorescence intensity = 100 with Poisson noise $N_a = 10$. Each noise+signal image contains additional 2D Gaussian signal intensity with the max. center value ranging from 0 to 180 and Poisson noise N_s equals to the square root of the signal.

To compute the local S/N by using Eq. 1, we pick the intensity of the center pixel in our $7X7$ pixel model image as C_{on} and the border pixels as the background. Both CC and SAD are used as the metrics for template matching. We use a $7X7$ pixel 2D Gaussian signal model with σ ranging from 1.4 to 2.4 as templates. The best-fit value from different templates will be collected as the final assessment for each search image.

B. Experimental Results

Let us analyze the results obtained from this experiment. First, we focus on the accuracy of our estimated local S/N compared to the ideal measured one. As described in Sec. III-A, we use the variance of the border pixels and an estimate of peak intensity of the images to compute their associated S/N . Fig. 2 shows, while $S/N \leq 4$, our S/N estimation (Eq. 1) closely predicts the S/N to the idea measured value, which is computed as $signal/\sqrt{N_s^2 + N_a^2 + N_e^2}$. As $S/N > 4$, our approach tends to underestimate the S/N . This finding indicates that our framework will conservatively assign ψ values to regions which have higher local S/N due to the fact that ψ is governed by the local S/N .

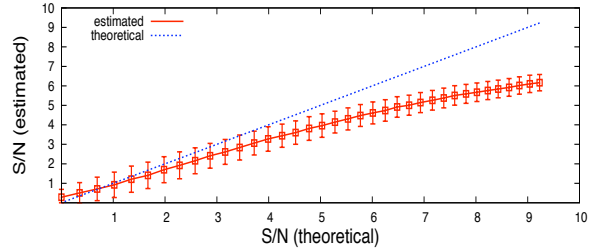


Figure 2. Average estimated and theoretical S/N from the synthetic noise+signal images.

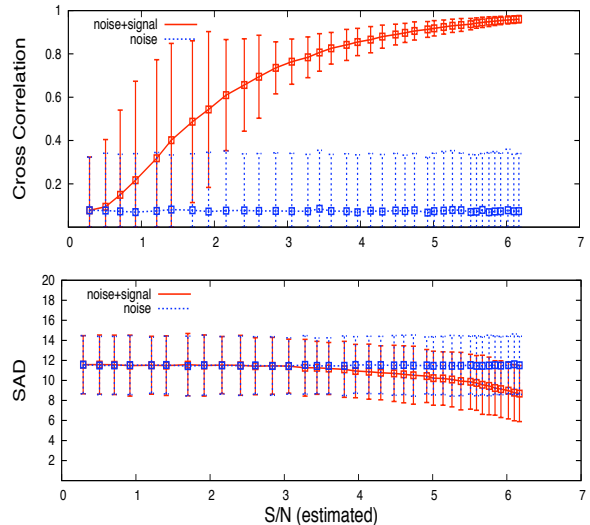


Figure 3. Average fitting score of synthetic noise and noise+signal images using CC (top) and SAD (bottom) methods.

To illustrate how the actual search performance changes with different fitting approaches, we apply CC and SAD to both the noise and the noise+signal images. Representative data plots (Fig. 3) show the mean \pm second standard deviation for a simulation corresponding to moderate auto-fluorescence in our specimens. We found that CC is more sensitive (effective to a lower S/N) than the SAD using a noise model that more accurately depicts our specimens.

IV. EXPERIMENT WITH LIVE-CELL IMAGES

A. Experimental Setup

To validate that our proposed framework can work with real data, we conduct an experiment with a sequence of images taken from living mammalian cells. To build the hypothesis testing model for ad hoc threshold fitting, we randomly select a $7X7$ pixel region from the images as a noise model and then cluster the selected image based on its estimated S/N . We continue this random sampling process until all the S/N groups between 0.5 and 10 have 1000 different images. Since the number of real blobs in images is small, we do not consider the effect that some sampled images may contain signals. For the noise+signal model,

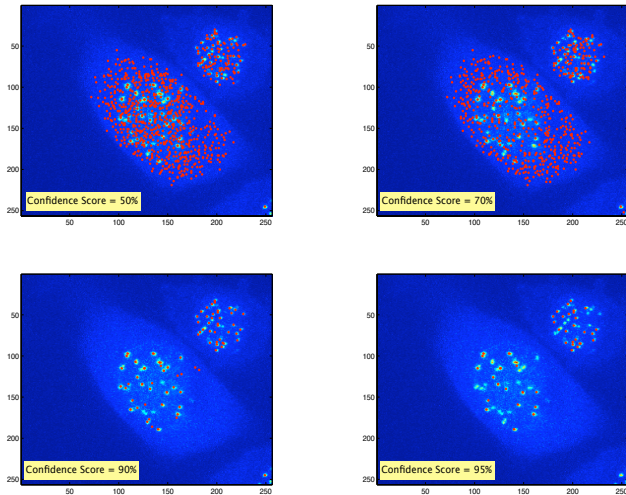


Figure 4. Blob centers prediction with confidence score $\psi = 50\%$, 70% , 90% , and 95% from one image of a live-cell data.

the random sampling images are modified by adding into each image a 2D Gaussian signal with the max. center value ranging from 250 to 5000. We then cluster images based on their new estimated S/N . We continue this process until all the S/N groups have 1000 different images.

Only CC is used as the matching approach for our blob search since it is more sensitive to our data type (Ref. Sec. III-B). We use a 2D Gaussian signal model with σ ranging from 1.4 to 2.4 and with size ranging from 5 to 7 pixels as matching templates. The best-fit value from different templates will be collected as the final assessment for each pixel in search images.

B. Experimental Results

To illustrate the quality of search results obtained through our proposed framework at different degree of confidence, we filter out the identifying results by using the S/N vs. ψ mapping table W^o with $\psi = 50\%$, 70% , 90% , and 95% as shown in Fig. 4. From these plots, we can see how selecting different ψ will impact the search results. Using a filter with a low ψ value (e.g., 50%), we will end up with too many candidates. In contrast, using a filter with a high ψ value (e.g., 95%), we will reject some real blob centers. Fig. 4 also shows, while setting $\psi = 90\%$, the search performance of our proposed framework can reach recall as high as 0.91 and precision as high as 0.88. Precision refers to the fraction of correct blob center locations identified compared to the total number of locations marked by our framework and recall refers to the fraction of correct blob center locations identified by our framework compared to the total number of real blob center in the image.

V. DISCUSSION

In this paper we explored the idea that the criteria for selecting threshold values for correct object identification based on the template matching will be inherently regulated by the local signal to noise properties of the image. The experimental results suggest that the local S/N estimation (Eq. 1) can be successfully integrated into our auto-thresholding algorithm to provide solid means for dynamically choosing appropriate threshold values for fitting at a non-arbitrary degree of confidence. Several issues are under investigation. We would like to explore different strategies for choosing the matching template in our proposed model. The way to decide the signal and noise strength used to compute the local S/N in Eq. 1 can be further studied. Additionally, we plan to study the use of spatial information for identifying possible blob center not only with their individual intensity but also that of their neighborhoods.

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