Robust modeling for the automated analysis of synaptic vesicle recycling assays

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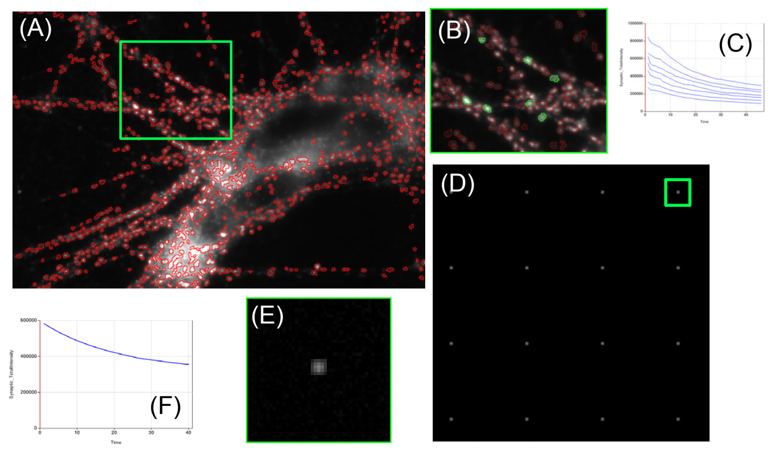
Introduction

The use of time-lapse subcellular microscopy to assay the exo-endocytic recycling of synaptic vesicles provides an important tool for understanding presynaptic function. The development of automated image analysis software for the recognition of fluorescently labeled axon terminals to characterize the kinetics of fluorescence destaining are beneficial for improving the speed of analysis, increasing the experimental sample size, and removing subjective bias from manual analysis. However, like many assays involving subcellular and temporal phenotypes, the synaptic vesicle recycling assay signal is often weak and unstable due to corruption by noise. This represents a significant challenge to the analysis algorithm and therefore limits the quality of assay outcomes. We have previously evaluated the use of a robust recognition method based on confidence maps as opposed to binary masks, and determined that the robust recognition method improves the rate of detection, the accuracy of object definition, and the reliability of assay measurements. In this study, we evaluated methods to improve the robustness and accuracy of kinetic characterization using the exponential dissociation model parameter τ. These methods include the spatial and temporal regulation of confidence maps, the use of robust image measurements, τ fitting with iterative improvement, and τ fitting with outcome directed data integrity assurance. Our results show that these methods can improve the robustness and accuracy of assay measurements. The use of confidence maps has the biggest positive impact, and the use of confidence maps in combination with iterative fitting yields the best overall performance.

Materials and Methods

Figure 1. One Real and Two Simulated Image Sets Were Created. (A,B) Eighteen image sets from synaptic vesicle recycling assays using fluorescent FM dye are used for this study ("Real Image Set"). The time-lapse images show the destaining of individual synapses of a small number of rat hippocampal neurons in microisland culture. (C) Each series has 65 images acquired at a rate of 1 Hz. A manual definition of synaptic boundaries was made and used as a "gold standard truth" from which measurements were made. Despite having true synaptic boundaries, true τ cannot be known in the real images. (D, E) Thus we created a single time-series image containing sixteen synthetic puncta that "destain" with a known rate of τ = 20 (F). A second simulated image set (not shown) was created where each of the synthetic objects is subjected to a random, sub-pixel shift, along with four levels of zero mean Gaussian noise. Here we refer to these simulated images as the "Simulated Without Shift", and "Simulated With Shift" images respectively (two sets of five movies). Four levels of simulated zero mean Gaussian noise was added to each of the real and simulated image sets for performance evaluation. The main performance metric, τ error, is defined as the absolute difference between the true τ and the estimated τ.



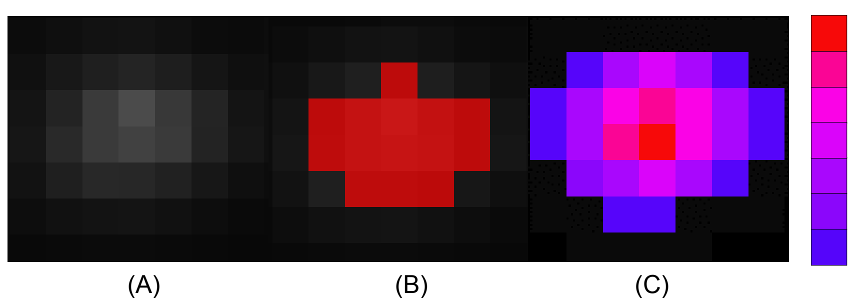
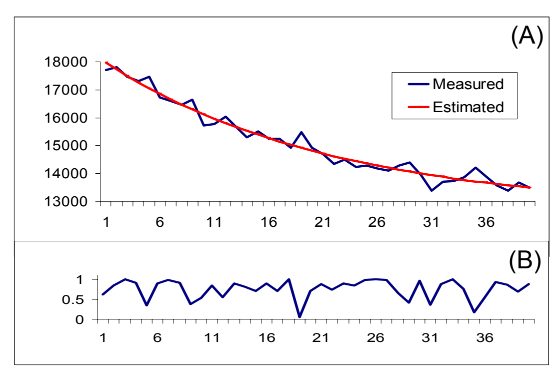


Figure 2. The Spatial-Temporal Regulation of Confidence Maps Was Evaluated. (A) Original image grayscale intensity values showing an axon terminal labeled with FM dye. (B) Binary masks make a crisp on / off association of pixels to objects. (C) In contrast, confidence maps make a probabilistic association of pixels to objects using a confidence function. Non-binary, the confidence map encodes a confidence score between 0 and 255. The confidence map values are used to weight confidence based measurements. For example, the median measurement typically defined as $m_t = Median\{I(x,y)\,|\,\forall(x,y)\in O\}$ where *(x,y)* are pixels in object *O*, can be weighted as follows using confidence map values C(x,y): $m_{tc} = Median\{C(x,y)*I(x,y)\,|\,\forall C(x,y)\in O\}$

Figure 3. An Iterative Fitting Method for Temporal Regulation of Confidence Maps Was Evaluated. Multiple iterations of model fitting refinement could progressively reduce the effect of data variation and thereby increase the accuracy and repeatability of the model fitting result. An equal weight τ fitting is performed at the first iteration. After each iteration, the error of each data point from the current τ predicted value is determined. The weight of each point in the series is adjusted given the error (lower weight for large error). The process is repeated until a stop criteria is met. (A) Shows an example measured data and estimated τ curve for a synthetic object from a "Simulated without Shift" image with noise σ = 4. (B) Shows the weights calculated for each time point. τ is an exponential dissociation model parameter which we calculated through a non-linear, iterative fitting of the measured data to the first order exponential decay function , where A is the amplitude and B is the offset. A is constrained to be greater than 1, and B is constrained to be greater than or equal to 90% of the max of either the last value in the series, or the average of the minimum value and the last value in the series. Weights are calculated as a function of the error and the variance of the data series as follows: $w_t = (1-\frac{1}{2}\cdot\ln(2\pi)-\frac{\varepsilon_t^2}{2\sigma^2})\cdot e^{\frac{\varepsilon_t^2}{2\sigma^2}}$. This formula was derived with the use of entropy (information theory framework[1].



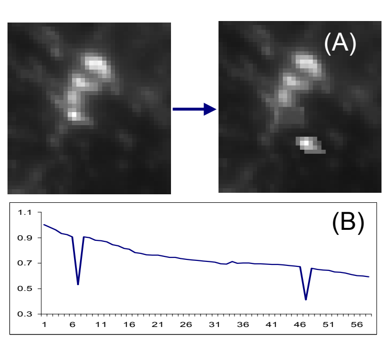


Figure 4. A Data Integrity Assurance Method for Error Correction Was Evaluated. In the synaptic vesicle recycling assay, FM dye labeled axon terminals may sometimes shift position causing large measurement error. The data integrity assurance method uses the temporal measurement profile to identify and distinguish between measurement errors and outliers. Outliers are removed from fitting consideration, and measurement errors are corrected. (A) Illustrates a synthetic image where a labeled axon terminal has been artificially shifted. (B) shows the uncorrected measurement error caused by two such shifts at frames eight and forty-seven. In addition to the image sets described above, three additional sets were created by implanting two such artificial movements in the two simulated image sets and one real image set, also subject to Gaussian noise; referred to as "Outlier" image sets (3 sets of five movies).

Results

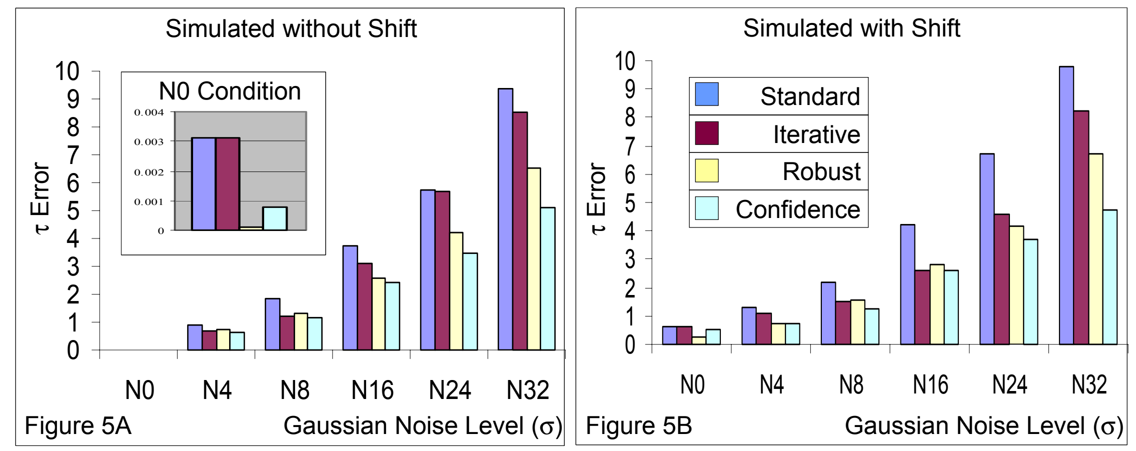
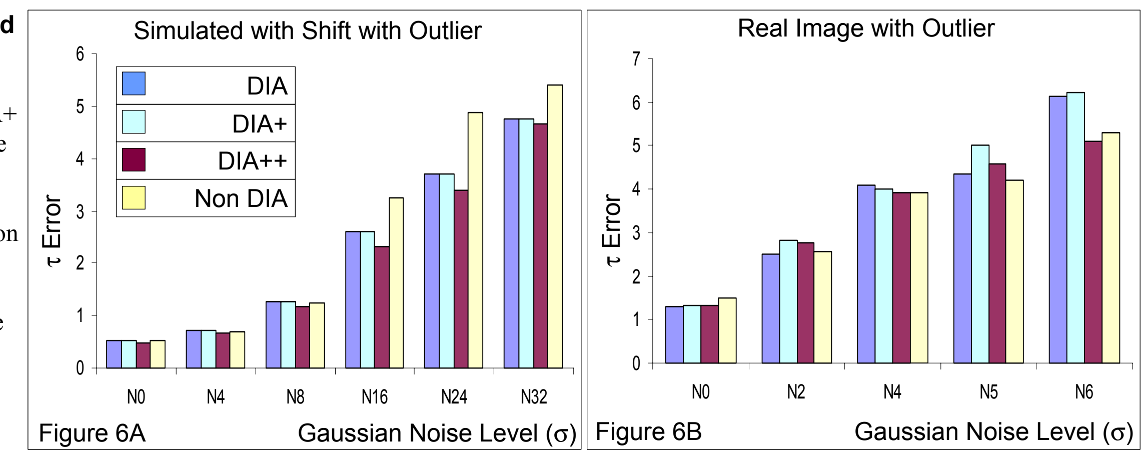


Figure 5. Confidence Maps Improve τ Measurement Error and are Robust to Noise and Sub-pixel Shifts. All evaluated methods show an improvement over the standard method (16 objects per condition). The confidence method generally has the least τ error across noise levels. The standard method calculates τ using binary masks, mean intensity measurement, and non linear regression. The iterative method applies the iterative fitting approach to the mean intensity measurement made with binary masks. The robust method uses a robust measurement: the mean intensity of the 80% brightest object pixels (all subsequent references to the robust measurement use this particular measurement). The confidence method uses the mean intensity measurement calculated from spatially regulated confidence maps.

Figure 6. The Data Integrity Assurance (DIA) Method for Error Correction May Not be Needed for Real Image Application. The DIA method is applied to confidence maps using the mean intensity measurement. DIA+ method applies the DIA method to confidence maps using the robust measurement. DIA++ applies the DIA method to confidence maps with the robust measurement subject to iterative fitting. We compare these three DIA methods to a non DIA method combining confidence maps, the robust measurement and iterative fitting. The DIA++ method has superior performance in the simulated image set at high noise levels, however it does not perform much better than the non DIA method in most cases, including the real image case shown here. We conclude that the non DIA method may not add a lot of value for typical real world application.



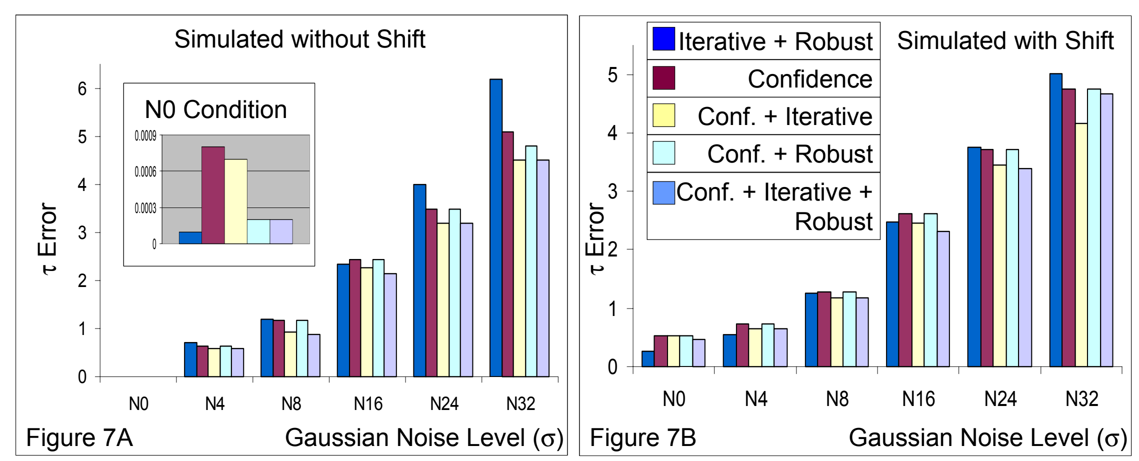
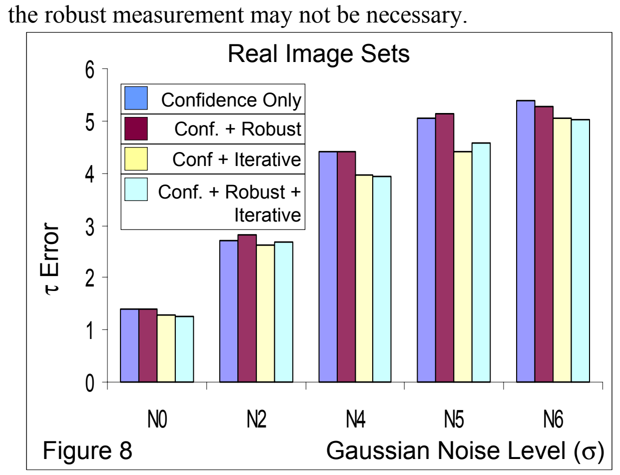


Figure 7. Using Confidence Maps with Other Methods in Combination Can Improve Performance. Confidence map based method alone is superior in performance to a non confidence based method using binary masks, the robust measurement and iterative fitting. Combining confidence maps with the robust measurement may improve performance slightly. Combining confidence maps with iterative fitting, or iterative fitting and the robust measurement combined does improve performance versus confidence maps alone.

Figure 8. Combination of Confidence Maps with Iterative Fitting Performs Best on Real Images. Multiple combinations of confidence map methods were compared using 18 real image sets (92 objects in total) at each noise level. The results show that combining confidence maps with iterative fitting can improve performance. Use of the robust measurement may not be necessary.



Literature cited

Robert M. Gray. 1990. Entropy and Information Theory, Springer-Verlag.
Zhang et al. 1999. Simple Statistical Parameter for Use in Evaluation and Validation of High Throughput Screening Assays. J of Biom Scr 4(2):67-73.
Roquemore et al. 2003. Is Z' factor the best assessment for the quality of cellular assays delivering higher content? Poster Presentation to the Society for Biomolecular Screening Conference. Portland, OR USA.

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Conclusions

- The use of confidence maps improves τ estimation, and is more robust to noise and sub-pixel shifts than the other evaluated methods
- The data integrity assurance method does improve errors resulting from moving objects. However, measurement errors resulting from moving objects can also be addressed with a combination of confidence maps, the robust measurement and iterative fitting. We conclude that the use of this method is not necessary.
- Using confidence maps in combination with iterative fitting yields the best overall performance