Batch image processing in synaptic membrane biology

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Time-series fluorescence microscopy is a tool commonly used in the research of synaptic membrane physiology and synaptic vesicle recycling function \cite{2,3}. The goal of this type of imaging is to record the changes in fluorescence of a population of synapses from cultured central nervous system neurons in response to changing stimuli. These fluorescence changes are then used to infer activity occurring within the synapses.

The analysis of the raw time series image data requires significant work to obtain these final synapse fluorescence measurements, with a large proportion of this work invoked manually (see Figure 1). Image processing tools such as FIJI/ImageJ\cite{4,5} are commonly used to carry out the individual operations. The system described here is our current progress in automating some of this analysis work.

The existing steps in image processing can include the following, after the time-series image data has been acquired:

1. **Image registration:** The frames of the time-series of images are aligned to correct for drift if necessary using FIJI and StackReg/TurboReg\cite{6}.
2. **Region Of Interest (ROI) candidate selection:** Potential synapses are identified and marked manually using FIJI.
3. **Measure ROI intensities:** The intensities of the set of ROIs over the time-series of frames is extracted using FIJI.
4. **Filter ROIs:** The ROI set is filtered to exclude unhealthy synapses and image artefacts, based on the observed intensity profile of each candidate ROI.
5. **Obtain normalised mean ROI fluorescence:** The mean intensity profile of the filtered ROI set is calculated.
6. **Correct for photo-bleaching:** Fluorescent markers can decay over time and in response to imaging. This effect is corrected by subtracting an exponential fluorescence decay curve from the mean intensity profile.
7. **Aggregate assays:** Multiple replicates of the same assay are then aggregated to provide mean and standard error ROI intensity profiles for synapse behaviour. Protocol being tested.

The aggregated result is then subject to further statistics and processing, depending on the experiment being performed. While some of the above processing steps could easily be automated, there is sufficient human input required that the resulting ROI intensity profiles would be suboptimal. Our work here has been both to develop components which automate steps of the sequence above, and to develop means of quantifying the effectiveness of the automation results.

To this end, we have developed a batch processing system to run image analysis routines over a large set of previously acquired imaging data from the Cousin group. As this data has already been processed manually, the effectiveness of our automation can be scored against the existing processing results.

The system currently uses both raw and aligned time-series image data, with matching manually acquired ROI sets. From these it allows the researcher to rapidly explore choices in ROI dimensions to obtain the cleanest signal in the resulting ROI intensity profiles.

We are currently also automating the ROI candidate selection step. We are exploring a range of approaches here and testing them against the available manually selected ROI sets. Robust solutions found here will further be integrated into the batch processing system.

In addition to improving the throughput of image analysis, our software allows for multiple analysis techniques to be attempted on each dataset, to rapidly search for a set of operations which best suits the data. For example, there are a number of options for image registration, the best of which to use depends on the dataset, and is difficult to judge quantitatively before the downstream filtered mean ROI intensity profiles had been obtained. This can be prohibitively time-consuming to do manually.

Finally, the batch processing system generates intermediate, exploratory and final results in a browsable web format, allowing the researcher to view the results of the available processing choices, and quickly review an archive of prior experimental results.

The system is Java based, making it platform independent and able to natively use components of the also Java based ImageJ/FIJI.

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