A quantifiable approach to filtering and analysis of cellular fluorescence imaging

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Introduction:

Cellular bioimaging has become standard practice in one form or another for its ability to attain detailed structural and functional information at the cellular level. As imaging systems and computer capabilities have evolved, image resolution, imaging speed and volume of collected data has increased dramatically. With increased imaging data, especially in the case of functional data, comes the need for timely, more consistent quantitative measurement and analysis techniques applicable to multiple cellular imaging methods and cell types. Integrity of data is essential, and some image and video filtering and analysis techniques not only have the potential to hide important quantitative information, but to actually distort the information that has been obtained. This paper presents consistent and reproducible image and video filtering techniques for use in the analysis of cellular bioimaging data, concentrating specifically on fluorescence imaging techniques.

Methods:

Filtering Techniques: Linear mean filtering algorithms are commonly employed to remove noise from imaging data. Whilst linear and non-linear mean filtering have merit based mainly in short analysis time and strong noise reduction, it has an inherent problem of incorporating noise into the image rather than removing it. Mean filtering also blurs spatial image segment interfaces within a frame, and may distort temporal activity when applied across multiple frames of a video, so reducing the effective sampling rate of the collected data. Non-linear ranked filtering methods such as median filters employ a more complex form of filtering, however have the essential benefit of avoiding both spatial and temporal blurring of data segments by rejecting impulse noise (Sabri *et al.*, 1997), resulting in strong noise rejection whilst maintaining signal and data integrity.

Automatic Image Segmentation and Cell Detection: Manual cell counting in cellular imaging is a common and time consuming process, there are a variety of image segmentation algorithms to remove the majority of the manual labor involved in this process. These techniques may also be applied to functional and structural fluorescence imaging data to identify specific cells of interest in the culture, slice, or whole animal.

Functional Quantification: Much functional imaging data is presented in a qualitative or inconsistent quantitative fashion that varies markedly between research groups. The quantifiable properties of stochastic noise, namely confidence levels of the normal distribution, may be utilized to quantify functional fluorescence signals in terms of cellular activation level above baseline at given confidence intervals.

Correlation of Activity Between Cells: Correlation of activity between cells may be achieved after applying the combined cell detection and functional quantification methods discussed above. Detection of networks and cellular information transmission through investigation of cellular activity correlation allows determination of network formation and quantification of function, critical aspects of true physiological condition of biological tissue. Timing, strength, and reliability of cell-cell interactions may, using the methods above, be quantified without excessive analysis time, allowing the lag between cells activity to be determined. These methods may well allow investigation of neurotransmitter function, synapse formation and efficiency, and the effects of receptor agonists or other pharmacological interventions on cell-cell interactions.

Conclusion:

Cellular bioimaging techniques currently employed have the potential to limit accurate quantification of data and hide vital information from researchers. Using non linear median filtering along with the known properties of stochastic noise allow a more robust, consistent and highly quantitative methodology for cellular imaging. This has the potential to improve both structural and functional imaging procedures and assist in the testing and development of pharmacological interventions at the intra- and inter-cellular level. These techniques may be applied equally well to traditional two dimensional and three dimensional confocal microscopic techniques.

Sabri S, Richelme F, Pierres A, Benoliel A-M, Bongrand P. (1997) *Journal of Immunological Methods* **208:** 1-27.