Automated 3–D quantification of neurodegeneration-associated changes in
a subcellular compartment of neurons
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Background/Aim: Recent studies have linked endo-lysosomal abnormality to neurodegenerative diseases in humans [1]. Blue cheese (bchs) [2] is a neurodegenerative mutant in Drosophila that is involved in endo-lysosomal trafficking [3], and that affects the morphology of degradative organelles in neurons (Wang, Kumar and Kraut in prep.). Rigorous, yet rapid quantification of the altered characteristics of these organelles would allow the efficient detection and comparison of neurodegenerative phenotypes in different mutant models. Here, we report a 3–D image processing scheme for robustly quantifying the distribution in the sizes of the endo-lysosomal compartments at the neuromuscular junction of the third larva instar. We also characterize the differences in size distribution between the bchs mutant and the wild type. This quantification method could be of use in drug screening for neurodegenerative diseases since it is much faster and more statistically reliable than traditional manual methods of phenotypic quantification.

Methods: Two transgenic lysosomal markers, spinster–GFP and LAMP–GFP (gifts of S. Sweeney & H. Kramer, respectively) were used as specific markers for the endo-lysosomal compartments. The neuromuscular junctions (NMJ) of the third lava instar were imaged on a DeltaVision wide-field fluorescence microscope at multiple focal planes to generate a stack of images, which were then deconvolved. The compartments manifest as punctate fluorescence spots on the 2–D images. The spots were enhanced via local and global normalization of the images with respect to mean and standard deviation. Spots are defined as image locations with sufficiently high coefficients for both local and global normalization. Image stacks comprising the binary spots at different focal planes were visualized in 3–D space with each spot identified using binary connectivity and its size information obtained by computing its volume. The spot size distribution in an NMJ is given by a histogram where the x-axis shows 11 size categories and the y-axis represents the percentage of spots for each category. Support vector machine (SVM) is then used to classify the NMJs as either mutant or wild type based on the spot size distribution.

Results: The SVM classifier can differentiate the mutant from wild type with an accuracy of 88.1% and 95.0% for the spinster–GFP and LAMP–GFP respectively. The difference in spot distribution between the mutant and wild type is statistically significant (p<0.0001).

Conclusions: The 3–D image processing scheme is robust to variations in image intensity, noise and spot density. The size distribution of a neurodegenerative mutant neuron is different from the wild type and this is detectable with high accuracy by the automated feature classification algorithm. This type of analysis will allow rapid, unbiased evaluation of degenerative changes in different models and may be applied to quickly screen the effects of drug candidates.

References: