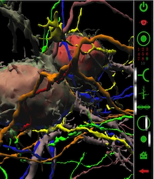
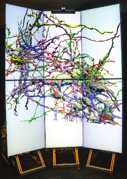
**Aim 1: Classify cells of the mouse cochlear nucleus by structural features.**

*Step XX: Classify neuron morphology by human and automated methods. Human classification* takes advantage of our development of a user interface to an immersive virtual reality (IVR) system to investigate high-resolution 3D models of neurons, called BrainTrek (see (Morehead et al. 2014); Fig. 1 and Facilities and Resources section). This system allows experts to explore, inspect, compare, and interact with cell structure in similar fashion to how cells have been historically categorized, but leverages BrainTrek to implement the advantage of systematically viewing large numbers of cells. In brief, 9 human viewers (3 groups of 3 people) initially inspect 100 randomly selected ground truth neurons in randomly selected groups of ten cells. A single reference cell is randomly selected, but used for all groups, and all remaining cells are sequentially rated for similarity on a 1-5 scale (1 = most similar). Next, a consensus cell maximally dissimilar to the reference cell is used as the reference, followed by another cell maximally dissimilar to both previous groups, and so on until all cells with at least one 5-score have been tested. From these data, groups will be constructed with probabilistic assignment of each neuron. Each observer will then inspect cells as groups and indicate improperly assigned cells. This process will be evaluated for the number of iterative classifications to develop statistically reliable classes and then tested with the same observers on the remaining ground truth neurons. Protocols for assigning cell type labels will be designed as part of this program. The dataset of 3D models and their classification will enter a MySQL database for the indexing and retrieval of models and the storage of associated labels. This dataset will not only be interpreted as human classification data, but also as training and testing labels for the automated classification approaches developed in this program. Eventually, the human classification and BrainTrek will become part of an *active learning framework* (see Fig. 3), aimed at the simultaneous discovery of new cell types, as well as the training of the corresponding automatic classifiers.

  
Figure 1: **BrainTrek**. Left: CAVE displaying nine cell models. Center: Close-up view with on-screen menu that allows for modification of the cell scene. Right: Tablet layout of a Nexus 7, which allows for modification of the scene with sliders and buttons.

*Automated classification and discovery.* We propose a computer vision approach for the automated discovery of cell types, as well as the classification of cells in the CN from high-resolution 3D reconstructions of neurons, generated in Step YY. Previously, only skeletonized versions of neurons have been used for clustering analysis and classification applied to skeletonized pyramidal neurons of the somatosensory cortex in the neuromorpho.org database (Schierwagen et al. 2010; Scorcioni et al. 2008); and a supervised learning model has been applied to wild-type motor neurons in the larval ventral nerve cord of the Drosophila, but using 2D images (Chang et al. 2014). A state-of-the-art review that relates morphological cell classification to the physiological and biochemical characterizations can be found here (Armañanzas & Ascoli 2015).

*Neuron representation.* A model for the representation of the 3D shape of neurons that is tailored for recognition will be used. Current approaches (Bustos et al. 2005; Funkhouser et al. 2005; Tangelder & Veltkamp 2007), convert the 3D point cloud into a “signature model” describing the cloud. Two relevant methods for generic 3D signatures include those based on *descriptors* (Heider et al. 2011; Johnson & Hebert 1999; Sun et al. 2009; Kazhdan et al. 2003; Osada et al. 2002; Chen et al. 2003), and on *graphs* (Tangelder & Veltkamp 2007; Hilaga et al. 2001; Sundar et al. 2003; Cornea et al. 2007; Chang & Kimia 2011). We will use signatures based on *local descriptors* (Heider et al. 2011; Johnson & Hebert 1999; Sun et al. 2009), because they are parsimonious, computationally efficient, and very effective for recognition. Graph-based methods (e.g. (Hilaga et al. 2001; Sundar et al. 2003; Cornea et al. 2007; Chang & Kimia 2011) may have greater representational power usually at a greater computational cost (the edit distance is NP-hard (Zhang et al. 1994), and computing the maximal common subgraph is even NP-complete (Garey & Johnson 1990)). In particular, from a 3D point cloud we will randomly select up to points and around those we will compute local descriptors called spin images (Johnson & Hebert 1999). Therefore, a neuron will be represented by the descriptor vectors .

The set of spin images vectors will then be encoded into an improved Fisher vector (IFV) (Sánchez et al. 2013), which is a state-of-the-art, highly discriminative representation that guarantees top recognition rates. Specifically, we will model the distribution of the spin images vectors by fitting a Gaussian mixture model (GMM) to a set of descriptor vectors computed from a training set of neuron 3D point clouds. The Fisher vector (FV) of a spin image , is computed as , where are the parameters of the GMM, and is the Fisher information matrix, here used for whitening the data. The FV expresses how much affects the maximum likelihood estimation of the parameters . The IFV representing a neuron is a normalized average of the Fisher vectors computed from each of the spin images vectors. The representation based on IFV has two main advantages with respect to other effective models, such as the bag-of-words model (Tang & Godil 2012). In particular, it provides better recognition accuracies, and it is more scalable because it can work very effectively with machine learning techniques based on the simple Euclidean distance, without requiring to define an ad-hoc distance with higher computational requirements.

Figure 2: **CellCompare**. Left: CAVE displaying BrainTrek running the CellCompare tool for cell comparison and similarity evaluation.

*Cell types discovery.* We will use an unsupervised learning technique for discovering the presence of potentially new cell types, as characterized by the same morphological features. Specifically, we will randomly sample a training set of neurons, uniformly from the CN volume, and we will characterize each of them by an IFV. We will then apply a clustering technique to the training set of IFV’s. However, we cannot apply a standard k-means clustering, because it requires *a priori* knowledge of the number of clusters, and is sensitive to the initialization conditions. Instead, we use a technique called affinity propagation (Frey & Dueck 2007). This one can estimate the number of clusters automatically, and is insensitive to the initial conditions. In particular, since we use IFV’s, we define the similarity between two neurons and , as . Finally, 3 neuroscientists will use a tool called CellCompare, which we have already developed in house, and that allows to visually compare a pair of cells, randomly selected from two different clusters, by using our IVR BrainTrek system, for an enhanced immersive comparison experience in 3D. CellCompare allows to interactively record similarity scores from 1 to 5 between two cells. We then compute the average similarity score between every pair of clusters, and if higher than a threshold, the two will be merged. The process will be repeated until no more clusters will be in need to be merged. The resulting clusters will represent the cell types that have been discovered.

*Cell classification.* Since every neuron in the training set has been assigned a cell type label, we can use a supervised learning approach to classify the remaining cells in the CN volume. In particular, we are going to use two approaches. The first one, will use the labeled training set as the prior knowledge for classifying other neurons based on a k-nearest neighbor (KNN) approach, and we will use approximate nearest neighbor techniques based on k-d trees for the efficient assignment cell type labels (Muja & Lowe 2014). In the second approach, we are going to train a multi-class support vector machine (SVM) (Schölkopf & Smola 2001), and since we are using IFV’s we can efficiently leverage linear kernels, as it is done in computationally efficient library LIBLINEAR (Fan et al. 2008).

|  |  |  |  |
| --- | --- | --- | --- |
|  | Discovery Accuracy | | |
| # Modes |  |  |  |
| Age 2 |  |  |  |
| Age 3 |  |  |  |
| Age 4 |  |  |  |
| Age 6 |  |  |  |
| Average |  |  |  |

Table 1: **Discovery Accuracy**. Maximum percentage of cells aggregated into the correct age range.

*Preliminary results.* We have a preliminary implementation of the entire cell classification pipeline. We have a dataset with the point clouds of XX mouse MNTB neuron cells of the same type but from 4 age groups (postnatal day 2, 3, 4, and 6) (Holcomb et al. 2013), extracted from stacks of images of SBEM data (Helmstaedter et al. 2011). We have tested the pipeline in 5-fold cross-validation. From each cell we have pooled YY local descriptors, and we have learned a GMM with YY, YY, and YY modes. Table 1 reports the accuracy with which we discover cell ages by aggregating the cell clusters. The similarity threshold for cluster aggregation was set to YY. Table 2 reports the classification accuracy on the testing set for the KNN (with K=YY), and the SVM approaches. As it can been seen these preliminary results are very encouraging and suggest that this is a viable approach for processing the neurons in the entire CN volume.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | KNN Accuracy | | | | SVM Accuracy | | |
| # Modes |  |  |  |  | |  |  |
| Age 2 |  |  |  |  | |  |  |
| Age 3 |  |  |  |  | |  |  |
| Age 4 |  |  |  |  | |  |  |
| Age 6 |  |  |  |  | |  |  |
| Average |  |  |  |  | |  |  |

Table 2: **Classification Accuracy**. Accuracy with which we classify cell age.

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