Inference of survival states in induced motor neurons of neurological diseases

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Introduction

Patient-derived cell models imaged by time-lapse microscopy could elucidate the pathogenic mechanisms of neurological diseases. To discover disease predictive phenotypes across a large representative patient sample, a systematic, unbiased approach is needed to mine time-lapse microscopy image sequences, patient clinical and concomitant data for robust phenotypes.

The current kinetic microscopy studies are overly simplistic, assessing metrics such as survival endpoints alone, which fails to account for spatial-temporal patterns and associated dynamic events. No analysis tools could effectively evaluate the large volume of dynamic data from in vitro induced motor neurons (iMNs) cultures with diverse patient populations to detect subtle spatial-temporal pattern differences with high sensitivity and specificity. There is a critical need for informatics tools to enable the discovery of disease predictive phenotypes robust to patient variations.

We are developing a kinetic informatics discovery (KID) tool. The first prototype performs kinetic cellular state inference. It is applied to infer the survival states of iMNs from a panel of ALS patients. The objective of this study is to assess the inferred cellular states and state transitions in the motor neuron degeneration process.

Kinetic Cellular State Inference Informatics

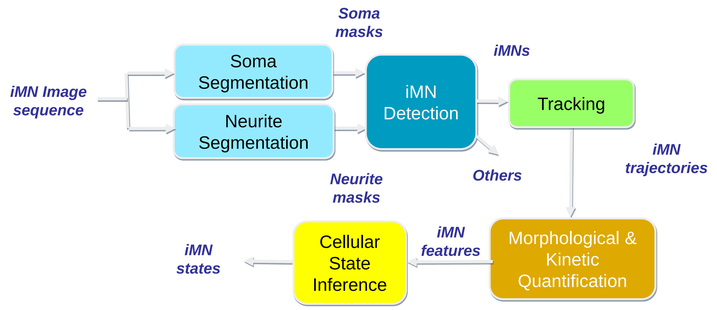

Fig 1. Kinetic Cellular State Inference Informatics: The informatics inputs an iMN image sequence. The "soma segmentation" and "neurite segmentation" steps detect and segment soma and neurite masks from each frame of the image sequence. These are followed by an iMN detection step which calculates features from soma and neurite masks and classifies detected objects into iMNs and others (non iMNs or artifacts). The detected iMNs are tracked by a tracking step that creates iMN trajectories for each successfully tracked iMNs. The morphological and kinetic quantification step measures morphological and kinetic iMN features. A cellular state inference step infers iMN states from the iMN features.

Segmentation

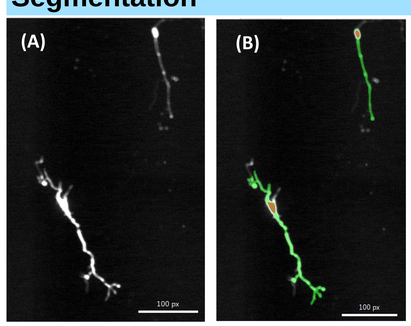

Fig 2. Multiscale vessel enhancement filters[1] enhances cell body (soma) and neurites separately. Soma and neurite are segmented by thresholding on each of the filter enhanced images. (A) One frame of an iMN image sequence containing two neurons. (B) Showing the soma segmentation and neurite segmentation results. The resulting soma masks are overlaid in Peru color and neurite masks are overlaid in green over the input image.

iMN Detection

Latency in the iMN conversion process produces a mix of fibroblasts and iMNs with similar morphology. To distinguish these populations with high sensitivity and specificity, we used a machine learning approach to train a classifier for iMN detection. Features are calculated from soma and neurite masks. The features are used to train a random forest classifier. Using the trained classifier, each segmented object is scored. Based on the score, the objects are classified as iMN or others (non-iMNs, artifacts, etc.). Only iMNs are analyzed.

Tracking

The tracking step is performed on iMN soma masks using a modified SVCell nuclei tracking recipe. In iMN survival assay, there are fewer objects in later frames than in earlier frames. In stead of forward tracking, we started from last frame back tracked to the first frame. Since later frames have fewer objects, this reduces match candidates between consecutive frames and improves the tracking accuracy. We used Hungarian algorithm for match making between consecutive frames. This yields robust tracking results even when time resolution is low resulting in large changes between consecutive frames.

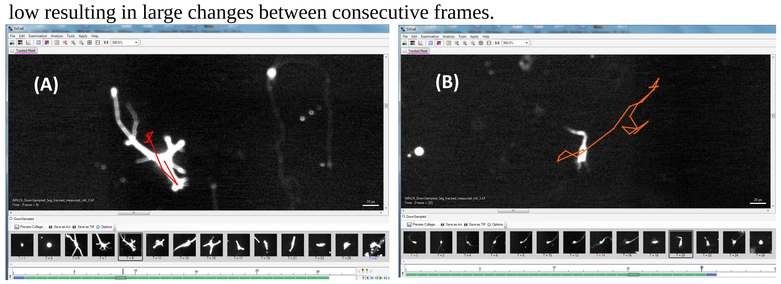

Fig 3. (A) An example showing the trajectory (in red) of a tracked iMN. The regions of the tracked iMN is shown as a filmstrip in the bottom (the regions of every other frame are shown). Since the tracking is backward in time, the regions on the right are from earlier frames and the regions on the left are from later frames. (B) shows another example of a tracked iMN.

Morphological and Kinetic Quantification

Morphological and kinetic features of each tracked motor neuron are automatically quantified and used for the cellular state inference step. Morphological features include soma area, soma shape circularity, neurite count, total neurite length, average neurite length, and neurite branch point count. Kinetic features include trajectory velocity and acceleration.

Cellular State Inference

In the preliminary study with limited data, only neurite length and soma area are used for cellular state inference step by unsupervised learning. To provide smooth cluster (state) boundaries, a Gaussian mixture model is used for state inference. In the future when more data are available, we will try non-parametric clustering algorithm on higher feature dimensions to infer clusters and their boundaries.

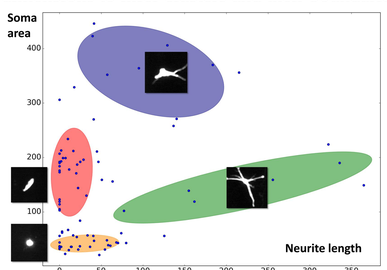

Fig 4. Four inferred states are shown as colored Gussian clusters in a 2D scatter plot. The horizontal axis of the plot corresponds to neurite length (in pixels) and the vertical axis corresponds to soma area (in pixels). Representative image crops of the iMNs clusters are shown near their corresponding states (clusters).

Study Materials and Methods

By expressing 7 motor neuron-specific genes in human skin fibroblasts, Ichida lab converted them into bona fide spinal motor neurons termed induced motor neurons (iMNs). This study used iMNs derived from patients with the C9ORF72 mutation, the most common form of ALS. Ichida lab have verified that these cells recapitulate in vitro surviving phenotypes[2].

Imaging and Software

The iMNs are labeled with the lentiviral HB9::RFP motor neuron reporter for imaging at Nikon Biostation CT, an integrated cell culture observation system using 10X objective lens. Time-lapse image sequences are acquired every 12 hours for 14 days during the iMN survival experiments using 96 well plates. SVCell with a special version of recipe is used to detect and track the survival of individual iMNs in culture and perform cellular state inference.

Study Data

3 time-lapse image sequences are used for the study. The image size is 9620x9620x28 that covers the whole well of a 96 well plate. The images are aligned and down-sampled by a factor of two for the study.

Results

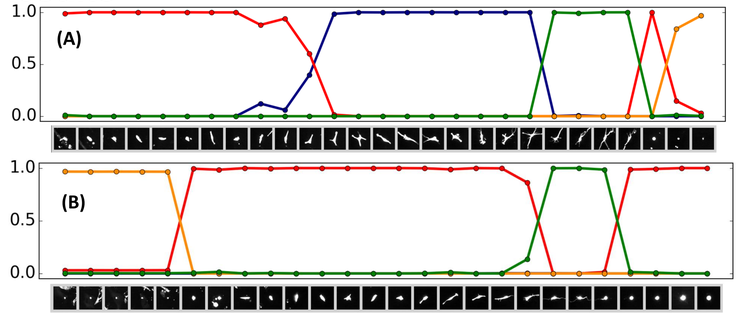

Fig 5. (A) An example showing a tracked iMN filmstrip and its state membership values (states are colored per Fig. 4 convention) at each time point (time increases from left to right). (B) Another example of a tracked iMN having different states and state transition.

Discussions and Conclusion

The preliminary results show promising cell states inferred from neuronal structures by our informatics tool. As the next step, the informatics will quantify state transitions using the hidden Markov model to study the iMN survival dynamics and compare models of disease vs. healthy patients to discover kinetic phenotypes that are disease predictive.

In the future, we will infer states and dynamic events using multichannel microscopy multiplexing reporters for spatial-temporal functional dynamics (e.g. neuronal firing, mitochondrial activity, protein degradation, etc.) and cell fate readouts.

References

1. **Frangi, AF**., et al. Multiscale vessel enhancement filtering. International Conference on Medical Image Computing and Computer-Assisted Intervention. Springer Berlin Heidelberg, 1998; 130-37
2. **Li Y, Shi Y, Alworth SV, Lee JSJ, Ichida J.** A phenotypic screen using induced motor neurons from C9ORF72 amyotrophic lateral sclerosis patients identifies pathways that modulate excitotoxicity and protein trafficking as therapeutic targets. Poster presented at the 2014 International Society for Stem Cell Research meeting, Vancouver BC, Canada.

Acknowledgments

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