Automatic Classification of C-Elegans Mitochondria

Machine Learning Project Proposal

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Prof. Barbara Conradt from Cell and Developmental Biology from LMU and her research group are investigating cellular organelles called mitochondria. These organelles generate energy currency of the cell called ATPs by a series of chemical reactions in the body. ATP can then be readily absorbed by the cell to meet its energy demands. In doing so, mitochondria also generate free radicals mostly in the form of reactive oxygen species as a by product of the reactions. These free radicals are harmful to the cell. Saroj Regmi, a student from Professor Conradt's research group, is investigating how mitochondria might change its morphology with age in the free-living transparent worms called C. elegans. Transparent body, short life span and easy genetics has made C elegans an excellent biological model to study physiological processes. The hypothesis in the field is that mitochondrial production of ATP over time damages cells and brings about aging. As a preliminary evidence, Saroj Regmi saw that mitochondria look fragmented with age. Thus, he is now looking at the morphology of the mitochondria under microscope and classifying them into three categories. This experiment is being done with wildtype "normal" worms and mutant worms that either live longer than normal or live shorter than normal. By comparing the morphologies and the life-spans of different kinds of worms, he hopes to find a whether there is a correlation between mitochondrial morphology and aging.

Worms with fluorescent mitochondria are put under a fluorescence microscope and 10 to 20 pictures of it are generated. As the worm is transparent, photographing it with different focuses yields to photographs of different levels of the worm. After that, each "slice" of the worm is scrutinized on the computer screen to find just one picture where cells are clearly visible. Using computer imaging software, the photographs are discretized until mitochondria can be separated from background fluorescence. Afterwards, another routine of this program computes different attributes of the shape of the areas refined during the previous step. These are attributes like "circularity" (shape compared to a perfect circle), length of one mitochondria, kind of clustering, etc. These data are used to classify the morphology of the mitochondria into three classes: *fragmented*, *tubular* and *in between*. As this process involves human based classification, it is prone to biases based on the hypothesis of the experiment. The goal of our project is to use machine learning to automate this process of classification.

We plan to employ both SVM and neural network for this classification problem. Relative performance of each of the two methods will be evaluated. Since data is somewhat rare, around 120 now and 20 images more per week until February 2012, we plan to do cross validation using leave-one-out or k-fold CV to make most out of this scarce data in order to find the hyper-parameters.

Once the image stacks are obtained from the microscope, picking the appropriate stack and

grayscale threshold is done manually. A stack and grayscale value is chosen in which the mitochondria are distinct. In other words, an image with the best contrast is chosen. The software then converts the noisy image into a binary image, which is then used to extract relevant features. Measurements of the dimensions of mitochondria are done using this binary image. The researchers were eager to possibly automate this process too because it is time consuming and error-prone. Hence, if time permits, we proposed to experiment with a supervised learning algorithm which would silently run in the background and learn how an operator picks the best grayscale values for an image, and over time, it would be able to select image with the best contrast itself. Structure detection using self-organizing-maps (SOM) could be an option.

The researchers study how mitochondrial fragmentation might affect lifespan of the worms using MS Excel and usual 2D plots. However, they suspect that other factors like length and breadth of mitochondria, though correlated with fragmentation, could also change with life span. We proposed that they could use weighted regression to create a more complex model that would enable them to study how the factors collectively affect aging in worms. This would be yet another extension to the project.

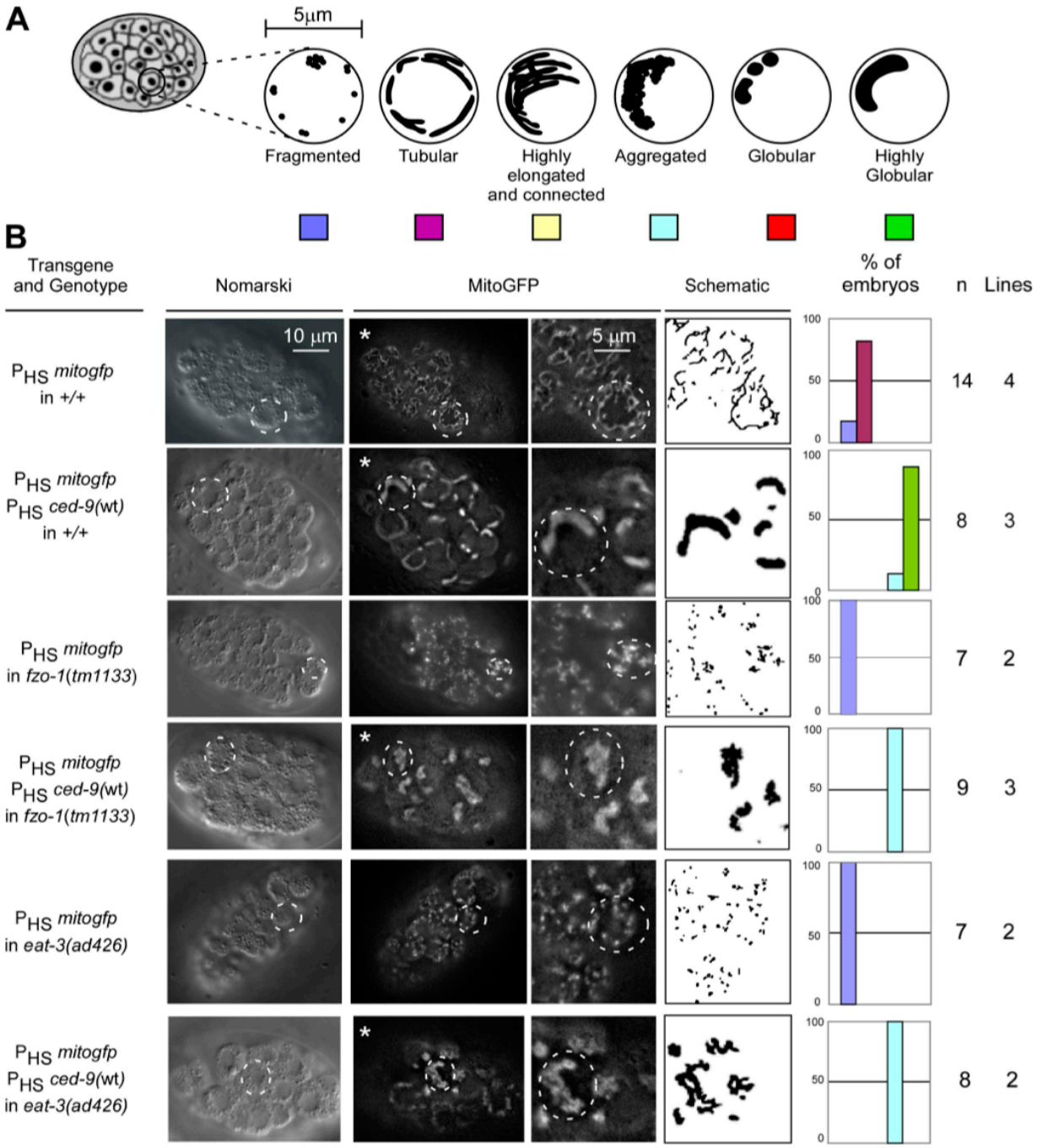

Figure 1. **CED-9 promotes FZO-1– and EAT-3–dependent mitochondrial fusion.** (A) Schematic of the mitochondrial morphologies observed in embryos. (B) Wild-type (+/+), *fzo-1*(*tm1133*), or *eat-3*(*ad426*) transgenic lines expressing *mitogfp* alone (P_{HS}*mitogfp*) or in the presence of *ced-9*(wt) (P_{HS}*mitogfp* + P_{HS}*ced-9*(wt)) were analyzed by Nomarski optics and fluorescent microscopy. Quantification of the different mitochondrial morphologies observed and the number of embryos and transgenic lines analyzed are indicated. The outlines of representative cells are indicated with dashed circles. Videos 1–4 show 3D reconstructions of the embryos marked with asterisks.