FARSIGHT: A Framework for Automated Quantification of 2D and 3D Multi-Parameter Images of Biological Tissues

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Abstract

We present a systematic ‘divide and conquer’ methodology for analyzing three-dimensional (3D) multi-parameter images of brain tissue to delineate and classify key structures, and compute quantitative associations among them. Automated 3D segmentation and tracing algorithms were utilized to delineate cell nuclei, vasculature, and cell processes. The cells are then classified into four major classes (Neurons, Microglia, Astrocytes and Endothelial cells) by using a two-step semi-supervised classifier.

Associative Image Analysis Framework

➤ The image is decomposed into 5 channels
➤ Each channel is segmented/analyzed independently

Input

Output

Association And Feature Extraction

Cell Classification

Details of the Nuclear Segmentation Algorithm

• Image Binarization
  • The image is initially binarized using the Poisson distribution-based minimum error thresholding [1].
  • The normalized image histogram is modeled by a mixture of Poisson distributions:
    \[ p(i) = \sum_{j} p_{ij} p(i|j) = \sum_{j} p_{ij} \frac{\mu_j^{i}}{i!} e^{-\mu_j} \]
  • The goal then is to find the threshold \( t \) that minimizes the error criterion function:
    \[ f(t) = \mu - \int p(i) \ln p(i) + p(i) \ln p(i|t) - p(i|t) \ln p(i|t) \]
  • The initial binarization is refined using Graph-Cuts [2].

• Seeds Detection and Initial Segmentation
  • Cells are detected using a scale-normalized Laplacian of Gaussian (LoG) filter
  • The filter is used on different scales:
    \[ \log_{scale}(x,y,\sigma) = \frac{\partial^2 G(x,y,\sigma)}{\partial x^2} - \frac{\partial^2 G(x,y,\sigma)}{\partial y^2} \]
  • Assuming the distance from a point \( x \) to the background is \( Dmap(x) \)
  • The response at that point in the resulting LoG-image response \( R(x) \) is given by:
    \[ R(x) = \arg \max \{ \log_{scale}(x,y,\sigma) \} \]
  • \( R(x) \) gives a topographical surface, where a cell is represented by a Gaussian blob
  • Each blob (nucleus) has one local maximum point called “seed point”
  • Local maximum clustering [3] is used to assign each foreground point to one seed

• Graph Coloring and Segmentation Refinement
  • Initial segmentation is refined using a Graph-Cuts based approach \( \sigma \rightarrow \sigma_{new} \) [4]
  • The number of alpha-expansions required is equal to the number of labels (cells)
  • Problem: Impractical when the number of labels in a connected component is large
  • Solution: Reduce the number of labels using Graph Coloring

  1. Build a Region Adjacency Graph (RAG) from the initial segmentation
  2. Use a sequential graph coloring algorithm to assign different colors to adjacent cells

Cell Classification

Initial Fuzzy c-means clustering

Omitting graph coloring

Experimental Results and Validation

• Classification Results
  • Cell classification results after 5 manual corrections to the training set

References


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