MORPHIAS: Molecular Phenotyping Image Analysis System

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Abstract. Visualizing metabolic phenotypes, tracking phenotype dynamics, and screening molecular interventions all require comprehensive, quantitative measures of micromolecular patterns across cell classes. In this project we develop an easy-to-use interface that permits the same sequence of operations as described in Marc and Jones[1] and is based on Cell Kit developed by Marc's lab. Work products for this project include: user application software (Matlab), user manual and if possible, a web portal interface to allow internet access and application of the system. The MORPHIAS system will be implemented by means of a Matlab function which creates a GUI which allows the user to import molecular signature images (in TIFF format), and then to perform classification using a clustering algorithm, and to then perform data analysis on the result.

1 Introduction

Unique cell classes can be distinguished by their small molecule or micromolecular signatures. Micromolecular signatures serve as formal metabolic phenotypes[1], track physiologic and pathologic metabolic transformations[2–4], and uncover novel cell classes [Marc2002,Marc2002a,Marc1995]. Visualization of these signatures by computational methods[1, 5] in all eukaryotic Kingdoms whose taxa develop complex heterocellular tissues (Plantae, Fungae, and Animalia from Platyhelminthes to Chordata) reveals unexpectedly diverse metabolic phenotypes across cell classes within tissues. Metabolic phenotype variants are challenging to interpret, as they arise from genetic and epigenetic demands: maintaining group transfer potentials for molecular synthesis, vectorial molecular transport processes, osmoregulation, redox and reactive oxygen species (ROS) control, energetics regulation, intercellular signaling and coupling, cell and tissue growth, and protein synthesis[6]. No coherent theory of metabolic differentiation predicts these phenotypes, but once visualized, micromolecular phenotypes constrain functional models and modes of pharmacologic intervention. These measures that can be uniquely acquired via computational molecular phenotyping (CMP) with anti-hapten IgG libraries[1, 5, 4]. The ultimate biological goal of our work is the construction of a micromolecular phenotype atlas of the C57b/6J

mouse using a basis set of molecular signals. technical goals include: (i) development of large-scale probe libraries; (ii) realization of high throughput scaling, analysis, and management resources; and (iii) creation of a normative mammalian tissue CMP atlas with single cell resolution and comprehensive coverage.

Every cell exists in an N-dimensional metabolic space of intracellular concentrations constrained by cell-autonomous and non-cell-autonomous factors (e.g., precursor supply). No theory of metabolism predicts, a priori, the micromolecular profile of any metazoan cell in its vectorial tissue context, partly because no uniform, high-resolution measure of metabolite composition has been available to test or inform models. These critical values are known as ground truth in remote sensing. Metabolite patterns can be quantitatively mapped with hapten-specific IgG reagents that selectively bind free or conjugated small molecules with high fidelity and signal strengths proportional to free intracellular levels[7] and micromolecular profiling in neural, muscular, hepatic, renal, digestive tract, immune, vascular, endocrine and reproductive tissues reveals extensive metabolic variation. Metabolic diversity is best documented in the vertebrate nervous system where massive differences in aliphatic amine contents exist across neuronal, glial and vascular cell classes[6]. Such immunochemical tools have rarely been exploited to characterize non-neural tissues[8]. When combined with true multispectral imaging and pattern recognition, computational molecular phenotyping (CMP) [1, 5, 7] based on micromolecular detection enables visualization of more than 10 metabolites in every cell. CMP provides the ability to concurrently discover and phenotype cell classes, track cell state, and map disease or environmental challenge sequelae with single-cell resolution in any tissue or organism. CMP provides ground truth for cell physiology.

2 MORPHIAS

Figure 1 shows the layout of the interactive Graphical User Interface (GUI).

The interface is divided into 3 sections: (1) the top section allows the user to specify the files (multiple image channels); this can be done by typing them in or browsing; (2) the middle classification section in which the user specifies parameters or options in applying the K-means algorithm, and (3) the lower section in which the user may visualize various aspects of the data.

3 Discussion

The goal of this project is to make a versatile, portable image and data analysis tool available to the CMP community. We have a prototype Matlab version developed, and have as a goal the development of a web-based interface as well. The current MORPHIAS system allows the user to:

- load N 8-bit grayscale TIF images
- perform simple K-means clustering (user-specified k)
- produce indexed TIFF of classified pixels

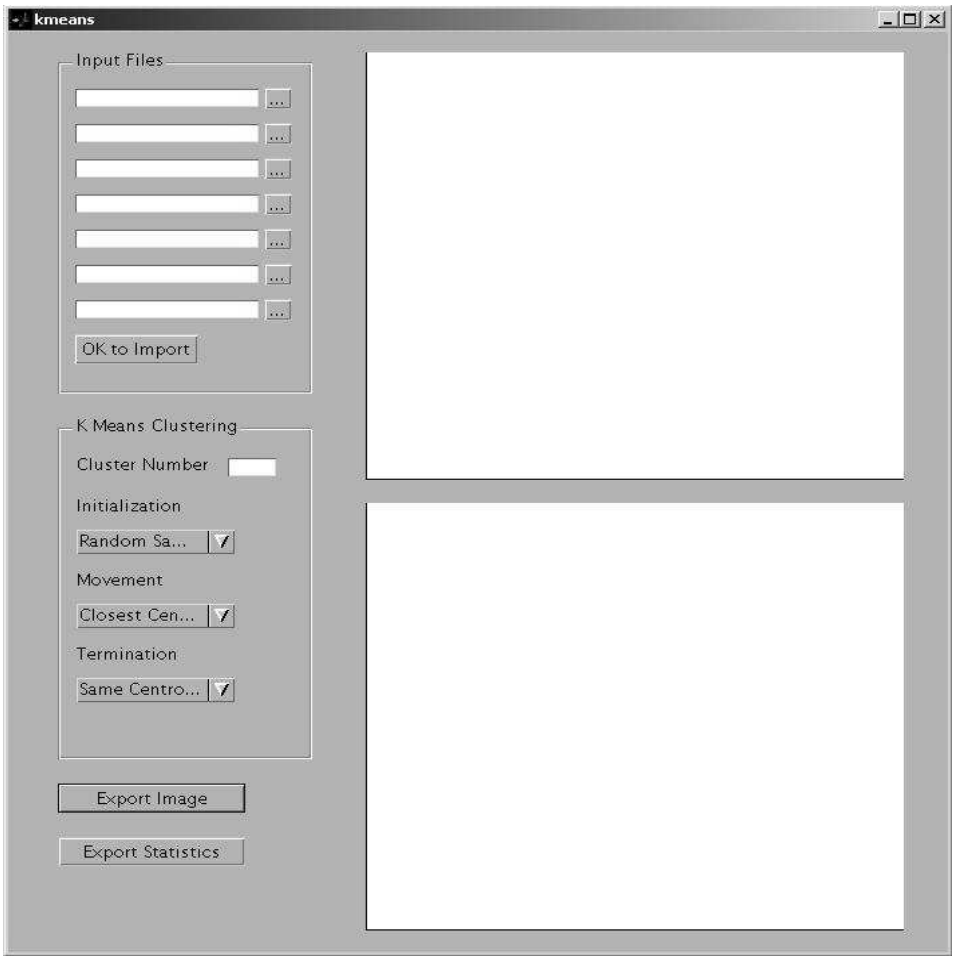

Fig. 1. MORPHIAS GUI Layout.

– produce text file with cluster means and moments
– explore alternative methods of initial cluster specification:
  • k on N-D diagonal
  • user specified
  • random
– and K-means convergence:
  • No change in clusters
  • Sum distance to closest center does not change
  • centroids don't move

We are also exploring various cluster quality measures (centroid separation, variance, cluster sphericalness (function of normalized eigenvalues of covariance matrix), point density in cluster, etc.)

References

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