Unsupervised Cell Identification on Multidimensional X-Ray Fluorescence Datasets

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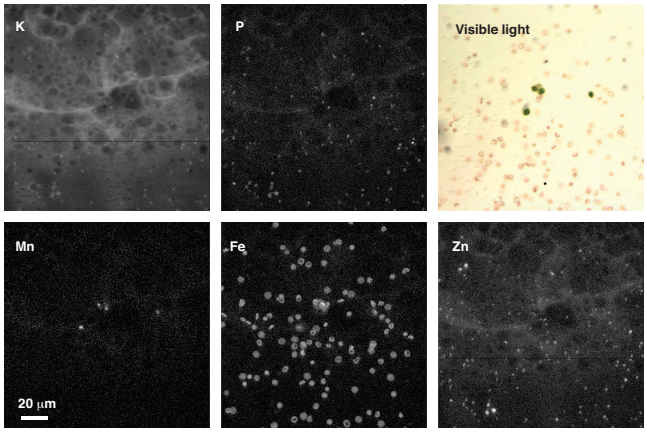

Figure 1: *Data used for subsequent analysis. A mixture of three cell types was prepared, as shown in the visible light micrograph: red blood cells, algae, and yeast. The maps of particular elements (K, P, Mn, Fe, and Zn) obtained from X-ray fluorescence images hint at the characteristics of the different cell types: Mn is prevalent in algae and Fe in red blood cells, while Zn and P are indicative of yeast cells. The visible light micrograph was acquired at one focal plane and thus does not show all cells; separate slight distortions in relative cell positions between the X-ray fluorescence maps and the visible light micrograph were not adjusted for.*

1 Introduction and Motivation

X-ray fluorescence microscopy is a powerful technique to map and quantify trace element distributions in biological specimens. It is perfectly placed to map nanoparticles and nanovectors within cells, at high spatial resolution. Advances in instrumentation, such as faster detectors, better optics, and improved data acquisition strategies are fundamentally changing the way experiments can be carried out, giving us the ability to more completely interrogate samples, at higher spatial resolution, higher throughput and better sensitivity. Yet one thing is still missing: the next generation of data analysis and visualization tools for multidimensional microscopy that can interpret data, identify and classify objects within datasets, visualize trends across datasets and instruments, and ultimately enable researchers to reason with abstraction of data instead of just with images.

We will present a novel approach to locate, identify, and refine positions and whole areas of cell structures based on elemental contents measured by X-ray fluorescence microscopy. We show that by initializing with only a handful of prototypical cell regions, this approach can obtain consistent cell populations, even when cells are partially overlapping, without training by explicit annotation. It is robust both to different measurements on the same sample and to different initializations. This effort provides a versatile framework to identify targeted cellular structures from datasets too complex for manual analysis, like most X-ray fluorescence microscopy data.

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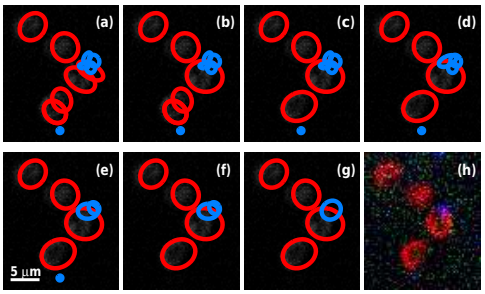

Figure 2: *Example of the progress of group merging and deleting operations. (a) Initial configuration; (b),(c) merging two red blood cell groups; (d),(e),(g) merging two yeast cell groups; (f) deleting a yeast cell group. The overlay of Mn (green), Fe (red), and Zn (blue) elemental maps (h) shows that this area contains four red blood cells and one yeast cell, which overlaps one of the red blood cells. The end configuration in (g) shows that the estimation procedure identifies this configuration correctly. Group boundaries are shown as ellipses for illustration only; all operations are based on taking the union of pixels.*

2 Our Approach

We start by thresholding pixels into foreground/background components based on their elemental content, then obtain an initial guess of the cells based on segmentation of the foreground pixels. We then use a generalized likelihood ratio test to improve the cell configurations and to refine these putative cell areas with respect to the multiple elemental distributions simultaneously. One of the strengths of this algorithm is its ability to identify and distinguish even overlapping objects (several recent methods can handle only samples that are at most touching at the boundaries [Arteta et al. 2012; Bergeest and Rohr 2012]). We will demonstrate the approach on a dataset with three cell types shown in Fig 1 we acquired at beamline 2-ID-E of the Advanced Photon Source at Argonne National Laboratory. In this dataset, we identified around 320 cells with many regions of strong overlap.

References

ARTETA, C., LEMPITSKY, V., NOBLE, J. A., AND ZISSERMAN, A. 2012. Learning to detect cells using non-overlapping extremal regions. In *Proceedings of the 15th International Conference on Medical Image Computing and Computer-Assisted Intervention (MICCAI12)*, Springer-Verlag, Berlin, 348–356.

BERGEEST, J.-P., AND ROHR, K. 2012. Efficient globally optimal segmentation of cells in fluorescence microscopy images using level sets and convex energy functionals. *Medical Image Analysis 16*, 7 (Oct.), 1436–1444.