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Micro-Magellan: open-source, sample-adaptive, acquisition software for optical microscopy

Henry Pinkard^{1,2,3}, Nico Stuurman^{4,5}, Kaitlin Corbin^{1,2}, Ronald Vale^{4,5}, and Matthew F Krummel^{1,2}

Henry Pinkard: hbp@berkeley.edu

¹Department of Pathology, University of California, San Francisco, San Francisco, California, USA

²Biological Imaging Development Center, University of California, San Francisco, San Francisco, California, USA

³Computational Biology Graduate Group, University of California, Berkeley, Berkeley, California, USA

⁴Howard Hughes Medical Institute, University of California, San Francisco, San Francisco, California, USA

⁵Department of Cellular and Molecular Pharmacology, University of California, San Francisco, San Francisco, California, USA

To the Editor

The past decade has seen an explosion of new techniques in optical microscopy with the potential to reveal the complex orchestra of biological processes across large scales of space and time¹. Scalable usability of any new optical technique requires widespread dissemination of hardware and software for data acquisition and of software for data analysis. Commercial software and, more recently, open-source software packages have begun to meet many needs for visualization and analysis of terabyte-sized imaging volumes^{2–4}. As the options for data analysis continue to expand and evolve, it becomes increasingly difficult to make ideal use of the full capabilities of widefield, confocal, and multiphoton microscopes already present in many labs because of a lack of automated and customizable acquisition software⁵.

To fill this gap, we have developed μ Magellan, open-source software (Supplementary Note 1) for reproducible, high-throughput imaging of biological samples across heterogeneous scales of space and time. μ Magellan provides several high-level automation capabilities for the collection of tiled 3D volumes (Supplementary Note 2) that dramatically reduce the

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

AUTHOR CONTRIBUTIONS

H.P. and M.F.K. conceived of the project. H.P. created the software with guidance from N.S. H.P. and K.C. performed beta testing. H.P., K.C., and M.F.K. wrote the manuscript. H.P. and K.C. wrote the user guide and recorded screencasts. M.F.K. and R.V. provided administrative and financial support.

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amount of time and effort researchers must expend to perform complex experiments. Because it utilizes the hardware abstraction layer of μ Manager⁶, μ Magellan can be used with a diverse set of components or complete microscopes from different vendors (Supplementary Note 3), enabling many new types of experiments with thousands of existing instruments. In addition, data written by μ Magellan can be read directly or imported easily by several commercial and open-source software packages for the visualization and analysis of large volumetric imaging data, including BigDataViewer, Vaa3D/TeraFly, and Imaris (Supplementary Note 4 and Supplementary Video 1).

μ Magellan enables users to efficiently map biological samples of unusual shape and unknown spatial organization in three dimensions with ‘explore acquisitions’. These present an interactive Google Maps-like user interface that enables fast sample navigation and high-resolution tiled imaging in user-specified shapes and directions (Fig. 1a and Supplementary Video 2). μ Magellan’s multiresolution pyramid file format (Supplementary Note 5) allows users to pan and zoom through 2D slices of samples in real time. After sample exploration, volumes can be specified for conventional acquisition of 3D cuboids using tiled z-stacks, or user-generated surfaces can be specified to bound acquisition volumes of arbitrary shape (Fig. 1b,c and Supplementary Videos 3 and 4). Surfaces are interpolated from user-specified points using an algorithm based on Delaunay triangulations, which allows both arbitrary precision and sublinear scaling of calculation time with the number of points (Supplementary Fig. 1 and Supplementary Note 6).

In addition to allowing for complex, non-cuboidal imaging volumes, μ Magellan permits surfaces to be used to automatically control imaging parameters on the basis of sample morphology using covariant pairings. These pairings enable automated variation of a particular hardware setting (such as excitation power, detector gain, exposure, etc.) based on either another hardware setting or, in relation to sample morphology, a calculation involving the geometry of a particular surface (Fig. 1d, Supplementary Note 7, and Supplementary Video 5). Coupling morphological information with acquisition settings in such a way facilitates reproducibility and comparisons across heterogeneous biological samples.

μ Magellan is designed to be able to adapt to dynamic biological processes and allows almost all settings (such as spatial regions, time point spacing, automated excitation calculations, etc.) to be altered during acquisition (Supplementary Note 8 and Supplementary Video 6). To compensate for focus drift, which often occurs during long time-lapse experiments, μ Magellan provides an algorithm for automated drift compensation based on a designated fiducial channel (Supplementary Fig. 2 and Supplementary Note 9).

Finally, μ Magellan provides automation to run multiple acquisitions in series or in parallel. This can be used, for example, to image multiple tissue sections sequentially on a single slide for hours or days at a time, or to monitor multiple sites in a sample or multiple organs from the same animal simultaneously to compare conditions while minimizing biological variability.

μ Magellan fills an important niche in the open-source bioimaging software ecosystem by empowering many existing microscopes for automated, reproducible, high-throughput

applications. Its open-source code also makes it an ideal platform for the development and dissemination of new technologies, thereby increasing the ease with which they can be put into practice to reveal the mysteries of biological systems. μ Magellan comes bundled with μ Manager and can be accessed in the plug-ins menu under “Acquisition tools.” The μ Magellan source code and user guide can be found in the supplementary material (Supplementary Software and Supplementary Software Guide). The latest versions of these materials can be found at <https://micro-manager.org/wiki/MicroMagellan>.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Overview of μ Magellan's capabilities for exploring samples and running automated, sample-adaptive acquisitions. (a) 'Explore acquisitions' present a simple click-and-drag interface for acquisition of tiled high-resolution images. A multiresolution file format allows image panning and zooming in real time. Upper and lower focus limits can be set to collect tiled z -stacks. (1), (2), and (3) indicate sequential acquisitions, beginning with exploration of a 2D slice and subsequently tracing the branching airways in a mouse lung in 3D without imaging irrelevant areas of the sample. (b) The user can create interpolated surfaces to encode sample morphology (the cortex of a mouse popliteal lymph node is shown here) by marking points on 2D slices to build a 3D point distribution, which is then interpolated into a smooth surface. (c) Two surfaces used to bound the acquisition volume of an airway (white dashed lines) in a 400- μ m lung slice, compared to the entire bounding cuboidal volume. (d) Surfaces and covaried settings used to set gradients of increasing excitation power at different xy positions, which begin at the surface marking the top of the sample for each position. Red indicates higher excitation power.