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Introduction to ImageJ for Light Microscopy

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This presentation introduces ImageJ as a tool for microscopy processing and analysis. I cover a range of topics including aspects of the history, development, and usage of ImageJ; managing its complex array of plugins; and the various uses of ImageJ for light microscopy.
ImageJ is an open-source Java-based image-processing and -analysis package. Its core functionality has been significantly expanded with the addition of over 300 user-written plugins. These plugins have been made freely available for download by the authors, from either the ImageJ website and/or the authors' website.

The user-written plugins fulfil specific image acquisition, processing, and analysis functions, ranging from medical imaging, macro-imaging, astronomy, material sciences, and, of course, biological light microscopy. However, collecting and organising all of the plugins relevant to a specific user (or group of users) is time-consuming and potentially confusing. This problem, combined with ImageJ's requirement of a java runtime environment, inspired the development of the WCIF_ImageJ bundle, now re-released as MBF_ImageJ (www.macbiophotonics.ca). This is a free, one-stop version of ImageJ, and includes those plugins most useful for biological light microscopy. These 130-plus plugins have been organised into sub-menus to correspond to the online ImageJ manual found at the McMaster Biophotonics website (www.macbiophotonics.ca/imagej).

The Windows version of this bundle contains the core ImageJ program (ij.jar), the runtime environment, macros, plugins, and a preference definition file to match the menus lists and hotkeys to the online ImageJ for Microscopy manual (www.macbiophotonics.ca/imagej). The "plugins only" bundle, available at MacBiophotonics, contains plugins, macros, and preference files that users with ImageJ already installed may download. The latter bundle is suitable for users with ImageJ already installed or users with non-Windows operating systems.

This presentation will describe how ImageJ is used at MacBiophotonics, illustrating ImageJ's versatility in a multi-user, broad-based research facility, which generates a wide variety of image formats. Following a brief discussion of ImageJ's history, I will move on to other relevant topics, including installation, image import, 3D reconstruction (Fig 1), deconvolution (Fig 2), colocalisation analysis, particle analysis (Fig 3), intensity over time analysis (Fig 4), and image processing. This section will also consider batch processing and the automation of processing steps, macro development, and provide a description of how ImageJ is being integrated into our High Content Screening platform.

The open source, multi-platform nature of ImageJ has generated a large community of ImageJ developers across the world. This expertise is accessible to both established and new users alike through a prompt and friendly mailing list. I will cover some guidelines for new users to help them get the most from this pool of experts.

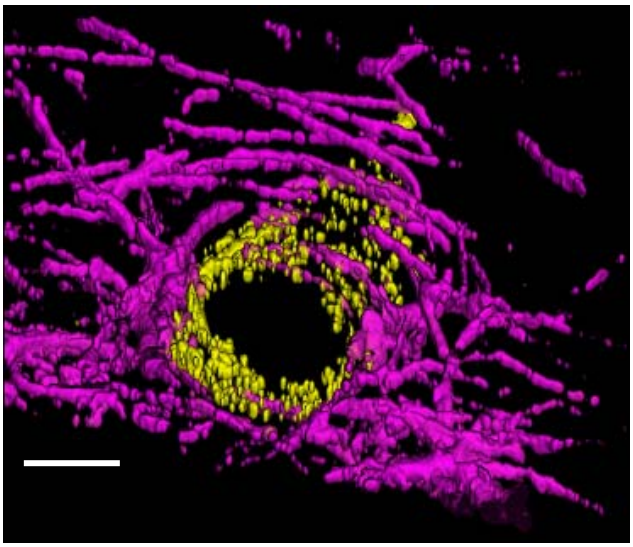

Figure 1. 3D reconstruction. Image of the blood-brain barrier characterized by microvascular cerebral endothelial cells showing Factor VIII positivity (yellow) surrounded by astrocytic foot processes (magenta). The image is a 3D surface render processed with the VolumeJ plugin (M. Abramoff) for ImageJ. Scale = 10 µm.

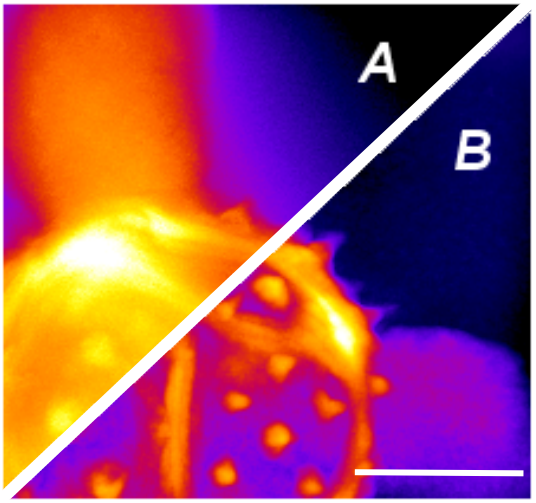

Figure 2. Deconvolution. Maximum intensity z-projection of raw (A) and deconvolved (B) pollen grain. Widefield image of pollen grain stack was iteratively deconvolved (30 iterations) with the "Iterative Deconvolve 3D" plugin using a theoretical PSF generated by the "Diffraction 3D PSF" plugin (both plugins by B. Dougherty). Scale = 5 µm.

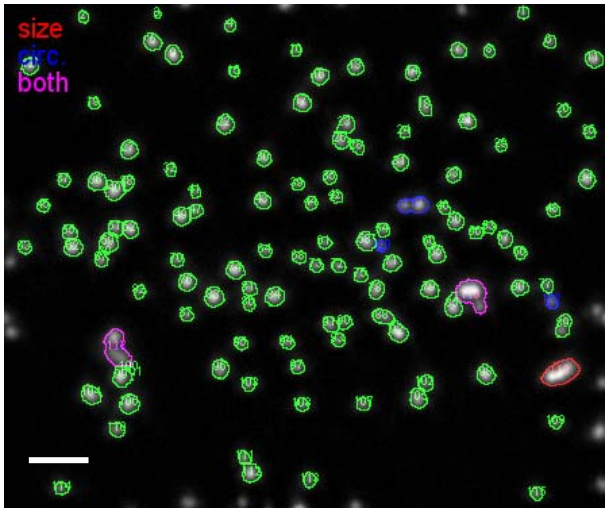

Figure 3. Particle Analysis. DAPI stained *E. coli* were quantified using the "*E.coli* Analysis" macro (T. Collins), which automates several core functions of ImageJ. Cells included in the analysis are outlined in green, while those excluded due to their size, or circularity or both are coloured red, blue or magenta respectively. Scale = 2 µm.

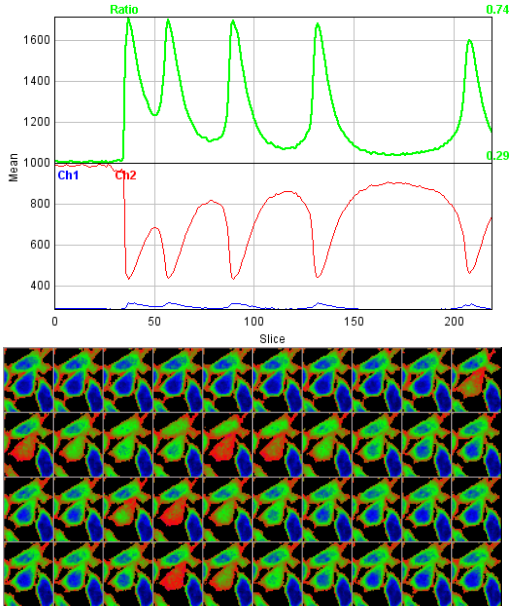

Figure 4. Intensity vs. Time analysis. Cytoplasmic calcium oscillations in HeLa cells loaded with Fura-2. (A) Intensity oscillation on the central cell in panel (B) quantified using the "Ratio Profiler" plugin (T. Collins). Red trace is the intensity of the 380 nm excitation; blue trace the 340 nm excitation; and green trace the ratio of 340:380 nm. B. Montage of selected frames taken from time series shown in (A) processed in ImageJ