

ADVANCED FLUORESCENCE ACQUISITION (AFA™)

An Image-Pro® Plus v4.5 Plug-In

Manage All Combinations of Acquisition Modes and Image Sets

- Time
- Channel (wavelength)
- Focus (Z-stack)
- Stage Position

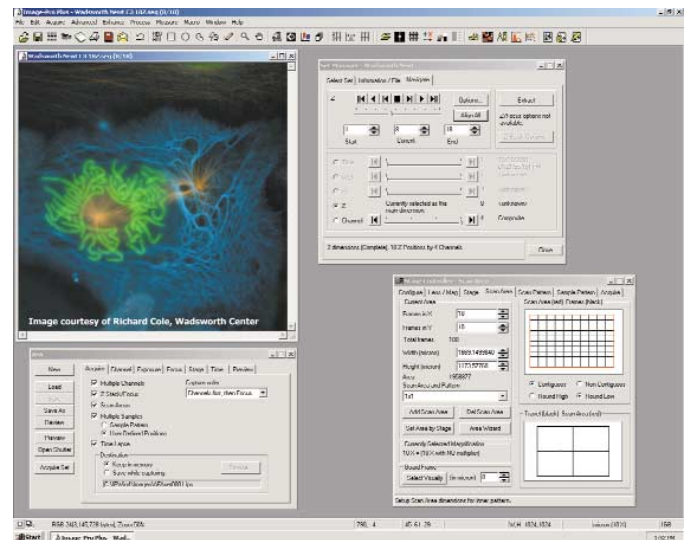
Overview

The nature of research is to push the envelope. What seemed impossible a few years ago is now routine. Digitizing and processing an image for a single time point, wavelength, and stage position for a microscope was a major chore. In recent years, a Z-series or time-lapsed movie added welcomed dimensions for complex or changing samples. But a vast storehouse of information may be exposed when one is able to collect 6D and 7D image sets within a single experiment.

AFA (Advanced Fluorescence Acquisition) Plug-In is for research microscopists who need to automate and manage complex acquisition setup parameters, user feedback display, and subsequent sorting into sets for analysis. Unlike other automation products, AFA becomes an integral piece of the analysis process by sharing information with all analysis modules.

Acquisition

AFA plugs into Image-Pro Plus v4.5 and Scope-Pro®. AFA uses the microscope and peripheral control capabilities of Scope-Pro to configure the system for each image captured in the series. The user interface is laid out in a logical flow of work via tab dialogs from left to right. The acquisition parameters may be saved to disk or in a database, recalled, reported, or accessed via macro programming.



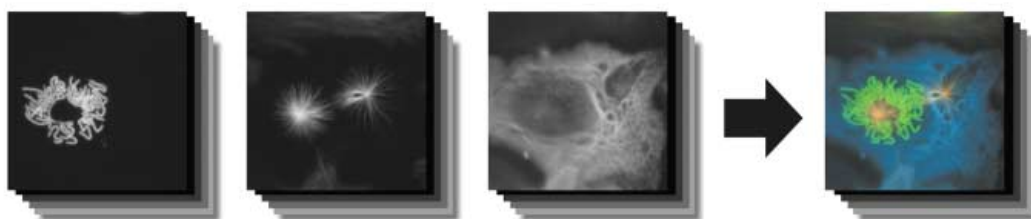
Manage all combinations of acquisition modes and image sets with AFA

Channel Management

Using Scope-Pro configuration settings files, images can be directed to individual channels. While fluorescence wavelengths are the most common channel type, DIC, RGB, and other modes can be defined. Descriptive input settings (e.g. Numerical Aperture or Refractive Index) are stored and can be read and used automatically by the SharpStack™ deconvolution and 3D Constructor™ plug-in modules.

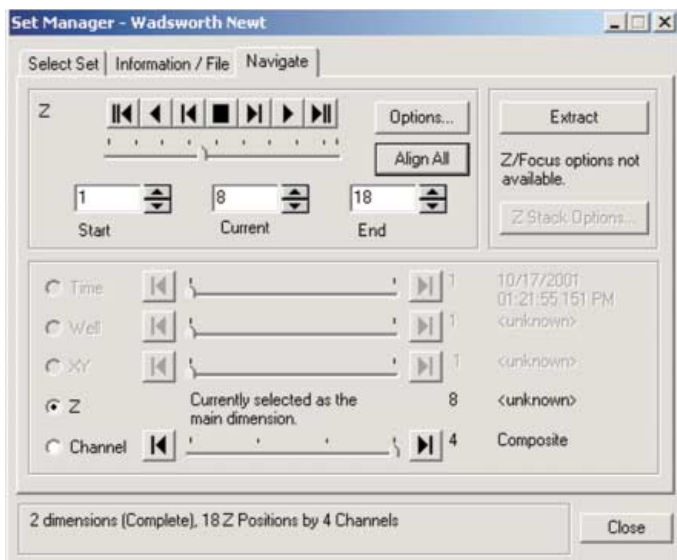
Exposure

Full exposure control is available individually across all channels. The user may specify a color composite to be built automatically as the image set is acquired. Convenient testing options ensure that no time is wasted in method development and an optimum image set is produced. For best results, background correction may be implemented.



Example of a composite image derived from sixteen Z-stack sets from three fluorescent channels using AFA
*Composite image courtesy of Richard Cole, Wadsworth Center.

FROM IMAGES TO ANSWERS[®]



AFA's Set Manager

Focus

Extended Depth of Field (EDF) gives the operator numerous options for control of Z-stacks that can adjust automatically over the run. A full stack can be returned for later deconvolution and rendering. Or a single best-focused frame can be returned. Focus drift can be corrected through tracking the best starting plane.

Stage

Landmarks can be assigned and used for alignment or random location acquisition. Alternatively, a regular pattern (such as a 96-well plate) can be configured. Tiling may be specified to build large images from location acquisitions limited by field of view.

Time

Specify the number of timed cycles at selected intervals or have the fastest cycle time calculated automatically. Once again, testing in advance ensures success of the run.

Preview

Multiple image sets can take a long time to collect. You do not want to discover at the end of a run that the parameters were not quite right. The preview function reassures the user that the desired settings are selected.

Set Manager

Organizing, extracting, and displaying multiple image sets is a tremendous challenge. The elegant, simple interface of Set Manager unlocks the information within those sets. Use it to play a movie showing changes of any single dimension. The currently active data set can be extracted as a separate sequence and stored in .seq or .avi file formats that can be shared with other software for presentation and reporting. It provides clear parameter display so the user may understand how the data was collected (or assembled from separate sources) and documented. Set information may be stored and recalled via a database or via files.

AFA System Requirements

- Image-Pro Plus v4.5 or higher
- Pentium III or Athlon CPU, running at 450 Mhz or higher
- Microsoft Windows 98/ME/NT/2000
- 256 MB of RAM (512 MB recommended)
- 3 GB disk drive with available disk space accommodate two times each image size (four bytes/pixel) within each set
- Color monitor displaying 16-bit high color (24 or 32-bit color preferred)



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