

Image-Pro[®] Plus Product Note

Application Advantages for Using Automated Fluorescence Analysis (AFA[™]) in Addition to Scope-Pro[™]

Introduction

Techniques in microscopy are increasingly complex in nature. Advanced applications such as deconvolution, FRET and ion ratio imaging require sophisticated routines for controlling automated microscopes and peripheral devices such as filter wheels, shutters, automated stages and cameras. Managing the control of these devices through a computer interface can become very involved, as these components may require the use of integrated computer interfaces or proprietary interface cards. A software component to manage complicated hardware and imaging requirements is invaluable when considering the myriad issues involved with these complex tasks.

Description

Scope-Pro and AFA[™] (Automated Fluorescence Analysis) are two plug-in modules that have been developed for use with Image-Pro Plus version to manage automated microscopes and microscope peripherals. While Scope-Pro has been designed to work as a stand-alone plug-in, the AFA module requires the presence of Scope-Pro to operate, and has been designed specifically for fluorescence-based imaging. AFA does have large crossover appeal however, and can be used for industrial applications like semiconductor wafer inspection.

Scope-Pro contains functions for controlling most of the microscopy hardware components found in today's imaging lab. Included with this plug-in module are drivers to control automated microscopes, stages, z-steppers, filter wheels and shutters. Routines also exist for software or hardware autofocus, to create composite, z-stack or tiled images, and capture wells in 96- and 384-well microtiter plates.

AFA has been developed to make the Scope-Pro module even more powerful. An intuitive interface has been designed to encapsulate and integrate the functions of Scope Pro and the Image-Pro Plus/Image-Pro Discovery acquisition modules. It contains functions that integrate these components to perform multi-dimensional imaging, including x/y/z image

capture, multiple x/y/z position, multiple wavelength imaging, as well as time-lapse.

System Requirements

Image-Pro Plus version 5.0 or higher OR
Image-Pro[®] Discovery
Scope-Pro v.5.0 or higher
Pentium 166 or higher
96 Mb RAM
Windows 98, 2000, ME, NT v. 4.0, XP

Automated Microscopes Supported

Olympus Provis AX-70/80, BX Series, IX-70/80 Series
Leica DM RXA, DM IRBE, DM RXA2, DM RA2, DM LA2, DM IRE2
Nikon E1000, TE 2000
Zeiss AxioPlan 2

Automated Stages, Filter Wheels and Shutters Supported

Media Cybernetics Pro-Series Automation Controller Kits
Prior H128/129 Series Controller and Stages
Prior OptiScan Controller and Stages
Ludl BioPoint Controller and Stages
Ludl MAC 2000/5000 Controller for Stages, Filter Wheels, and Shutters
Märzhäuser MultiControl 2000 Controller and Stages
Märzhäuser L-Step Controller and Stages
Sutter Lambda 10-2 and 10-C Filter Wheels and Shutters
Vincent Associates Shutters
Nikon Remote Focus Motor Attachment

Selecting What's Best

The following matrix is intended as a guide for assisting you in selecting which combination of AFA and Scope-Pro will be best for your application. Keep in mind that if you want to use AFA, you must also have Scope-Pro installed on your computer. The matrix is intended only to be used as means for selecting which software interface is best for controlling the hardware used for image acquisition.

The modules are not intended or designed to perform any image processing or manipulation routines (deconvolution, FRET, etc.). If you are not sure which product will work best for you, please contact Media Cybernetics or your local reseller.

	Scope-	AFA/ Scope-	Image- Pro Discovery	Image- Pro Plus
Wavelength	•	•		
Wavelengths	•	•		
probes		•		
Multiple z-planes	•	•		
Multi-Well Plates	•	•		
Multiple x,y positions		•	•	
Time Lapse/Multiple z-planes		•		
Time Lapse/Multiple z-planes/Multiple x,y positions		•		
x,y positions, Multiple z-planes, Multiple fluorescent probes		•		

Table 1.- The AFA/Scope-Pro selection matrix

Some Technical Information

It is impossible to adequately describe every application that may be suited for AFA or Scope-Pro. But each application will fall into a general category like those listed in Table 1,. The following descriptions will be helpful when making your determination as to which plug-in is best for you.

Multiple Z-Planes

Single Wavelength- Applications such as materials QA, color and monochrome brightfield applications requiring through-focus, and single wavelength

fluorescence can all make use of the through-focus option in Scope-Pro.

Multiple Wavelengths- Applications in fluorescence microscopy that require the acquisition of more than one wavelength through multiple z-positions can make the best use of AFA. It has been designed to allow for different exposure times for different fluorescent probes, accommodates peripheral devices such as z-steppers and fluorescent cube turrets, and manages the acquired images through the use of the Set Manager- a unique tool that allows you to look at image planes and acquisition parameters both before and after acquisition.

Multiple x/y Positions

Adjacent Areas- If you need to acquire an image area larger than the optics of your system allow, a means for moving the sample is necessary. This is usually accomplished through a motorized stage. Scope-Pro is used in this case, as it is a straightforward application. Adjacent areas can be scanned and then ‘tiled’ together to create a much larger image. Applications of this type include histology and pathology experiments, materials QA/QC and semiconductor inspection.

Non-Adjacent Areas- There are cases where non-adjacent areas need to be collected and analyzed. In these cases, AFA is the better selection as it has a built-in function that allows you to select different areas of a sample and collect them. Scan patterns can be saved for subsequent image sampling, reducing the amount of time you spend creating experimental protocols. Applications making use of this type of random-position acquisition include materials inspection and histology and pathology.

Multi-Well Plates

This specialized application is accomplished most easily through Scope-Pro. The most popular 96- and 384-well plates are accommodated through pre-defined scan patterns. You can also indicate the positions of standards and wells of interest using a convenient scan pattern wizard. Scope-Pro also contains an auto-focusing routine that allows you to automatically focus on clusters and objects within each cell.

Time Lapse

Applications in time lapse are many. Depending on the complexity of your application, you may require both

¹ The camera control software in Image-Pro Plus and Image-Pro Discovery contains the ability to perform time-lapse functions. Therefore, neither Scope-Pro or AFA are necessary to complete this operation. Image-Pro Express also contains a time-lapse function, but is not mentioned in the table, as it does not support either Scope-Pro or AFA.

Scope-Pro and AFA to accomplish the task. However, there are cases where neither is necessary.

Time Lapse Only- If all you need to do is capture a time-lapse series of events, you don't need either Scope-Pro or AFA to complete the task. Time-lapse functions are included in camera drivers written specifically for Image-Pro Plus, Image-Pro Discovery and Image-Pro Express. Techniques that can make use of this tool include cell division/motility and embryonic development.

Time lapse at multiple positions- This application requires the Scope-Pro/AFA combination. AFA contains a function that allows you to acquire images from locations within the sample and return to those locations at specified intervals to acquire additional images. Alternatively, you can acquire a time-lapse series at one location, and then move to another location to perform a similar acquisition. AFA has the ability to move to any combination of x, y and z axes to complete this task. The functionality of this tool is quite nice, as it allows you to look at more than one cell division or other time-lapse event that may be occurring simultaneously within the same sample.

Time lapse and multiple wavelength- AFA along with Scope-Pro is the preferred method for this type of application. Functions are included in AFA that allow you to select multiple wavelengths and different

exposure times for each wavelength. Including time-lapse then makes techniques such as FRET and ion-ratio imaging very possible.

See Also

Media Cybernetics Product Note- "Extended Depth of Field"

Media Cybernetics Product Note- "Automated Microscope Setup in Image-Pro Plus"

Media Cybernetics Product Note- "The Advanced Microscopy Suite"

Media Cybernetics Product Note- "Choosing the Correct Digital Camera for Your Application"

Related Products

Media Cybernetics Stage Kits

Media Cybernetics Digital Camera Kits

SharpStack™ Deconvolution Plug-In Module

3D Constructor™ 3D Reconstruction Plug-In Module

Image-Pro Plus MDA (Multi Dimensional Analysis)

Image-Pro Plus AMS (Advanced Microscopy Suite)

How To Order

For more information on Image-Pro Plus, and to locate a Media Cybernetics' reseller in your area, visit our website at www.mediacy.com.

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