

# Media Cybernetics Product Note

## Configuring the Advanced Microscopy Suite

### Introduction

Modern microscopy involves an ever-increasing array of specialized microscopes, automated hardware accessories and application-specific software and ultra-sensitive cameras. New methods in microscopy based research require the need for more hardware and software integration. And on top of all this, the complex systems used for these high-performance applications are used for more than one type of application. So at any time, a microscope system may be required to perform such complex analyses as multi-wavelength fluorescence acquisitions, time-lapse or FRET studies, or even three dimensional image reconstructions. The possibilities are vast.

To operate in this multitasking environment, it is vital to have software and hardware that can operate harmoniously to facilitate research and provide results. The Media Cybernetics Advanced Microscopy Suite (AMS) is a solution that integrates the complex needs of imaging applications into a purpose-built and scalable package designed for the changing needs inherent in present day research.

Configuring these components to work together is a task made much easier by improved computer speed and optimization. Still, it is always helpful to have a few pointers in hand to

make the whole configuration process a little less mystifying. The notes contained here are designed as a general reference for installing the components of the AMS. Detailed instructions may be found in the installation guides provided with each of the hardware and software components comprising the Suite.

### The Components Involved

Several software and hardware components make up the AMS, and may be ordered through your local Media Cybernetics dealer ([www.mediacy.com](http://www.mediacy.com) and click 'Dealers'). Each component is sold with an initial 90-day technical support policy. If you would like additional support, extended technical support policies may be purchased directly through Media Cybernetics or your local dealer.

The Advanced Microscopy Suite may be configured from combinations of the following components:

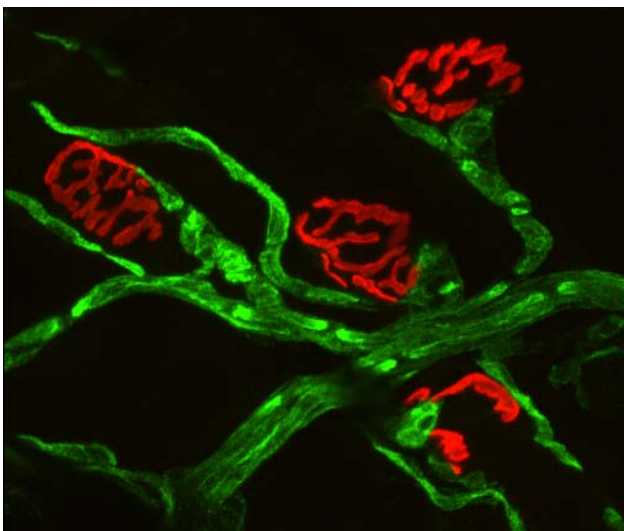
#### Software

Image-Pro<sup>®</sup> Plus- Image Analysis  
 Image-Pro<sup>®</sup> Discovery- Image Analysis  
 Advanced Fluorescence Acquisition (AFA<sup>™</sup>)-  
 Experiment Environment Automation  
 Scope Pro<sup>®</sup> - Microscope Automation  
 SharpStack<sup>®</sup> - Deconvolution and Deblurring Algorithms  
 3D Constructor<sup>®</sup> - Three Dimensional Image Reconstruction and Measurement  
 IQBase<sup>™</sup> - Information and Image Management Software

#### Hardware

Media Cybernetics' Pro-Series Automation Controller Kits  
 Evolution<sup>™</sup> QEi- High Sensitivity CCD Camera  
 Evolution<sup>™</sup> MP- High Resolution CCD Camera

The exact components you will require depend largely on your applications. Your dealer is a great source for suggestions, as is our Media Cybernetics Applications staff. As your needs change it is always possible to add more components to your system. The entire AMS is modular, which enables you to expand over time and as your experimental needs require.



Double staining of mouse intercostal neuromuscular junction for acetylcholine receptors (red) and Kv1.1 potassium ion channels (green). Courtesy of Jennifer Freund, Burnham Institute, La Jolla, CA.

**Compatibility**

In general, the software plug-in modules comprising the AMS do not have compatibility issues with older versions of Image-Pro Plus and Image-Pro modules. Table 1 shows compatibility for each software component with Image-Pro Plus and Image-Pro Discovery. Compatibility is indicated by a ●.

	Scope Pro v. 4.0 v. 5.0	3D Constructor v. 4.0	3D Constructor v. 5.0	SharpStack v. 5.0
IPP v. 5.0	●	●	●	●
IPD v. 4.5	●	●	●	●

Table 1. Compatibility of AMS modules with Image-Pro software.

**Minimum Requirements**

The following minimum computer requirements are needed to successfully configure the AMS:

**Computer**

- Intel® Pentium III or AMD Athlon™ CPU 1.0 GHz Processor
- 512 MB RAM
- Microsoft® Windows 2000 or XP Professional
- OpenGL® Accelerated Graphics Card with 128 Mb RAM<sup>1</sup>
- 100 MB Free Hard Disk Space

**Hardware**

- Serial Port
- PCI Slot

**Before Installation**

Before installing any software or electronic hardware, it is important to make sure you have the correct mounting hardware available. It is also important that the microscope is in good operating condition.

**The Microscope**

As with any microscopy application, it is a requirement that the microscope be in excellent working order and designed with the application in mind. The proper objective lenses for the application must be present and must be clean and free from debris. This holds true for the entire optical path of the

microscope as well. Illumination must be properly aligned, whether it is for brightfield or fluorescence applications.

**Selecting Mounting Hardware for Your Automation Kit**

When ordering the hardware for attaching stages, steppers and cameras to the microscope, it will be necessary to know exactly which microscope and which camera will be used with the system. Microscope stage mounts and focus knobs vary by manufacturer, so it is important to specify the proper adapters when ordering automation kits. Please contact Media Cybernetics or your dealer prior to ordering so we can assist you in ordering the correct mounts for your hardware.

**Selecting a Camera Coupler**

Depending on the camera you choose, the appropriate camera coupler must be selected. Camera mounts vary by microscope manufacturer. And different sizes of CCD chips in the cameras mean certain criteria must be met in order to achieve the best overall image quality. Sometimes this means placing a reduction lens in place to adequately collect and focus light from the microscope's optical path onto the surface of the CCD. Knowing which coupler to use will dramatically improve the quality of the image. Use Table 2 to help you determine which magnification coupler to use with your system.

Camera	CCD Size	Fluorescence	Brightfield
on QEi	2/3"	0.63X	0.63X
Evolution MP	2/3"	0.63X	0.63X

Table 2. Selecting the proper camera coupler.

Once the proper adapters have been selected, you can mount the hardware to the microscope using the manufacturer's instructions supplied with the components.

**The Computer**

A dedicated computer should be used for the AMS. Although computers nowadays are quite stable, it is always best to have a computer system dedicated to image analysis.

As many users will no doubt be using the same computer for their imaging applications, it is useful to have default settings for each of the components in the Suite. It is also useful to have backup information

<sup>1</sup> Required for the 3D Constructor Module. Please contact Media Cybernetics for a list of compatible cards.

available should the system become corrupted. A useful macro is available on our Solutions Zone ([www.solutions-zone.com](http://www.solutions-zone.com)) that helps you create backup environments for your component and individual user settings, as well as system environments used to control the different components. This solution can be downloaded at no cost.

**Solution #1929 - Processing Settings Vault**

We have an additional technical overview for maintaining demonstration computers that our technical support staff has written. If you would like a copy of ‘Maintaining a Demo Computer’, please refer to the ‘General Imaging’ link under the ‘Applications’ link on our web site- [www.mediacy.com](http://www.mediacy.com).

**If You Are Upgrading**

If you happen to be upgrading software from an earlier version, you will be asked to supply an Upgrade Authorization Code (UAC) for each of the upgraded items. These UAC’s are available from Media Cybernetics Customer Service. You will need to have your Image-Pro Plus or Discovery serial number and software version number when calling for your upgrade. Customer Service may be reached by phone at 301.495 3305 or via e-mail at [customerservice@mediacy.com](mailto:customerservice@mediacy.com).

**Configuring the System**

In general, the mechanical components (stages, stepper motors, shutters, filter wheels, etc.) should be installed in a separate step. It is best to refer to the component’s operating instructions for complete details. The computer software, driver and interface card installation is an entirely separate issue and instructions must be followed exactly.

The software, modules, drivers, interface cards, etc., should be installed in a particular order. The order in which you install may change slightly depending upon your particular configuration:

- Image-Pro Plus or Image-Pro Discovery
- SharpStack and/or 3D Constructor
- Scope Pro
- AFA
- Firewire camera interfaces
- Firewire camera drivers

Once the applications software, device drivers and hardware interfaces have been installed, you can shut down the computer and attach cabling from each component to its interface.

**Some Comments About the AMS Components**

Each part of the AMS relies on the others for information about the imaging environment. As a result, it is important to keep in mind certain details about the components when you are installing and configuring the system. This section provides insight for each item, gained from practical experience. Useful information about the use of each component is also provided, in the form of White Papers, Applications Notes, additional Product Notes and Solution Notes. This additional information is available on our website- [www.mediacy.com](http://www.mediacy.com) under the ‘Applications’ link.

**Image-Pro Plus/Discovery**

It is important for Image-Pro Plus or Discovery to be installed first before any module, hardware or camera kit. It is the operating environment in which all the components of the AMS operate. Without the core software present, it will not be possible to operate the software modules or hardware components.

**3D Constructor**

**Video Card-** It is absolutely necessary to have an accelerated video card that supports the OpenGL environment. Several cards are available that meet this criteria and are listed in Table 3.

The ability of a display card to successfully operate in the 3D Constructor environment is highly dependent on the quality of the software drivers. In our experience, the drivers provided by NVIDIA® for their GeForce series of display cards (GeForce2 through GeForce FX) provide the most robust OpenGL support.

Although 64 Mb is the minimum video RAM necessary to view and rotate image stacks, it is always better to have more, as image stacks can become quite large.

**NVIDIA Drivers-** If you are using the NVIDIA graphics card, image rotation may appear non-fluid, even though you have plenty of memory. This problem can be solved by downloading the very latest video

driver software from the NVIDIA website ([www.nvidia.com](http://www.nvidia.com)).

**Additional Reading**

Product Note- Two and Three Dimensional Fluorescent Imaging Using Image-Pro Plus and SharpStack.

Solution Note #1275- 3D Colocalization

**SharpStack**

**Computer Memory-** The most important aspect of the SharpStack configuration is computer memory. Although a minimum of 512MB is necessary, it is best to have at least 1GB. When SharpStack is operating, it can require up to five times the amount of available memory, based upon the size of the image being processed. If the amount of available memory is exceeded, the algorithm can be potentially slowed down while the volume is broken into smaller pieces, processed, and then reassembled.

**Calibrated Objectives-** It is extremely important that the correct calibration files be used when performing deconvolution using SharpStack. Make sure you have calibrated each of your objective lenses before attempting any deconvolution with SharpStack. Scope Pro 5.0 includes a micrometer to assist in calibrating your lens. Each objective should be calibrated at 1x1 binning and any variable intermediate magnification lens at 1x.

**Additional Reading**

Product Note- Two- and Three-Dimensional Fluorescent Imaging Using Image-Pro Plus and SharpStack

**IQBase**

**Overwriting the Existing IPP or IPD Database-** If you are installing IQBase in an environment in which Image-Pro Plus or Image-Pro Discovery are being used, you will be asked if you want to overwrite the existing database. It is best to do so; otherwise IQBase will not be able to automatically archive measurements generated by the AMS. IQbase can be used as a stand-alone database, but additional steps will be necessary to complete the integration with Image-Pro Plus if you choose to use it in stand-alone mode.

**Scope Pro**

**Wizards-** Scope-Pro version 5.0 has both setup and experiment configuration wizards that make installation and use very simple. The wizards take into

account your exact hardware configuration and then configure each component to work properly with the others. This saves you the hassle of trying to configure and test each component separately.

**Up-to-Date Drivers-** It is very important to have the most up-to-date drivers for any hardware component you may be using. The Solutions-Zone ([www.solutions-zone](http://www.solutions-zone)) is the best place to check for these updates. You will be prompted on installation to check for them, and be provided instant access to the Solutions-Zone when doing so.

If your hardware component is an automated microscope, please read the next section carefully. It will help you resolve most of the problems encountered during initial installation and configuration.

**Calibrations-** Improved calibration methods have been implemented in Scope-Pro version 5.0. It is now possible to use calibration files created using Image-Pro's Spatial Calibration dialog or wizard.

As other modules in the AMS require precise calibration information to perform their tasks (3D Constructor, SharpStack) it is necessary to have all your calibration files created prior to imaging. If they are not present, it will not be possible to acquire image stacks in either Scope-Pro or AFA, nor will you be able to deconvolve or deblur images in SharpStack.

**Microscope Specific Downloads-** Certain automated microscopes require additional downloads to complete the Scope-Pro installation. While we try to keep our software as up-to-date as possible, microscope manufacturers will occasionally introduce new automated microscopes or update their existing hardware and software. To keep up with these changes, we issue our updates on our Solutions Zone. Check below to see if the scope you are configuring requires an update. If it does, just type in the provided Solutions Number and follow the download instructions.

**Leica Microscopes**

2 Series- Solution #1609  
DMRXA and DMIRBE- Solution #688

**Olympus Microscopes**

IX-81- Solution #1432  
BX-2- Solution #694

AX-80- Solution #684

**Nikon Microscopes**

TE-2000- Solution #1431  
E1000M- Solution #691

**Zeiss Microscopes**

AxioVert 100M Controller- Solution #481  
AxioVert Microscope- Solution #1487  
AxioPlan- Solution #682  
Micro ToolBox- Solution #1488

**Additional Reading**

Product Note- Using AFA with Scope-Pro

**AFA**

**Scope-Pro Configuration-** Installing AFA is quite straightforward. The most important part of the configuration process is making sure all the components specified in the Scope-Pro configuration are working properly.

AFA requires the presence of settings files that are produced in Scope-Pro to create the unique environments required by complex imaging applications. So it's important that all the components are configured correctly and operating properly. The installation manual for AFA and Scope-Pro contain detailed instruction for creating these environments. In addition, some automated microscope controllers require you to provide information about the microscope environment. Both Scope-Pro and AFA use this information to configure themselves, so it is important the microscope controller be properly configured.

**Stage-Pro Configuration-** As with Scope-Pro, it is vital to have your automated stage components configured correctly. Especially important is the need for spatial calibration files. These calibration files are necessary for proper deconvolution in SharpStack and for proper volume reconstruction in 3D Constructor.

**Wizards-** AFA version 5.0 has both setup and experiment configuration wizards that make using AFA quite easy. The wizards take into account your exact imaging environment and allow you to create complex imaging applications in a straightforward manner.

**Capture Configuration-** The camera settings used for AFA applications can be created and stored using the Capture options in Image-Pro Plus and Image-Pro Discovery. Once these files have been created, they can then be recalled directly into the AFA environment.

It's important to be able to recreate a default state for AFA, especially if many people are using the system. Please use the 'Vault Settings' macro (Solution #1929) found on the Solutions-Zone.

**Additional Reading**

Product Note- Using AFA with Scope-Pro

**Media Cybernetics Camera Kits**

The camera kits in the AMS run through Firewire interfaces, making them quite easy to configure and maintain. Each of the camera kits has a dedicated driver which simplifies and standardizes operation of the camera.

**Order of Installation-** Since Firewire cards are recognized by the Windows operating environment, you should install the board first, and then the Media Cybernetics driver. After installing the card, reboot the system and the correct Firewire drivers will be loaded by the Windows operating system. Then install the Media Cybernetics driver.

**Additional Reading**

Product Note- Choosing the Correct Digital Camera for Your Application.

**Media Cybernetics Automation Kits**

**Adapters-** Since there are so many different combinations of microscopes, cameras and automation hardware that can be run through the AMS, it is important the correct adapters be specified. Please be sure to indicate which microscope you will be running the AMS on when ordering your components from us.

**Serial Interface-** Our automation kits run through a serial interface. It is important that the interface be properly set up. Please refer to the Scope-Pro documentation to properly configure the serial port.

**Additional Reading**

Product Note- Using AFA with Scope-Pro

## The Solutions Zone ([www.solutions-zone.com](http://www.solutions-zone.com))

Our Solutions Zone contains a useful collection of downloadable macros, camera drivers and automated microscope modules. Many of these solutions are free of charge and contain accompanying Solution Notes. If you have a unique imaging requirement, or are looking for updated camera or microscope drivers, the Solutions Zone is the place to look.

## Who to Contact

**Technical Support-** <http://support.mediacy.com>.

**Applications-** [applications@mediacy.com](mailto:applications@mediacy.com)

**Service Contracts-** [customerservice@mediacy.com](mailto:customerservice@mediacy.com)

**Sales-** [info@mediacy.com](mailto:info@mediacy.com)

## For More Information

Three excellent sources of information are available to you. These are our Solutions Zone, Support and Applications Links. All can be accessed from our

website- [www.mediacy.com](http://www.mediacy.com). They contain quite a bit of useful information, and can answer many of the questions you may have about configuring your system.

## Further Reading

Russ, John C. The Image Processing Handbook. 4th Ed. CRC Press (2002)

Matsumoto, Brian - Cell Biological Applications of Confocal Microscopy- 2<sup>nd</sup> Ed. Academic Press (2002).

## How to Order

For more information about the AMS, and to locate a Media Cybernetics' reseller in your area, visit our website at [www.mediacy.com](http://www.mediacy.com).

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