Computer Control of Microscopes Using μManager

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ABSTRACT

With the advent of digital cameras and motorization of mechanical components, computer control of microscopes has become increasingly important. Software for microscope image acquisition should not only be easy to use, but also enable and encourage novel approaches. The open-source software package μManager aims to fulfill those goals. This unit provides step-by-step protocols describing how to get started working with μManager, as well as some starting points for advanced use of the software. *Curr. Protoc. Mol. Biol.* 92:14.20.1-14.20.17. © 2010 by John Wiley & Sons, Inc.

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INTRODUCTION

Light microscopy is an essential tool for many biologists, since research in molecular and cell biology, molecular biophysics, and histology all require the use of optical microscopes. The development of synthetic fluorescent stains and genetically encoded fluorescent tags has permitted labeling of specific biomolecules, thus enabling the researcher to observe molecular locations in living and dead cells. Fluorescence microscopy also enables the observation of single molecules for biophysical observations. In clinical as well as research settings, bright-field and fluorescence microscopy are standard techniques for the examination of slices of multicellular tissues.

For decades, control of optical microscopes was a laborious process involving manipulation of many hardware components. Historically, images were first recorded using hand drawings, and later film. The development of digital cameras, along with computer control of other microscope components, has drastically increased ease of use and expanded the possibilities for researchers. Computerized control of motorized shutters, filter wheels, and other devices now permits users to precisely and repeatedly control the color, intensity, and duration of illumination light impinging on a sample. Motorized Z-stages enable automated focusing and rapid acquisition of image planes along the optical axis. Motorized XY-stages are essential to high-throughput imaging, where the researcher can obtain images from many fields or wells in a multiwell plate. Digital cameras allow the researcher to acquire huge numbers of images, each precisely quantifying photon flux at every pixel location. Digital image storage and processing give users the opportunity to save, annotate, and analyze these large image collections.

Software is required to orchestrate the actions of all these microscope components, and the importance of microscope control software in microscopy-based research and diagnostics is continually increasing. The software should have an easy-to-use interface

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that works well with the most common imaging modalities. It should work with all microscope hardware available, and its functionality should be easily expanded to enable novel microscope imaging approaches. A number of commercial microscope control software applications are available, including IPLab (BD Biosciences), MetaMorph (Molecular Devices), Nikon NIS Elements, Olympus SlideBook, Openlab (PerkinElmer), QED Imaging (MediaCybernetics; *http://www.mediacy.com/*), Simple PCI (Hamamatsu), Volocity (PerkinElmer), and Zeiss AxioVision, but these are specific to a small range of instruments or have limited customizability. National Instruments' LabView, a generic instrumentation software package, is highly customizable, but requires substantial programming for users setting up a new microscope system.

Since 2005, several members of Ron Vale's laboratory at UCSF, with the assistance of contributors worldwide, have been developing μManager: a free, open-source software package for controlling automated microscope equipment (Stuurman et al., 2007). μManager offers a universal standard for computer control of microscopes, cameras, stages, and other peripheral equipment from a diverse set of manufacturers, and runs on Windows, Mac, and Linux computer systems. The application includes an easy-to-use, window-based graphical user interface (GUI) for everyday microscope users, but also offers advanced tools for customization. To easily combine acquisition with analysis, μManager is designed as a plugin for ImageJ (*http://rsbweb.nih.gov/ij/*), a free and open-source image analysis program that is used by many biologists. Because μManager supports a wide range of hardware, it is possible for researchers to combine devices of different brands and control them together on a single microscope system. μManager currently supports various motorized microscopes (Leica, Nikon, Olympus, Zeiss), cameras (ABS, Andor, Apogee, Diagnostic, IIDC Firewire, Hamamatsu, Photometrics, PCO, Stanford Photonics, QImaging, Scion, Sensicam, TWAIN), stages (ASI, Ludl, Märzhäuser, Mad City, Physik Instrumente, Prior), shutters, filter wheels, and illuminators (ASI, Cobolt, Coherent, Conix, CoolLED, Ludl, Nikon, Prior, Spectral, Sutter, Thorlabs, Vincent), data acquisition boards (Arduino, DataTranslation, Heka/InstruTECH, and Velleman), and other accessories (spatial light modulators, Yokogawa confocal spinning disk units, the Pecon incubation system controller). A full list of devices and instructions for installing and using each of them is available at *http://micro-manager.org/devices.html*.

As well as improving the effectiveness of expert microscopists, μManager has the potential (now partly realized) to make microscopy more accessible to nonexpert users. μManager's relatively self-explanatory computer-based user interface can help to hide the complex details of microscope hardware. Users who have become familiar with μManager can easily control an unfamiliar camera, stage, or microscope.

The acquisition of an image in a modern biological microscope often involves a complex sequence of steps. A typical imaging sequence might include rotating a color filter wheel, changing the intensity of a lamp, moving to a position on a specimen slide, changing the focus position, setting a camera exposure time, opening a shutter, starting the camera exposure, finishing the exposure, closing the shutter, retrieving the image from the camera, and storing the image. μManager automates such sequences to make them easily repeated and reproducible.

μManager consists of several modules: a collection of "device adapter" libraries for controlling specific hardware components, a "core" hardware control system that coordinates these devices, a window-based, graphical user interface (GUI) for everyday microscope users, a "script panel" for writing custom automated protocols, and a "plugin" mechanism for advanced microscopy applications. These modules may be accessed by programmers (and scientists who enjoy programming) through a collection of APIs (application programming interfaces). The Device API allows developers to implement device adapters for new hardware not yet supported by μManager. The Core API interface gives programmers control of all hardware via a set of commands that is the same for any device of a given type. The GUI API is a Java-based API that gives programmers the ability to modify and extend all aspects of the user interface. Both the GUI and Core APIs can be accessed by scripts in the μManager script panel and by μManager plugin code, as well as by MATLAB (a commercial numerical computing application). Additionally, the μManager core API can be accessed by the Python programming language. Because of its highly modular software architecture, it is straightforward for outside developers to add functionality to μManager, and the current code contains contributions from at least 25 developers (outside of the μManager team) working either in industry or academia.

This unit presents a series of short protocols for using different parts of μManager. First, instructions are given on how to begin using the application: Basic Protocol 1 describes how to download and install the software, and how to practice using μManager with virtual (software-simulated) equipment (no hardware needed). Then, example protocols are presented for setting up custom configuration settings and presets, running a multi-dimensional acquisition, and viewing acquired images. Next is an explanation of common hardware connectors found in digital microscope equipment, followed by a description of how to use the hardware configuration wizard that connects real equipment to μManager. Finally, two brief tutorials are provided for advanced users on the scripting capabilities of μManager.

GETTING STARTED WITH μMANAGER

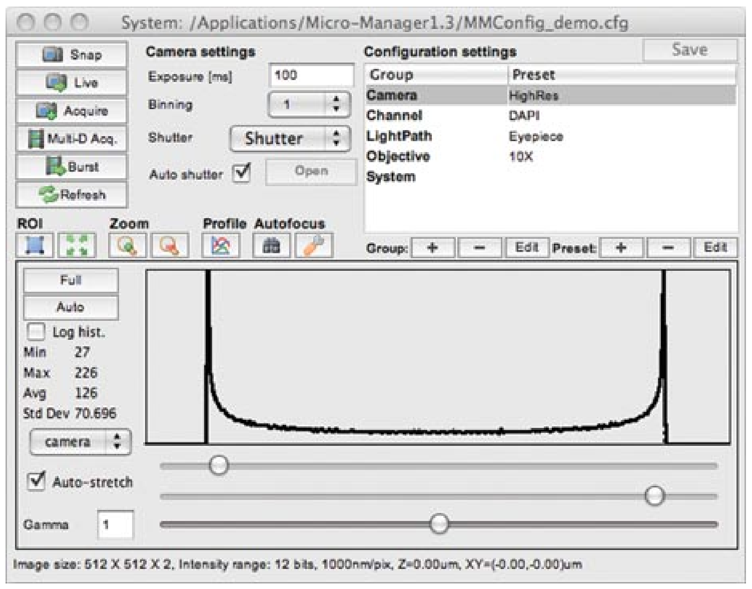
BASIC PROTOCOL 1

μManager will run on nearly any computer running Windows (XP and later, 32-bit only at present), Mac OS X (10.4 and later, PPC and Intel processors, the latter at both 32 and 64 bits), or Ubuntu/Debian Linux (it can be compiled for other Linux distributions as well). Make sure that the microscope hardware is currently supported by μManager by checking the current list of supported devices (*http://micro-manager.org/devices.html*).

1. Download μManager from *http://micro-manager.org/downloads.php*. Installers are available for Windows, Mac, and Ubuntu/Debian Linux. Follow the instructions that come with the μManager installer for the desired operating system. If the goal is to examine μManager image files acquired elsewhere, and not to run actual hardware or experiment with demo (virtual) equipment, then download the μManager Reader plugin for ImageJ.

2. Start μManager by using the shortcut provided by the installer. On startup, μManager shows a splash screen, prompting for the configuration file to be loaded for this session. This configuration file contains information about the hardware attached to the computer, as well as presets and pixel size calibration data. The user will eventually need to make such a configuration file specific for the particular equipment setup (see Hardware Configuration below), but at this point choose the file `MMConfig_demo.cfg` that contains definitions for "virtual," emulated demonstration equipment. This demo configuration makes it easy to experiment with μManager software. At any time during a μManager session, a different configuration can be loaded using the menu command: **Tools > Load Hardware Configuration. . .**.

   Once a configuration file is loaded, there will be two windows visible: one is the main μManager window, the other is the main ImageJ window. Each of these windows offers its own set of menu commands. Switch between them by clicking on the respective main window. Images acquired using the **Snap** and **Live** buttons in μManager's main window are ordinary ImageJ windows, and all of ImageJ's image visualization and analysis tools can be applied to these images.



System: /Applications/Micro-Manager1.3/MMConfig_demo.cfg

Camera settings

Exposure [ms] 100
Binning 1
Shutter Shutter
Auto shutter ✓

Configuration settings

Group	Preset
Camera	HighRes
Channel	DAPI
LightPath	Eyepiece
Objective	10X
System	

Log hist.
Min 27
Max 226
Avg 126
Std Dev 70.696
camera
Auto-stretch ✓
Gamma 1

Image size: 512 X 512 X 2, Intensity range: 12 bits, 1000nm/pix, Z=0.00um, XY=(-0.00,-0.00)um

Figure 14.20.1 The main µManager window, where the application's primary controls are located.

Using the main window

3. The main µManager window (Fig. 14.20.1) serves two functions: the top half lets the user snap images, control the state of the microscope system, and set up experiments. The bottom half of the main window shows a histogram of the last acquired image, and has tools that allow adjustment of contrast and brightness on the image display. The top left quadrant of the window contains controls to set the camera exposure time, binning (grouping of camera pixels before camera readout), the shutter to be used (for those systems that have multiple shutters), and whether or not the software will automatically open the shutter before a camera exposure and close it afterwards (autoshutter check box). When the autoshutter feature is turned off, toggle the state of the active shutter with the main window's open/close button.

4. A row of icon buttons allows the user to set and unset regions of interest (ROIs) on the camera, zoom in and out of the image, view a live line profile, and operate autofocus (both hardware and image-based autofocus methods are available).

   The **Snap** button will capture an image using the current settings and display it in an ImageJ window. The **Live** button will start continuous image acquisition from the camera (stream images) and display the incoming images in the same window. The **Acquire** button snaps an image and adds this to a µManager image viewer window (see µManager Image Viewer, below). The **Multi-D Acq** button opens the multi-dimensional acquisition window (see Basic Protocol 3), which can be used to set up 5-D imaging experiments. The **Burst** button enables high-speed image acquisition, with frame rates up to the maximum speed supported by the camera hardware.

   Cameras typically have two different modes: image snapping and live streaming. When snapping an image, the sensor is exposed and accumulated charge is moved to an unexposed area of the chip and eventually converted into digital intensity values. Only after the image is completely transferred to the computer's memory (a process that can take more than a second on some slow-scan cameras) can a new image be acquired. In streaming mode, exposure of a new image starts immediately after transfer of accumulated charge to a dark area on the chip. Exposure of the new image and transfer to the computer happen simultaneously, which increases the speed with which images can be acquired. Moreover, when the camera snaps images, it can often take some time before the actual exposure starts; such delays do not happen once a camera is streaming images. μManager uses the "snap" mode when executing multi-dimensional acquisition and the streaming mode for live imaging and burst mode.

The top right quadrant of the main window contains a user-defined table of **Configuration Groups** and **Configuration Presets**, created separately for each microscope system. These configuration settings are explained in more detail below.

To get an idea of the workflow in μManager, load the demo configuration (or use a real camera if there is one available that can be controlled by μManager) and execute the following steps:

5. Click the **Snap** button in the top left corner.
6. Uncheck the Auto-stretch check box in the bottom left corner. Move the three sliders below the histogram to affect the display (but not the actual pixel values) of the image. The top slider sets the pixel value that will be displayed as black, the middle slider sets the pixel value that will be shown as white, and the bottom slider will change the gamma (a parameter for adjusting contrast).
7. Change the exposure time to 25 msec and snap another image. Inspect the effect on pixel values (look at the histogram; you can also place the mouse cursor in the image to show the pixel value in the ImageJ window), and display intensity (try pressing the **Auto** and **Full** buttons in the histogram part of the main window).
8. Change the binning to "2," snap an image, and note the change in image size, pixel intensities and display intensity.
9. Press the **Burst** button. In the MM Fast Acquisition Plugin window, set the sequence length to 50, set **Output** to **Screen**, and press the **Start Acq** button. This should result in a μManager viewer window with 50 images, acquired at the highest speed possible.

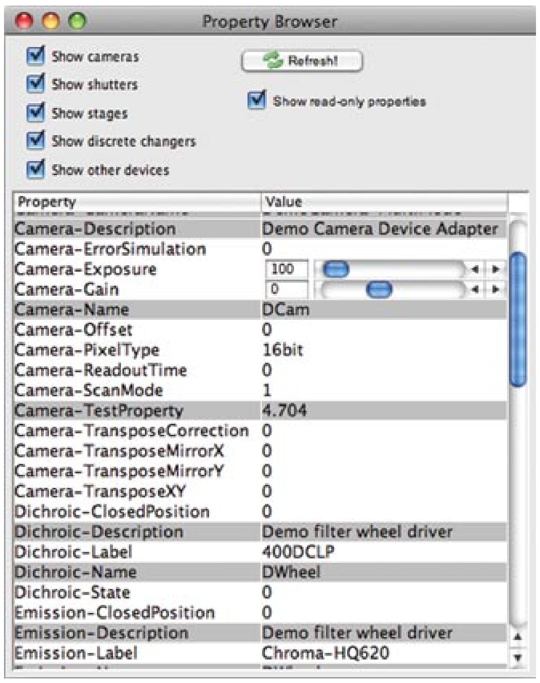
WORKING WITH DEVICES, PROPERTIES, AND CONFIGURATION SETTINGS

BASIC PROTOCOL 2

The **Device/Property Browser** window (available in the **Tools** menu; see Fig. 14.20.2) lets the user examine and change the state of all hardware components of the system. Each device has a set of properties, each with a unique name. Properties may be text or numbers. Gray properties are read-only, while white properties can be changed by the user.

Properties can be grouped together in **Configuration Groups**, which behave as a collection of simple shortcuts for the user to view and change important settings in the hardware. The following is a short protocol for creating a Configuration Group and its **Presets** (i.e., choices), using the demo configuration as an example. Suppose the goal is to set up filter sets for a FRET pair: a green-emitting donor and a red-emitting acceptor. By creating presets that provide different filter and dichroic combinations, direct fluorescence can be observed from either donor or acceptor, or an energy transfer image can be acquired.

1. If the demo configuration has not already been loaded, select the menu **Tools > Load Hardware Configuration. . .** and select the configuration file named `MMConfig_demo.cfg`.


Property Browser

- [✓] Show cameras
- [✓] Show shutters
- [✓] Show stages
- [✓] Show discrete changers
- [✓] Show other devices
- [✓] Show read-only properties

Property	Value
Camera-Description	Demo Camera Device Adapter
Camera-ErrorSimulation	0
Camera-Exposure	100
Camera-Gain	0
Camera-Name	DCam
Camera-Offset	0
Camera-PixelType	16bit
Camera-ReadoutTime	0
Camera-ScanMode	1
Camera-TestProperty	4.704
Camera-TransposeCorrection	0
Camera-TransposeMirrorX	0
Camera-TransposeMirrorY	0
Camera-TransposeXY	0
Dichroic-ClosedPosition	0
Dichroic-Description	Demo filter wheel driver
Dichroic-Label	400DCLP
Dichroic-Name	DWheel
Dichroic-State	0
Emission-ClosedPosition	0
Emission-Description	Demo filter wheel driver
Emission-Label	Chroma-HQ620

Figure 14.20.2 The property browser window. To reach it, choose **Tools > Device/Property Browser. . .**. All major hardware properties and their values are included in this table.

2. On the Configuration Pad in the Main Window, create a new Configuration Group by pressing the + button next to the word **Group**.

3. The **Group Editor** window will open. Name the group `FRETChannel` and select the Use in Group? check boxes for Dichroic-Label, Emission-Label, and Excitation-Label.

4. Next, the **Preset Editor** will open to let you edit the first Preset in this Group. Name the Preset `Transfer` and set the following settings:

   Dichroic-Label: Q505LP
   Emission-Label: Chroma-HQ700
   Excitation-Label: Chroma-D360.

5. Press the Preset + button; the Preset Editor opens again. This time name the Preset `QuenchedDonor` and set the following settings:

   Dichroic-Label: 400DCLP
   Emission-Label: Chroma-D460
   Excitation-Label: Chroma-D360.

6. Press the Preset + button once more, name the Preset `AcceptorDirect` and set:

   Dichroic-Label: Q505LP
   Emission-Label: Chroma-HQ700
   Excitation-Label: Chroma-HQ570.

7. Thus, three Configuration Presets have been created in the FRETChannel group: `Transfer`, `AcceptorDirect`, and `QuenchedDonor`. These Presets are available in a drop-down menu in the **Configuration Settings** panel on the main window. Open the **Device/Property Browser**, choose one of the Presets in the FRETChannel

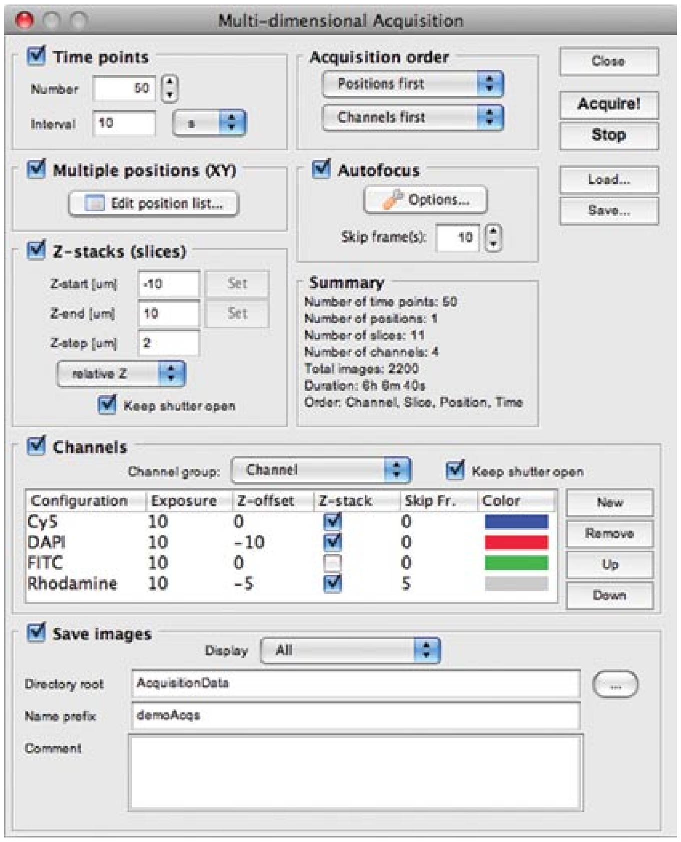
group, and then press **Refresh** in the **Device/Property Browser** to see how the relevant property values have been changed as a result. Note that you can also hover the mouse pointer over a Configuration Preset to reveal a tool tip showing the properties set by the Preset.

RUNNING A MULTI-DIMENSIONAL ACQUISITION

BASIC PROTOCOL 3

μManager's Multi-Dimensional Acquisition (MDA) allows the user to set up 5-D (*x*, *y*, *z*, wavelength, and time) acquisitions at multiple positions. A large number of options are offered (Fig. 14.20.3), most of which are self-explanatory. It is straightforward to set up experiments that run for days and generate many gigabytes of data, but before doing so it is important to become familiar with the MDA controls.

The Acquisition Order can have a profound influence on the efficiency of MDA. When executing an MDA using both *z* stacks and multiple channels, the user can decide whether to change channels at each *z* height (**Channels first** as **Acquisition order**) or to take complete *z* stacks for each channel individually (**Slices first**). The best choice depends on the specifics of the hardware. For example, if the available hardware can switch between channels very quickly, it is likely better to use the **Channels first** option, whereas if channel switching is relatively slow but the Z-drive is fast and reliable, the **Slices first** option is preferable. When carrying out acquisition at multiple stage positions (which are marked using the **Stage-position List** window accessible through the **Edit position list...** button), the user can decide to either move the stage between positions at each



Time points ✓
Number 50
Interval 10 s

Acquisition order
Positions first
Channels first

Multiple positions (XY) ✓

Autofocus ✓
Skip frame(s): 10

Z-stacks (slices) ✓
Z-start [um] -10
Z-end [um] 10
Z-step [um] 2
relative Z
Keep shutter open ✓

Summary
Number of time points: 50
Number of positions: 1
Number of slices: 11
Number of channels: 4
Total images: 2200
Duration: 6h 6m 40s
Order: Channel, Slice, Position, Time

Channels ✓
Channel group: Channel
Keep shutter open ✓

Configuration	Exposure	Z-offset	Z-stack	Skip Fr.	Color
Cy5	10	0	✓	0	🟦
DAPI	10	-10	✓	0	🟥
FITC	10	0	☐	0	🟩
Rhodamine	10	-5	✓	5	⬜

Save images ✓
Display All
Directory root AcquisitionData
Name prefix demoAcqs
Comment

Figure 14.20.3 The Multi-Dimensional Acquisition window. Use the check boxes to select which of the five dimensions you want to include in an acquisition: Time points, Multiple positions (XY), Z-stacks (slices), and Channels.

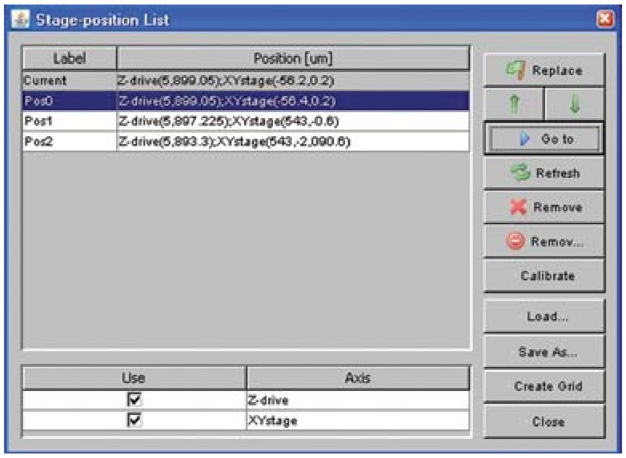
time point (choose **Positions first** as **Acquisition order**) or to consecutively execute the full 5-D acquisition at each position (choose **Time first** as **Acquisition order**).

Channels can be selected from the **Channel group**, which is one of the Configuration Groups that was previously defined. Channel definitions can include any property available on the system: most often used is a Channel group that defines positions of dichroic mirrors and emission and excitation filters. For each individual channel (added using the **New** button), it is necessary to set the exposure time as well as the color to be used to display this channel in the μManager Image Viewer.

Multiple positions can be defined with the **Stage-position List** window (Fig. 14.20.4), accessible from the menu entry **Tools>XY List. . .** and also from the **Edit position list. . .** button in the MDA window. Move the XY stage and Z-drive into the desired position using the joystick, dial, or other input device, and click the **Mark** button (when a position is selected, this button changes into a **Replace** button). Use the check boxes in the bottom of the window to determine which of the stages attached to the system will be recorded. Select a recorded position and click on the **Go to** button to move all stages to the selected position.

To experiment with MDA acquisition, load the demo configuration and click on the **Multi-D Acq.** button in the main window. Use the MDA window (Fig. 14.20.3) to execute the following steps:

1. Check the **Time points** check box and select three time points at 5-sec intervals. Uncheck the **Multiple positions (xy), Autofocus**, and **Z-stacks (slices)** check boxes. Check the **Channels** check box. Select **Channel** as the **Channel group**. Use the **New** button to add a DAPI and FITC channel. Click on the **Color** column and select a blue color for DAPI and green for FITC. Set the exposure time to 20 msec for DAPI and 50 msec for FITC. Press **Acquire** to run the acquisition. Inspect the resulting acquisition window and make sure that acquisition is executed as expected.
2. In the MDA window, check the **Z-stack (slices)** check box. Choose **relative Z**. Set **Z-start [μm]** to -2, **Z-end [μm]** to 2 and **Z-step [μm]** to 0.1. Set **Acquisition order** to **Slices first**. Press **Acquire**. Inspect the resulting acquisition window and make sure that acquisition is executed as expected.
3. Change the **Acquisition order** to **Channels first** and run the acquisition again.



Label	Position [um]
Current	Z-drive(5,899.05);XYstage(-56.2,0.2)
Pos0	Z-drive(5,899.05);XYstage(-56.4,0.2)
Pos1	Z-drive(5,897.225);XYstage(543,-0.6)
Pos2	Z-drive(5,893.3);XYstage(543,-2,090.6)

Use	Axis
✓	Z-drive
✓	XYstage

Figure 14.20.4 The **Stage-position List** window. Manage acquisition stage positions in 2 or 3 dimensions in the position list table. Each position can refer to one or more stage axes

USING THE µMANAGER IMAGE VIEWER

The **µManager Image Viewer** (Fig. 14.20.5) is based on the Image5D plugin for ImageJ written by Joachim Walter (*http://rsbweb.nih.gov/ij/plugins/image5d.html*). The viewer contains scroll bars for quick navigation through *z* slices (***z***) and time-points (***t***). The controls on the right hand side of the viewer will change depending on the display mode chosen. µManager defaults to overlay (***ovl***) mode in which the channels are shown superimposed, each channel in its own color. Each channel contains a radio button to select the active channel. ImageJ operations, such as setting brightness/contrast or applying filters, work on the channel selected with this radio button. Each channel also has a check box that provides a quick way to switch the channel display on and off. In ***color*** display mode, the channel controls on the side are replaced by a slider that selects the active channel (which is also the only one shown). The ***gray*** display mode works similarly, but displays the channel as a monochrome image.

The controls on the bottom of the window (from left to right) serve to:

save the acquired data
stop an ongoing acquisition
pause an acquisition
open the image metadata
open the ImageJ brightness/contrast tool
play the acquired time points as a movie.

µManager keeps track of image metadata coming from the hardware, as well as metadata the user might add (such as comments or display settings). The metadata can always be accessed from the Image Viewer by pressing the **Display metadata** button. For each image, the application remembers image-specific data (such as the "Channel used", "Exposure time", and "Slice number" under the **Image** tab), as well as all property values for all devices in the microscope system (under the **State** tab). **Comments** can be edited at any time (note that comments can also be added in the MDA window, before the start of acquisition). The **Save** button in the image metadata window (Fig. 14.20.6) will save

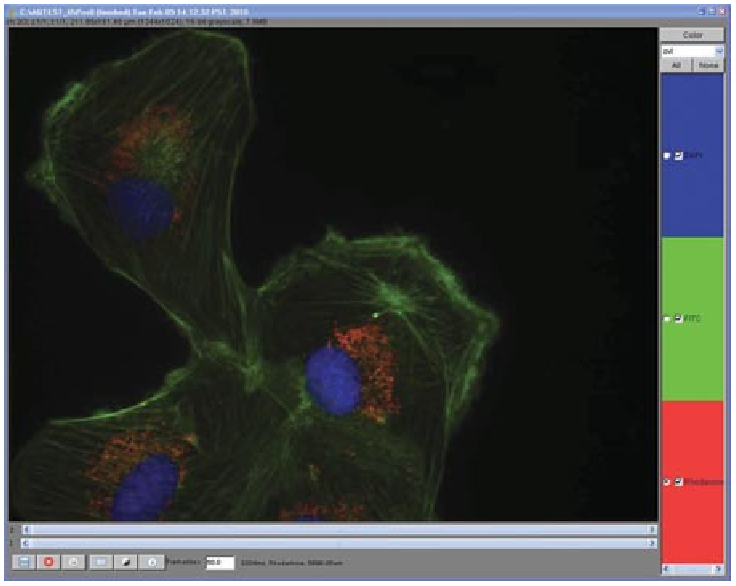
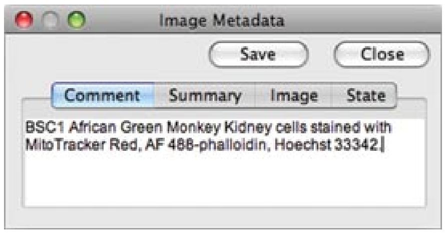


Figure 14.20.5 The 5D Image Viewer of µManager. Use the ***z*** and ***t*** sliders to view different slices and time points; use the boxes at the right to show and hide **channels**; use the buttons at bottom to save files, halt acquisition, examine metadata, adjust contrast, and play back time-lapse sequences.



BSC1 African Green Monkey Kidney cells stained with MitoTracker Red, AF 488-phalloidin, Hoechst 33342

Figure 14.20.6 The Image Metadata window. Experimental annotations can be entered in the **Comment** tab.

changes in the comments as well as the brightness/contrast settings of each channel (pixel data and other metadata are not affected).

To get started working the µManager image viewer, perform the following steps:

1. Acquire a 5-D data set using the MDA window as described in Basic Protocol 3.
2. Press the bottom left button to save the data now displayed in the viewer window.
3. Toggle the check boxes for each of the channels and notice the change in the displayed image.
4. Select one of the channels and open the ImageJ Brightness/Contrast tool (press the fifth button from the left at the bottom row). Click **Auto**. Move the **Minimum** and **Maximum** slider and notice the effect on the display.
5. Activate the radio button in front of another channel. Go back to the Brightness/ Contrast tool and adjust the now active channel.
6. Observe other *z* slices and time points by moving the *t* and *z* sliders.
7. Open the metadata window (4th button from the left at the bottom row). Add desired comments. Click on the other tabs and inspect the data presented (note that the **Image** and **State** data will change with the image currently displayed in the viewer). Click the **Save** button to save the changes made in the comment and display settings.
8. Close the Image Viewer window and open the data saved on disk again using the µManager menu command: **File > Open Acquisition Data as Image5D. . .**.

   ImageJ does not "see" the µManager viewer images as normal ImageJ data. It is therefore necessary to convert the data before working with them in ImageJ. The µManager menu **Image5D** contains several tools for this purpose (**duplicate, crop, make montage, Z project, copy to Stack**, etc.). For instance, to make an AVI movie file of a time series as displayed in the µManager viewer, select the viewer window, choose **Image5D > Copy to RGB Stack(t)** from the µManager menu, and then use the **File > Save As > AVI. . .** ImageJ menu command to save the file. It is also easy to convert the µManager viewer data into an ImageJ Hyperstack using the ImageJ menu command **Image > Hyperstacks > Stacks to Hyperstack. . .**.

BASIC PROTOCOL 5

CONNECTING AND CONFIGURING HARDWARE

Connecting the hardware

Before using the µManager application to control microscope equipment, it will first be necessary to connect the hardware to a computer. There are many different types of communication interfaces between the computer and microscope hardware. Some knowledge of these interfaces is essential for configuring a microscope system. Here, the most commonly used interfaces and their connectors are described.

Serial (RS232) interface

In serial communication, bytes are sent one at a time through a single wire. The archetypal serial interface is specified by the RS232 standard. Bits are transferred at an agreed-upon speed, called the baud rate (the μManager Wiki, *http://micro-manager.org/devices.html*, lists what baud rate to use for each specific device). The number of bits per data unit, as well as the number of stop bits, can vary, but current equipment almost invariably uses 8 data bits and 1 stop bit. Extra wires can be used for handshaking (used to signal that the device is ready to receive or send data). Currently, most RS232 cables have 9-pin connectors (DB-9). Some devices need to be connected through a null-modem cable in which the send and receive wire have been cross-linked (such cables are clearly labeled). Since modern computers generally do not include DB9 RS232 ports, a USB-to-serial converter or an add-on (PCI) card will be needed (the latter solution is more reliable and highly favored). Although it may seem archaic, the RS232 interface is still a simple and robust method of communication between computer and device, and many newer devices conceal an RS232 interface inside a USB connection.

USB interface

The Universal Serial Bus is a top-down serial interface that is driven by the "top" (the computer). Connections to other equipment can be made through hubs, resulting in a branched network. Sending and receiving of data is always initiated by the "top" computer. Three generations of the USB protocol exist, with data rates up to 480 Mbit/sec (USB 2.0) or even 4 Gbit/sec (USB 3.0). On most operating systems, specific drivers are needed for each device. In microscopy, the USB is increasingly used to send simple commands to equipment. Also, a number of cameras make use of the USB interface.

Firewire/IEEE1394

The IEEE1394 interface (commercialized as Firewire by Apple) is another high-speed serial interface. Unlike USB, any device, not only the computer, can initiate communication. Many scientific-grade cameras make use of the IEEE1394 interface. However, not all those cameras use the same protocol to send data and commands through the IEEE1394 interface. A number of camera manufacturers use a standard protocol, IIDC, that is supported by μManager's Mac and Linux versions.

CameraLink

CameraLink is a serial communication protocol for vision equipment maintained by the Automated Imaging Association (AIA). The base CameraLink implementation provides data speeds up to 2.04 GBit/second. Many scientific-grade cameras use the CameraLink interface and they are usually sold together with a matching CameraLink interface card for the computer.

TTL

Transistor-Transistor Logic (TTL) is used here to mean TTL-compatible logic levels, where 0 to 0.8 V corresponds to a low (inactive) state, and 2 V and higher to the high (active) state. TTL signals are used in microscope equipment to (for instance) open and close shutters, signal when a camera is exposing, trigger movement to a new stage position, etc. TTL signals are usually run through coaxial cable and often use BNC connectors (alternatives are SMA and SMB connectors). Modern computers do not have direct TTL connections to outside equipment (in the past, some pins on the parallel port as well as handshaking signals on RS232 interface were used for this purpose), but several low-cost solutions exist on μManager (e.g., the Arduino board, *http://arduino.cc/*, can be used for TTL communications).

Analog voltage

Some device controllers have connections that respond to an analog voltage. For instance, the position of a piezo stage can sometimes be set using a voltage differential, or the intensity of a diode laser can be controlled by an applied voltage. Analog voltages are most often transmitted using coaxial cable with BNC connectors. Computers usually do not directly expose outputs that generate voltage differentials, but many expansion boards (some of which can be connected using USB) exist that contain Digital-Analog (DA) conversion chips that translate a digital signal into an analog voltage.

Constructing a configuration file using the Hardware Configuration wizard

Once the equipment is correctly connected to the computer and the connection tested with the manufacturer's software tools, a configuration file can be created. Before doing so, check the instructions for the specific devices on the μManager Web site (*http://micro-manager.org/devices.html*) to find information such as the appropriate serial port settings.

1. Start the Hardware Configuration Wizard using the μManager menu command **Tools > Hardware Configuration Wizard. . .**. The first page (step 1) lets the user decide whether to start a new configuration file or to continue work on an existing configuration.

2. Step 2 lists all serial ports available on the system. It will be necessary to know which serial port is connected to which piece of equipment, and to know the serial port settings (especially the baud rate). Identifying serial ports is often difficult since they are often not, or only poorly, labeled. With USB-serial adapters, the hardware configuration wizard can be started with or without the adapter plugged in to determine under which name it appears.

3. In step 3 of the Hardware Configuration Wizard, the devices in the system are added. Pressing the **Add** button opens a list of all devices that μManager supports. Find the camera, stage, microscope, shutters, etc. that are connected to the computer and press the **Add** button. The name of the device can be changed as desired. In some cases, if it is difficult to match a certain piece of equipment with names in the device list, check the documentation for the equipment and the μManager Web site, or try figuring it out through trial and error.

4. Step 4 of the wizard asks the user to set pre-initialization settings for the various devices. For instance, devices that communicate through a serial port will require a port name (configured in step 2). Pressing the **Next** button at this point will start the initialization sequences of all devices. This is the most critical part of the configuration. When it completes, the computer will be properly connected to all devices.

5. In step 5, select the default camera, shutter, and focus stage. μManager can work with multiple cameras, shutters, and focus stages, but expects one of each to be selected as default. These defaults can be changed later by changing the Core-Camera, Core-Shutter, and Core-Focus properties in the Property Browser. Also, the shutter drop-down menu in the main window directly controls the Core-Shutter property.

6. In step 6, enter "delays" for equipment items that are not designed to signal when they are busy. For example, before acquiring an image, it may be necessary for μManager to wait for a particular delay interval while a shutter opens. If prompted to set delays for devices, it is important to determine these delays empirically. Setting

this value too high may result in long waits and useless exposures of the sample to light; setting it too low may result in images that are taken while components are still moving. Luckily, most of the newer devices can signal whether they are moving and setting delays for them is not necessary.

7. In step 7, select devices to be synchronized with image capture. Before taking an image, μManager will repeatedly query the selected devices and wait until they are not "busy." Most devices need not be synchronized; consult the Web site for specific devices.

8. In step 8, define labels for state devices. Click on any of the state devices listed in the left side column. Now edit the Label for each of the positions of the State device. In some cases, these labels are read automatically from the device. You can force μManager to read them again by pressing the **Read** button. Labels are intended to help the user remember what each of the positions of a state device contains.

9. Save the configuration in Step 9 of the wizard.

10. Construct appropriate **Configuration Groups** and **Presets** as described above. A special **Configuration Group** can be created by naming it System. Within the System group, the presets Startup and Shutdown will be applied during μManager startup and shutdown, respectively, setting any included device properties to specific values. For example, a common practice is to set camera exposure time in the Startup configuration Preset. Save the configuration file (using the **Save** button in the top right corner of the main window), or using the **Tools** > **Save Configuration Settings. . .** menu command.

CALIBRATING PIXEL SIZE

BASIC PROTOCOL 6

To measure the physical dimensions of a specimen from a digital microscope image, one needs to know the size of pixels at the specimen plane. μManager has a mechanism to store calibrated pixel size data (similar to Configuration Groups), in which each pixel size is matched to a particular set of device settings. When the state of the system matches the state described in one of the stored, calibrated pixel sizes, μManager will automatically record that pixel size in the image metadata. To enter pixel size data into μManager's table, it is first necessary to decide which device settings of the system influence pixel size (usually the objective and tube-lens/optovar), and measure the pixel size using the various settings of those devices. Pixel size can be measured either by using a calibration slide or by moving the stage over a known distance (the latter method requires that the stage has been accurately calibrated).

Materials

Optional: calibration slide, available from many suppliers. These slides typically bear a photo-etched calibration grid on one surface. Many variations exist (for instance: Electron Microscopy Sciences 68045-29); be sure that the distance between the center of the lines of the repeating pattern is known (10 μm for the EMS 68045-29).

1. Place the components (objectives, tube-lens, etc.) that will influence magnification in the desired configuration.

2. Obtain an image of the calibration slide. Focus on the repeating grid pattern of the calibration slide using transmitted light illumination. Direct the light to the camera and use the μManager **Snap** button to snap an image. Measure the center-to-center distance between the grid lines. To do so, select the ImageJ line tool, and draw a straight line from the left side of the leftmost grid line to the left side of the rightmost

grid line on the image. Read the distance in pixels in the ImageJ status line (or select **Analyze** > **Measure** from the ImageJ menu) and count the total number of grid spacings, *n*, that are covered with the line. Pixel size is calculated as: $(d\ n)/p$, where *d* is the distance between the grid lines, and *p* is the length of the line in pixels.

3. An alternative method to measure pixel size does not require a calibration slide, but relies instead on the accuracy of stage movements. Press the **Snap** button to take an image in bright field of an object that has a clearly defined edge. Use the ImageJ Point Selection tool to mark the XY position (in pixels) of a clearly recognizable point in the image. Open the μManager script panel (**Tools** > **Script Panel. . .**). In the lower right-hand panel type the following commands:

```
p = gui.getXYStagePosition();
  gui.setXYStagePosition(p.x + 10, p.y);
```

   Press the Enter key to execute each command. Together they move the XY stage by 10 μm. Snap another image. Use the ImageJ Point Selection tool to mark the XY position (in pixels) of the same point. Calculate the distance between the old position and the new position in pixels using the Pythagorean theorem. Divide 10 μm by the distance in pixels that was calculated to obtain the pixel size in microns.

4. Select **Tools > Pixel Size Calibration. . .** from the μManager menu. Click the **New** button in the **Pixel Size calibration** window. In the **Use** column, select the device properties that affect magnification (e.g., Objective-Label, TubeLens-Position), name the calibration (e.g., 10× to 1.5×), and enter the pixel size that was previously determined. Press **OK**.

5. Repeat step 4 for all other combinations of settings for which the pixel size was measured.

   Note that μManager is aware of the pixel size whenever the state of the microscope system matches one of the calibrated settings. The status line at the bottom of the main window will show the pixel size in nm/pixel.

BASIC PROTOCOL 7

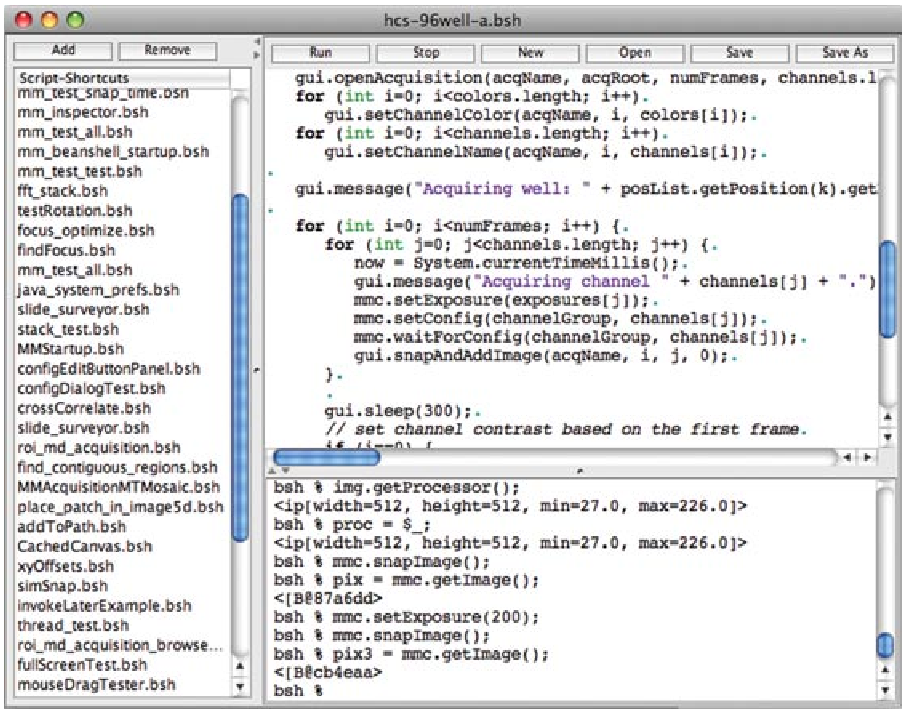
WRITING AND RUNNING μMANAGER SCRIPTS

The μManager script window (Fig. 14.20.7) offers the user a platform for developing automated acquisition and analysis routines, to make elaborate protocols straightforward. The window contains three sections: a table of script shortcuts, an editor for the current script, and an interactive command panel. The scripts and interactive commands employ the same scripting interface (Beanshell, a scripting language for Java, *http://beanshell.org*), to give the programmer access to all functionality of the hardware, the user interface, μManager plugins, and image processing analysis routines supplied by ImageJ and its plugins.

1. Three objects are immediately available in the scripting panel: `mmc` (the μManager Core for hardware control), `gui` (the graphical user interface), and `acq` (the acquisition engine). A full list of functions offered by each of these objects is given on the μManager Web site, *http://micro-manager.org/documentation.php*. To become familiar with the μManager scripting interface, start by typing a command in the lower-right interactive command panel. For example:

```
gui.snapSingleImage();
```

   This command snaps an image from the default camera and displays it onscreen in an ImageJ window. The result is equivalent to that obtained by pressing the Snap button on the main window.



```
mm_test_snap_time.bsh
mm_inspector.bsh
mm_test_all.bsh
mm_beanshell_startup.bsh
mm_test_test.bsh
fft_stack.bsh
testRotation.bsh
focus_optimize.bsh
findFocus.bsh
mm_test_all.bsh
java_system_prefs.bsh
slide_surveyor.bsh
stack_test.bsh
MMStartup.bsh
configEditButtonPanel.bsh
configDialogTest.bsh
crossCorrelate.bsh
slide_surveyor.bsh
roi_md_acquisition.bsh
find_contiguous_regions.bsh
MMAcquisitionMTMosaic.bsh
place_patch_in_image5d.bsh
addToPath.bsh
CachedCanvas.bsh
xyOffsets.bsh
simSnap.bsh
invokeLaterExample.bsh
thread_test.bsh
roi_md_acquisition_browse...
fullScreenTest.bsh
mouseDragTester.bsh
```

```
   gui.openAcquisition(acqName, acqRoot, numFrames, channels.l
   for (int i=0; i<colors.length; i++).
      gui.setChannelColor(acqName, i, colors[i]);.
   for (int i=0; i<channels.length; i++).
      gui.setChannelName(acqName, i, channels[i]);.
.
   gui.message("Acquiring well: " + posList.getPosition(k).get
.
   for (int i=0; i<numFrames; i++) {.
      for (int j=0; j<channels.length; j++) {.
         now = System.currentTimeMillis();.
         gui.message("Acquiring channel " + channels[j] + ".")
         mmc.setExposure(exposures[j]);.
         mmc.setConfig(channelGroup, channels[j]);.
         mmc.waitForConfig(channelGroup, channels[j]);.
         gui.snapAndAddImage(acqName, i, j, 0);.
      }.
      .
      gui.sleep(300);.
      // set channel contrast based on the first frame.
```

```
bsh % img.getProcessor();
<ip[width=512, height=512, min=27.0, max=226.0]>
bsh % proc = $_;
<ip[width=512, height=512, min=27.0, max=226.0]>
bsh % mmc.snapImage();
bsh % pix = mmc.getImage();
<[B@87a6dd>
bsh % mmc.setExposure(200);
bsh % mmc.snapImage();
bsh % pix3 = mmc.getImage();
<[B@cb4eaa>
bsh %
```

Figure 14.20.7 The **Script** Window. Scripts are written in Beanshell and allow fine-grained control of all aspects of μManager.

2. Now try controlling the camera via the core with the following two lines:

```
mmc.snapImage();
pix = mmc.getImage();
```

The first command tells the camera to acquire a new image; the second command returns an array of pixels containing the image data.

3. An important element of scripting is the control of properties. Each device's table of properties and values, which is displayed in the Device/Property Browser (described above), can be read and written by two core methods. For example, type:

`mmc.getProperty("Camera","Gain");`
to obtain the current Gain value of the demo camera, and:
`mmc.setProperty("Camera","Gain","4");`
to change the Gain setting.

4. Other useful shortcuts include setting the stage positions. Read the current stage position:

```
pos = gui.getXYStagePosition();
```

5. Now move the stage by 1 μm up and 2 μm left as follows:

```
pos = gui.setXYStagePosition(pos.x - 1, pos.y + 2);
```

6. To see a list of all methods and properties (with their values) for any object, use the inspect function. For example:

```
inspect(gui);
```

will give a long list of methods and the current state of many of the application's internal variables.

7. To create a new script, press the New button. To edit an existing script, click once on the script name in the shortcuts panel, or click the Open button to select an existing file.

BASIC PROTOCOL 8

USING EXTERNAL PROGRAMMING LANGUAGES TO CONTROL μMANAGER

In addition to the built-in Beanshell-based scripting interface, two other programming interfaces (Python and MATLAB) are available to advanced users who wish to combine image acquisition and hardware control with numerical analysis routines. MATLAB is a widely used commercial numerical analysis package, and numpy and scipy are numerical libraries for the Python programming language.

Python

The Python interface is a wrapper around the C++-based μManager core that provides low-level controls for the hardware, and can be used instead of the GUI. Here is how to get started with the μManager Python library:

1. μManager should be already installed in the computer. Additionally, install the latest versions of:

   Python 2.6.x (*http://python.org*; Python 3.x is not yet supported)
   numpy (*http://numpy.org*)
   scipy (*http://scipy.org*, optional, good for advanced image processing)
   matplotlib (*http://matplotlib.sourceforge.net*, useful for displaying images)
   ipython (*http://ipython.scipy.org*, optional, a nice interactive Python environment)
   PIL (Python Imaging Library, *http://www.pythonware.com/products/pil/* optional, image processing and file saving).

2. Start Python inside the μManager directory. Enter the following commands at the prompt:

```
import MMCorePy
mmc = MMCorePy.CMMCore()
```

3. The `mmc` object offers low-level hardware control for all hardware supported by μManager. Individual devices can be loaded and initialized. Here is an example using the DemoCamera:

```
mmc.loadDevice("cam","DemoCamera","DCam")
mmc.initializeDevice("cam")
```

4. Then snap and retrieve an image from the camera:

```
mmc.snapImage()
img = mmc.getImage()
```

5. The image is stored in the img variable as a 2-D numpy array (matrix). The values can be inspected in the image. For example, use the following command to list intensities in a 5 × 5 pixel square in the top left corner:

```
img[1:5,1:5]
```

6. Display the image using the matplotlib plotting library:

```
from pylab import *
ion() # Activate interactive mode
```

```
figure()
imshow(img,cmap = cm.gray)
```

7. Proceed with image processing. For example, try rotating the image by 90°:

```
imgr = rot90(img)
imshow(imgr,cmap = cm.gray)
```

MATLAB

The use of MATLAB with μManager is very similar to the Python example given above, although setup is somewhat more complicated. Full instructions on using MATLAB are given on the μManager Wiki, at *http://micro-manager.org/matlab.html.*

COMMENTARY

Background Information

These protocols are intended to help users get started with μManager, and should make its capabilities clear. Users needing additional help with any part of the system can address their questions to the μManager mailing list, available on the Web site.

μManager continues to be under ongoing development to improve its usability, expand its features, and increase the range of its device support. Planned enhancements to the basic application include a facility for automatic hardware configuration that will be able to detect newly connected hardware, extended support for color cameras, an improved system of error messages, a context-based help system, and a macro recorder.

As an open-source platform, μManager has the potential to bring advanced microscopy methods into mainstream research. Many new microscopy techniques (including STORM/PALM, structured illumination microscopy, fringe-based optical sectioning, photobleaching and photoactivation, single-molecule tracking, high-throughput imaging, intelligent acquisition, and optical tweezer methodologies) are still difficult or impossible to implement for most research labs, either because the required algorithms are complex, or because the equipment needed is too expensive. μManager's modularity (that is, device control is separated from the application level) means that it will be possible to develop plugins for μManager that enable users to use advanced techniques on the hardware of their choice. This gives labs developing new technology a direct way of making their innovations quickly available to others.

Further development of μManager device adapters will also offer opportunities for education and low-cost research. By extending device support to inexpensive cameras, stages, and illumination systems, students and researchers will have new, expanded capabilities.

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Literature Cited

Stuurman, N., Amodaj, N., and Vale, R.D. 2007. Micro-Manager: Open source software for light microscope imaging. *Microsc. Today* 15:42-43.

Internet Resources

http://micro-manager.org

The μManager Web site contains further documentation, download links, and links to a μManager news group through which you can obtain community support.

http://rsbweb.nih.gov/ij/

ImageJ is a public-domain cross-platform application for image analysis. Hundreds of plugins extend its capabilities. μManager runs as an ImageJ plugin.

http://beanshell.org

Beanshell is a Java-like scripting language used in the μManager script panel.