AxioCam MR

Installation + Reference - 12.2008

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Safety

Refer to the safety notes and instructions in the manuals of all necessary devices (e.g. microscope peripherals, cameras, computers, computer additionals, etc.) before installing and using the software.

Disposal and Recycling

This product has been developed, tested and manufactured in accordance with the applicable environmental provisions and directives of the European Union.

- The product and its accessories comply with EU directives 2002/95/EC (RoHS) and 2002/96/EC (WEEE), insofar as these apply to this product.

- We have implemented a take-back and recycling process that ensures that proper recycling is carried out in accordance with the aforementioned EU directives.

- Please contact your Carl Zeiss sales/service organization for details relating to disposal and recycling.

- This product must not be disposed of with domestic waste or using municipal waste disposal services. In the event of resale, the seller must inform the buyer of the need to dispose of the product appropriately.

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IMPORTANT NOTE

ZEISS

⚠️ FireWire (IEEE 1394 a) Interface

The FireWire (IEEE 1394 a) interface is defined as a "hot plugging" interconnection, i.e. peripherals (cameras, drives, etc.) may be connected or disconnected while the computer is powered up. In connection with this "hot plugging" feature in isolated cases damages being caused to interface components were reported, presumably due to a discharge of static electricity during the insertion of the cables.

To avoid this kind of damage, we urge you to take careful note of the following points before inserting or removing the **AxioCam MR's** FireWire cable:

- Avoid hot plugging with devices that are powered via the FireWire bus, i.e. do not insert or remove the camera while the computer is switched on.
- Use only high-quality and undamaged FireWire cables.

If you cannot avoid hot plugging, please take the following information into account:

Sequence for cable insertion:

- When you connect the camera to the PC, insert the cable into the PC first, and only then into the camera head.
- When you disconnect the FireWire camera from the PC, remove the cable from the camera head first, and only then from the PC.

In this way the cable provides protection in the event that static electricity is discharged.

Additional interface card: As an added precaution, we also recommend that you establish the FireWire connection via FireWire interface PCI cards rather than the onboard FireWire interfaces. Alternatively, a FireWire hub can be connected between the camera and PC.

Carl Zeiss is unable to accept any liability for damage that results from failure to comply with these safety recommendations.

1. Installation

The AxioCam MR is a professional, high-resolution digital camera with FireWire® interface for universal light-microscopy applications.

There are two versions available: **AxioCam MRc** (color camera) and **AxioCam MRm** (b/w camera).

To make it easier for you to set up the camera, please follow the instructions in these chapters step by step.

In this manual the interface between AxioCam MR and the computer is called FireWire. Synonym the terms IEEE 1394 a and i.LINK® are also used for this interface.

Note:
- In the description that follows the term **AxioCam HR** is used for both the color camera and the black/white camera. A distinction is only made where this is necessary for technical reasons.

1.1 Safety Regulations

Please read this chapter carefully and observe the regulations in order to ensure your safety and the intended operation of the system. Please observe the warnings and notes printed in this manual and on the unit.

The digital camera AxioCam MR has been manufactured and tested by Carl Zeiss according to the regulations specified in CE und cUL and has left the manufacturer's premises in perfect working order.

In order to ensure that this condition is maintained and to avoid any risks when operating the system, the user must comply with any notes and warnings contained in this manual.

Exemption from statutory liability for accidents

The manufacturer shall be exempt from statutory liability for accidents should the operator fail to observe the safety regulations.

Limitation of liability

No warranty shall be assumed by Carl Zeiss during the warranty period if the equipment is operated without observing the safety regulations. In any such case, Carl Zeiss shall be exempt from statutory liability for accidents resulting from such operation.

Exemption from warranty

Carl Zeiss shall be exempt from any warranty obligations should the user fail to observe the safety regulations. Carl Zeiss only guarantees the safety, reliability, and performance of the system if the following safety regulations are closely observed.

➢ The electrical installations of the room where the system is to be set up must conform to IEC requirements.

   Warning:
   Any interruption of the PE conductor, either internally or externally, or removal of the earthed conductor will make the system unsafe to use. Any intentional interruption of the earthed conductor is illegal.

   Attention:
   To interrupt the power supply simply disconnect the data cable.

➢ Setup, expansions, re-adjustments, alterations, and repairs must only be carried out by persons who have been authorized by Carl Zeiss.

➢ Use only those cables supplied by Carl Zeiss.

➢ Do not allow any cables, particularly power cords, to trail across the floor, where they can be snagged by people walking past.

General notes

Please ensure the notes described below are adhered to when setting up and operating the AxioCam MR camera.

➢ All connectors must be firmly and securely attached.

➢ Please protect the data cable from excessive heat (e.g. halogen lamps, microscope fluorescence illumination).

➢ Only use the camera in a clean and dry location.

➢ The camera must be protected against mechanical impact. External damage may affect the operation of inner components.

➢ Keep chemicals and fluids away from the camera. To avoid the risk of fire, do not use near inflammable liquids or gases.

➢ Make sure there is sufficient ventilation of the camera head. Avoid direct exposure to sunlight and locations near to heat sources (radiators, stoves). Overheating can cause noisy images.

➢ The camera housing can be cleaned using normal microscope cleaning material.

In case of repair please contact your local Carl Zeiss representation.

Backup copies

We strongly recommend that all users save the data they create, such as images, measurement data, archives, reports, forms and documents, at regular intervals on an external medium. Otherwise it cannot be excluded that access to this data may be lost as a result of operational errors or hardware defects. Carl Zeiss accepts no liability for consequential damage resulting from insufficient data protection.

1.2 System Requirements

The appropriate system requirements relating to hard- and software equipment can be found in the file "Installation Manual.PDF" on the actual product DVD.

Contents of delivery

- AxioCam MR camera head
- FireWire data cable (6-pin to 6-pin) for power supply to the AxioCam MR and for data transmission between camera and computer
- DVD-ROM with device driver for AxioVision as well as this Installation+Reference Guide as a PDF file.

Camera, data cable

Setting up the camera system

The standardized FireWire interface allows the camera to be used for a variety of applications. The following graphic provides an overview of the possible hardware configurations and the required accessories:

```
┌──────────────────┐
│ Notebook with    │
│ FireWire PCcard  │ ◄── additional 6-pin to 6-pin digital FireWire cable
│ (CardBus)        │      (included in FireWire Hub package)
│ Interface        │
│                  │          ┌──────────────────────┐
│ order number:    │          │ FireWire Hub         │
│ 000000-0452-037  │ ───────► │ (with external power  │ ◄── 6-pin to 6-pin digital FireWire cable
└──────────────────┘          │  supply)             │      (included in AxioCam MR package)
                              │ order number:        │
                              │ 000000-0452-376      │
┌──────────────────┐          └──────────────────────┘
│ Notebook with    │ ◄──                                    ┌──────────────────┐
│ FireWire         │    additional 4-pin to 6-pin digital   │                  │
│ interface        │    FireWire cable                      │   AxioCam MR     │
│ "onboard"        │    (included in FireWire Hub package)  │                  │
└──────────────────┘                                        └──────────────────┘
┌──────────────────┐
│ PC with          │ ◄── 6-pin to 6-pin digital FireWire cable
│ FireWire PCI-Bus │      (included in AxioCam MR package)
│ Interfaceboard   │
│                  │
│ order number:    │
│ 000000-0452-036  │
└──────────────────┘
                        6-pin to 6-pin digital FireWire cable
                        (included in AxioCam MR package)
┌──────────────────┐
│ PC with          │ ◄──
│ FireWire         │
│ interface        │
│ "onboard"        │
└──────────────────┘
```

When it is being operated on a PC, power is supplied to the AxioCam MR via the 6-pin to 6-pin FireWire cable. On a notebook, a FireWire hub is also required to supply power to the camera.

The table below provides an overview of the order numbers of the accessories:

Accessory	Order Number
FireWire hub	000000-0452-376
FireWire PCI bus interface card	000000-0452-036
FireWire PCcard (CardBus) interface card	000000-0452-037

Operating the camera on a PC

Depending on your system specifications, you can choose between two forms of connection:

- connection of the camera to the FireWire PCI bus interface card
- connection of the camera to the PC's integrated (onboard) FireWire interface

In both cases the AxioCam MR is connected to the PC using the FireWire cable (6-pin to 6-pin) supplied with the camera. Power is supplied to the camera through the PC.

Operating the camera on a PC using the FireWire PCI bus interface card

Installing the PCI bus interface card on the PC

⚠ Static electricity can damage electronic components. To protect electronic components against static electricity, do not touch them until you have earthed yourself to the casing of the device. Never touch the contacts of electronic components. We also recommend that you work only on an antistatic mat.

⚠️ For installation of the FireWire PCI bus interface card, please also take into account the notes in the interface card documentation. Only the basic principles of interface card installation are described in this manual.

⚠️ The FireWire PCI bus interface card (order number: 000000-0452-036) also has integrated USB connections.

FireWire PCI bus interface card

To ensure that power is supplied to the AxioCam MR, no other devices should be connected to the FireWire or USB ports of the FireWire PCI bus interface card.

If you wish to operate other FireWire or USB devices, you can configure the FireWire PCI bus interface card in accordance with the manufacturer's documentation.

Switch off your PC and all connected peripherals. Disconnect the PC and the peripherals from the mains. The FireWire PCI bus interface card can now be fitted into the appropriate slot on your computer.

⚠️ Special details on opening your computer and on the slots it contains can be found in the relevant documentation from your PC's manufacturer.

Connecting the camera to the PC

⚠️ Lay the data cable carefully between the camera and the PC. Make sure that the cable is a safe distance from hot light sources on the microscope, to prevent it being damaged by heat.

To connect the camera to a PC, you require a FireWire cable with a 6-pin connector at both ends. This is supplied with the camera.

Connect the FireWire cable of the AxioCam MR to the PCI bus interface card.

Connecting the AxioCam MR to the FireWire PCI bus interface card

➢ Power is supplied to the AxioCam MR via the FireWire interface card. If you switch off the PC, the camera is also switched off.

➢ The AxioCam MR has been designed for continuous operation. During operation the camera may heat up. This is quite safe, and does not in any way impair the functioning of the device.

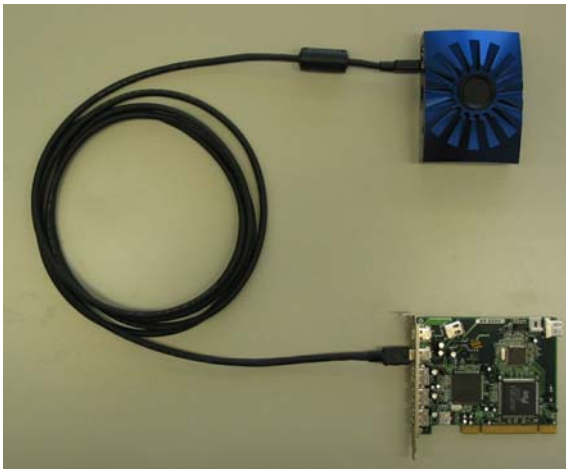## *Operating the camera on a PC with onboard FireWire interface*

Connecting the camera to the PC

⚠ Lay the data cable carefully between the camera and the PC. Make sure that the cable is a safe distance from hot light sources on the microscope, to prevent it being damaged by heat.

To connect the camera to a PC, you require a FireWire cable with a 6-pin connector at both ends. This cable is supplied with the camera.

Connect the FireWire cable of the AxioCam MR to the onboard FireWire interface of the PC.

Connecting the AxioCam MR to the onboard FireWire interface of the PC

➢ Power is supplied to the AxioCam MR via the FireWire interface. Before operating the AxioCam MR for the first time, you should therefore check that the specifications of your PC's FireWire interface correspond with the power requirements of the AxioCam MR.

➢ If you switch off the PC, the AxioCam MR is also switched off.

➢ The AxioCam MR has been designed for continuous operation. During operation the camera may heat up. This is quite safe, and does not in any way impair the functioning of the device.
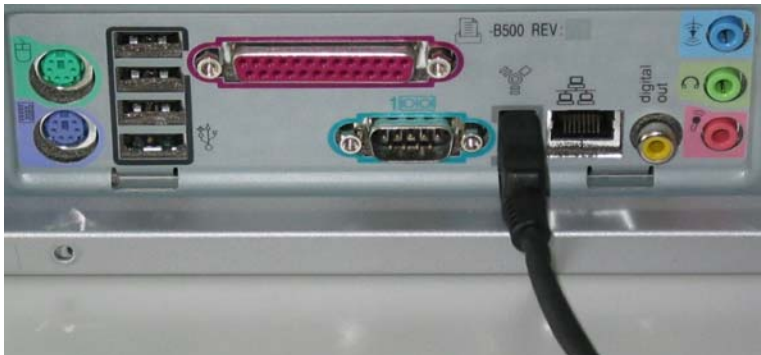
Operating the camera on a notebook

Depending on your notebook's specifications, you can choose between two forms of connection:

▪ connection of the camera to the FireWire PCcard (CardBus) interface card

▪ connection of the camera to the notebook's integrated (onboard) FireWire interface

In both cases the FireWire hub is also required to supply power to the AxioCam MR. The AxioCam MR is connected using the cables supplied with the camera and the hub.

Operating the camera on a notebook using the FireWire PCcard (CardBus) interface card

Installing the PCcard (CardBus) interface card on the notebook

Static electricity can damage electronic components. To protect electronic components against static electricity, do not touch them until you have earthed yourself to the casing of the device. Never touch the contacts of electronic components.

⚠ For installation of the FireWire PCcard (CardBus) interface card, please also take into account the notes in the interface card and notebook documentation. Only the basic principles of interface card installation are described in this manual.

FireWire PCcard (CardBus) interface card

Switch off your notebook and all connected peripherals. Insert the FireWire PCcard (CardBus) interface card into the appropriate slot on the notebook.

Connecting the camera to the notebook

⚠ Lay the data cable carefully between the camera and the notebook. Make sure that the cable is a safe distance from hot light sources on the microscope, to prevent it being damaged by heat.

⚠ To ensure the efficient use of energy resources, the FireWire slots on the notebook are not normally designed to supply power to peripheral devices. A FireWire hub should therefore be used to supply power to operate the AxioCam MR on a notebook.

The following components are required:

- FireWire hub with power supply (order number: 000000-0452-376)

- 6-pin to 6-pin FireWire cable (supplied with the AxioCam MR) to connect the AxioCam MR to the FireWire hub.

- A second 6-pin to 6-pin FireWire cable (supplied with the FireWire hub) to connect the FireWire hub to the PCcard (CardBus) interface card.

Connecting the AxioCam MR to the FireWire PCcard interface card

➢ The camera is powered by the FireWire hub. The hub must therefore be connected to the power supply. If you switch off the FireWire hub, the camera is also switched off.

➢ When the power supply of the FireWire hub is connected, the red LED of the FireWire hub lights up. The green LED of the FireWire hub lights up as soon as a FireWire device is connected.

➢ The AxioCam MR has been designed for continuous operation. During operation the camera may heat up. This is quite safe, and does not in any way impair the functioning of the device.

Operating the camera on a notebook with onboard FireWire interfaces

Connecting the camera to the notebook

⚠ Lay the data cable carefully between the camera and the notebook. Make sure that the cable is a safe distance from hot light sources on the microscope, to prevent it being damaged by heat.

⚠ To ensure the efficient use of energy resources, the FireWire slots on the notebook are not designed to supply power to peripheral devices. A FireWire hub should therefore be used to supply power to operate the AxioCam MR on a notebook.

The following components are required:

▪ FireWire hub with power supply (order number: 000000-0452-376)

▪ 6-pin to 6-pin FireWire cable (supplied with the AxioCam MR) to connect the AxioCam MR to the FireWire hub.

▪ 4-pin to 6-pin FireWire cable (supplied with the FireWire hub) to connect the FireWire hub to the notebook.

Connecting the AxioCam MR to the onboard FireWire interface of the notebook

➢ The camera is powered by the FireWire hub. The hub must therefore be connected to the power supply. If you switch off the FireWire hub, the camera is also switched off.

➢ When the power supply of the FireWire hub is connected, the red LED of the FireWire hub lights up. The green LED of the FireWire hub lights up as soon as a FireWire device is connected.

➢ The AxioCam MR has been designed for continuous operation. During operation the camera may heat up. This is quite safe, and does not in any way impair the functioning of the device.

Mounting the camera onto the microscope

To mount the camera onto your microscope's TV port, use a 1.0x or a 0.63x C-mount adapter.

Suitable adapters (example):

	Port 44	Port 60	Port 60N
1,0x	No. 452995 (44 C2/3")	No. 456105 (60 C2/3")	No. 426114 (60N C2/3")
0,63x	No. 452997 (44 C2/3")	No. 1069-414 (60 C2/3")	No. 426113 (60N C2/3")

For the Axiovert 25 please use adapter 451268.

The adapters are not supplied with the camera.

To mount the camera onto the microscope, remove the dust cap from the camera's C-mount port. Screw the adapter in as far as it will go. Then mount the camera onto the microscope's TV port. Please ensure that no dust enters the opening on the camera.

⚠ Never touch the surface of the infrared barrier filter or CCD sensor with your fingers. If you remove the camera from the microscope, make sure you immediately cover the C-mount opening with the dust cap provided, to prevent dust getting in.

Function indicator

After you have switched on the PC or the FireWire hub, the LED on the camera lights up in red for a short time, before changing to green. If the LED goes out instead of turning green, this indicates that the camera's firmware is not functioning correctly. In this case please consult the notes in the section "Resetting the AxioCam MR firmware".

As a rule, if the LED is green, this indicates that power is being properly supplied to the AxioCam MR via the data cable. During continuous image acquisition, the color of the LED switches between red (exposure) and green (ready).

Expansion socket

In addition to the socket for the data cable, the camera also has an additional socket. This socket can be used to open and close an external shutter via trigger output signals.

Second FireWire socket

This FireWire socket may be used to cascade FireWire devices. This feature should only be used, if you are sure that the power specification of your computer's FireWire interface fits the needs of all connected devices.

Checking the equipment has been set up correctly

Check the following connections:

➢ The AxioCam MR is mounted onto the microscope and is receiving light.

➢ The AxioCam MR is connected to the PC or notebook via the FireWire data cable. If you are operating the AxioCam MR on a notebook, the FireWire hub is also connected.

Now start your PC or notebook.

If the LED on the AxioCam MR does not turn green now, please check that you have carried out all of the above steps correctly.

Installing the driver software

In order to operate the AxioCam MR the AxioVision software is needed.

First connect the AxioCam MR to your PC (see the section "Setting up the camera system"), and then install the driver software.

General

Firstly we would like to emphasize a number of important points:

➢ Allow sufficient time for installation. As a rule this will take just over five minutes, although in exceptional circumstances considerably more time may be necessary. The actual time you need will depend on the current status of your computer's Microsoft® Windows operating system.

➢ Carl Zeiss's image processing products have been developed using the most up-to-date software tools and methods. This means that you must first update your computer's operating system to this same standard to ensure that things will run smoothly. The files to be installed are contained in the enclosed installation program and are standard Microsoft products or updates.

➢ For some of the Microsoft update components the installation instructions are often only available in English. If you are installing a Carl Zeiss product in English then this presents no problems. If, however, you are installing a Carl Zeiss product in German or another language, then the language will change during installation if, for example, a Microsoft update component is installed.

➢ During installation you will have to answer a sequence of questions. We have kept such questions to a minimum. Take sufficient time to read through the questions carefully and only then decide which answer applies.

➢ When installing the components it is possible that some of the files to be installed may already exist. You are informed if this is the case. You are then asked if you want to retain or overwrite the existing files. Always select the answers suggested by the installation program.

Installing the camera driver with the hardware wizard

Notes:
- For installation on a computer running under the operating system Microsoft Windows XP Professional and Microsoft Windows Vista, you need administrator rights. Enter the system using a user name with administrator rights, or consult your system administrator.

- The installation procedure is displayed in this Installation Guide with screenshots of Microsoft Windows XP Professional.

⚠️ If you have fitted a PCI bus or a PCcard (CardBus) interface card into your computer, you first need to install these with the help of the documentation enclosed by the manufacturer. Depending on your operating system, this installation may not be necessary, as the drivers may already be integrated into the operating system.

⚠️ In addition to installing the AxioCam MR driver, to carry out full installation of the camera you also need to check and, if necessary, update the camera's firmware. These tasks are performed by the AxioCam MR installation program. This program must also be called up after installation of the camera driver using the hardware wizard (see the section "Installing the camera driver using the setup program").

This section describes installation using the hardware wizard. If you want to update the camera installation or if you have interrupted the driver wizard, follow the instructions in chapter "Installing the camera driver with the setup program".

Once you have connected the AxioCam MR, the **Found New Hardware Wizard** dialog appears on your screen. Please ensure that during all the steps below, the DVD-ROM containing the AxioCam MR driver is in your PC's DVD drive.

Select the option **Install from a list or specific location (Advanced)**. Click on **Next**.
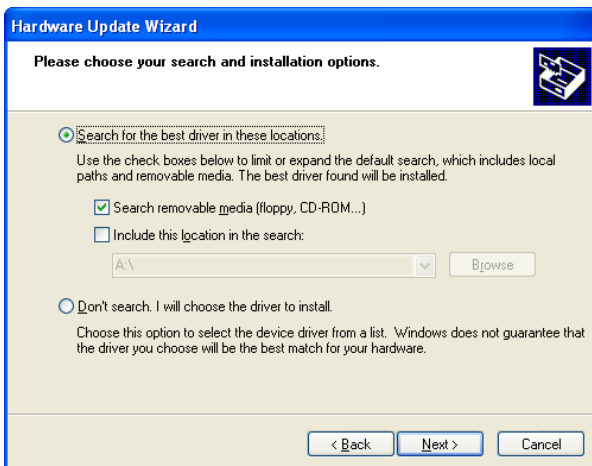
Select the option **Search removable media floppy, DVD-ROM…)**. Click **Next**.

Under Microsoft Windows XP Professional this dialog window is displayed.

Click on **Continue Anyway**.

The last dialog window of the hardware wizard is displayed.

Click **Finish** to close the hardware wizard. To complete the installation follow the steps in the next chapter.

Installing the camera driver using the setup program

Notes:

- For installation under Microsoft Windows XP Professional and Microsoft Windows Vista, you need to log on with administrator privileges. If you do not have access to administrator rights on your computer, please speak to your system administrator.

- In this section, installation is explained using screenshots from Microsoft Windows XP Professional.

- During the installation of the AxioCam MR, the camera's firmware is checked and, if necessary, updated. The AxioCam MR must therefore be connected to the PC and be in 'ready' mode (green LED) during the installation procedure.

⚠ If you have fitted a FireWire PCI bus or a PCcard (CardBus) interface card into your computer, you first need to install these with the help of the documentation enclosed by the manufacturer. Depending on your operating system, this installation may not be necessary, as the drivers may already be integrated into the operating system.

⚠ In addition to installing the AxioCam MR driver, to carry out full installation of the camera you also need to check and, if necessary, update the camera's firmware. These tasks are performed by the AxioCam MR installation program, which is described in this section.

After you have inserted the DVD into the DVD drive installation starts automatically.

If the automatic start function has been switched off on your system (**Autorun**) you must start the installation program manually in the following way:

➢ Double-click on the desktop icon **My Computer**

➢ Double-click on the **DVD-ROM Drive** icon

After the start procedure (automatically resp. manually) this image will be displayed on your monitor:
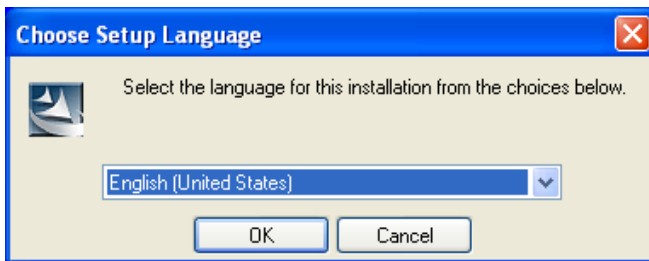
Select the language you want to install (English or German) and click **OK**.

Follow the instructions of the setup program until the window is displayed, where you can customize your setup. In this window you can also choose to read the **ReadMe** files.

It is recommended that you carefully read the **ReadMe** file. This file provides complementary or up-to-the-minute information concerning AxioCam MR hard- and software. The text window can be closed by selecting this Window's symbol.

Set the entry "Cameras and Framegrabbers" - "Carl Zeiss AxioCam" to **This feature will be installed on local hard drive**. Check the other settings and continue by clicking **Next**.

Under Microsoft Windows XP Professional this dialog window will be displayed during installation of the AxioCam MR.

Click **Continue Anyway**.

Once the AxioCam MR driver has been installed, the installation program checks the camera's firmware and if necessary starts updating the firmware.

This procedure may take a few minutes.

While the firmware is being updated, under no circumstances must the power supply or the data connection to the AxioCam MR be cut off. This would result in the incorrect functioning of the AxioCam MR. If this situation nevertheless arises, please read the section "Resetting the AxioCam MR firmware".

The system prompts for a restart, depending on the used operating system. After the restart the installed driver will be activated.

Start the systems Device Manager to check the installation:

➢ Click **Start → Control Panel**.

➢ Double-click the **System** icon. The **System Properties** dialog will be displayed.

➢ Select the property page **Hardware** and here the **Device Manager** button. Now double-click the entry **Imaging devices** to check the entry **Carl Zeiss AxioCam MR**.
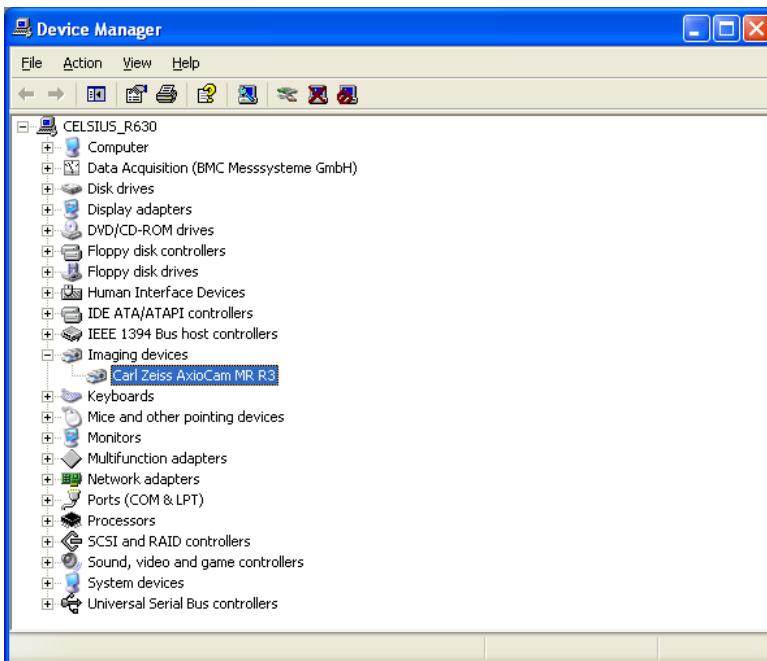
## *How to correct the driver entry in the device manager*

Note:

- In this chapter the correction of the driver entry is described with Microsoft Windows XP Professional screen shots.

If the entry in the **Device Manager** must be corrected, i.e. if a question mark or an exclamation mark is in front of the **AxioCam MR** entry, proceed as follows. Please make sure that during all the steps described below, the DVD-ROM containing the AxioCam MR driver remains inserted in the DVD-ROM drive of your computer.
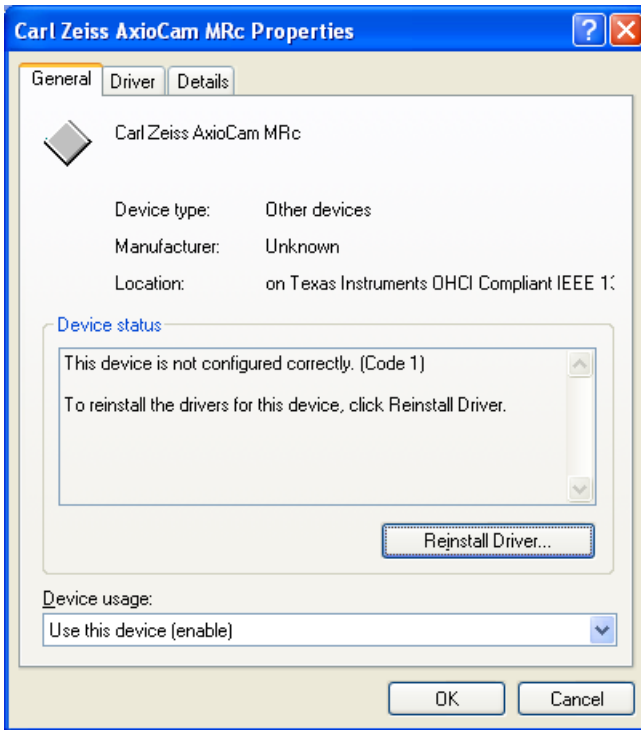
Start the systems **Device Manager**:

➢ Click **Start → Control Panel**.

➢ Double-click the **System** icon. The **System Properties** dialog will be displayed. Select the property page **Hardware** and click the **Device Manager** button.
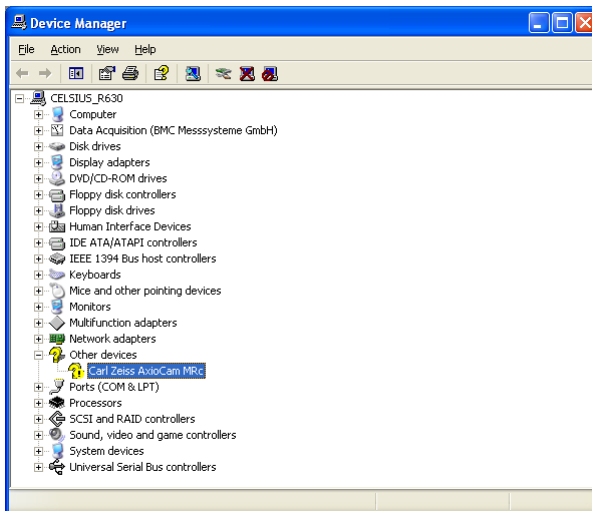

➢ Under **Other devices** double-click on **Carl Zeiss AxioCam MR**.

➤ The property page **General** opens.

In this case, reinstall the AxioCam MR using the installation program (see section "Installing the camera driver using the setup program").

After the camera driver has been installed and (depending on the operating system you are using) your computer has been restarted, the **Device status** of the AxioCam MR should be **This device is working properly.**.
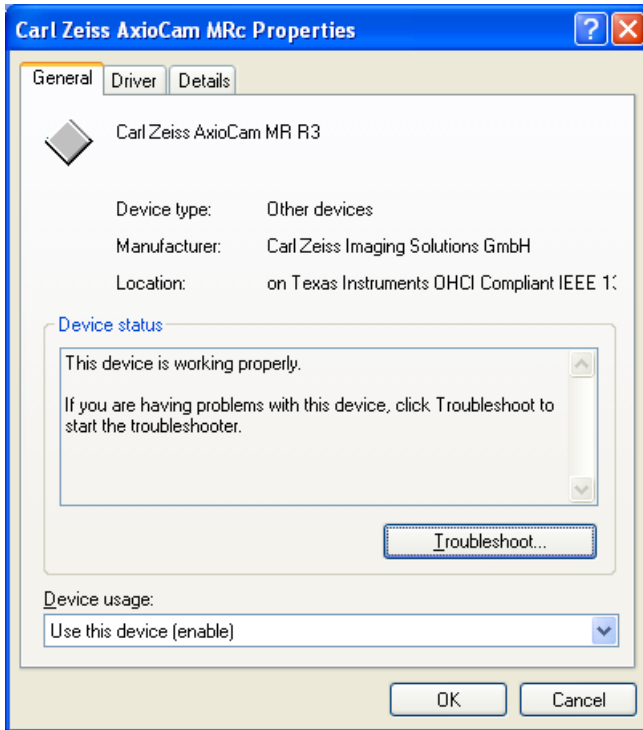
Resetting the AxioCam MR firmware

If the color of the LED on the back of the AxioCam MR does not immediately change from red to green but goes out after the insertion of the FireWire cable, this means that the AxioCam MR's firmware is not functioning properly.

This error can occur, for example, if the data transmission or power supply to the camera has been cut off during the firmware recording procedure.

If this happens, you need to record the firmware of the AxioCam MR again:

➢ Connect the AxioCam MR to your computer in accordance with the description in the section "Setting up the camera system". The LED on the back of the camera lights up in red briefly and then goes out.

➢ Press the button next to the expansion socket of the AxioCam MR using a pointed object (e.g. a pencil). Keeping the button depressed, briefly remove and then reinsert the FireWire connector on the camera. Now the LED lights up red and then changes to green.

⚠ Do not press down hard on the button. This can damage the camera's electronics.

➢ Update the firmware of the AxioCam MR by running the installation program. Here follow the description in the section "Installing the camera driver using the setup program". If necessary, at the end of the installation program the firmware of the AxioCam MR must be updated. Follow the instructions on the monitor.

1.3 Notes on Maintenance

Camera electronic

Camera, interface and power supply are maintenance free.

Optical system

The internal optical components of the camera should always be protected. If no lens or TV adapter incorporating optics is screwed into the camera's C-Mount thread, the camera's sensor and IR-filter must be protected by screwing the protective cap into the camera's C-Mount thread.

Cleaning the infrared barrier filter

Contamination of the infrared filter has an adverse effect on the quality of the resulting image (dark points, cloudy structures in the image). If there is dry dust on the front side of the infrared filter, you can remove it with a soft brush or with cotton (wool) after unscrewing the lens or TV adapter.

Important note on the use of C-mount objectives

The camera can be mounted onto microscopes with standard TV-adapters with C-mount connections. Carl Zeiss supplies the camera complete with a pre-mounted infrared barrier filter, which offers the optical advantage for use on microscopes of a lower sensitivity to dust.

The IR barrier filter is situated 5 mm behind the outer edge of the C-mount opening in the camera.

⚠ Due to this pre-mounted IR barrier filter, C-mount objectives that screw more than 5 mm into the thread cannot be screwed into the camera.

2. Image Acquisition

2.1 General

The AxioCam MR is a professional, high-resolution digital camera. It has been specially optimized for routine and research applications in the area of light microscopy. This is shown among other by the fast live image, which is displayed on a PC monitor to allow the simple focusing and setting of the sample.

This extremely compact camera is connected using a single cable for the power supply, control information and image data, making it very simple to operate.

There are two versions available: **AxioCam MRc** (color camera) and **AxioCam MRm** (b/w camera).

The color camera is suitable for all situations where stained or naturally colored samples need to be acquired with very high levels of quality.

The black/white version of the camera has been optimized in particular for acquisition when light intensities are low. In these cases it is important for the widest possible spectral response and the highest possible sensitivity to be achieved. Due to the elimination of the color filter mask, there is no need for the IR filter usually required with color cameras.

The camera's very low level of background noise (supported by active Peltier cooling of the sensor), and possible exposure times ranging from 1 ms up to several sec, make it suitable for an extremely wide range of applications in the field of microscopy.

2.2 Quick Guide to the First Image with AxioVision

If you are using several cameras on your system you must always make the AxioCam MR camera the active camera before proceeding.

➢ Start AxioVision by double-clicking on the corresponding symbol.

➢ Select from the **Acquisition** menu the **Camera selection** ⇒ **AxioCam MR3** function.

The image acquisition can also be easily done via the toolbar. Open the

corresponding menu by clicking on **Workflow**.

The text below describes how to achieve your first image in AxioVision with just a few clicks of the mouse.

The **Standard** workflow is the easiest way to control image acquisition. If the workflows are not displayed, in the **View** menu select the **Windows** function, and there select the **Workflow** command.

Note:

- The workbar may in fact be open but covered by the work area. If that is the case, simply click on the **Workflow** tab at the bottom edge of the

  work area ☐ Workarea ⬛↕ Workflow .

The workflow Standard

Standard
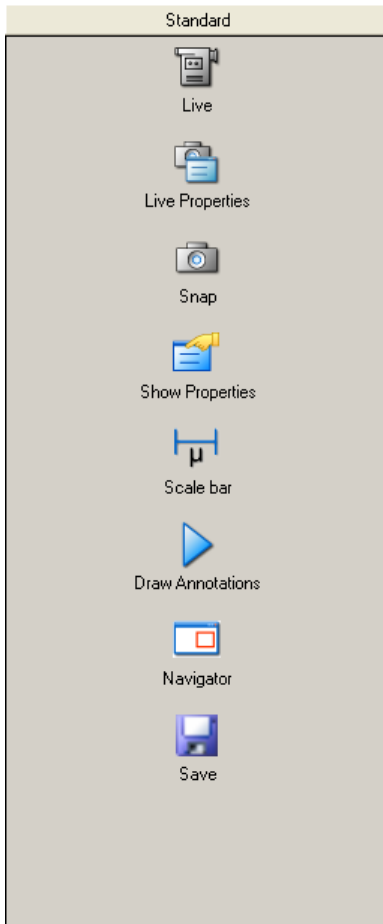
Live

Live starts and closes the live window.

Live Properties

Show live window properties opens and closes the window for adjusting the display characteristic curve and for controlling the camera (exposure time, white balance etc.).

Snap

Snap acquires an image using the active camera.

Show Properties

Show Properties adapts the image display using a characteristic curve and shows the parameters of the image.

Scale bar

Scale bar inserts a scale bar.

Draw Annotations

Annotations inserts a caption and scale bar into the acquired image.

Navigator

Navigator opens the **Navigator** window for zooming.

Save

Save saves the image.

As the icons are identical on all menus, the following description applies both to operation via the toolbar and to the workflow.

Step by step to the first image

➢ Select the camera you want from the drop-down list box.

➢ Set the light path to the camera.
Then click on the live image icon. The
live window is opened to display the
camera image

➢ This function can also be called up via
the **Acquisition** ⟹ **Live** menu.

➢ Now focus the camera image and
select the frame you want to acquire.

➢ In the footer you can select a suitable
scaling for the objective you are using
from the **List of available scalings.**
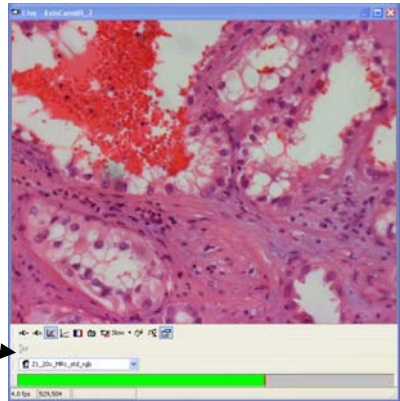
➢ Clicking on this icon overexposed (too
bright) areas will be displayed in red
in the live image.

➢ Clicking on the gamma icon in the
bottom line of the live image
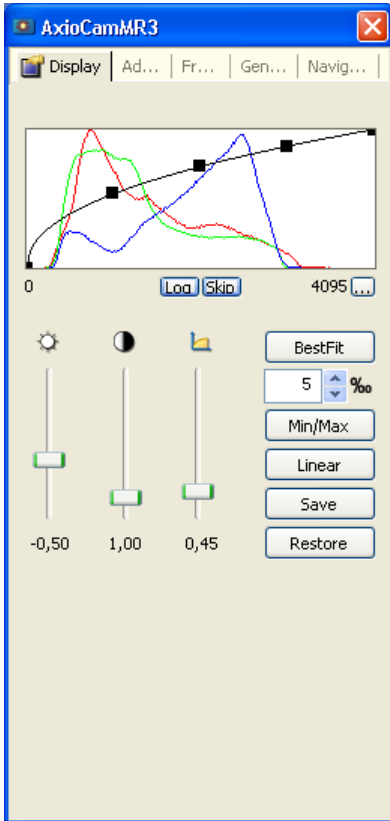accomplishes the optimum color
reproduction.
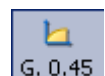
➢ In this drop-down list box you can
select the speed of the live image
(**Slow, Medium, Fast**).

➢ Click on the icon **Live properties** to
open the live properties.

Here you can see the settings for the display of the image like **Brightness**, **Contrast** and **Gamma**. Settings made here are passed on the acquired image.
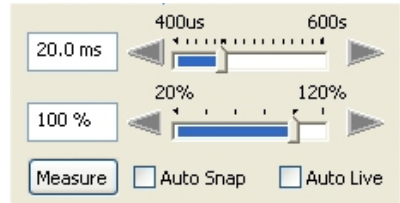

Note:

- Only the display is adapted, the camera data remain unchanged.

Make changes for the camera settings on the following controls:

➢ The central element is the setting for the exposure time on a digital camera. The **Measure** button can be used to determine an optimum exposure time. Check the quality of the exposure in the live image.

➢ The arrow keys and sliders can be used for fine adjustment of the exposure time.

➢ Exposure time can be edited via the keyboard.

➢ **Auto Snap** performs an exposure measurement before the acquisition of each image. **Auto Live** adjusts the exposure time continuously in the live image.

➢ If you are using a color camera, you need to perform a white balance for the image. If you select the automatic balance (**Automatic** button), the camera tries to determine an optimum value itself.
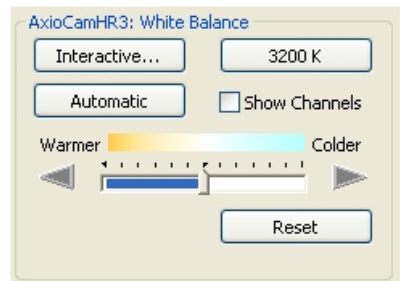
➢ Using the **Warmer/Colder** slider you can shift the target value of the white balance to warmer ($\rightarrow$ redder) or colder ($\rightarrow$ bluer) tones.

➢ The **Show Channels** check box allows you to switch to an alternative setting option if you wish to influence the target value of the white balance manually for each channel.

➢ If you are using a color camera, you need to perform a white balance for the image. If you select the automatic balance (**Automatic** button), the camera tries to determine an optimum value itself.
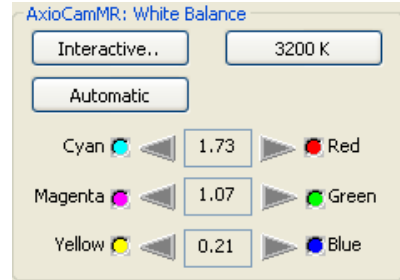
➢ Move the slider to increase or decrease the color intensity of the acquired images.

➢ Enter further settings for the selected camera on the **Adjust**, **Frame** and **General** property pages.

➢ Click on the camera icon to acquire an individual image, or in the **Acquisition** menu click on **Snap**.

➢ Click on the **Properties** icon and activate the **Display** tab. Any changes you make here are displayed in real time in the image window.
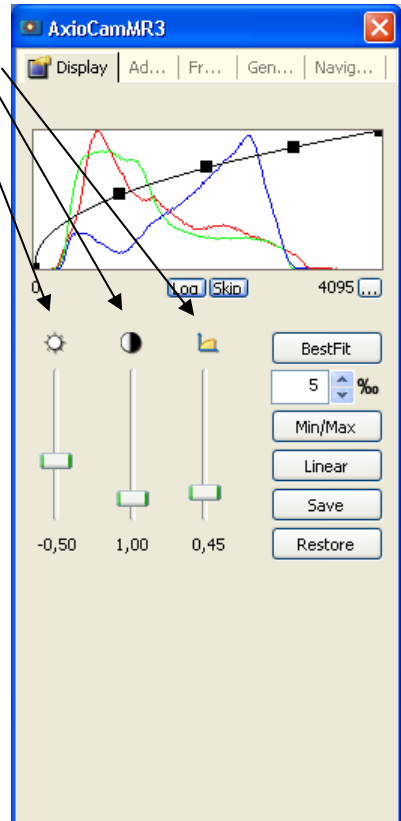
➤ Move the sliders

Gamma
Contrast
Brightness

up or down to adjust the image. Good color reproduction on a monitor is achieved with a gamma value of around 0.45.

➤ To find the optimum setting for contrast and brightness, click on **Min/Max**.

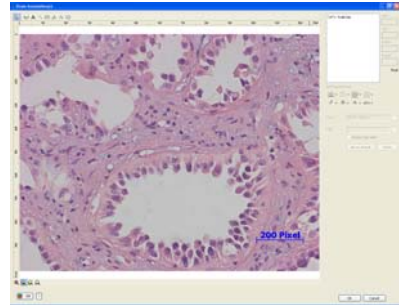➤ To undo all your changes, click on **Linear**.

➤ You can use this icon to insert a scale bar into your image. The appropriate scaling must have been generated and selected in advance.

➤ This icon is used to open the window for generating annotations.
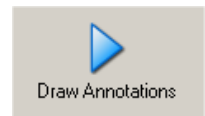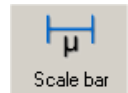
➢ Lettering and marking can be inserted in the **Draw Annotation(s)** window.

➢ In the dialog window **Draw Annotation(s)** the icons **scalebar**, **text**, **line**, **rectangle**, **outline**, **curve** und **ellipse** are available in the menu line.

➢ In the dialog window **Draw Annotation(s)** a field for **Settings for selected items** as well as for **Font** are available. If **Display tag name** is activated, you can use the ⌐…⌐ icon in the drop-down list box **Tag**. The dialog window **Select tag** opens where parameters for lettering can be selected.
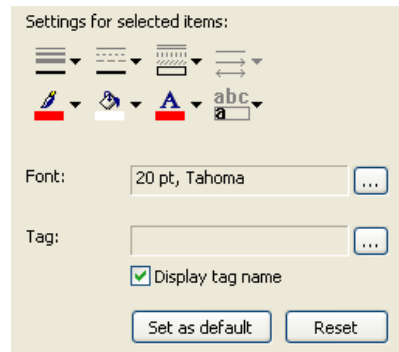
➢ In the dialog window **Draw Annotation(s)** zoom symbols and two buttons to toggle the color channels are available at the bottom of the image window.

➢ Using the Navigator you can magnify or reduce the image and position the displayed image area in the overview window.

➢ Click on this icon to save the image you have generated to the computer's hard drive.
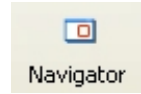
Notes:

- Once you have found a good camera setting, you can save it with the **Settings Editor** in the **Tool** menu and load it again automatically in the future. This allows you, for example, to acquire particular samples under the same conditions every time.

- To allow correct scalings to be selected in the footer of the live image during acquisition, you need to have generated these before you start your work. If you are using a motorized or encoded microscope, the scalings can be allocated automatically. For further information see Chapt. 9 "Configuration".

2.3 Adjust Property Page

This property page contains a summary of the most important camera
settings, which are constantly used during operation.
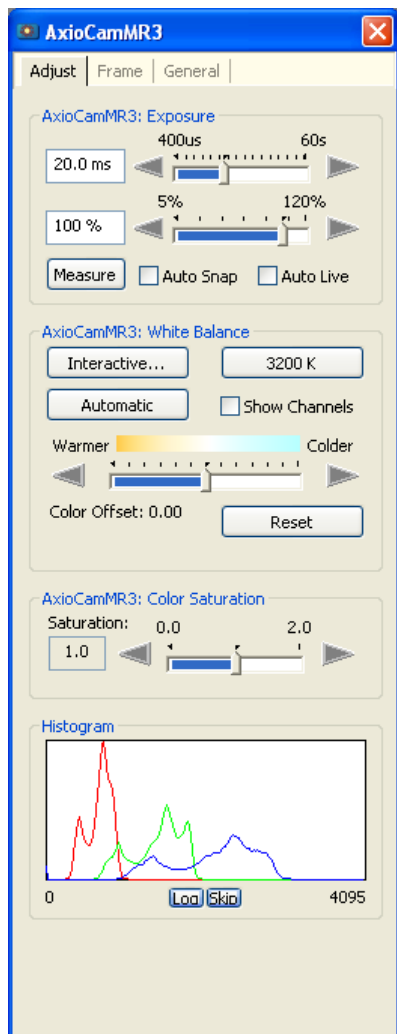
The property page is splitted in the fields **Exposure**, **White Balance** (with color settings) and **Histogram** (for the display of an image histogram).

Exposure

The camera's exposure time can be adjusted to the ligting conditions on the microscope using a slider or by entering an exposure time directly into the input field. When entering the exposure time manually you can state the value range directly using the abbreviations "ms" for milliseconds and "s" for seconds.

Exposure time measurement

The program supports you in calculating an optimum exposure time, at which the camera will be maximally saturated without being overexposed. To perform this calculation, click on the [Measure] button.

The exposure time calculated is available for subsequent images. It can be changed at any time by moving the slider or by entering a new exposure time manually.

This measurement function uses all the image information from the camera sensor for the exposure measurement as standard (integral measurement).

Note:
- More detailed information of the live image you can find in chapter 2.6 "Practical Notes on Operation in AxioVision" - " Speed of the live image".

Weighting of exposure time measurement

During an exposure time measurement an attempt is made to expose the camera in such a way that the sensor is not overexposed in any area of the image. The value calculated therefore corresponds to 100% of the camera's maximum exposure range.

The exposure time measurement can also be adjusted to particular lighting conditions. If under certain acquisition conditions the exposure time measurement does not achieve satisfactory exposure of the areas of the

image in which you are interested, it is possible to influence the result of this measurement by adjusting the weighting.

A setting of 120% means that 120% of the exposure time set in the display window or measured by pressing the appropriate button is used for actual acquisition.

A setting of 50% means that 50% of the exposure time set in the display window or measured by pressing the appropriate button is used for actual acquisition.
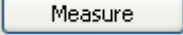
The setting range is between 5% to 120%.

EXAMPLE:
A thick sample is acquired in bright field. The empty image background is much brighter than the darker sample. The measurement produced an exposure time of 50 ms, at which the background would not be overexposed during acquisition. Setting a correction factor of 120% extends the effective exposure time to 60 ms, which ensures that the darker area of the sample is exposed more brightly. The brightest area of the image, which is not of interest, is overexposed here by 120%.

Automatic exposure

The software sets the exposure automatically for each individual shot when the check box **Automatic** is activated. This makes it possible to generate acquisitions with the correct exposure without having to enter manual presettings. For each individual acquisition the correct exposure time is calculated and set in advance.

If the **Auto Live** check box is activated, the software continuously sets the exposure during the live image. In this way it is possible to generate images with the correct exposure without having to enter manual presettings. The exposure time currently calculated in the live image is used for each individual image.

The exposure measurement button [Measure] can be pressed at any time. This is useful if you want to update the exposure time for a current live image. If you deactivate **Automatic**, the program will continue to use the last setting calculated.

Note:

- The automatic exposure time adjustment operates in a range from 1 ms to 1.5 s.
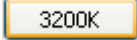
Note:

▪ In this mode it is not possible to edit the exposure times in the multichannel mode for the individual channels.

White balance

In order to achieve a color display which is neutral and close to reality it is necessary to balance the individual color channels against one another. For this, you need to use the pointer to click in the live image on an area of the microscope's specimen, which is white, neutral and illuminated as evenly as possible. *The scene must not be overexposed.*
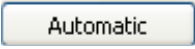
It is important to set the exposure correctly before carrying out the white balance. This can be carried out via automatic exposure measurement, for example.

If the measurement cannot be performed because of unsuitable lighting conditions an error message is issued. In this case the amount of light must be reduced or increased accordingly.

The [3200K] icon allows you to switch back to the basic white balance setting. This setting is optimized for a halogen light temperature at 3200 K, which can be preselected at the touch of a button on many high-quality microscopes.

Note:
- Setting the white balance to 3200 K generally leads to image reproduction that is closest to the impression of the image in the microscope's eyepiece, if the microscope has also been adjusted to this value. Note that it is recommended to set the gamma curve for the image display on a value of 0,45 (please find more information in Chapter 2.6 "Practical Notes on Operation in AxioVision" - "Display characteristic curve").

Clicking on the [Automatic] button starts an attempt to automatically adjust the white balance. This requires the presence of sufficient neutral-tint areas in the image.

Notes:
- With fluorescence samples or image areas that are completely filled, this automatic method can lead to color distortion. In this case use the 3200 K standard setting or the manual color adjustment function.

- The color setting is only available for the color camera AxioCam **MRc** and not for the AxioCam **MRm**.

Color adjustment

On a microscope, however, the brightness of a halogen lamp is often adjusted by changing the lamp voltage. This changes the color of the light emitted at the same time, though.

By moving the [Wärmer ◀ ▭ ▶ Kälter] slider it is possible to adjust the target color value of the white balance to the color distribution of halogen illumination.

At the middle setting, the color white (R=B=G) is taken as a neutral value. This neutral value can also be achieved at any time by clicking on the **Reset** button.

Alternatively, you can influence the color channels individually by activating **Show Channels**:

Clicking on the buttons [◄] and [►] allows you to change the weighting of the individual color channels manually. Using this method a white balance you have generated can be subsequently changed. Each click on the arrows shifts the weighting of the colors in the direction indicated. These changes can be observed immediately in the live image and in the histogram display.

Note:
- Further information on setting optimum color reproduction in AxioVision can be found in Chapter 2.6 "Practical Notes on Operation in AxioVision" - "Notes on optimum color reproduction in AxioVision".

A setting found using this method can be saved as a setting with a name of your choice and subsequently reloaded. Activate the corresponding parameters (White Balance Red, White Balance Green, White Balance Blue) in the menu **Tools ⇒ Settings Editor**.

Color saturation

Using the **Saturation** slider, it is possible to increase or decrease the color intensity of the acquired images.

Setting the slider at a value of 1 achieves the most natural color reproduction (recommended default setting). If set at values higher than 1, the color intensity is increased and, if set at values lower than 1, it is decreased.

Note:
- This function supersedes the **Enhance Color** function from previous releases. Setting the slider to 0 corresponds to the **Enhance Color Off** setting. Setting the slider to 1 corresponds th the **Enhance Color On**.

Histogram

A histogram indicates the intensity distribution of the image signal in the live image and the acquired image. It provides a clear representation of the brightness distribution in the current image. The horizontal axis corresponds to the scale of possible intensity values from dark to light and the vertical axis shows the number of pixels calculated from the current image which possess this intensity value on the x axis.

With the color camera the value distribution is displayed separately for each color channel in three lines (red, green and blue).

If an image is overexposed, at least one curve on the histogram is cut off at the right-hand edge of the display area, where it forms a maximum. Reduce the exposure time or insert gray density filters into the microscope's beam path to reduce the amount of light. It is also possible to reduce the lamp voltage of the microscope illumination. However, this changes the basic color of the illumination (color temperature) and requires a new white balance.

If an image is underexposed, the histogram does not reach the right-hand edge of the display area. As a result optimum use is not made of the camera's resolvable gray value range. You should therefore increase the exposure time or the amount of light in the microscope.

Log: By clicking on this button it is possible to influence the histogram display. You can toggle between a linear scaling and a logarithmic display of the signals represented in the histogram.

The logarithmic display makes it possible to view small amplitudes and high peaks at the same time in a single overview. Otherwise, because of the automatic scaling of the display, small signal values are often not visible in the diagram.

Skip: This function can be used to influence the automatic scaling of the histogram. If an image contains an extremely bright glare or extremely dark features the result will be overexposure or underexposure of the image in these areas. All of these pixels possess the maximum / minimum numerical value which can be displayed (e.g. in an 8-bit image 255 for overexposure or 0 for underexposure). Consequently, for the gray value 255 the histogram has to display an extremely high number of pixels with the same value. As the scaling of the histogram curves is automatically adjusted to this high value, smaller values can no longer be resolved in the display.

By using the **Skip** function the maximum values at the upper and lower end of the histogram are omitted during the scaling of the display.

2.4 Frame Property Page

The **Frame** property page can be used to select a frame for the image acquisition and is splitted in the fields **Camera Mode** and **Frame**.
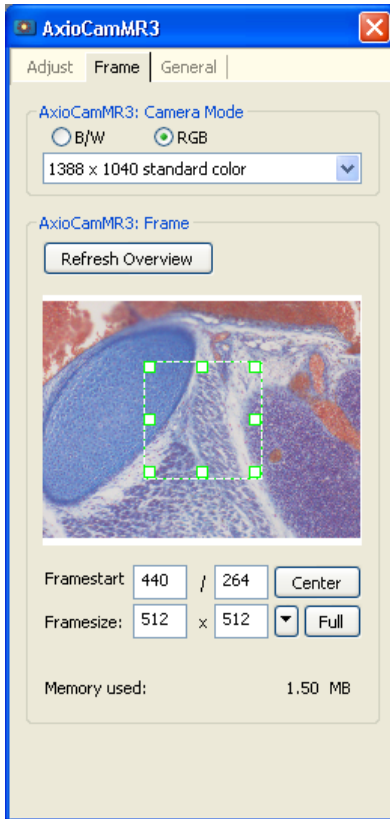
Camera mode

RGB and **B/W**: Two types of AxioCam MR are produced; one with a CCD sensor with a color mask on the chip (AxioCam **MRc**), and the other with a black and white sensor (AxioCam **MRm**).

With the color variant (AxioCam **MRc**), you can use these setting options (**RGB** or **B/W**) to determine whether the camera should supply a color or a black and white image. This may be desirable for fluorescence images, or to reduce the amount of data. The camera will always acquire a color image, which is then converted to a black and white image.

In **RGB** mode only odd Binning modes, in **B/W** mode only even Binning modes are available. This is caused by the structure of the color filters on the pixels.

AxioCam **MRm** works always in the b/w mode. All Binning modes are always available.

Resolution

Optical adaption of the camera

The camera resolution can be selected between Binning and Standard.

The camera has a 2/3" CCD sensor with dimensions of 8.8 mm x 6.6 mm. This corresponds to an image diagonal of approx. 11 mm.

To optimize the size of the camera's field of view a camera adapter with a magnification factor of 0.63x is recommended. In this case approx. 42% of the intermediate image is acquired with a microscopical field of view of 20 mm in diameter.

To adapt the camera in the best possible way to the resolution of the objectives a camera adapter with a magnification factor of 1.0x is recommended.

The color quality is comparable with that of 3-chip CCD video cameras, without the need for an expensive beam splitter.

In the *color mode* the following resolutions are available:

276 x 208	Binning mode 5x5
460 x 344	Binning mode 3x3
1388 x 1040	Single image, color interpolation

In the *black and white mode* of the AxioCam **MRc** only the following image resolutions are available:

1388 x 1040	Interpolated image
692 x 520	Binning mode 2x2
344 x 260	Binning mode 4x4

For the AxioCam **MRm** (*black and white camera*) the following image resolutions are available (**camera version 2.0**)

276 x 208	Binning mode 5x5
344 x 260	Binning mode 4x4
460 x 346	Binning mode 3x3
692 x 520	Binning mode 2x2
1388 x 1040	Single image

Note:

- With the AxioCam **MRm** the omission of the color filter mask on the CCD sensor and the associated generation of monochrome images offers the following advantages:

    - The acquisition speed in scanning modes is increased by a factor of four, as fewer positions have to be located.

    - The light sensitivity is increased.

    - The visible spectrum for the camera expands, because no IR barrier filter is required.

    - Even single-frame exposures produce images with the full basic resolution of the CCD sensor.

    - The quantity of image data is reduced by a factor of 3 with the same spatial resolution, as no RGB color channels are acquired.

Binning

The process of **Binning** ensures that the information in neighboring pixels, in both a horizontal and vertical direction, is summarized on the camera's sensor. This increases image brightness in accordance with the number of pixels summarized.

However, this process also reduces the sensor's spatial resolution accordingly. Since the amount of data to be transferred is reduced, the potential image rate is increased.

EXAMPLE:
In the case of 2 x 2 binning, out of 1400 x1000 pixels 700 x 500 pixels remain.

Size of the resulting files

The size of the resulting files depends on the choice of resolution and gray value scaling.

The following table gives an overview:

	Image size	8 bit	12 bit
AxioCam **MRc**	1388 x 1040	4,3 MB	8,6 MB
AxioCam **MRm**	1388 x 1040	1,4 MB	2,8 MB

In AxioVision, images in 12 bits per pixel or in 3 x 12 bits per pixel mode can be saved in ZVI image format. When exporting the images to other image formats, (TIF, BMP, JPG etc.) this option is not available. With these image formats the image data are converted from 12 bit to 8 bit data in accordance with the graduated display characteristic curve.

Frame

In this field you can specify whether the frame or the entire area of the sensor is used for the acquisition.

Refresh overview: This button makes it possible to insert an overview image into the area where the frame can be positioned. This makes it extremely easy to target your specific area of interest.

In the displayed overview image a frame can be positioned by using the mouse, which corresponds to the selected frame of the acquired image. The shape and size of the frame can be changed by clicking on and dragging the edges of the frame while holding down the left-hand mouse button. It is also possible to move the frame by clicking inside it and keeping the button pressed as you change its position.

Center: By clicking on **Center** you can automatically center the selected frame. This can be useful as interesting parts of the sample are usually moved to the center of the view area. In addition the center of the view area is the place of the highest optical quality.

Framestart / **Framesize**: The start point and the selected frame size will be displayed in these input fields. Alternatively you can enter the values manually. It is also possible to select default frame sizes from the drop-down list box and position it in the center of the image by using the **Center** button.

Memory used: Memory used shows the memory size of the acquired image, which depends on the camera resolution, the image size of the selected image frame and of the selected gray value scaling.

2.5   General Property Page

On this property page you enter the basic settings for the camera.
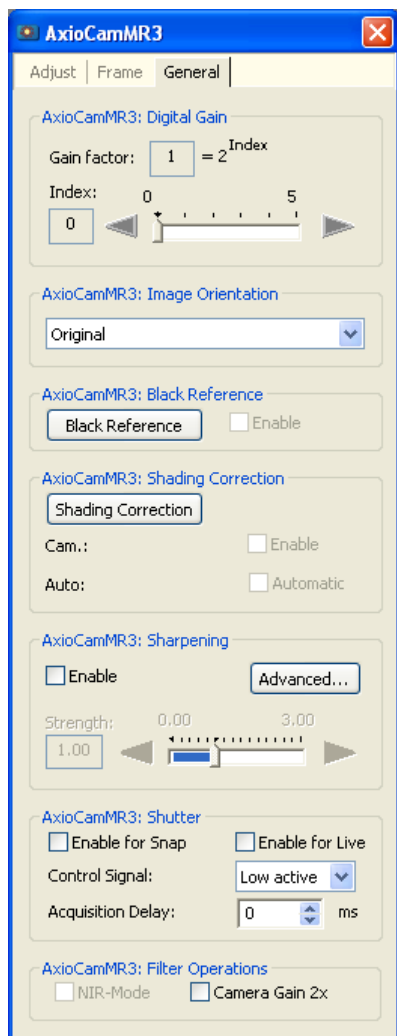
Digital gain

Using the slider it is possible to change the interpretation of the brightness of the image data received. This allows you to display dark images as very bright images, which means you can acquire images with very high contrast using short exposure times.

he disadvantage of this technique, however, is that noise is greatly intensified, and the potential dynamic range of the corresponding image data is reduced. You should therefore carefully weigh up the benefits of using digital signal gain before actually employing the technique. For this reason the recommended value is gain by a factor of 1.

From a technical viewpoint, this technique involves multiplying the data by a corresponding factor. The maximum numerical pixel value generated is always limited to the maximum value of the analog/digital converter you are using (12 bits per pixel per color channel in the case of the AxioCam MR).

Note:
• You can also achieve a bright image display with short exposure times by making the display characteristic curve steeper.

Image orientation

An AxioCam MR image is adjusted in such a way that the image orientation corresponds to the image in the eyepiece when mounted on an upright microscope with the front of the camera facing you.

This starting position can be adapted via this menu to suit your requirements. If, for example, the camera's cable routing needs to be changed on an inverse microscope, the camera can be mounted at a different angle, without the correspondence between the image orientation and the eyepiece being lost.

Black reference

Setting a black reference ensures that the image background retains a uniform darkness, even with exposure times in excess of several seconds. This setting is therefore particularly recommended for the acquisition of weak fluorescence or other images with weak light intensity and consequently *long* exposure times (starting from approx. 3 seconds).

Note:
- Perform this black-value calibration approx. 15 minutes after switching on the camera, as the camera will then be in a thermally balanced condition.

For this calibration process make sure that the camera is viewing a completely black image. For example, close the light path to the camera on the microscope. In exceptional cases it may be necessary to remove the camera from the microscope for this calibration process and to cover the sensor with the cap provided.

Calibration takes approx. 20 seconds. At the end of this period it will continue to be available even if the software is restarted. Under certain circumstances it may be necessary to repeat this calibration process from time to time.

Notess:
- The black reference is needed to correct deviations in the dark current for individual pixels (dark current compensation). This measure is additional to the camera's available Peltier cooling, and allows a uniformly dark and even image background to be achieved, even with long integration times.

- The ubiquitous phenomenon of environmental radiation can cause individual bright pixels (hot pixels) on CCD sensors (especially during air travel). Such pixels are recognized and compensated for by the black reference. This compensation functions as soon as a measurement has been carried out, even if the black reference is deactivated.

- Activating the black reference can reduce the maximum possible speed of the live image.

Shading correction

Activate this function if you notice, e.g. with bright-field images, a reduction in image brightness, caused by the optics, at the image edge. For this calibration process make sure that the camera is viewing a completely empty image. To ensure that this is the case, move your sample on the microscope completely out of the field of view.

Shading correction to improve image quality

In microscopy, numerous side effects can have an undesirable influence on image quality. Dust and particles in the beam path, for example, can leave dark spots in the image, and, under certain conditions, diaphragms and optics can cause slight shadowing that increases towards the image edge, due to unavoidable physical effects.

For all AxioCam models it is possible to acquire a shading reference image and apply this automatically each time an image is acquired. On the camera property page **General** you will find the **Shading Correction** button. This can be used to trigger the acquisition and saving of a shading reference image.

If you are using a motorized microscope or a microscope with an encoded nosepiece, it is possible to acquire a separate reference image for each objective. These images are then applied automatically for the objective that has been selected.

Procedure for generating objective-specific shading correction

Prerequisites:

- Encoded or motorized microscope stand
- Camera belonging to the AxioCam family

The following steps are required to set and apply shading correction in the optimum manner:

➢ Activate the **Automatic** check box on the camera property page **General**.

➢ Make sure that all motorized and encoded microscope components are correctly configured in the Micro Toolbox. For the automatic application of shading correction, the correct configuration of the available objectives and the cameras is particularly important (please also refer to the "*MTB2004 Configuration*" manual).

➢ Set the light intensity with which you want to work.

➢ To do this, set a suitable exposure time and the white balance of the camera.

➢ Set Köhler illumination on the microscope (if using transmitted light) in order to create a reproducible image-acquisition situation.

➢ Create a test image of a specimen with quality that is as high as possible.

➢ Check the quality of the Köhler setting for each objective with which you want to generate automatic shading correction.

➢ Now move the sample out of the camera's field of view and bring the microscope clearly out of focus.

➢ Generate a shading reference image for each objective, one after the other, by clicking on the **Shading Correction** button on the camera property page **General**.

➢ If you are using a fully motorized microscope, it is very easy to save and reload the configuration of the light path setting in a hardware setting:

- Light intensity
- Gray density filter
- Setting of the condenser aperture diaphragm and field diaphragm

➢ You can now refocus the microscope and work with the automatic shading correction.

Notes:

- The reference images generated are saved on a user-specific basis. All users who have their own login must therefore generate their own set of reference images.

- Automatic shading correction only takes into account the changing of the objective. Changes to the settings of other optical components, such as an Optovar, the camera adapter or the condenser, are not taken into account and will make it necessary to repeat the generation of the reference images.

- If several cameras are operated on a system (e.g. an AxioCam MRm and an AxioCam MRc5), the reference images for each camera will managed separately.

- In the **Multidimensional Acquisition** function, a function for fluorescence-channel-specific shading correction is offered under **Extended parameters** on the **C (Channel)** property page.

- Activating shading correction can reduce the maximum possible speed of the live image.

Applying object-specific shading correction

Activate the **Automatic** check box on the camera property page **General**.

Note:

- Make sure that the shading reference images generated are still having the desired effect if the images were generated some time ago. They will need to be generated again in any case if the optical settings of the microscope have been changed. This applies to the following settings in particular:

  - Lamp adjustment
  - Köhler setting
  - Optovar
  - Camera adapter
  - Camera port

Applying shading correction manually

With non-motorized and non-encoded microscopes the automatic shading correction can not be activated. But it is possible to carry out shading correction using a previously generated standard reference image. This image is applied irrespective of the objective set.

To generate this file, follow the procedure described above. However, this function can only be carried out for one objective. As a compromise for all available objectives, you can perform this using an objective with a medium magnification. For special requirements you will need to carry out shading correction again, specifically for the objective you are using, prior to the acquisition of an image.

Shading correction via image processing

For advanced users it is also possible to carry out shading correction via an image processing function. On the **Processing** menu $\Rightarrow$ **Adjust** $\Rightarrow$ **Shading Correction** you will find a function that allows you to perform targeted subsequent processing. This function can be used in a script to allow you, for example, to generate and apply an image with an assigned shading correction for each objective.

Sharpening

With this function, it is possible for images to receive further processing using a sharpness algorithm immediately after acquisition by the camera without an image-processing function having to be started manually. The impression of sharpness in the image is increased and it is therefore possible for extremely attractive images that are rich in detail to be displayed on the monitor without delay.

The technique applied is employed by many image-processing programs and is known as **Unsharp Masking**. Here, the contrasts in small structures and edges are increased in a targeted way, leading to an enhanced impression of sharpness.

This functionality is also offered in AxioVision as a subsequent processing function on the AxioVision menu **Processing** $\Rightarrow$ **Sharpen** $\Rightarrow$ **Unscharp Masking**. This allows you to decide whether you wish to save the original data and optimize the impression of sharpness as necessary, or whether the image data should be processed immediately upon acquisition.

Important note:

- This function processes and changes image data that are supplied by the camera. The images displayed therefore differ from the original acquisition situation, depending on the parameters set.

- The parameters applied must be selected appropriately with consideration for the respective image content, microscopic contrast technique and structure sizes.

- Any form of image processing also potentially increases the noise. This means that this technique is not recommended for acquisition situations where there is little light.

➢ **Enable**

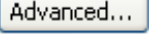Activating this check box enables you to switch the function on or off.

➢ **Strength**

As a weighting factor, **Strength** determines how much of an increase in contrast is added to image edges and small structures. This increases the impression of sharpness accordingly. In order to retain a realistic impression, the parameter should be adjusted so that it is as small as possible but, nevertheless, as large as necessary. Typical values are between 0.5 and 1.5.

1 = Default.

➢ **Extended Parameters**

Further parameters are available. Clicking on the $\boxed{\text{Advanced...}}$ button opens a dialog window, where you can select additional settings.

> ➤ **Color Mode**

This parameter can be switched between **RGB = 0** (Default) and
Luminance = 1.

In **RGB** mode the **Unsharp Masking** is calculated individually for each color
channel. The color saturation and color of sharpened structures may be
changed and color noise may occur.

If this is unwanted, it is possible to switch to **Lumincance** mode. Here, the
sharpness calculation is only carried out on the brightness signal determined
for this purpose (**Lumincance**). This mode does not display any color noise
and alters the color saturation accordingly.

> ➤ **Radius** and **Strength**

Radius determines the width of structures that are to be sharpened.

A small radius intensifies smaller details. If the radii are too large, halo effects
may occur at the edges. **Radius** and **Strength** interact. A smaller radius
allows a greater strength and vice versa.

At a resolution of 1388 x 1040 pixels, value 2 is an appropriate setting. If you
switch to a higher resolution for the same image, you can increase this value
in order to obtain an impression of the image comparable to the basic
resolution.
Value range: 1-11
The radius is stated in pixels: value 1 = 3 pixels and value 2 = 5 pixels

> ➤ **Auto Contrast**

Increasing the contrasts changes the distribution of intensities in the image.
As a result, the histogram becomes broader.

This parameter limits the change in the distribution of intensities during the
sharpness calculation. The purpose of this is to try to maintain the existing
minimum and maximum values of the input image.

This parameter can be switched between On=1 (Detault) or Off=0.

➢ **Auto Contrast Tolerance**

This parameter determines how much the distribution of intensities is permitted to be changed by the **Auto Contrast** mode. This is helpful if there are particularly strong contrasts in the image which would otherwise lead to a considerable shift in intensities as a result of the **Auto Contrast** adjustment.

Value 0 retains the input value of the histogram limits.

Using the tolerance threshold it is possible to make the sharpening more robust in relation to pixel anomalies. This means that extreme values are not clipped.

Value 5 (Default) tolerates 5% anomalies at both ends of the histogram.

The following parameters are only available in the image processing function "Unsharp Masking" in the AxioVision menu Processing ⇒ Sharpening as additional parameters, as their effect needs to be precisely tailored to the respective image content.

They make it possible to limit the application of **Unsharp Masking** to certain areas of the image. This allows homogeneous areas to be excluded from the processing, for example, in order to minimize image noise there.

➢ **Level Low**

This threshold value determines the lower limit from which existing contrast structures are changed.

Default: 0

➢ **Level High**

This threshold value prevents existing strong contrasts in the image from being unnecessarily increased any further.

Default = 100

➢ **Threshold Mode**

This value allows a smooth transition between sharpened image areas and image areas excluded by **Level Low** / **Level High**. It is only effective when threshold values are set.

This parameter can be switched between **Binary**=1 (Default) or **Linear**=0.

➢ **Clip To Valid Bits**

This parameter limits the bit range of the image to the original bit range following the increase in sharpness.

This parameter can be switched between On=1 (Default) or Off=0.

➢ **The parameters set for the sharpening function are saved as a tag in the AxioVision image format. The image tags documented in the image properties and can be inserted as annotations in the image.**

The 9 parameters of the implemented method **Unsharp Masking are indicated**:

The meaning of the 9 values, which are separated by a comma, are described above.

Shutter control

Via a supplementary cable the AxioCam MR offers a TTL control pulse, which can be used, for example, to control a mechanical shutter (e.g. "Uniblitz") in synchronism with the camera's exposure time.

As soon as this output is activated you can select the polarity that you want to set for this signal:

➢ **Enable for Snap**

If this check box is activated, the shutter pulse is only generated during image acquisition.

➢ **Enable for Live**

If this check box is activated, the shutter pulse is only generated during the live image.

If you want a trigger signal to be generated both during the live image and during image acquisition, both check boxes must be activated at the same time.

➢ **Control Signal**

High active means that the **Control Signal** jumps from 0V to 5V when the camera's exposure begins and returns to 0V when exposure is complete.

Low active means that the **Control Signal** jumps from 5V to 0V when the camera's exposure begins and returns to 5V when exposure is complete.

Please carry out configuration for this procedure in accordance with the requirements of the device you have connected.

Note:
- Simultaneous control of the external Uniblitz shutter by AxioVision via a serial interface and by the shutter control of the camera is resulting in the following behavior:

    - Shutter closed via AxioVision → than AxioCam MR shutter control.
    - Shutter open via AxioVision → than AxioCam MR shutter control does not work.

➢ **Acquisition Delay**

Due to the inertia of the masses being moved, a mechanical shutter requires a certain amount of time to change from the closed to the open position after the control signal has been initialized.

To make sure that when the sensor is exposed it does not record this transition state and is not thereby exposed unevenly it is possible to delay the start of actual acquisition. Please set a value here in accordance with the data for the device you have connected.

Filter operations

➢ **NIR-Mode**

The designation "NIR" stands for "Near Infrared". If you activate this mode, it is possible to achieve a further increase of up to 20% in the light sensitivity of the sensor in the range of red and near infrared wavelengths (from approx. 650 nm). However, this makes the CCD sensors more susceptible to overexposure (so-called "blooming"). We recommend that you use this mode for particularly weak fluorescence samples in this wavelength range, to reduce the exposure times required.

Note:
- This mode is **only** available for the black/white version of the AxioCam. With color cameras an infrared barrier filter blocks light above approx. 710 nm. This filter has been permanently installed into the color cameras, as it is essential in order to acquire colors correctly.

➢ **Camera Gain**

Enableling this check box activates the analog gain of the image data.

If this check box is activated, analog gain by a factor of approximately 2 is applied to the image data. Weak signals are therefore displayed more brightly.

Notes:
- This function is available from camera models HR and MR, from Revision 3 onwards.

- The use of gain reduces the maximum dynamic range that can be achieved by the camera. As camera noise is also intensified as a result, no actual increase in sensitivity is achieved.

2.6   Practical Notes on Operation in AxioVision

Below you will find a few practical notes on the speed of the live image, color adjustment, working with exposure times, fluorescence images and configurable toolbars.

Reset camera to a default state

To reset the numerous camera parameters to a reproducible default state, reset functions are available.

Select the **Status** function in the **Acquisition** menu. In the **Status** dialog window click on the **Reset** button to reset the following settings of the active camera.

If you click on the **Reset** button, the following settings of the active camera are reset:

Camera parameters for all cameras (as available):

Exposure time	20 ms
White balance	3200 K (only color cameras)
Color Offset	0
Auto Snap	Off
Auto Live	Off
Sensitivity Range	100%
Gain	1
Analog Gain	Off
Frame (ROI)	Full frame
Image Orientation	No changes
Black Reference	No changes
Shading Correction	Off
USM	Off
HDR	Off
Shutter (Trigger Out)	No changes
Trigger In	Off
NIR Mode	No changes
Color Saturation	1
Binning	1x1 (sensor basic resolution)

With the function **Load Factory Defaults** (context menu of the live image) you can reset camera settings in addition to the settings for the live display:

Spot Meter / Focus Bar	No changes
Spot Meter	No changes
Live Speed	No changes
Auto Best Fit	Off (in case of color cameras), No changes (in case of b/w cameras)
Auto Min/Max	Off (in case of color cameras), No changes (in case of b/w cameras)
Display Curve	Full range
Gamma	0.45 (in case of color cameras), Linear (in case of b/w cameras)
White balance	No changes (supplied in the driver)
Overexposure	Off
Zoom	Fit into window
Ruler	No changes
Cross Bar	No changes
Live Staus bar	No changes
Close after Snap	No changes

Speed of the live image

There are three speed modes available for the AxioCam MR live image. If you use the pointer to click on the Slow icon at the bottom edge of the live image, a pop-up menu with the possible setting options appears. The current setting is displayed at the bottom edge of the live image. Each mode has its own particular compromises.

Live mode	Description
Fast	Greatly reduced resolution, fast frame rate
Medium	Medium resolution, moderate frame rate
Slow	High resolution, slow frame rate

The image rate can be further increased by deactivating certain AxioVision features:

- Deactivating the focus bar.

- Deactivating the live image scaling (1:1 representation of the image).

- Activating a frame for the live image (**Size**) on the **Frame** property page.

- Set image orientation to original on the **General** property page (no rotation).

Notes:
- Note that the above mentioned notes are only valid for <u>short</u> exposure times, as for very long exposure times the speed of the acquisition is mainly dominated by the exposure time.

- For very long exposure times, the live image will appear grainy, as automatic amplification is active. However, this will not influence the quality of the final image.

Notes on optimum color reproduction in AxioVision

Below you will find a detailed description of how best to set up AxioVision to achieve optimum color reproduction with the AxioCam MR.

Note:
- For an optimum color reproduction the monitor first has to be adjusted. To do so please use a well-suited image.

Adjusting the display characteristic curve in AxioVision

In order to achieve optimum reproduction of color images of the AxioCam MRc data on a monitor, the display characteristic curve must be used to set "gamma compensation". The advantage here is that only the display is influenced and the actual data remain unchanged. Further details on this can be found below. The settings can be done as follows:

➢ With the mouse click on the icon G. 0,45 in the footer of the live image. This adjusts the display characteristic curve automatically for an optimum color reproduction of the monitor (gamma = 0.45).

➢ In the **Properties** dialog window, set the gamma characteristic curve in the histogram image on the **Display** property page.

Notes:
- This also influences the reproduction of the color saturation.

- For fluorescence images, background correction can also be carried out simply by shifting the offset of the characteristic curve.

- Clicking on this icon Linear returns to a linear display of the data.

2.7   Information for Displaying 12 Bit AxioCam Images in AxioVision

A PC graphics card cannot display more than 3 x 8 bits per pixel. However, as we have already mentioned, the AxioCam MR is able to generate 3 x 12 bits per pixel. So that intelligent use can be made of this feature, the display's characteristic curve can be altered in AxioVision.

To do this, right-click in the image acquired and select from the context menu the **Properties** function. The dialog window **Properties** is displayed on the screen:
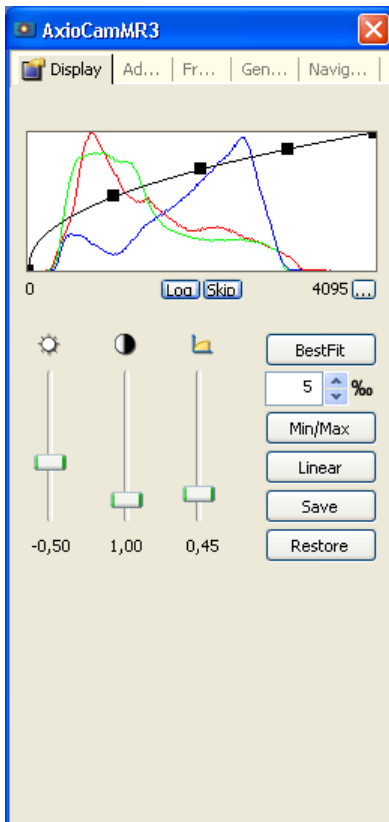
You can now select the area to be displayed by dragging the black rectangles along the characteristic curve or carry out automatic optimization by clicking on the corresponding buttons.

A histogram of the gray and color values will be displayed which makes the gray and color distribution visible.

The **Best Fit**, **Min/Max** and **Linear** buttons can be used to quickly adjust the histogram display. You can influence the display by clicking on the **Log** or **Skip** buttons.

Best Fit calculates in the current histogram the values which exclude 1‰ of the pixels contained in the image. The color/color range calculated in this way is displayed on the screen. (You can adjust the value of 1‰ to suit your requirements).

Min/Max displays on the screen the smallest and largest gray or color value present in the image.

Linear displays on the screen the total possible value range.

Log displays the histogram in a logarithmic scaling, allowing you to effectively evaluate histograms of images with high dynamics.

Skip: By using the **Skip** function the maximum values at the upper and lower end of the histogram are omitted during the scaling of the display.

The ☼, ◑ and ⌐ scrollbars can be used to change the brightness, contrast and gamma of the display. This can also be achieved by dragging the individual "handles" of the characteristic curve in the histogram. You can adjust the brightness and contrast by changing the left or right-hand end points of the characteristic curve individually or by shifting the entire line using the center "handle". The gamma value can be changed by shifting both "handles".

Click the **Save** button to store the current settings. You can use these settings when displaying another image by clicking the **Restore** button. This enables you to compare different images.

If you activate the **Apply to all T** check box the settings in all the acquired instants of exposure are applied to the relevant fluorescence channel.

Note:
- You can get the best display of colored objects, if the gamma setting is in the range of approx. 0,45.

Recommended camera settings

Activate the **Enhance Color** check box on the **General** property page to improve the color reproduction quality (this slows down the live image rate slightly as additional calculations are required).

The standard setting installed for 3200 K (**3200 K** button on the **Adjust** property page) often offers the camera the most natural reproduction of the image situation on the microscope. Here the image background is reproduced as it appears on the microscope. This setting is also useful in acquisition situations where no neutral-tint area is available to carry out a balance manually, e.g.: fluorescence.

If the image background in the acquired image is to be displayed with a neutral tint (gray), a manual white balance is required. Click on the **White Balance** button; then click on an area in the image that has a neutral tint and is not overexposed.

If the lamp voltage selected on the microscope is too low, the color of the image background may have been shifted a long way into the red. If a white balance is carried out in this event, the result may be visible deviations in the display of the color of the current sample. Correct this situation by increasing the lamp voltage to achieve a more neutral background, and insert gray filters into the microscope's illumination beam path to adjust the intensity.

Recommended monitor settings

The output medium has a significant influence on color perception!

Use a familiar specimen to adjust the brightness and contrast rendering. If possible also set the color temperature of the monitor to approx. 5000 K or 6500 K.

2.8 Background Information

Gamma setting

This setting is required to compensate for the inherently non-linear behavior of the image tube ("gamma"). It also influences the quality of the color display.

You can get the best display of colored objects, if the gamma setting is in the range of approx. 0,45.

Many video cameras have built-in gamma compensation, as they are intended for use on video monitors.

Linearity

All versions of AxioCam MR have been designed to determine linear, quantitative measurement data, as such data is frequently used for tasks in the field of image analysis. Consequently, internal gamma compensation has been deliberately omitted from the AxioCam MR.

Configurable toolbars for operating the camera

AxioVision allows you to configure your own toolbars. This means that the procedure can be tailored to your own requirements, and thus optimized. The size of the toolbar buttons can also be adjusted, helping you to work quickly with the mouse. A detailed description of how to create toolbars can be found in Chapt. 9 "Configuration" - "Adapting Toolbars" in the AxioVision Manual.

3. Trouble Shooting

If errors occur, check first the setup instructions of the camera.

3.1 Software

Please also pay attention to all the comments in the file **ReadMe.TXT**, which is displayed during the driver installation.

➢ **AxioCam MR does not appear in the menu of selectable cameras**

- Make sure that the AxioCam MR files have been installed in accordance with the installation of the software.

- For Microsoft Windows XP Professional and Microsoft Windows Vista: make sure that the camera drivers have been installed with administrator rights.

➢ **No camera image on your screen**

- Check the light setting of the microscope.

- Is the green control light on the camera on? If not, check the cable connections between camera and computer.

- Execute an automatic exposure measurement (Autoexposure).

- Check display adjustments for the live image Properties .

- Check the aperture diaphragm of the microscope.

- Check the position of the beam splitter between the ocular and the TV port.

➢ **The color of the image is green and not realistic coloring**

- Check white balance and, if necessary, repeat white balance.

3.2   Image Quality

➢ **Sudden jolt, vibration or moving objects**

- If the microscope is jolted whilst you are taking a shot, the image will show periodic interference.

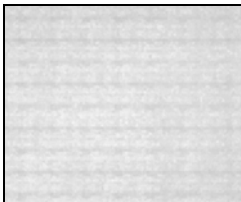- Repeat the shot and take care to ensure the conditions remain calm.
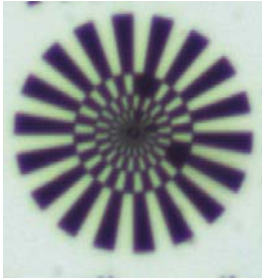
An example of a shaky shot   *Compare: An error-free image*
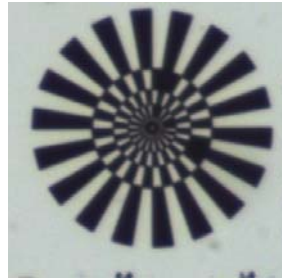
➢ **Fluctuations in illumination**

- If the light source has a fluctuating quality of light intensity, stripes or blotches will show up in the image.

4. Technical Data

4.1 AxioCam MRc (color version)

Sensor	Sony ICX 285, progressive readout, with RGB filter mask
CCD basic resolution	1388 x 1040 = 1.4 megapixels
Pixel size	6.45 µm (h) x 6.45 µm (v)
Sensor size	Chip area 8.9 mm x 6.7 mm, equivalent 2/3 "
Spectral range	Approx. 400 nm-700 nm, BG 40 IR protection glass
Dynamic range	Typical > 1 : 2200 (> 66.8 dB)
Full well	Typical 17 Ke
Readout noise	Typical < 7.7 e
Dark current	Typical 0.7 e/pixels/s, dark current compensation for maximum low light performance
Readout speed	24.57 MHz pixel clock
Live image frame rates	H x V Mode / Binning Max. frame rate* 1388 x 1040 slow / 1 13 images/s 460 x 344 middle / 2 26 images/s 276 x 208 fast / 3 38 images/s
Resolution and frame rates for time lapse images in AxioVision module Fast Acquisition (High Speed Color mode**)	H x V Binning Max. frame rate* 1388 x 1040 1 x 1, RGB / b/w 14 images/s 692 x 520 2 x 2, b/w 26 images/s 460 x 344 3 x 3, RGB 35 images/s 344 x 260 4 x 4, b/w 42 images/s 276 x 208 5 x 5, RGB 48 images/s
Max. file size per image	Approx. 8.6 MB at 1388 x 1040 at 3 x 12 bit (36 bit color depth)
High speed operation modes for AxioVision module Fast Acquisition	• Five preloadable exposure time parameters in camera head for high-speed multichannel acquisition*** • Continuous mode for fast triggered acquisition • Overlapping exposure and readout of the sensor in fast time lapse images****
Color interpolation	High Speed Color- or High Quality Color Mode selectable
Hard disk recording	Inline recording of image data directly to hard disk at all speeds with AxioVision module Fast Acquisition

Readout of sub frames (ROI)	Freely selectable
Signal amplification	Analog: 2x, digital 32x
Digitization	12 bit
CCD cooling	One stage Peltier cooling
Interface	FireWire 1394a (400 megabits/s)
Range of integration time	1 ms up to 60 s
Signal output connectors	2 x TTL-Out: exposure time and readout time (i.e. for driving external electric shutters), 1 x Trigger-In to start an acquisition
Optical interface	C-mount
Housing	Blue anodized aluminum, with cooling fins, ¼" connection for tripod mount, 11 cm x 8 cm x 4.5 cm / 370 g
Operating system	Microsoft® Windows 2000 Professional
	Microsoft® Windows XP Professional
Registration	CE, cUL
Power supply	10-33 V, DC, 4 W power supply provided by FireWire bus from PC (external power supply only for Notebook operation required)
Ambient condition (operation)	+5° ... +35° Celsius, max. 80% relative humidity, no condensation, free air circulation required
Order number	426508-9901-000

Above frame rates are supported by the camera electronics. Computer hardware, operating system and application software may decrease the frame rates. Selecting a part of the sensor area can increase the frame rate. All specifications are subject to change without notice.

* Frame rates depend on exposure time and readout mode.

** Image rates when recording onto hard drive in High Speed Color mode.

*** In Continuous mode the maximal exposure time is 819 ms per channel.

**** In basic resolution mode the sensor readout time is 69 ms. Below this value, the frame rate is only determined by readout time. Above this value, the frame rate is determined by exposure time, only. With activated binning mode, the readout time is shorter, respectively.

4.2 AxioCam MRm (monochrome version)

Sensor	Sony ICX 285, progressive readout, without filter mask
CCD basic resolution	1388 x 1040 = 1.4 megapixels
Pixel size	6.45 µm (h) x 6.45 µm (v)
Sensor size	Chip area 8.9 mm x 6.7 mm, equivalent 2/3"
Spectral range	Approx. 350 nm - 1000 nm, BK 7 protection glass without IR filter (IR filter BG 40 can be inserted)
NIR mode	Mode for higher sensitivity, especially for near IR
Dynamic range	Typical > 1 : 2200 (> 66.8 dB)
Full well	Typical 17 Ke
Readout noise	Typical < 7.7 e
Dark current	Typical 0.7 e/pixels/s, dark current compensation for maximum low light performance
Readout speed	24.57 MHz pixel clock
Live image frame rates	H x V Mode / Binning Max. frame rate* 1388 x 1040 slow / 1 13 images/s 692 x 520 middle / 2 23 images/s 460 x 344 fast / 3 32 images/s
Resolution and frame rates for time lapse images in AxioVision module Fast Acquisition	H x V Binning Max. frame rate* 1388 x 1040 1 x 1 14 images/s 692 x 520 2 x 2 26 images/s 460 x 344 3 x 3 35 images/s 344 x 260 4 x 4 43 images/s 276 x 208 5 x 5 50 images/s
Max. file size per image	Approx. 2.8 MB at 1388 x 1040 at 12 bit
High speed operation modes for AxioVision module Fast Acquisition	• Five preloadable exposure time parameters in camera head for high-speed multichannel acquisition** • Continuous mode for fast triggered acquisition • Overlapping exposure and readout of the sensor in fast time lapse images***
Hard disk recording	Inline recording of image data directly to hard disk at all speeds with AxioVision module Fast Acquisition
Readout of sub frames (ROI)	Freely selectable

Signal amplification	Analog: 2 x, digital 32 x
Digitization	12 bit
CCD cooling	One stage Peltier cooling
Interface	FireWire 1394a (400 megabits/s)
Range of integration time	1 ms up to 60 s
Signal output connectors	2 x TTL-Out: exposure time, readout time (i.e. for driving external electric shutters), 1 x Trigger-In to start an acquisition
Optical interface	C-mount
Housing	Blue anodized aluminum, with cooling fins, ¼" connection for tripod mount, 11 cm x 8 cm x 4.5 cm / 370 g
Operating system	Microsoft® Windows 2000 Professional
	Microsoft® Windows XP Professional
Dual camera operation	Possible
Registration	CE, cUL
Power supply	10 - 33 V, DC, 4 W power supply provided by FireWire bus from PC (external power supply only for Notebook operation required)
Ambient condition (operation)	+5° ... +35° Celsius, max. 80% relative humidity, no condensation, free air circulation required
Order number	426509-9901-000

Above frame rates are supported by the camera electronics. Computer hardware, operating system and application software may decrease the frame rates. Selecting a part of the sensor area can increase the frame rate. All specifications are subject to change without notice.

* Frame rates depend on exposure time and readout mode.

** In Continuous mode the maximal exposure time is 819 ms per channel.

*** In basic resolution mode the sensor readout time is 69 ms. Below this value, the frame rate is only determined by readout time. Above this value, the frame rate is determined by exposure time, only. With activated binning mode, the readout time is shorter, respectively.

4.3 Spectral Sensitivity

Normalized to maximum amplitude

Spectral Sensitivity AxioCam HRc/MRc Rev 2.x, incl. BG 40 IR Filter
und AxioCam HRm/MRm Rev 2.x, without IR Filter
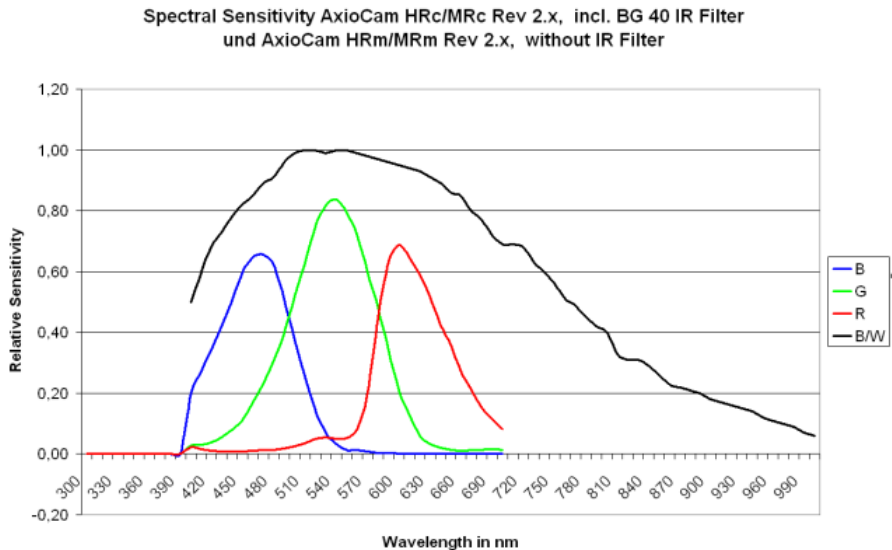
The use of the AxioCam **MRc** (color version) without infrared barrier filter is not recommended as color is only defined for visual spectral range.

The AxioCam **MRm** (black/white version) is used without infrared barrier filter. If necessary, an additional infrared barrier filter can be inserted between the microscope adapter and the camera.

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