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# Mehr braucht man nicht

## A simple and convenient program to document microscope pictures

**by Christian Hennig and Ralf Schnabel**

## Handbook (Version, 01_09_2010)

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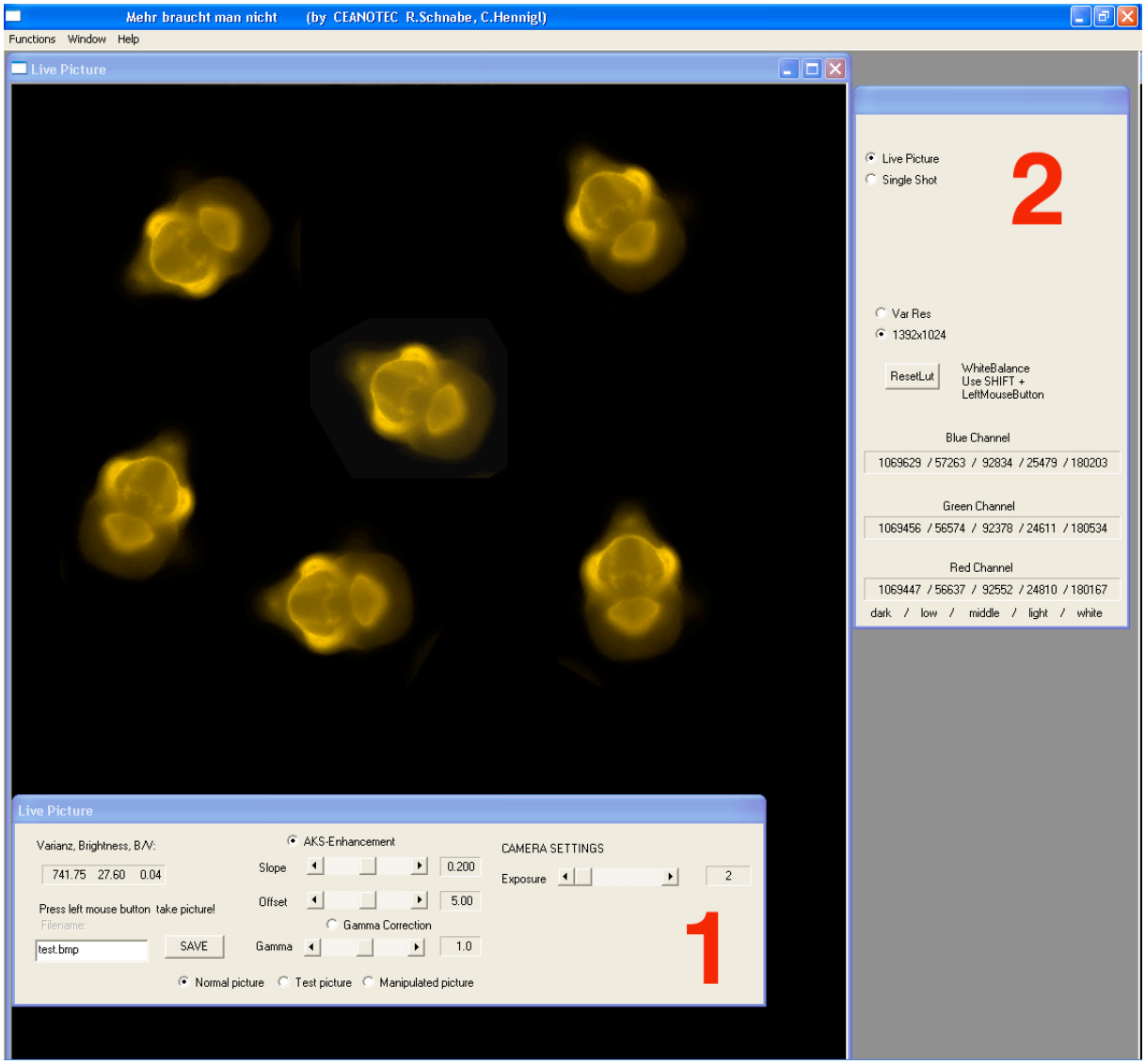

## Installation

The installation CD (or Zip-file in case of email) contains the following files:

**- manbrauchtnichtmehr.exe** (This is the program. Make a new folder in "Programs" on your computer and copy the exe into it. Put an alias on the desktop)

**- hdd32.exe** (This program installs the dongle driver when started from any location).

For Windows NT or XP the following DLLs must be dropped into the System folder C:/Windows/System32 and for Windows2000 in C:/WINNT/System32

**-lwf215p.dll**
**-MFC42D.dll**
**-MSVCRTD.dll**
**-MFCO42D.dll**

**To get started**
Connect your camera to the computer

Copy the manbrauchtnichtmehr.exe into the "Program" folder of your computer and make an alias on the surface of your desktop. Install with the hdd32.exe the driver for the dongle Also install the DLLs from the CD into the system folder as specified above. Set the resolution of the graphic display on 1280x1024 and plug in the hardware dongle. Make a folder "Pictures" on hard drive C:. Allow network access to the folder. Please note that the program crashes when pictures should be saved and these folder is not existing in the right location. Finally restart your computer.

The program may crash (or may take for ever to start) if the camera is not switched on.

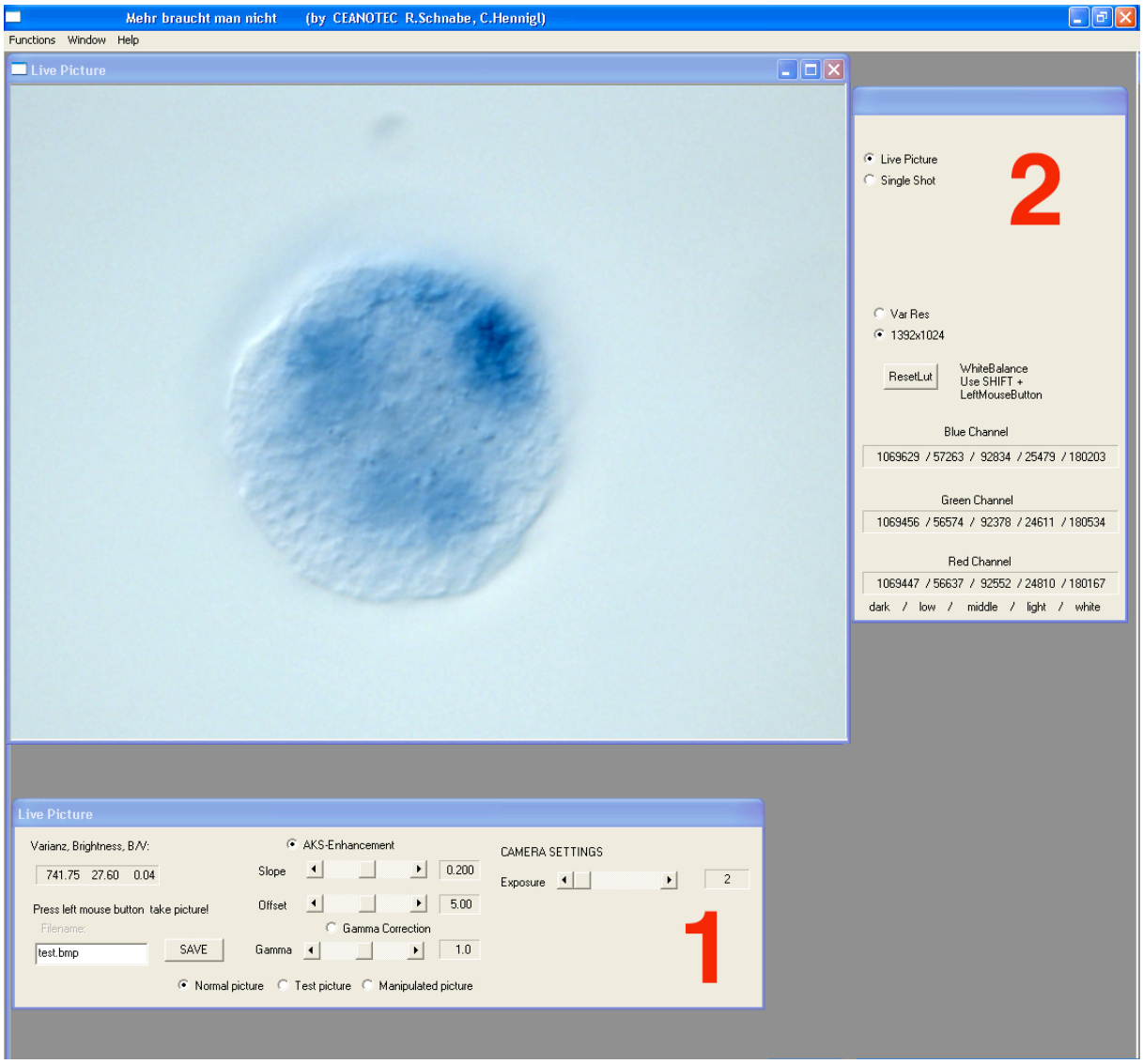


Fig. 1 The main windows with the two control windows

## Video camera

The program is currently designed to function with the pixelfly (PCO) black and white or colour camera since we find these cameras to be very good. On request we can also implement other cameras. However, we learned that this is a lot of effort and we have to have the camera in house to do so.

## Start the program

Double click the alias for the Mehr braucht man nicht-program. The program opens and you will see the main window (Fig 1), however, the Video window will be white and the window for the camera control (window 1) will be still missing. To open the capturing of the video camera pull down "window" in the top panel and activate "*win1*". To open the camera control window pull down "Functions" in the top panel and activate "*Video Dlg*".

Mount your specimen onto the microscope and activate the appropriate lamp and bright light or fluorescence channel for your specimen and search for a specimen. Open the shutter for the camera and now you should see the specimen, if the light intensity is properly adjusted at the lamp and the camera exposure time is set properly in window 1a (Live Picture). The picture may have a strong colour fault, which can be easily corrected using the feature "White balance" in window (2b). Just aim with the mouse pointer at a part of the picture, which should be white press shift and the left mouse button. Just repeat the procedure at a different region if the result is not perfect yet

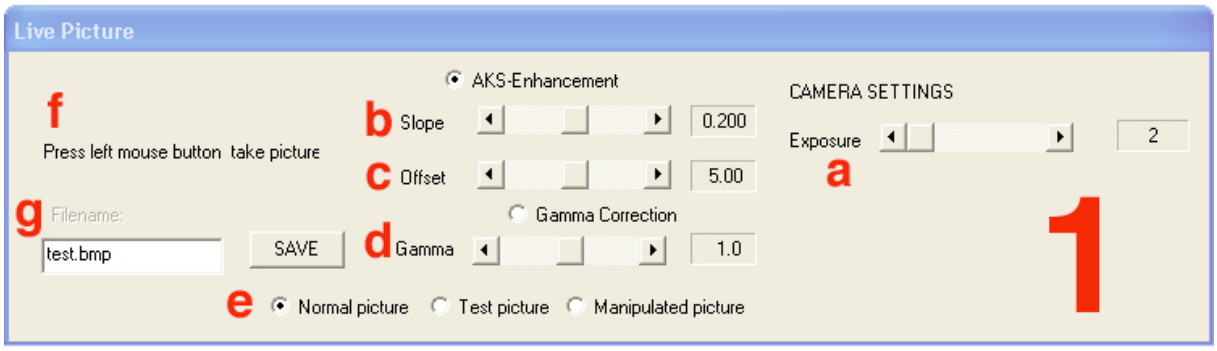


**Fig. 2 Window Live Picture (1). Camera control and advanced video functions**

Exposure time of the camera is adjusted in Window 1 (a). Make sure to use an appropriate setting of your white light source to have a correct light temperature. Avoid too strong fluorescent light to minimise bleaching of your fluorochrome. The camera is very sensitive if high exposure times e.g. 1000 ms are used. Get your own experience. The feature "Variance, Brightness, B/V" (b) will assist you to get an optimal DIC (Nomarski) picture by reading out the variance of the brightness values of the pixels and the mean brightness of the "measurement Window" (100 x 100 pixels). Pictures will be exposed properly if the brightness is around 40 to 90. Get your own experience, this depends on the brand and settings of the computer screen used. The ratio of Brightness/Variance represents the contrast in the picture. It thus helps to set the DIC contrast properly. A value of B/V of < 0.2 corresponds to a very good DIC picture, if you look at an upper plane of the embryo. The feature "picture analysis" in window (2c) serves a similar function for fluorescence pictures (see below).

**Taking pictures**

If you want to take a picture just click with the left mouse button in the "Live Picture" (video) window. The picture will be saved with the system time down to the second as the file name, which thus creates an unique document in the laboratory. Alternatively you may enter a file name in the field "file name" (1d) in the "Live Picture" window and save the file into the folder Pictures. Pictures are saved in the BMP format, which proved to be the most stable format if different computer systems are used.

**Real time picture enhancement**

Usually animals can be seen really well using just the proper DIC settings, light level, camera settings and an optimal stop field width. However, there may be situations where you are unhappy with the picture quality (bad mounting or if you use a difficult animal), therefore the program offers two "real time" picture processing routines. A function I (RS) call "AKS Enhancement" (b) since Anja-Kristina Schulz made the algorithm and a gamma function (c).

This functions cope with the problem that a digital video picture has a much smaller dynamic range than a classical video picture on a video screen. The gamma function suppresses brightness (value >1) or darkness (value <1), which may be useful to dim down very bright or to brighten up very dark structures. The AKS function is much more flexible and better than the gamma function, with the two parameters "Slope" and "offset" you can manipulate the representation of the pixels like in the "graduation curve" of Photoshop. For example you can make an S-curves with different shapes. Anyway the theory may be not that important here you will see that you may get very nice improvements of the pictures. Please be aware that these manipulations require complex calculations on every pixel of the pictures and that this may slow down the picture representation.

Since it is sometimes very difficult to decide whether a picture manipulation indeed improves a picture we made a function "Test picture". When the corresponding button (e) is activated the picture is split in the middle and the manipulation is only applied to the upper part. Thus the consequence of the manipulation can be compared to the unmanipulated picture. Now you can use the gamma or the AKS function to find out which one gives the better result. With the gamma function you pull the slider (d) to alter the picture. With the AKS function you may play with both sliders "Slope" and/or "offset". In my experience pulling the slider "Slope" to the left improves the clarity of the picture very much. Please play with both functions around to get your own experience. If you want to use the manipulation to take a picture or a recording activate button "Manipulated picture" (e) to apply the manipulation to the whole picture. The picture can now be saved as described before. If you could not improve the picture significantly return to the "Normal picture".


**Fig. 3 Window 2**

### Scaling picture size

The digital camera has a very high resolution (1376x1024 pixels), which can not be used properly with high magnifications when the specimen is not large enough. Thus huge pictures, which do not contain much meaningful information would be collected. Therefore, you may rescale the video window by activating the button "Var Res" in window (2a Fig. 3). Then you point with the mouse pointer at the upper corner of the new video window you want to create and then you press and hold the right mouse button while you move to the lower right corner. You can see the new window created as a frame drawn into the picture. When the mouse button is released the picture will be rescaled automatically. By activating the button "1392 x 1024" again you return to the full resolution.

### Setting the white balance

As mentioned before DIC colour pictures, for example of a ß Galactose staining, need a colour correction to get a nice picture representing the colours as we see them with the eye. Aim with the mouse pointer at a part of the picture, which should be white press shift and the left mouse button. If you are unhappy with the result you just try a different region. By clicking at the field "ResetLUT" you return to the normal setting of the colour representation.

### Getting the perfect exposure for fluorescent pictures

Photoshop offers unique opportunities to improve pictures. However, it is impossible to correct pictures properly if they contain white pixels and are thus overexposed. The feature in window (2c) is a real time analysis of the pixel brightness distribution of the picture shown in the video window while the camera is running. You see the values for the three colour channels divided in five brightness classes. Our analyses

of many pictures showed that "nice" pictures have almost no pixels in the range of white and only few in light (below 3000). Thus setting the light or the exposure time such that this criterion is fulfilled almost guarantees a "good" picture, which can be still improved for example in Photoshop.

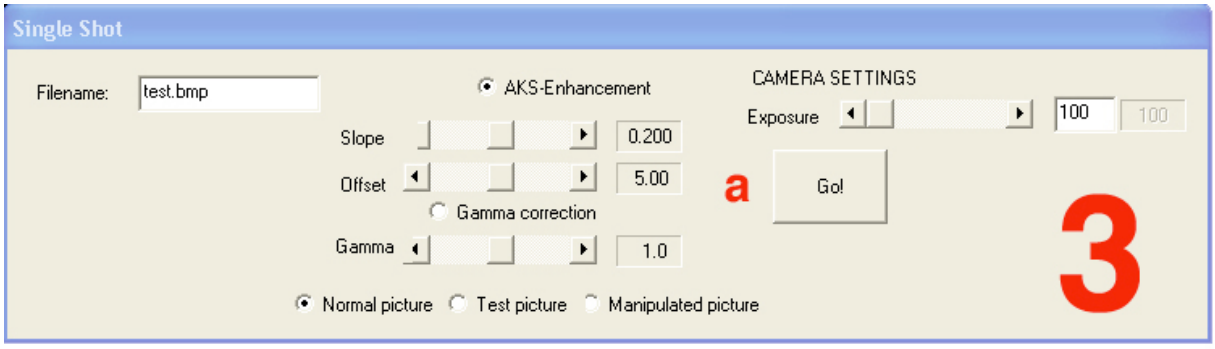


**Fig. 4 The Single Shot camera window (3)**

### Single shots

If the light intensity of fluorescent analyses gets very low – it is possible to picture fluorescence signals which cannot be seen anymore by the eye (exposure times >1000ms to 10 sec) – the delay created by the collection of the photons by the camera blocks the program. Thus you have for example a hard time to change the exposure time again or to navigate the specimen through the picture. This problem is solved by using the button "Single Shot" in window (2d). Now window Live Picture (1) is exchanged against the window Single Shot (3 Fig. 4). You can navigate with the lowest exposure time which helps you to orient yourself or to search for specimen with the DIC optics. Then you set a very high exposure time and click the field "Go!" (3a). The program will then take one exposure and the picture will remain in the window. You can either save the picture or preview how a different exposure will look like by pulling the slider in a different position and then take a new exposure by activating "Go!" again. The session is stopped by activating again the button "Live picture" in window (2d) again.

We hope you find our program - "Mehr braucht man nicht" useful - Please do not hesitate to contact us if you have problems or to suggest improvements of the program.

Have fun!

Ralf Schnabel and Christian Hennig