

PICOLAY *Tutorial* [Download this as pdf file](#)

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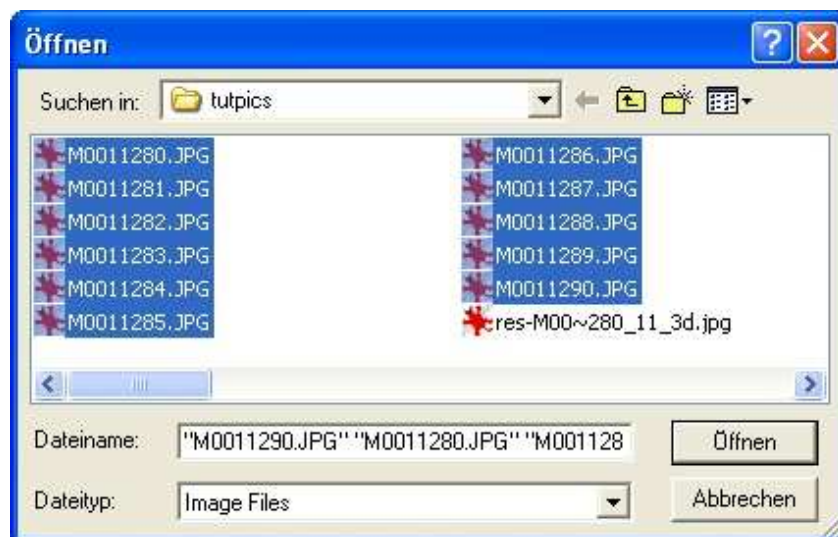
Installation

In order to install PICOLAY on your computer you only have to download a small file, **picolay.exe**, and save it in any directory (or even on a USB stick). Don't forget to place a link to the file on your desktop in order to start it easily. That's all installation you need. There is NO CHANGE of any system file on you computer.

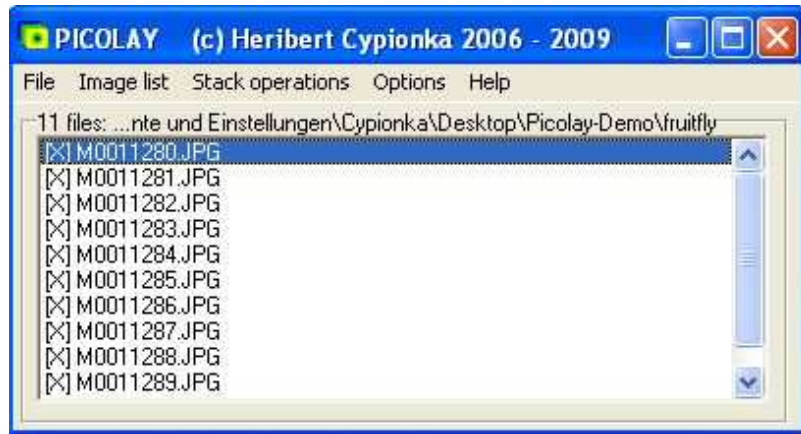
Note: Even if you are are not online and did forget to print out this tutorial, this text is available within the program under 'Help'.

Select files

Te first thing you have to do is select a set of image files. Click on 'File' and then 'Select pictures' to open a new window. Click on the first image file you want to select, then press the shift-key and hold it pressed, click on the last file of your selection (the selected File names turn blue) and then click on 'Open'. Alternatively, one can keep the Ctrl-key pressed and click on each image sequentially.



A list of the selected images is shown in the listbox, and the first image of the list is displayed.

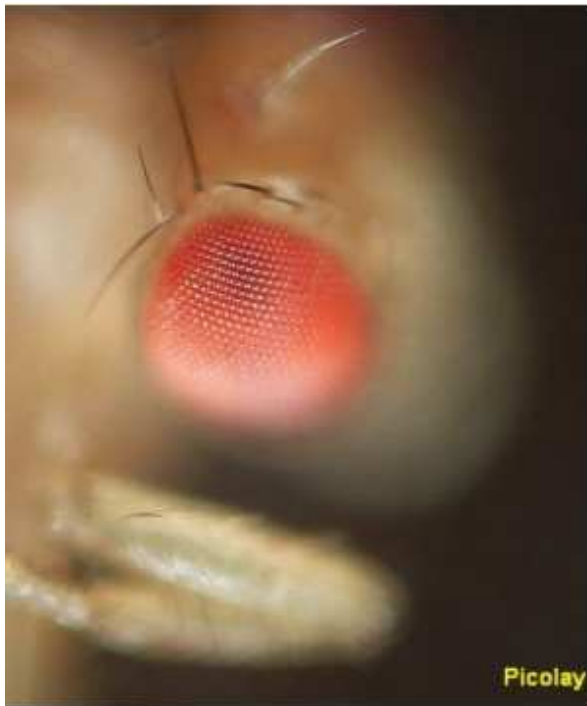


To browse through the list just click on the names. You can zoom in and out ([+] and [-] on the image frame), and you will have the same position with all images. To unmark or mark images, **double-click** a name, the 'X' in at the left will disappear (or re-appear, if you double-click again), and the corresponding image will not be regarded during the following stack operations.

Note: All pictures should have the same dimensions and have the JPEG or Bitmap format. Tif files might work also, but this is not guaranteed. The names of the files should have the same alphabetical sequence as the layers they were taken. You can reverse the order under 'Image list' depending on whether you started with the uppermost or lowermost layer. PICOLAY assumes that the first images shows the uppermost layer and requires the image names in the corresponding alphabetical order.

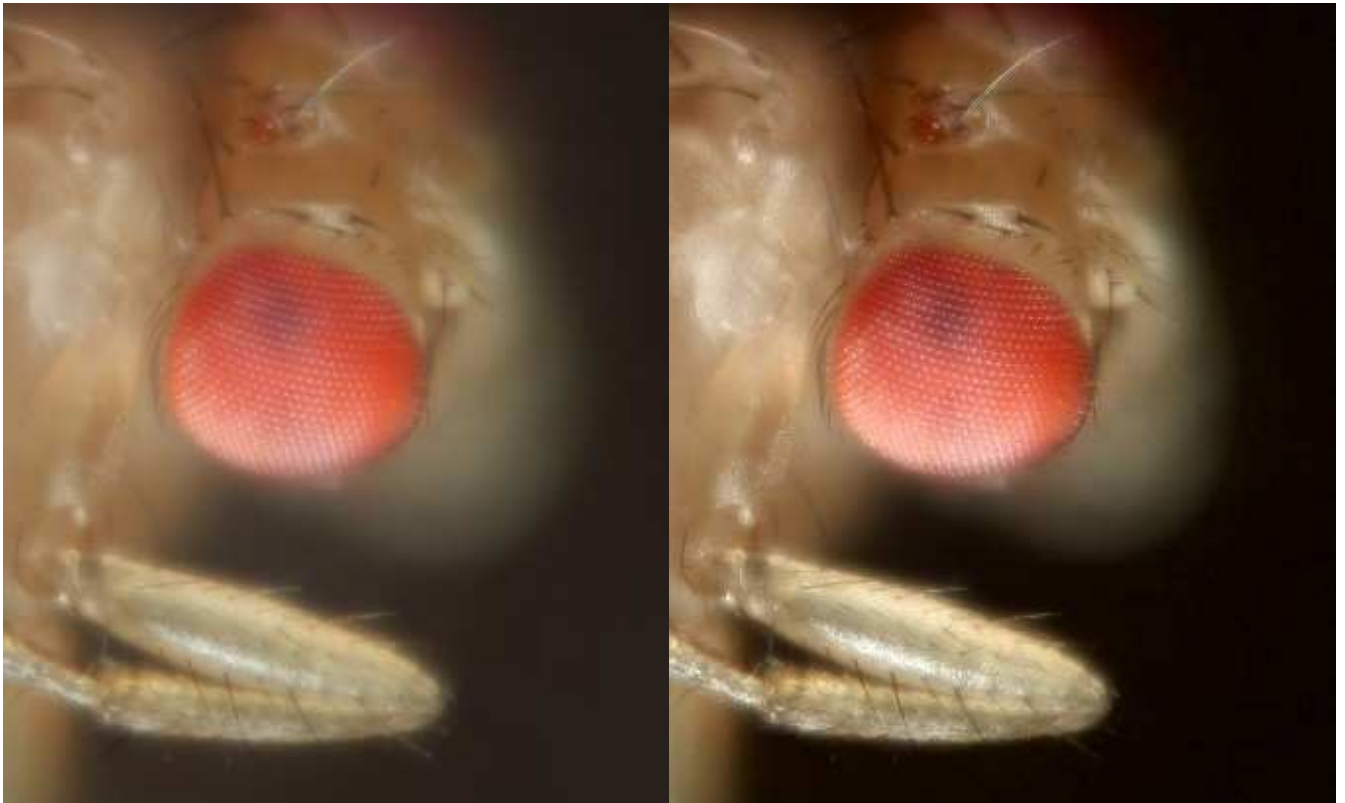
PICOLAY will not change the original files. However, automatically generated files will overwrite older versions. Next time you start PICOLAY the last picture directory will be remembered.

In order to practice, [download here a series of pictures](#) taken at different focus levels and extract them into a new directory. Here are four of the eleven pictures used for this tutorial:



Averaging images

A very simple operation you can make is averaging the images of your list. Just click on 'Go' in the 'Average' field and you will get the result shown at the left:

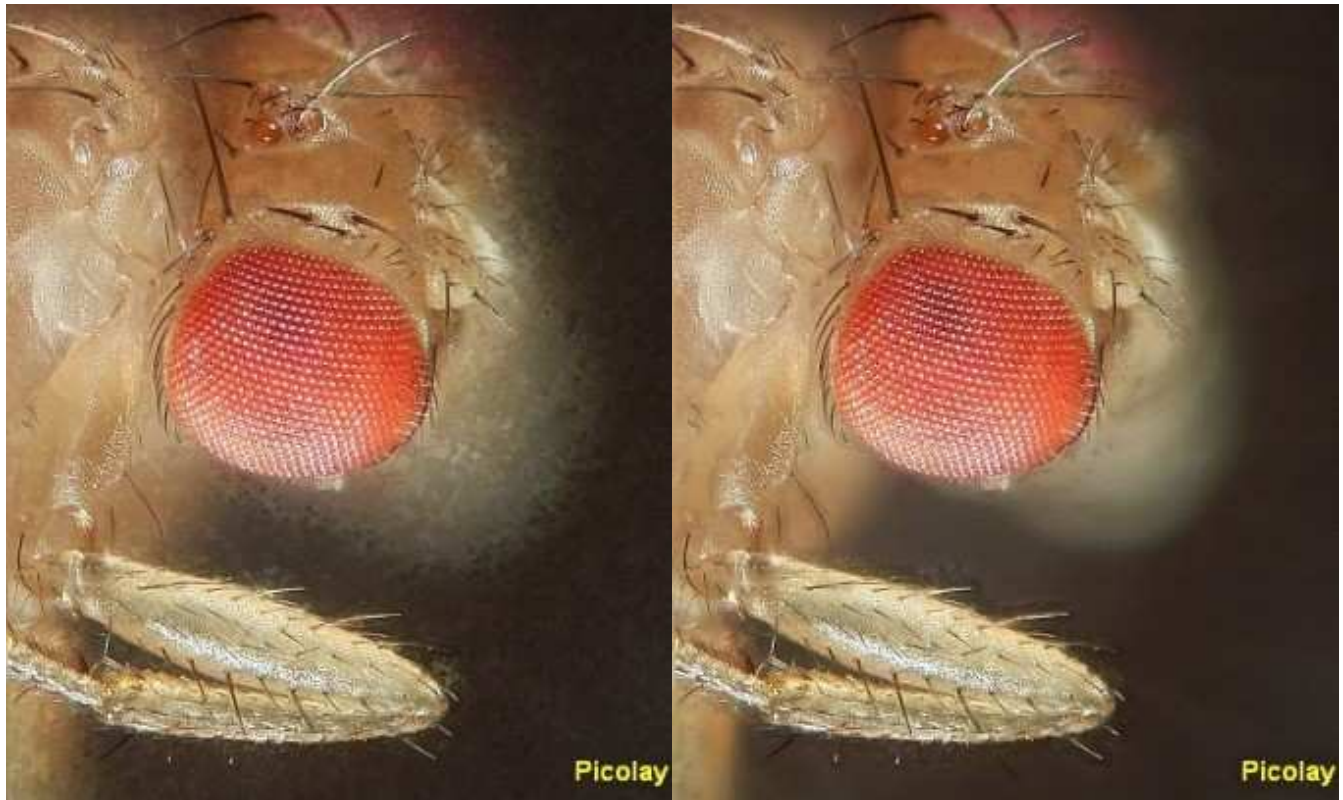


After clicking on the file name in the list and then [Enhance image] when it is displayed, you can test (and finally apply) changed parameters, until you get a result like the one shown at the right:



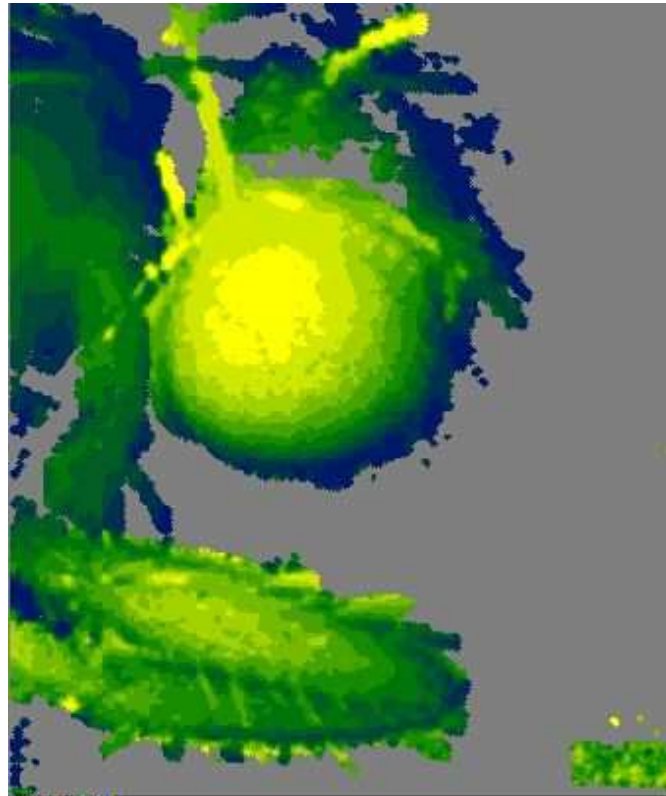
Stacking the images to select sharp areas

A main feature of PICOLAY is stacking. This means filter out the sharp areas from your image stack and compose it to 2-D or even 3-D images. Just hit the F1-key or click on 'Stack operations' and 'Stacking based on sharp areas' to get the result shown at the left:



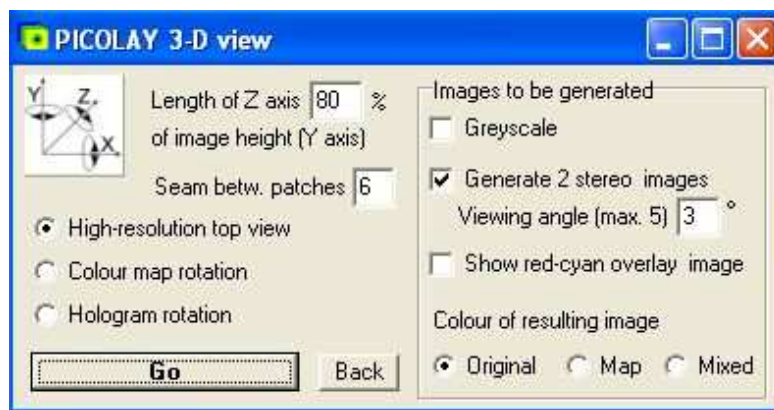
This has more brilliance than the first results, but still some ugly stains. These are the result of the stacking parameters applied. If we change the 'Filter frame size' to 5 (which means that sharpness is defined by the difference over a larger distance), and set the minimum contrast to 11 (that means pixels with a lower contrast than this value will be set to the average), and check 'Smooth 2-D map' we get the result on the right.

This picture shows much more details than that derived from the average. And it has a big advantage for scientific use: It is composed of original (or averaged) pixels found in the z-stack, exclusively. A colour map of the layers from which the pixels are derived is generated, too, and can be seen by clicking on '[Flip view=] Result:

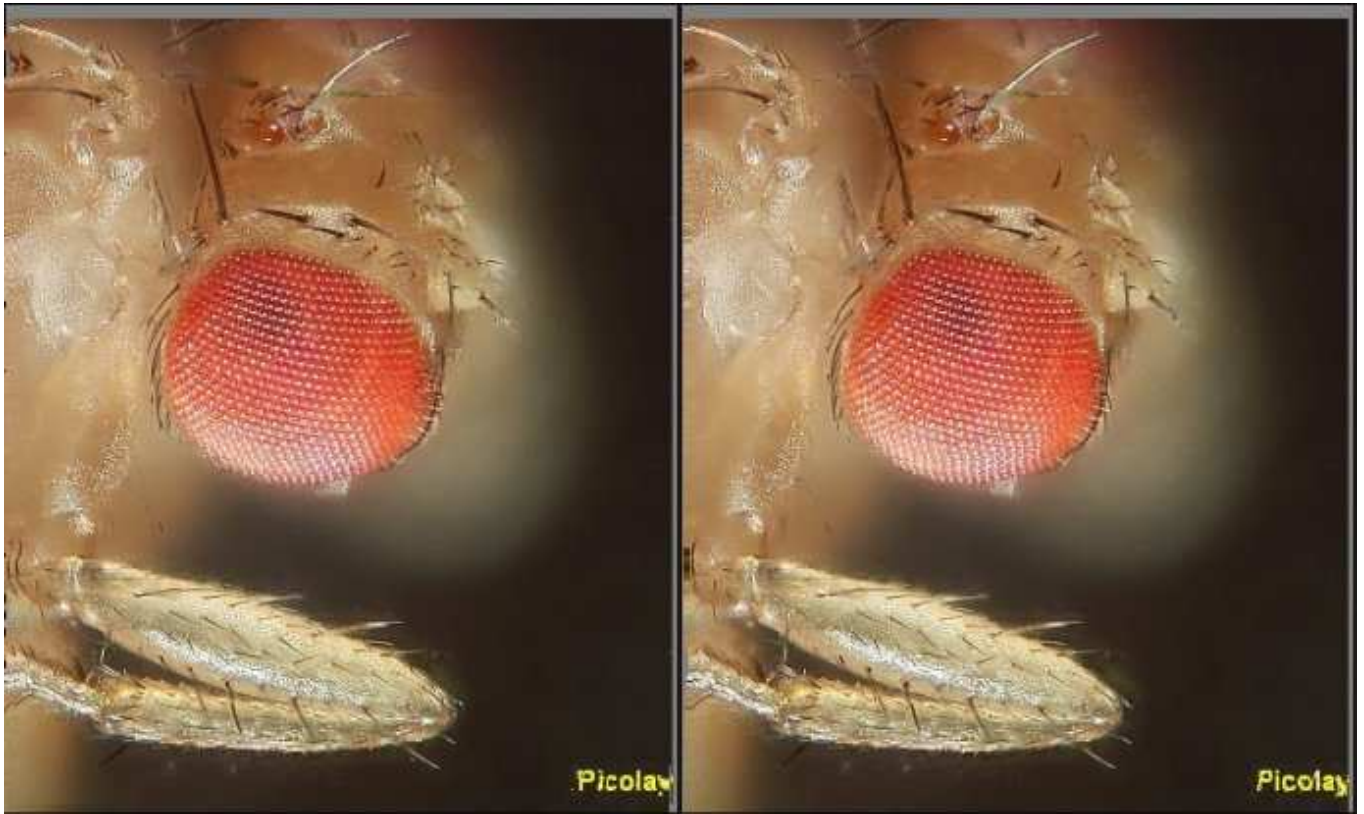


How to generate three-dimensional pictures

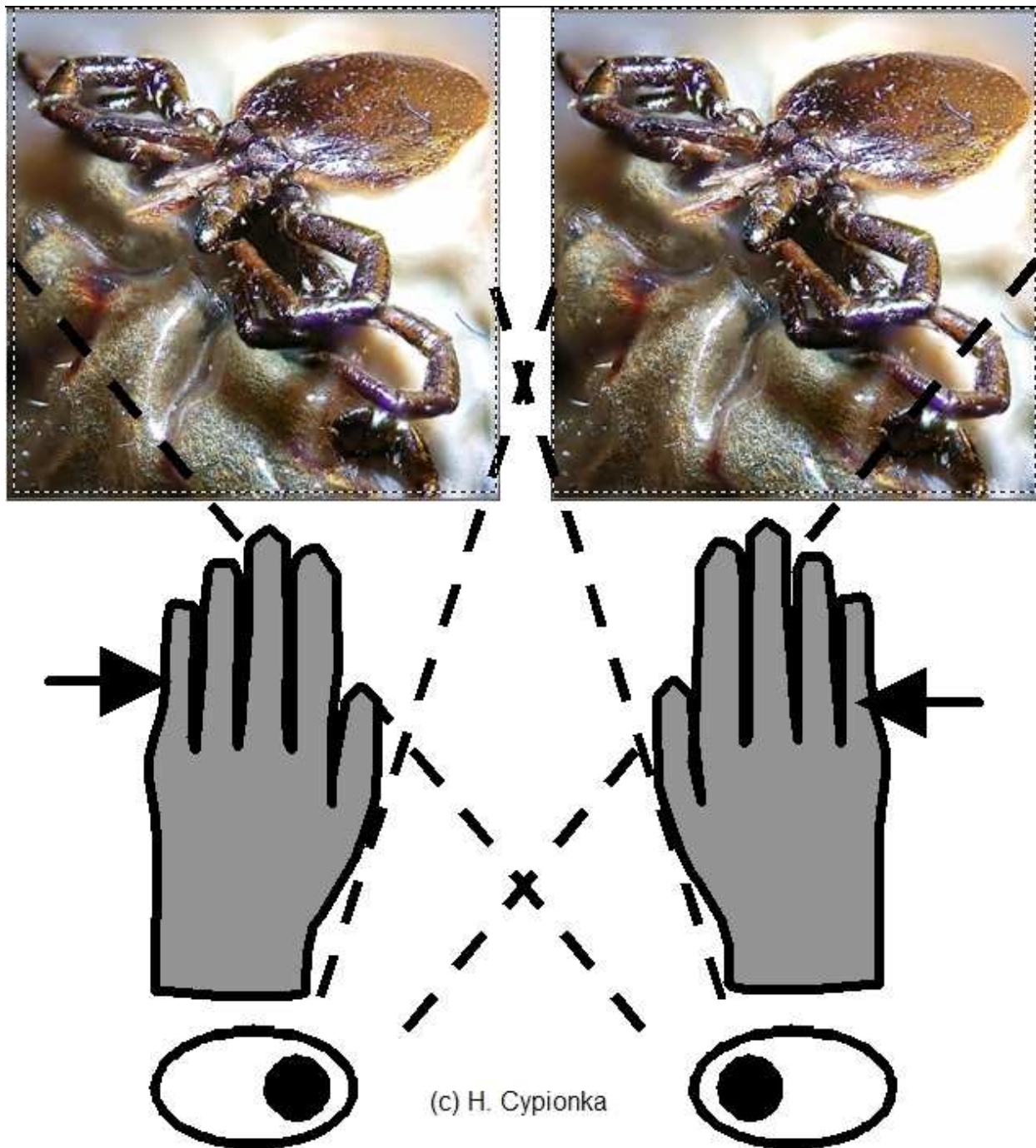
A unique feature of PICOLAY is that the information on the layer usage in a single stack take from one constant perspective can be used to generate true three-dimensional pictures as well as rotating models. Just click on '3-D view' to get the following window:



Check 'Generate 2 images (+/- 2°)' in order to get the following files (which are automatically saved and displayed in your image list):

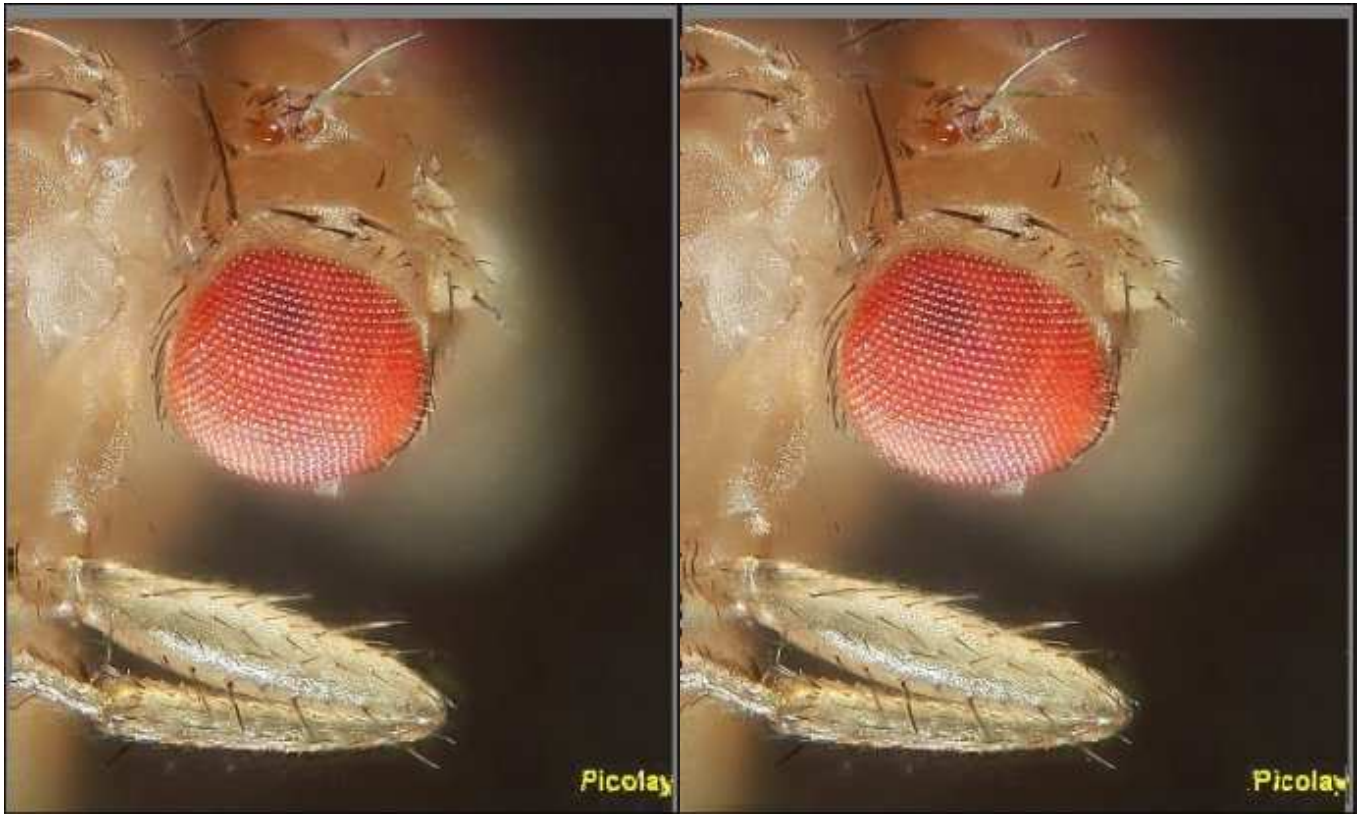


At a first glance these picture do not look very different from those above, even a bit less sharp. However, they are showing two different perspectives. And you can get a three-dimensional impression when you look at them as described here:



Just keep your hands in front of your face in a manner that forces the right eye to see the left image and vice versa ('cross-eyed' viewing). Then wait a bit while playing with the focus of your eyes. (The tick images also were generated by PICOLAY from a single stack)

For some people, it is easier to see 3-D images by parallel ('wall-eyed') viewing. These should look at the next example, while they prevent the left eye to see the right image and vice versa:

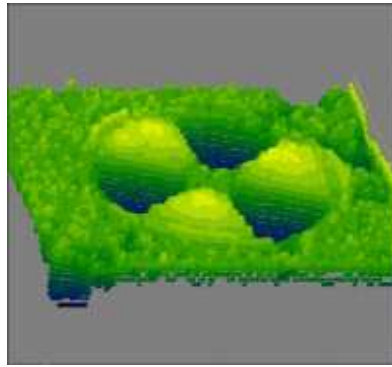


For those who still don't have the fantastic 3-D impression at this point, a red-cyan (or red-green) glasses might help as the final solution. The image is generated after checking the corresponding item in the 3-D view window (plus 'Go'):



Look at it through the cyan or green glass on the right, and the red glass on the left eye.

For those unhappy people who still didn't get it (and for those interested in other 3-D aspects of their object) the layer model can be rotated in any direction around the x- y- and z-axis [thanks to Jöran März for help with the cos and sin calculations!], as shown here for the shell of a diatom:



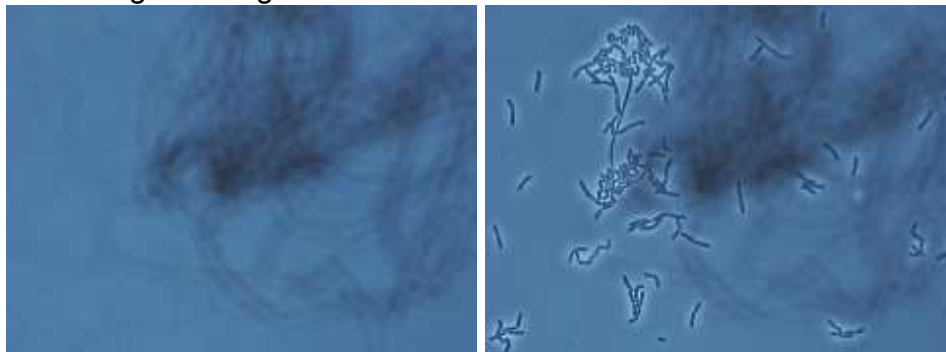
The rotation can be varied stepwise and saved automatically in order to generate images that can be combined in an animation.

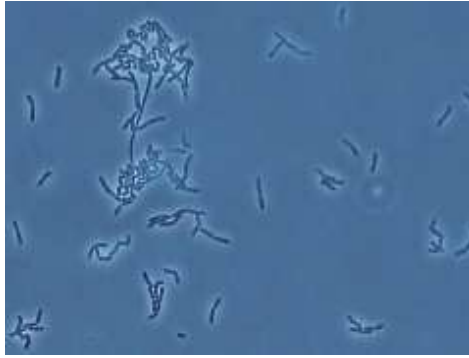
Select for colours

The colour-analysing function can be used to find out the lightest and darkest layers for every pixel. This function is helpful for scientific purposes, e.g, if a certain dye has to be followed through the stack. The routine also allows to strip off all non-target colours. In order to adjust the target color enter the RGB values or simply click with the RIGHT mouse button in the original image.

Adding and subtracting images

This routine adds the RGB values of the first image on the list to all following images. Negative factors result in subtraction. The images are automatically saved with a 'enh#' preceding the old name. The 'Set background' function allows to correct for disturbing elements not caused in the the object area (dust, dead pixels in your camera etc.). If you have a background picture (left) without your object as the first item in your image list, the background will be subtracted from your original (middle). Concomitantly, the background will be set to the value adjusted in the 'Colour' panel (right). In order adjust the background color you can just click with the RIGHT mouse button in the original image.





Finally

Good luck and many great images! Please let me know of any possible problems. And don't forget to check for new versions (look at your Version Date under 'Help'/'Info'), since I am still developing the program.

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