

(c) Heribert Cypionka

[This text is displayed in the help window of PICOLAY]

Please use the online help (Short reference, tutorial, tips & tricks) to get support with workflows and examples. You can find this in PICOLAY under 'Help | Short reference (HTML)' or at www.picolay.de under 'Manual' and 'Tutorial'.

PICOLAY (from **P**ICture **O**ver**L**AY) is a program for Windows (TM) computers designed for the generation of improved images and three-dimensional projections from digital picture series.

PICOLAY is freeware and comes without any warranty. The author is not responsible for loss of data or any other damage caused by PICOLAY. Always keep a backup of all images you process with PICOLAY. The copyright of the program remains with the author.

Please cite the website ([**www.picolay.de**](http://www.picolay.de)) when you use PICOLAY for the production of published images.

[==>] **Main features**

Although PICOLAY is a small program and very easy to use, it has powerful functions. It is designed for

- Focus stacking: Generate sharp images with increased depth of focus from picture series taken at sequential focus levels (so-called z-stacks).
- Stacking based on target colours instead of sharp areas.
- Average images.
- Add or subtract images.
- Auto-align positions and size if images are not perfectly congruent.
- Auto-adjust brightness of the pictures in a stack.
- Generate a flat field with adjustable background or white balance.
- Add text and a scale bar to pictures
- PICOLAY generates animations of picture series either as slide show or as animated gif file.
- There are many more retouching functions to improve images by changing parameters like sharpness, contrast, size etc.

Special features of PICOLAY are various routines to generate three-dimensional views, projections and animations:

- Generation of three-dimensional images from a single stack taken at a constant perspective.
- Generation of rotating 3D images, red-cyan overlay images and images for crossed-eyes or parallel viewing, and MPO files or interlaced images for 3D-TVs.
- 'Hologram stacking', a routine making structures visible that are hidden by the normal stacking process.

Thus PICOLAY gives you a little bit of 'confocal microscopy' for free. It also has several functions for changing general image parameters like sharpness, contrast, brightness, colour saturation, gamma correction, resizing and cropping.

Please check www.picolay.de for more information.

[==>] **Installation**

Download the newest version of PICOLAY from www.picolay.de. PICOLAY is a portable app. The installation routine can install the program into any folder, on SD card or usb sticks. Example images and help files are installed in a subfolder. If the help files are not found during runtime PICOLAY will seek them on the internet. The installation does not change any system files.

ATTENTION

It is recommended to install PICOLAY into a subfolder different from of the Windows program folder. Otherwise there might be conflicts with administrator rights or you have to start the program by a right-click on 'Run as administrator'.

[==>] picolay.ini

The last folder from which you loaded images, is automatically saved under 'picolay.ini' and will be used again in following session.

[==>] **Quick Focus stacking for the impatient user**

- Select images (File | Add images or Ctrl-A)
- Click on Stack operations | Stack with current parameters or press the Ctrl-F1 to start the stacking routine with the pre-adjusted standard parameters.
- If necessary, vary the parameters as described below.

[==>] **Main window: File menue**

'Add image(s) | Ctrl-A'

- Allows for selecting a list of images to be processed. All images have to be selected from the same folder and resulting images will be saved there as well.

'Clear list'

- will remove all images from the list without deleting them.

'Stack multiple sub-folders'

- allows for stacking images in several sub-folders upon a single click. Results will be saved in the upper-level folder. Take care to set the parameters as 'image alignment' and 'Save depth maps' prior to starting this routine.

'Save Result as', 'Save depth map as' and 'Print result window as'

- allows for saving and printing the results specifically.

- Exit PICOLAY | Ctrl-Q

- will quit the program. You will be asked whether new files generated by PICOLAY shall be kept or deleted from disc.

[==>] **Image formats PICOLAY can handle**

PICOLAY can (theoretically) handle up to $2^{16}-1 = 65535$ images at once.

It can open and save images with the following format:

- JPEG (*.jpeg, *.jpg)

- Bitmaps (*.bmp)
- PNG (*.png)
- MPO (*.mpo)

MPO files can be generated from the first two marked jpg images in the list. If you open an MPO file, it will be split and the two jpg files in it will be displayed separately.

- GIF (*.gif)

PICOLAY can generate animated gif files but not show the animation.

- RAW (*.dng, *.Cr2, *.nef)

For RAW images you need to have installed the appropriate CODEC for Adobe, Canon or Nikon. Please check the corresponding websites for download.

- TIF images (*.tiff or *.tif) can be opened, but will be changed to 24 bit bitmaps internally. PICOLAY cannot save tif files.

[==>] **Setting the file format for saving**

The file format for saving is set under 'Options' (see below).

It is easy to transform a list of images from one format to another: Just click on 'Options' and select the target format under [Save as=]. Then click on 'Enhance', (eventually change some parameters,) and carry out 'Apply to all marked images'.

[==>] **Resulting images generated by PICOLAY**

Most of the PICOLAY routines generate files that are automatically saved and added (unmarked) to the image list (see below). Most of the new files have names beginning with 'py'. PICOLAY will not overwrite original images, but will overwrite py-files without warning! The resulting files are generated in the same directory as the analyzed images. Therefore it is **STRONGLY RECOMMENDED** to copy images of each stack to be analysed into a separate folder.

When you exit PICOLAY you will be asked whether all 'py' files in your list should be erased (leaving no residues on disk). If you erase them, be sure to have saved before your final result under a name not beginning with 'py'. (Can be achieved under Image list | Rename marked files.)

Exceptions: clip, enh, xy and by files

If you decide to crop a rectangle from ALL MARKED IMAGES (edit | crop all marked images) the results will be saved under 'clip' + original file name.

Similarly, series of enhanced images will be saved under 'enh' + original file name, images processed by the auto-align x-/y-positions or auto-adjust brightness subroutines will be saved under 'xy' and 'by'+ original filename, respectively.

If selected under 'Options' the original name of the first marked image in the list will be placed in front of the py names generated by PICOLAY.

[==>] **Select pictures**

The first step is to select pictures. Click on 'File' and 'Add pictures'. Then click on the first image of the series, keep the SHIFT key pressed, and click on the last image file of your series. In order to select only part of the files from your directory, you can keep the CTRL key pressed and click on each file separately.

Once you have selected images, their names are displayed in the listbox at the left. PICOLAY sorts the files in alphabetical order. Take care that the names have the same sequence as the layers of your z-stack. The sequence can be reversed under 'Image list'.

Tip 1: In order to get familiar with PICOLAY use small images first (and copy them into a separated directory before you start!).

[==>] **Browsing the image list**

You can display the selected images by clicking in the listbox or by means of the up- and down-arrow keys. By DOUBLE-clicking on the names one can mark [X] ... or unmark [_] ... files for further processing. Alternatively one can toggle all marks, delete selected images, clear the list etc. under the menu item 'Image list'.

One can zoom in and out by clicking on [-] or [+] on the image window. Of course, PICOLAY will not change the original images in this case. A click on the number in the middle brings you back to the 100% view.

A special feature of PICOLAY is the following: When you have zoomed in or you have selected a certain part of an image, the position and size will be kept constant during browsing other images in the list.

[==>] **Handling the 'Image list'**

Under the menu item 'Image list' one can reverse the order of the image files. This is relevant because PICOLAY assumes that the first image shows the uppermost layer, and because only the first image can be added or subtracted from the rest of the list. (Eventually you'll have to rename the file to be added before selecting it.)

It is also possible to flip the mark (by double-clicking) and to delete some or all of the selected files from disk. Furthermore, one can rename marked files by replacing characters in the name string. This function is helpful, e.g. to escape the automatic deletion of 'py...' files when finishing the program.

It is possible to clear the list (without deleting any files), or to add new images, or to generate mpo files for displaying them, e.g., on a 3D-TV.

[==>] **Generate an MPO file**

For this function the first two marked jpg images in the list will be used. The new mpo file is saved in the current folder without showing the name in the list. (Prior to generate an MPO file you'll have to use the methods available in the 3d-view window to obtain the suited jpg images for the left and right eye view.)

[==>] **Generating an animated gif image**

The slide show can be transformed into a single animated gif file. Please regard that a large number of images results in large files (file size increasing with the square of the image dimensions!). Don't give it a name without the '.gif' at the end.

Animated gif images can be opened, but the animation will not (yet) be displayed by PICOLAY. Internet browsers can show the animation without requirement of a special driver.

[==>] F12 **Slide show of selected images**

With the F12 key or under 'Image list | Start slide show' you can get an animated presentation of all marked images. Under 'Options' it can be adjusted how fast the images are displayed. Furthermore, you can set whether the show goes back or starts from the first image when the end of the list is reached. Clicking on the image or on the list will stop the show. This function can be used for the original stack as well as for stepwise 3D rotations generated by PICOLAY, or for any other images series.

[==>] **Image window: Display modes**

[==>] **[Full screen] / [Half screen]**

Clicking on [Full screen] or [Half screen] switches the display size.

[==>] **[Fit to window] / [Set to 100%]**

Clicking on [Fit to window] or [Set to 100%] switches the images size.

[==>] **Zoom in or out**

Clicking on [+] of [-] on the image window (as well as on the result window) will change the zoom factor.

[==>] **Image window: Edit functions**

Under 'Edit' one can copy an image or parts of it to the clipboard. Other functions are 'Crop' or paste an image from the clipboard.

'Crop all marked images' can be used to select the interesting area in your z-stack for further processing. In this case the new file names will be 'clip' + <original name>.

The contents of the image window can be copied to the result window either as 'Result' or as 'Depth map'. This function can be used to make changes to the stacking results or depth map, before they are used for 3D projections.

[==>] **Image window: Enhance image**

When a selected image is displayed you can change a series of image parameters by clicking on 'Enhance image' on the upper window edge. First check the result, then apply the changes. There is no 'Undo'-function as in other routines.

However, a new file will be generated. If you 'Apply' the enhancement to a single image, the result will be saved automatically under 'pyenhanced-01.jpg'(with increasing numbers at the end). Additionally, one can save the displayed result by selecting 'Save result as' or Ctrl-S).

The selected transformations can be applied to all marked images if the corresponding box is checked. In this case the results will be saved under 'enhy' + <original name>.

Please select the file format of the generated images under 'Options | Save as'.

[==>] **Image window: Mouse functions**

The standard function is 'Rectangle' used to select areas for cropping or copying to the clipboard.

Tip: Set the zoom factor to 100% or 50% to get the fastest display of the mouse actions.

There are several other 'Mouse tools' selectable:

[==>] **Mouse: Retouching with paintbrush**

'Paintbrush' is used to paint on your image. RIGHT-clicking on the image will feed the pipette with the colour underneath the tip, which is indicated in the little tool window. Moving the mouse with the left mouse button (or the Ctrl-key!) pressed will paint on the image with fading edges. You can restore the image back to the last mouse-down situation by clicking on 'Undo' (located on top of the image) or by pressing 'Ctrl-Z'.

The thickness of the brush line indicated by a dashed circle, and is adjusted in the additional tool window. Finally you can choose whether the changes should be 'Saved with a new name' that gives a new file named 'pyenhanced-...' or 'Overwrite current image'.

[==>] **Mouse: Clean background**

This function helps to embed an object into a homogeneous background without very accurate use of the paintbrush being necessary. First, RIGHT-click onto the colour in your picture you want to set as background. Then (left-)click on the 'Background colour' block from the 'Mouse Paintbrush | Paintbrush' tool to define this colour as background. Now set the paintbrush width to a rather high value (e.g., 16 to 64) and surround your object with the left mouse key pressed. Whenever patches of the background area are not overpainted, RIGHT-click on these patches and try again. If parts of your object are overpainted by mistake, you can use the Undo-function and you can set the tolerance ranges for brightness and colour deviation to lower values.

[==>] **Mouse: 'Clone to result image'**

'Clone to result image' copies the area underneath the mouse circle in the image window to the same position of the result window (indicated by a ring in both windows). This allows manual stacking. The depth map will keep the information about the layer used for cloning, thus allowing a correct 3D projection. Only the original images used for stacking should be marked during cloning to the result window. For exact positioning the brush width can be adjusted as described above, and one can zoom in by clicking on [+].

[==>] **Mouse: 'Clone within image'**

This function allows to clone areas inside the same image, e.g., in order to overwrite disturbing items with undisturbed background. First RIGHT-click on the target position. Then left-click on the source and gently move the mouse through the source area while observing changes in the target zone, which is indicated by a second ring.

[==>] **Mouse: 'Insert text'**

This opens a new window for the selection of a text string, font, colour and direction (vertical or horizontal).

A subsequent click places the text on your image. Use 'Undo' and repeat placing the text until you are happy with the outcome. Then 'Save' the result.

You can add the text to all marked images if you check the corresponding box.

[==>] Mouse: 'Insert scale bar'

This opens a new window for the definition of dimensions and colour of a rectangle. A subsequent click on your image places the bar. Use 'Undo' and repeat placing the bar until you are happy with the outcome. Then 'Save' the result. You can add the scale bar to all marked images if you check the corresponding box.

[==>] Stack operations

Once you have selected images various tasks are enabled. Stack operations can be started by means of the Ctrl-F1, F2...F12 keys of from the corresponding menu items. Operations are only applied to marked images.

[==>] Stacking based on sharp structures

This is the normal focus stacking. PICOLAY can do 'Colour-based' or 'hologram-stacking' as well (see below).

[==>] Ctrl-F1 Stack with current settings

The Ctrl-F1 key or clicking on 'Stack with current parameters' will start the stacking routine using the current parameters. On the right window you can follow how the sharp result is sequentially composed. When done an overlay of the sharp image and the depth map is displayed in the left window. This should help you to assess the performance.

The result will not always represent the optimum. Often one would like to change some of the parameters to improve it. This can also be done before you start the stacking routine.

[==>] F2 Edit stacking parameters

Press F2 'Set stacking parameters' in order to adjust the parameters prior to stacking. A new panel at the right opens, where you can adjust some parameters. Here the parameter list and their effects:

[==>] 'Noise suppression'

is set to a value that allows separation between a smooth background and areas with sharp structures. This avoids that minor disturbances or noise is selected instead of filtering it out. Noisy (low-contrast) pixels will be averaged, giving a smooth background. You might lose detail if the value is too high.

[==>] 'Narrow or widen patches'

will move the transitions between sharp and unsharp areas. This function becomes visible only at minimum contrast values above 1. Positive values are used to fill unsharp gaps inside an object. Negative values will shrink the outer edge of a structure.

[==>] 'Filter:' (Smart or 1 - 10)'

defines the filter for the detection of image details. With 'Smart' the filter will be flexible depending on the structures in different image areas. You also can fix the filter to a value between 1 (fine, high-pass) and 10 (coarse, low-pass).

[==>] 'Prefer bottom or top frames'

defines to which degree structures in upper frames should cover those located deeper in the stack (and in the visualised object). Negative values move low-contrast areas to the back.

[==>] **'Align images'**

corrects displacements and size variations of your images. It must be done if your stack was generated at low magnification and particularly, if it was taken on a stereomicroscope. There are two options: '1x' will perform the alignment during the stacking process, starting with the last image in the list. '2x' will first generate aligned images, starting with the middle of the stack. The aligned images will be saved with new names (xy...). Under 'Options' you can switch off size correction and/or switch on additional correction of rotation in your stack.

[==>] **'Test 4 filter settings'**

generates four different stacking results by varying filter size and noise suppression. This might help to find the perfect parameters easily...The applied parameters are documented in the file names (see next paragraph).

[==>] **'Auto-enhance'**

increases sharpness and contrast of your result.

Upon clicking on 'Go' the stacking routine starts. You can follow the process in the result window. Finally, an unmarked file named e.g.

'pysharp_min6_pa3_pr5_fil5_en.jpg' will be saved and added to the listbox with 'pysharp' meaning sharpness-based PICOLAY file, and 'sup6_pa3_pr5' describing the applied parameters for noise suppression, patch enlargement, and preference of upper frames, respectively. '_en' indicates that the resulting image was automatically enhanced.

[==>] **Depth map**

Concomitantly with the sharp image, a map is generated showing from which layer each pixel is derived. The depth map is helpful for finding the perfect stacking parameters. Grey areas indicate where the pixels were averaged as no sharp structures were detected. The information in the map is also used to generate three-dimensional pictures and projections (see below). To automatically save the depth map, activate this function on the stacking parameter panel.

To see the map and switch between the stacked image and the depth map click on '[Flip view=] Result' (or '[Flip view=] Depth map', respectively). Yellow indicates uppermost and blue the lowest levels of the stack. If selected under 'Options', the depth map is saved automatically as 'pymap...' and added to the image list (unmarked).

Again: One can improve the resulting image by clicking on [Enhance image] as described above.

[==>] **F3 Colour-based stacking**

Starting 'Stacking based on colours' in the main window or 'F3' opens the colour-based stacking routine. A new panel 'Set target colours' is showing up. You can define a target colour by clicking in the original image with the RIGHT mouse button, or by entering value of the RGB channels in the corresponding fields on the panel, or by clicking on the red, green, blue, black and white squares.

Especially black and white (R/G/B = 0/0/0 and 255/255/255, respectively) give interesting information about your image stack!

Clicking on 'Go' will produce an image composed of those ORIGINAL pixels from the Z-stack that are closest to the target colour. This feature is especially helpful for phase contrast images and stained objects.

Colour-based stacking also generates a depth map (see above) and opens the three-dimensional possibilities described below.

If you check the box with 'Strip non-target part', your target colour will be filtered out from the stack while other colours are neglected.

The resulting image is saved as 'pycolour_r0_g0_b0.jpg' (with numbers indicating the RGB values) and added to the image list (unmarked). If selected under 'Options' the depth map is saved as 'pycolour2Dmap_r0_g0_b0.jpg'.

[==>] F4 **Average of images**

The mean image is obtained by hitting the F3-key of from the 'Stack operations' in the main window. The resulting image is saved as 'pymean.jpg' and added to the image list (unmarked). [Again: Older files with the same name will be overwritten!]

To enhance the quality of the result one can increase sharpness and contrast using [Enhance image]. In some cases this gives an excellent output, comparable to sharpness-based stacking.

Averaging might also be helpful, if you want to combine different images that were produced by PICOLAY. For example, try out what happens if you average the resulting images for the darkest and lightest pixels...

[==>] F5 **Insert intermediate images**

This function generates additional images inserted between your originals. This can be useful for smooth transitions in a slide show or for 3D projections, if you would like to increase the number of layers. The name of the new images will get an 'i' added at the end.

[==>] F6 **Auto-align images and resize**

If some of your original images are displaced with respect to their horizontal and vertical positions, rotation, or if the object size varies, you can compensate this by using the auto-align function. This routine will especially be helpful for pictures taken through a stereomicroscope, where the object appear moving with the focus, as well as for macro images, where the objects appear larger when the lens comes closer. The resulting images will be saved under 'xy' + <original file name>. Use this function prior to stacking.

Under 'Options' the resize function can be inactivated or switched on again. If a rotation correction is necessary, this has to be switched on under 'Options' as well.

TIP: If the alignment is not perfect, a second run might give a better result.

TIP: If you have any hot pixels on your chip or dust in your optical system, it is recommended that you take unsharp images above and underneath the object, average these and subtract them via the 'Set background/flat field' routine (F10), see below. Otherwise those particles could grow out to disturbing lines.

Note: If the images in your stack are not displaced the 'Auto-align and resize' function might impair the stacking result.

[==>] **F7 Auto-adjust brightness**

If some of the pictures in your stack differ in brightness, try 'Auto-adjust brightness' to compensate for that. The resulting images will be saved under 'by'+ <original file name>. Use this function prior to stacking.

[==>] **F8 Set white balance**

If you use this function you can correct the white balance of your picture/s. Just click with the RIGHT mouse button into the background and then on 'Go'. The corrected images will be saved automatically with 'py' inserted in front of the original file name. These files can be used for further PICOLAY processing after changing the mark correspondingly.

[==>] **F9 Add or subtract an image**

PICOLAY allows to add (or subtract) the first image in your list to all following images. Take into account that RGB values above 255 and below 0 are not allowed and will be cut off automatically.

[==>] **F10 Set background /flat field correction**

If you select this function and a 'Factor' of -1 (a negative factor means subtract) this function can be used to eliminate disturbing elements that are present in all pictures of your stack (e.g. 'hot pixels' or dust particles on your lenses). The function does not simply subtract the first image (which would result in nearly black images) but adds RGB values required to reach the background colour you have selected by clicking in the image with the RIGHT mouse button. The best result is usually obtained when you select the natural background colour.

[==>] **F11 Divide by 1st image**

This function will divide all marked images by the first marked image in the list. This is done colour-based by using the RGB values 0-255 for each of the channels. E.g., if the first image (divisor) has a value of 127 in the red channel then the R channel of the other images will be divided by $255/127$ (i.e. multiplied by $127/255 = 0.5$).

[==>] **Mount 2 images side by side**

Generates and saves a bitmap named 'sbs...(original name).bmp' from the first two marked images in the list. The function maybe helpful for combining the left and right view of stereo images.

[==>] **Main window: Options**

'Save as'

- sets which format is used for images generated by PICOLAY (bmp, jpg, gif, png, or same as original).

'Name files with time stamp'

- adds a 5-character time code at the beginning of the filename (increasing alphabetically every 1/10 sec)

'Add original name to py file'

- adds the name of the first marked image in the list to the py file

'Resize during alignment'

- allows for switching off the size correction during alignment, which is helpful with the macro- but not with micro-photography stacks. (Standard setting is: ON)

'Correct rotation w/alignment'

- should be switched on if your images need rotation corrections in you stack. (Standard setting is: OFF)

'Cropping ratio width:height'

- allows for manual cropping (pre-set) or fixed ratios of width and height, when you crop out a single or all marked images of your list.

'Background'

- lets you set a dark blue or grey background to PICOLAY windows.

'Slide show parameters'

- lets you set how long each of the images of a slide show (and an animated gif) is displayed (in msec), and whether the show starts from the first slide or goes backwards, when the end of the list is reached.

'Flip depth map'

- will reverse the depth map colours from top-down to bottom-up. (Similar results you'll get when you use 'Image list | Reverse order of images' prior to the stacking process.)

'Save depth map as grey-scale bitmap'

- will generate a grey-scale depth map that might be usable by other programs like Stereophoto Maker.

'Anaglyph mode'

- allows for handling colour issues of anaglyph images. You might reduce the colour intensity by half or apply the Dubois algorithm that shifts the colours to a more comfortable (but not realistic) pattern.

[==>] **Main window: Help**

This manual is displayed under 'Help | Manual'. You can switch between the English, German and French version. For further hints and examples check the 'Short reference (HTML)' or check www.picolay.de.

- Please, take care to download the newest version. The release date of the PICOLAY version you are using is displayed on top of the main window.

- I am still developing the program. Let me know any comments, problems and suggestions.

[==>] **Result window**

The right window is used to display results. One can change window size and image resolution as with the (left) image window.

'Flip view'

- Clicking on [Flip view=] switches the display between stacking result and depth map.

'Edit'

- The 'Edit' functions are restricted to copying the image to the left (image) window or clipboard.

'Synopsis'

- [Synopsis=] ON forces the right window to the same position and zoom factor as the image window at the left. This is particularly helpful if you have separate images for the left and right eye and want to zoom in.

'3D view'

- '3D view' opens a new window that allows for setting the 3D parameters.

[==>] **Generating three-dimensional images**

As described above the stacking routines (both, sharpness- and colour-based) generate depth maps (from blue to yellow, background pixels in grey), indicating which layer is used at each position. The depth information in this map can be used to produce various three-dimensional projections and virtual 3D images.

[==>] **Introduction to the 3D functions**

While 3D projections are quite common and easy to look at, true 3D images require the generation of two different pictures for the right and left eye, respectively, and a special technique for the observation. However, they give a completely different and fascinating perception!

Some people can stare with parallel or crossed eyes on the two images presenting the object at slightly different angles (2 - 4°). After some time they see three images, the one in the middle giving a 3D effect. Depending on parallel or crossed eyes, fore- and background appear exchanged.

With parallel eyes it is difficult to look at images broader than the distance between our eyes (about 6.5 cm). Crossed-eye viewing allows to see the effect also with larger images. Many people are unable to see the 3D effect in two adjacent images. In this case red-cyan (anaglyph) glasses and an overlay of the different views can be the solution.

Based on nothing but a single image series taken at a constant perspective, PICOLAY generates freely rotatable 3D projections that can give a natural spatial impression (especially if animated) as well as images suited for wall- and cross-eyed viewing and red-green overlays.

A special feature is 'Hologram stacking' - the visualisation of structures that are covered by the common stacking procedures: If an object has more than one layer with interesting structures usually only that with the highest contrast remains visible. By using 3D projections with changing viewing angle this problem can be overcome by means of PICOLAY.

[==>] **Using PICOLAY for the generation of 3D views**

Clicking on 'Generate 3D views' in the main menu or on [3D view] in the Result window (after stacking operations) opens a new window that allows to produce different kinds of 3D images: pairs of true stereo images for cross-eyed or wall-eyed viewing, red-green overlay for the use of red-green glasses, MPO files for 3D-TVs, freely rotatable 3D landscapes derived from the depth map, and so-called hologram stacking.

The dimensions (width and height) of the 3D images will be the same than those of the original images. However, the projections will leave some space unused. This will be filled by the background colour you select at the corresponding panel.

[==>] **Length of Z axis**

The Z axis (object depth) is defined in comparison to the height of the image (Y axis). If the lowest and top-layer of your z-stack have a distance of 100 μm and the height of your image is 200 μm the correct value would be 50 (%). A value of 0 will not give any 3D effect. Too large value will result in separation of layers of the object.

[==>] **Enlarge pixel depth**

This function is used to fill horizontal and vertical fissures in 3D images. Larger values for the length of Z axis, and viewing angle (right panel) require larger values and vice versa.

[==>] **Projection based on depth map**

The generated 3D projections are based on the depth map generated during the previous stacking process.

[==>] **Hologram stacking**

This will require a new reading of all images -> see below.

[==>] **Images to be generated**

Defines the format of 3D-images to be generated. If 3D-view is deactivated, single images will be produced that, however, can be rotated around the 3 axes.

Pictures produced are named, e.g., 'pyrecy_x0_y0_z0.jpg' with the numbers after x, y and z indicating the rotation angles of the three axes.

[==>] **Stereo**

If you check 'Stereo', two images with slightly different viewing angles (for your left and right eye) will be generated. The viewing angle is set on the right.

The produced images can be overlain as red-cyan images. Alternatively one can produce two separated images, combine them as MPO or jps image, or as 2 or 3 combined images or a panel with 4 half-sized images for the right and left, respectively left and right eye combined. The latter has the advantage that one can see 3D effects independent of whether the observer is staring with crossed or parallel eyes on the panel. The upper images will give a convex impression, the lower a concave - or vice versa.

[==>] **Relief**

- The relief function adds light (bright pixels coming from top-left) and shadows to the projection. This might be helpful if a stereo display is not possible. Shadowing is based on the depth map, which can be overlain with the structure (in black and white). Dot density and shadow intensity are adjusted in a separate window.

[==>] **Viewing angle**

- Our 3D viewing angle is larger when we are sitting close to a monitor screen than in a lecture hall. Therefore a value of 3° might be good for a TV monitor, while for a bigger audience the value should be reduced to 1.5 or 2°.

[==>] **Distance**

- PICOLAY places the mid layer of an image stack on the screen level. Closer structures will appear in front of the screen. To avoid that those are touching the image windows (3D image violation) one should increase the distance value, which moves the object into the back.

[==>] **Anaglyph images**

- Anaglyphs are images showing a 3D effect by using different colour filters for the left and right eye. These can cause colour deviations, particularly if colours similar to those of the filters are present in the object. To compensate this issue, one can try three different filter sets [Full colour, Half colour and Optimised (Dubois)], which are selectable under Options.

[==>] **Hologram stacking (sharpness-based)**

- Hologram stacking requires a threshold value to make sure that only pixels with a minimum sharpness remain visible while others are suppressed. Often the minimum contrast should be set to a higher value than used for normal sharpness-based stacking. You also have to set a filter size ('smart' filter is not usable here). To make all layers visible in a series of resulting images, 'Stepwise rotation' should be used.

[==>] **Hologram stacking (colour-based)**

- For colour-based hologram stacking the target colour and tolerance have to be defined. To set the colour, click with the RIGHT mouse button into the original image or use the colour definition function in the colour-based stacking window.

If you are selecting the black or white as target colour, a large tolerance value might be necessary. Again, you can also produce pairs of images for stereo viewing or red-cyan overlay images.

[==>] **Background**

As background used for pixels below the minimum contrast one can use either the average of all images or a selected colour. After clicking on 'Colour' you can set this either by a RIGHT-click in an original image or after clicking on the colour bar. A fixed background colour is helpful, if you would like to rotate the specimen in space.

[==>] **Acknowledgements**

Eberhard Raap has induced many improvements of the program by constructive critic and helpful user suggestions. I thank Jöran März, Wolfgang Ebenhöf and Cora Kohlmeier for mathematical support, Stefan Layer for several good suggestions, and Wolfgang Franke for valuable support with the deciphering of the MPO format. Bernard Jenni has suggested many improvements of the manual and generated the French manual version – many thanks!

Heribert Cypionka
Institut für Chemie und Biologie des Meeres
Universität Oldenburg, Germany

[==>] **e-mail:** [heribert.cypionka\[at\]uni-oldenburg.de](mailto:heribert.cypionka@uni-oldenburg.de)

[==>] **Internet:** www.picolay.de and www.microbial-world.com