

# DAIME Changelog

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## 1 Changes from version 1.3 to 1.3.1

This is mainly a bugfix release.

### 1.1 Improved features

- The display of borders around segmented objects can be toggled by using a new checkbox in the Object Editor.

### 1.2 Bug fixes

- Some functions crashed DAIME when called with RGB images. Fixed.
- File:Export, when called for RGB images, exported greyscale instead of color images. Fixed.
- In the Object Editor, borders around defined objects were not drawn when custom object colors were shown. Fixed.
- Edit:Copy could crash DAIME if the target stack/series contained more images than the source stack/series. Fixed.

### 1.3 Polishing

- Old-style LCD displays of object number and size (in the Object Editor) have been replaced by text labels, which show the total number of objects, the numbers of selected and rejected objects, and the size of the object under the mouse pointer.

## 2 Changes from version 1.2 to 1.3

### 2.1 New features

#### 2.1.1 General

- Added support for color RGB image import. Color images can be imported either as color or as greyscale images (RGB channels are split in the latter case, like in previous versions of DAIME). The user is asked to decide how color images should be imported. Color images are displayed in color in the main window stack list.
- New STK file format (version 10) that stores monochrome (8 bits/pixel) or color (8 bits per plane; i.e. 24 bits/RGB pixel) image stacks/series. This format also uses a better compression method (Deflate, provided by libz via Qt abstraction) than previous STK file versions, for both image and segmentation data. The better compression leads to smaller STK files. All previous STK file versions can still be read by strict import filters.
- Image view dialogs display color images in color or in luminance (left mouse-click with SHIFT pressed down switches between color and luminance display mode). Especially useful for object editor, where either original colors or luminance with selected/rejected indicator colors can be displayed. Where appropriate, threshold mask mode is also possible for color stacks (blue/red/"luminance grey" are shown in this mode).

### 2.1.2 New editing features

- Extract color channels (from RGB images; optionally convert extracted color channel to greyscale)
- Convert greyscale images to color (RGB) images; applies a user-defined base color

### 2.1.3 New segmentation features

- “Whole” image mode for magic wand. Detects pixels (voxels) similar to the seed in a whole 2D image or 3D image stack. These similar pixels/voxels will then become parts of newly detected 2D or 3D objects.
- Color magic wand that compares colors (RGB) to find pixels/voxels similar to the seed. In contrast to the old magic wand tool, intensity is not used (instead all colors are internally normalized to midtone intensity prior to comparison). The user can now choose between two magic wand tools: the old intensity-based one and the new color-based one. However, with grayscale images only the old intensity-based magic wand tool is enabled.

### 2.1.4 New visualization features

- Perspective projection, improves fly-through and spatial impression. Parallel projection can still be used, too (new checkbox in Visualizer selects the projection mode).
- Rotation of viewer now implemented. Movement of viewer in  $z$  direction actually is movement along current viewing vector now. Faster moving along  $z$ : turn mouse wheel; slower: turn mouse wheel+SHIFT key. Together with viewer panning (improved, see below), entire scene rotation and persp. projection, this enables nice fly-through. Viewer rotation either by arcball widget+dials, or by mouse movement in GL widget (together with SHIFT key). New RESET button for viewer rotation added, too. Resets only rotation, not position of viewer. In contrast, the reset button at panning sliders resets rotation AND position of viewer.
- New reset button for the whole-scene rotation.
- New rendering engine implemented that uses 3D textures and GLSL pixel shaders. Offers many more rendering features and interactive editing, local/hemisphere lighting, and so on. Complete feature list would be too long for this changelog. Refer to the user manual. GUI added for the new features, too.
- Keyframe editor and keyframe animation. Keyframe sequences can be defined and animation steps between keyframes are automatically interpolated. This makes the old movie interface (rotation around axes) obsolete.
- Coordinate systems: numbers at tickmarks and grid (can be switched on/off).
- User can set an arbitrary center of rotation for the 3D scene. GUI: New buttons for “Show”, “Set” and “Reset” the center of rotation. Center is drawn like a star in yellow (center inside volume) or orange (center outside volume). Center is moved along current viewing vector by turning the mouse wheel with ALT or SHIFT+ALT pressed down (coarse/fine movement).
- Vis file version 3, which stores also all the new features of entries and sessions. Versions 1 and 2 are retained as strict import filters.

## 2.2 Improved features

- All image-editing features: “Apply” now works incrementally (like previously only in the math. morphology dialogs). Thus, multiple operations can be performed in direct sequence, and “OK” confirms them all at the end. “Reset” brings back the original unmodified stack. “OK” is disabled until the stack has been modified at least once, and becomes disabled again when “Reset” is clicked. This seems far more logical than the behaviour of those dialogs in older DAIME versions.
- Interactive opacity editing (3D volume rendering) works now with RGBA-based textures, too (thus: with RGB stacks and with segmented stacks); GUI (dlgcoloralpha) adapted accordingly

- Shadows are now drawn by texture-based 3D renderers for RGBA textures, too (DAIME version 1.2: only for lumA textures)
- Depth cueing in interactive 3D rendering mode adapts to current viewer position
- When a Visualizer session is loaded it checks whether a stack with the same name AND path has already been loaded previously (also separately from the session). If yes, this stack is not loaded again into memory.
- User panning (in Visualizer, 3D rendering modes) takes user rotation into account. Thus, the panning behaviour always is correct regardless of the viewer's position and rotation state.
- Whole-scene rotation (in Visualizer, 3D rendering modes) takes user rotation into account. Thus, the rotation behaviour always is correct regardless of the viewer's position and rotation state. This also applies if the center of rotation was re-defined by the user (see above, new features).
- LZW compression is used in all exported TIFF files, including Visualizer snapshots and keyframe animations. This compression format is lossless, i.e. accuracy of exported image data is not compromised.
- The object editor (i.e. class `RendererDispWidget2D`) now draws an orange border around all selected objects and a purple border around all rejected objects. The border makes it easier to spot (small/dark) objects and to distinguish adjacent objects, and to spot objects at all in RGB color mode.
- Edit:Copy now checks stack types when building the source/target lists for the combo box dialog. This avoids copying e.g. RGB into grayscale stacks or vice versa.

## 2.3 Removed features

- High-quality volume rendering (raytracing); functionality is replaced by quick volume rendering with pixel shaders.
- Old vertex-based volume rendering engine (DAIME 1.1).
- Transfer function “shaded”: all other transfer functions except MIP can now be surface shaded anyway (with the GLSL-based rendering engine).
- Old movie interface (for rotation around main coordinate axes) in Visualizer. Replaced by the much more powerful keyframe editor and keyframe-based animation interface.

## 2.4 Optimization & other changes of existing code

- API of `Vis::Session` simplified after removal of HQ `Renderer`. 3D shadow flags unified for all 3D renderers.
- All calls to `rand()` and `srand()` replaced by calls to the thread-safe and platform-independent Qt functions `qrand()` and `qsrand()`.

## 2.5 Bug fixes

- Stack list widgets (e.g. in main window): center slice was not shown for newly added stacks, if these stacks were empty upon addition to the list and were filled later with data. Fixed: The widget now handles these cases appropriately and shows the center slice when the stacks are not empty anymore.
- Visualizer: Intensity cutoff was applied only with luminance-alpha textures. Fixed: now it is applied with RGBA textures, too.
- `Plot2DWidget/Graph2D`: Automatic axis scaling did not put the origin at (0,0). This caused graph elements to be drawn at wrong positions in some cases. Fixed: The origin is now at (0,0) for all axes when automatic scaling is used.
- Spatial arrangement analysis (2 populations): Warning about overlapping objects was not shown if overlaps were the only detected problem. Fixed.
- Temporary stack saving/loading did not work correctly in extended object editor. Fixed.

## 2.6 Polishing

- “Close” button removed from session entry editing dialog (volume rendering engines without shaders). Now all accessory dialogs of the Visualizer are closed only via their system menu or the close button of the window border.

## 3 Changes from version 1.1 to 1.2

### 3.1 New features

#### 3.1.1 Main Window

- Added a check box to every stack entry in the main window's stack list. The check boxes are used to "select" stacks for the "extended object editor".
- Added "Close all" option to the "File" menu. Closes all loaded images (series, z-stacks) at once.

#### 3.1.2 New editing features

- Histogram stretching (not morphological); 2D and 3D
- 2D edge detection: added gradient filter
- 2D and 3D morphology: added morphological gradient (structuring element can be chosen for this)
- 2D and 3D morphology: added skeleton of influence zone (structuring element can be chosen for this)
- 2D illumination (vignetting) correction: adapted from Leong et al., J. Clin. Pathol. 56:619-621 (2003)
- 2D blur and subtract filter (used for background reduction)
- 2D local average subtraction (used for background reduction)

#### 3.1.3 New segmentation features

- Local thresholding (in: automatic segmentation; conversion to object mask). Subdivides an image and determines intensity thresholds for individual regions of the image. Segments images with (very) bright and (very) dark objects.
- Size of "small objects" (which are to be ignored during segmentation) can now be changed in the automated segmentation dialog.
- Object editor: currently edited images can be saved to and loaded from a temporary file on disk. This is kind of a workaround for the still missing "undo" feature.
- Object editor: added new tools for unregistering dark or very small objects (probably noise).
- Extended object editor: can be opened from main window. Can handle as many image series/stacks as computer memory permits. Contains tools for artifact rejection and for selecting objects (such as cells) that were detected by multiple stains (e.g., FISH probes and also DAPI).

#### 3.1.4 New image analysis features

- New 2D object features can now be measured:
  - Min/Max/Mean/Median/std.dev. intensity without internal holes
  - Min/Max/Mean/Median/std.dev. intensity with (including) internal holes
  - Sizes in pixels of whole object (including holes) and of internal holes
  - Total area (including holes) and area of holes only
  - Outer perimeter and perimeter of internal holes
  - Ratio hole perimeter:outer perimeter
  - Ratio hole area:total area
  - Centroid (x,y) coordinates in pixel and  $\mu\text{m}$  space
  - Min. and max. Feret's diameter of object

- Ratio of Feret’s diameters of object
- New 3D object features can now be measured:
  - Min/Max/Mean/Median/std.dev. intensity without internal holes
  - Min/Max/Mean/Median/std.dev. intensity with (including) internal holes
  - Sizes in voxels of whole object (including holes) and of internal holes
  - Total volume (including holes) and volume of holes only
  - Outer surface and surface of internal holes (=of the walls that surround the holes)
  - Ratio hole surface:outer surface
  - Ratio hole volume:total volume
  - Centroid (x,y,z) coordinates in pixel and  $\mu\text{m}$  space
  - Min. and max. Feret’s diameter of object
  - Ratio of Feret’s diameters of object
- User can indicate the precision (number of decimal places) for the object measurements.
- New object classification interface with extended functionality (compared to DAIME 1.1).
- Object classification based on template objects, user-defined values, or on automatically determined object similarities.
- “Count objects”: determines and reports numbers of selected and rejected objects in 2D or 3D-segmented images. For 2D-segmented image series, the numbers in each image and the total numbers (whole series) are reported.

### 3.1.5 New visualization features

- New “Split view” mode of 2D image renderer (up to 16 session entries can be viewed as tiled images)
- Previous “High-quality volume rendering” mode is now called “Raytracing”. Image quality of previous “quick volume rendering” mode is greatly improved now.
- Added a button for global renderer settings (3D renderers), for example diffuse lighting, volume shading and antialiasing in Raytracing mode.
- 2D texture based volume renderer. Yields much higher image quality at higher frame rates than old (vertex-based) quick volume renderer.
- 3D texture based volume renderer. Yields much higher image quality at higher frame rates than old (vertex-based) quick volume renderer.
- Both 2D/3D texture renderers: image stacks which are too large to fit into texture memory are silently scaled down, so that they can be rendered (this affects the x and y resolution only; z is never scaled down).
- Added Visualizer preferences dialog: user can select the rendering engine.
- Added support for creating movies. This means that animations are exported as series of TIFF images, which can be used by third-party video authoring software to create movie files for presentations.
- Interactive color and opacity editing. Works with 2D/3D texture-based quick volume rendering.
- Interactive pseudo-shadows. Shadows can be cast onto the background only or onto the background and objects. User can choose the shadowing mode (no shadows, only on background, background and objects). Works with 2D/3D texture-based quick volume rendering.
- Interactive shadow intensity and x/y shadow offset editing.
- Interactive clipping with up to 6 clipping planes. Clipping can be switched on/off for each session entry individually. Clipping planes can be (de)activated and their orientation can be reversed.

- The Visualizer permanently stores the following settings on disk (in the Windows registry or in a hidden file on Linux systems):
  - The volume rendering engine to be used
  - Vertically flip snapshots (flag)
  - Take snapshots as screenshots (flag)
  - Enforce max. 3D texture size of 512x512xZ texels (flag). Some OpenGL drivers report wrong hardware capabilities; this results in rendering errors in 3D texture mode. This flag limits the max. 3D texture size independently from what the OpenGL driver claims.
- A new “Vis” file format. Backwards compatibility to DAIME 1.1 is maintained for loading old “Vis” files.
- Zooming and panning in the Visualizer are now possible by turning the mouse wheel (zooming) and moving the mouse with the middle button or wheel pressed down (panning).
- The background color can be changed in the quick volume rendering mode.
- All x/y/z rotation dials can be adjusted to 90 degree steps (90,180,270,360 degrees) by using the PageUp and PageDown keys when a dial has got the keyboard focus.
- A new Visualizer can be opened by double-clicking at images in the list of loaded images (main window).
- Added new feature ”2D Projection”: Easy way to obtain a static 2D projection of a 3D image stack without using the Visualizer.

## 3.2 Improved old features

### 3.2.1 Image editing

- 3D edge detection uses a different algorithm that yields much nicer edges than the old implementation.
- 2D/3D Morphology: The number of iterations can be indicated for operations where this feature makes sense.

### 3.2.2 Image segmentation

- Edge detection segmentation. Algorithm re-implemented. Obsolete user interface elements, which do not make sense with the new edge detection approach, have removed from the ”automatic segmentation” dialog.
- Object splitting (2D/3D) uses a new algorithm that yields better results in many situations.
- Automatic segmentation dialog suggests a size of “small” objects, which may be noise and should be ignored during segmentation, based on the image or z-stack size. This size threshold for “small” objects is editable.

### 3.2.3 Image analysis

- Object measurements:
  - User can now select the object features to be measured and to be shown in the results dialog.
  - 2D “shape” descriptor replaced by “circularity”, which is rotation-independent (except a small error due to the rectangular shape of pixels).
  - Results dialog shows box plots instead of histograms. Median, mean, and standard error of the mean are reported, too.
- Biovolume fraction quantification:

- Biovolume fraction is now calculated as the ratio of TOTAL pixel sums (probe-target population area/general probe pop. area) over ALL image pairs. This minimizes biases due to images that contain only little biomass vs. images that contain much biomass.
  - More results are reported: the total pixel sum ratio (see above) with congruency, and also the area ratios of individual image pairs with their median, mean, std.dev. and var.coefficient.
  - Now a cumulative fraction graph is shown that helps the user to assess whether enough image pairs have been analyzed. Alternatively, a box plot is shown that informs on the distribution of the individual image pair area fractions. This helps the user to assess how evenly the probe-target population is distributed in the sample.
- Spatial arrangement analysis (with dipole probes): User can now select between analysis mode “scan whole reference space” (=DAIME 1.1) or “Random dipoles” (=test a fixed number of randomly positioned & oriented dipoles for each distance). The number of tested dipoles for the latter mode can be adjusted by the user, too. This new mode is much faster but the precision and speed depend on the number of tested dipoles; this enables the user to do preliminary and fast tests with a relatively small number of dipoles and to perform the final analysis either with a very large number of dipoles or by scanning the whole reference space (which is always the most exact and slowest mode).
  - Spatial arrangement analysis (with dipole probes): User can now select a “reference space mask” (which is an object mask). This mask defines the reference space for the analysis (without any mask the whole image is the reference space). Images containing only small pieces of biomass (e.g. flocs or thin biofilm strips) were a problem for spatial arrangement analysis, because the physical concentration of biomass to some image parts could bias the results (“artificial co-aggregation” of organisms). This problem is solved by using a mask, which restricts the analysis to image regions that actually contain biomass and ensures that all other (“empty”) image regions are ignored.
  - Spatial arrangement analysis (with dipole probes): The algorithms now perform some consistency tests (e.g. they detect very dark or small objects in images) and warn the user if a possible source of bias has been identified.
  - “Convert to isotropic scale” now uses nearest neighbour interpolation. Therefore, object intensities measured in original and isotropic (=converted) images are almost the same now (this was not the case in DAIME 1.1).

### 3.2.4 Visualization

- All image displays use hardware-accelerated (2D texture mapping) rendering and offer a better zooming and panning functionality.
- Raytracing-based renderer (previously called “high-quality volume rendering”): Models for specular and diffuse lighting have been improved. Volumetric shading and antialiasing work correctly now. Image quality has been further improved. The highest quality uses a sampling rate that satisfies the Nyquist theorem (but means a long rendering time).
- The opacity (“global” opacity of a visualizer session entry) now affects all rendered voxels (independent of the transfer function).
- The checkbox “Use these settings” has been removed from the transfer function parameters dialog, because these parameters are always used now and are combined with the global opacity of the respective session entry to calculate the final opacity of a voxel.
- Visualizer snapshots: User can adjust some parameters that overcome typical hardware-related problems with off-screen rendering.

### 3.2.5 General usability

- Import/export functions now “remember” the current working directory on disk.
- Color channel splitting during TIFF color image import: new images are created only for those color channels that actually contain data.

- Image import: Single images, whose file names end in a number, can now be imported (program does not longer complain that the image does not belong to an image series).
- Image import: New filter for Leica file name style added (e.g., name\_z000\_ch00.tif).

### 3.3 Removed old features

- “Convert to object mask (high background)” is obsolete with the new 2D editing functions for background reduction and illumination correction.
- “Magnifying glass” is obsolete with new zooming/panning functionality of image displays.
- “Nearest neighbour” feature for spatial arrangement analysis. This is not included anymore, because the precision of the results depended too much on the range of analyzed distances. Accordingly, the color-coding feature has also been removed.
- “Aspect ratio” measurement for 2D and 3D objects (replaced by Feret’s diameters as less orientation-dependent descriptors).
- “Split objects” feature is obsolete in automatic segmentation dialog. Objects can still be split by using the respective tool of the object editor.
- The extra menu entry for “Extended focus” has been removed. This feature is now integrated in the new option “View->2D Projection”.
- “File->New” is obsolete and has been removed.

### 3.4 Modernization of code

- DAIME has been completely ported to Qt version 4. Qt version 3 is not supported anymore.

### 3.5 Bug fixes

- Recursive repaint events that could cause program crashes (due to stack overflow) are now prevented by recursion guards.
- 2D/3D Conditional dilation code has been rewritten and now works like a region filling algorithm. This change improves edge detection-based segmentation and 2D/3D hole filling.
- Measure objects: Rounding errors with floating-point numbers have been fixed.
- Spatial arrangement test: removed a bug, which caused fatal crash in debugging mode and with images of resolution 256x256.
- Morphology: Pixels/voxels outside image (stack) boundaries are now filled with the alues of the border pixels (voxels) and not anymore with zero. This eliminates a black border after erosion.
- Fixed problem with volume rendering mode “Surface”.
- 3D-Visualization did not work correctly on graphics cards with NVIDIA GeForce chips. This bug has been fixed.
- Maximum projection works now in quick volume rendering mode on hardware that supports the GL\_ARB\_imaging or GL\_EXT\_blend\_minmax extensions of OpenGL. This became possible by including the GLEW library into the main source tree of DAIME.
- Monochrome TIFF images that contained the pixel data in another but channel #1 were not imported correctly. This bug has been fixed.
- Images with file(path) names in non-Western scripts can now be imported by DAIME for Windows (under Linux this depends on the version of LIBTIFF, which must support Unicode strings for this feature to work).
- Spatial arrangement parameters dialog: Weird behaviour of ”Every nth” spin box has been fixed.
- Rejected objects in segmented z-stacks were rendered as black blocks in the quick volume rendering mode. This bug has been fixed.

### 3.6 Polishing/streamlining of the user interface

- Icons now have transparency masks (white background removed, looks better).
- All old icons have been replaced by newly designed icons, which are free of third-party copyrights.
- Red background of toggle buttons in “on” state has been removed in the Linux version of DAIME (this background never appeared in the Windows version).
- More key accelerators in the main menu.
- Toolbar and status bar in the main window cannot be switched off anymore (options removed).
- The appearance of tick numbers and the automated definition of tick intervals in 2D graphs have been improved.
- The Visualizer does not open a “status box” anymore. Instead, a progress bar and a stop button have been integrated into the “3D” tab.