BioImageXD – Getting started

Updated 2012-07-01 by Pasi Kankaanpää

Introduction

Welcome to **BioImageXD**, free open source software for biomedical image post-processing, visualization and analysis. This guide will help you get started, providing an overview of the most important features and functions. Please send us your feedback and questions to info@bioimagexd.net.

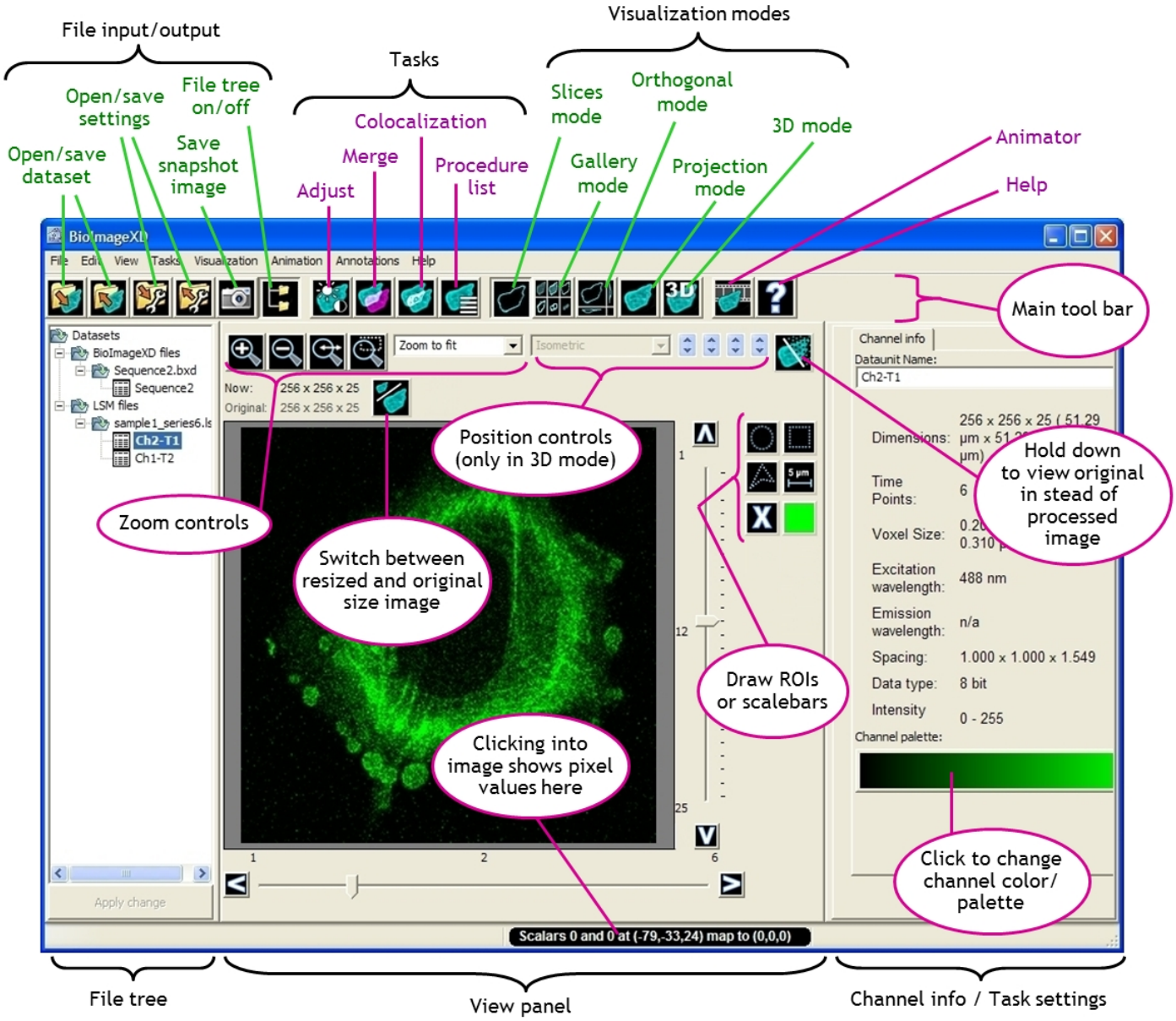
Remarks

- It is recommended to give BioImageXD time to finish things "in peace" before "clicking further", as some tasks are demanding for regular computers.
- With some older 32-bit operating systems it may be advisable to restart the program between demanding processing steps, as these operating systems have somewhat problematic memory handling.
- In problem situations first restart the software and try again; if the problem persists, tell us about it (send email or click **Report bug** in the **Help** menu)
- Important terminology:
  - **Dataset** = multidimensional image file that can consist of multiple *slices* (such as the optical sections from a confocal microscope that make up a 3D volume), multiple *timepoints* and multiple *channels*
  - **Channel** = subset in a dataset; all channels show the same image data but somehow differently, for instance different fluorescent markers can constitute different channels.

User interface

In the main window there is a *file tree* of loaded files on the left, currently active visualization in the middle (called the **View panel**) and settings for currently active *task* on the right (displaying *channel information* if no task is selected). At the top is the *main toolbar* with buttons in four groups (from left): file input/output functions, tasks, visualization modes, special features. Some tools and settings have a reddish color – these are for more advanced use and are mostly not needed in basic use.

Selecting data. From the file tree data is selected both for viewing and for processing it with tasks. More than one channel ("file") can be selected by CTRL/SHIFT-clicking. Different visualization modes and different tasks accept/require different numbers of channels to be chosen. All visualization modes except 3D mode display only one channel, even if several are chosen (except if some task is active, which allows displaying more than one channel). Different tasks accept channels as follows: Adjust – only one channel allowed; Merge – two or more channels required; Colocalization – two channels required; Procedure list – typically one channel, but does accept more; Batch processor – unlimited, accepts also several datasets at the same time. See sections below for more details on different visualization modes and tasks.



Opening files

Click **Open dataset** to open volume datasets in any of the supported file formats, meaning especially microscopy file formats like LSM (Zeiss), OIF (Olympus) or LIF (Leica), and BioImageXD's own file format BXD.

For opening "regular" image files like JPEG and TIFF, use **Import images** (also handles volume stacks and timepoints):

1) Go to the folder containing the images from the **Browse** button, and choose one image (doesn't matter which one) from among the ones you wish to import. *Tip: All images you want to import as one dataset must be in the same directory and have the same dimensions, and they should all have the same file name prefix, with a running number starting from 1 (or another small number) at the end of the file name.*
2) Choose whether to import "All files in same directory" or "Files following pattern" (in the latter case alter the suggested file name pattern if necessary) and click **Update**. *Tip: it might be easiest to put the images for each dataset into their own folders (with no other content in the folders) and use the "All files in same directory" option.*
3) "List of Input Data" lists the images about to be imported. If you do not wish to import all of them, choose the one(s) you want by clicking them (choose several by SHIFT/CTRL-clicking).
4) Specify "Volume information". This information is required for correct image visualization and processing, and must be entered by the user when opening regular image files, from which it cannot be read automatically.
5) Click OK, and a BioImageXD dataset (BXD) is created from the chosen images, and opened into BioImageXD. If importing RGB color images, the dataset will contain 4 channels (1 color channel + 3 greyscale channels for the components R, G and B). Note that many features of BioImageXD require a greyscale channel, rather than the color channel, to be used as input.

Opened datasets appear into the internal file tree, with each channel as a separate "file". Datasets in the tree are not read into memory upon loading, but only as needed while working with them. It is therefore possible to instantly open numerous files into the tree. The tree can be toggled visible/invisible from its tool bar button. Datasets can be removed from the tree by right-clicking them. When exiting the program, you are asked whether you want to save the file tree for the next time the program starts (note: this does not save any of the datasets themselves, only information of which datasets are listed in the file tree).

Viewing files

Choose any one channel from the file tree, and it is visualized in the view panel. Choose between any of the five visualization modes: **Slices** (default), **Gallery**, **Orthogonal, Projection** or full **3D rendering**. One (and only one) of these is always selected. Sliders on the sides of the image are used to switch between timepoints and z-slices when applicable, and controls for zooming are at the top. For some visualization modes additional settings appear to the left side of the view panel.

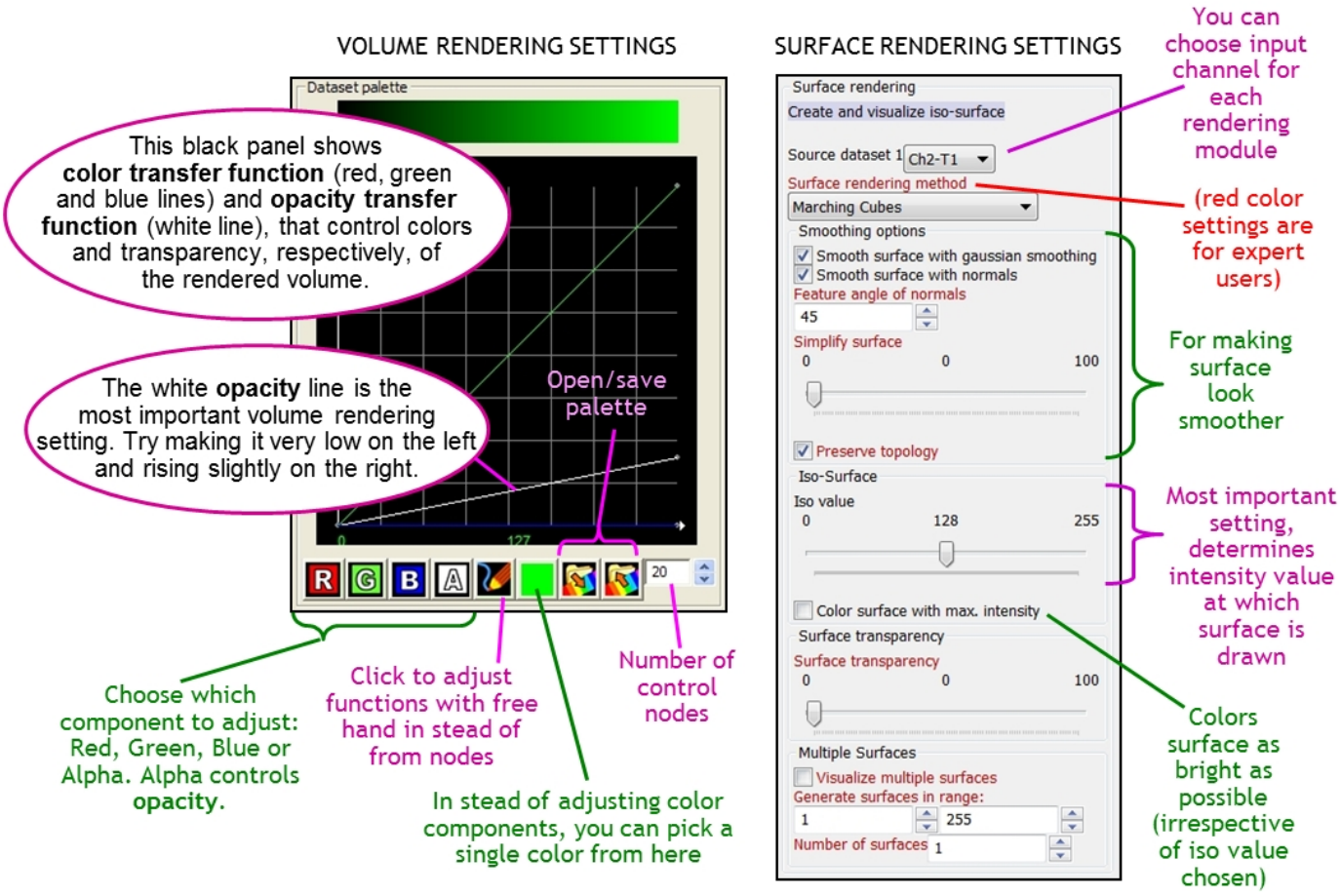
Creating 3D rendering

The 3D mode is based on rendering modules, listed at the top of the settings panel appearing to the left of the view panel. Choose a module from the drop-down menu and click **Load** to add it to the list. Several modules can be active at the same time. Click on a loaded module and its settings appear. Click **Apply** at the bottom to see the effect of any changes to the settings, if the screen is not automatically updated. The most typical modules are **Volume rendering** and **Surface rendering**, and their most important settings are shown below.

To visualize multiple channels at the same time: Choose the channels from the file tree, then go to 3D mode, where you can specify which channel is used for input for each rendering module. You can for instance visualize one fluorescence channel as volume rendering and another as surface rendering.

Tasks

Tasks are used to do something to the selected images, not just to visualize them. The four main tasks are **Adjust, Merge**, **Colocalization**, and **Procedure list**. Pressing its button activates the task, pressing it again deactivates it. Only one task can be active at a time, but no task has to be selected. The settings of the active task appear to the right. Click **View result** at the bottom to see the effect of any changes to the settings, if the screen is not automatically updated. To keep any changes made, save them as a new dataset from **Save dataset** (if you don't do this, the changes are not kept once you exit the task). This creates a new BXD file that automatically appears into the file tree. **Slices** and **Projection** visualization modes are recommended starting points when working with tasks. When a task is active you can change the dataset(s) it processes by choosing them from the file tree and clicking **Apply change**.



Adjust task. Used to change the *intensity transfer function*, which adjust parameters like brightness and contrast, for a single channel. The transfer function is shown graphically and the most important controls are the sliders around it for **Contrast**, **Brightness** and **Gamma**.

Merge task. Used to combine several channels, mainly for two purposes: 1) to view several channels simultaneously (by activating the task) and 2) to create 32-bit RGBA color datasets (by **Save dataset** when the task is active). The settings have a *graphical intensity transfer function* very similar to the **Adjust** task, for adjusting how bright etc. each channel will appear in the merged image. Channel selection buttons at the top determine the channel whose transfer function is being adjusted, whereas similar channel selection buttons on the right side of the image toggle the display of the channels on and off. *Tip: an RGBA color dataset can be volume rendered in 3D mode, but not surface rendered.*

Colocalization task. Used to analyze colocalization between *two* channels. First adjust thresholds manually at the top, or click **Calculate thresholds** to adjust them automatically. Then click **Calculate statistics** to see the quantitative result, which can be saved as a CSV file from the

Export button. If you want to calculate also P-value, choose the appropriate method before clicking Calculate statistics. The **2D histogram** can be saved by right-clicking it. The three channel selection buttons on the right side of the image toggle the display of the original channels and the colocalization map on and off.

Procedure list task. Does "everything else", from noise filtering to segmentation to tracking. Can take as input one or several channels. Create a list of procedures by selecting them from the categorized menu buttons below the list. Clicking on a procedure displays its settings, and a brief description of the procedure, including acceptable inputs and outputs. Click **View result** to test the execution of the whole list, and **Save dataset** to execute the list into a BXD file. The inputs and outputs vary between procedures, and both can consist of image data and/or quantitative results in the form of CSV files. Procedure lists can be saved and loaded by clicking **Presets**. The channel selection buttons on the right side of the image toggle the display of the original channels and the outcome of the procedure list on and off. *Tip: If you want the resulting dataset to contain more than one channel (for instance if you want to crop all channels of a dataset to a defined region of interest), use the Batch processor, because the Procedure list task can only output datasets with one channel.*

Segmentation

Typically segmentation consists of several steps in the procedure list, for instance as follows:

1. **Hybrid median 2D** (or some other noise filtering).
2. **Threshold** (or some other segmentation method that separates image material of interest from the background; the settings for this are critical for successful segmentation).
3. **Connected component labeling** or **Object separation** (these separate the segmented material into objects that are given identifying colors and that can be quantified; Object separation is more complex of the two, capable of separating objects that touch each other).
4. **Analyze segmented objects** (quantitatively analyzes the segmented objects for number, volume, area, intensity etc.).

Saving files

- To save a multidimensional volume dataset (can only be done in the BXD file format), click **Save dataset**. This needs to be done to save the results of a task before closing/changing it. A dataset may also be saved without any task active, for instance to just save it with a different color palette (see *Color palette* below)

- To save the image visible in the View panel at any time, click **Save snapshot image** (the camera button). This saves a 2D color image of the whole visualization with the zoom level specified (also image areas that do not fit into view are saved)
- To save a dataset as a series of 2D images (PNG, JPEG, TIFF or OME-TIFF) use **Export images** in the **File** menu
- To save a movie file of a 3D rendering, use the Animator (see *Creating an animation* below)

Tracking

Motion tracking is an example of a more complicated procedure that requires several different procedure lists. It is carried out as follows (each step is its own procedure list):

1. Segment and analyze (see box above) the objects whose motion over time you wish to track.
2. Perform tracking with **Create motion tracks**. In its settings first choose the results file from the previous step into **Objects file**, then click **Read objects** and finally click **Calculate tracks and export result**. The settings for tracking are critical to its success, and are mostly determined by trial and error.
3. Analyze the tracks for quantitative results such as speed of movement with **Analyze motion tracks**. In its settings, choose the results file from the previous step into **Tracks file**. Perform calculation and save results as csv file by clicking **Analyze tracks and export statistics.**
4. If you want to visualize the tracks in a 2D view mode, choose **Visualize motion tracks**. In its settings, choose the results file from step 2 into **Tracks file**, and then click **Read tracks**. Choose the tracks to visualize from the list.
5. If you want to visualize the tracks in 3D, **Procedure list** task is not used. Simply load the module **Visualize motion tracks** in the 3D mode. Its settings are similar to those of 2D **Visualize motion tracks** above.

Batch processor

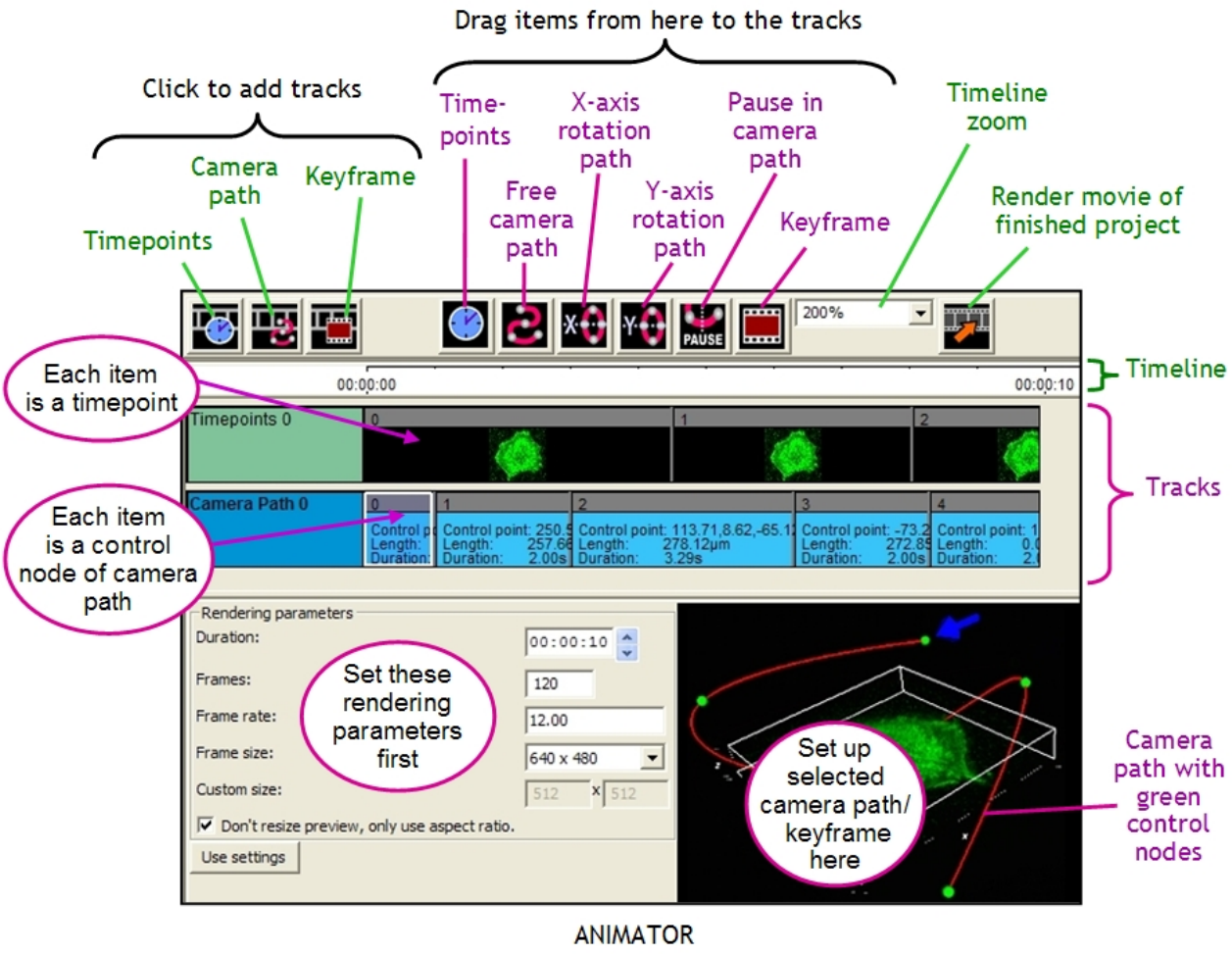
Used for processing more than one dataset in one go (can also be used to process several channels of a single dataset). Choose the datasets you want to process from the file tree, then activate Batch processor from the **Tasks** menu or by clicking CTRL-B. The batch processor is very similar to the **Procedure list** task, except that you can specify several different procedure lists, for instance to process different channels differently.

How to use Batch processor

- Choose **Each channel separately** for all channels to be processed separately through every Procedure list, or **All channels as input** for all channels to be given as input to every Procedure list. Channels can be grouped into datasets by ticking the appropriate check-box.
- Click **Add** to add the required number of Procedure lists. Click on the list to give it a name and to specify its procedures as you would in Procedure list task. Check input for every Procedure list.
- If the desired output for a Procedure list is a quantitative result, click on the **(Click to define)** to see a list of possible variables. Choose the ones you want in your aggregated results file.
- Start batch processing by clicking **Run!** (The program asks for a name for the file into which possible quantitative results are aggregated. If there are none, ignore this.) The processing may take a long time and the program may seem unresponsive during it.
- You can save and open Batch analyses from the **File** menu in the Batch processor.

Creating animation

Make sure no task is selected, then go to 3D mode and set up the visualization you want to animate. Then activate the animator. First set **Rendering parameters** like duration and frame rate, then click **Use settings**. The animator is based on three kinds of *tracks*: **Timepoints**, **Camera Path** and **Keyframe**, the latter two being two different animation techniques. Click on the buttons to add tracks as required. Then drag and drop items to the desired positions in these tracks from the corresponding buttons. Items in the tracks can be moved and resized, and additional controls are available in the **Animation** menu. When the animation is set up, click **Create animation** to create the video. The PAL-DVD and NTSC-DVD presets are the easiest options here. Click **Ok** to render the video, and wait for the computer to finish the process. Animation projects can be saved and opened from the **Animation** menu.



Two animation techniques

- In **Camera Path** animation a red curve illustrates "flight path" of the camera, which can be adjusted from the green control nodes that correspond to items in the Camera Path track. The whole path can be moved and zoomed by grabbing it elsewhere than at the nodes. Easy method for creating impressive animations quickly.
- In the more traditional **Keyframe** animation one moves the image manually to a position, then drags an item for this position to the Keyframe track, then moves the image to another position, drags an item for that and so on. The computer then calculates frames between these *keyframes* the user has specified.

Additional features

Resize. To change the amount of pixels/voxels in a dataset, choose **Resize dataset** from the **Tasks** menu. You can make for instance a large dataset smaller to make its processing faster. A resized dataset appears red and marked with * in the file tree. You can toggle between using the original or the re-sized dataset from the button at the top of the view panel. This way you can for instance use a smaller version of a dataset while finding out settings for its processing, but then switch to the original size for the actual processing. If you want to save the dataset with the new size, click **Save dataset**. (If you want to change the bit depth of a dataset, for instance from 16-bit to the recommended 8-bit, choose **Change bit depth** from the **Tasks** menu.) From **Preferences** in the **Edit** menu you can set up BioImageXD to automatically resize large datasets upon loading.

Region Of Interest (ROI). Using the circular, rectangular and polygonal drawing tools on the right side of the View panel you can draw ROIs onto an image (recommended visualization modes: Slices or Maximum Intensity Projection). You can then use the **Procedure list** task to for instance create a smaller dataset consisting of the ROI only (**Extract a subset**) or to analyze the pixels/voxels within the ROI (**Analyze ROI** or **FRAP analysis**). *Tip: If you want the ROI processing to cover all the channels in a dataset, you have to use the **Batch processor** in stead of the **Procedure list** task.*

Palette. Typically BioImageXD operates on greyscale images that are pseudo-colored using palettes (*look up tables*). You can change the palette by clicking on the **Channel palette** color strip in **Channel info** (visible on the right when no task is active). This opens a *color transfer function* editor, which is basically the same as the corresponding one used for volume rendering (see above), except that there is no alpha channel. The new palette is activated when you close the editor. If you want a channel to have the new palette by default the next time it is opened, click **Save dataset**, which creates a new dataset that includes palette information.

Immediate updating. BioImageXD often updates the screen automatically when you change some settings. This can be switched off by un-ticking **Immediate view panel updating** from the **Edit** menu. This is advisable for large datasets or complex procedures especially with slower computers. When switched off, the screen is only updated when an **Apply** or **View result** button is pressed.

Saving and opening settings

- When in any task, click **Save settings**, and current settings are saved. They can be opened from **Open settings** while in the same task again.
- Several features of BioImageXD have their own additional possibilities for saving and opening settings:
    - **Color transfer function editor** in volume rendering or when adjusting channel palette: Save/open palette (color look-up table)
    - **Procedure list** task: Save/open procedure lists as **Presets**
    - **Batch processor**: Save/open batch analysis from **File** menu
    - **Animator**: Save/open animation project from **Animation** menu.

Reference and further information

BioImageXD was published in the July 2012 issue of *Nature Methods*. Please use this as a reference to BioImageXD in publications and other work where BioImageXD has been used:

Kankaanpää P, Paavolainen L, Tiitta S, Karjalainen M, Päivärinne J, Nieminen J, Marjomäki V, Heino J, White DJ. 2012. BioImageXD: an open, general-purpose and high-throughput image-processing platform. Nat Methods. 9(7): 683-689. doi: 10.1038/nmeth.2047.

The paper and its supplementary material offer a lot of further information on BioImageXD, such as sample data, detailed procedure lists and instructions for advanced segmentation and motion tracking, and screen capture videos illustrating how BioImageXD is used.

Additional further information is available on the BioImageXD web site and wiki: www.bioimagexd.net.