

## BioImageXD – New Open Source Free Software for the Processing, Analysis and Visualization of Multidimensional Microscopic Images

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### Solving modern problems in advanced microscopy with BioImageXD

With the recent success of the human genome project, researchers must now discover biological functions of the tens of thousands of proteins encoded in the genome. Molecular cell biology and structural biology methods are required for this work.

Modern *multidimensional bioimaging methods*, such as laser scanning confocal microscopy and other light microscopic techniques, have emerged as a major research methodology in this new era of informational biology. These methods all produce vast amounts of digital information and require specialized *computer software* to process and analyze it. Several programs intended for this purpose have become available in recent years, but microscopists around the world frequently discover them unsuitable for many of their required tasks. The reasons for this are varied, and include: extensive bugs that take too long to fix, prohibitively high prices (also for updates and bug fixes), algorithms that are undisclosed and as such unacceptable for accurate scientific work, limited sets of features dispersed into separate “modules”, complex and difficult user interfaces, and lack of flexibility in adapting to specific research situations. Indeed, it can be argued that the lack of good post-processing software is a major obstacle for conducting advanced microscopy today. Our multidisciplinary team from the universities of Turku and Jyväskylä in Finland has implemented the new open source software philosophy, resulting in *BioImageXD* (Kankaanpää et al., under preparation), to solve this problem.

### What is BioImageXD and how does it work?

The starting point for the development of BioImageXD was *four dimensional laser scanning confocal microscopy*, a relatively new technique that our team was among the first in Europe to apply. In this technique, living biological samples, such as cells, are imaged in all three spatial dimensions by acquiring optical sections through them with a scanning laser beam. Such three dimensional image stacks can be repeatedly obtained from the same sample over time, resulting in what are called *four dimensional images* (*time* is the fourth dimension). **BioImageXD is a new computer program for the processing, analysis and visualization of this type of multidimensional image data.** “XD” in the name refers to “X dimensions”, meaning that the software is applicable for two, three and four dimensional data. Any such image data, whether from a confocal microscope or some other device, is supported. Despite the name, BioImageXD can therefore be used also in other fields besides biology, such as biomedicine, structural biology and materials science.

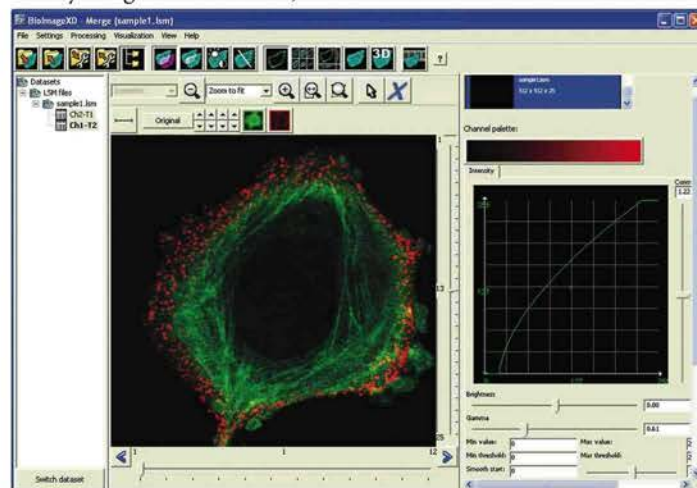
BioImageXD is *free* and has *open source code*. This ensures low development costs and fast, flexible responses to changing research situations and requirements. It also guarantees anyone free access to the algorithms and functions for checking or altering them. During

the development of the program, specific attention has been paid to the fact that the user always has full control of all relevant parameters and features, and graphical representations of some of the image processing functions have been added for extra clarity. The key idea with BioImageXD is that the user always knows exactly what is done to the data, and can freely control and change that. We believe this to be a requirement for accurate scientific work - a requirement that is too seldom fulfilled with commercial software.

BioImageXD is written in Python (<http://www.python.org>), with C++ being used for all heavier calculations and wxPython (<http://www.wxpython.org>) for the graphical user interface. The software also utilizes the Visualization Toolkit (<http://www.vtk.org>) for many image processing and rendering tasks, and includes many algorithms previously published in scientific journals, for instance for statistical calculations. All these tools are free and open source.

BioImageXD is intended to contain everything necessary for the post-processing of modern, multidimensional microscopic images in a single, easy to use package. Today, numerical and statistical analyses are growing in importance, but it has been pointed out that the human brain is still the best computer to analyze microscopic images. BioImageXD attempts to deliver the best of both worlds, providing tools for computer-based analyses as well as for creating high quality visualizations of multidimensional image data. The user interface of the software is based on a single, large window (Figure 1). It is divided into several parts, such as the visualization panel in the middle, adjustments for the currently chosen task on the right, a file tree on the left and toolbars and menus at the top. All relevant features, functions and adjustments can be accessed directly from the main window, in particular with the grouped buttons of the main toolbar.

The basic work flow with BioImageXD is as follows: multidimensional image data is first loaded into the *internal file tree*. Numerous files of different types can be loaded at the same time, and simply clicking a file name in the tree chooses it for operation. (Currently common confocal microscopy file formats from Zeiss, Olympus and Leica are supported, as well as the import of numbered stacks of ordinary images such as Tiffs.) When the desired file has been selected,



**Figure 1.** The BioImageXD graphical user interface is based on a single, large window. All relevant features and adjustments are directly accessible, and the program has been designed to be intuitive and easy to use. The view panel in the middle shows the desired visualization for a dataset chosen from the internal file tree on the left. Here, a two-channel three dimensional confocal microscopy dataset of a living bone cell is shown (red = integrins, adhesion and signaling molecules on the cell surface, green = actin filaments, intracellular support and transport structures). On the right are settings for the chosen task mode; shown here is the advanced graphical intensity transfer function for adjusting image features such as brightness, contrast and gamma.

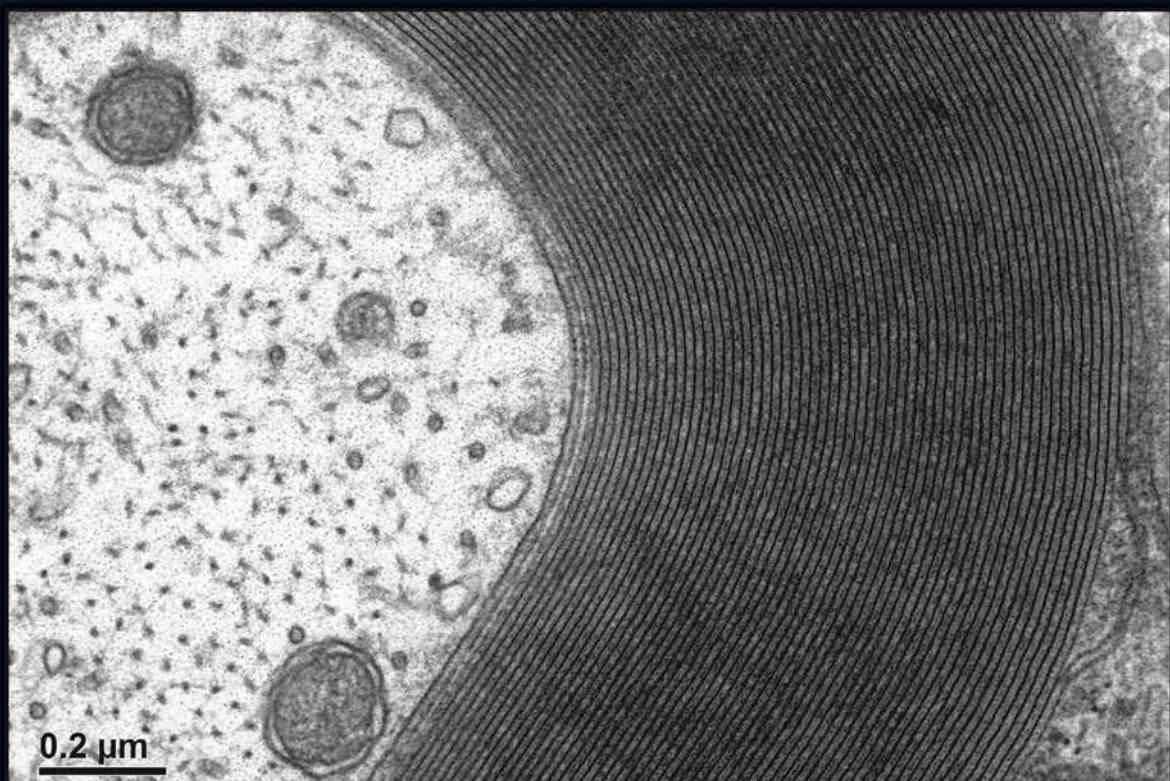
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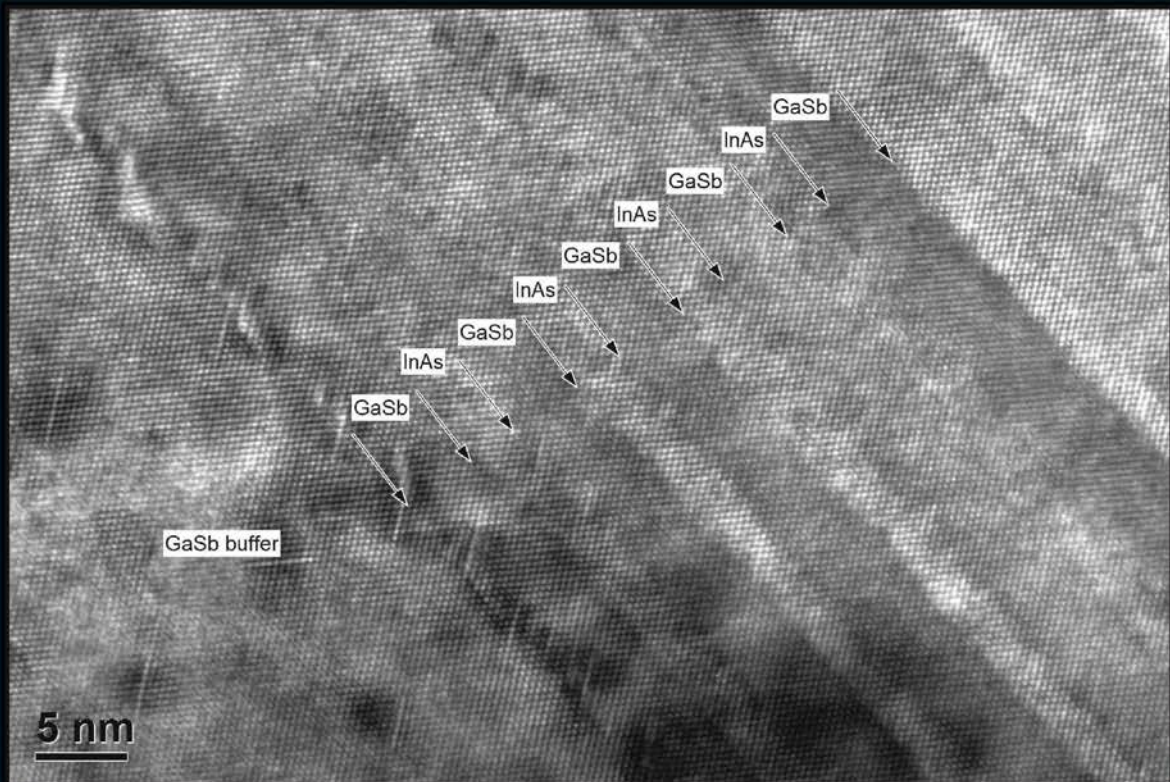
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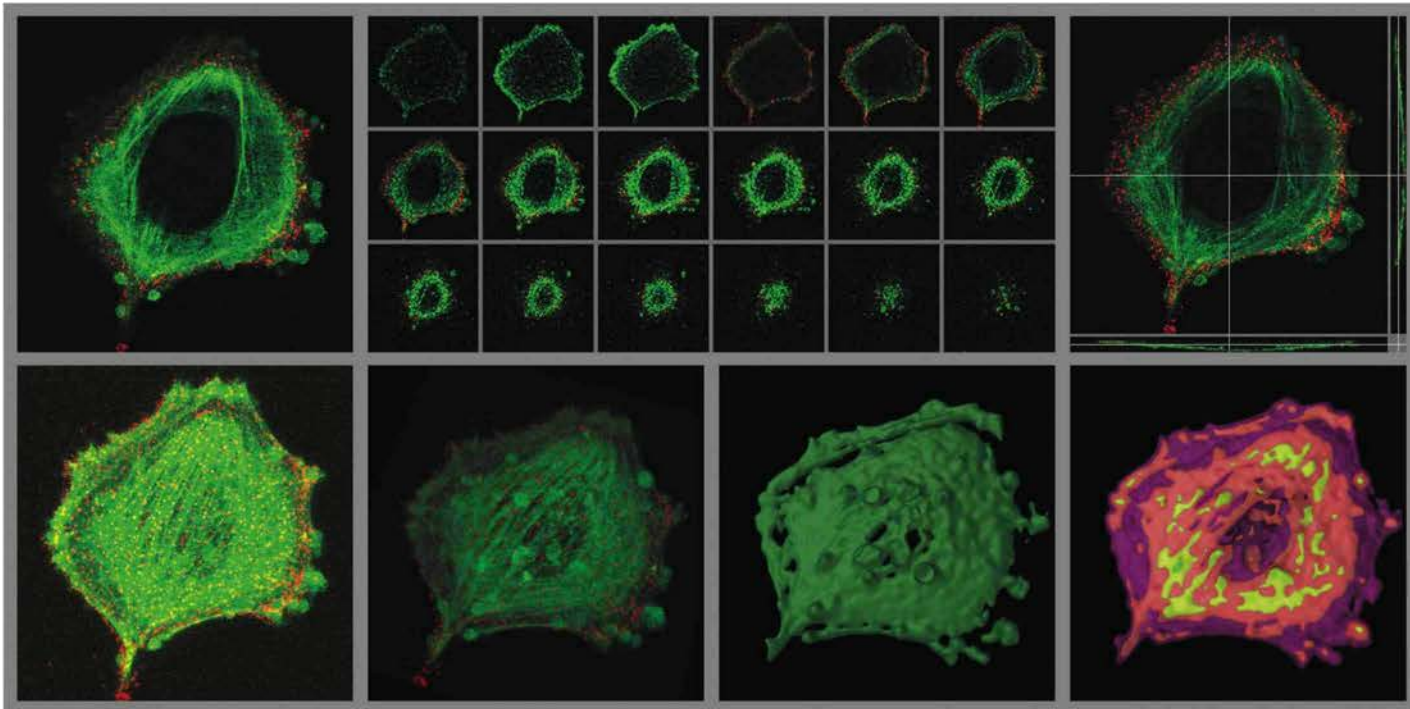


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 (Top) Image of myelinated nerve recorded with ORIOUS™ SC1000W (35mm mount) CCD camera. TEM magnification = 85kx at 80kV. Image courtesy of Kenneth L. Tiekotter, University of Portland. (Bottom) HREM image of GaSb/InAs superlattice on GaSb substrate. Image was recorded with ORIOUS™ SC1000B (bottom mount) CCD Camera on a LaB6 TEM at 200kV. Image courtesy of C.H. Lei and J.G. Wen at the Center for Microanalysis of Materials (CMM) at the University of Illinois Urbana-Champaign, USA.



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**Figure 2.** BioImageXD offers multiple visualization modes and methods, illustrated here with the same dataset as in Figure 1. Top row: optical slices viewing mode, gallery mode for simultaneous observation of all optical slices, orthographical mode for visualizing cut-through sections. Bottom row: maximum intensity projection view for fast and easy viewing of a multidimensional dataset, interactive three dimensional volume rendering (here ray casting), interactive three dimensional surface rendering, interactive three dimensional multiple surfaces rendering.

a task mode is chosen. These currently include channel merging, colocalization analysis, image adjustment and image processing. Any task mode can be combined with any visualization mode, which determines how the image data is being visualized. Currently supported modes (Figure 2) are optical slices viewing mode, gallery mode for simultaneous observation of all optical slices, orthographical mode for visualizing cut-through sections, maximum intensity projection view for fast and easy viewing of a multidimensional dataset and an interactive three dimensional rendering mode. The latter includes several different rendering methods, such as ray casting and texture mapping volume rendering and surface rendering. At any point, settings for all tasks and visualization modes can be saved (or recalled), or the currently displayed visualization saved as a regular image file. Also, the full dataset or part of it can be saved, for instance to create an RGBA multicolor dataset from several grayscale datasets. When the image has been analyzed and numerical/statistical results saved, desired parameters adjusted and new datasets created, final visualizations and movies can be created with the built in animation generation tool.

#### A closer look at some of the features

*Graphical intensity transfer function for advanced image parameter adjustment and channel merging.* For adjusting image parameters BioImageXD offers one of the most advanced intensity transfer functions available (Figure 1). The intensity transfer function, ITF, describes how all the possible intensity values an image pixel (or voxel) can have are altered when the data is processed by any method that is based on changing these intensities (examples of such methods include “linear” adjustments like brightness and contrast and “non-linear” adjustments like gamma). The BioImageXD ITF is always graphically represented, allowing the user to, for instance, get a clear perception of what part of the data is lost when adjustments are made. It also allows both conventional “sharp” as well as “smooth” thresholds to be specified, for instance, for cutting off low intensity noise, and it can be interpolated between time points to compensate fluorescence

bleaching over time. It can also be used when combining several channels (such as images of different fluorescent markers obtained from the same sample) into a single 24-bit color image for fast multicolor three dimensional rendering.

*Colocalization tool for visualizing and analyzing structures occupying the same spatio-temporal location.* Colocalization studies are very common in confocal microscopy, and aim at determining whether two proteins, for example, share the same spatial and temporal location in a cell, indicating possible interaction. Traditionally colocalization studies have been carried out simply by superimposing images of the two proteins, typically one in red and the other one in green, and observing the amount of yellow color in the resulting image. This approach, of course, is rather imprecise and subjective. Many programs now include tools for the numerical and statistical analysis of colocalization, and BioImageXD offers one of the most versatile such tools available. With the BioImageXD colocalization task (Figure 3), colocalizing voxels can be visualized separately just as any other image data, histograms displayed, and a variety of numerical parameters, such as various coefficients, obtained. Also included is the recently published algorithm for automatic threshold determination (Costes *et al.*, 2004, *Biophys J.* 86:3993), enabling objective colocalization analysis, as well as several algorithms for calculating the statistical significance of the observed colocalization (Costes – Fay – van Steensel).

*The most versatile animation generation tool available.* Creating clear and illustrative animations and movies of three and four dimensional datasets is imperative in successfully representing such datasets in two dimensional media, such as on a computer screen. To our knowledge, BioImageXD offers the most versatile animation generation tool available in the microscopy field (Figure 4). Animations can be created with two alternative methods, key frame animation and camera flight path control, or a combination of both. In key frame animation, a rendering of the sample is manually rotated and zoomed to the desired position and a key frame saved. The same is

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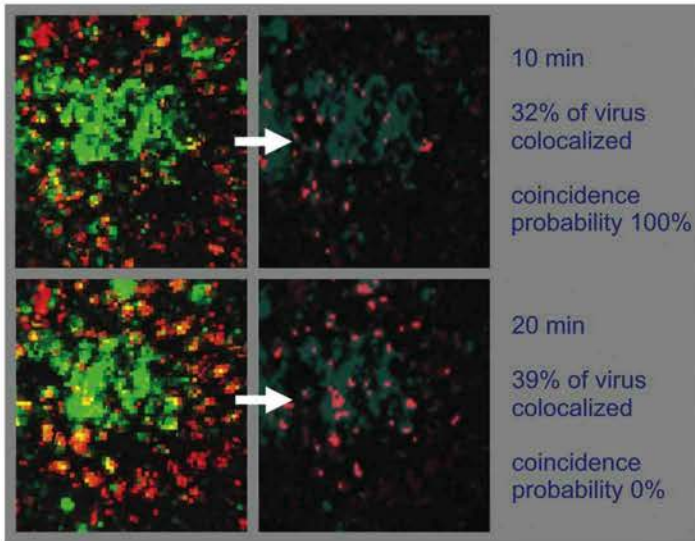
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**Figure 3. BioImageXD enables advanced colocalization analysis.**

In this simplified example the colocalization of two fluorescent dyes, one showing a human virus (red) and the other showing a potential intracellular target for it (green), is examined. Volume rendering close-ups of part of the cytoplasm of a living cell are shown, 10 and 20 minutes after viral infection. Traditionally, colocalization has been analyzed by visually inspecting the amount of yellow color in superimposed red and green images, such as the ones shown here on the left. BioImageXD offers more advanced visualization possibilities, such as the images on the right, showing colocalizing voxels in bright red, making them more clearly discernible. Built-in numerical and statistical analyses provide valuable additional information, such as the percentage of colocalization and its statistical significance. Here the amount of colocalization is notable already at the 10 min time point, but it is not statistically significant. 10 minutes later the colocalization amount has increased only moderately, but it has become statistically significant. Thus it could be argued, that the virus in question is targeted to the intracellular compartments studied within 20 minutes. (It would be impossible to draw this conclusion reliably just by looking at the images on the left.)

repeated a few times, and the computer will then calculate frames between the specified key frames. In camera flight path control a line representing the flight path of the camera (Figure 4) is rendered into the preview scene together with the sample. The flight path has control points, which can be moved around in three dimensional space, thus specifying a flight path for the camera. The final animation is put together with a video editor type interface that includes several tracks for time point changes, animation key frames and camera flight path control points. Common movie formats, such as MPEG, are supported.

### Who can use BioImageXD and how?

BioImageXD is licensed under the GNU GPL (<http://www.gnu.org/copyleft/gpl.html>) license. This means that *everyone* is free to use, modify, distribute and develop it. However, the license requires that the source code remain open and that any modifications and improvements be shared with all other users. It also states that our original development team will remain as the copyright holder.

The BioImageXD software itself, its source code and a sample file package can all be downloaded from our website, <http://www.bioimagexd.org>. The installation is light, the program easy and intuitive to use, and a comprehensive user manual will become available shortly. All this should enable any interested

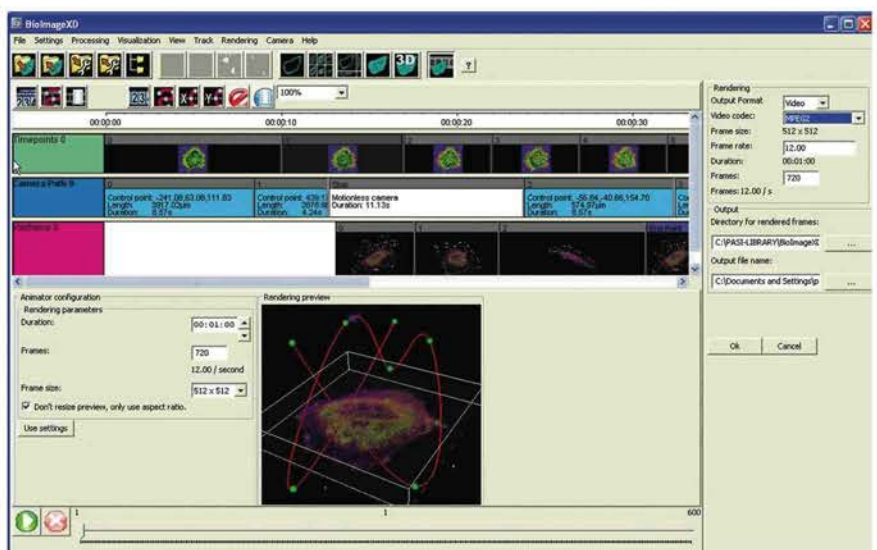
microscopist to begin using BioImageXD straight away. In addition, our development team can be reached by email at [info@bioimagexd.org](mailto:info@bioimagexd.org), and we will be glad to help with any problems or answer any questions regarding the software. Anyone interested in helping to develop BioImageXD is also encouraged to contact us to join the collaborative development team.

We would like to point out that at this stage only the first *beta* version of BioImageXD has been released, and it is by no means complete or bug free. Currently only a Windows compatible version is available, but Mac OS X and Linux versions are soon to follow. Since the release of the first beta in February 2006, there have been thousands of downloads of BioImageXD, and with the help of collaborators and beta testers worldwide we are working toward the first production quality release. We would like to invite everyone to try out BioImageXD, send us feedback and join our fast growing community!

### The future of BioImageXD

BioImageXD already contains a wealth of features, but many more are needed before it is as comprehensive as planned. The program is under active development, with a constantly expanding international team hard at work. Many commercial parties have also shown great interest in the project, and negotiations are underway (the software will, however, remain free and open source). Future development plans include a myriad of features; including the development of segmentation based analyses and support for more file types, ranging from atomic force microscopy and electron microscopy to positron emission tomography. Compatibility with the Open Microscopy Environment (<http://www.openmicroscopy.org>) is also being planned, as well as the implementation of a graphical pipeline user interface for creating customized work flows, and a simple plug-in architecture for users to write their own functional modules.

The great interest shown toward BioImageXD long before it was even released indicated a clear need for this type of software. Our own team and collaborators now use BioImageXD almost exclusively, and we believe it has the potential to become a widely used tool in the world microscopy community. Certainly, it can do its part in advancing open source software development in the field! With the current feature set we believe BioImageXD can already rival and even out-perform commercial competitors. ■



**Figure 4. The BioImageXD animation generation tool is perhaps the most versatile available.** Shown here is the animator user interface. At the top are video editor like tracks for time points, camera paths and key frames. The rendering preview shows the camera flight path as a red line, interactively adjustable in three dimensions from the green control points.

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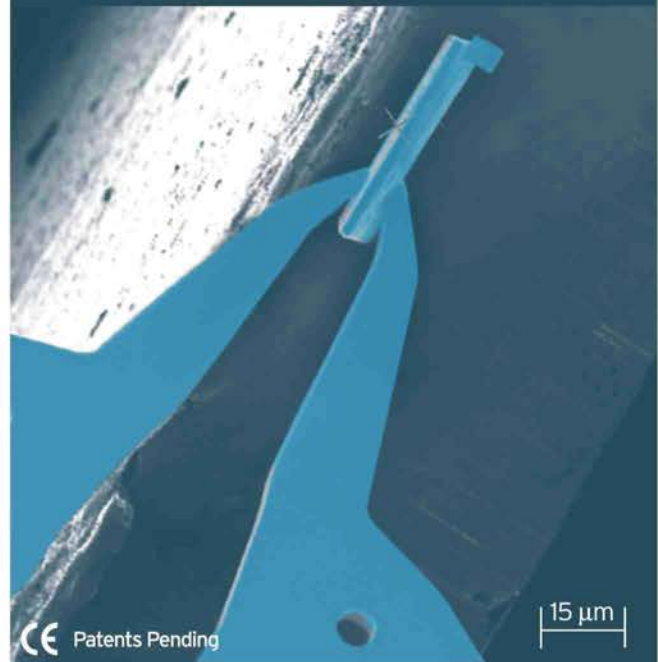


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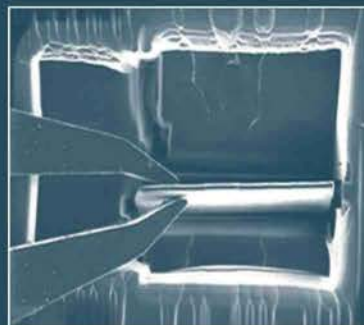


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