

Technical Note #2600

Comprehensive Image Processing with LuxBundle Software and the Luxendo Image Processor

Light-sheet fluorescence microscopy (LSFM) generates very large image data (multiple terabytes) that require efficient processing software to give users fast results. Therefore, efficient software is constantly evolving to be able to handle and process LS data.¹ Bruker's LuxBundle software for Luxendo light-sheet microscopes integrates flexible microscope control (LuxControl), image viewing (Image Viewer), and powerful post-processing (Luxendo Image Processor). This technical note discusses the powerful, yet user-friendly, post-processing provided by the Luxendo Image Processor. This image processor, which was developed with extensive feedback from researchers and imaging facilities, provides effective processing, is easy to use, and comes with comprehensive documentation to enable customization to specific application requirements.

Efficient Management of Image Processing

There are several components that enable users to effectively process their images, such as the intuitive setup and configuration of tasks, an easy overview and management of all processing steps, and the ability to queue and batch tasks for large-scale projects. Often, since data processing is not done on a single day, it also helps to have the ability to save tasks for future reproducibility and to pause tasks to temporarily free your computer for other tasks. The Luxendo Image Processor incorporates features that make it well equipped to meet these requirements, including:

- Registration of multi-view, multi-tile, and multi-channel LSFM data, including meta-data-based, content-based, and landmark-based approaches;
- Multi-view image fusion and deconvolution;
- Blended tile stitching for very large samples (hundreds of tiles); and
- Integration with other LuxBundle components and third-party software, such as Fiji,² Bitplane Imaris (Oxford), or ZEISS arivis Pro (Carl Zeiss Microscopy Software Center Rostock GmbH).

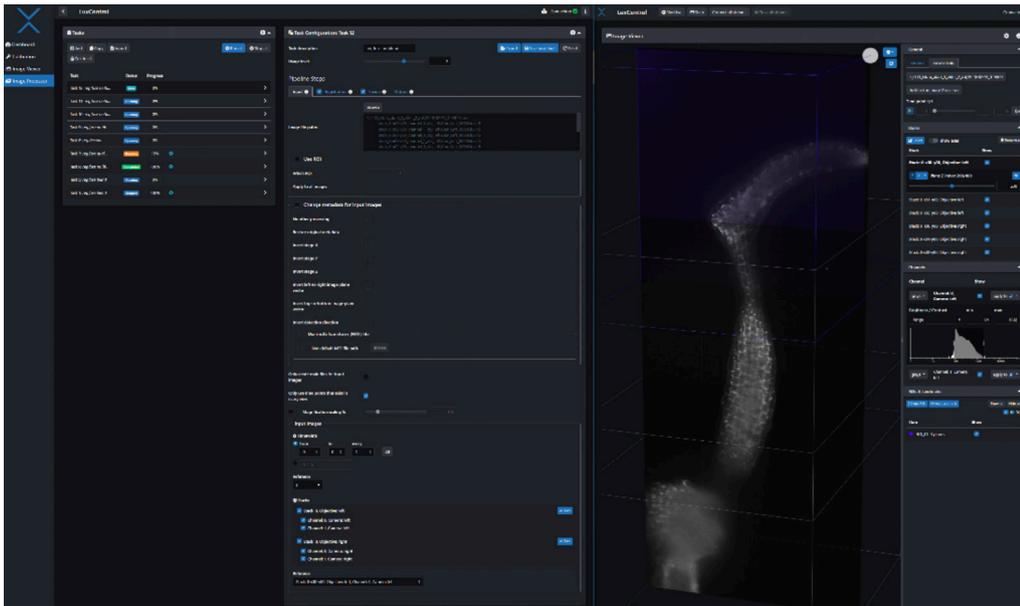


FIGURE 1

Luxendo Image Processor and Image Viewer side by side.

Image Registration

When images or image stacks of an object are taken from different viewpoints, the object appears at different positions and in different orientations in the coordinate system of respective fields of view (FOVs). Image registration is the process of determining how two or more datasets can be brought into one overlapping and aligned spatial coordinate system in such a way that when overlaying the modified datasets, the features and structures of the object appear at the same position for all datasets. Generally, one dataset and its viewpoint and coordinate system is called the reference, while the others are the moving images. In medical imaging, this is widely used to register datasets from different patients or data from the same patient acquired with different imaging modalities, such as MRI and CT.³ In biomedical imaging, registration is used to align data from different samples, different views, or different color channels. Together, registration can be a computationally difficult task depending on the presence of distinguishable sample structures, features, and properties of the acquired image data (e.g., signal-to-noise ratio).

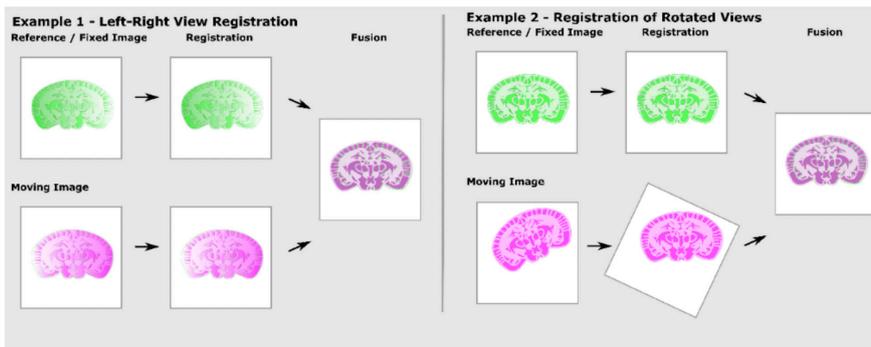


FIGURE 2

After selecting a reference or fixed image (or template), the other images, called moving images, are registered to the reference (e.g., from the left view — green, better image quality left — and from the right — magenta, better image quality on the right). After registration, images are fused. Example 1 is left-right fusion between opposing views (e.g., MuVi multiview acquisition). Example 2 is registration to register images from different views into one spatial coordinate system.

The Image Processor can perform registration using the coordinates inscribed into the metadata, user-defined landmarks (i.e., features that the user recognizes and highlights in the different datasets) or the overall content of image areas that overlap, most of which depends on the images. This allows for fully automated registration that can be refined using manual landmarks. Using a multi-level approach from coarse to fine implemented in a proprietary algorithm, the Image Processor registration reaches good levels of robustness and accuracy. Another impressive aspect is that the Image Processor was developed to deal with very large datasets, making it capable of accurately stitching large arrays of hundreds of 3D tiles with terabytes of image data. Importantly, the Image Processor was also designed with speed in mind, using Fast Fourier Transform and running on GPUs. It only takes a few seconds per registration and allows thousands of pair-wise registrations to be performed in a reasonable amount of time, as is needed for example when stitching a dataset of several hundred tiles.

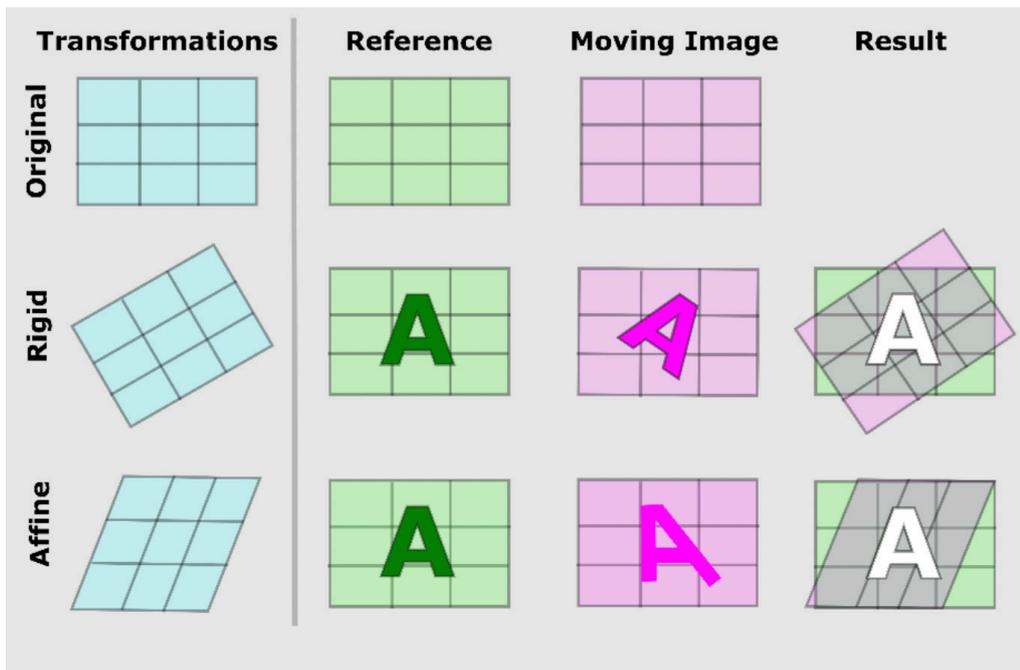


FIGURE 3

Registration approaches differ in their degrees of freedom of registering the moving image to the reference or fixed image. Rigid registration allows translation and rotation, while affine registration also allows for anisotropic scaling, and shearing. In LuxBundle, landmark-based registration allows alignment by 3D affine transform, which may give better alignment than the rigid transform obtained from content registration.

Image Fusion

Following image registration, data can be merged. This is what happens during the image fusion step. Image fusion is particularly critical for large samples, where one FOV would not be large enough to capture the whole image. Thus, multiple overlapping so-called tiles are acquired to cover the whole sample. The subsequently required fusion of tiles is commonly referred to as stitching. Very often, neighboring tiles differ in brightness or contrast, resulting in visible tile boundaries, but this can be overcome by blending between the tiles.^{4,5} In addition to tiling, individual images can be chopped into smaller image chunks, also allowing terabytes-large sets of tiles to be stitched even with small RAM requirements.

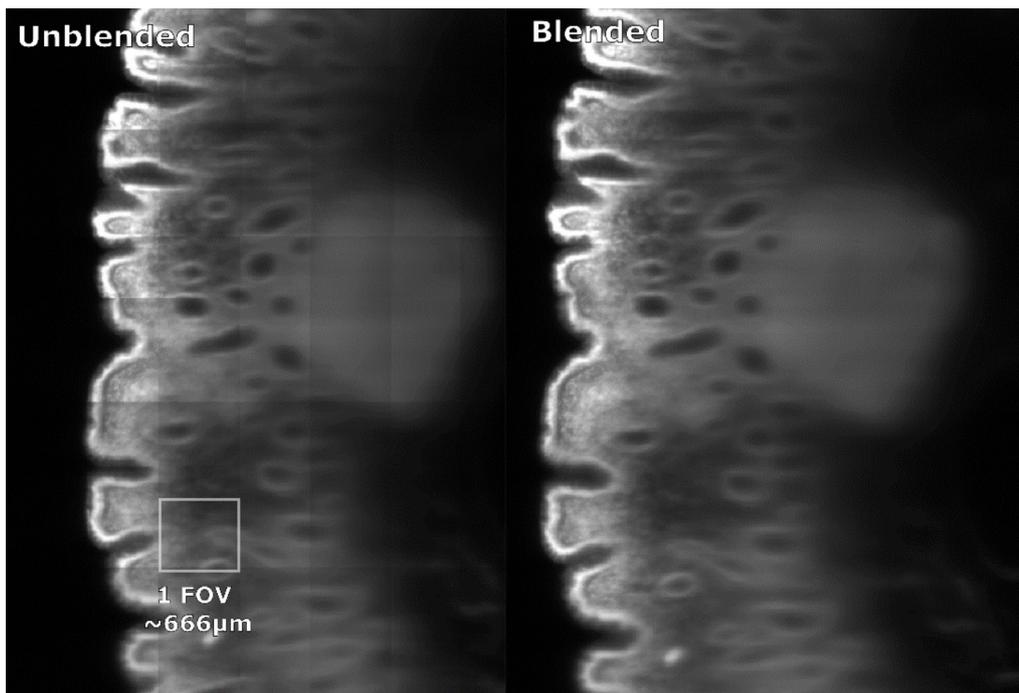


FIGURE 4

Large intestine imaged with the Luxendo MuVi SPIM Light-Sheet Microscope (ClearLight Biotechnologies); 20x magnification, 666 μm FOV, and fused without and with blending.

3D Viewer

The Image Processor is tightly integrated with the Image Viewer, both of which are comprised in the LuxBundle software, allowing researchers to inspect the entire dataset directly after acquisition. The Image Viewer software gives users control over their data with key capabilities, including 1) the handling of both raw and post-processed images; 2) the ability to turn tile stitching on or off; 3) fast viewing of multi-terabyte datasets; and 4) flexible options to draw and annotate regions and landmarks.

Regions of Interest and Landmarks

The Regions of Interest (ROIs) set in the Image Viewer can be used for the cropping of input images and the definition of output volumes in the Image Processor. Landmarks can be used for annotation of relevant points in the sample as well as for landmark-based registration. Both ROIs and landmarks are saved to a flexible and simple human-readable JSON file that can also be easily accessed from other software or custom user scripts.

User-Friendliness

Getting started

When beginning to use LuxBundle and the Luxendo Image Processor, software is delivered pre-set for the specified system, and users get in-depth hands-on training. An up-to-date user guide that walks the user step by step through the most common and relevant use cases is directly linked in the software. Also, helpful information can be found when hovering over labels of the respective settings.

Once defined and set up, multiple data processing tasks can be queued and run by themselves to maximize system usage and prioritize user time (i.e., multiple tasks can be set up and left to run by themselves). Furthermore, to increase reproducibility and save time, processing tasks can be personalized, stored, reused, and adapted. Users can also batch their processing of datasets in one folder, and output data formats (.tiff, .hdf5, .ims) are

compatible with common image processing software, such as Imaris, Aivia, BigDataViewer, Python (Napari), and MATLAB.

Advanced User Interactions

LuxBundle was designed by users for users and therefore includes a variety of ways to interact with the processor, such as:

- A slider allows the selection of a user expertise level to enable advanced users to access more options and parameters for fine-tuning of processing tasks;
- The open interface implementation allows for custom microscope state readouts and issuing commands to the microscope;
- Image processing can be run in headless mode from the terminal, which can be useful, for example, to distribute Image Processor tasks over multiple computers or a compute cluster;
- The LuxLogger logs all messages being sent to and from the microscope on the fly, making it very easy to understand and test how to communicate programmatically with the Image Processor; and
- Any microscope control that can be done through the GUI can also be done through a “scriptable” interface, meaning users can customize processes easily. This also allows users to write their own (automated) microscope control and smart microscopy applications.

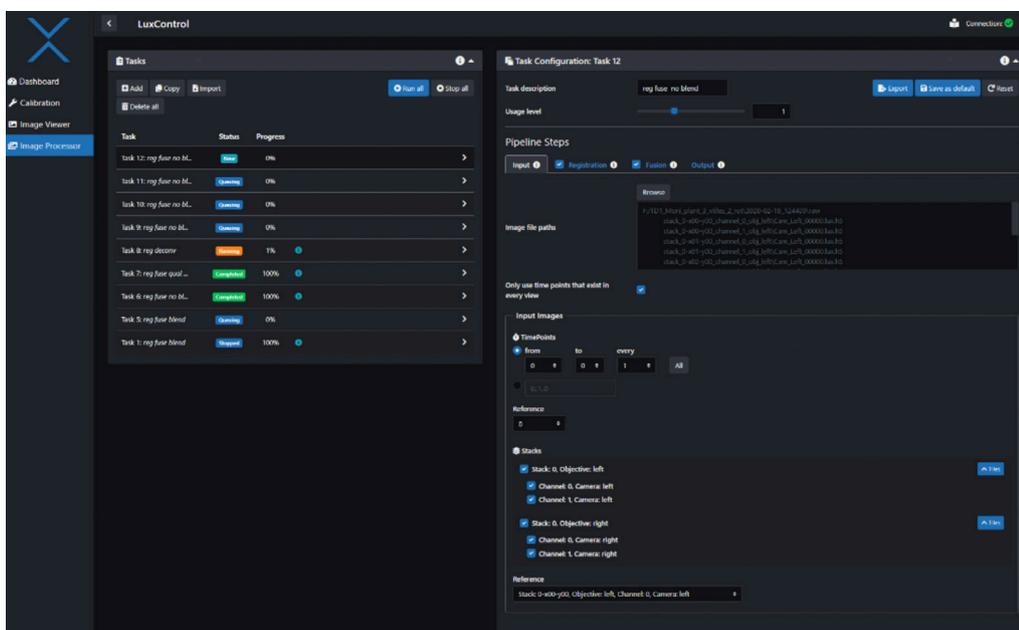


FIGURE 5

Image Processor GUI, with “Input” tab.

Constant Improvement with the User in Mind

Bruker is consistently looking for ways to improve all components of LuxBundle and strives to make the software as user-friendly and intuitive as possible by creating an ergonomic product for users of all backgrounds and expert levels. To accomplish this, users have the option to be part of the beta-testing team to get the newest features faster, or the option to subscribe to stable releases only. Furthermore, software and application teams are constantly improving LuxBundle with a focus on actual use cases. We encourage customers to contact our application scientists with any questions about LuxBundle or our other light-sheet technologies.

References

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