# Supplementary Images



Supplementary Fig. . The initial screen of the *Batch Deconvolution* Graphical User Interface.

# Supplementary Data

## Pseudocode

1. Read parameters.
2. *LOOP* for all files in the input folder:
3. Read a file.
4. Split the file into z-stacks of separate positions, frames, and channels.
5. Collect data of pixel and image sizes for every file for further PSF calculations (if selected).
6. Save split files.
7. *LOOP* for pixel and image sizes data (if a PSF is not provided):

Option I: calculate a PSF for every image.

Prepare a PSF config file (see *PSF Generator instruction*) for every channel (excluding brightfield) of every file.

Option II: calculate a PSF once for similar images.

Prepare a PSF config file for every channel of every voxel size, taking the PSF image dimensions from maxima of images of a common voxel size.

1. *IF* a PSF is provided, load it, split into channels, and save as separate files.
2. *LOOP* for all the files in the input folder:
3. *LOOP* for all the split files from one position:
4. If the image is of a brightfield channel, copy the image into the intermediate deconvolved images folder.
5. Calculate a PSF using *PSF Generator* if was not calculated earlier or not provided.
6. Deconvolve the image using *DeconvolutionLab2*. Save the deconvolved image to the intermediate deconvolved images folder.
7. (*IF* option chosen) Delete the split file.
8. Combine all files from the single position (channels, frames), and save it to the output folder.
9. (*IF* option chosen) Delete expendable images from the intermediate deconvolved images folder (single-frame, single-channel images).
10. *IF* a PSF is to be calculated for every file, delete expendable PSFs (*IF* option to delete files chosen).
11. Create the condense Log file
12. (*IF* option chosen)Delete all intermediate files.

## Further troubleshooting

1. For parameters optimization we recommend first to work with stand-alone *PSF Generator* and *DeconvolutionLab2* separately and then to input settings into *BatchDeconvolution* for the batch processing.
2. *BatchDeconvolution* reads a voxel size from an image metadata. Such information must be provided for a proper work of the software.
3. Pixel calibration can be set in any units. Their relation to the SI units has to be provided first in *units.dat* file within the *BatchDeconvolution* folder. Some most common units are already provided.
4. If a PSF file is provided, it either has to match the channel structure of the image file (including brightfield channel(s) – though, these channels will not be taken into account) or has to have the same number of fluorescence channels as the image file. *BatchDeconvolution* software will account for the different channel structure resulting from missing brightfield channel(s).
5. *Log.dat* file (in *Log* folder) contains condense information about deconvolution process. Check it after processing for eventual errors.
6. The list of file formats supported by *BatchDeconvolution* is included in *extensions.dat* file within the *BatchDeconvolution* folder. We tried to include all the file formats readable by *BioFormats* library. If the library supports an additional file format that is not included in *extensions.dat* file, adding the extension to the data file may allow *BatchDeconvolution* to read the image file.