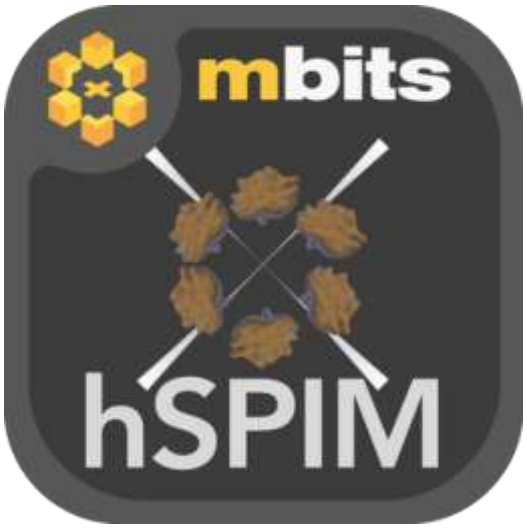
mbits

mbits hSPIM analyzer

user manual



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mbits hSPIM analyzer
Version: 1.0

Image analysis and viewing for Selective Plane Illumination Microscopy

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1 INTRODUCTION

The **mbits hSPIM viewer** application has been optimized for processing Dual-view Inverted Selective Plane Illumination (diSPIM) acquisitions of spheroids containing cells. It may not work with different input.

Realtime deconvolution and fusion of images

Given a beads image the application can calculate the imaging system's Point Spread Functions (PSF) and the registration between two perpendicular input images. By using a multi-view deconvolution the sharpness of the images is improved while allowing a simultaneous fusion of the two inputs.

Fast visualization of 3D DISPIM data – The Live-Viewer

After a calibration has been saved, it can be used to quickly create a live 3D preview and visualization of currently captured image data.

Analysis of cells

Use the application to calculate Haralick feature sets of cells for advanced analysis of your DISPIM data. The calculation can be used in long term experiments with multiple time steps.

Create video sequences of samples over time

Create a video of sample evolution on already recorded image data. Utilize the different visualization modes of the Live-Viewer component to make impressive videos.

2 SYSTEM REQUIREMENTS

The **mbits hSPIM analyzer** is only available for Windows. Other requirements are:

Requirements	
CPU	Intel multi-processor system with 16 or more cores clocked at 3ghz
Memory	16 GB
Hard drive	256GB SSD (depending on your experiments)
Graphics Hardware	NVidia Titan X or comparable, with 6GB OpenCL memory (no multiple GPUs or cards)

3 INSTALLATION

The application is provided as a "portable executable". This mean, everything you need to run the application is included in the zip file. There is no installation required. You can extract the zip file to any location on your PC.

Please be aware that the deconvolution and visualization only works with nVidia GPUs supporting OpenCL.

4 GENERAL FUNCTIONS

This section explains the general aspects of using the **mbits hSPIM analyzer**. The application has been designed for following three main use cases:

- Live-Viewer
  - Quickly viewing for "what is currently under the microscope"
- Streaming-Mode
  - Fast segmentation and feature extraction for high content microscopy
- Movie-Maker
  - Create a movie from a preselected position over time

Those three modes will be described later in the following chapters. The application is a command line based tool to allow automation and easy integration into other analysis frameworks. You can start the command line by right-clicking the windows start menu icon and selecting "Command Prompt"

Use the command **cd** to switch the current path of the command line to the decompressed folder. Example (assuming you decompressed the zip file to C:\hspim):

```
cd c:\hspim
```

The following sections are assuming that the command line is in this path.

Instead of using the command prompt in the following examples, there are following alternatives:

Another possibility is to associate the **.ini** files with the hspim.exe application. This can be done by using "open with" in the explorer right-click context menu on an **.ini** file. So you can simply start a process by double-clicking the respective **.ini** file in the explorer.

Also you can drag the **.ini** file upon the hspim.exe applicaton to start the process.

5 LIVE-VIEWER

The Live-Viewer provides fast visualization of 3D DISPIM image data in real-time. The next sections will give you an overview of this mode.

These configuration files reside in the visualization directory. You can enter it in the "Command Prompt" by:

```
cd c:\hspim\visualization
```

5.1 How to calibrate the viewer

The calibration routine is invoked by:

```
..\bin\hspim calibration.ini
```

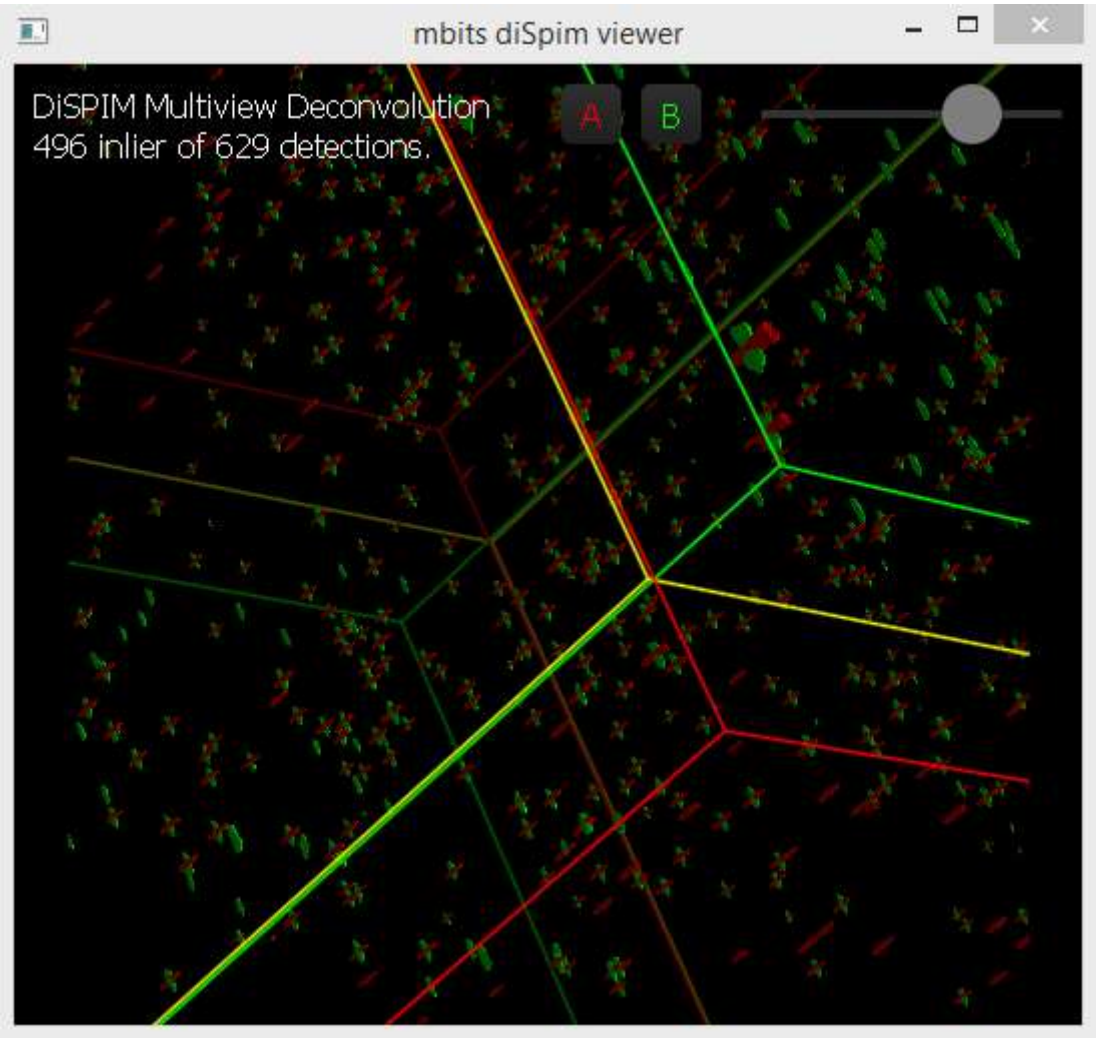
A file popup dialog will be shown, which should be pointed to the beads image. This image should be a 16bit dual-view acquisition TIFF image containing the beads image data.

A calibration file containing the PSF and the registration will be written to the .\data\calibration.hspim file. In our example this should reside in:

c:\hspim\visualization\data\calibration.hspim

If a successful registration can be computed, a 3D visualization appears of both stacks for manual verification of the registration and the PSF. This calibration user interface behaves like the normal viewing mode described below.

Visualization after a successful registration:



5.2 How to start the viewer

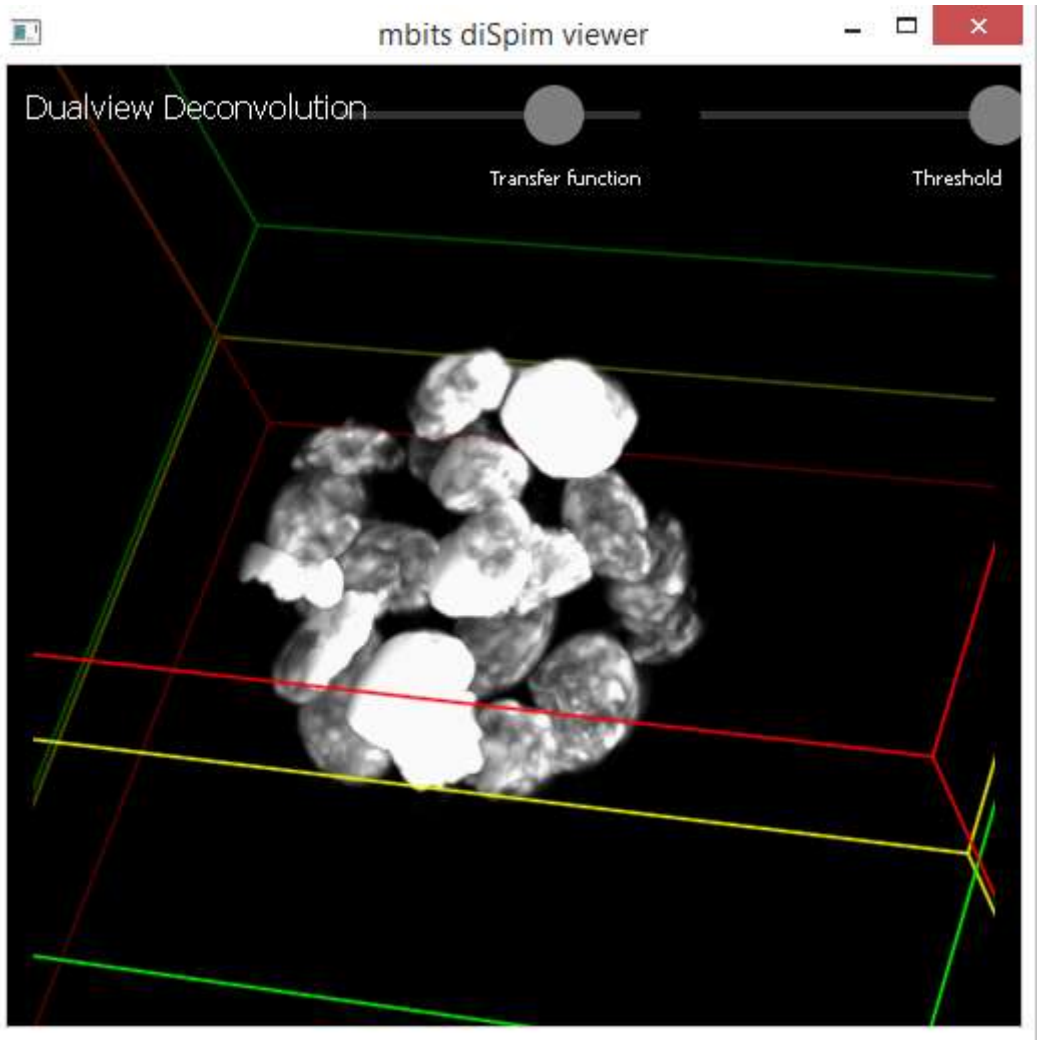
The viewer can be started by:

```
..\bin\hspim visualization.ini
```

A file popup dialog will be shown, which should be pointed to 16bit dual-view acquisition TIFF image containing the image data to be visualized.

A calibration file containing the PSF and the registration (created in previous step) will be read from the **.\data\calibration.hspim** file.

The viewer will open with a 3D-visualization:



5.3 Live-Viewer user interface

Use the mouse to navigate within the 3D volume:

Interaction	Action
Left button + move	Rotate 3D Camera
Right button + move	Zoom In/Out

There are several keyboard shortcuts:

Shortcut	Action
1-8	Switch between available view modes: - Multiple 3D-volume rendering techniques - 2D-View of fused image if available - 2D-View of label image if available - 2D-View of up sampled dual-view stacks - 2D-View of up sampled PSF images
W	Move forward in 3D view

A	Move left in 3D view
S	Move backward in 3D view
D	Move right in 3D view
R	Move up in 3D view
F	Move down in 3D view
Q	Rotate left in 3D view
E	Rotate right in 3D view
Z / X / C / V / Space	Reset view to axes or microscope
+ or mouse wheel up	One slice up in a 2D view
- or mouse wheel down	One slice down in a 2D view
M	Save current view for movie generation

UI elements in 3D views
There are sliders for the threshold used for the 3D volume rendering as well as a slider for the transfer function. The transfer function is not present for all view modes.

6 STREAMING MODE (ANALYSIS MODE)

The image analysis mode segments the image into background and cells and calculates features of each cell within the sample. This gives you the ability to do further calculations like classification of a cell in other frameworks.

The streaming mode consists also of two steps: a calibration and processing step.

The corresponding .ini files are located in:

c:\hspim\streaming

Like in the visualization mode the calibration file is stored under .\data\calibration.hspim, in our example it would be:

c:\hspim\streaming\data\calibration.hspim

6.1 How to calibrate the streaming mode

The calibration routine is invoked with no visualization at end of processing by:

```
..\bin\hspim stream-calibrate.ini beadsImagePath
```

Or with visualization by:

```
..\bin\hspim stream-calibrate-show.ini
```

A file popup dialog will be shown, which should be pointed the beads image.

beadsImagePath: path to a 16bit dual-view acquisition TIFF image containing the beads image data

A 3D visualization appears only with the **`stream-calibrate-show.ini`** configuration file to allow for manual verification.

6.2 How to process a dataset in the streaming mode

The calibration routine is invoked with no visualization at end of processing by:

```
..\bin\hspim stream-process.ini [imagePath] [featuresPath]
```

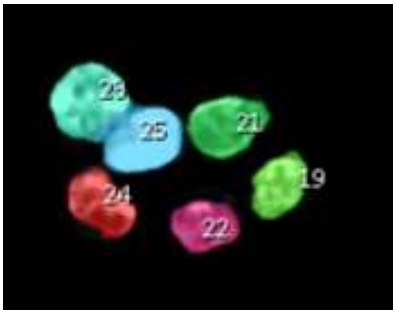
Or with visualization by:

```
..\bin\hspim stream-process-show.ini [imagePath] [featuresPath]
```

imagePath: path to a 16bit dual-view acquisition TIFF image containing the image data to be visualized.

featuresPath: path to output csv file containing a table of the features of each cell. See following chapter its contents

The features will be saved under the specified path. A 3D visualization appears when the **`stream-calibrate-show.ini`** configuration is used. An additional 2D-view mode will be available for inspecting the label image.



6.3 Calculated feature set

The calculated features are saved as a standard .csv file (comma separated values).

Overview of features:

- Cell volume (given in number of voxels)
- Center of mass (given as an X/Y/Z coordinate in the fused image)
- Average grey value
- Haralick texture features

7 MOVIE MAKER

The Movie Maker mode generates a movie from your diSPIM image data when multiple image acquisitions are made over time. In contrast to the other modes, the movie maker mode consists of two steps.

7.1 How to calibrate the movie maker

The calibration routine is invoked by:

```
..\bin\hspim 1.calibration.ini
```

A file popup will appear to select the beads image.

If a successful registration can be computed, a 3D visualization appears of both stacks for manual verification of the registration and the PSF.

7.2 How to setup the 3D camera

The viewer can be started by:

```
..\bin\hspim 2.set-camera.ini
```

A file popup will appear to select a 16bit dual-view acquisition TIFF image containing the image data to be visualized.

The viewer will open with a 3D-visualization of the first frame. You can press "m" to start the movie generation from the current 3D-mode and parameters.

7.3 How to setup the 3D camera and generate the movie

The viewer can be started by:

```
..\bin\hspim 3.create-movie.ini
```

A directory popup will appear to select the home directory. After that the deconvolution batch process will begin.

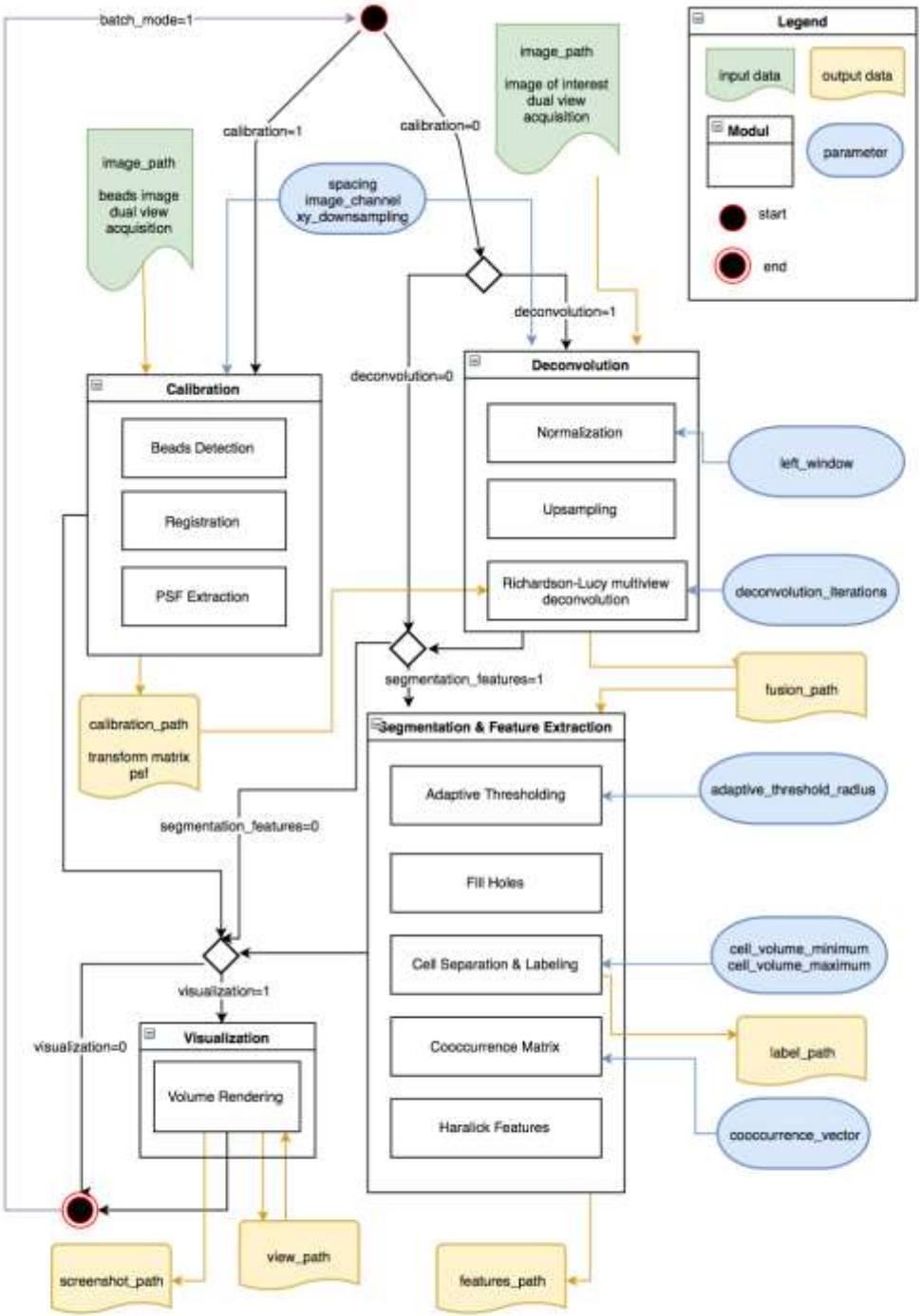
The configuration file has to be adapted, following parameters needs to be adapted: image_path, batch_start, movie_mode and batch_end

After successful processing the movie should be generated under the path .\data\movie.mp4

8 DETAILED INSTRUCTIONS

Those previous configuration templates provide only a quick start into the application. They can be adapted to your microscope setup.

The following diagram shows the processing pipeline of the application, and how it is influenced by the configuration file.


batch_mode=1
image_path
image of interest
dual view
acquisition
calibration=1
calibration=0
image_path
beads image
dual view
acquisition
spacing
image_channel
xy_downsampling
Legend
input data
output data
Modul
parameter
start
end
deconvolution=1
deconvolution=0
Calibration
Beads Detection
Registration
PSF Extraction
Deconvolution
Normalization
Upsampling
Richardson-Lucy multiview
deconvolution
left_window
deconvolution_iterations
segmentation_features=1
fusion_path
calibration_path
transform matrix
psf
Segmentation & Feature Extraction
Adaptive Thresholding
adaptive_threshold_radius
segmentation_features=0
Fill Holes
Cell Separation & Labeling
cell_volume_minimum
cell_volume_maximum
visualization=1
visualization=0
Visualization
Volume Rendering
Cooccurrence Matrix
label_path
Haralick Features
cooccurrence_vector
screenshot_path
view_path
features_path

8.1 Executing the application

The application is intended to be executed via command line or batch file. Thereby the executable can be called with other command line parameters that are explained in the following sections.

8.1.1 THE CONFIGURATION (INI) FILE

mbits hSPIM analyzer utilizes an configuration/parameters file to switch between its main modes. This file is also used to configure the application with several parameters for algorithms. You can find templates of configuration files for each use case in later sections. It is recommend to save a dedicated configuration file for each use case. The path of the desired configuration file can be specified via the first command line parameter:

c:\hspim\bin\hspim.exe my_config.ini [parm1] [parm2] ...

parm1 will be put in the {1} placeholder, **parm2** in the {2} placeholder of the configuration file. A total of 9 parameters are possible.

Please note that the parameters have to be consistent across multiple configuration files (i.e. spacing etc.)

The entries within the configuration (.ini) file are:

parameter	description
batch_mode, **batch_start**, **batch_end**	If **batch_mode** = 1 then the process will be started multiple times. It will be iterated from **batch_start** to **batch_end**. In all other parameters that contain a file path, the placeholder **{b}** while be replaced for the current iteration. If batch_start and batch_end contain leading zeros, the placeholder will retain the leading zeros. This mode is internally used by the movie maker and can be used for batch processing multiple datasets
batch2d_mode, **batch2d_start**, **batch2d_end**	If **batch_mode** = 1 and **batch2d_mode** = 1 then the batch process will be started multiple times. It will be iterated from **batch2d_start** to **batch2d_end**. In all other parameters that contain a file path, the placeholder **{b2}** while be replaced for the current iteration. If batch2d_start and batch2d_end contain leading zeros, the placeholder will retain the leading zeros. This mode is internally used by the movie maker and can be used for batch processing multiple datasets

force_fusion_size_x, force_fusion_size_y, force_fusion_size_z	If different registrations are used in multichannel images, the resulting fused channel images may not be exactly the same size. With this parameter a fixed output size for the fusion image can be forced.
calibration	Enables calibration mode (registration and extraction of psf). In this mode **image_path** is read and the calibration is saved under **calibration_path.** **deconvolution** and **segmentation_features** parameters have no effect in this mode
visualization	If this mode is 1 the application will not exit after the processing, instead it tries to show visualizations of all available data.
deconvolution	If this mode is 1 the application will read **image_path** and will write the image under **fusion_path** if given. **calibration_path** will be read for the psf and the transformation of the two stacks. If this mode is 0 the application will just read an already fused image under path **fusion_path**
segmentation_features	If this mode is 1, a segmentation and feature extraction is performed on the fused image and saved under the path **features_path**
image_path	Input image (16bit) path for calibration or deconvolution modes
calibration_path	Path to input/output calibration file. Is used in calibration and deconvolution modes
fusion_path	Optional path to write out the fused image (8bit)
report_path	Path for optional report file, where the result are saved.
features_path	Path for the output features .csv file. Used in the **segmentation_features** mode.
spacing_x, spacing_y, spacing_z	Spacing of the input image (specified in **image_path**)
num_channels, select_channel	**num_channels** specifies number of channels in the **image_path**, **select_channel** specifies the channel to process.
cooccurrence_vector_x, cooccurrence_vector_y, cooccurrence_vector_z	When the **segmentation_features** mode is enabled, this parameters sets the difference vector between two samples for the Haralick features computation
xy_downsampling	If this is set to 1, the input image will be halved in X- and Y-direction
num_iterations	Set the number of deconvolution iterations, 25 is the default
adaptive_threshold_radius	Sets the adaptive threshold radius measured in voxels
left_window	Defines in percent the distance of the left border of

	the normalization window, default is 100 percent
right_window	Defines in percentage the distance of the right border of the normalization window, default is 250 percent left_window and right_window can be effectively used to change the brightness/contrast of the resulting fusioned image
cell_volume_minimum	Defines the minimum cell volume in voxel. Cells with volume below that will be discarded as artifacts.
cell_volume_maximum	Defines the maximum cell volume in voxel. Cells with a volume above will cause the segmentation algorithmn to run more iterations till that cell is split and falls below the threshold.
movie_mode	The movie mode can be used together with batch_mode. The parameter specifies the fps (frames per second) for the movie generation.
view_path	Under this path the input/output camera view parameters are saved for movie generation
screenshot_path	This path specifies where the individual screenshots for movie generation should be saved. This parameter needs to contain a {b} to work properly.
movie_path	The final output movie file path (should end with .mp4)
request_home	If this parameter is 1 a file requester popup will appear. If this parameter is 2 a directory requester will appear. The placeholder {h} will be filled with the selected file or directory.
home	If request_home is 0, a default home path for {h} can be set here

8.2 Data specification

8.2.1 INPUT

Input image data
The input is required in TIFF format with 16 bit unsigned values containing a TIFF directory for each image slice.
The dual view acquisition is assumed to be stored in a single TIFF file. Within the file the whole first image stack should be followed by the second image stack.

8.2.2 OUTPUT

Fused image

The fused and deconvoluted image is stored and read as an 8 bit unsigned TIFF image. All image slices are stored in separate TIFF directories.

Video
Video files are stored in mp4 format which is supported by vast majority of video player applications.

Calibration
The calibration file is stored in a custom format and contains the transformation matrix and psf prepared for the current GPU. So this file is not transferrable from a computer to another computer.

Feature set
The features are stored as table in a .csv file readable by many tools.
The first line contains the headers of the column. Current columns are:

- Label: index of cell
- NumVoxels: volume of cell in voxels
- CenterX, CenterY, CenterZ: position of cell in the fused image
- Average: average grayvalue across the cell voxels
- f1-f13: haralick features computed over the cell

ID	Name	Equation
1	Angular Second Moment (Energy)	$f_1 = \sum_i \sum_j \{p(i,j)\}^2$
2	Contrast	$f_2 = \sum_{n=0}^{N_g-1} n^2 \{\sum_i \sum_j p(i,j)\}_{\lvert i-j\rvert=n}$
3	Correlation	$f_3 = \frac{\sum_i \sum_j (ij)p(i,j) - \mu_x \mu_y}{\sigma_x \sigma_y}$
4	Variance (Sum of Squares)	$f_4 = \sum_i \sum_j (i-\mu)^2 p(i,j)$
5	Inverse Difference Moment	$f_5 = \sum_i \sum_j \frac{p(i,j)}{1+(i-j)^2}$
6	Sum Average	$f_6 = \sum_{i=2}^{2N_g} i p_{x+y}(i)$
7	Sum Variance	$f_7 = \sum_{i=2}^{2N_g} (i-f_8)^2 p_{x+y}(i)$
8	Sum Entropy	$f_8 = -\sum_{i=2}^{2N_g} p_{x+y}(i) \log\{p_{x+y}(i)\}$
9	Entropy	$f_9 = -\sum_i \sum_j p(i,j) \log(p(i,j))$
10	Difference Entropy	$f_{10} = variance\ of\ p_{x-y}$
11	Difference Variance	$f_{11} = -\sum_{i=0}^{N_g-1} p_{x-y}(i) \log\{p_{x-y}(i)\}$
12	Information Measures of Correlation	$f_{12} = \frac{HXY - HXY1}{\max\{HX,HY\}}$
13	Information Measures of Correlation	$f_{13} = (1 - exp[-2.0(HXY2 - HXY)])^{1/2}$

9 NOTES

- Input image data sets are required in TIFF format with 16 bit unsigned short pixel values.
- Only file paths can contain a placeholder e.g. **{1}** or **{b}**
- All entries used to perform the calibration routine should stay the same for other use cases of the application.
- If there is no image shown in the de-convoluted view, you might have used an over saturated beads image. Thus no PSF can be extracted and no deconvolution is available.