

## **Requirements:**

64-bit Operating System (Linux, Mac OS X or Windows)

Nvidia graphics card

## **Installation:**

Download and run the “MVDApp\_Installer\_web.exe” and follow the instructions.

## **Usage:**

Files must be named as follows:

Scheme: „filename\_c=wavelength\_r=angle\_t=number.tif”

Examples: “WT\_h2aEGFP\_c=561\_r=000\_m=1\_t=0000.tif”;

“WT\_h2aEGFP\_c=561\_r=090\_m=1\_t=0001.tif”; “WT\_h2aEGFP\_c=561\_r=180\_m=1\_t=0002.tif”;

“WT\_h2aEGFP\_c=561\_r=270\_m=1\_t=0003.tif”

In the **Data Handling** box, click “*Select Folder*” and browse for the folder containing your data. A list of all files will be displayed in the table. Select the time point, channel and angle. Click “*Load Data*” to load the image into the working memory.

In the **Device Information & Program Status** box, memory usage of CPU and GPU are displayed alongside the current status of the program.

**Workspace** displays all images in the working memory.

Use the **Preprocessing** tab to interpolate your stacks along the axial direction to achieve isotropic voxel size and then zero-pad them to cubic shape.

In the **Registration** tabs, choose a channel, a reference angle and a moving angle. You can then find the required affine transformation that generates overlap of your volumes. The parameters of the transformation can be adjusted manually. Alternatively, use an evolutionary search algorithm that automatically identifies the proper transform. The checkboxes specify which parameters will be optimized (e.g., translation in the x and z directions only). You can specify the search radii to improve speed and accuracy. Using “*Resolution Levels*” will lead to a faster convergence by first looking for a coarse estimate on downsampled data sets.

In the **2D+1D MVD** tab, we recommend using Bayesian optimization with roughly 7 iterations (depending on sampling and noise level) and entropy weighting with an entropy neighborhood size between 5 and 11 (odd numbers only). A median filter can be applied to the weights to remove outliers, which might help to improve the result. Three iterations of the 1D deconvolution step are recommended (again depending on sampling and SNR).

The **Time-lapse Processing** tab opens the routine for automatic processing of multiple time points. You have to process the first time point manually and make sure that all settings are correct. Specify the range of time points that you want to process and hit “*Run*”. The time points will automatically be loaded, preprocessed, registered (using the previous result), fused and finally saved. Be aware that this process can take several hours for data sets containing many images.

**Publication:**

Manuel Hüpfel, Manuel Fernández Merino, Johannes Bennemann, Masanari Takamiya, Sepand Rastegar, Anja Tursch, Thomas W. Holstein, G. Ulrich Nienhaus “Two plus one is almost three: A fast approximation for multi-view deconvolution”, *Biomed. Opt. Express* **XX**, xxx-xxx (2021).

**Troubleshooting:**

In case you have questions about the software, send an email to [manuel.huepfel@kit.edu](mailto:manuel.huepfel@kit.edu).