MotionTracking 3D Image Processing Guide

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1997-2012

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August 2012

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1 Introduction

MotionTracking provides a large variety of tools to work with stacks of images that can be interpreted as 3d images. This document is intended to provide some basic explanation how to process such stacks and calculate objects and structures in 3d. Since MotionTracking is under constant development and functions are added and optimized often, this guid is by no means an exhaustive manual bur rather an overview. It is assumed that the reader has a basic understanding of MotionTracking and the 2d functionality. Refer to the MotionTracking manual for an introduction to MotionTracking itself.
The processing itself can be done on any machine, but the 3d view requires a up-to-date graphic card with proper drivers installed (see Requirements for 3d).

1.1 Requirements for 3d

First it is recommended to update your display driver.
For NVidia Graphic Cards you need to install the CUDA developer drivers from http://developer.nvidia.com/cuda/cuda-downloads. These include the CUDA toolkit, the developer drivers and the GPU Computing SDK. Follow the installation isttructions provided by NVidia.

1.2 File Structure of 3d Images

The file structure is slightly different than in 2D image sequences or movies. The whole stack of images is handled as a single image with multiple layers with the same X, Y coordinates but different Z values. Instead of saving each layer as single .mtf image files as it is the case with movies all the raw image data is saved as one single .MTST file. As before, the images can have up to 4 different color channels. A major difference is that most of the processing is done directly on the color channels of the stack rather than

it's objects. To provide some space for processing there are up to 8 artificial, temporary channels called prc1 to prc8 where the partly processed data can be stored. The first 4 prc channels are called processed stack 1 and the other 4 processed stack 2. In general they are not saved but they can be saved, loaded or exported by accessing File→ZStack→Save(or Load or Export)→Processed Stack 1 (or 2) manually if necessary.
In general the processing leads to the calculation of objects such as triangulation meshes, central lines and so on which are handled as objects similar to the treatment of vesicles in 2D. Due to the multitude of different operations it is not clear when to save the objects automatically. Therefore, after finishing all calculations, it is **very important to save the objects manually** by clicking File→ Objects→ Save Objects in Current Frame.

2 First Steps

2.1 Import Stacks

2.2 3d View

The stack of images can be visualized in 3d. This has no practical purpose from the calculations point of view, but it is very useful to visualize the actual data. To access the 3d view select either the checkbox labelled "3D" above the image. The first time you access the 3D view after loading the project you will be asked which channels you want to transfer to 3D view. After that it won't recalculate the 3D even if the data changed due to processing. To update the 3D view got to "Processing→Process ZStack→Transfer Stack to 3D". This transfer is necessary because the visualization requires some calculation time. Therefore it would be counter-productive to recalculate everything if it is not desired by the user.

3 Functions and Scripts

The processing of the stack is usually a sequence of operations. An example: Smoothing, then Segmentation, then Triangulation to get a mesh, finding central lines and finally copying central lines back into the original channel as objects (See Fig. 1). Each operation can be started alone by selecting "Processing→Process ZStack→Operation over Stack". On the other hand, since most operations are a part of a sequence, it is much more convenient to use scripts. Scripts basically list all selected operations in order of execution. They can be loaded and saved to process multiple projects in the same way. To access scripts select "Processing→Process ZStack→Stack Operation Script". It will always load the last script used. Scripts can be executed from the beginning by selecting "Start" or from the selected entry by "Start from Selected Step".

To create a script simply click "Add" and add an operation. A new line will appear in the script list in the format [A -> B] OPERATION (PARAMETERS):
A is the source channel, from which the data is taken.
B is the destination channel where the processed data is stored.
OPERATION is the name of the applied operation.
PARAMETERS is a list of all parameter values of the operation.

It is advised to use prc channels for all operations and only copy to final result in to the real color channel in the end to preserve the original data. In addition it is useful to use another prc channel after each time-consuming operation like segmentation to save time on recalculation if something unexpected happens. See the example for clarification.
To modify the parameters of an operation click "Edit".
The order of operations can be modified by selecting an operation and clicking "move up" or "move down". "Clear" clears the scripts and removes all operations.

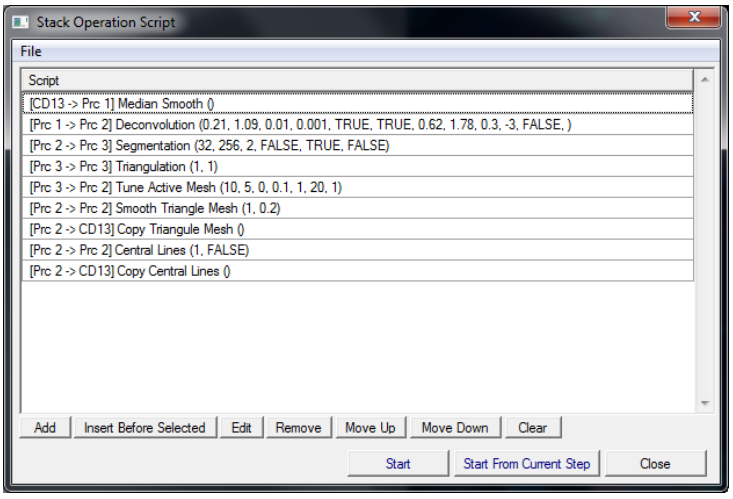


Figure 1: Example of a script. It processes the channel with the CD13 marker which is named "CD13". In the end the triangle mesh and the central lines of that mesh are copied back into the color channel as objects.

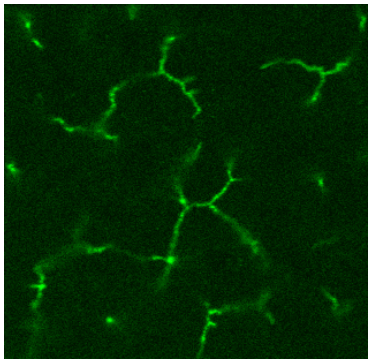
4 Common procedures and order of operations

The common procedure to find a triangular has the following concept:

1. Smoothing of original data - optional, but recommended.
2. Segmentation
3. Triangulation
4. Smoothing and/or fine-tuning of triangulation mesh - optional but recommended.
5. calculation of central lines
6. copy mesh and central lines as objects back into original color channel

Important Note: Triangulation can only be applied on Segmented channels

The following examples are working procedures for different types of objects



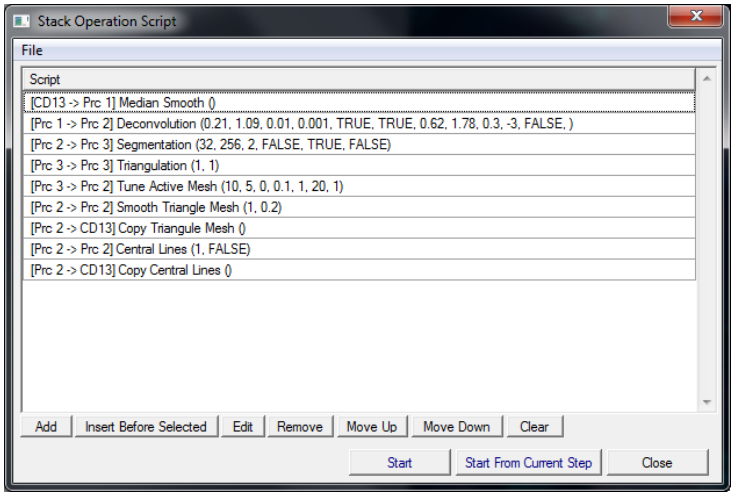


Figure 2: Script for a channel with a tube like structure

The inflation/deflation process in the nuclei script (Fig. 3) is supposed to close the inner holes of the nuclei mesh because the nuclei calculation requires filled objects, not surfaces.

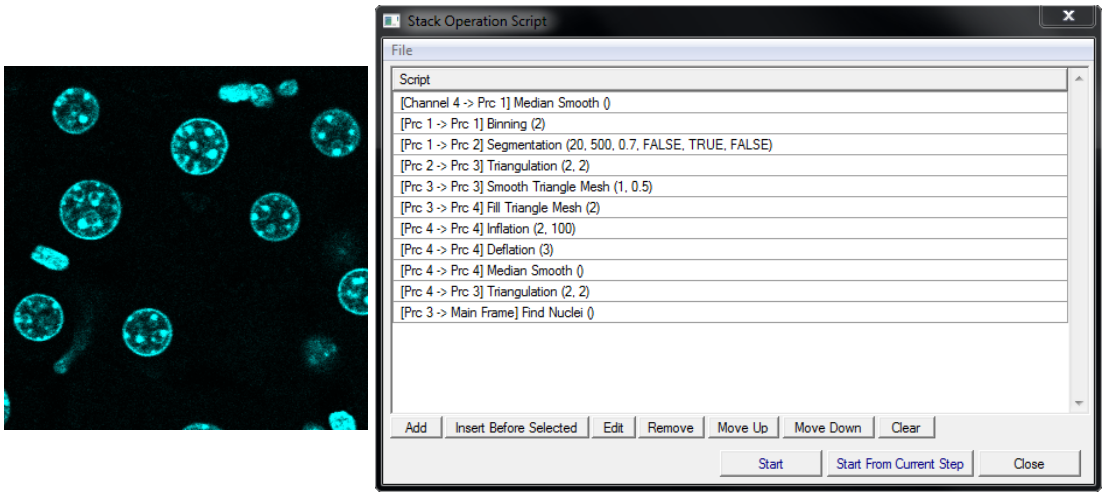


Figure 3: Script to find nuclei. After calculation they are copied back to the image as objects.

It should be obvious that some operations can be only executed after others, for example smooth thriangle mesh is possible only after the triangle mesh is calculated.

5 List of Functions

- Apply ROI Mask - apply either the normal or the inverse mask based on Regions of Interest (ROI). See ROI in MotionTracking manual.
- Apply Segmented Mask - apply the mask calculated by segmentation on a channel to a different channel.
- Arithmetics - performs basic arithmetic operations on the channel.
- Binning - compresses the image by the specified factor in each dimension (X, Y, Z). Useful for Nuclei and Cell calculation where level of precision is not that high.
- Blur - blurs the image with a Gaussian smooth with the size specified in the parameter.
- Central Lines - calculates the central lines of the triangle mesh. Estimated tube radius should be roughly accurate to ensure proper behaviour on branching points.
- Copy Cells - operation is used to copy cell objects from one channel to another.
- Copy Central Lines - operation is used to copy central line objects from one channel to another.
- Copy Channel Data - operation is used to copy channel data from one channel to another.
- Copy Nuclei - operation is used to copy nuclei objects from one channel to another.
- Copy Triangle Mesh - operation is used to copy the whole triangle mesh from one channel to another.
- Correct Dark Frames
- Deconvolution - Reverse operation of convolution - optical distortions can be modelled as such and therefore this can be used to correct distortions.
- Deflation - decreases the size of a filled mesh in every direction by X pixels.
- Deflation->Inflation - decreases and then increases the size of a filled mesh in every direction by X pixels.
- Delete Small Objects - deletes all objects that are made of less than a certain number of voxels. A voxel is the equivalent of a pixel in 3D.
- Diffusion - diffuses a channel with the given formula.
- Fill Nuclei- if Nuclei type objects have holes inside this operation fill them.
- Fill Triangle Mesh - fills the mesh so that it becomes an object rather than a surface. Parameter: Binning (similar to Binning operation, see above).
- Find Cells - calculates cells on basis of nuclei, triangle meshes and a unprocessed color channel with data related to cell boundaries. A higher Intensity Gradient means that the intensity of the channel repels the cell boundaries stronger. A lower value for accuracy means more precise boundaries. It can be defines which meshes are impassable for the cell contours.
- Find Nuclei - calculated nuclei on basis of a triangle mesh.
- Free Channel Memory - removes everything from the selected prc channel and frees up the memory. Very useful for machines with low memory.
- Inflation - increases the size of a filled mesh in every direction by X pixels and fills the new pixels with the value Y.
- Inflation->Deflation - increases the size of a filled mesh in every direction by X pixels and fills the new pixels with values taken from the selected channel and then decreases it by X pixels again.
- Laplasian - applies the Laplace operator (divergence of the gradient) on a channel.
- Median Smooth - smooths the original image data (intensity values) of one channel.

- Process Central Lines
- Resample Active Mesh - recalculates the triangle mesh based on a new sample of control points.
- SQRT LUT
- Segmentation - creates a mask on each layer to seperate objects. Parameters need to be fine tuned for each channel and the result of the segmentation has to be verified manually. Typical parameters for tube-like structures are
- Shift - shifts a channel relative to all other channels by dx, dy, dz.
- Smooth Cells - smooths the surface of the Cell objects.
- Smooth Nuclei - smooths the surface of the Nuclei objects.
- Smooth Triangle Mesh - smooths the triangle mesh N times with a weight W.
- Triangulation - calculates the triangle mesh. Parameters specify how man pixels in each dimension should be taken into account. Typical values are 2, 2.
- Tune Active Mesh - performs a fine tuning of the triangle mesh by reprocessing the surface.
- Tune Cells
- UnBinning - reverse of Binning (see above).

6 Stack Statistics

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