CAS3D DOCUMENTATION

V4.5

DOMINIK SCHNEIDEREIT, INSTITUTE OF MEDICAL BIOTECHNOLOGY

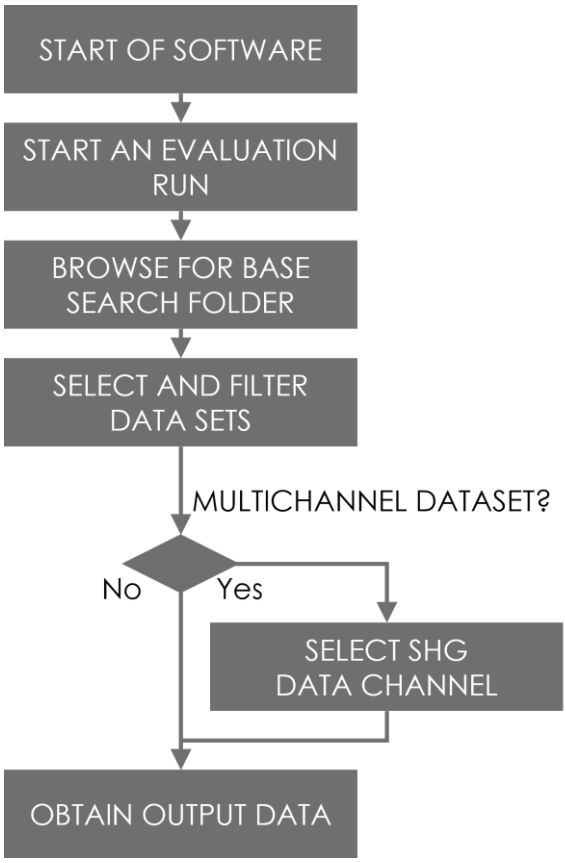
INTRODUCTION

This documentation offers an overview of the capabilities and workflow of the CAS3D software package while also providing installation and setup instructions. A set of step-by-step instructions provides detailed guidelines for using the software.

INSTALLATION

Execute the installer package on Windows 10, 7 SP1 with a double click, and follow the installation instructions. If the Matlab 2019a runtime libraries are not already present on the machine, the installer will download and install the required files (Internet connection required). A startup shortcut is placed on the desktop if the respective option is selected, and the default installation folder is "C:\Program Files\Institute of Medical Biotechnology\CAS3dTool". The software can be started by double-clicking the desktop shortcut or the CAS3dTool.exe (the default location of the executable file is: C:\Program Files\Institute of Medical Biotechnology\CAS3dTool\application).

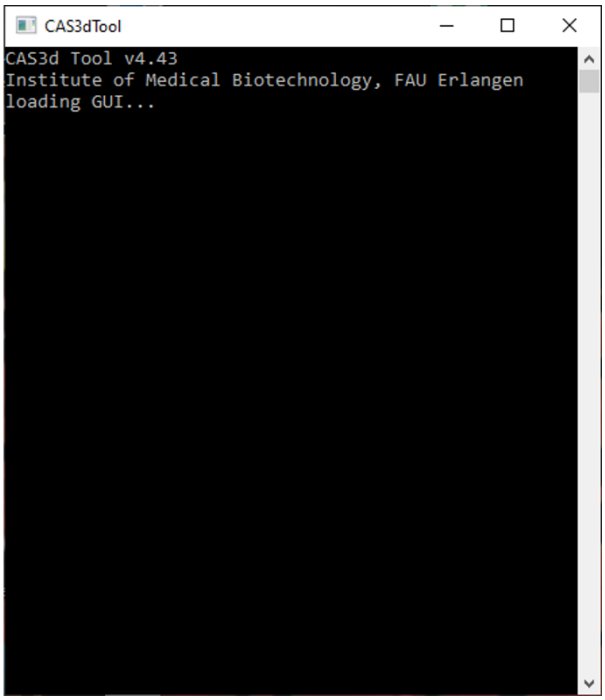
USAGE FLOWCHART



USAGE STEP-BY-STEP

START OF SOFTWARE

The software is launched by double-clicking the desktop icon. After a short loading time that depends on system specifications, a console window is displayed, which will provide basic information and feedback while the software is running, initially displaying the software version. Closing the console window will exit the software, terminating all running processes.

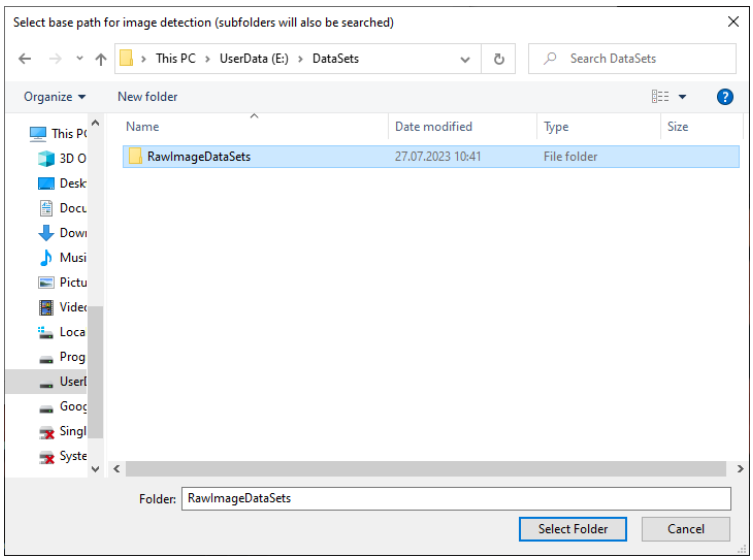


THE LAUNCHER WINDOW

The launcher window appears alongside the console window shortly after starting the software. An evaluation run can be started and this documentation can be accessed from the launcher window. If the launcher window is kept open, multiple evaluation runs may be performed in sequence without the need to re-load the Matlab runtime engine. Closing the launcher window will exit the software, terminating all running processes.

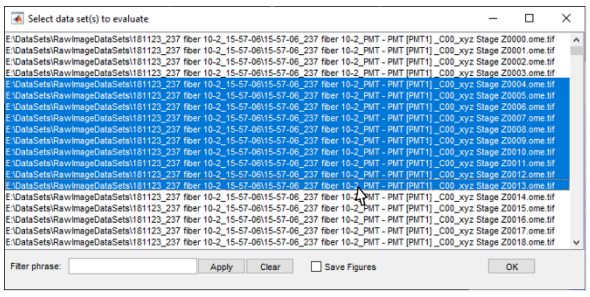
BROWSE FOR THE BASE SEARCH FOLDER

A file browsing dialogue is displayed where a folder is to be selected. The folder marks the base search folder, and the whole folder tree within the marked folder or drive will be scanned for data files in the .ome.tiff, tiff or .tif format, which will be listed in the next step. The dialogue will open in the folder location that was last indicated for an evaluation. When the folder no longer exists, or the software is restarted, the folder to start browsing from will default to "C:\".

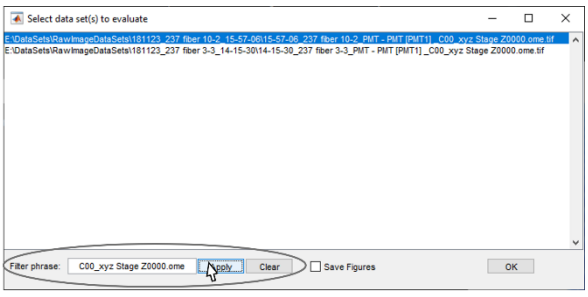


SELECT AND FILTER DATA SETS

The list of detected files is displayed, showing their full path to allow for unambiguous identification of each file. One or multiple files may be selected for evaluation by a combination of the common drag-click, shift-click, and control-click commands that are applicable in most file browser dialogues. Additionally, a filter string may be specified in the lower left of the panel that, when applied, will show a reduced list of files that contain the specified string in their name or path. The previously mentioned multi-selection methods may still be applied to the filtered file list. An applied filter string will be retained and applied automatically when the software is executed the next time but can be changed or cleared if desired. **Be sure to only select one slice per image stack, if the image slices of your stacks are saved as individual files. Also, be sure the selected slice contains the stack metadata (usually this is the first slice of a stack). Otherwise, the evaluation will fail or repeat the stack evaluation for every selected slice.** The current selection is confirmed, and the software will proceed to the next dialogue with a click on OK. If the "save figures" checkbox option is selected a summary figure is created and saved for each evaluated data set. Unchecking this option will skip the generation of the output figures and reduce the required runtime for an evaluation run.



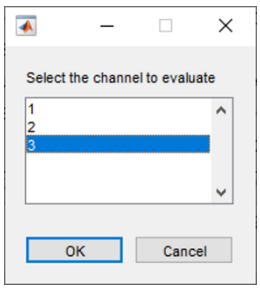
UNFILTERED



FILTERED

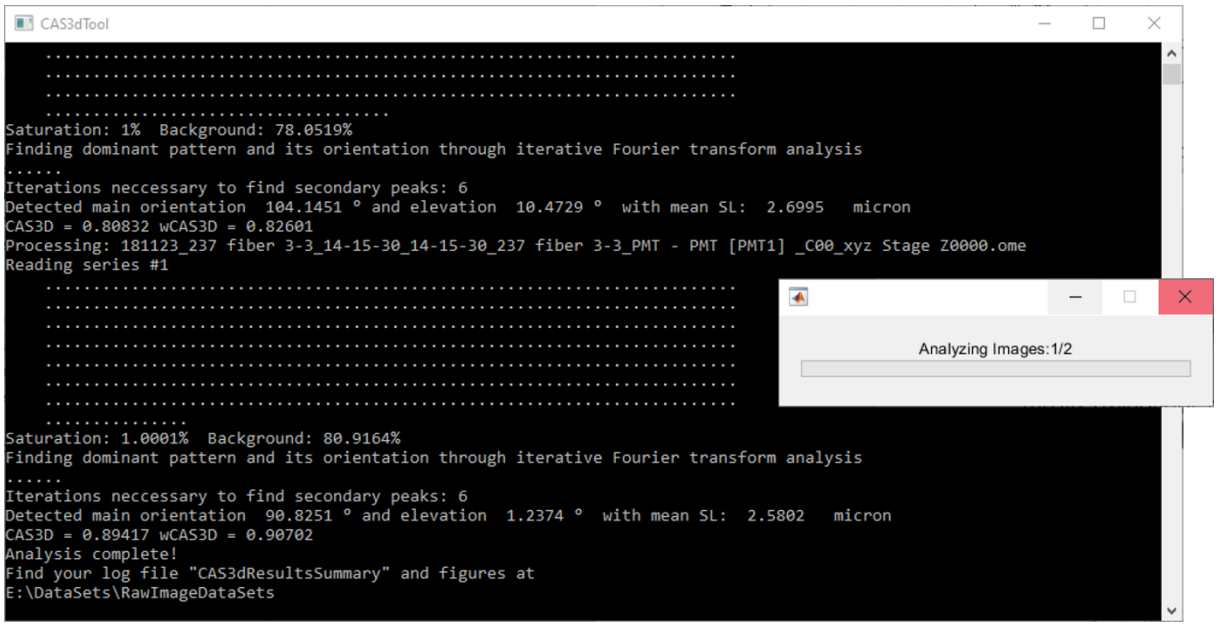
SELECT CHANNEL

If multiple channels are detected in the selected data set, the software will prompt the user to pick the channel that contains the SHG signal to be used for evaluation. Other channel data are not relevant to the evaluation and will be ignored. Confirm the relevant channel by double-clicking or selecting it and clicking the OK button.



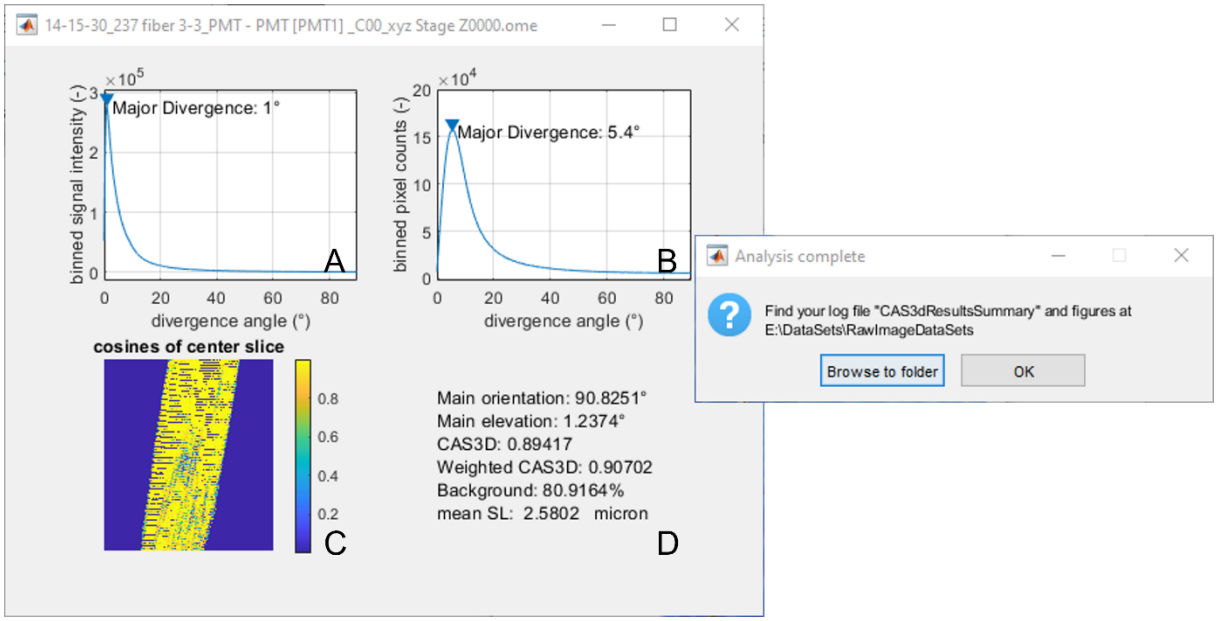
ANALYSIS PROGRESS

During the analysis process, the progress through the data sets is displayed in a progress bar. Intermediate results and progress steps are displayed in the console window. Warnings and error messages are also displayed in the console window. An output graph for each data set is displayed after a successful evaluation.



ANALYSIS RESULTS AND OUTPUTS

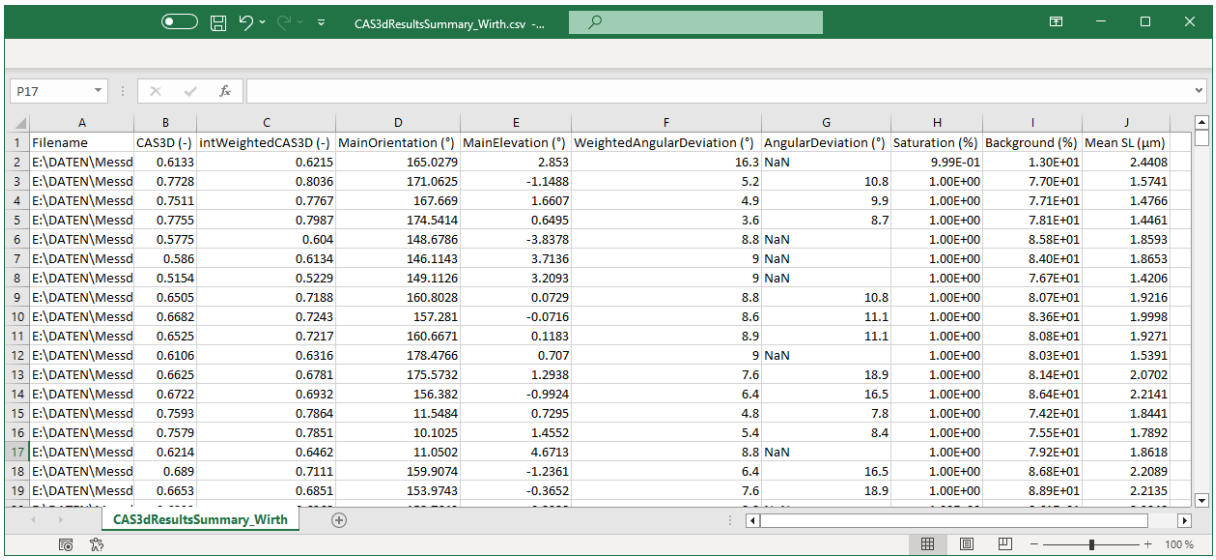
After the evaluation run is complete and all data sets have been processed. A dialogue window informs of the successful completion and the number of warnings during execution. The dialogue also indicates the location of the results log file. When clicking "Browse to folder", a windows file browser window is opened at the location of the log file. The log file is a spreadsheet containing all evaluated parameters of all data sets. An output graph summarizes the results of each analyzed data set. The output graphs are saved alongside the log file in .png and Matlab .fig format. The summary graphs show a histogram of voxel gradient orientation divergence unweighted (A) and weighted by voxel intensity (B). It shows the centre image slice of the data set with the cosine angle of each voxel shown in a false colour scale. Additionally, the key output parameters of the evaluation are listed in the output graph and the file name of the data set is displayed in the window header.



LOG FILE STRUCTURE

The log file, named "CAS3DResultsSummary" is provided as a basic spreadsheet in comma-separated values format(.csv). The separator character is defined by your Windows system settings. Depending on your region settings, the separator may be a different character, such as a semicolon or a tab stop. CSV files are compatible with most spreadsheet software such as Excel, OpenOffice Calc or Google Tables. Each measurement run creates a new log file. In case of duplicate file names, an incrementing four-digit number is appended to the end of the file name.

The log file contains a simple data structure in which one line represents the results of a single volumetric image, and each column represents a descriptor or output value. The Filename column contains the full folder path that points to the evaluated volumetric image and serves as a unique identifier. The evaluated parameters CAS3D, intensity weighed CAS3D (intWeighedCAS3D), dominant Orientation Angle (MainOrientation), dominant elevation angle (MainElevation), mean angular deviation (AngularDeviation), mean intensity weighed angular deviation (WeighedDeviation), and mean sarcomere length (Mean SL) are provided as fixed point values with four-digit precision. The image saturation (Saturation) and image background (Background) are provided as floating point values with four-digit precision.



	A	B	C	D	E	F	G	H	I	J
1	Filename	CAS3D (-)	intWeightedCAS3D (-)	MainOrientation (°)	MainElevation (°)	WeightedAngularDeviation (°)	AngularDeviation (°)	Saturation (%)	Background (%)	Mean SL (µm)
2	E:\DATEN\Messd	0.6133	0.6215	165.0279	2.853	16.3	NaN	9.99E-01	1.30E+01	2.4408
3	E:\DATEN\Messd	0.7728	0.8036	171.0625	-1.1488	5.2	10.8	1.00E+00	7.70E+01	1.5741
4	E:\DATEN\Messd	0.7511	0.7767	167.669	1.6607	4.9	9.9	1.00E+00	7.71E+01	1.4766
5	E:\DATEN\Messd	0.7755	0.7987	174.5414	0.6495	3.6	8.7	1.00E+00	7.81E+01	1.4461
6	E:\DATEN\Messd	0.5775	0.604	148.6786	-3.8378	8.8	NaN	1.00E+00	8.58E+01	1.8593
7	E:\DATEN\Messd	0.586	0.6134	146.1143	3.7136	9	NaN	1.00E+00	8.40E+01	1.8653
8	E:\DATEN\Messd	0.5154	0.5229	149.1126	3.2093	9	NaN	1.00E+00	7.67E+01	1.4206
9	E:\DATEN\Messd	0.6505	0.7188	160.8028	0.0729	8.8	10.8	1.00E+00	8.07E+01	1.9216
10	E:\DATEN\Messd	0.6682	0.7243	157.281	-0.0716	8.6	11.1	1.00E+00	8.36E+01	1.9998
11	E:\DATEN\Messd	0.6525	0.7217	160.6671	0.1183	8.9	11.1	1.00E+00	8.08E+01	1.9271
12	E:\DATEN\Messd	0.6106	0.6316	178.4766	0.707	9	NaN	1.00E+00	8.03E+01	1.5391
13	E:\DATEN\Messd	0.6625	0.6781	175.5732	1.2938	7.6	18.9	1.00E+00	8.14E+01	2.0702
14	E:\DATEN\Messd	0.6722	0.6932	156.382	-0.9924	6.4	16.5	1.00E+00	8.64E+01	2.2141
15	E:\DATEN\Messd	0.7593	0.7864	11.5484	0.7295	4.8	7.8	1.00E+00	7.42E+01	1.8441
16	E:\DATEN\Messd	0.7579	0.7851	10.1025	1.4552	5.4	8.4	1.00E+00	7.55E+01	1.7892
17	E:\DATEN\Messd	0.6214	0.6462	11.0502	4.6713	8.8	NaN	1.00E+00	7.92E+01	1.8618
18	E:\DATEN\Messd	0.689	0.7111	159.9074	-1.2361	6.4	16.5	1.00E+00	8.68E+01	2.2089
19	E:\DATEN\Messd	0.6653	0.6851	153.9743	-0.3652	7.6	18.9	1.00E+00	8.89E+01	2.2135