## **Evaluation software**

The evaluation software for detection (DET), segmentation (SEG), and tracking (TRA) accuracy is written in C++ and released as three command-line executables to help the participants with the self-evaluation and refinement of their algorithms during the training period. The same routines are used to evaluate the submitted results in the competition phase of the challenge. The executables can be run on several Windows, Linux, and Mac platforms and require three mandatory input parameters:

- dir Path to a dataset directory (e.g., Fluo-C2DL-MSC),
- seq Sequence identifier (e.g., 01),
- num\_digits The number of digits used for encoding temporal indices (e.g., 3).

After specifying these parameters, each routine returns the value of a particular measure on the standard output, and creates a log file (DET\_log.txt, SEG\_log.txt, or TRA\_log.txt) in the directory with evaluated results. Note that the software requires the evaluated results to be saved in the format as described in Naming and file content conventions.pdf.

To verify proper functionality of the software, the provided testing directory (the subdirectory "testing\_dataset") can be used. It contains some reference annotations and results for three sequences:

- 01 Example of 2D results for the sequence 01 from the Fluo-C2DL-MSC dataset, dir=testing\_dataset, seq=01, num\_digits=3, SEG measure: 0.232874,
- 02 Example of 3D results for the sequence 01 from the Fluo-C3DL-MDA321 dataset, dir=testing\_dataset, seq=02, num\_digits=3, SEG measure: 0.443686,
- 03 Image data that corresponds to Figure 2 in <a href="http://dx.doi.org/10.1371/journal.pone.0144959">http://dx.doi.org/10.1371/journal.pone.0144959</a>, dir=testing\_dataset, seq=03, num\_digits=3, TRA measure: 0.622980, dir=testing\_dataset, seq=03, num\_digits=3, DET measure: 0.688000.

It is important to note that the DET evaluation software accepts the fourth, optional input parameter, penalize\_extra\_detections. This parameter governs how all extra detected objects are dealt with. Its default value is 1, leading to penalization of all extra detected objects. By contrast, these objects are not penalized at all after setting penalize\_extra\_detections to 0:

dir=testing\_dataset, seq=03, num\_digits=3, penalize\_extra\_detections=0, DET measure: 0.700000.

The non-default option is used only for evaluating segmentation-only results for the Fluo-N3DL-DRO, Fluo-N3DL-TRIC, and Fluo-N3DL-TRIF datasets.

## Windows users

The 64-bit executables for Windows users can be found in the subdirectory "Win", including mandatory dll files. They were dynamically built using the Microsoft Visual Studio C++ 2017 compiler. Thus, please make sure that the Microsoft Visual Studio 2017 Redistributable (x64) package has been installed on your machine before running the executables. Without this package installed, the executables will not run properly, producing no output. They may even complain about a missing msvcp140.dll file. The software was tested on workstations running Windows 7, Windows 8, and Windows 10 operating systems.

## Linux users

The 64-bit executables (statically linked ELF 64-bit LSB executable) for Linux users can be found in the subdirectory "Linux". They were statically built using the Ubuntu GCC-7.5.0 compiler. The software was tested on workstations running Ubuntu 18.04.3 LTS, Red Hat Enterprise Linux Server release 7.8 (Maipo), and CentOS Linux release 7.8.2003 (Core) operating systems.

## **Mac users**

The 64-bit executables for Mac users can be found in the subdirectory "Mac". They were dynamically built using the Apple LLVM-8.0 compiler. The software was tested on workstations running Mac OS X 10.10.5 (Yosemite), 10.11.6 (El Capitan), 10.12.6 (Sierra), and 10.13.6 (High Sierra) operating systems.