Fluctuation Statistics – User Guide

Malte Wachsmuth

Cell Biology and Biophysics Unit
European Molecular Biology Laboratory
Meyerhofstrasse 1
D-69117 Heidelberg, Germany
E-mail: malte.wachsmuth@embl.de

Christian Conrad

Theoretical Bioinformatics
German Cancer Research Center/BioQuant
Im Neuenheimer Feld 267
D-69120 Heidelberg, Germany
E-mail: c.conrad@dkfz-heidelberg.de

Introduction

The Fluctuation Statistics software tool allows to statistically analyze and summarize large-scale fluorescence auto- and cross-correlation spectroscopy (FCS/FCCS) data. It reads results files from the accompanying Fluctuation Analyzer software, applies required calibrations, normalizations and corrections and creates HTML-formatted report sheets. It also allows to interactively explore the biophysical parameters extracted from larger numbers of cellular FCS/FCCS measurements.

It is developed as a National Instruments LabVIEW project to run on Microscoft Windows XP, Vista, 7 and 8 operating systems where LabVIEW 2010 SP1 (or higher) is installed. However, we cannot guarantee any compatibility and we exclude any liability. The code is provided as is.

Installation and start

Unpack the downloaded package file to a directory of choice. The “FluctuationStatistics” folder contains the project file “FluctuationStatistics.lvproj” that you must open with LabVIEW. Double-click “Main.vi” in the LabVIEW project manager and press the arrow button to start execution.

How to work

Upon start, the window shows up as illustrated in **Figure 1**.

1. Here, you can select the **Root path**. The software then searches for subdirectories, which contain Fluctuation Analyzer results files with the name given in **Results file** 2. In addition, the subdirectory names contain the pattern defined in **Clone ID** 3
4. For additional information about this clone, construct or condition and for automatically processing a larger number of **Clone IDs**, a **Clone info file** can be selected. An example file is provided with the installation
5. Next, a list of **Sessions** is generated, each defined by the names of the last two hierarchical directory levels, the latter of which must contain the **Clone ID** 4. If necessary, click **Update session list** 6
7. When you click **Update table** or when you change the selection in the **Session** list 5, the **Statistics overview** table 8 containing a set of biophysical and biochemical parameters in two versions, as raw value and as value corrected for photobleaching, background and crosstalk, for two cellular localization classes, **Nuclear** and **Cytplasmic**, is filled with mean values (ave) and an error (sd), either the **Standard Deviation** or the **Standard Error**, depending on the selection in **error mode** 9
10. As quality control or **Selection criteria**, two columns of the Fluctuation Analyzer results file for the auto- and one for the cross-correlation results can be taken. Typically, the adjusted R squared value must exceed a certain threshold as quality control criterion for the goodness of the fit and thus the noise of the correlation curves. Also, the bleaching correction factor must exceed a certain threshold to rule out slow cellular movements resulting in increasing fluorescence signal during acquisition. In general, any column head and corresponding threshold value can be used here.
11. In order to transform numbers of molecules and diffusion times into concentrations and diffusion coefficients, the **Focal dimensions** must be known. The values here are read from the “Focus.txt” file in the installation directory. They can be changed manually after starting the program or programmatically by changing the content of ”Focus.txt”
12. In addition to the **Crosstalk correction** implemented as correction factors in the Fluctuation Analyzer, an alternative and more accurate crosstalk correction can be performed by activating **Manual** and providing a **Crosstalk Ch1 -> Ch2** factor
13. In order to compute and visualize the statistical properties of the parameters shown in table 8, two parameters can be selected in the table that are highlighted in red and blue. When changing the selection in the table or when clicking **Update histograms**, the distributions of the selected parameters across all measurements are shown in 14 and 15. The binning can be changed by changing the **Bar height** parameter 16
17. In addition to the mean values and standard deviations in 14 and 15, some **percentiles** are also provided
18. This scatter plot shows the correlation between the two selected parameters. Obviously, this cannot be done on the basis of single measurements but is determined on the basis of single cells, i.e., each circle represents an individual cell. Correlation coefficients are also caculated.
19. Using the information from the **Session** results files as described above and from the **Clone info file** 4, a predefined selection of histograms and scatter plots is generated and compiled to an HTML report sheet together with the table in the directory defined in **Output path** 21
20. When clicking **Create clone list reports**, for the whole list of Clone IDs provided in the **Clone info file** 4 the HTML report sheets are generated

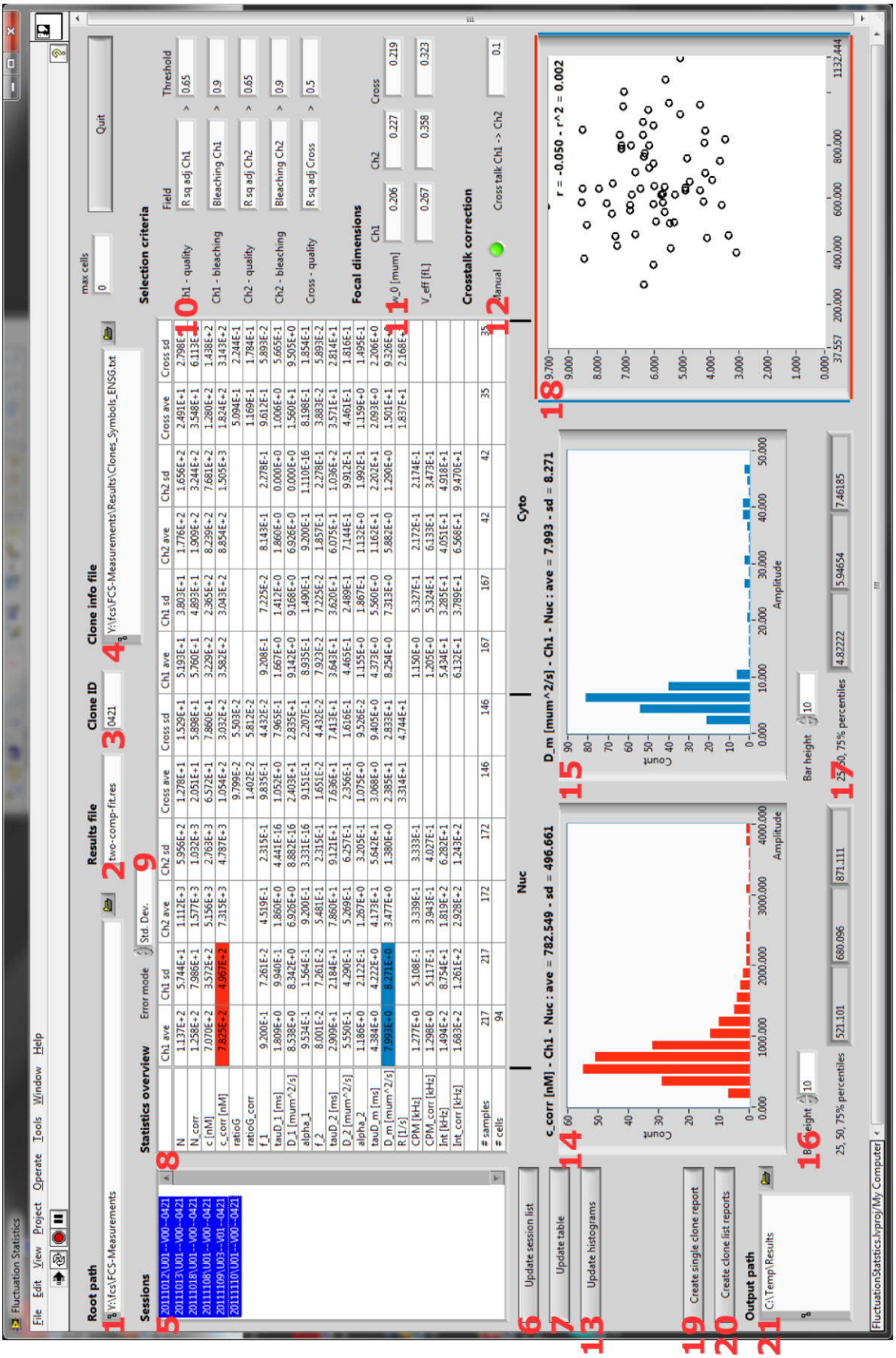

Figure 1: Fluctuation Statistics main window