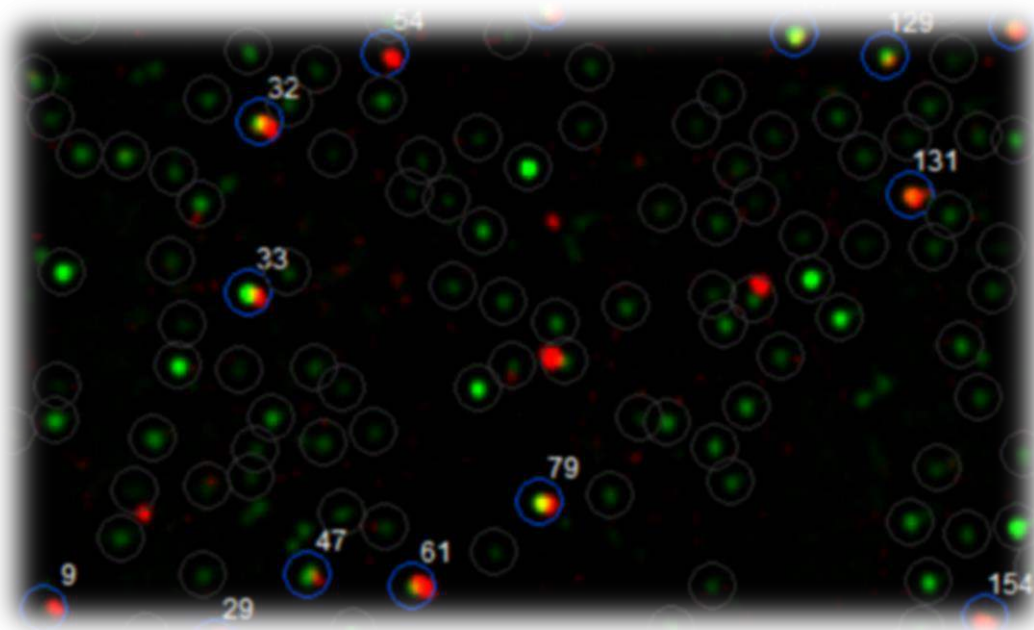# FluoroTensor v6.6.8r

Quick-Start Reference Guide

Introduction

This document is not a substitute for the full user guide. The purpose of this quick-start reference guide is to point new users in the direction of the right chapters and subchapters to read in order to get started immediately without needing to read the whole of the full guide. Having said this, we recommend beginner and expert users alike should take the time (approx. 1 - 2 hours) to read the full user guide and familiarise themselves with the functions the program has to offer. A full understanding of the program is not necessary for routine use but encouraged such that users have a firm grasp of the potential features and limitations of the software. The Tracking program can be considered a separate system from the colocalization and step detection program. Thus, users that wish to focus on Colocalization and step detection need not read this information. Likewise, Users that wish only to use single-particle tracking may ignore the rest of the guide.

The full user guide is laid out logically in chronological way in the order that analysis should be carried out in the program. The full user guide documents the functions of the software in more detail than is needed for routine analysis. Thus, this guide is intended as reference for which chapters and subsections are required knowledge to perform routine analysis.

Chapter 1 – Getting Started

Chapter 1 in the full user guide covers requirements and prerequisites as well as how to run the program and should be read in full before use to avoid surprises with setup and compatibility.

Calibration

Before beginning an experiment, the software should be calibrated to ensure chromatic aberration is corrected.

Read **Chapter 3**, sections **3.2** and **3.3** in the full user guide to familiarise yourself with the method for calibration. Sections **3.4** and **3.5** are optional but should be covered if using automatic solving of the chromatic aberration correction parameters.

Colocalization

This section covers how to get straight into analysing raw .tif movies, extracting the traces of colocalized spots in different channels and analysing the step distributions.

Read **Chapter 2**, sections **2.1**, **2.2**, and **2.3** to learn how to open the raw data analysis interface and load a TIF file. To view the raw movie to find the frame boundaries of the channels and ensure the fluorophores are in the right channels, read section **2.4**. Read sections **2.7** and **2.12** to learn how detect spots automatically and calculate the traces of colocalized molecules. Repeat the steps from section **2.3**, **2.4**, **2.7**, and **2.12** for all the files from the same dataset you wish to analyse.

Step Detection & Trace Fitting

Read **Chapter 5**, sections **5.1** and **5.2** to learn how to import traces from the raw data analysis window into the main interface and understand its layout. Sections **5.4** and **5.5** cover how to use AI accelerated step detection and trace fitting.

Exporting Data

Read **Chapter 6**, section **6.1** to learn how to export the analysed data to a preformatted Excel spreadsheet. Sections **6.2** through **6.6** cover in detail all of the information that is exported. For step distributions and statistics read sections **6.3** and **6.5**.

Automation

To learn how to automate analysis of multiple TIF files Read **Chapter 4**. Section **4.2** covers quality control to select only data that is in focus and clean for automation. For examples of experiments utilising automated colocalization analysis, see **Chapter 7**

Tracking

Read **Chapter 8**, sections **8.1**, **8.2**, and **8.3** to learn how to launch the TrackXpress add-in for single-molecule tracking from the main interface, the layout of the GUI and how to load a TIF file into the interface. Read sections **8.5**, **8.7**, and **8.8** to learn how to set up the parameters for particle detection and tracking. Read section **8.9** to track the particles in the movie.

MSD Fitting and Diffusion Analysis

Read **Chapter 9**, section **9.1** to fit the MSDs of each track in the dataset. Section **9.5** covers how to plot the diffusion coefficient distribution of the dataset. Section **9.6** covers how to load several tracking experiment files and pool the results into a single diffusivity distribution. Section **9.7** covers how to export the fitted diffusivity distribution into an Excel spreadsheet.

Chapter 10 details how to export tracking results including coordinates and other time series of each track into an Excel Spreadsheet. Sections **10.2** and **10.4** cover how to save and load the entire interface, settings, data, and analysis to and from the 'Tracking Experiment' (.txp) file format. Section **10.7** covers how to save figures from the interface. **Chapter 11** details how to set up and run automated tracking on a set of raw data files.