Shape-Out 2 Documentation

Release 2.0.3

Paul Müller and Christoph Herold

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Shape-Out 2

Shape-Out 2 is the successor of Shape-Out, a user interface for data analysis in real-time deformability cytometry (RT-DC). This is the documentation of Shape-Out version 2.0.3.

This documentation is also available as a PDF.

CHAPTER 1

Getting started

1.1 Installation

Shape-Out 2 can be installed via multiple channels.

1. **Windows installer:** Download the latest version for your architecture (e.g. `Shape-Out_X.Y.Z_win_64bit_setup.exe` for 64bit Windows) from the official release page.
2. **macOS:** Download the latest version (`Shape-Out_X.Y.Z.dmg` or `Shape-Out_X.Y.Z.pkg`) from the official release page.
3. **Python 3.6 with pip:** Shape-Out can easily be installed with pip:

   ```
   pip install shapeout2
   ```

   To start Shape-Out, simply run `python -m shapeout2` or `shapeout2` in a command shell.

1.2 Update

Shape-Out automatically searches for updates (you may opt-out via the Help menu) and notifies the user when a new version is available.

1. **Windows installer:** The older version of Shape-Out will be automatically uninstalled when installing a new version.
2. **macOS:** The older version of Shape-Out will be automatically uninstalled when installing a new version.
3. **Python 3.6 and pip:**

   ```
   pip install --upgrade shapeout2
   ```

CHAPTER 2

RT-DC Basics

This section conveys the basic understanding necessary for analyzing and interpreting RT-DC data. If you have the feeling that something is not covered here, please create an issue on GitHub.

2.1 Working Principle

In Real-time deformability cytometry (RT-DC), small objects, such as cells or beads, are flushed through a narrow channel at high speeds. The flow profile inside the channel causes hydrodynamic stresses that deform these objects [MOG+15], [MMM+17]. For each event (detected object), an image is recorded and analyzed in real-time [ORM+15]. The analysis consists of the extraction of several features from the event images, such as deformation, projected area, or average brightness. These features can then be used to identify and characterize sample populations; For instance, major blood cells and their pathological changes in disease conditions [THO+17].

2.2 Measured Features

A multitude of features can be extracted from the data recorded during an RT-DC measurement. These features are mostly computed live during data acquisition and are stored alongside the raw image data. Here, only the most important features are discussed. A full list of the features available in Shape-Out is maintained in the dclab documentation.

2.2.1 Area and porosity

The area is the projected object area which is determined via the contour of the binarized event image. Shape-Out differentiates between two types of area, area of the measured contour ("Measured area [px]") and area of the convex contour ("Convex area [px]" and "Area [µm^2]"). The convex contour is the convex hull of the measured contour and enables a quantification of porosity (convex to measured area ratio). The porosity is often used for filtering, e.g. to *remove high-porosity dirt particles in a preprocessing step*.

[1] *Detection Of Human Disease Conditions By Single-Cell Morpho-Rheological Phenotyping Of Whole Blood* by Toepfner et al., licensed under CC BY 4.0 [THO+17].

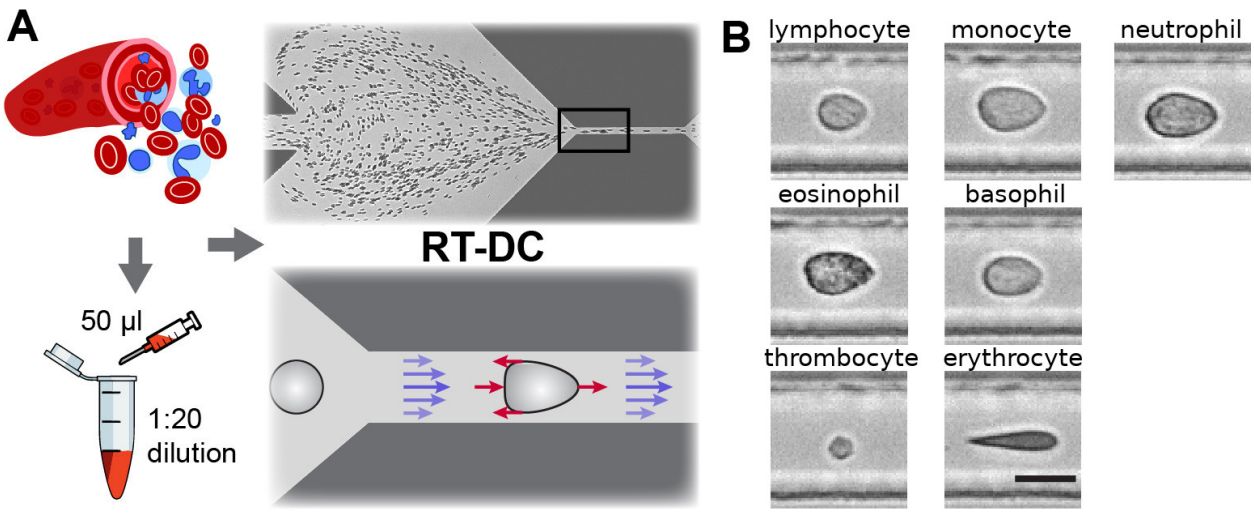


Fig. 2.1: Single-cell, morpho-rheological phenotyping of blood. (A) Analysis of whole, diluted blood. Hydrodynamic shear forces (red arrows) induce deformation of cells passing through a microfluidic channel (20x20 µm^2) at speeds of more than 30 cm/s (blue arrows). (B) Representative images of blood cell types acquired. Scale bar is 10 µm. Figure and caption adapted from Toepfner et al.[1].

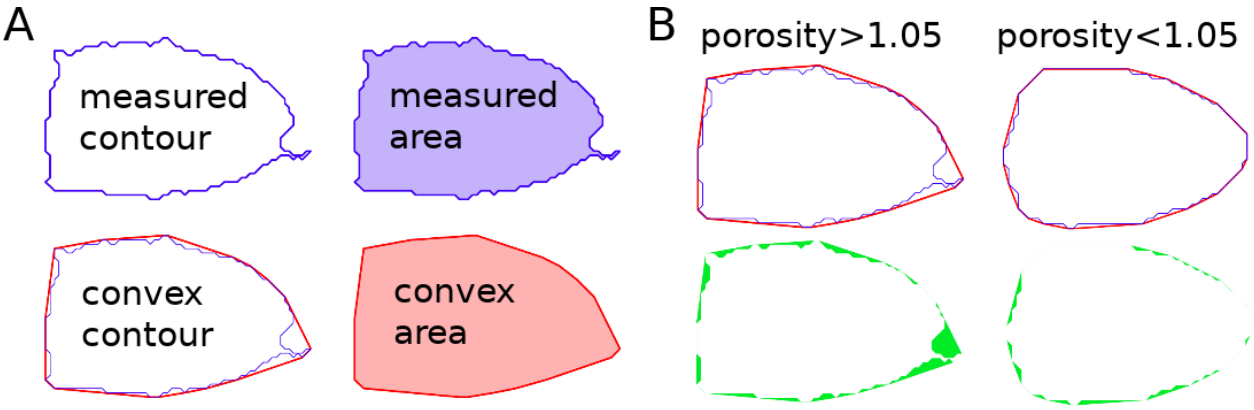


Fig. 2.2: Visualization of porosity. (A) The measured contour (blue line) defines the measured area (blue shade). The convex contour (red line) results in an area (red shade) that is usually larger than the measured area. (B) The porosity is the ratio between measured and convex contour. The difference (the "pores") between the measured and convex areas is indicated in green. Porosity is often used to remove events with non-physical contours, e.g. for cells all events with a porosity above 1.05.

A porosity of 1 means that the measured contour is convex. Note that the porosity can only assume values larger than 1. Also note that the convex contour/area is computed on the same pixel grid as the measured contour/area and is, as such, subject to pixelation artifacts.

2.2.2 Bounding box

The bounding box of an event image is the smallest rectangle (with its sides parallel to the x and y axes) that can hold the event contour. The aspect ratio of the bounding box is the rectangle's side length along x divided by the side length along y. The size of the bounding box along x and y as well as its aspect ratio are often used for filtering.

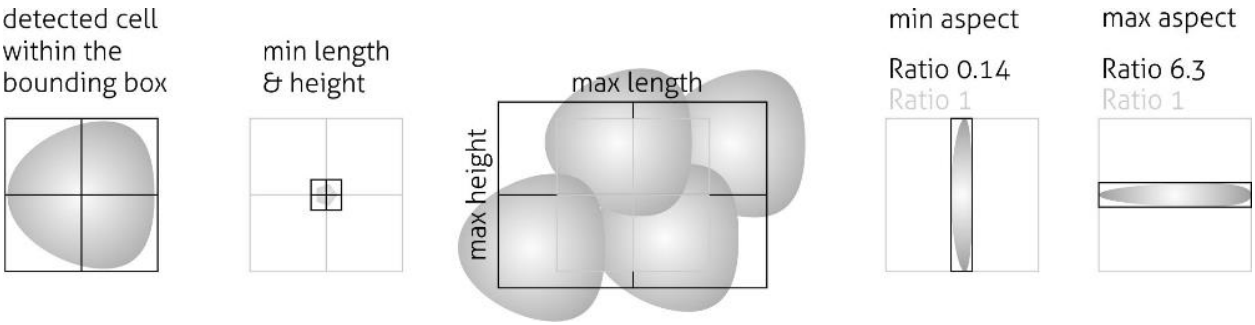


Fig. 2.3: Illustration of the event bounding box and its use cases. From left to right: definition of the bounding box, exclusion of small objects (e.g. debris) via the bounding box size, exclusion of clusters via the bounding box size, exclusion of objects elongated perpendicular to the channel axis, exclusion of objects elongated along the channel axis.

2.2.3 Brightness within contour

Quantifying the brightness values within the image contour yields information on object properties such as homogeneity or density. For instance, it has been shown that the quantities "mean brightness" and "convex area" are sufficient to identify (and count) all major blood cells in a drop of blood [THO+17].

In addition to the average brightness values, Shape-Out also has access to the standard deviation of the brightness in each image.

2.2.4 Deformation and elasticity

The deformation describes how much an event image deviates from a circular shape. It is defined via the circularity:

$$\begin{aligned} \text{deformation} &= 1 - \text{circularity} \\ &= 1 - 2\sqrt{\pi A}/l \end{aligned}$$

with the projected area A and the contour length of the convex hull of the event image l. The contour length is computed from the *convex* hull to prevent an overestimation due to irregular, non-convex event shapes. It has been shown that the knowledge of deformation and area allows to derive a value for elasticity in RT-DC [MOG+15] [MMM+17]. As a convenient measure for elasticity, isoelasticity lines are often employed to visualize stiffness.

Note that it is also possible to directly *access the Young's modulus in Shape-Out*.

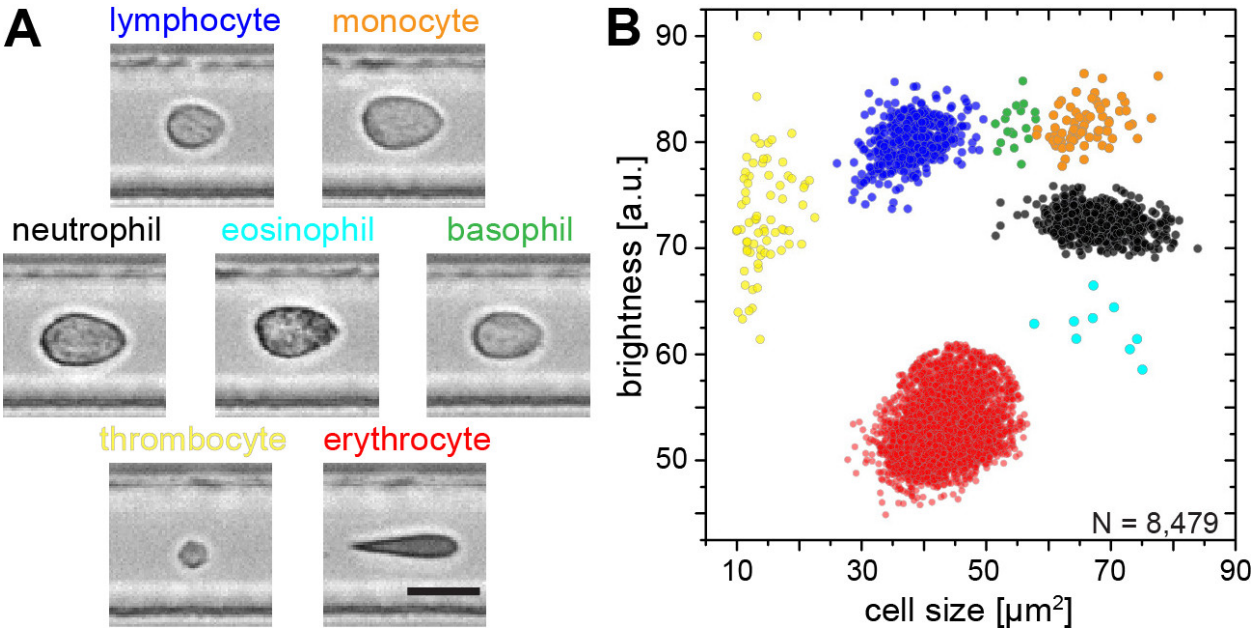


Fig. 2.4: Blood-classification with event brightness and cell size. (A) Representative images of blood cell types acquired. Scale bar is 10 µm. (B) Brightness versus cell size (area) scatter plot with cell types labeled according to the color scheme in (A). Figure and caption adapted from Toepfner et al.[1].

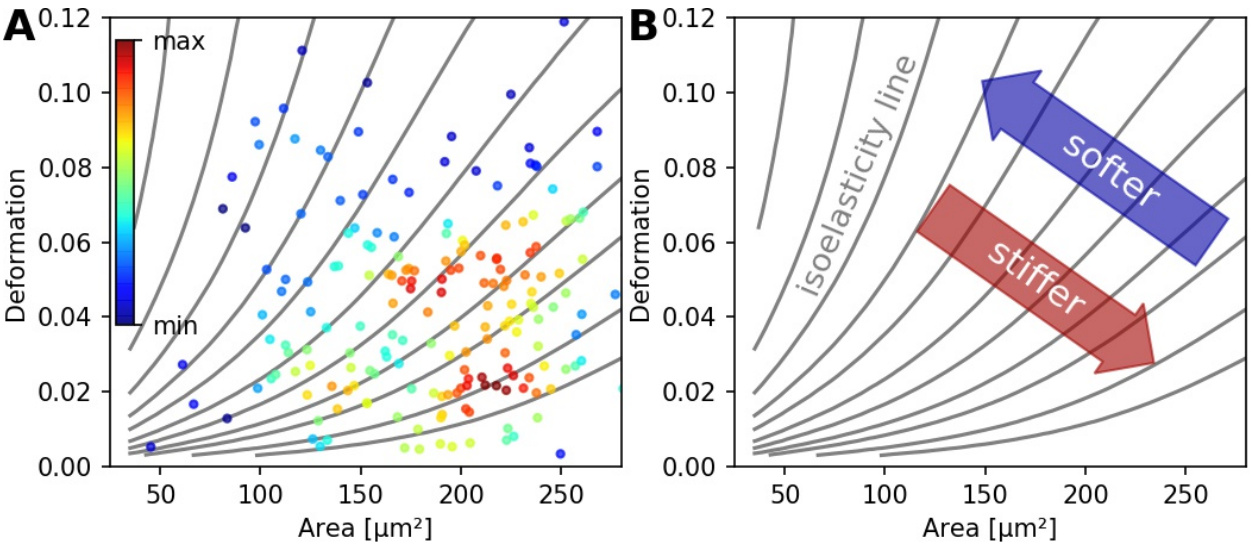


Fig. 2.5: (A) Typical deformation versus cell size scatter plot. The color scale indicates event density. (B) Isoelasticity lines derived from numerical simulations indicate trends in stiffness.

2.2.5 Fluorescence

Real-time fluorescence and deformability cytometry (RT-FDC) records, in addition to the event images, the fluorescence signal of each event [RPJ+18]. The raw fluorescence data consists of the one-dimensional fluorescence intensity trace from which features such as peak fluorescence or peak width can be computed. For more advanced applications, RT-FDC also supports multiple fluorescence channels.

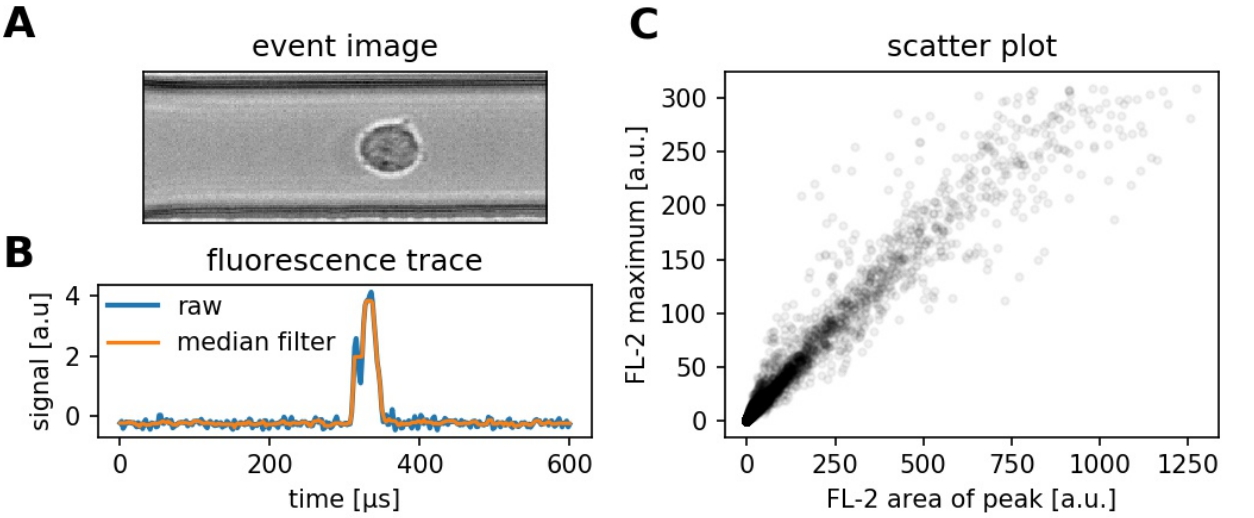


Fig. 2.6: (A) Event brightfield image. (B) Fluorescence trace of the event. The raw fluorescence data and the fluorescence data filtered with a rolling median filter (from which the parameters are computed) is shown. (C) Scatter plot of two parameters extracted from the fluorescence trace.

2.2.6 Inertia ratio

The inertia ratio is the ratio of the second order central moments along x and y computed for the event contour. Thus, the inertia ratio is a measure of deformation. In comparison to deformation, the inertia ratio has a low correlation to porosity. Shape-Out also allows to compute the principal inertia ratio which is the maximal inertia ratio that can be obtained by rotating the contour. Thus, the principal inertia ratio is rotation-invariant which makes it applicable to reservoir measurements where e.g. cells are not aligned with the channel. To quantify the alignment of the measured objects with the measurement channel, Shape-Out can additionally quantify the tilt of the contour relative to the channel axis.

2.2.7 Volume

Shape-Out can compute the volume from the event contour under the assumption of rotational symmetry, i.e. it is assumed that the projection of the cell volume onto the detector plane does not change when the cell is rotated, with a rotational axis parallel to the flow direction. The computation of the volume is based on a full rotation of the upper and the lower halves of the contour from which the average is then used [HWT02]. Volume has the advantage to be less correlated to deformation when compared to the projected area and it is therefore a better measure of cell size in the channel.

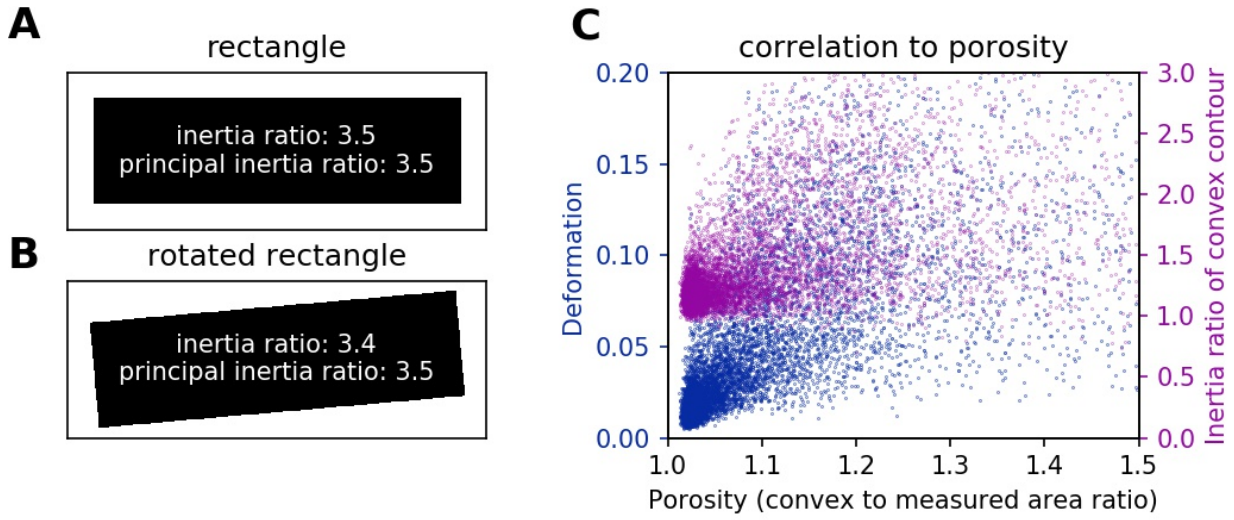


Fig. 2.7: (A) For a rectangle that is aligned with the coordinate axes, the inertia ratio and the principal inertia ratio are identical. (B) If the rectangle is rotated, the inertia ratio changes, but the principal inertia ratio does not. (C) Comparison between deformation and inertia ratio when plotted against porosity for an exemplary RT-DC experiment. Deformation exhibits a higher correlation to porisity.

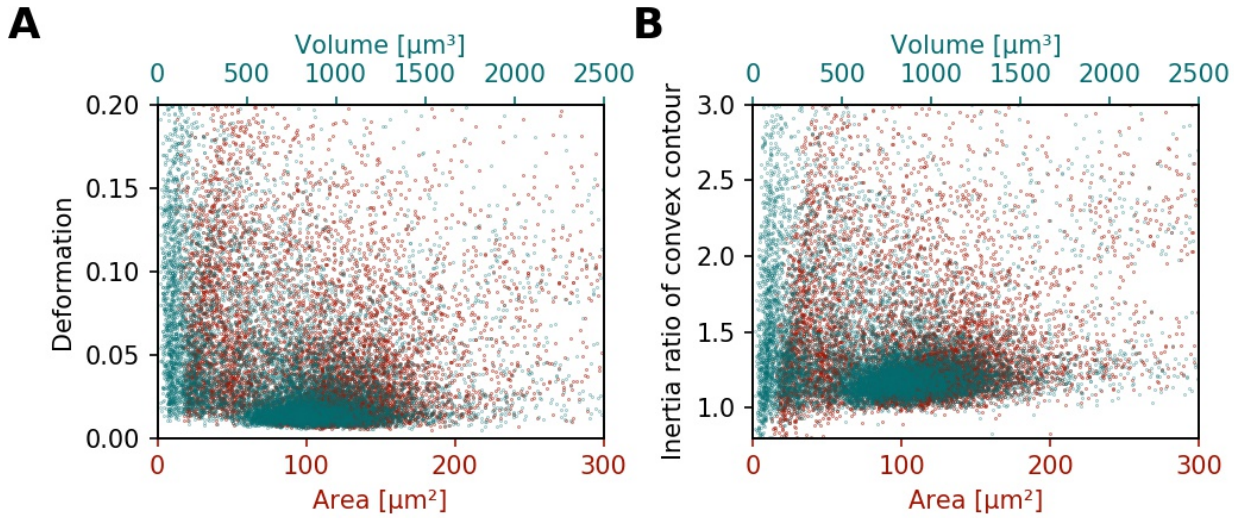


Fig. 2.8: (A) Deformation versus area (red) and volume (cyan) for an exemplary RT-DC dataset. There is a correlation between area and deformation, at least for small (<50µm^2) areas. (B) Inertia ratio versus area and volume. A correlation between inertia ratio and area is visible as well.

CHAPTER 3

User Interface

3.1 Differences to Shape-Out 1

Shape-Out 2 is a complete rewrite of the user interface from scratch. The overall user experience is much better compared to Shape-Out 1. The main reasons for the development of Shape-Out 2 were:

- The graphical user interface (GUI) of Shape-Out 1 was designed to be flexible with regard to new configuration parameters. While this played well for the beginning of RT-DC development, the GUI now appears not very thought-out for a mature measuring technique. The workflow of Shape-Out 2 has been designed to meet most of its users needs.
- Shape-Out 1 is condemned to Python 2 (which reached its end of life January 1st 2020), because it relies on the combination of chaco (for plotting) with wxPython 3 (for the GUI). Chaco is now focusing on PyQt and thus does not support the new wxPython 4. However, wxPython 3 does not support Python 3, so we are stuck with Python 2.
- For Shape-Out 1, there appeared a weird issue which is probably related to the old wxPython library and some other software installed on some Windows systems.

Both versions of Shape-Out rely on dclab which provides the core functionalities for RT-DC data analysis. There are, however, a few breaking changes that might prevent you from moving your data analysis from Shape-Out 1 to Shape-Out 2:

- Shape-Out 2 does not anymore support the .tdms file format. If you would like to use .tdms data in Shape-Out 2, you have to convert those data to the .rtdc file format first. You can do just that with DCKit, which provides a convenient GUI for several other RT-DC data management tasks as well. The .rtdc file format is faster, occupies less space on disk, and consists of only one file per measurement (no more para.ini, etc.).
- Shape-Out 1 sessions cannot be opened in Shape-Out 2. This is mostly caused by the different approach to data analysis. In principle, it could be possible to convert sessions (including the corresponding .tdms files), but the effort in doing so would probably exceed the effort required to just rebuild a clean analysis session in Shape-Out 2.
- Shape-Out 2 does not provide a linear mixed effects models (LMM) analysis. LMM analysis in Shape-Out 1 is done using R/lme4 and thus requires a full R distribution shipped with Shape-Out 1. This blows up the installation size and makes it more difficult to deploy. Furthermore (and we are not saying that LMM Analysis

is “bad”) we are also looking into other methods for determining statistical significance which might be more intuitive to understand.

3.2 Terminology

Shape-Out 2 introduces several terms in the user interface that also play a role in data analysis and are consequently used in the entire code base.

block matrix The *Block Matrix* is a visualization of the analysis pipeline. It is divided into data matrix (filters) and plot matrix (plots).

dataslot or slot A slot holds all information about a measurement: the path to the .rtdc file, fluorescence labels, display color, etc. (see the *Dataset* tab in the *Analysis View*).

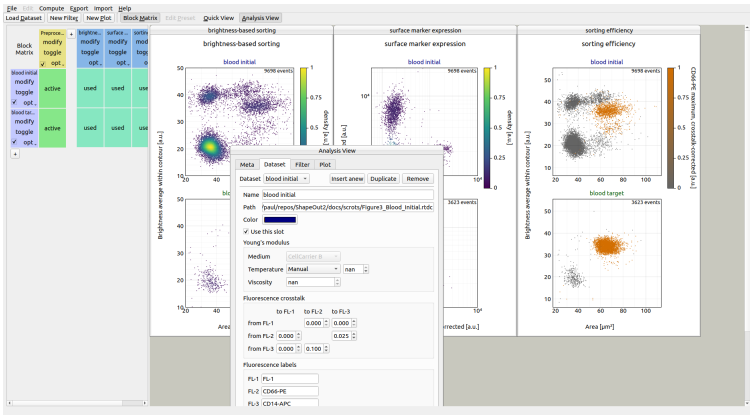
filter A filter is a set of filtering options (box filter, polygon filter, downsampling, etc.) that can be applied to a slot (see the *Filter* tab in the *Analysis View*). Filters can be exported and imported again in Shape-Out 2 (.sof file format).

filter ray A filter ray is a list of filters that can be applied to a slot. In Shape-Out 2, each row in the *Block Matrix* contains one filter ray.

pipeline A pipeline consists of all filters, slots, plots, and the filter rays (the filter selection) applied to each slot. A Shape-Out 2 session file (.so2) stores all information necessary to rebuild a pipeline.

plot A plot is a user-defined visualization of a slot/filter ray combination.

3.3 Basic usage

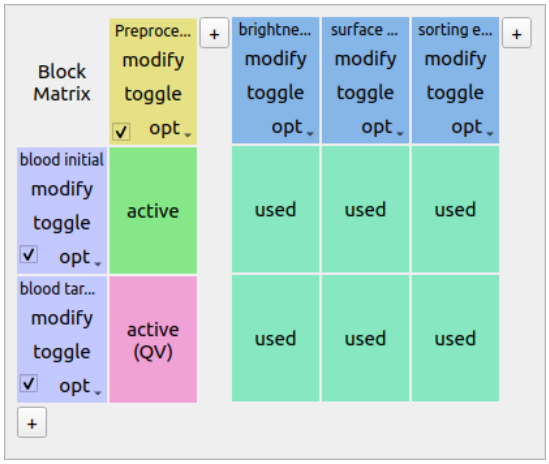


The user interface is split into several parts: the menu bar and the tool bar at the top, the Block Matrix on the left, and the Workspace on the right (example data taken from [NUH+19a], [NUH+19b]).

3.3.1 Menu bar and tool bar

The menu bar is used for session management (File menu) and makes additional functionalities available, such as data statistics, data export, plot export, or filter import/export. The tool bar contains shortcuts for adding new datasets, filters, or plots (left part) and allows to hide/show the *Block Matrix* as well as the *Quick View* and *Analysis View* windows (right part).

3.3.2 Block Matrix



The Block Matrix gives an overview of the current analysis pipeline. Each row represents a dataset (purple). The columns represent either filters (yellow) or plots (blue) of your pipeline.

You can perform dataset operations in the purple rectangular area at the beginning of each row: To modify a dataset, click on the *modify* button. You can duplicate, insert anew (unmodified), or remove datasets using the dropdown menu. You can also exclude a dataset from an analysis via the check box.

Filters can also be modified, copied, removed and disabled. By default, all filters are disabled when they are created. To apply a filter to a dataset, click on the corresponding matrix element. The element changes its color from gray (incactive) to green (active). In Shape-Out, all filters that are applied to a dataset are called a **filter ray**. In the above example, the filter ray only consists of a single filter for each dataset. Filter rays may be different for each dataset.

By holding down the *Shift* key while clicking on a matrix element, you can activate the *Quick View* for the specific dataset (with filters applied up until the selected column). The block matrix element is then colored pink.

To add a plot, click on the *New Plot* button in the tool bar. This adds a plot column with a blue header to the Block Matrix and creates an empty plot window. You can add datasets to your plot by clicking on the corresponding matrix elements. In the above example, both datasets are being used in all three plots.

The modification of datasets, filters, and plots is discussed below.

3.3.3 Workspace

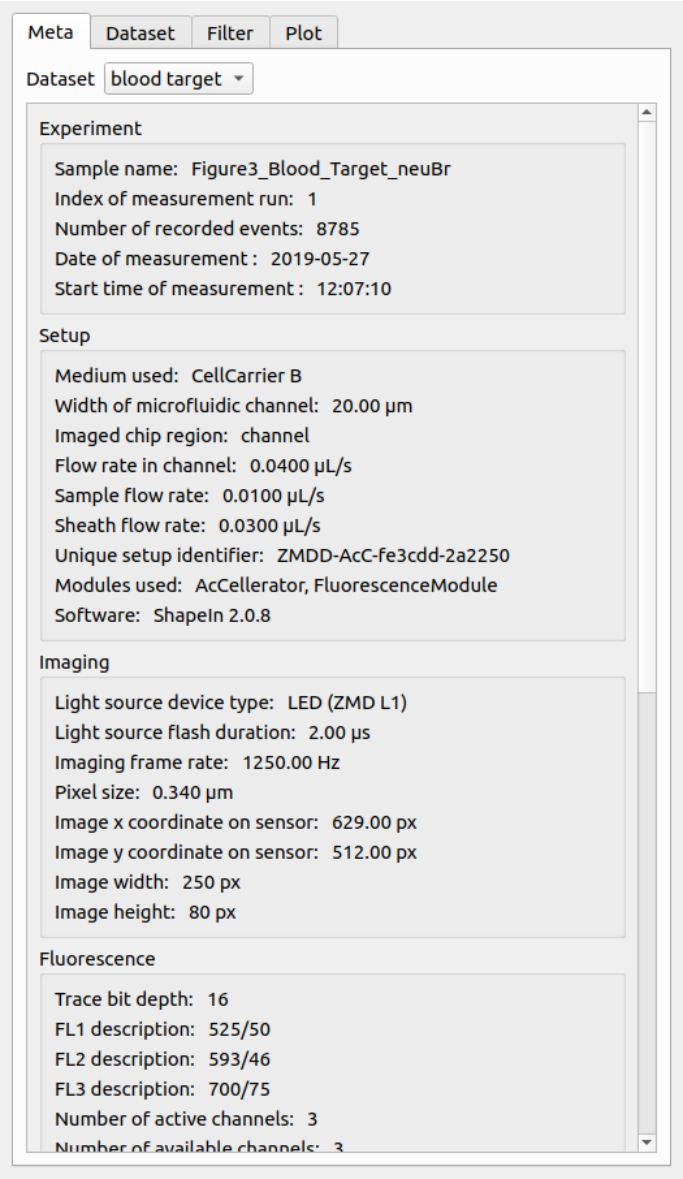
The Workspace is designed as an infinite scrollable area and contains all plot windows as well as the *Quick View* and Analysis View windows.

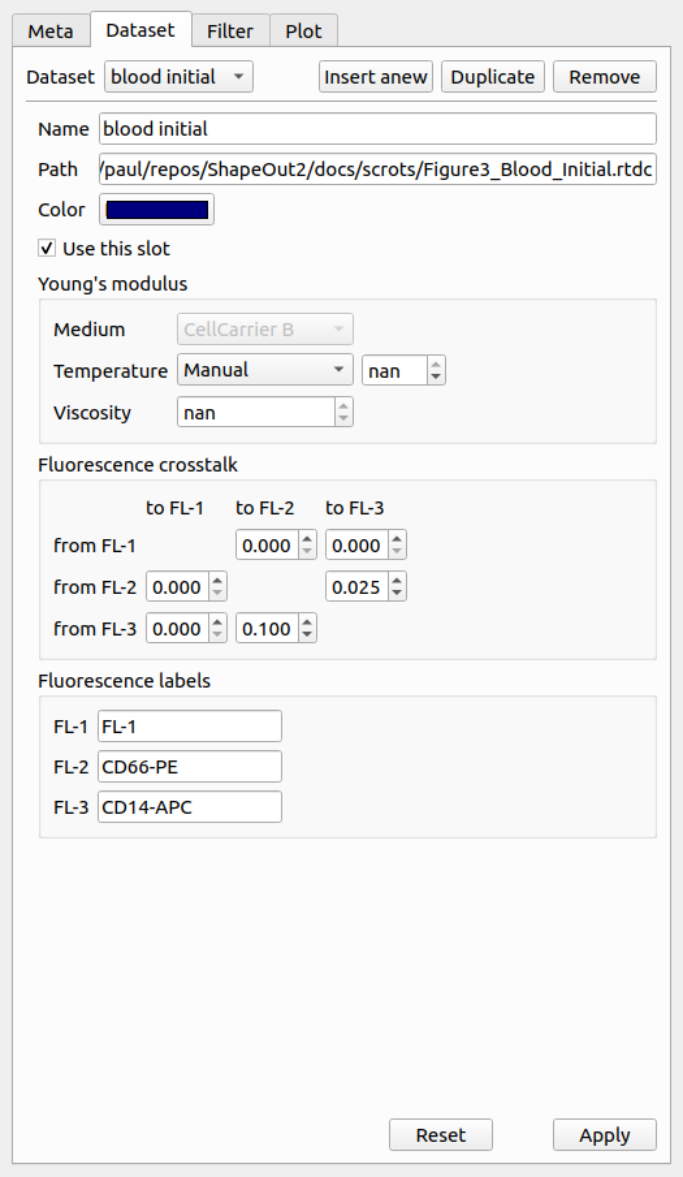
3.3.4 Analysis View

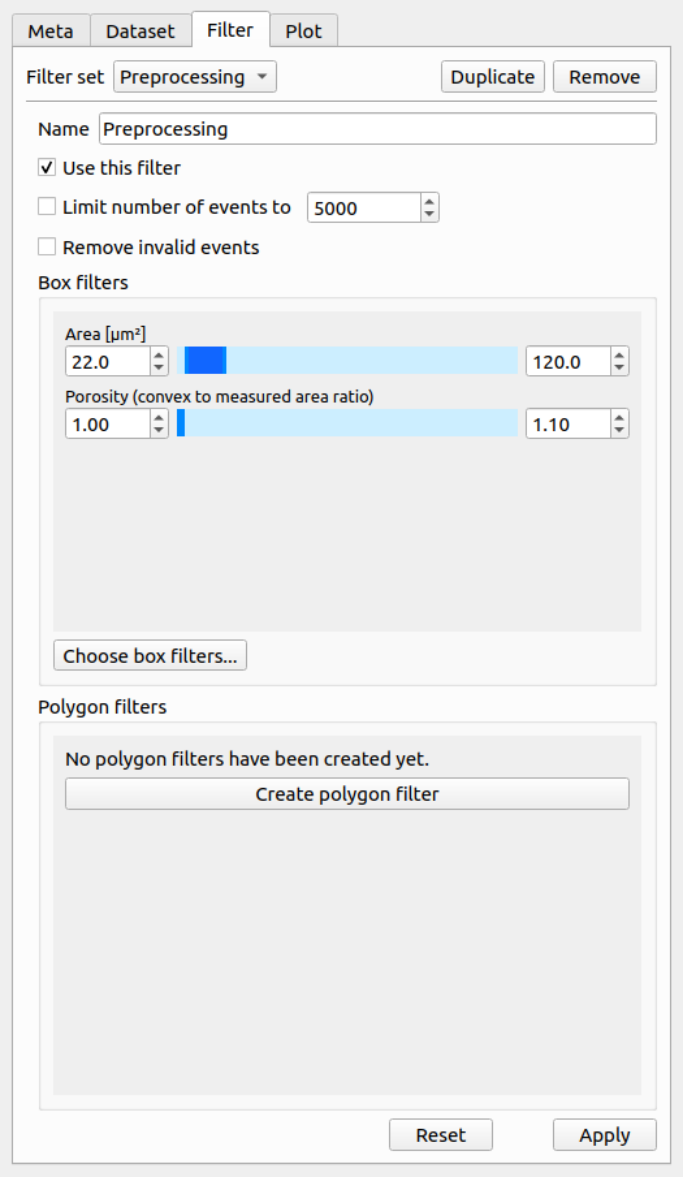
The analysis view is separated into four tabs (see screenshots below).

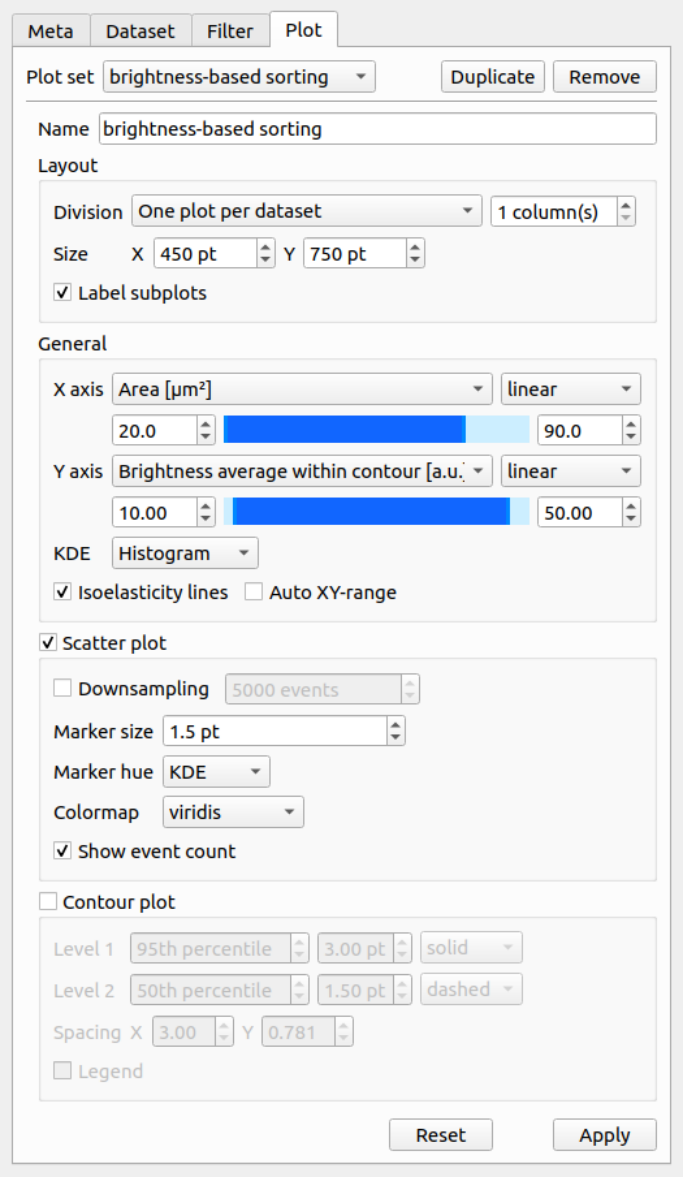
- The **Meta** tab displays all metadata of the selected dataset that is stored in the original .rtdc file.
- The **Dataset** tab allows to specify additional metadata, such as unique colors used for plotting and additional metadata for computing the Young's modulus or correcting for fluorescence cross-talk. It also allows to specify fluorescence channel labels that will then be used for labeling the axes of plots.
- The **Filter** tab is used to modify the filters of the pipeline. New box filters can be added by selecting *Choose box filters...*. Polygon filters are created in the *Quick View* window.

- The **Plot** tab allows to specify all plotting parameters. Please take special note of the *Division* option in the *Layout* section (defines the arrangement of the subplots) and the *Marker hue* option in the *Scatter plot* section (allows you the specify whether the scatter data points are colored according to a kernel density estimate (KDE), another feature dimension, or the dataset color specified in the *Dataset* tab). In this example, contour plots are not used.




Meta
Dataset
Filter
Plot
Dataset
blood initial
Insert anew
Duplicate
Remove
Name
blood initial
Path
/paul/repos/ShapeOut2/docs/scrots/Figure3_Blood_Initial.rtdc
Color
Use this slot
Young's modulus
Medium
CellCarrier B
Temperature
Manual
nan
Viscosity
nan
Fluorescence crosstalk
to FL-1
to FL-2
to FL-3
from FL-1
0.000
0.000
from FL-2
0.000
0.025
from FL-3
0.000
0.100
Fluorescence labels
FL-1
FL-1
FL-2
CD66-PE
FL-3
CD14-APC
Reset
Apply

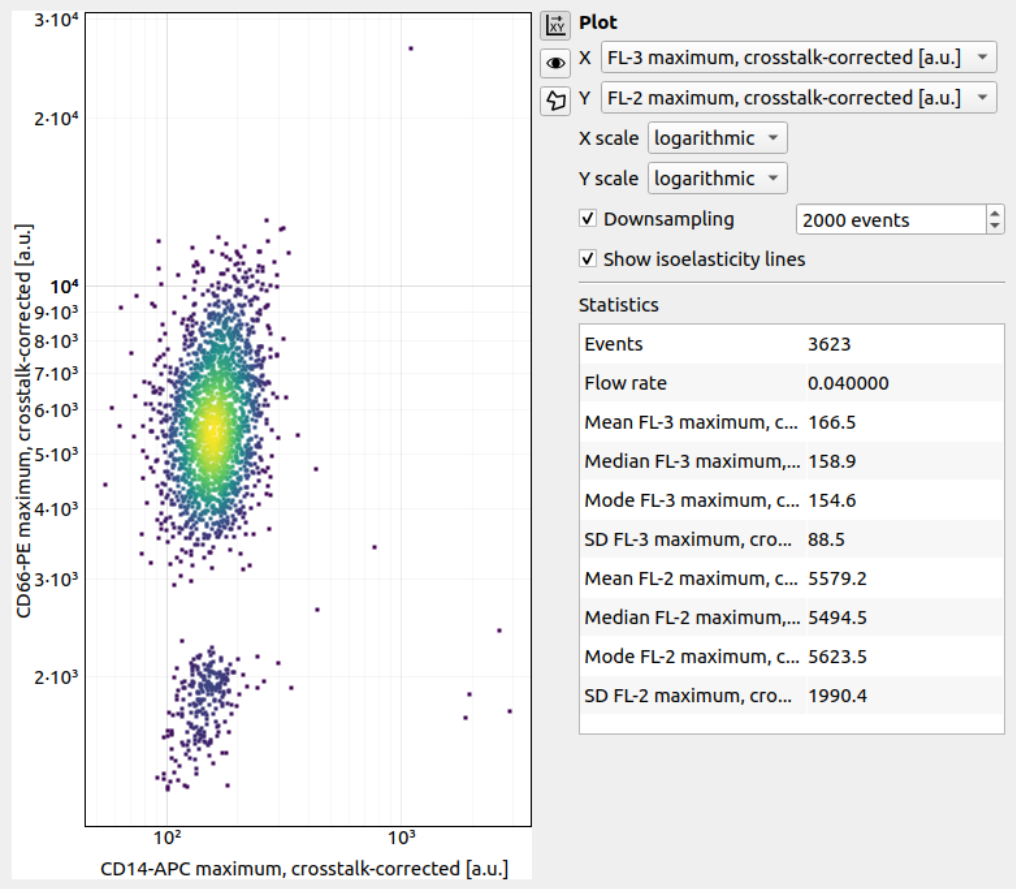

Meta
Dataset
Filter
Plot
Filter set
Preprocessing
Duplicate
Remove
Name
Preprocessing
Use this filter
Limit number of events to
5000
Remove invalid events
Box filters
Area [µm²]
22.0
120.0
Porosity (convex to measured area ratio)
1.00
1.10
Choose box filters...
Polygon filters
No polygon filters have been created yet.
Create polygon filter
Reset
Apply



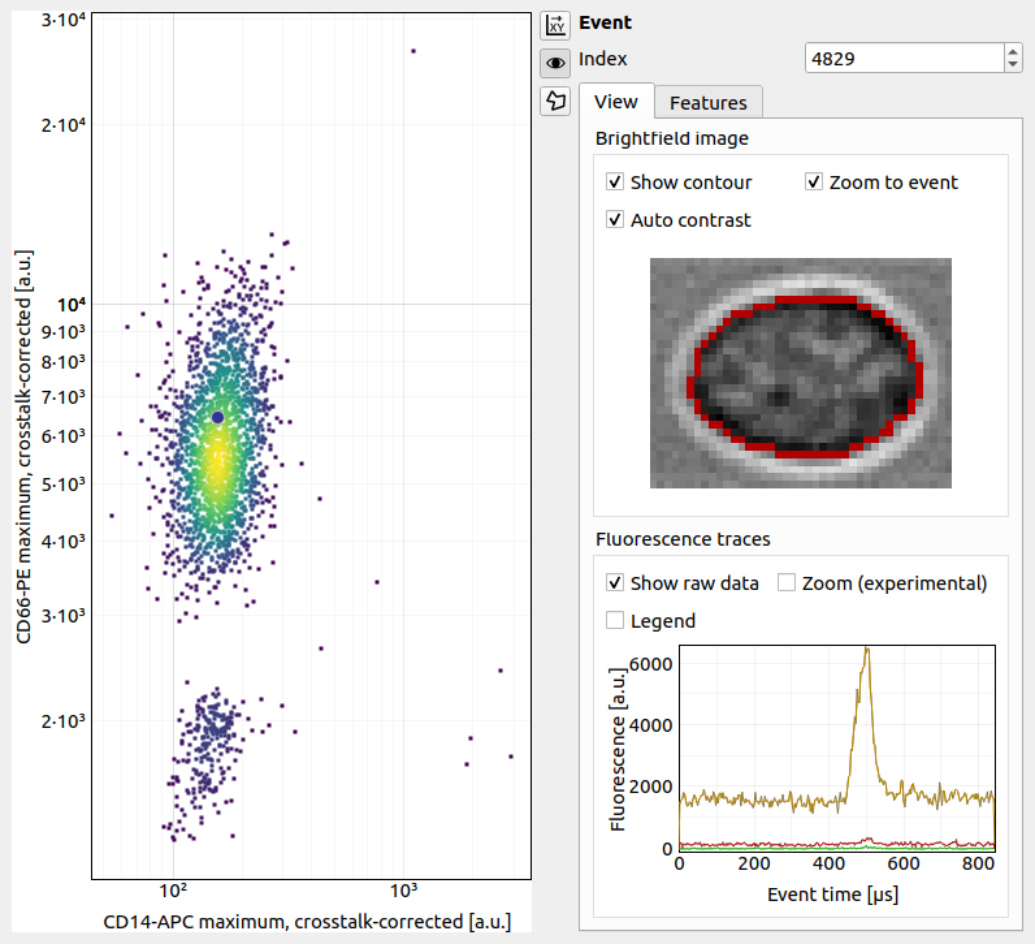
3.3.5 Quick View

The Quick View is meant for dataset exploration. It consists of a scatter plot on the left and a set of tool panels that are accessible via the corresponding tool buttons on the right.

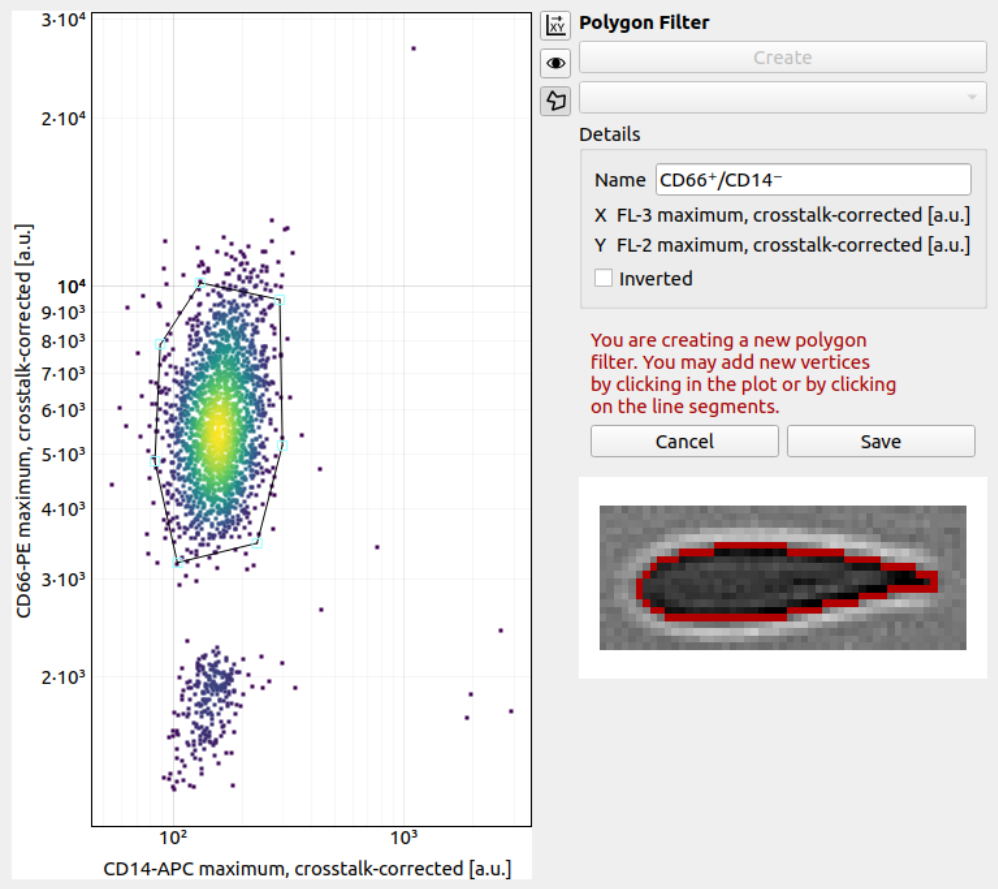
Use the **Plot** panel to define all plot parameters. It also displays common statistics of the two features plotted.



The **Event** panel displays all parameters of an individual event. You can select single events by clicking in the scatter plot or by scrolling through *Index* spin control. If available, the event image is shown alongside the fluorescence trace of the event. All features of the event are listed in a separate tab.



The **Polygon Filter** panel allows you to create and modify polygon filters. When the panel is active you can move the mouse pointer across the scatter plot and the image of the event closest to the mouse pointer is displayed.


3·10⁴
2·10⁴
10⁴
9·10³
8·10³
7·10³
6·10³
5·10³
4·10³
3·10³
2·10³
CD66-PE maximum, crosstalk-corrected [a.u.]
10²
10³
CD14-APC maximum, crosstalk-corrected [a.u.]
Polygon Filter
Create
Details
Name CD66⁺/CD14⁻
X FL-3 maximum, crosstalk-corrected [a.u.]
Y FL-2 maximum, crosstalk-corrected [a.u.]
Inverted
You are creating a new polygon filter. You may add new vertices by clicking in the plot or by clicking on the line segments.
Cancel
Save

CHAPTER 4

Quick Guides

4.1 Checking experimental data for consistency

- **Trends over time:**

  To verify that the sample "behaves" stationary during the measurement, it is helpful to plot a few selected features versus time. No systematic deviations should be observable, as in the figure below.

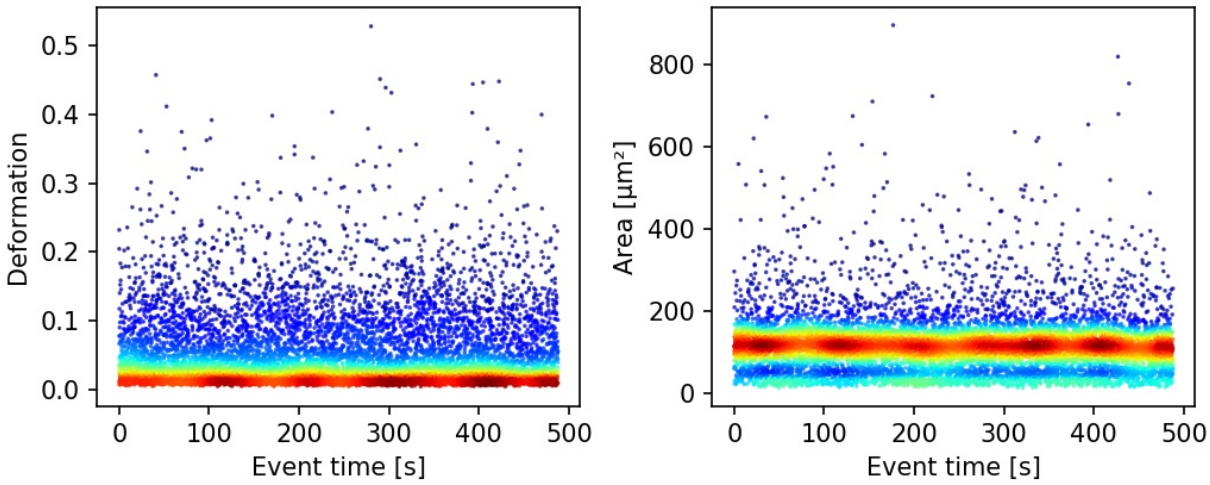


Fig. 4.1: Deformation and event area versus time. There is no systematic trend over time, indicating stable measurement conditions.

- **Countrate:**

  Trends over time do not directly visualize the count rate (measured events per second). The count rate is the slope of the event index versus event time plot and should remain constant.

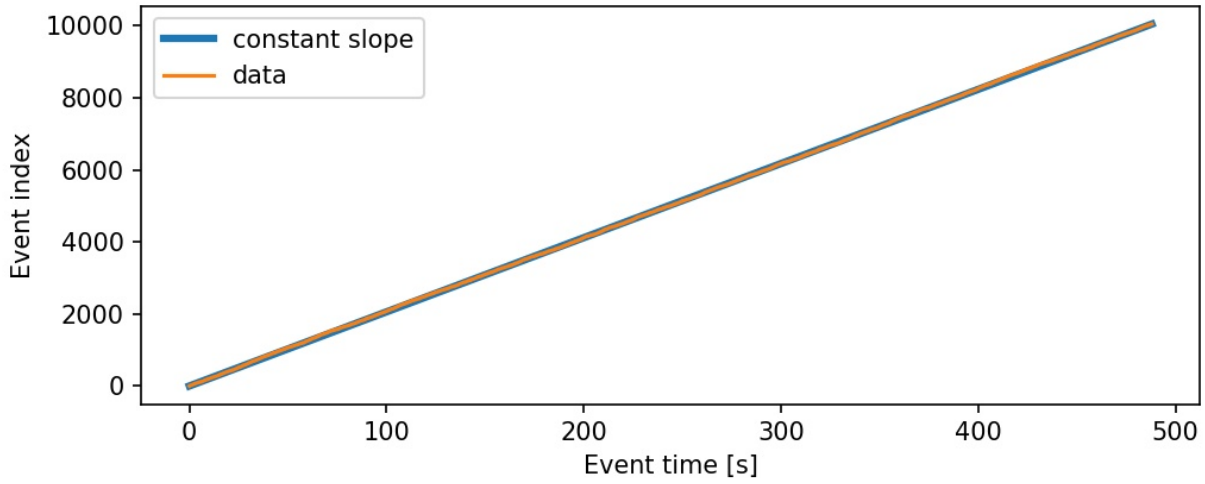


Fig. 4.2: The event index versus event time plot shows a constant slope, an indicator for stable measurement conditions.

4.2 Excluding invalid events

Filters can be used to exclude invalid events, such as cell debris, cell doublets or larger aggregates, or cells that are not intact, from an analysis.

- **Area:**

  The event area (the area defined by the event contour) can be used to filter cell aggregates (larger area), dead or degenerate cells, as well as cell debris (small area). Use the deformation versus area plot to identify exclusion candidates by clicking on an event and visualizing it in the event area.

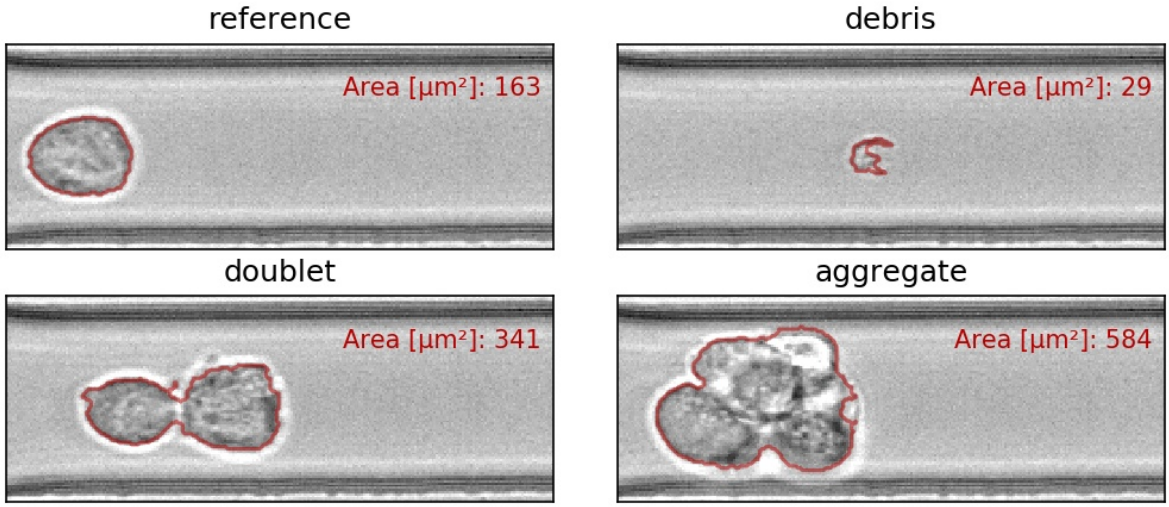


Fig. 4.3: Examples of events with small or large areas.

- **Aspect and inertia ratio:**

  The aspect ratio of the bounding box and the inertia ratio of the contour can be used to filter cell aggregates and otherwise invalid events. For instance, an aspect ratio below 1 (elongation perpendicular to the channel axis) is

most-likely debris and can be excluded from the analysis. An inertia ratio below 1 also indicates invalid events.

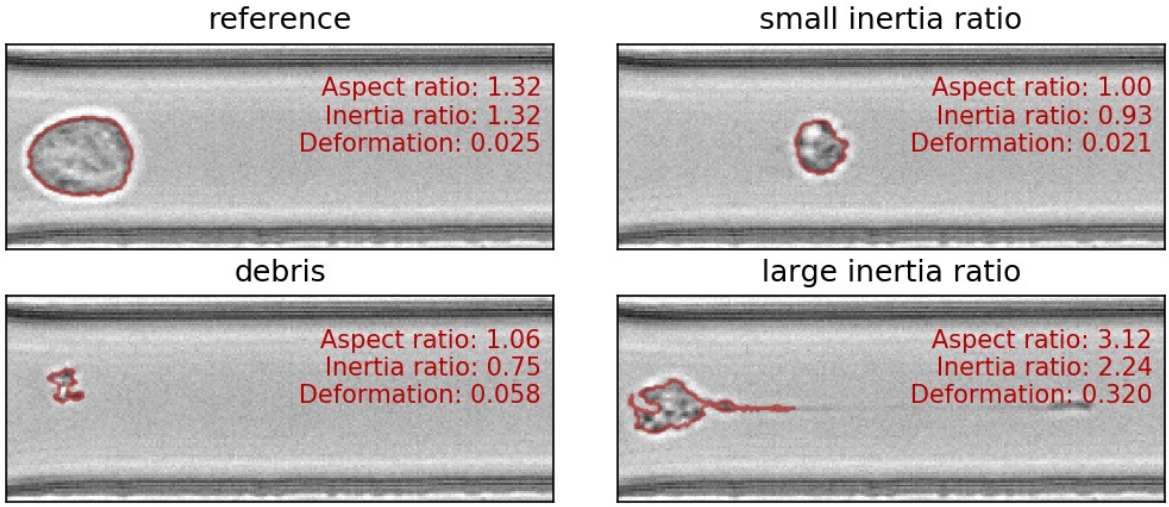


Fig. 4.4: Examples of events with various aspect and inertia ratios.

- **Porosity:**

  The porosity is the ratio between measured contour and the convex contour. Porosity is commonly used to remove events with non-physical contours, e.g. for cells, all events with a porosity above 1.05.

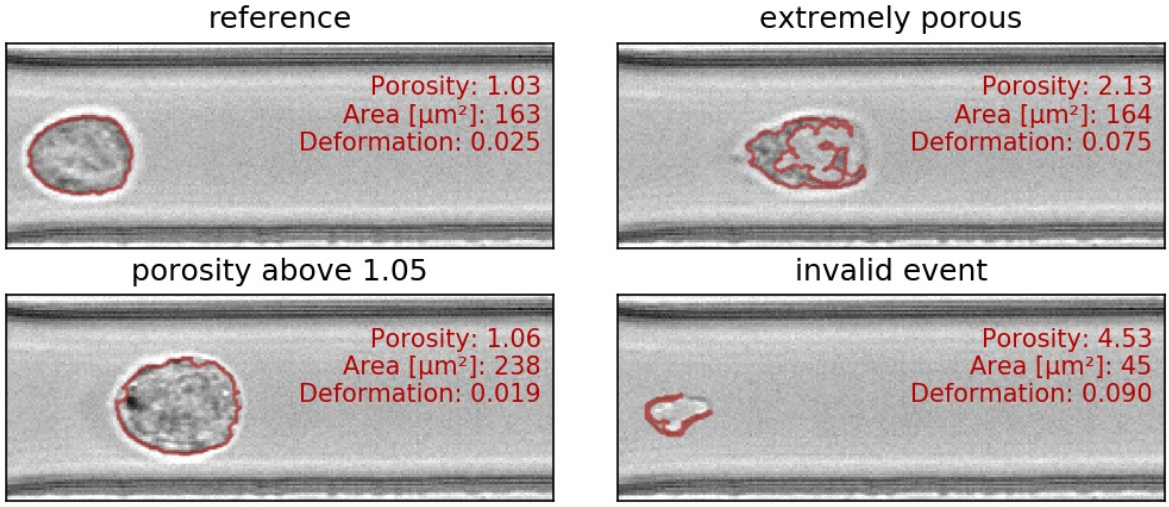


Fig. 4.5: Examples of events with various porosities.

4.3 Young's Modulus

With Shape-Out, it is possible to translate the measured area and deformation to the Young's modulus based on numerical simulation for fully elastic spheres according to Mokbel et al. [MMM+17].

4.3.1 Usage

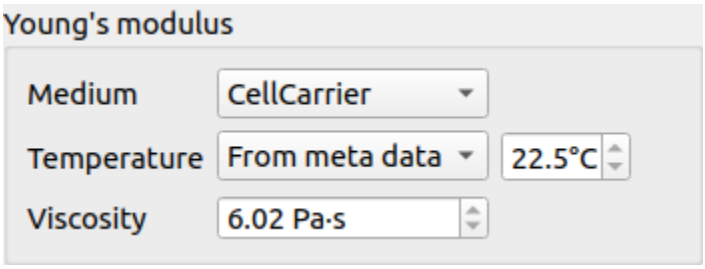


Fig. 4.6: Temperature taken from the meta data.

The parameters for computing the Young's modulus can be set in the *Dataset* tab of the *Analysis View*. The Young's modulus is computed using a precomputed look-up table and additionally depends on channel width, flow rate, pixel size (pixelation correction), and viscosity. For known media, such as CellCarrier, the viscosity can be derived from channel width, flow rate, and temperature. In some RT-DC setups, the chip temperature is recorded during the measurement. For instance, in Fig. 4.6, the average chip temperature of 22.5°C from the [setup] meta data section is used. The value of the resulting viscosity is shown below. If the chip temperature is recorded for each event, then the user may select the *From feature* option (Fig. 4.7). In this case, the Young's modulus is computed from the individual viscosities for each event.

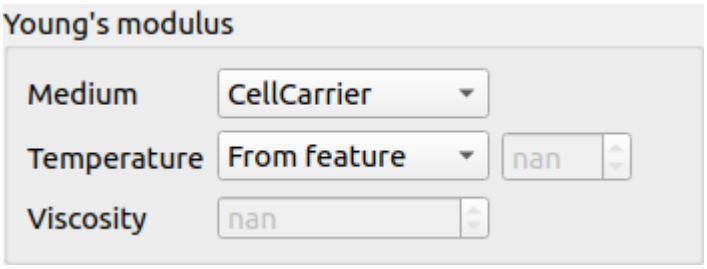


Fig. 4.7: Use the temperature (temp) feature.

If the temperature is not given as a feature or as meta data, then you may select the temperature manually. This case is visualized in Fig. 4.8. Here, the temperature is purposely set outside of the known range defined in [Her17], which is highlighted by coloring the viscosity red.

You may also set the viscosity manually by selecting *other* as a medium (Fig. **??**). In this case, the values for temperature are irrelevant. Please only use this option if you know what you are doing (e.g. you have considered shear-thinning [Her17]).

Click *Apply* for any changes to take effect. The Young's modulus is then available for the selected dataset.

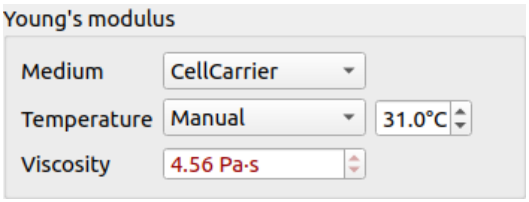


Fig. 4.8: Temperature outside of known range.

4.3.2 Validity

The computation of the Young's modulus is valid only for objects that initially have a spherical shape. In addition, the deformation and size values must be in a "valid region". Events outside this region will have a *nan*-valued (not a number) Young's modulus. Note that as a result, these events will be excluded from all plots when *remove invalid events* is checked in the *Filter* configuration tab.

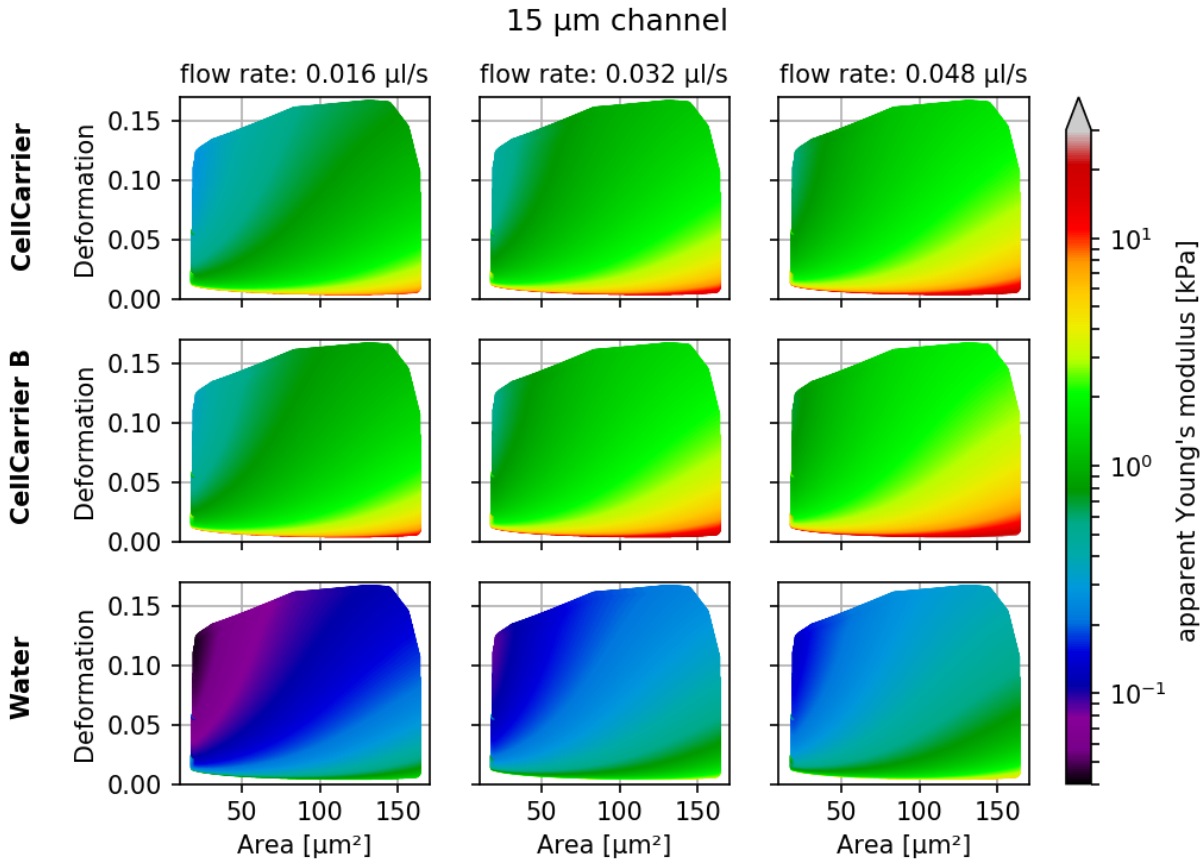
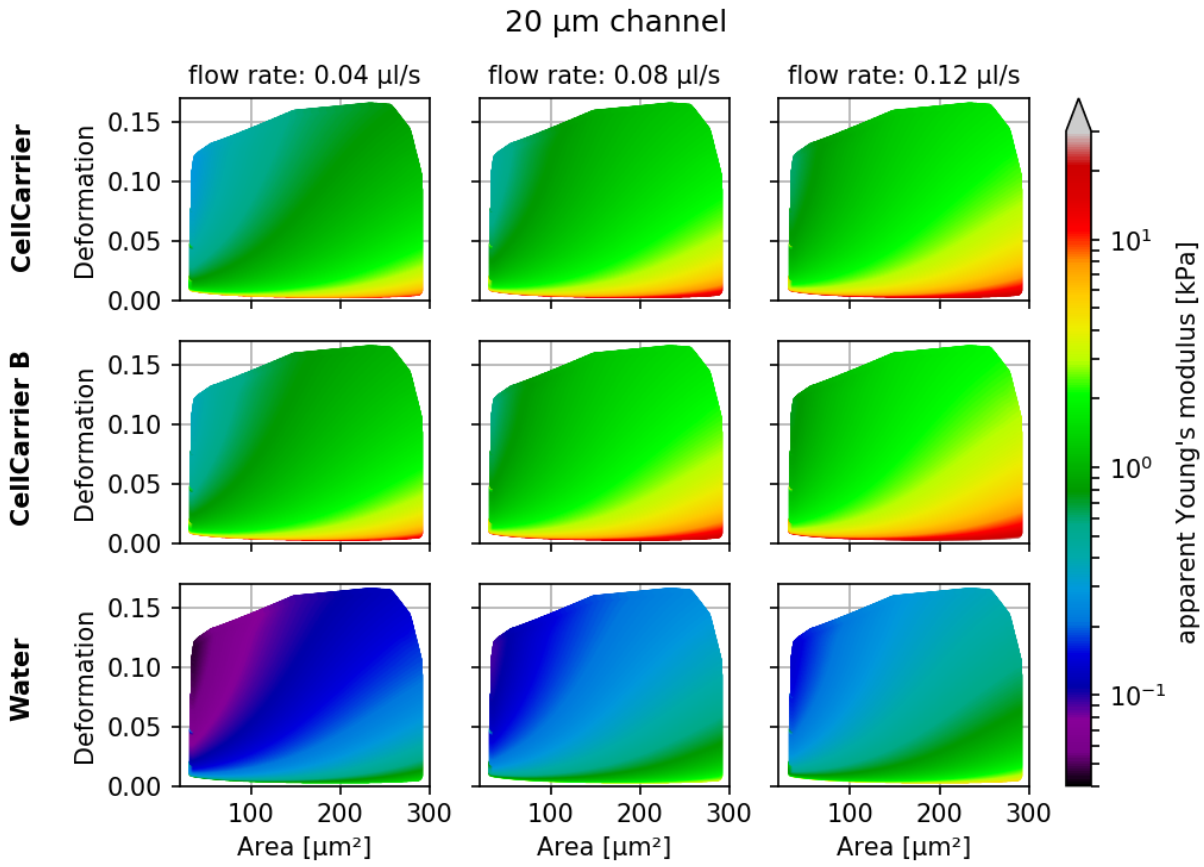


Fig. 4.10: Visualizations of the support and the values of the look-up table (LUT) used for determining the Young's modulus from deformation and cell area. The values of the Young's moduli in the regions shown depend on the channel size, the flow rate, the temperature, and the viscosity of the medium [MOG+15]. Here, they are computed for a 15 µm wide channel at 24°C with an effective pixel size of 0.34 µm. The data are corrected for pixelation effects according to [Her17].

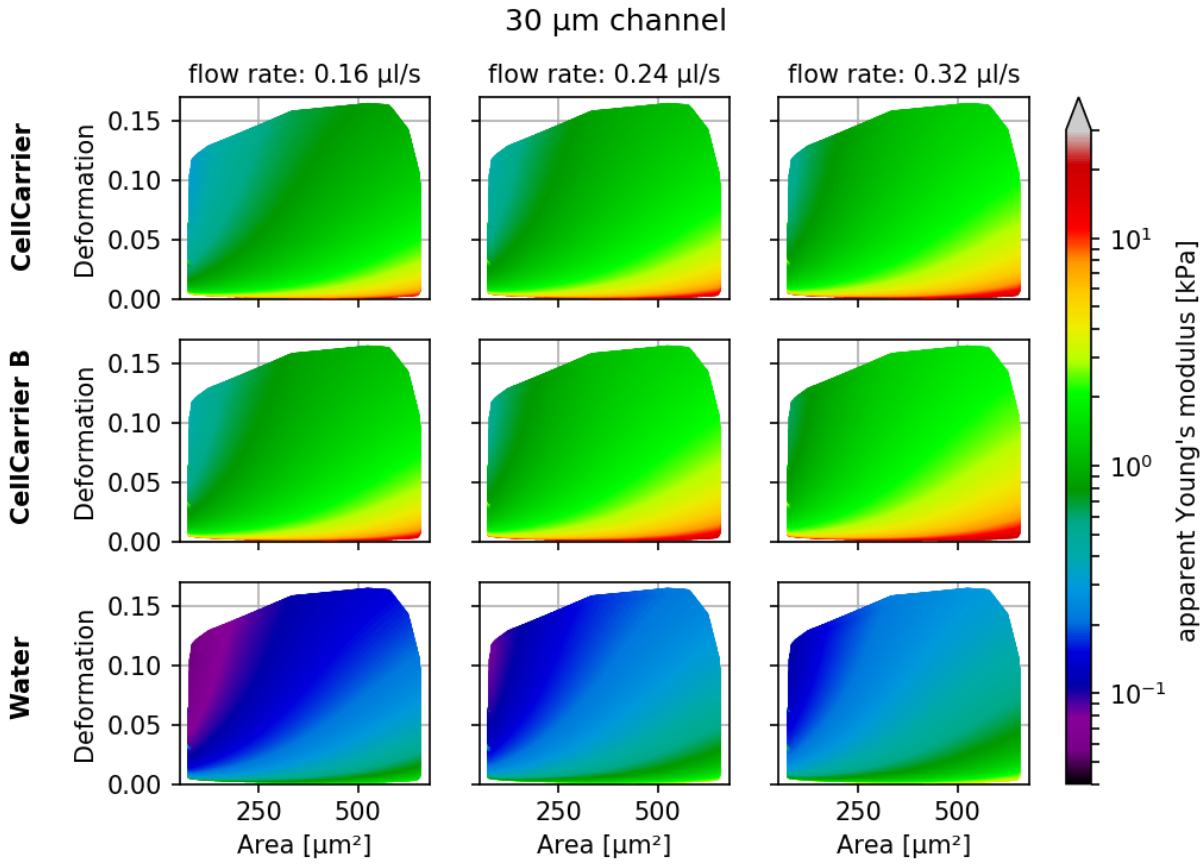
The invalid regions (white in the figure above) include objects that are very small or objects with very low deformation values. The reason for that is a very steep increase of the Young's modulus with little decrease in deformation that could potentially result in very large simulation errors. In addition, regions with high deformation are invalid as well, because the simulations do not converge (objects simulated with lower Young's moduli become more and more elongated until they rupture). In practice, this means that the channel size has to be selected carefully to match the object sizes. Larger object sizes require wider channels if meaningful values for the Young's modulus are to be computed.

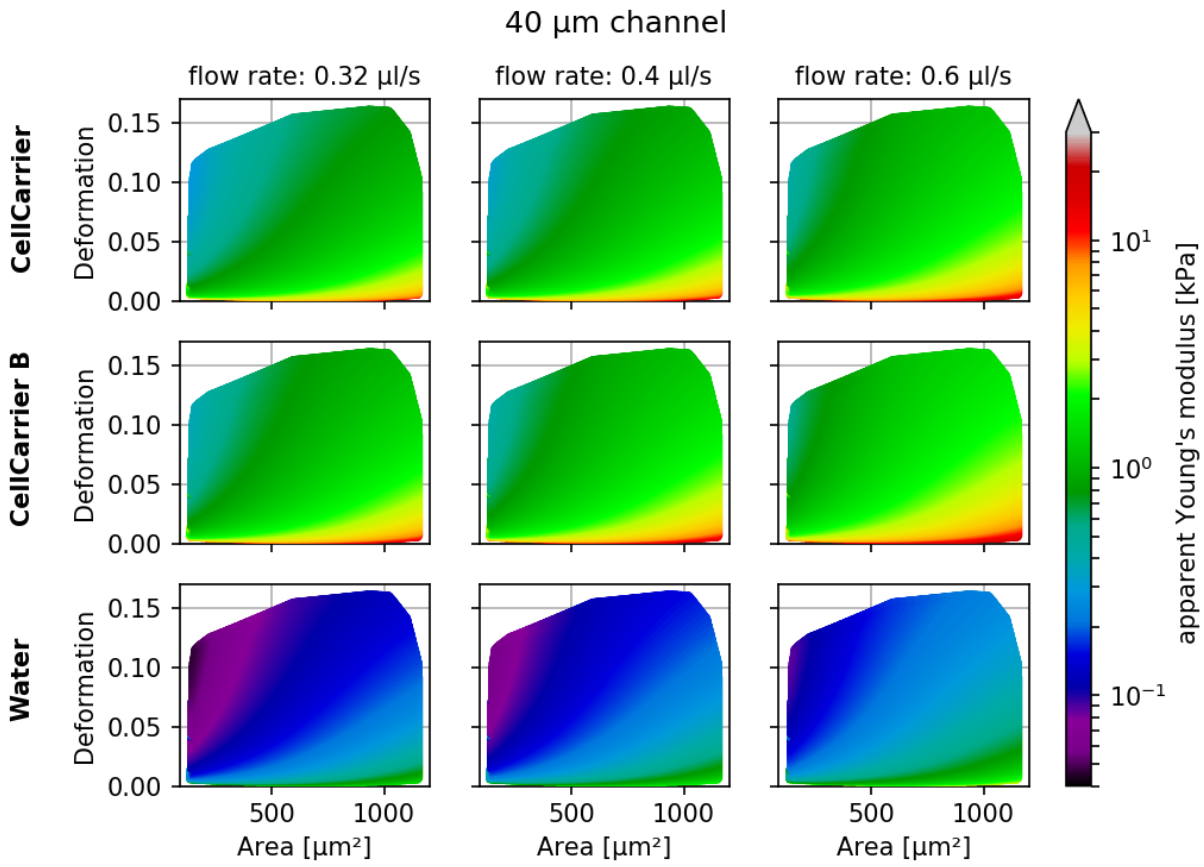
In the following, additional visualizations for commonly used channel sizes and flow rates are shown:



4.3.3 Implementation

As described above, the Young's modulus can be derived in multiple ways, for known media and global or event-based temperature values. The underlying implementation is described in the


30 µm channel
flow rate: 0.16 µl/s
flow rate: 0.24 µl/s
flow rate: 0.32 µl/s
CellCarrier
CellCarrier B
Water
Deformation
0.15
0.10
0.05
0.00
250
500
Area [µm²]
apparent Young's modulus [kPa]
10^1
10^0
10^{-1}


40 µm channel
flow rate: 0.32 µl/s
flow rate: 0.4 µl/s
flow rate: 0.6 µl/s
CellCarrier
CellCarrier B
Water
Deformation
0.15
0.10
0.05
0.00
500
1000
Area [µm²]
apparent Young's modulus [kPa]
10^{1}
10^{0}
10^{-1}

dclab docs.

4.4 Compute statistics

The *Quick View window* quantifies the statistics for the features shown there. If you need access to statistics for more features of a dataset or if you need to quantify the statistics for multiple datasets on disk, you can use the *Statistics* tool available via the *Compute* menu.

There are two modes of operation. The first mode exports statistical data from the current session (Fig. 4.11). Statistics are always computed after the filter ray has been applied. You may select the features in the table on the left and the desired statistics in the table on the right.

The second mode computes statistics for .rtdc data on disk (Fig. 4.12). Select the *All datasets in a directory* option and (optionally) a filter ray from the current session that you would like to apply before computing the statistics.

Note that statistics may not be available for all selected features in all datasets. In such a case, statistics are exported as *nan* values in the resulting .tsv file.

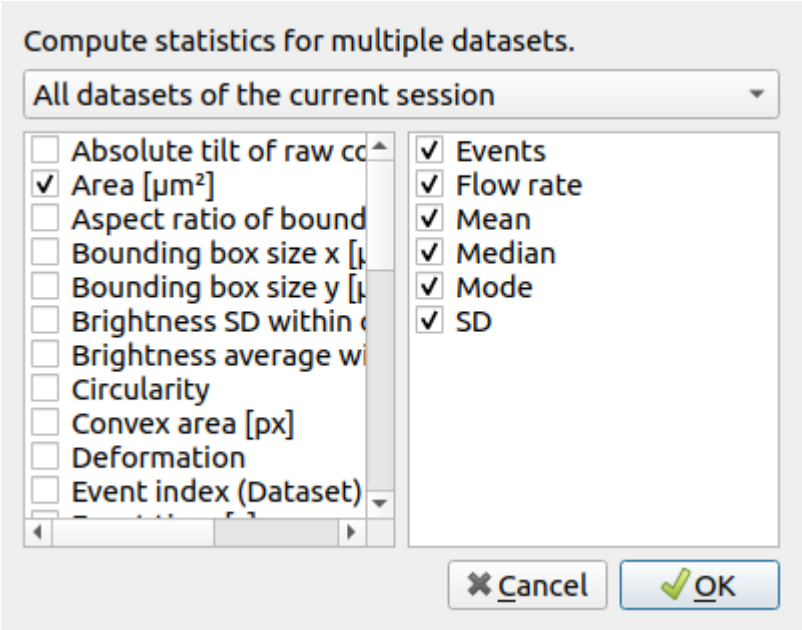


Fig. 4.11: Statistics export of current session

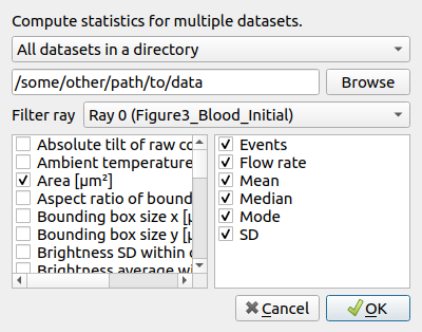


Fig. 4.12: Compute statistics for other data

CHAPTER 5

Changelog

List of changes in-between Shape-Out releases.

5.1 version 2.0.3

- setup: bump dclab from 0.20.3 to 0.21.1
- docs: add terminology section (#12)

5.2 version 2.0.2

- fix: IndexError when removing a plot (#36)
- fix: ValueError when filtering all events in Quick View (#37)
- fix: TypeError when removing datasets and opening Quick View (#38)
- fix: RuntimeError when removing datasets and opening Quick View (#38)
- fix: ValueError when removing activated datasets and opening Quick View

5.3 version 2.0.1

- fix: correctly distinguish prereleases when checking for new versions
- enh: allow loading data via drag&drop

5.4 version 2.0.0

- initial release

CHAPTER 6

Bilbliography

CHAPTER 7

Imprint/Impressum

7.1 Imprint and disclaimer

For more information, please refer to the imprint and disclaimer (Impressum und Haftungsausschluss) at https://www.zellmechanik.com/Imprint.html.

7.2 Privacy policy

This documentation is hosted on https://readthedocs.org/ whose privacy policy applies.

CHAPTER 8

Indices and tables

- genindex
- modindex
- search

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