
Shape-Out Documentation

Release 0.9.0

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Jan 29, 2019

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Shape-Out

Shape-Out is a user interface for data analysis in real-time deformability cytometry (RT-DC). This is the documentation of Shape-Out version 0.9.0.

Warning: This documentation is under construction and its existence thus not yet communicated publicly. Use it at your own risk.

1.1 Installation

Shape-Out can be installed via multiple channels.

1. **Windows installer:** Download the latest version for your architecture (e.g. ShapeOut_X.Y.Z_win_64bit_setup.exe for 64bit Windows) from the official [release page](#).
2. **Python 2.7 with pip:** Shape-Out can easily be installed with `pip`:

```
pip install shapeout[GUI]
```

Note that ShapeOut requires wxPython 3 and chaco. You might have to manually install these for your system if they cannot be installed automatically via pip. To start Shape-Out, simply run `python -m shapeout` in a command shell.

1.2 Update

Shape-Out automatically searches for updates (you may opt-out via the preferences 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. **Python 2.7 and pip:**

```
pip uninstall shapeout  
pip install shapeout[GUI]
```

1.3 Basic usage

Will be updated when new GUI is ready.

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

Describe microfluidic setup; describe forces [MOG+15], [MMM+17]; measured quantity are bright-field images as seen in figure below; several features can be extracted for each cell, such as area, deformation, or brightness. These can be used to visualize populations in a subsequent analysis step.

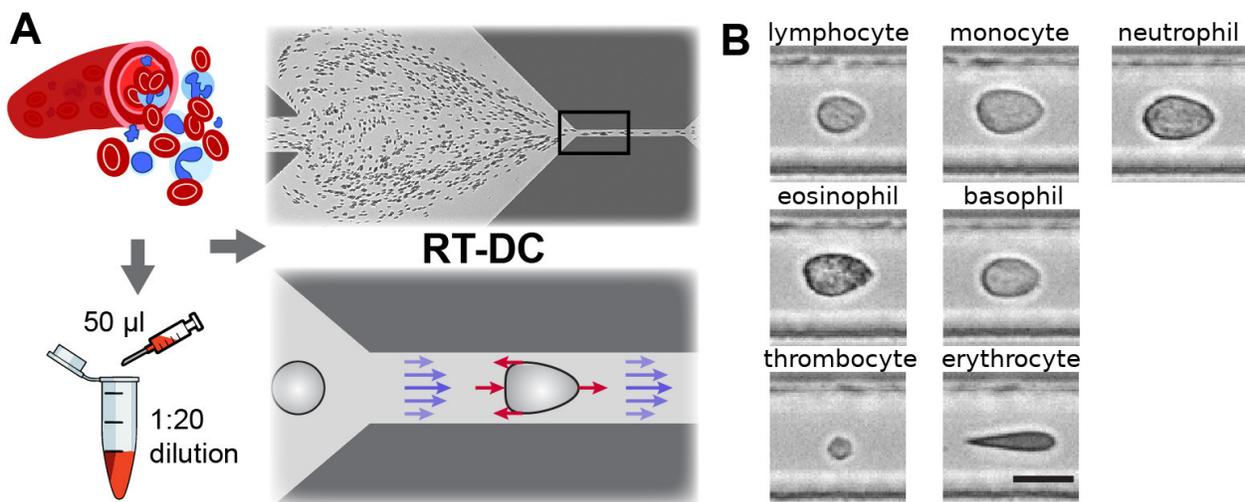


Fig. 1: Single-cell, morpho-rheological phenotyping of blood. (A) Analysis of whole, diluted blood. Hydrodynamic shear forces (red arrows) induce deformation of cells passing through a microfluidic channel ($20 \times 20 \mu\text{m}^2$) at speeds of more than 30 cm/s (blue arrows). (B) Representative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

RT-DC enables a morpho-rheological (MORE) analysis of suspended cells and can be used to identify major blood cells, characterize their pathological changes in disease conditions [THO+17], etc...

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 stored alongside the raw data. Here, only the most important features are covered. A full list of the features available in Shape-Out is maintained by the [dclab documentation](#). Please note that some of the features are only available in expert mode (accessible via the preferences menu).

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.

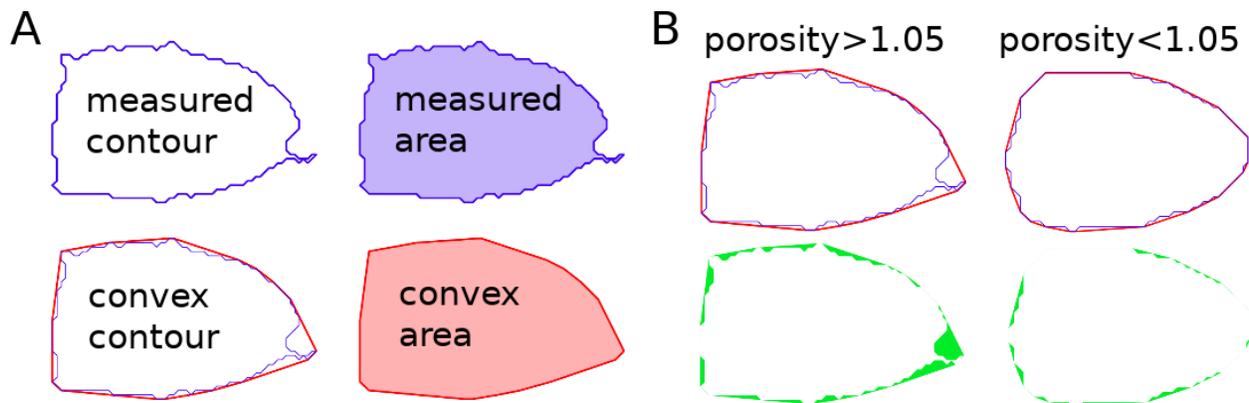


Fig. 2: Visualization of porosity. (A) The measured contour (blue line) defines the measured area (blue shade). The convex contour (red line) results in an area (red shade) that is usually larger than the measured area. (B) The porosity is the ratio between measured and convex contour. The difference (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.

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].

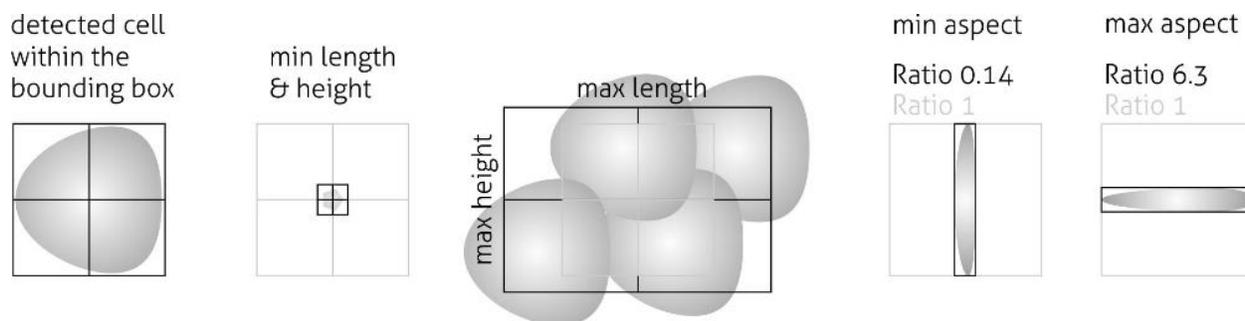


Fig. 3: Illustration of the event bounding box and its use cases. From left to right: definition of the bounding box, exclusion of small objects (e.g. debris) via the bounding box size, exclusion of clusters via the bounding box size, exclusion of objects elongated perpendicular to the channel axis, 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].

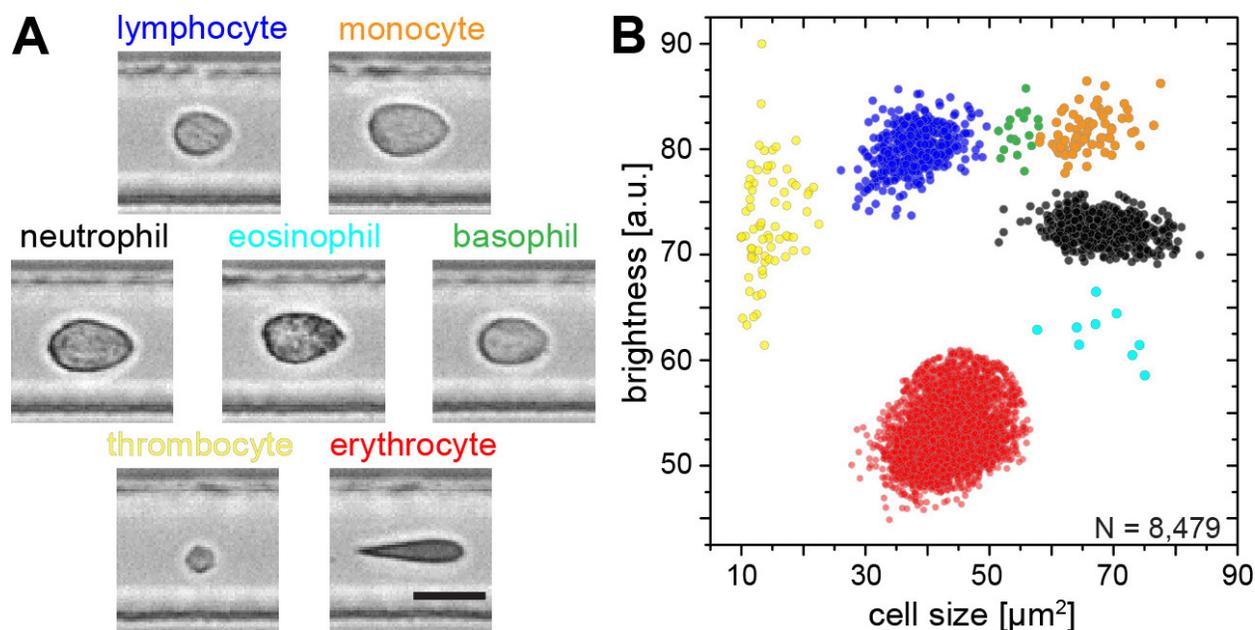


Fig. 4: Blood-classification with event brightness and cell size. (A) Representative images of blood cell types acquired. Scale bar is 10 μm . (B) Brightness versus cell size (area) scatter plot with cell types labeled according to the color scheme in (A). Figure and caption adapted from Toepfner et al.¹.

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.

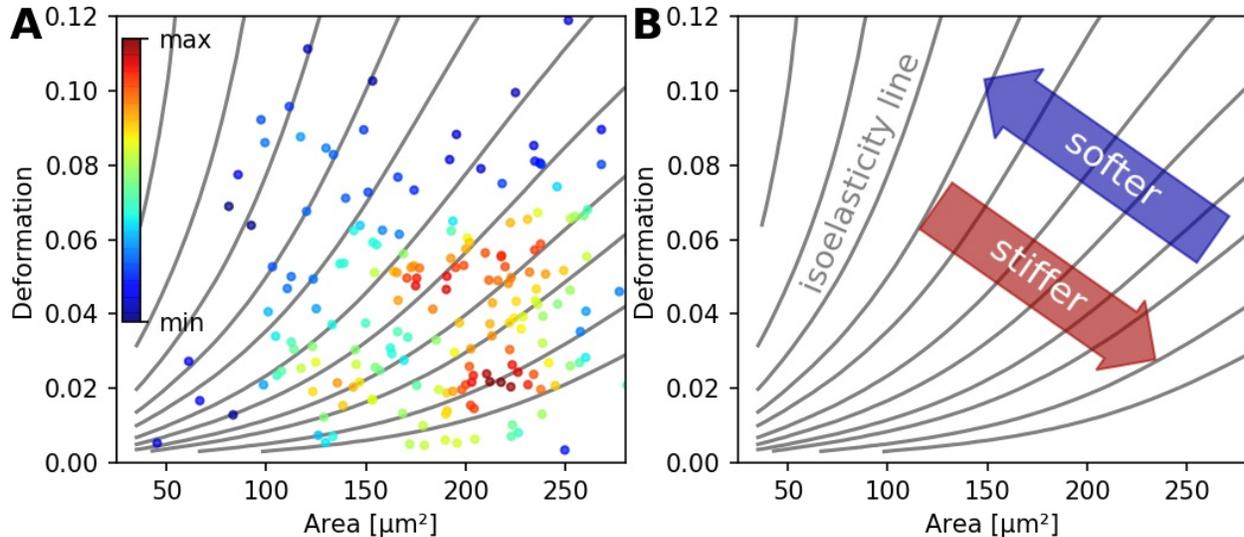


Fig. 5: (A) Typical deformation versus cell size scatter plot. The color scale indicates event density. (B) Isoelasticity lines derived from numerical simulations indicate trends in stiffness.

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

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.

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.

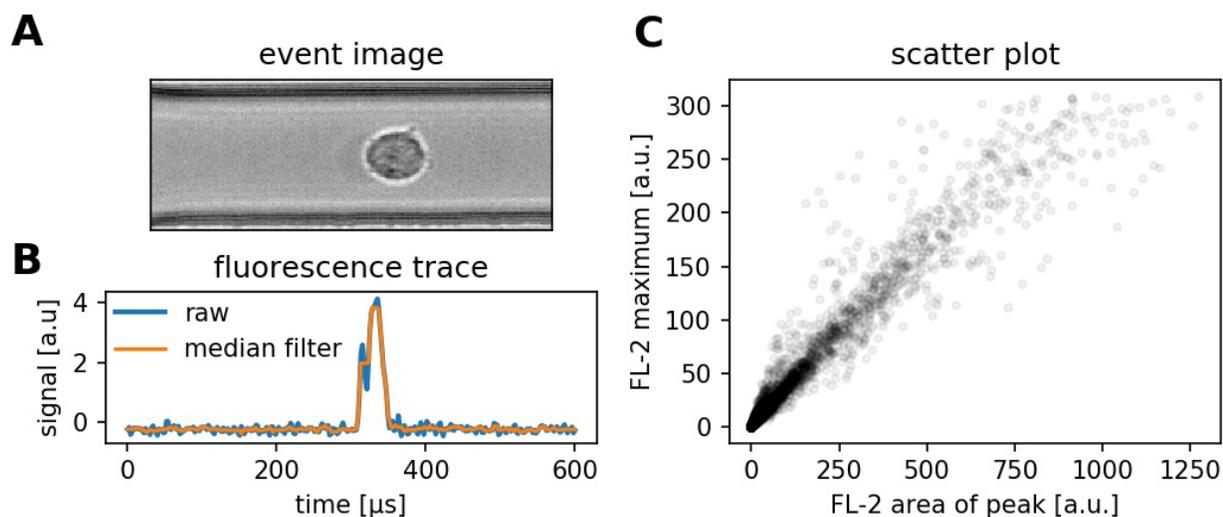


Fig. 6: (A) Event brightfield image. (B) Fluorescence trace of the event. The raw fluorescence data and the fluorescence data filtered with a rolling median filter (from which the parameters are computed) is shown. (C) Scatter plot of two parameters extracted from the fluorescence trace.

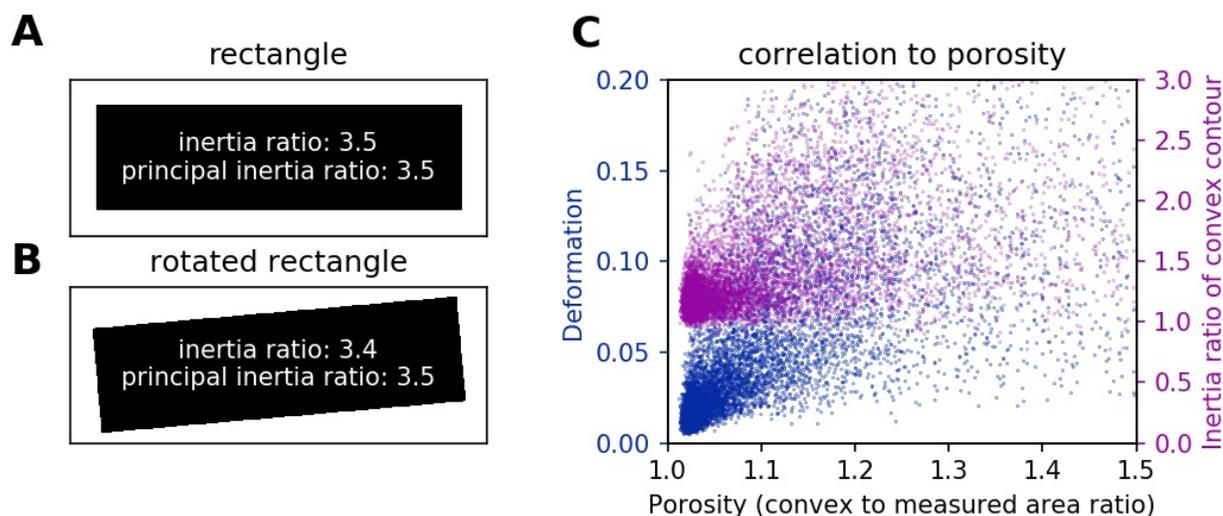


Fig. 7: (A) For a rectangle that is aligned with the coordinate axes, the inertia ratio and the principal inertia ratio are identical. (B) If the rectangle is rotated, the inertia ratio changes, but the principal inertia ratio does not. (C) Comparison between deformation and inertia ratio when plotted against porosity for an exemplary RT-DC experiment. Deformation exhibits a higher correlation to porosity.

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.

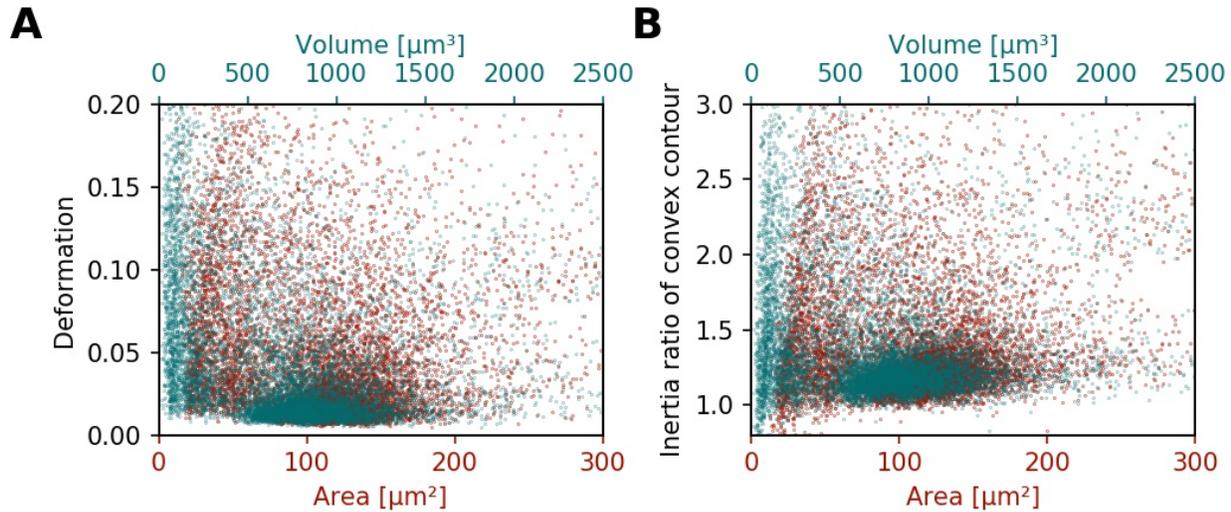


Fig. 8: (A) Deformation versus area (red) and volume (cyan) for an exemplary RT-DC dataset. There is a correlation between area and deformation, at least for small ($<50\mu\text{m}^2$) areas. (B) Inertia ratio versus area and volume. A correlation between inertia ratio and area is visible as well.

3.1 Menu bar

- **File:**
 - Find Measurements: opens a project (including all samples with all measurements) or a single sample (including all measurements). Open and choose the “YYMMDD_Project Leader_Sample” folder to open a whole project or a “SampleName” folder to open all measurements of a sample
 - Add Measurement: adds another project or sample to the already loaded measurements
 - Clear Measurements: removes all samples or measurements, which are not selected in the measurement browser.
 - Save session: saves the status of the analysis
 - Load session: loads a saved session
 - Quit: quits the program
- **Export:**
 - All event data (*.tsv): tool to export datasets (filtered, unfiltered, specified parameters only)
 - Graphical plot (*.pdf): exports all plots in a pdf file
 - Computed statistics (*.tsv): exports the statistics in a table
- **Batch:** Batch analysis of datasets
- **Help:** Help and information for debugging

3.2 Measurement browser and data selector

The browser shows the opened projects and the corresponding measurements (in an expandable tree structure). The name of the measurement is composed of the measurement number, the measurement region and the flow rate. For

easy selection of measurements, you can use the buttons below the browser (e.g. you can select all data for a certain flow rate).

Analyze button: The Analyze button plots all selected data either as raw data or using the filter settings.

3.3 Session tabs

3.3.1 Information tab

Shows all saved measurement properties. Several properties are only highlighted if a single measurement is analyzed. If properties differ between single measurements this is indicated by “(multiple)”. Use the scroll bars or adjust the pane or increase the size of the pane by dragging of the edge to see the full table.

General

Cell Number: number of recorded cells. In ShapeIn this number is shown on the front panel “Number of cells measured”.

Channel Width: dimension of the channel. This number is not detected automatically by the system, but has to be set by hand in ShapeIn on the front panel under “Channel dimension [μm]”.

Flow Rate: is the sum of sheath and sample flow in $\mu\text{l/s}$. ShapeIn records this number when the measurement starts and shows it on the front panel under “Flow Rate [$\mu\text{l/s}$]”.

Measurement Number: is the number of the measurement within the opened project. In ShapeIn this number is shown on the front panel under “current/next #”

Region: shows the position of the Region-of-interest during the measurement. This is not detected automatically by the system, but has to be set by hand in ShapeIn on the front panel under “Region of interest in the ...”.

Sample Flow Rate and Sheath Flow Rate: are the flow rates of the sample and sheath flow in $\mu\text{l/s}$, respectively.

Image

Cell Aspect Max: gating parameter for maximum aspect ratio = length/height. Here, length defines the extension of cell in flow direction and the height the extension of the cell transverse to the channel.

Cell Aspect Min: gating parameter for minimum aspect ratio = length/height. Here, length defines the extension of cell in flow direction and the height the extension of the cell transverse to the channel.

Cell Max Height / Length / Min: shows the gate that was set in ShapeIn to exclude cells larger / smaller than this number. In ShapeIn these numbers are shown in the normal tab under Gating tools.

Pix Size: resolution of the microscope-camera-system in [$\mu\text{m}/\text{pixels}$]. In ShapeIn this number is shown in the expert tab under Expert settings and “Resolution [$\mu\text{m}/\text{pix}$]”.

Thresh: grey value used as a threshold for each pixel of the image after subtracting the background image. All pixels above the threshold form the binary image. In ShapeIn under Expert settings “min grey threshold”.

Trig Thresh: minimum number of pixels within the binary image to be further processed in cell analysis. In ShapeIn under Expert settings “min pixels threshold”.

Framerate

Frame Rate: of the camera during the measurement. ShapeIn records this information when the measurement starts and shows it in the Expert tab under Camera tools and “Framerate [Hz]”.

ROI

Height / Width: of the region of interest in pixels. ShapeIn records this information when the measurement starts. It is set automatically or manually in the Expert tab under Camera tools and “Height [pix]”/ “Width [pix]”.

3.3.2 Filtering tab

In this tab, you can filter your data by excluding values beyond certain limits. Select the Enable Filters check box on the right and hit the Apply/Reset button to apply/reset the settings. The Box Filters limit the range of parameters; the Polygon Filters allow freely defining a gate by a polygon in the current plot. The filters are applied to the whole data set, independently of the actual shown plot. At least one event has to be within the limits or the program shows an error.

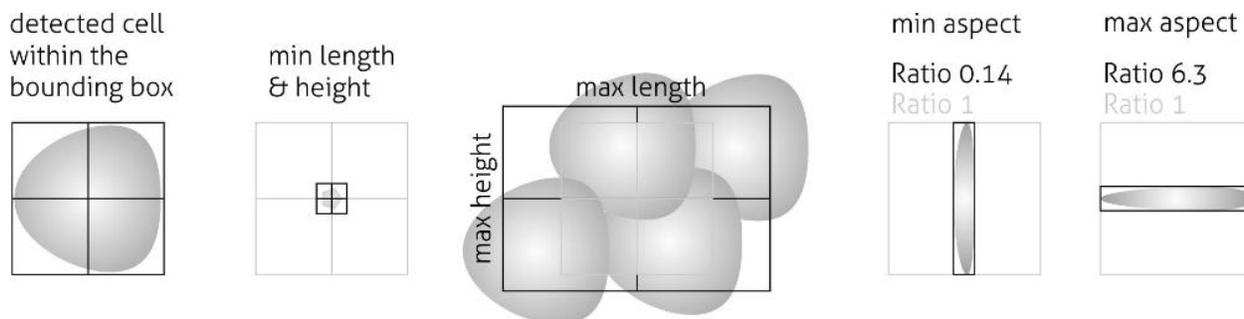
Box Filters

Minimum and maximum values for box filters are set to 0.0 by default. As long as min and max values equal 0.0, the filter is not applied. On the left of the table the minimum value is set, on the right the maximum. If you set a minimum, you have to set a maximum as well, otherwise all data points are gated out.

Range Area: limits the cell size in [μm^2].

Range Area Ratio: defines the relative difference between convex hull area and cell area. “1”: convex hull and area contour are the same, “<1”: forbidden, because the convex hull cannot be smaller than the contour. The minimum value is only effective “>1”.

Range Aspect: limits the ratio of height and length of the bounding box around the contour of the cell (see Figure below).



Range Defo: limits the deformation

Range Pos Lat: limits the lateral position of a contour in the region of interest

Range Pos x: limits the position in direction of channel axis in the region of interest

Range Time: limits a number of time frames within the measurement in [s].

Range x-size: limits the size in x direction (width)

Range y-size: limits the size in y direction (height)

Polygon Filters

TODO: brief explanation; See the tutorial 1 for details.

3.4 Plot Window

Will be updated when new GUI is ready.

4.1 Checking experimental data for consistency

4.1.1 Changes with time

any trend is bad; e.g. brightness: change in focus; area: sample changed

4.2 Filtering

4.2.1 Aspect ratio

remove cell clusters

4.2.2 Orientation

only cells aligned with channel

4.2.3 Porosity

Remove dirt

4.3 Young's Modulus

TODO: helpful screenshots when the new GUI is ready.

With Shape-Out it is possible to convert deformation values to values of the Young's modulus based on the numerical simulation work for fully elastic spheres by Mokbel et al. [MMM+17].

The “Calculate” tab allows you to obtain the Young’s modulus for the samples you selected for plotting.

Currently, the only model available is the elastic sphere. After choosing the type of measurement medium you must set the right temperature or – in case you choose “Other” – the correct viscosity. For CellCarrier media, the correct viscosity is automatically calculated according to the shear thinning behavior as analyzed in [Her17].

Once “Compute elastic modulus” is clicked, a plotting option for the Young’s modulus will become available.

Note: A conversion can only be carried out for deformation and size values in a “valid region” that will be described in more detail below.

Events outside this region will disappear from the plot – also if the Young’s modulus is not selected for plotting. To plot the complete sample in those cases again, the checkbox “remove invalid events” in the “Filter” tab needs to be unchecked.

4.3.1 Valid Conversion Region

This section is meant to guide an experimental strategy to obtain results that can be converted to a Young’s modulus. Numerical simulations [MMM+17] have yielded a valid region for the conversion in the space of deformation and cell size shown with a color gradient for a 20 μm channel.

It is limited by regions A and B for objects too small and objects too large for reliable conversion. It is further limited for very small deformation values in region C. The reason for that is a very steep increase of E with little decrease in deformation that would yield potentially very large errors. Finally, it is limited by region D at larger deformation. In this region, simulations did not reach a stationary shape for the softer objects to be found there. Instead they became more and more elongated until they disintegrated by rupturing.

Therefore, as an experimental strategy, the goal of the experiment must be to choose the suitable channel size and to vary the flow rate such, that the results fall well within the valid region.

In order to make this process more comfortable, in the following, the valid regions are shown for the four standard channel sizes available. Those representations include an offset shift in deformation that would be expected in the experimental results due to the pixelation of the image as described in [Her17].

The values of the Young’s moduli in those regions will depend on the specific flow rate and the viscosity of the medium [MOG+15]. Note that in the illustrations that follow they merely represent a relative scaling and are not to be compared between illustrations.

List of changes in-between Shape-Out releases.

5.1 version 0.9.0

- Introduce new online documentation at <http://shapeout.readthedocs.io>
- Consistently use the term Shape-Out
- Code cleanup and compatibility with dclab 0.8.0
- Enhancements:
 - Order input fields in measurement browser according to measurement number (#229)
 - Support new features “temperature” and “ambient temperature” (dclab 0.9.0)
- Bugfixes:
 - Displaying the contour of .rtdc files that do not contain the “mask” feature resulted in an error.
 - ValueError in settings.py when stored data does not contain a key-value pair or when the value is missing.
 - IndexError when clicking on an event that has no image data
 - When the titles of all measurements are identical, the measurement selection in the combobox does not have any effect on the displayed measurement when creating a new polygon filter.
 - Possible errors when loading sessions containing empty datasets

5.2 version 0.8.8

- Bugfixes:
 - “Mean of empty slice” error when attempting to plot bad data

- New ancillary features in expert mode:
 - Principal inertia ratio (dclab 0.6.3.dev1)
 - Absolute tilt of raw contour (dclab 0.6.3.dev2)
- Add computation of Young’s modulus for water medium (dclab 0.6.3.dev14)
- Show fluorescence metadata in Information tab
- Display data paths as tool tip in title editor (Contour Plot tab) (#227)

5.3 version 0.8.7

- Bugfixes:
 - Search for missing datasets was falsely extended to directories not chosen by the user
 - Add recording date/time tooltip in the analysis tab to avoid ambiguities (#223)
 - Allow arbitrary number of repetitions for mixed model analysis with a spin control (#222)
 - Setting the treatment to “None” for (g)lmm analysis did not exclude it from the analysis, but introduced it as a different treatment (#224)
 - Channel width not correctly identified for old tdms files (dclab 0.6.2.dev7)
 - Do not draw isoelastics in contour plot if measurements have different channel widths
- Automatically identify treatment and repetition for mixed model analysis (#222)
- Automated release to PyPI with appveyor
- Begin migration of docs to sphinx/readthedocs.org

5.4 version 0.8.6

- Refactoring:
 - Use pathlib instead of os.path for
 - * All session handling/conversion tasks
 - * Configuration file management
 - * Measurement/Analysis management
 - Support all changes to dclab.definitions (dclab 0.4.0)
 - Support all changes to dclab.features (dclab 0.5.0)
- Bugfixes:
 - Opening sessions containing paths to data with a hash “#” failed
 - AttributeError during autosave when no measurement data is available
 - Exporting to .fcs files failed due to NaN-valued events; Events containing NaN values are now ignored (fcswrite 0.1.3.dev22) (#215)
 - Improve displayed float values for selected configuration keys (#216)
 - Unicode error when searching for data sets on disk (#218)

- Unicode error when performing LMM analysis on some setups (#217)
- IndexError when obtaining meta data from previous, broken analysis
- KeyError when starting a new analysis with unavailable plotting axes
- Ignore broken .rtdc data files

5.5 version 0.8.5

- Update plotting range when switching to log scale (#198)
- Allow to fix plotting range to retain previous behavior (#198)
- Initial plotting range is determined from min/max values of all plots (#213)
- Improved standard contour and kde accuracies using Doane’s formula (#147)
- Bugfixes:
 - Support opening tdms files with capitalized “userDef” column names (dclab dclab 0.3.2.dev5) (#212)
 - Wx colors and chaco not compatible anymore (Windows appveyor build)
- Refactoring:
 - Use pathlib when saving and opening sessions and do not use os.chdir

5.6 version 0.8.4

- Add pixelation-corrected numerical and analytical isoelastics (#203)
- Update emodulus look-up table (dclab 0.3.1.dev3) (#202)
- Improved handling of polygon filters (partly #95)
- Updated R version to 3.2.5 due to build errors
- Bugfixes:
 - Young’s modulus computation is wrong due to faulty FEM simulations (dclab 0.3.1.dev20) (#207)
 - Image/trace panel shows wrong event when the mouse pointer hovers from one scatter plot to another
 - Hierarchy children: image, contour, and trace data were not displayed in the image/trace panel (dclab 0.3.1.dev1) (#100)
 - Hierarchy children did not support manual filters (dclab 0.3.1.dev2)
 - Regression: Setting plotting range manually not possible (#201)
 - Pixel size not read from tdms-based data (dclab 0.3.1.dev13) (#205)
 - “limit events” filtering must be integer not boolean (dclab 0.3.2.dev3) (#210)

5.7 version 0.8.3

- Prefer sample name from para.ini over data folder name (dclab 0.3.0.dev15)
- Support new .rtdc file format (dclab 0.3.0.dev20) (#151)

- Bugfixes:
 - Automatically fix inverted plotting range
 - Enable scrolling in data export dialogs
 - Ignore missing features during data export
 - Plotting circularity with isoelastics failed (dclab 0.3.0.dev21) (#197)
 - Plotting errors with log-scale axes (#194, #195)
 - SHIFT+ENTER shortcut did not update filters
 - Fixed memory leak (#187)

5.8 version 0.8.2

- Add fluorescence crosstalk correction (#185)
- Bugfixes:
 - Regression: Session autosaving broken (#191)
 - Young’s modulus not updated (dclab 0.2.9.dev9) (#190)
 - UnicodeDecodeError on fcs-export (fcswrite 0.1.3.dev15) (#189)
 - Mouse cursor stuck in wait when plotting hierarchy data (#188)
- Code cleanup:
 - Use the term “feature” consistently (dclab 0.2.9.dev2)
 - Use file format agnostic fluorescence trace keys (dclab 0.2.9.dev4)

5.9 version 0.8.1

- Update internal configuration parameter names (dclab 0.2.8)
- Cache event counts of tdms-based data sets on disk (#179)
- Bugfixes:
 - KeyError when isoelastics not defined for combination of axes (#177)
 - Wrong channel width when loading ShapeIn 2.0.1 tdms data (dclab 0.2.9)

5.10 version 0.8.0

- Remember directory for searching missing measurement data
- Bugfixes:
 - Cannot open old session files with hierarchies when the measurement data location has changed (#174)
 - Cannot open polygon filters exported versions < 0.7.6 (#174)
- Code cleanup:
 - Move program-relevant data to “shapeout” directory

- Move computation of isoelastics to dclab (dclab 0.2.6.dev23)
- Create separate directory for appveyor recipe
- Remove unused/incomplete support for internationalization

5.11 version 0.7.9

- Add on-the-fly computation of contour inertia ratios
- Add menu options to save current event image (#164)
- Bugfixes:
 - Isoelastics are not shown (#145)

5.12 version 0.7.8

- Introduce unique identifiers for measurements (dclab 0.2.5.dev4)
- Include ffmpeg in the Windows installer (#165)
- Bugfixes:
 - dclab 0.2.6.dev2: Correctly identify contour files (#166, #169)
 - dclab 0.2.5.dev11: Allow contour data indices larger than uint8 (#167)
- Refactoring:
 - dclab 0.2.5.dev10: Event images are returned as 2D ndarray

5.13 version 0.7.7

- Add “Preferences” menu:
 - Allow to (dis/en)able session autosaving
 - Allow to (dis/en)able checking for updates
 - Allow to (dis/en)able expert mode (#157)
- Bugfixes:
 - Hidden axes (“area_cvx”, “area_msd”, “frame”) visible in analysis tab
 - Display of fluorescence traces broken (#159)
 - Display of contour plots broken (#160)
 - Error when loading sessions created with version 0.7.6 on Windows
 - dclab 0.2.5.dev1: Automatically fix ancillary column sizes
 - dclab 0.2.5.dev2: Correctly distinguish b/w hierarchy children of same parent

5.14 version 0.7.6

- Add on-the-fly computation of average and SD for brightness
- Bugfixes:
 - Save sessions with deep (>1) hierarchies correctly
 - Better backwards compatibility for old session files
- Refactoring (dclab 0.2.5):
 - New standard column names
 - New class for handling filtering
 - Minor speed-up when dealing with hierarchies

5.15 version 0.7.5

- Move from OpenCV to imageio (#129)
- Allow to select region and flow rate in batch processing (#132)
- Allow to create an inverted copy of a polygon filter (#127)
- Define new configuration file path with appdirs
- Add on-the-fly computation of volume (dclab 0.2.3.dev23)
- Change: Disable “remove invalid events” in filtering to avoid computation of ancillary columns (performance)

5.16 version 0.7.4

- Add de-/select all buttons to Batch | Statistical analysis
- Add de-/select all buttons to export all events data (#148)
- Bugfixes:
 - Young’s modulus not computed in batch analysis (#149)
 - dclab 0.2.3.dev4: Loading old session files with polygon filters (#152)
 - Sessions saved with Windows cannot be opened on Linux (#153)
 - Regression: save button did not work

5.17 version 0.7.3

- Add interface to calculate elastic modulus
- Bugfixes:
 - Regression analysis not working for some Windows regional settings (#142)
 - Filters not applied when loading a session
 - Regression: Not possible to manually set plotting range

- Excluded events were plotted despite downsampling limit
- Support columns containing NaN and infinite values
- dclab 0.2.3:
 - Add filtering option “remove invalid events” to remove nan/inf
 - Add methods to compute elastic modulus
- fcswrite 0.1.3.dev6
 - Range for fluorescence data is always $2^{*}15$
 - Range for Deformation/Circularity is 100 or 1.0

5.18 version 0.7.2

- Set histogram-based kernel density estimator as default (#133)
- Regression analysis:
 - Added generalized linear mixed effects model (#126)
 - Remember treatment and repetition for each measurement (#134)
- Bugfixes:
 - Same default location for find/add measurements dialogs (#139)
 - Opening saved sessions with more than nine data sets failed (#141)

5.19 version 0.7.1

- Improved speed by optimization of GUI functions
- Hash checks: Replace exception with a user-friendly warning (#128)
- Change: Renamed config key “kde multivariate” to “kde accuracy”
- Bugfixes:
 - Contour plot displays unused contour accuracy parameters
 - Persistent busy cursor when trace or video data is missing
 - Directory selections not always remembered correctly
- dclab:
 - Add histogram-based kernel density estimator
 - Refactoring to improve code quality

5.20 version 0.7.0

- Allow to rename polygon filters (#119)
- dclab:
 - Traces are loaded lazily (on demand)

- Add “Event index” column (#124)

5.21 version 0.6.9

- Disable kernel density estimation and contour plot by default (#116)
- Add avi export option
- Bugfixes:
 - Fluorescence traces not shown for some data sets (#118)
 - Prefer the R package that is shipped with the executable over a system installation (#120)
 - Image plot and fluorescence traces were not always updated correctly
 - Regression: Contour plot offset by one frame in some cases (#121)
- dclab:
 - Add user defined data fields 0..9
 - Add principal components 1 and 2
 - Change Cell size to Area

5.22 version 0.6.8

- Speed-ups:
 - search for measurements (#113)
 - dclab 0.1.9.dev10: contour handling

5.23 version 0.6.7

- Minimize memory usage for batch processing (#108)
- Allow export to flow cytometry standard file format (#54)
- dclab 0.1.9.dev2: add support for “User Defined” column in tdms files
- dclab 0.1.9.dev7: compute default parameters for contours (#55, #110)

5.24 version 0.6.6

- Hide unimportant data in Info tab (#102)
- Disable/Enable controls dependent on values of other controls (#103)
- Bugfixes:
 - Regression: Error when batch analysis started (#105, #106)
 - Contour error when plot contains no events (#104)

5.25 version 0.6.5

- Sessions are now autosaved every 60s (#97)
- Bugfixes:
 - Loading of sessions with hierarchy children failed (#99)
 - Reduced a memory leak for new analyses that resulted in frequent memory errors (should be less frequent now)

5.26 version 0.6.4

- Bugfixes:
 - Batch analysis: Individual measurement parameters not preserved (#96)
 - Legend plot: >9 measurements cannot be displayed in legend at the same time. Introduced new config key “Legend Autoscaled” (#91)
- New filter hierarchy allows to investigate subpopulations (#87, #63)
- dclab
 - Add InertiaRatio and InertiaRatioRaw to tdms files (typo)

5.27 version 0.6.3

- Bugfixes:
 - Regression: Excluded events plotted on top of filtered events (#86)
 - PDF plot export: Scatter inspector event highlighted (#92)
- Implement differential (w.r.t. reservoir) linear-mixed models analysis with bootstrapping (#88)

5.28 version 0.6.2

- Add batch filter processing
- Move statistics computation to dclab (0.1.7.dev12):
 - New columns: “Flow rate”, “%-gated”
- Bugfixes:
 - Windows: Saving session on different drive than data causes ValueError
 - Memory leak: Re-plotting scatter plots (*ControlPanel.OnChangeFilter*)
 - Handle plots where all events are filtered correctly
 - Scatter plots: False filtered events shown in gray
 - dclab (0.1.7.dev9): Faulty hashing strategy in *RTDC_DataSet.GetDownSampledScatter*
 - dclab:
 - * Corrections/additions in definitions (fRT-DC)

5.29 version 0.6.1

- Load session: Allow user to select missing data files (#80)
- Sessions: Store relative paths in index file for user convenience (#80)
- Bugfixes:
 - Setting order of plots not possible in Windows (#79)
 - Inability to search measurements due to broken data files (#81)

5.30 version 0.6.0

- Compute mode in statistics tab using Freedman-Diaconis rule (#72)
- Add channels: bounding box x- and y-size
- Display number of events in measurement browser (#70)
- Bugfixes:
 - “KeyError: ‘Plotting’” when filtering (#73)
 - “AttributeError” when plotting 2col 1row without legend (#76)

5.31 version 0.5.9

- dclab add channel: distance between two first peaks
- Allow logarithmic plot axes (#56)
- Display fluorescence curve of events (#65)
- Bugfixes:
 - OpenCV version 2 vs 3 variable name differences (CV_CAP...)
 - Gray dots in PDF export too large (#69)

5.32 version 0.5.8

- dclab add fluorescence channels: peak area, peak position, number of peaks
- dclab add position along channel axis from img
- dclab (0.1.6.dev2): Allow to disable KDE computation
- Allow to change order of plots (#51)
- Implement indexing of events (#49)
- Allow to exclude single events (#47)
- Allow to show excluded events in scatter plot (#52)
- Display cell images in the polygon selection window (#64)
- Bugfixes:

- Settings panel not redrawn in Ubuntu (#66)
- Order of plots not reproduced from session (#51)
- OpenCV version 2 vs 3 changes (#45)

5.33 version 0.5.7

- Bugfixes:
 - Filtering: Plot selection resets to last item unexpectedly
 - User-defined titles were not saved in session
 - Remove ghost images of cells (#18)
 - dclab (0.1.5.dev13): Correct determination of video file
 - dclab (0.1.5.dev14): Multivariate KDE did not work
 - dclab (0.1.5.dev15): “Contour Fix Scale” did not work for Defo

5.34 version 0.5.6

- Add menu option for data export (#22)
- Add shortcut SHIFT+ENTER for “Apply” controls (#48)
- Bugfixes:
 - Define “Video Frame Offset” parameter to correctly index frames (#46)

5.35 version 0.5.5

- Bugfixes:
 - String formatter did not work (#40)
 - Regression in dclab<0.1.5 (#43)

5.36 version 0.5.4

- Changes in dclab:
 - Support for 3-channel fluorescence data (add corrections 21:42)
 - Fix behaviour with empty columns (#1 dclab)

5.37 version 0.5.3

- Fixes in dclab:
 - Use correct lateral position (from channel walls) (#37)

- Compute aspect ratio according to elongation along flow (#38)
- Allow to change thickness of contour lines (#34)
- Display number of events in statistics tab (#35)
- Improve visual representation in statistics tab

5.38 version 0.5.2

- Bugfix: PDF export did not work (#33)
- Fix disappearance of scrollbars upon hitting apply in controls

5.39 version 0.5.1

- New logo (#25)
- Bugfix: Analysis tab reset button (#30)
- Display correct axis names in Plotting tab
- Fix appveyor build script with pinning and downgrades

5.40 version 0.5.0

- Include full R installation with lme4 package in Windows installer
- Added Analysis tab for application of linear mixed-effects models (#15)
- Allow individual polygon filters for each scatter plot
- Bugfix: Changing filters did not update statistics tab (#24)

5.41 version 0.4.9

- Bugfix: statistics panel head was in wrong order
- Allow to fix contour plot scaling (#9)
- Ordered parameters in control panels

5.42 version 0.4.8

- New statistics tab (#6)
- Minor improvements of GUI
- Update from dclab: errors when using MV KDE (#20)

5.43 version 0.4.7

- Fix loading of sessions (#17)

5.44 version 0.4.6

- Limit number of events for data analysis (#11)
- Automatically set number of events for multiple data sets (#11)
- Display number of used events on plots (#10)

CHAPTER 6

Bibliography

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`
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