# dclab Documentation

Release 0.7.0

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Dclab is a Python library for the post-measurement analysis of real-time deformability cytometry (RT-DC) datasets. This is the documentation of dclab version 0.7.0.

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# CHAPTER 1

Getting started

## 1.1 Installation

Dclab depends on several other Python packages:

- fcswrite (.fcs file export),
- h5py (.rtdc file support).
- imageio (.tdms file support, .avi file export),
- nptdms (.tdms file support),
- numpy,
- scipy,
- · statsmodels.

In addition, dclab contains code from OpenCV (computation of moments) and scikit-image (computation of contours) to reduce the list of dependencies (these libraries are not required by dclab).

To install dclab, use one of the following methods (the above package dependencies will be installed automatically):

- from PyPI: pip install dclab
- from sources: pip install . or python setup.py install

Note that if you are installing from source or if no binary wheel is available for your platform and Python version, Cython will be installed to build the required dclab extensions. If this process fails, please request a binary wheel for your platform (e.g. Windows 64bit) and Python version (e.g. 3.6) by creating a new issue.

## 1.2 Use cases

If you are a frequent user of RT-DC, you might run into problems that cannot (yet) be addressed with the graphical user interface ShapeOut. Here is a list of use cases that would motivate an installation of dclab.

- You would like to convert old .tdms-based datasets to the new .rtdc file format, because of enhanced speed in ShapeOut and reduced disk usage. What you are looking for is the command line program tdms2rtdc that comes with dclab. It allows to batch-convert multiple measurements at a time. Note that you should keep the original .tdms files backed-up somewhere, because there might be future improvements or bug fixes from which you would like to benefit.
- You would like to apply a simple set of filters (e.g. polygon filters that you exported from within ShapeOut) to every new measurement you take and apply a custom data analysis pipeline to the filtered data. This is a straight-forward Python coding problem with dclab. After reading the basic usage section below, please have a look at the *polygon filter reference*.
- You would like to do advanced statistics or combine your RT-DC analysis with other fancy approaches such as machine-learning. It would be too laborious to do the analysis in ShapeOut, export the data as text files, and then open them in your custom Python script. If your initial analysis step with ShapeOut only involves tasks that can be automated, why not use dclab from the beginning?
- You simulated RT-DC data and plan to import them in ShapeOut for testing. Once you have loaded your data as a numpy array, you can instantiate an RTDC\_Dict class and then use the Export class to create an .rtdc data file.

If you are still unsure about whether to use dclab or not, you might want to look at the *example section*. If you need advice, do not hesitate to create an issue.

# 1.3 Basic usage

Experimental RT-DC datasets are always loaded with the new\_dataset method:

```
import numpy as np
import dclab

# .tdms file format
ds = dclab.new_dataset("/path/to/measurement/Online/M1.tdms")
# .rtdc file format
ds = dclab.new_dataset("/path/to/measurement/M2.rtdc")
```

The object returned by *new\_dataset* is always an instance of RTDCBase. To show all available features, use:

```
print (ds.features)
```

This will list all scalar features (e.g. "area\_um" and "deform") and all non-scalar features (e.g. "contour" and "image"). Scalar features can be filtered by editing the configuration of ds and calling ds.apply\_filter():

```
# register filtering operations
amin, amax = ds["area_um"].min(), ds["area_um"].max()
ds.config["filtering"]["area_um min"] = (amax + amin) / 2
ds.config["filtering"]["area_um max"] = amax
ds.apply_filter() # this step is important!
```

This will update the binary array ds.filter.all which can be used to extract the filtered data:

```
area_um_filtered = ds["area_um"][ds.filter.all]
```

It is also possible to create a hierarchy child of this dataset that only contains the filtered data.

```
ds_child = dclab.new_dataset(ds)
```

The hierarchy child  $ds\_child$  is dynamic, i.e. when the filters in ds change, then  $ds\_child$  also changes after calling  $ds\_child$ . apply\_filter().

Non-scalar features do not support fancy indexing (i.e. ds["image"][ds.filter.all] will not work. Use a for-loop to extract them.

```
for ii in range(len(ds)):
    image = ds["image"][ii]
    mask = ds["mask"][ii]
    # this is equivalent to ds["bright_avg"][ii]
    bright_avg = np.mean(image[mask])
    print("average brightness of event {}: {:.1f}".format(ii, bright_avg))
```

If you need more information to get started on your particular problem, you might want to check out the *examples* section and the *advanced scripting section*.

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# CHAPTER 2

## Command line interface

## 2.1 tdms2rtdc

Convert RT-DC .tdms files to the hdf5-based .rtdc file format. Note: Do not delete original .tdms files after conversion. The conversion might be incomplete.

```
usage: dclab-tdms2rtdc [-h] [--compute-ancillary-features] tdms-path rtdc-path
```

## 2.1.1 Positional Arguments

tdms-path Input path (tdms file or folder containing tdms files)

rtdc-path Output path (file or folder), existing data will be overridden

## 2.1.2 Named Arguments

**--compute-ancillary-features** Compute features, such as volume or emodulus, that are otherwise com-

puted on-the-fly. Use this if you want to minimize analysis time in e.g. ShapeOut. CAUTION: ancillary feature recipes might be subject to change (e.g. if an error is found in the recipe). Disabling this option maximizes compatibility with future versions and allows to isolate the original data.

Default: False

# 2.2 verify-dataset

Check experimental datasets for completeness. Note that old measurements will most likely fail this verification step. This program is used to enforce data integrity with future implementations of RT-DC recording software (e.g. ShapeIn).

usage: dclab-verify-dataset [-h] path

# 2.2.1 Positional Arguments

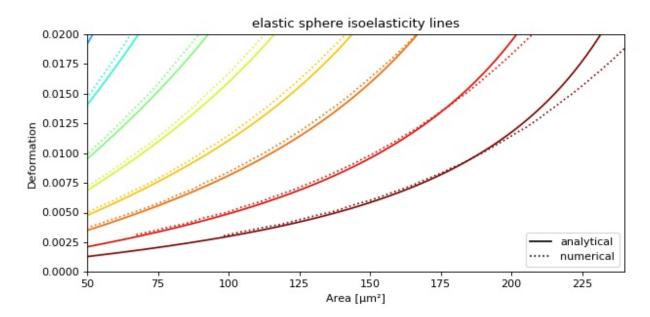
**path** Path to experimental dataset

# CHAPTER 3

Examples

# 3.1 Plotting isoelastics

This example illustrates how to plot dclab isoelastics by reproducing figure 3 (lower left) of [MMM+17].



isoelastics.py

```
import matplotlib.pylab as plt
import matplotlib.lines as mlines
from matplotlib import cm
import numpy as np
```

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```
import dclab
   # parameters for isoelastics
   kwargs = {"col1": "area_um",  # x-axis
             "col2": "deform", # y-axis
10
             "channel_width": 20, # [um]
11
             "flow_rate": 0.04, # [ul/s]
12
             "viscosity": 15, # [Pa s]
13
             "add_px_err": False # no pixelation error
14
15
             }
   isos = dclab.isoelastics.get_default()
   analy = isos.get(method="analytical", **kwargs)
   numer = isos.get(method="numerical", **kwargs)
19
20
   plt.figure(figsize=(8, 4))
21
   ax = plt.subplot(111, title="elastic sphere isoelasticity lines")
22
   colors = [cm.get_cmap("jet")(x) for x in np.linspace(0, 1, len(analy))]
23
   for aa, nn, cc in zip(analy, numer, colors):
24
       ax.plot(aa[:, 0], aa[:, 1], color=cc)
25
       ax.plot(nn[:, 0], nn[:, 1], color=cc, ls=":")
26
27
   line = mlines.Line2D([], [], color='k', label='analytical')
28
   dotted = mlines.Line2D([], [], color='k', ls=":", label='numerical')
   ax.legend(handles=[line, dotted])
32
   ax.set_xlim(50, 240)
  ax.set_ylim(0, 0.02)
33
   ax.set_xlabel(dclab.dfn.feature_name2label["area_um"])
34
   ax.set_ylabel(dclab.dfn.feature_name2label["deform"])
35
   plt.tight_layout()
   plt.show()
```

# Advanced Usage

This section motivates the design of dclab and highlights useful built-in functionalities.

# 4.1 Notation

When coding with dclab, you should be aware of the following definitions and design principles.

## **4.1.1 Events**

An event comprises all data recorded for the detection of one object (e.g. cell or bead) in an RT-DC measurement.

## 4.1.2 Features

A feature is a measurement parameter of an RT-DC measurement. For instance, the feature "index" enumerates all recorded events, the feature "deform" contains the deformation values of all events. There are scalar features, i.e. features that assign a single number to an event, and non-scalar features, such as "image" and "contour". The following features are supported by dclab:

scalar features	description [units]
area_cvx	Convex area [px]
area_msd	Measured area [px]
area_ratio	Porosity (convex to measured area ratio)
area_um	Area [μm <sup>2</sup> ]
aspect	Aspect ratio of bounding box
bright_avg	Brightness average within contour [a.u.]
bright_sd	Brightness SD within contour [a.u.]
circ	Circularity
deform	Deformation

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Table 1 – continued from previous page

applar factures	description [unite]
scalar features	description [units]
emodulus	Young's Modulus [kPa]
fl1_area	FL-1 area of peak [a.u.]
fl1_dist	FL-1 distance between two first peaks [μs]
fl1_max	FL-1 maximum [a.u.]
fl1_max_ctc	FL-1 maximum, crosstalk-corrected [a.u.]
fl1_npeaks	FL-1 number of peaks
fl1_pos	FL-1 position of peak [μs]
fl1_width	FL-1 width [μs]
fl2_area	FL-2 area of peak [a.u.]
fl2_dist	FL-2 distance between two first peaks [µs]
fl2_max	FL-2 maximum [a.u.]
fl2_max_ctc	FL-2 maximum, crosstalk-corrected [a.u.]
fl2_npeaks	FL-2 number of peaks
fl2_pos	FL-2 position of peak [µs]
fl2_width	FL-2 width [μs]
fl3_area	FL-3 area of peak [a.u.]
fl3_dist	FL-3 distance between two first peaks [µs]
fl3_max	FL-3 maximum [a.u.]
fl3_max_ctc	FL-3 maximum, crosstalk-corrected [a.u.]
fl3_npeaks	FL-3 number of peaks
fl3_pos	FL-3 position of peak [µs]
fl3_width	FL-3 width [μs]
frame	Video frame number
index	Event index
inert_ratio_cvx	Inertia ratio of convex contour
inert_ratio_prnc	Principal inertia ratio of raw contour
inert_ratio_raw	Inertia ratio of raw contour
nevents	Total number of events in the same image
pc1	Principal component 1
pc2	Principal component 2
pos_x	Position along channel axis [µm]
pos_y	Position lateral in channel [µm]
size_x	Bounding box size x [µm]
size_y	Bounding box size y [µm]
tilt	Absolute tilt of raw contour
time	Event time [s]
userdef0	User defined 0
userdef1	User defined 1
userdef2	User defined 2
userdef3	User defined 3
userdef4	User defined 4
userdef5	User defined 5
userdef6	User defined 6
userdef7	User defined 7
userdef8	User defined 8
userdef9	User defined 9
volume	Volume [µm <sup>3</sup> ]
	H 4

non-scalar features	description [units]
contour	Binary event contour image
image	Gray scale event image
mask	Binary region labeling the event in the image
trace	Dictionary of fluorescence traces

## Example: deformation vs. area plot

```
import matplotlib.pylab as plt
import dclab
ds = dclab.new_dataset("data/example.rtdc")
ax = plt.subplot(111)
ax.plot(ds["area_um"], ds["deform"], "o", alpha=.2)
ax.set_xlabel(dclab.dfn.feature_name2label["area_um"])
ax.set_ylabel(dclab.dfn.feature_name2label["deform"])
plt.show()
```

### Example: event image plot

```
import matplotlib.pylab as plt
import dclab
ds = dclab.new_dataset("data/example_video.rtdc")
ax1 = plt.subplot(211, title="image")
ax2 = plt.subplot(212, title="mask")
ax1.imshow(ds["image"][6], cmap="gray")
ax2.imshow(ds["mask"][6])
```

# 4.1.3 Ancillary features

Not all features available in delab are recorded online during the acquisition of the experimental dataset. Some of the features are computed offline by delab, such as "volume" or "emodulus". These ancillary features are computed on-the-fly and are made available seamlessly through the same interface.

## 4.1.4 Filters

A filter can be used to gate events using features. There are min/max filters and 2D *polygon filters*. The following table defines the main filtering parameters:

filtering	parsed	description [units]
enable filters	fbool	Enable filtering
hierarchy parent	str	Hierarchy parent of the dataset
limit events	fint	Upper limit for number of filtered events
polygon filters	fintlis	tPolygon filter indices
remove invalid events	fbool	Remove events with inf/nan values

Min/max filters are also defined in the filters section:

filtering	explanation
area_um min	Exclude events with area [µm²] below this value
area_um max	Exclude events with area [µm²] above this value
aspect max	Exclude events with an aspect ratio above this value

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**Example:** excluding events with large deformation

```
import matplotlib.pylab as plt
import dclab
ds = dclab.new_dataset("data/example.rtdc")
ds.config["filtering"]["deform max"] = .1
ds.apply_filter()
dif = ds.filter.all
f, axes = plt.subplots(1, 2, sharex=True, sharey=True)
axes[0].plot(ds["area_um"], ds["bright_avg"], "o", alpha=.2)
axes[0].set_title("unfiltered")
axes[1].plot(ds["area_um"][dif], ds["bright_avg"][dif], "o", alpha=.2)
axes[1].set_title("Deformation <= 0.1")</pre>
for ax in axes:
    ax.set_xlabel(dclab.dfn.feature_name2label["area_um"])
    ax.set_ylabel(dclab.dfn.feature_name2label["bright_avg"])
plt.tight_layout()
plt.show()
```

**Example: excluding random events** This is useful if you need to have a (sub-)dataset of a specified size. The down-sampling is reproducible (the same points are excluded).

```
import matplotlib.pylab as plt
import dclab
ds = dclab.new_dataset("data/example.rtdc")
ds.config["filtering"]["limit events"] = 4000
ds.apply_filter()
fid = ds.filter.all

ax = plt.subplot(111)
ax.plot(ds["area_um"][fid], ds["deform"][fid], "o", alpha=.2)
ax.set_xlabel(dclab.dfn.feature_name2label["area_um"])
ax.set_ylabel(dclab.dfn.feature_name2label["deform"])
plt.show()
```

## 4.1.5 Experiment metadata

Every RT-DC measurement has metadata consisting of key-value-pairs. The following are supported:

experiment	parsed	description [units]
date	str	Date of measurement ('YYYY-MM-DD')
event count	fint	Number of recorded events
run index	fint	Index of measurement run
sample	str	Measured sample or user-defined reference
time	str	Start time of measurement ('HH:MM:SS')

fluorescence	parsed	description [units]
bit depth	fint	Trace bit depth
channel 1 name	str	FL1 description
channel 2 name	str	FL2 description
channel 3 name	str	FL3 description
channel count	fint	Number of active channels
channels installed	fint	Number of available channels
laser 1 lambda	float	Laser 1 wavelength [nm]
laser 1 power	float	Laser 1 output power [%]
laser 2 lambda	float	Laser 2 wavelength [nm]
laser 2 power	float	Laser 2 output power [%]
laser 3 lambda	float	Laser 3 wavelength [nm]
laser 3 power	float	Laser 3 output power [%]
laser count	fint	Number of active lasers
lasers installed	fint	Number of available lasers
sample rate	float	Trace sample rate [Hz]
samples per event	fint	Samples per event
signal max	float	Upper voltage detection limit [V]
signal min	float	Lower voltage detection limit [V]
trace median	fint	Rolling median filter size for traces

fmt_tdms	parsed	description [units]
video frame offset	fint	Missing events at beginning of video

imaging	parsed	description [units]
flash device	str	Light source device type (e.g. green LED)
flash duration	float	Light source flash duration [μs]
frame rate	float	Imaging frame rate [Hz]
pixel size	float	Pixel size [µm]
roi position x	float	Image x coordinate on sensor [px]
roi position y	float	Image y coordinate on sensor [px]
roi size x	fint	Image width [px]
roi size y	fint	Image height [px]

online_contour	parsed	description [units]
bin area min	fint	Minium pixel area of binary image event
bin kernel	fint	Odd ellipse kernel size, binary image morphing
bin threshold	fint	Binary threshold for avg-bg-corrected image
image blur	fint	Odd sigma for Gaussian blur (21x21 kernel)
no absdiff	fbool	Avoid OpenCV 'absdiff' for avg-bg-correction

4.1. Notation

online_filter	parsed	description [units]
area_ratio max	float	Maximum porosity
area_ratio min	float	Minimum porosity
area_ratio soft limit	fbool	Soft limit, porosity
area_um max	float	Maximum area [μm <sup>2</sup> ]
area_um min	float	Minimum area [μm <sup>2</sup> ]
area_um soft limit	fbool	Soft limit, area [µm <sup>2</sup> ]
aspect max	float	Maximum aspect ratio of bounding box
aspect min	float	Minimum aspect ratio of bounding box
aspect soft limit	fbool	Soft limit, aspect ratio of bbox
deform max	float	Maximum deformation
deform min	float	Minimum deformation
deform soft limit	fbool	Soft limit, deformation
fl1_max max	float	Maximum FL-1 maximum [a.u.]
fl1_max min	float	Minimum FL-1 maximum [a.u.]
fl1_max soft limit	fbool	Soft limit, FL-1 maximum
fl2_max max	float	Maximum FL-2 maximum [a.u.]
fl2_max min	float	Minimum FL-2 maximum [a.u.]
fl2_max soft limit	fbool	Soft limit, FL-2 maximum
fl3_max max	float	Maximum FL-3 maximum [a.u.]
fl3_max min	float	Minimum FL-3 maximum [a.u.]
fl3_max soft limit	fbool	Soft limit, FL-3 maximum
size_x max	fint	Maximum bounding box size x [µm]
size_x min	fint	Minimum bounding box size x [μm]
size_x soft limit	fbool	Soft limit, bounding box size x
size_y max	fint	Maximum bounding box size y [µm]
size_y min	fint	Minimum bounding box size y [µm]
size_y soft limit	fbool	Soft limit, bounding box size y
target duration	float	Target measurement duration [min]
target event count	fint	Target event count for online gating

setup	parsed	description [units]
channel width	float	Width of microfluidic channel [µm]
chip region	lcstr	Imaged chip region (channel or reservoir)
flow rate	float	Flow rate in channel [µL/s]
flow rate sample	float	Sample flow rate [µL/s]
flow rate sheath	float	Sheath flow rate [µL/s]
identifier	str	Unique setup identifier
medium	str	Medium used
module composition	str	Comma-separated list of modules used
software version	str	Acquisition software with version

## **Example**: date and time of a measurement

```
In [1]: import dclab
In [2]: ds = dclab.new_dataset("data/example.rtdc")
In [3]: ds.config["experiment"]["date"], ds.config["experiment"]["time"]
Out[3]: ('2017-07-16', '19:01:36')
```

## 4.1.6 Analysis metadata

In addition to metadata, dclab also supports a user-defined analysis configuration which is usually part of a specific analysis pipeline and thus not considered to be experimental metadata.

calculation	parsed	description [units]
crosstalk fl12	float	Fluorescence crosstalk, channel 1 to 2
crosstalk fl21	float	Fluorescence crosstalk, channel 2 to 1
crosstalk fl31	float	Fluorescence crosstalk, channel 1 to 3
crosstalk fl31	float	Fluorescence crosstalk, channel 3 to 1
crosstalk fl32	float	Fluorescence crosstalk, channel 2 to 3
crosstalk fl32	float	Fluorescence crosstalk, channel 3 to 1
emodulus medium	str	Medium used (e.g. CellCarrierB, water)
emodulus model	lcstr	Model for computing elastic moduli
emodulus temperature	float	Chip temperature [°C]
emodulus viscosity	float	Viscosity [Pa*s] if 'medium' unknown

## 4.2 RT-DC datasets

Knowing and understanding the *RT-DC dataset classes* is an important prerequisite when working with dclab. They are all derived from *RTDCBase* which gives access to feature with a dictionary-like interface, facilitates data export and filtering, and comes with several convenience methods that are useful for data visualization. RT-DC datasets can be based on a data file format (*RTDC\_TDMS* and *RTDC\_HDF5*), created from user-defined dictionaries (*RTDC\_Dict*), or derived from other RT-DC datasets (*RTDC\_Hierarchy*).

## 4.2.1 Loading data from disk

The convenience function <code>dclab.new\_dataset()</code> takes care of determining the data file format (tdms or hdf5) and returns the corresponding derived class.

```
In [1]: import dclab
In [2]: ds = dclab.new_dataset("data/example.rtdc")
In [3]: ds.__class__.__name__
Out[3]: 'RTDC_HDF5'
```

## Working with other data

It is also possible to load other data into delab from a dictionary.

4.2. RT-DC datasets

## **Creating hierarchies**

When applying filtering operations, it is sometimes helpful to use hierarchies for keeping track of the individual filtering steps.

Note that calling ds1\_b.apply\_filter() automatically calls ds1\_a.apply\_filter() and ds1. apply\_filter(). Also note that, as expected, the size of each hierarchy child is identical to the sum of the boolean filtering array from its hierarchy parent.

## **Scripting goodies**

Here are a few useful functionalities for scripting with dclab.

```
# unique identifier of the RTDCBase instance (not reproducible)
In [14]: ds.identifier
Out [14]: 'mm-hdf5_ce6af29'
# reproducible hash of the dataset
In [15]: ds.hash
# dataset format
In [16]: ds.format
# available features
In [17]: ds.features
['area_cvx',
'area_msd',
'area_ratio',
'area_um',
'aspect',
'bright_avg',
'bright_sd',
'circ',
'deform',
'frame',
'index',
```

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```
'inert_ratio_cvx',
'inert_ratio_raw',
'nevents',
'pos_x',
'pos_y',
'size_x',
'size_y',
'time']
# test feature availability (success)
In [18]: "area_um" in ds
→ True
# test feature availability (failure)
In [19]: "image" in ds
→False
# accessing a feature and computing its mean
In [20]: ds["area_um"].mean()
49.728645
# accessing the measurement configuration
In [21]: ds.config.keys()
→dict_keys(['imaging', 'setup', 'filtering', 'experiment', 'online_contour'])
In [22]: ds.config["experiment"]
{'date': '2017-07-16',
'event count': 5000,
'run index': 1,
'sample': 'docs-data',
'time': '19:01:36'}
# determine the identifier of the hierarchy parent
In [23]: child.config["filtering"]["hierarchy parent"]
→ 'mm-hdf5_ce6af29'
```

# 4.3 Data processing

### 4.3.1 Visualization

For data visualization, dclab comes with predefined *kernel density estimators (KDEs)* and an *event downsampling* module. The functionalities of both modules are made available directly via the RTDCBase class.

### **KDE** scatter plot

The KDE of the events in a 2D scatter plot can be used to colorize events according to event density using the RTDCBase.get\_kde\_scatter function.

```
import matplotlib.pylab as plt
import dclab
ds = dclab.new_dataset("data/example.rtdc")
kde = ds.get_kde_scatter(xax="area_um", yax="deform")

ax = plt.subplot(111, title="scatter plot with {} events".format(len(kde)))
sc = ax.scatter(ds["area_um"], ds["deform"], c=kde, marker=".")
ax.set_xlabel(dclab.dfn.feature_name2label["area_um"])
ax.set_ylabel(dclab.dfn.feature_name2label["deform"])
ax.set_xlim(0, 150)
ax.set_ylim(0.01, 0.12)
plt.colorbar(sc, label="kernel density estimate [a.u]")
plt.show()
```

## KDE scatter plot with event-density-based downsampling

To reduce the complexity of the plot (e.g. when exporting to scalable vector graphics (.svg)), the plotted events can be downsampled by removing events from high-event-density regions. The number of events plotted is reduced but the resulting visualization is almost indistinguishable from the one above.

```
import matplotlib.pylab as plt
import dclab
ds = dclab.new_dataset("data/example.rtdc")
xsamp, ysamp = ds.get_downsampled_scatter(xax="area_um", yax="deform",
downsample=2000)
kde = ds.get_kde_scatter(xax="area_um", yax="deform", positions=(xsamp, ysamp))

ax = plt.subplot(111, title="downsampled to {} events".format(len(kde)))
sc = ax.scatter(xsamp, ysamp, c=kde, marker=".")
ax.set_xlabel(dclab.dfn.feature_name2label["area_um"])
ax.set_ylabel(dclab.dfn.feature_name2label["deform"])
ax.set_ylabel(dclab.dfn.feature_name2label["deform"])
plt.colorbar(sc, label="kernel density estimate [a.u]")
plt.show()
```

### **Isoelasticity lines**

In addition, dclab comes with predefined isoelasticity lines that are commonly used to identify events with similar elastic moduli. Isoelasticity lines are available via the *isoelastics* module.

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### **Contour plot**

Contour plots are commonly used to compare the kernel density between measurements. Kernel density estimates (on a grid) for contour plots can be computed with the function RTDCBase.get\_kde\_contour.

```
import matplotlib.pylab as plt
import dclab
ds = dclab.new_dataset("data/example.rtdc")
X, Y, Z = ds.get_kde_contour(xax="area_um", yax="deform")
Z /= Z.max()
ax = plt.subplot(111, title="contour lines")
sc = ax.scatter(ds["area_um"], ds["deform"], c="lightgray", marker=".", zorder=1)
cn = ax.contour(X, Y, Z,
                levels=[.03, .2, .75],
                linestyles=["--", "-", "-"],
                colors=["blue", "blue", "darkblue"],
                linewidths=[2, 2, 3],
                zorder=2)
ax.set_xlabel(dclab.dfn.feature_name2label["area_um"])
ax.set_ylabel(dclab.dfn.feature_name2label["deform"])
ax.set_xlim(0, 150)
ax.set_ylim(0.01, 0.12)
plt.clabel(cn, fmt="%.2f")
plt.show()
```

## 4.3.2 Statistics

The *statistics* module comes with a predefined set of methods to compute simple feature statistics.

(continues on next page)

(continued from previous page)

```
In [4]: dict(zip(*stats))
Out[4]:
{'Mean Aspect ratio of bounding box': 1.2719607,
   'Mean Deformation': 0.0287258,
   'Mode Aspect ratio of bounding box': 1.1091422,
   'Mode Deformation': 0.016635261,
   'SD Aspect ratio of bounding box': 0.25233853,
   'SD Deformation': 0.028740086}
```

Note that the statistics take into account the applied filters:

```
In [5]: ds.config["filtering"]["deform max"] = .1
In [6]: ds.apply_filter()
In [7]: stats2 = dclab.statistics.get_statistics(ds,
                                                   features=["deform", "aspect"],
   . . . :
                                                   methods=["Mode", "Mean", "SD"])
   . . . :
   . . . :
In [8]: dict(zip(*stats2))
Out[8]:
{'Mean Aspect ratio of bounding box': 1.2407207,
'Mean Deformation': 0.02476519,
'Mode Aspect ratio of bounding box': 1.1232222,
'Mode Deformation': 0.017006295,
 'SD Aspect ratio of bounding box': 0.15993708,
 'SD Deformation': 0.015638638}
```

These are the available statistics methods:

```
In [9]: dclab.statistics.Statistics.available_methods.keys()
Out[9]: dict_keys(['Mean', '%-gated', 'Mode', 'Events', 'Median', 'SD', 'Flow rate'])
```

## **4.3.3 Export**

The RTDCBase class has the attribute RTDCBase.export which allows to export event data to several data file formats. See *export* for more information.

Note that data exported as HDF5 files can be loaded with dclab (reproducing the previously computed statistics - without filters).

```
In [12]: ds2 = dclab.new_dataset("export_example.rtdc")
In [13]: ds2["deform"].mean()
Out[13]: 0.02476519
```

## 4.3.4 ShapeOut

Keep in mind that you can combine your dclab analysis pipeline with ShapeOut. For instance, you can create and export *polygon filters* in ShapeOut and then import them in dclab.

```
import matplotlib.pylab as plt
import dclab
ds = dclab.new_dataset("data/example.rtdc")
kde = ds.get_kde_scatter(xax="area_um", yax="deform")
# load and apply polygon filter from file
pf = dclab.PolygonFilter(filename="data/example.poly")
ds.polygon_filter_add(pf)
ds.apply_filter()
# valid events
val = ds.filter.all
ax = plt.subplot(111, title="polygon filtering")
ax.scatter(ds["area_um"][~val], ds["deform"][~val], c="lightgray", marker=".")
sc = ax.scatter(ds["area_um"][val], ds["deform"][val], c=kde[val], marker=".")
ax.set_xlabel(dclab.dfn.feature_name2label["area_um"])
ax.set_ylabel(dclab.dfn.feature_name2label["deform"])
ax.set_xlim(0, 150)
ax.set_ylim(0.01, 0.12)
plt.colorbar(sc, label="kernel density estimate [a.u]")
plt.show()
```

# CHAPTER 5

Code reference

# 5.1 module-level methods

#### **Parameters**

- data can be one of the following:
  - dict
  - .tdms file
  - .rtdc file
  - subclass of RTDCBase (will create a hierarchy child)
- **identifier** (*str*) A unique identifier for this dataset. If set to *None* an identifier is generated.

Returns dataset - A new dataset instance

Return type subclass of dclab.rtdc\_dataset.RTDCBase

# 5.2 global definitions

These definitionas are used throughout the dclab/ShapeIn/ShapeOut ecosystem.

# 5.2.1 configuration

Valid configuration sections and keys are described in: Analysis metadata and Experiment metadata.

dclab.dfn.CFG\_ANALYSIS

User-editable configuration for data analysis.

```
dclab.dfn.CFG METADATA
```

Measurement-specific metadata.

### dclab.dfn.config\_funcs

Dictionary of dictionaries containing functions to convert input data to the predefined data type

### dclab.dfn.config\_keys

Dictionary with sections as keys and configuration parameter names as values

### dclab.dfn.config\_types

Dictionary of dictionaries containing the data type of each configuration parameter

### 5.2.2 features

Features are discussed in more detail in: Features.

dclab.dfn.FEATURES\_SCALAR

Scalar features

dclab.dfn.FEATURES\_NON\_SCALAR

Non-scalar features

dclab.dfn.feature\_names

List of valid feature names

dclab.dfn.feature labels

List of human-readable labels for each valid feature

dclab.dfn.feature\_name2label

Dictionary that maps feature names to feature labels

dclab.dfn.scalar\_feature\_names

List of valid scalar feature names

## 5.2.3 parse functions

```
boolean

dclab.parse_funcs.fint(value)
    integer

dclab.parse_funcs.fintlist(alist)
    A list of integers

dclab.parse_funcs.lcstr(astr)
    lower-case string
```

dclab.parse\_funcs.fbool(value)

dclab.parse\_funcs.func\_types = {<function fbool>: <class 'bool'>, <function fint>: <class functions to their expected output types</pre>

# 5.3 RT-DC dataset manipulation

## 5.3.1 Base class

```
class dclab.rtdc_dataset.RTDCBase(identifier=None)
    RT-DC measurement base class
```

#### **Notes**

Besides the filter arrays for each data feature, there is a manual boolean filter array RTDCBase.filter. manual that can be edited by the user - a boolean value of False means that the event is excluded from all computations.

```
apply_filter(force=[])
```

Compute the filters for the dataset

get\_downsampled\_scatter (xax='area\_um', yax='deform', downsample=0)

Downsampling by removing points at dense locations

#### **Parameters**

- **xax** (str) Identifier for x axis (e.g. "area\_um", "aspect", "deform")
- yax (str) Identifier for y axis
- downsample (int or None) Number of points to draw in the down-sampled plot. This number is either
  - >=1: exactly downsample to this number by randomly adding or removing points
  - 0 : do not perform downsampling

#### Returns xnew, xnew

Return type filtered x and y

Evaluate the kernel density estimate for contour plots

#### **Parameters**

- xax (str) Identifier for X axis (e.g. "area\_um", "aspect", "deform")
- yax (str) Identifier for Y axis
- xacc (float) Contour accuracy in x direction
- yacc (float) Contour accuracy in y direction
- kde\_type (str) The KDE method to use
- kde\_kwargs (dict) Additional keyword arguments to the KDE method

**Returns** X, Y, Z – The kernel density Z evaluated on a rectangular grid (X,Y).

**Return type** coordinates

Evaluate the kernel density estimate for scatter plots

#### **Parameters**

- xax (str) Identifier for X axis (e.g. "area\_um", "aspect", "deform")
- yax (str) Identifier for Y axis
- **positions** (*list of points*) The positions where the KDE will be computed. Note that the KDE estimate is computed from the points that are set in *self\_filter*.
- kde\_type (str) The KDE method to use

• **kde\_kwargs** (dict) – Additional keyword arguments to the KDE method

**Returns density** – The kernel density evaluated for the filtered data points.

Return type 1d ndarray

### polygon\_filter\_add(filt)

Associate a Polygon Filter with this instance

Parameters filt (int or instance of PolygonFilter) – The polygon filter to add

### $polygon_filter_rm(filt)$

Remove a polygon filter from this instance

**Parameters filt** (int or instance of *PolygonFilter*) – The polygon filter to remove

#### config = None

Configuration of the measurement

#### export = None

Export functionalities; instance of dclab.rtdc\_dataset.export.Export.

#### features

All available features

#### filter = None

Filtering functionalities; instance of dclab.rtdc\_dataset.filter.Filter.

#### format = None

Dataset format (derived from class name)

#### hash

Reproducible dataset hash (defined by derived classes)

### identifier

Unique (unreproducible) identifier

#### title = None

Title of the measurement

# 5.3.2 Dictionary format

```
class dclab.rtdc_dataset.RTDC_Dict(ddict, *args, **kwargs)
    Dictionary-based RT-DC dataset
```

### **Parameters**

- **ddict** (dict) Dictionary with keys from dclab.definitions.feature\_names (e.g. "area\_cvx", "deform", "image") with which the class will be instantiated. The configuration is set to the default configuration of dclab.
- \*args Arguments for RTDCBase
- \*\*kwargs Keyword arguments for RTDCBase

## 5.3.3 HDF5 (.rtdc) format

#### **Parameters**

- h5path (str or pathlib.Path) Path to a '.tdms' measurement file.
- \*args Arguments for RTDCBase
- \*\*kwargs Keyword arguments for RTDCBase

#### path

pathlib.Path – Path to the experimental HDF5 (.rtdc) file

#### static parse\_config(h5path)

Parse the RT-DC configuration of an hdf5 file

dclab.rtdc\_dataset.fmt\_hdf5.MIN\_DCLAB\_EXPORT\_VERSION = '0.3.3.dev2'
 rtdc files exported with dclab prior to this version are not supported

## 5.3.4 Hierarchy format

A few words on hierarchies: The idea is that a subclass of RTDCBase can use the filtered data of another subclass of RTDCBase and interpret these data as unfiltered events. This comes in handy e.g. when the percentage of different subpopulations need to be distinguished without the noise in the original data.

Children in hierarchies always update their data according to the filtered event data from their parent when *apply\_filter* is called. This makes it easier to save and load hierarchy children with e.g. ShapeOut and it makes the handling of hierarchies more intuitive (when the parent changes, the child changes as well).

#### **Parameters**

- hparent (instance of RTDCBase) The hierarchy parent.
- \*args Arguments for RTDCBase
- **\*\*kwargs** Keyword arguments for *RTDCBase*

#### hparent

RTDCBase - Hierarchy parent of this instance

### 5.3.5 TDMS format

```
class dclab.rtdc_dataset.RTDC_TDMS (tdms_path, *args, **kwargs)
    TDMS file format for RT-DC measurements
```

#### **Parameters**

- tdms\_path (str or pathlib.Path) Path to a '.tdms' measurement file.
- \*args Arguments for RTDCBase
- \*\*kwargs Keyword arguments for RTDCBase

#### path

pathlib.Path – Path to the experimental dataset (main .tdms file)

dclab.rtdc\_dataset.fmt\_tdms.get\_project\_name\_from\_path(path, append\_mx=False)

Get the project name from a path.

For a path "/home/peter/hans/HLC12398/online/M1\_13.tdms" or For a path "/home/peter/hans/HLC12398/online/data/M1\_13.tdms" or without the ".tdms" file, this will return always "HLC12398".

#### **Parameters**

- path (str) path to tdms file
- append\_mx (bool) append measurement number, e.g. "M1"

```
dclab.rtdc_dataset.fmt_tdms.get_tdms_files(directory)
```

Recursively find projects based on '.tdms' file endings

Searches the *directory* recursively and return a sorted list of all found '.tdms' project files, except fluorescence data trace files which end with *\_traces.tdms*.

# 5.3.6 config

```
class dclab.rtdc_dataset.config.Configuration(files=[], cfg={})
    Configuration class for RT-DC datasets
```

This class has a dictionary-like interface to access and set configuration values, e.g.

```
cfg = load_from_file("/path/to/config.txt")
# access the channel width
cfg["setup"]["channel width"]
# modify the channel width
cfg["setup"]["channel width"] = 30
```

#### **Parameters**

- **files** (list of files) The config files with which to initialize the configuration
- **cfg** (dict-like) The dictionary with which to initialize the configuration

```
copy()
```

Return copy of current configuration

#### keys()

Return the configuration keys (sections)

save (filename)

Save the configuration to a file

update (newcfg)

Update current config with a dictionary

```
dclab.rtdc_dataset.config.load_from_file(cfg_file)
```

Load the configuration from a file

**Parameters** cfg\_file (str) – Path to configuration file

**Returns** cfg – Dictionary with configuration parameters

Return type CaseInsensitiveDict

## **5.3.7** export

avi (path, filtered=True, override=False)

Exports filtered event images to an avi file

#### **Parameters**

- path (str) Path to a .tsv file. The ending .tsv is added automatically.
- **filtered** (bool) If set to *True*, only the filtered data (index in ds.\_filter) are used.
- **override** (bool) If set to *True*, an existing file path will be overridden. If set to *False*, raises *OSError* if path exists.

#### **Notes**

Raises OSError if current dataset does not contain image data

fcs (path, features, filtered=True, override=False)

Export the data of an RT-DC dataset to an .fcs file

#### **Parameters**

- mm (instance of dclab.RTDCBase) The dataset that will be exported.
- path (str) Path to a .tsv file. The ending .tsv is added automatically.
- **features** (list of str) The features in the resulting .tsv file. These are strings that are defined in *dclab.definitions.scalar\_feature\_names*, e.g. "area\_cvx", "deform", "frame", "fl1 max", "aspect".
- **filtered** (bool) If set to *True*, only the filtered data (index in ds.\_filter) are used.
- **override** (bool) If set to *True*, an existing file path will be overridden. If set to *False*, raises *OSError* if path exists.

### **Notes**

Due to incompatibility with the .fcs file format, all events with NaN-valued features are not exported.

hdf5 (path, features, filtered=True, override=False, compression='gzip')

Export the data of the current instance to an HDF5 file

#### **Parameters**

- path (str) Path to an .rtdc file. The ending .rtdc is added automatically.
- **features** (list of str) The features in the resulting .tsv file. These are strings that are defined in *dclab.definitions.feature\_names*, e.g. "area\_cvx", "deform", "frame", "fl1\_max", "image".
- **filtered** (bool) If set to *True*, only the filtered data (index in ds.\_filter) are used.
- **override** (bool) If set to *True*, an existing file path will be overridden. If set to *False*, raises *OSError* if path exists.
- **compression** (*str or None*) Compression method for "contour", "image", and "trace" data as well as logs; one of [None, "lzf", "gzip", "szip"].

**tsv** (path, features, filtered=True, override=False)

Export the data of the current instance to a .tsv file

#### **Parameters**

- path (str) Path to a .tsv file. The ending .tsv is added automatically.
- **features** (list of str) The features in the resulting .tsv file. These are strings that are defined in *dclab.definitions.scalar\_feature\_names*, e.g. "area\_cvx", "deform", "frame", "fl1\_max", "aspect".
- **filtered** (bool) If set to *True*, only the filtered data (index in ds.\_filter) are used.
- **override** (bool) If set to *True*, an existing file path will be overridden. If set to *False*, raises *OSError* if path exists.

## **5.3.8** filter

```
class dclab.rtdc_dataset.filter.Filter(rtdc_ds)
     Boolean filter arrays for RT-DC measurements
           Parameters rtdc_ds (instance of RTDCBase) – The RT-DC dataset the filter applies to
     update (force=[])
           Update the filters according to self.rtdc_ds.config["filtering"]
               Parameters force (list) - A list of feature names that must be refiltered with min/max
                   values.
     all = None
           All filters combined (see Filter.update())
     invalid = None
          Invalid (nan/inf) events
     manual = None
          Reserved for manual filtering
     polygon = None
          Polygon filters
     rtdc ds = None
```

## 5.4 low-level functionalities

Instance of RTDCBase the filter applies to

## 5.4.1 downsampling

Content-based downsampling of ndarrays

dclab.downsampling.downsample\_rand(a, samples, remove\_invalid=True, retidx=False)

Downsampling by randomly removing points

#### **Parameters**

- a (1d ndarray) The input array to downsample
- **samples** (*int*) The desired number of samples

- remove\_invalid (bool) Remove nan and inf values before downsampling
- **retidx** (bool) Also return a boolean array that corresponds to the downsampled indices in *a*.

#### Returns

- **dsa, dsb** (1d ndarrays of shape (samples,)) The pseudo-randomly downsampled arrays a and b
- [idx] (1d boolean array with same shape as a) A boolean array such that a[idx] == dsa is all true

#### 5.4.2 features

```
dclab.features.contour.get_contour(mask)
```

Compute the image contour from a mask

The contour is computed in a very inefficient way using scikit-image and a conversion of float coordinates to pixel coordinates.

**Parameters mask** (binary ndarray of shape (M, N) or (K, M, N)) – The mask outlining the pixel positions of the event. If a 3d array is given, then K indexes the individual contours.

**Returns cont** – A 2D array that holds the contour of an event (in pixels) e.g. obtained using *mm.contour* where *mm* is an instance of *RTDCBase*. The first and second columns of *cont* correspond to the x- and y-coordinates of the contour.

**Return type** ndarray or list of K ndarrays of shape (J,2)

```
dclab.features.bright.get_bright(mask, image, ret_data='avg, sd')
```

Compute avg and/or std of the event brightness

The event brightness is defined by the gray-scale values of the image data within the event mask area.

#### **Parameters**

- mask (ndarray or list of ndarrays of shape (M, N) and dtype bool) The mask values, True where the event is located in *image*.
- **image** (ndarray or list of ndarrays of shape (M, N)) A 2D array that holds the image in form of grayscale values of an event.
- ret\_data (str) A comma-separated list of metrices to compute "avg": compute the average "sd": compute the standard deviation Selected metrics are returned in alphabetical order.

#### Returns

- **bright\_avg** (*float or ndarray of size N*) Average image data within the contour
- **bright\_std** (*float or ndarray of size N*) Standard deviation of image data within the contour

```
dclab.features.emodulus.get_emodulus(area\_um, deform, medium='CellCarrier', channel\_width=20.0, flow\_rate=0.16, px\_um=0.34, temperature=23.0, copy=True)
```

Compute apparent Young's modulus using a look-up table

#### **Parameters**

• area\_um (float or ndarray) - Apparent (2D image) area [µm²] of the event(s)

- **deform** (float or ndarray) The deformation (1-circularity) of the event(s)
- medium (str or float) The medium to compute the viscosity for. If a string in ["CellCarrier", "CellCarrier B"] is given, the viscosity will be computed. If a float is given, this value will be used as the viscosity in mPa\*s.
- **channel\_width** (*float*) The channel width [µm]
- flow\_rate (float) Flow rate [µl/s]
- px\_um (float) The detector pixel size [μm] used for pixelation correction. Set to zero to disable.
- temperature (float or ndarray) Temperature [°C] of the event(s)
- **copy** (bool) Copy input arrays. If set to false, input arrays are overridden.

Returns elasticity – Apparent Young's modulus in kPa

Return type float or ndarray

#### **Notes**

- The look-up table used was computed with finite elements methods according to [MMM+17].
- The computation of the Young's modulus takes into account corrections for the viscosity (medium, channel width, flow rate, and temperature) [MOG+15] and corrections for pixelation of the area and the deformation which are computed from a (pixelated) image [Her17].

#### See also:

```
dclab.features.emodulus_viscosity.get_viscosity() compute viscosity for known media
```

```
dclab.features.emodulus_viscosity.get_viscosity(medium='CellCarrier', chan-
nel_width=20.0, flow_rate=0.16,
temperature=23.0)
```

Returns the viscosity for RT-DC-specific media

#### **Parameters**

- **medium** (*str*) The medium to compute the viscosity for. One of ["CellCarrier", "CellCarrier B", "water"].
- channel\_width (float) The channel width in  $\mu m$
- flow\_rate (float) Flow rate in μl/s
- temperature (float or ndarray) Temperature in °C

**Returns viscosity** – Viscosity in mPa\*s

Return type float or ndarray

#### **Notes**

- CellCarrier and CellCarrier B media are optimized for RT-DC measurements.
- Values for the viscosity of water are computed using equation (15) from [KSW78].

dclab.features.fl\_crosstalk.correct\_crosstalk(fl1, fl2, fl3, fl\_channel, ct21=0, ct31=0, ct12=0, ct32=0, ct13=0, ct23=0)

Perform crosstalk correction

#### **Parameters**

- fli (int, float, or np.ndarray) Measured fluorescence signals
- **fl\_channel** (*int* (1, 2, or 3)) The channel number for which the crosstalk-corrected signal should be computed
- cij (float) Spill (crosstalk or bleed-through) from channel i to channel j This spill is computed from the fluorescence signal of e.g. single-stained positive control cells; It is defined by the ratio of the fluorescence signals of the two channels, i.e cij = flj / fli.

#### See also:

get\_compensation\_matrix() compute the inverse crosstalk matrix

#### **Notes**

If there are only two channels (e.g. fl1 and fl2), then the crosstalk to and from the other channel (ct31, ct32, ct13, ct23) should be set to zero.

dclab.features.fl\_crosstalk.get\_compensation\_matrix(ct21, ct31, ct12, ct32, ct13, ct23)
Compute crosstalk inversion matrix

The spillover matrix is

```
c11 c12 c13 l
```

c21 c22 c23 l

c31 c32 c33 l

The diagonal elements are set to 1, i.e.

```
ct11 = c22 = c33 = 1
```

**Parameters** cij (float) – Spill from channel i to channel j

**Returns** inv – Compensation matrix (inverted spillover matrix)

Return type np.matrix

```
dclab.features.inert_ratio.get_inert_ratio_cvx(cont)
```

Compute the inertia ratio of the convex hull of a contour

The inertia ratio is computed from the central second order of moments along x (mu20) and y (mu02) via sqrt(mu20/mu02).

**Parameters cont** (*ndarray* or *list* of *ndarrays* of *shape* (*N*, 2)) – A 2D array that holds the contour of an event (in pixels) e.g. obtained using *mm.contour* where *mm* is an instance of *RTDCBase*. The first and second columns of *cont* correspond to the x- and y-coordinates of the contour.

Returns inert\_ratio\_cvx - The inertia ratio of the contour's convex hull

**Return type** float or ndarray of size N

#### **Notes**

The contour moments mu20 and mu02 are computed the same way they are computed in OpenCV's moments.cpp.

#### See also:

```
get_inert_ratio_raw() Compute inertia ratio of a raw contour
```

```
dclab.features.inert ratio.get inert ratio raw(cont)
```

Compute the inertia ratio of a contour

The inertia ratio is computed from the central second order of moments along x (mu20) and y (mu02) via sqrt(mu20/mu02).

**Parameters cont** (*ndarray or list of ndarrays of shape* (*N*, 2)) – A 2D array that holds the contour of an event (in pixels) e.g. obtained using *mm.contour* where *mm* is an instance of *RTDCBase*. The first and second columns of *cont* correspond to the x- and y-coordinates of the contour.

Returns inert\_ratio\_raw - The inertia ratio of the contour

**Return type** float or ndarray of size N

#### **Notes**

The contour moments mu20 and mu02 are computed the same way they are computed in OpenCV's moments.cpp.

#### See also:

```
get_inert_ratio_cvx() Compute inertia ratio of the convex hull of a contour
```

```
dclab.features.volume.get_volume(cont, pos_x, pos_y, pix)
```

Calculate the volume of a polygon revolved around an axis

The volume estimation assumes rotational symmetry. Green's theorem and the Gaussian divergence theorem allow to formulate the volume as a line integral.

This is a translation from a Matlab script by Geoff Olynyk: http://de.mathworks.com/matlabcentral/fileexchange/36525-volrevolve

### **Parameters**

- **cont** (*ndarray* or *list* of *ndarrays* of *shape* (*N*, 2)) A 2D array that holds the contour of an event [px] e.g. obtained using *mm.contour* where *mm* is an instance of *RTDCBase*. The first and second columns of *cont* correspond to the x- and y-coordinates of the contour.
- **pos\_x** (*float* or ndarray of length N) The x coordinate(s) of the centroid of the event(s) [μm] e.g. obtained using *mm.pos\_x*
- **pos\_y** (*float or ndarray of length N*) The y coordinate(s) of the centroid of the event(s) [µm] e.g. obtained using *mm.pos\_y*
- px\_um (float) The detector pixel size in µm. e.g. obtained using: mm.config["image"]["pix size"]

**Returns volume** – volume in um<sup>3</sup>

#### **Return type** float or ndarray

#### **Notes**

The computation of the volume is based on a full rotation of the upper half of the contour to obtain the volume. Similarly, the lower part of the contour is rotated. Both volumes are then averaged.

The volume is computed radially from the the center position given by (pos\_x, pos\_y). For sufficiently smooth contours, such as densely sampled ellipses, the center position does not play an important role. For contours that are given on a coarse grid, as is the case for RT-DC, the center position must be given.

#### References

Advanced Mathematics and Mechanics Applications with MATLAB 3rd ed. by H.B. Wilson, L.H. Turcotte, and D. Halpern, Chapman & Hall CRC Press, 2002, e-ISBN 978-1-4200-3544-5. See Chapter 5, Section 5.4, doi: 10.1201/9781420035445.ch5

#### 5.4.3 isoelastics

Isoelastics management

```
class dclab.isoelastics.Isoelastics(paths=[])
```

add (isoel, col1, col2, channel\_width, flow\_rate, viscosity, method) Add isoelastics

#### **Parameters**

- isoel (list of ndarrays) Each list item resembles one isoelastic line stored as an array of shape (N,3). The last column contains the emodulus data.
- col1 (str) Name of the first feature of all isoelastics (e.g. isoel[0][:,0])
- col2 (str) Name of the second feature of all isoelastics (e.g. isoel[0][:,1])
- channel\_width (float) Channel width in µm
- flow rate (float) Flow rate through the channel in µl/s
- **viscosity** (float) Viscosity of the medium in mPa\*s
- method (str) The method used to compute the isoelastics (must be one of VALID\_METHODS).

### **Notes**

The following isoelastics are automatically added for user convenience: - isoelastics with col1 and col2 interchanged - isoelastics for circularity if deformation was given

```
static add_px_err (isoel, col1, col2, px_um, inplace=False)
```

Undo pixelation correction

Isoelasticity lines are already corrected for pixelation effects as described in

Mapping of Deformation to Apparent Young's Modulus in Real-Time Deformability Cytometry Christoph Herold, arXiv:1704.00572 [cond-mat.soft] (2017) https://arxiv.org/abs/1704.00572.

If the isoealsticity lines are displayed with deformation data that are not corrected, then the lines must be "un"-corrected, i.e. the pixelation error must be added to the lines to match the experimental data.

#### **Parameters**

- **isoel** (*list of 2d ndarrays of shape* (*N*, 3)) Each item in the list corresponds to one isoelasticity line. The first column is defined by *col1*, the second by *col2*, and the third column is the emodulus.
- **col2** (*col1*,) Define the fist to columns of each isoelasticity line. One of ["area\_um", "circ", "deform"]
- px\_um (float) Pixel size [µm]

static check\_col12(col1, col2)

#### **Parameters**

- **isoel** (*list of 2d ndarrays of shape* (*N*, 3)) Each item in the list corresponds to one isoelasticity line. The first column is defined by *col1*, the second by *col2*, and the third column is the emodulus.
- **col2** (col1,) Define the fist to columns of each isoelasticity line. One of ["area\_um", "circ", "deform"]
- channel\_width\_in (float) Original channel width [µm]
- **channel\_width\_out** (*float*) Target channel width [µm]
- flow\_rate\_in (float) Original flow rate [µl/s]
- flow\_rate\_in Target flow rate [µl/s]
- viscosity\_in (float) Original viscosity [mPa\*s]
- viscosity\_out (float) Target viscosity [mPa\*s]

### **Notes**

If only the positions of the isoelastics are of interest and not the value of the elastic modulus, then it is sufficient to supply values for the channel width and set the values for flow rate and viscosity to a constant (e.g. 1).

#### See also:

```
dclab.features.emodulus.convert() conversion method used
```

get (col1, col2, method, channel\_width, flow\_rate=None, viscosity=None, add\_px\_err=False,
 px\_um=None)
 Get isoelastics

#### **Parameters**

- **col1** (str) Name of the first feature of all isoelastics (e.g. isoel[0][:,0])
- col2 (str) Name of the second feature of all isoelastics (e.g. isoel[0][:,1])
- **method** (*str*) The method used to compute the isoelastics (must be one of *VALID\_METHODS*).

- channel\_width (float) Channel width in µm
- **flow\_rate** (float or *None*) Flow rate through the channel in µl/s. If set to *None*, the flow rate of the imported data will be used (only do this if you do not need the correct values for elastic moduli).
- **viscosity** (float or *None*) Viscosity of the medium in mPa\*s. If set to *None*, the flow rate of the imported data will be used (only do this if you do not need the correct values for elastic moduli).
- add\_px\_err (bool) If True, add pixelation errors according to C. Herold (2017), https://arxiv.org/abs/1704.00572
- px\_um (float) Pixel size [µm], used for pixelation error computation

#### See also:

dclab.features.emodulus.convert() conversion in-between channel sizes and viscosities

dclab.features.emodulus.corrpix\_deform\_delta() pixelation error that is applied to
 the deformation data

get\_with\_rtdcbase (col1, col2, method, dataset, viscosity=None, add\_px\_err=False)

Convenience method that extracts the metadata from RTDCBase

#### Parameters

- col1 (str) Name of the first feature of all isoelastics (e.g. isoel[0][:,0])
- col2 (str) Name of the second feature of all isoelastics (e.g. isoel[0][:,1])
- **method** (*str*) The method used to compute the isoelastics (must be one of *VALID\_METHODS*).
- dataset (dclab.rtdc\_dataset.RTDCBase) The dataset from which to obtain the metadata.
- **viscosity** (float or *None*) Viscosity of the medium in mPa\*s. If set to *None*, the flow rate of the imported data will be used (only do this if you do not need the correct values for elastic moduli).
- add\_px\_err (bool) If True, add pixelation errors according to C. Herold (2017), https://arxiv.org/abs/1704.00572

#### load data(path)

Load isoelastics from a text file

The text file is loaded with *numpy.loadtxt* and must have three columns, representing the two data columns and the elastic modulus with units defined in *definitions.py*. The file header must have a section defining meta data of the content like so:

# [...] # # - column 1: area\_um # - column 2: deform # - column 3: emodulus # - channel width [um]: 20 # - flow rate [ul/s]: 0.04 # - viscosity [mPa\*s]: 15 # - method: analytical # [...]

**Parameters** path (str) – Path to a isoelastics text file

class dclab.isoelastics.IsoelasticsDict

dclab.isoelastics.get\_default()
 Return default isoelasticity lines

## 5.4.4 kde methods

```
Kernel Density Estimation methods
```

```
dclab.kde_methods.bin_num_doane(a)
```

Compute number of bins based on Doane's formula

```
dclab.kde_methods.bin_width_doane(a)
```

Compute accuracy (bin width) based on Doane's formula

```
dclab.kde_methods.get_bad_vals(x, y)
```

dclab.kde\_methods.ignore\_nan\_inf(kde\_method)

Ignores nans and infs from the input data

Invalid positions in the resulting density are set to nan.

dclab.kde\_methods.kde\_gauss(events\_x, events\_y, xout=None, yout=None, \*args, \*\*kwargs) Gaussian Kernel Density Estimation

#### **Parameters**

- events\_y (events\_x,) The input points for kernel density estimation. Input is flattened automatically.
- yout (xout,) The coordinates at which the KDE should be computed. If set to none, input coordinates are used.

**Returns density** – The KDE for the points in (xout, yout)

**Return type** ndarray, same shape as *xout* 

### See also:

scipy.stats.gaussian\_kde

### **Notes**

This is a wrapped version that ignores nan and inf values.

dclab.kde methods.kde histogram (events x, events y, xout=None, yout=None, \*args, \*\*kwargs) Histogram-based Kernel Density Estimation

#### **Parameters**

- events\_y (events\_x,) The input points for kernel density estimation. Input is flattened automatically.
- yout (xout,) The coordinates at which the KDE should be computed. If set to none, input coordinates are used.
- bins (tuple (binsx, binsy)) The number of bins to use for the histogram.

**Returns density** – The KDE for the points in (xout, yout)

Return type ndarray, same shape as xout

#### See also:

numpy.histogram2d scipy.interpolate.RectBivariateSpline

#### **Notes**

This is a wrapped version that ignores nan and inf values.

Multivariate Kernel Density Estimation

#### **Parameters**

- **events\_y** (*events\_x*,) The input points for kernel density estimation. Input is flattened automatically.
- **bw** (tuple (bwx, bwy) or None) The bandwith for kernel density estimation.
- yout (xout,) The coordinates at which the KDE should be computed. If set to none, input coordinates are used.

**Returns density** – The KDE for the points in (xout, yout)

Return type ndarray, same shape as xout

#### See also:

statsmodels.nonparametric.kernel\_density.KDEMultivariate

#### **Notes**

This is a wrapped version that ignores nan and inf values.

```
dclab.kde_methods.kde_none (events_x, events_y, xout=None, yout=None)
No Kernel Density Estimation
```

#### **Parameters**

- **events\_y** (*events\_x*,) The input points for kernel density estimation. Input is flattened automatically.
- yout (xout,) The coordinates at which the KDE should be computed. If set to none, input coordinates are used.

**Returns density** – The KDE for the points in (xout, yout)

**Return type** ndarray, same shape as *xout* 

#### **Notes**

This method is a convenience method that always returns ones in the shape that the other methods in this module produce.

## 5.4.5 polygon filter

#### **Parameters**

- **axes** (tuple of str) The axes/features on which the polygon is defined. The first axis is the x-axis. Example: ("area\_um", "deform").
- **points**  $(array-like\ object\ of\ shape\ (N,2))$  The N coordinates (x,y) of the polygon. The exact order is important.
- **inverted** (bool) Invert the polygon filter. This parameter is overridden if *filename* is given.
- name (str) A name for the polygon (optional).
- **filename** (*str*) A path to a .poly file as create by this classes' *save* method. If *filename* is given, all other parameters are ignored.
- **fileid** (*int*) Which filter to import from the file (starting at 0).
- unique\_id (int) An integer defining the unique id of the new instance.

#### **Notes**

The minimal arguments to this class are either *filename* OR (*axes*, *points*). If *filename* is set, all parameters are taken from the given .poly file.

#### static clear\_all\_filters()

Remove all filters and reset instance counter

copy (invert=False)

Return a copy of the current instance

**Parameters** invert (bool) – The copy will be inverted w.r.t. the original

filter (datax, datay)

Filter a set of datax and datay according to self.points

```
static get_instance_from_id(unique_id)
```

Get an instance of the PolygonFilter using a unique id

```
static import_all(path)
```

Import all polygons from a .poly file.

Returns a list of the imported polygon filters

```
static instace_exists(unique_id)
```

Determine whether an instance with this unique id exists

```
static point_in_poly(x, y, poly)
```

Determine whether a point is within a polygon area

#### **Parameters**

- $\mathbf{y}(x,)$  The coordinates of the point
- poly (list-like) The polygon (PolygonFilter.points)

**Returns inside** – *True*, if point is inside.

Return type bool

### static remove(unique\_id)

Remove a polygon filter from PolygonFilter.instances

```
save (polyfile, ret_fobj=False)
```

Save all data to a text file (appends data if file exists).

Polyfile can be either a path to a file or a file object that was opened with the write "w" parameter. By using the file object, multiple instances of this class can write their data.

If *ret\_fobj* is *True*, then the file object will not be closed and returned.

```
static save_all (polyfile)
Save all polygon filters
```

```
instances = []
```

dclab.polygon\_filter.get\_polygon\_filter\_names()

Get the names of all polygon filters in the order of creation

#### 5.4.6 statistics

Statistics computation for RT-DC dataset instances

```
exception dclab.statistics.BadMethodWarning
```

```
class dclab.statistics.Statistics (name, method, req_feature=False)
```

A helper class for statistics.

All statistical methods are registered in the dictionary *Statistics.available\_methods*.

```
get_data (kwargs)
    get_feature (rtdc_ds, axis)
    available_methods = {'%-gated': <dclab.statistics.Statistics object at 0x7fbe30924828
dclab.statistics.flow_rate (mm)
dclab.statistics.get_statistics(rtdc_ds, methods=None, features=None)</pre>
```

#### **Parameters**

- **rtdc\_ds** (instance of *dclab.rtdc\_dataset.RTDCBase.*) The dataset for which to compute the statistics.
- **methods** (list of str or None) The methods wih which to compute the statistics. The list of available methods is given with dclab.statistics.Statistics.available\_methods.keys() If set to None, statistics for all methods are computed.
- **features** (list of str) Feature name identifiers are defined in dclab.definitions.scalar\_feature\_names. If set to None, statistics for all axes are computed.

#### Returns

- header (list of str) The header (feature + method names) of the computed statistics.
- values (list of float) The computed statistics.

```
dclab.statistics.mode(data)
```

Compute an intelligent value for the mode

The most common value in experimental is not very useful if there are a lot of digits after the comma. This method approaches this issue by rounding to bin size that is determined by the Freedman–Diaconis rule.

**Parameters data** (1d ndarray) - The data for which the mode should be computed.

**Returns mode** – The mode computed with the Freedman-Diaconis rule.

Return type float

# CHAPTER 6

# Changelog

List of changes in-between dclab releases.

# 6.1 version 0.7.0

- feat: add new ancillary feature: principal inertia ratio (#46)
- feat: add new ancillary feature: absolute tilt (#53)
- feat: add computation of viscosity for water (#52)

## 6.2 version 0.6.3

• fix: channel width not correctly identified for old tdms files

## 6.3 version 0.6.2

• ci: automate release to PyPI with appveyor and travis-ci

## 6.4 version 0.6.0

- fix: image export as .avi did not have option to use unfiltered data
- fix: avoid a few unicode gotchas
- feat: use Doane's formula for kernel density estimator defaults (#42)
- docs: usage examples, advanced scripting, and code reference update (#49)

## 6.5 version 0.5.2

- Migrate from os.path to pathlib (#50)
- fmt\_hdf5: Add run index to title

## 6.6 version 0.5.1

- Setup: add dependencies for statsmodels
- Tests: filter known warnings
- fmt\_hdf5: import unknown keys such that "dclab-verify-dataset" can complain about them

## 6.7 version 0.5.0

- BREAKING CHANGES:
  - definitions.feature\_names now contains non-scalar features (inlcuding "image", "contour", "mask", and "trace"). To test for scalar features, use definitions.scalar\_feature\_names.
  - features bright\_\* are computed from mask instead of from contour
- Bugfixes:
  - write correct event count in exported hdf5 data files
  - improve implementation of video file handling in fmt\_tdms
- add new non-scalar feature "mask" (#48)
- removed configuration key [online\_contour]: "bin margin" (#47)
- · minor improvements for the tdms file format

## 6.8 version 0.4.0

- Bugfix: CLI "dclab-tdms2rtdc" did not work for single tdms files (#45)
- update configuration keys:
  - added new keys for [fluorescence]
  - added [setup]: "identifier"
  - removed [imaging]: "exposure time", "flash current"
  - removed [setup]: "temperature", "viscosity"
- renamed feature "ncells" to "nevents"

## 6.9 version 0.3.3

- ref: do not import missing features as zeros in fmt\_tdms
- CLI:
  - add tdms-to-rtdc converter "dclab-tdms2rtdc" (#36)
  - improve "dclab-verify-dataset" user experience
- Bugfixes:
  - "limit events" filtering must be integer not boolean (#41)
  - Support opening tdms files with capitalized "userDef" column names
  - OSError when trying to open files from repository root

## 6.10 version 0.3.2

- CLI: add rudimentary dataset checker "dclab-verify-dataset" (#37)
- Add logic to compute parent/root/child event indices of RTDC\_Hierarchy
  - Hierarchy children now support contour, image, and traces
  - Hierarchy children now support and remember manual filters (#22)
- Update emodulus look-up table with larger values for deformation
- Implement pixel size correction for emodulus computation
- Allow to add pixelation error to isoelastics (add\_px\_err=True) (#28)
- Bugfixes:
  - Pixel size not read from tdms-based measurements
  - Young's modulus computation wrong due to faulty FEM simulations (#39)

## 6.11 version 0.3.1

- · Remove all-zero dummy columns from dict format
- Implement hdf5-based RT-DC data reader (#32)
- Implement hdf5-based RT-DC data writer (#33)
- Bugfixes:
  - Automatically fix inverted box filters
  - RTDC\_TDMS trace data contained empty arrays when no trace data was present (trace key should not have been accessible)
  - Not possible to get isoelastics for circularity

6.9. version 0.3.3 47

## 6.12 version 0.3.0

- New fluorescence crosstalk correction feature recipe (#35)
- New ancillary features "fl1\_max\_ctc", "fl2\_max\_ctc", "fl3\_max\_ctc" (#35)
- Add priority for multiple ancillary features with same name
- Bugfixes:
  - Configuration key values were not hashed for ancillary features
- Code cleanup:
  - Refactoring: Put ancillary columns into a new folder module
  - Refactoring: Use the term "feature" consistently
  - Unify trace handling in dclab (#30)
  - Add functions to convert input config data

## 6.13 version 0.2.9

- Bugfixes:
  - Regression when loading configuration strings containing quotes
  - Parameters missing when loading ShapeIn 2.0.1 tdms data

## 6.14 version 0.2.8

• Refactor configuration class to support new format (#26)

## 6.15 version 0.2.7

- New submodule and classes for managing isoelastics
- New ancillary columns "inert\_ratio\_raw" and "inert\_ratio\_cvx"
- · Bugfixes:
  - Typo when finding contour data files (tdms file format)
- Rrefactoring:
  - "features" submodule with basic methods for ancillary columns

## 6.16 version 0.2.6

- Return event images as gray scale (#17)
- Bugfixes:
  - Shrink ancillary column size if it exceeds dataset size

- Generate random RTDCBase.identifier (do not use RTDCBase.hash) to fix problem with identical identifiers for hierarchy children
- Correctly determine contour data files (tdms file format)
- Allow contour data indices larger than uint8

## 6.17 version 0.2.5

- Add ancillary columns "bright\_avg" and "bright\_sd" (#18, #19)
- Standardize attributes of RTDCBase subclasses (#12)
- · Refactoring:
  - New column names and removal of redundant column identifiers (#16)
  - Minor improvements towards PEP8 (e.g. #15)
  - New class for handling filters (#13)
- Bugfixes:
  - Hierarchy child computed all ancillary columns of parent upon checking availability of a column

### 6.18 version 0.2.4

- Replace OpenCV with imageio
- Add (ancilliary) computation of volume (#11)
- Add convenience methods for Configuration
- Refactoring (#8):
  - Separate classes for .tdms, dict-based, and hierarchy datasets
  - Introduce "\_events" attribute for stored data
  - Data columns (including image, trace, contour) are accessed via keys instead of attributes.
  - Make space for new hdf5-based file format
  - Introduce ancilliary columns that are computed on-the-fly (new "\_ancilliaries" attribute and "ancilliary\_columns.py")

## 6.19 version 0.2.3

- Add look-up table for elastic modulus (#7)
- · Add filtering option "remove invalid events" to remove nan/inf
- Support nan and inf in data analysis
- · Improve downsampling performance
- Refactor downsampling methods (#6)

6.17. version 0.2.5 49

## 6.20 version 0.2.2

- Add new histogram-based kernel density estimator (#2)
- · Refactoring:
  - Configuration fully handled by RTDC\_DataSet module (#5)
  - Simplify video export function (#4)
  - Removed "Plotting" configuration key
  - Removed .cfg configuration files

## 6.21 version 0.2.1

- Support npTDMS 0.9.0
- Add AVI-Export function
- Add lazy submodule for event trace data and rename RTDC\_DataSet.traces to RTDC\_DataSet.trace
- Add "Event index" column

## 6.22 version 0.2.0

- Compute sensible default configuration parameters for KDE estimation and contour plotting
- Speed-up handling of contour text files
- Add support for "User Defined" column in tdms files

## 6.23 version 0.1.9

- Implement hierarchical instantiation of RTDC\_DataSet
- Bugfix: Prevent instances of PolygonFilter that have same id
- Load InertiaRatio and InertiaRatioRaw from tdms files

## 6.24 version 0.1.8

- Allow to instantiate RTDC\_DataSet without a tdms file
- · Add statistics submodule
- Bugfixes:
  - Faulty hashing strategy in RTDC\_DataSet.GetDownSampledScatter
- Code cleanup (renamed methods, cleaned structure)
- Corrections/additions in definitions (fRT-DC)

## 6.25 version 0.1.7

- · Added channel: distance between to first fl. peaks
- Added fluorescence channels: peak position, peak area, number of peaks
- Allow to disable KDE computation
- · Add filter array for manual (user-defined) filtering
- · Add config parameters for log axis scaling
- Add channels: bounding box x- and y-size
- · Bugfixes:
  - cached.py did not handle None
  - Limiting number of events caused integer/bool error

## 6.26 version 0.1.6

- Added RTDC\_DataSet.ExportTSV for data export
- · Bugfixes:
  - Correct determination of video file in RTDCDataSet
  - Fix multivariate KDE computation
  - Contour accuracy for Defo overridden by that of Circ

## 6.27 version 0.1.5

- Fix regressions with filtering. https://github.com/ZELLMECHANIK-DRESDEN/ShapeOut/issues/43
- Ignore empty columns in .tdms files (#1)
- Moved RTDC\_DataSet and PolygonFilter classes to separate files
- Introduce more transparent caching improves speed in some cases

## 6.28 version 0.1.4

• Added support for 3-channel fluorescence data (FL-1..3 max/width)

## 6.29 version 0.1.3

- Fixed minor polygon filter problems.
- Fix a couple of ShapeOut-related issues:
  - https://github.com/ZELLMECHANIK-DRESDEN/ShapeOut/issues/17
  - https://github.com/ZELLMECHANIK-DRESDEN/ShapeOut/issues/20

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- https://github.com/ZELLMECHANIK-DRESDEN/ShapeOut/issues/37
- https://github.com/ZELLMECHANIK-DRESDEN/ShapeOut/issues/38

## 6.30 version 0.1.2

- Add support for limiting amount of data points analyzed with the configuration keyword "Limit Events"
- Comments refer to "events" instead of "points" from now on

# 6.31 version

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Bilbliography

# CHAPTER 8

Imprint/Impressum

# 8.1 Imprint and disclaimer

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

# 8.2 Privacy policy

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

# CHAPTER 9

# Indices and tables

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