

# ShapeOut

Software version: 0.7.3

Instructions version: 1.2

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The software is under constant development, so there might be deviations between the current version and the manual. We try to update frequently.

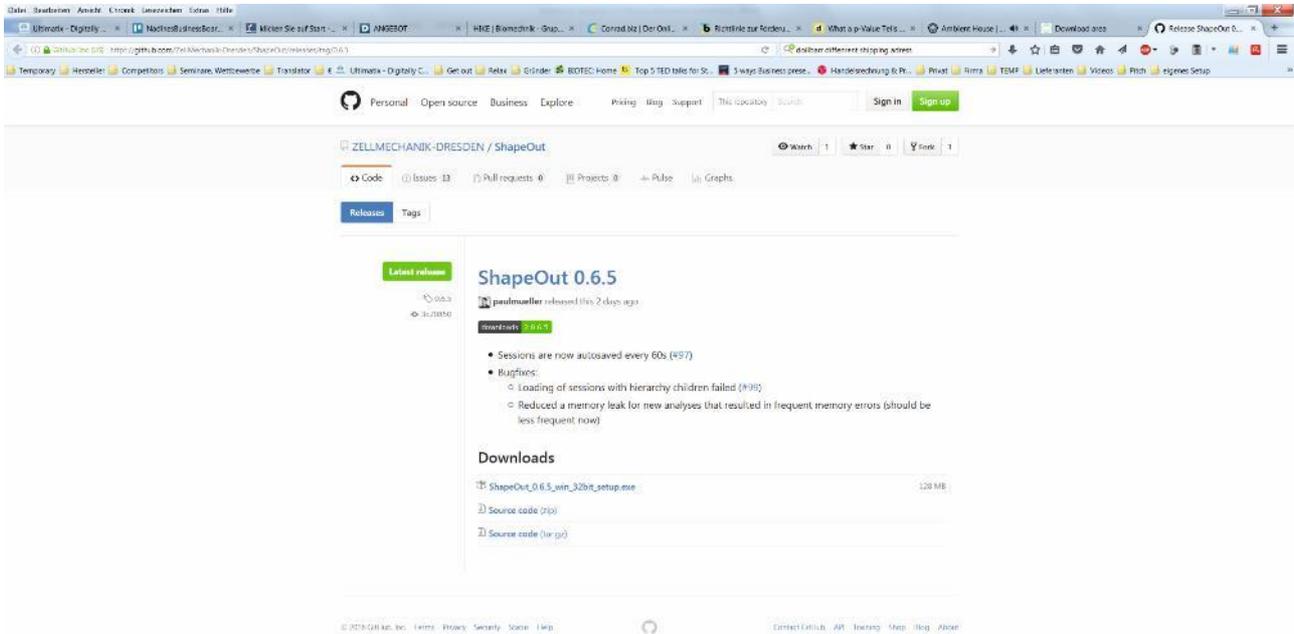
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## Installation of the software

Download the latest ShapeOut version from Github, following this link:

<http://www.zellmechanik.com/Download.html>



Install the software, following the instructions.

The software is under constant development, so check out for updates from time to time. The installer automatically uninstalls older versions of **ShapeOut**.

## Folder structure of *Shapeln*

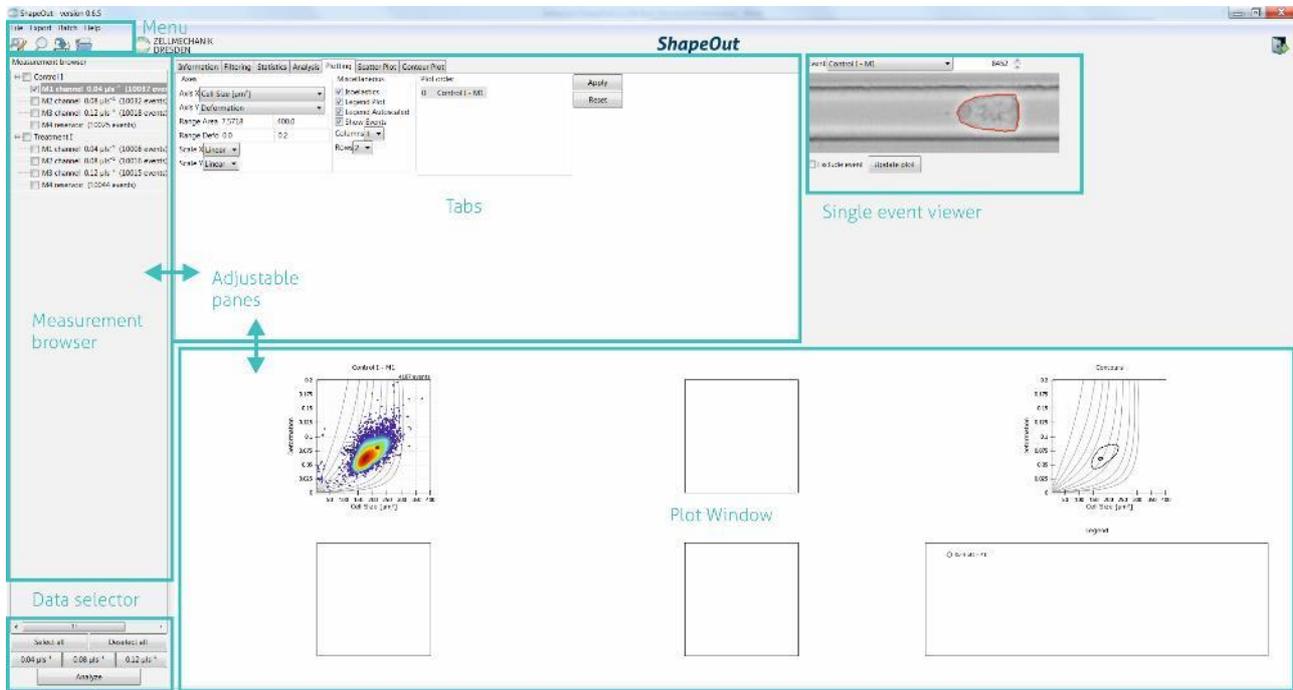
[Hard drive]:\Main project folder\YYMMDD\_ProjectLeader\_ProjectName\SampleName\Offline and Online folder.

### Example

D:\ Experiments\ 160223_Tom_CellLineA\	Control\	Offline
		Online
160311_Eva_PrimCellB\	Control\	Offline
		Online
	Test1\	Offline
		Online

The Online folder contains all the measurement files. The file names start with M and a number (successive numbering during data acquisition with **Shapeln**) M1\_..., M2\_..., M3\_....

## Layout of *ShapeOut*'s main window



## The menu

**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 ShapeOut



- 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 functions

## 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.

## The tabs

### **Information tab**

Shows all saved measurement properties, which are stored in the corresponding \*.ini and \*.tdms files. 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 **Shapeln** 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 **Shapeln** on the front panel under "Channel dimension [ $\mu\text{m}$ ]"

**Flow Rate:** is the sum of sheath and sample flow in  $\mu\text{l/s}$ . **Shapeln** 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 **Shapeln** 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 **Shapeln** 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 **Shapeln** to exclude cells larger / smaller than this number. In **Shapeln** 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 **Shapeln** 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 **Shapeln** under Expert settings "min grey threshold".

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

#### Framerate

**Frame Rate:** of the camera during the measurement. **Shapeln** 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. **Shapeln** 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]".

### **Filtering tab**

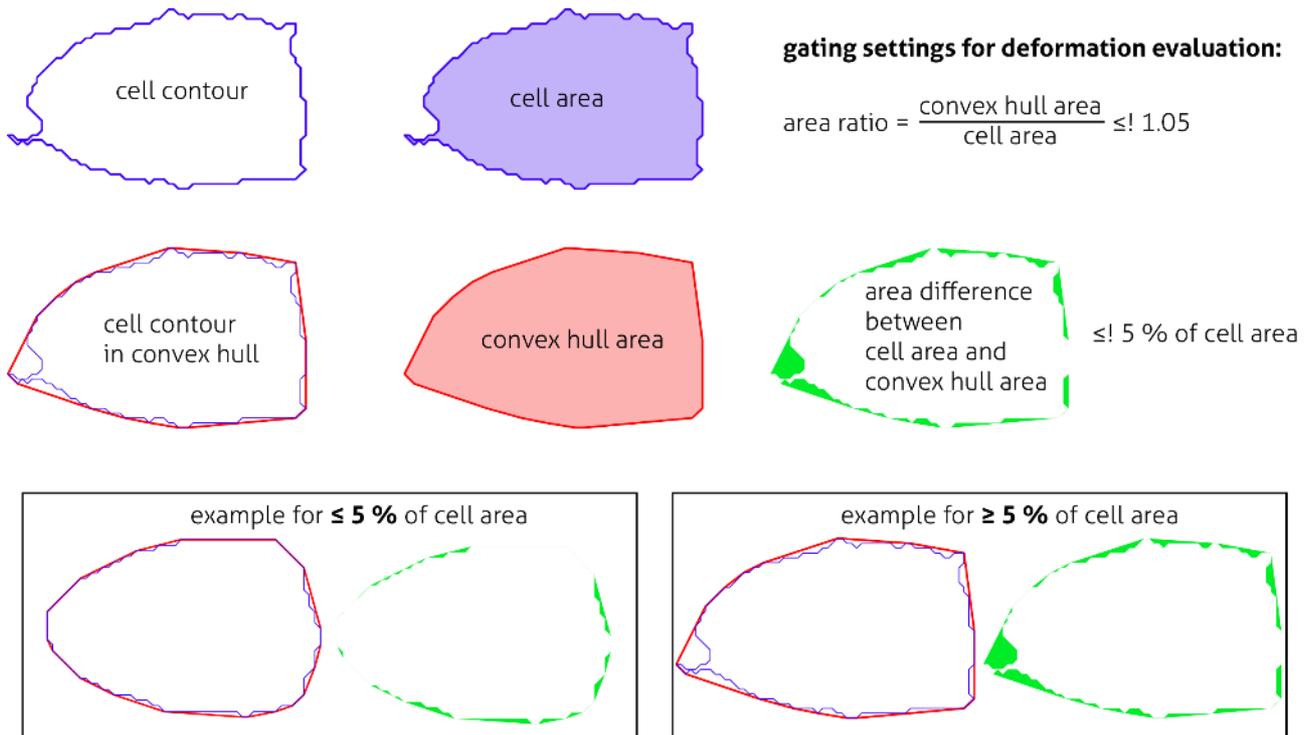
In this tab, you can filter your data by excluding values beyond certain limits. Select the **Enable Filters** check box on the right and hit the **Apply/Reset** button to apply/reset the settings. The Box Filters limit the range of parameters; the Polygon Filters allow freely defining a gate by a polygon 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

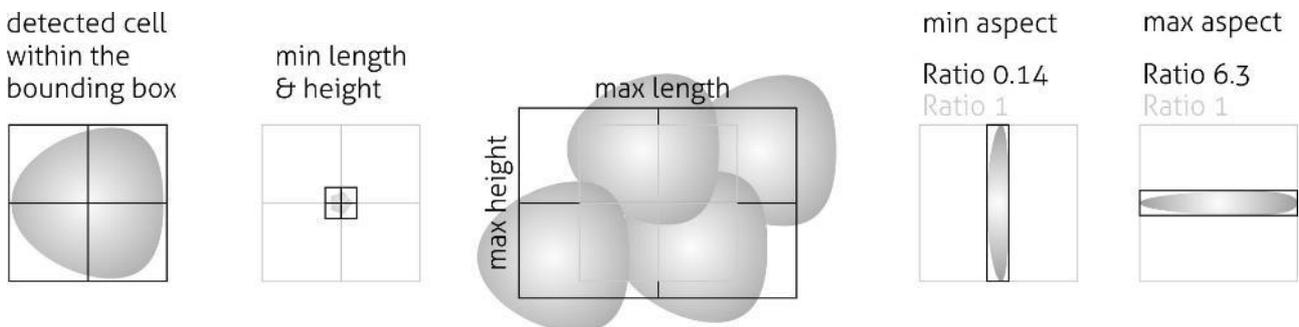
Minimum and maximum values for box filters are set to 0.0 by default. As long as min and max values equal 0.0, the filter is not applied. On the left of the table the minimum value is set, on the right the maximum. If you set a minimum, you have to set a maximum as well, otherwise all data points are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

**Range Area:** limits the cell size in [ $\mu\text{m}^2$ ].

**Range Area Ratio:** defines the relative difference between convex hull area and cell area (see Figure below). "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)

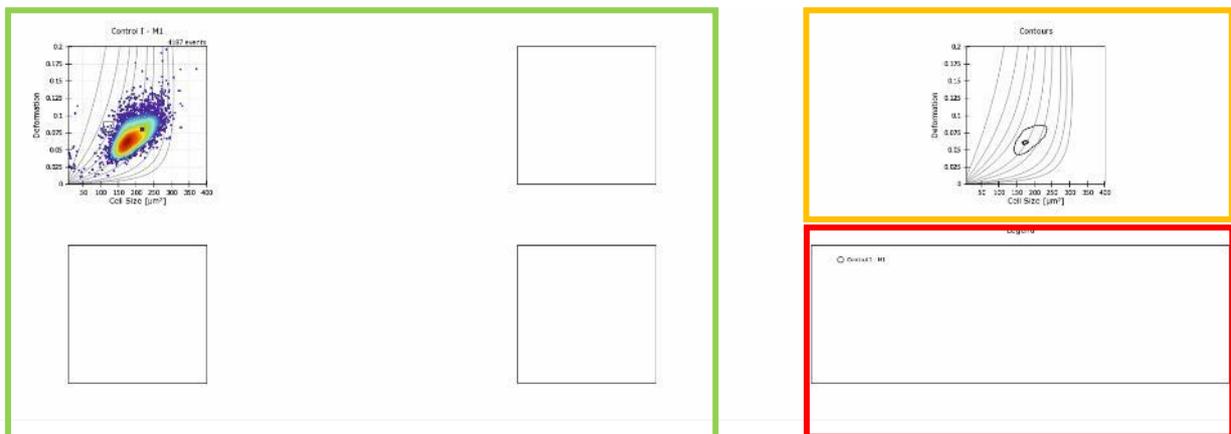
**Range y-size:** limits the size in y direction (height)

## Polygon Filters

See the example procedure for details.

**Statistics tab, Analysis tab, Plotting tab, Scatter plot tab, Contour plot tab** are explained in the example procedure.

## The Plot Window



The plot window contains the plots of **single measurements**, a plot with an **overlay of all contours** and a **legend for the color code of the contour plots**.

The whole panel is a grid of rows and columns, which can be extended or reduced in the *Plotting tab*. The example above shows three columns and two rows.

## Example procedure of analyzing

This chapter shows a general workflow for evaluating data with **ShapeOut**. As an example, we use experimental data acquired with the **AcCellerator**. On the next pages you will learn the main features, but there are many more. Just play around!

This is the experiment we use as example:

Day1:

- one sample, called "Treatment I", measured at flow rates of 0.04, 0.08 and 0.12  $\mu\text{l/s}$  and one measurement in the reservoir
- one control, called "Control I", measured at flow rates 0.04, 0.08 and 0.12  $\mu\text{l/s}$  and one measurement in the reservoir

Day2:

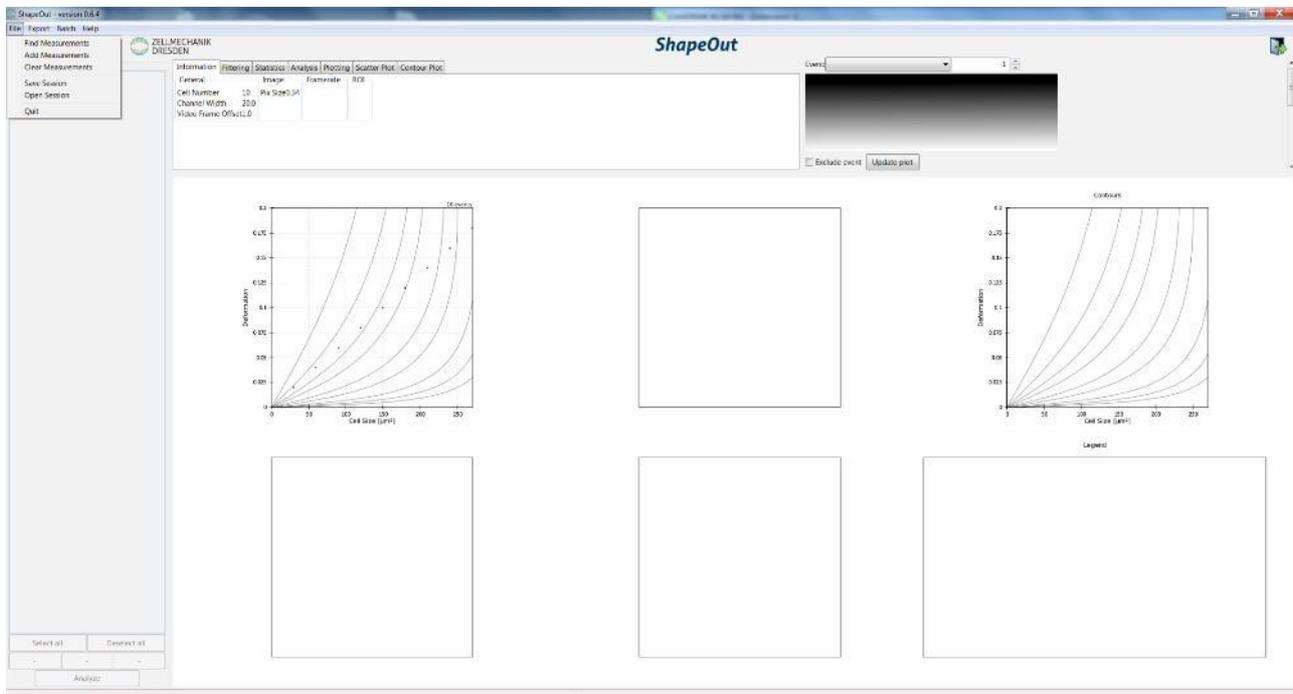
- two samples, called "Treatment II" and "Treatment III", measured at flow rates 0.04, 0.08 and 0.12  $\mu\text{l/s}$  and one measurement in the reservoir
- two controls, called "Control II" and "Control III", measured at flow rates 0.04, 0.08 and 0.12  $\mu\text{l/s}$  and one measurement in the reservoir

## Load data

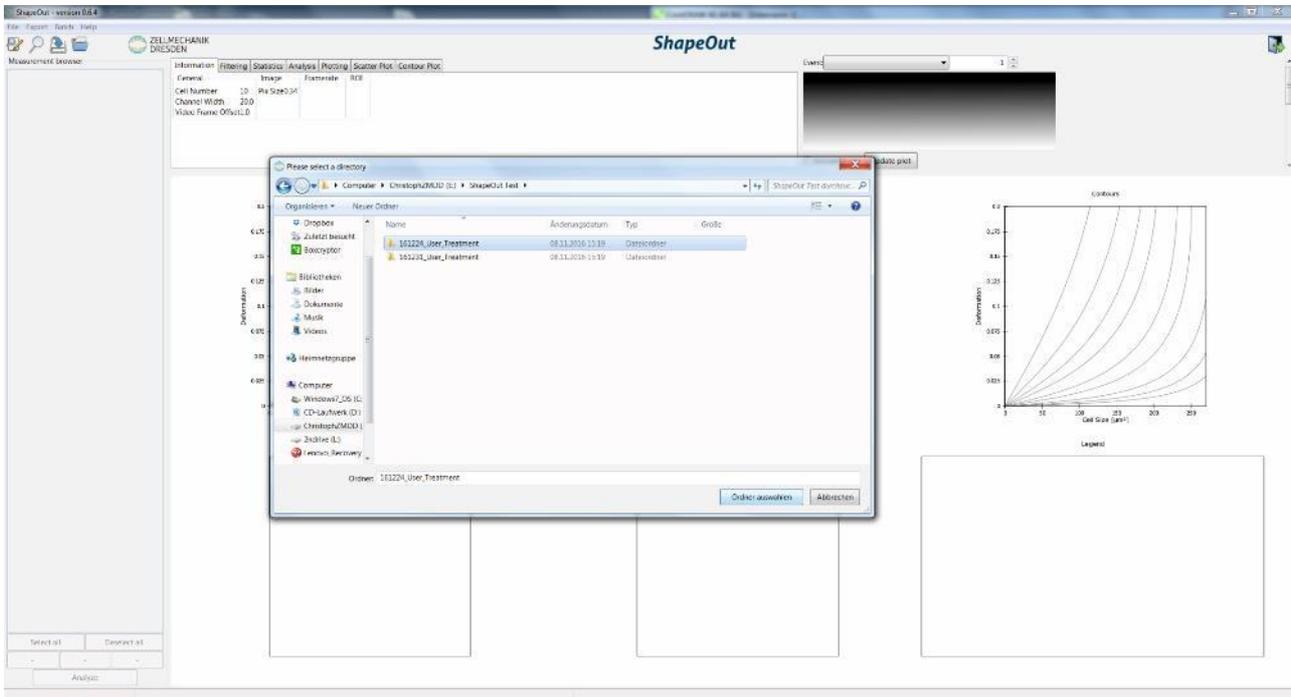
Download, install and start **ShapeOut**. (<http://www.zellmechanik.com/Download.html>)



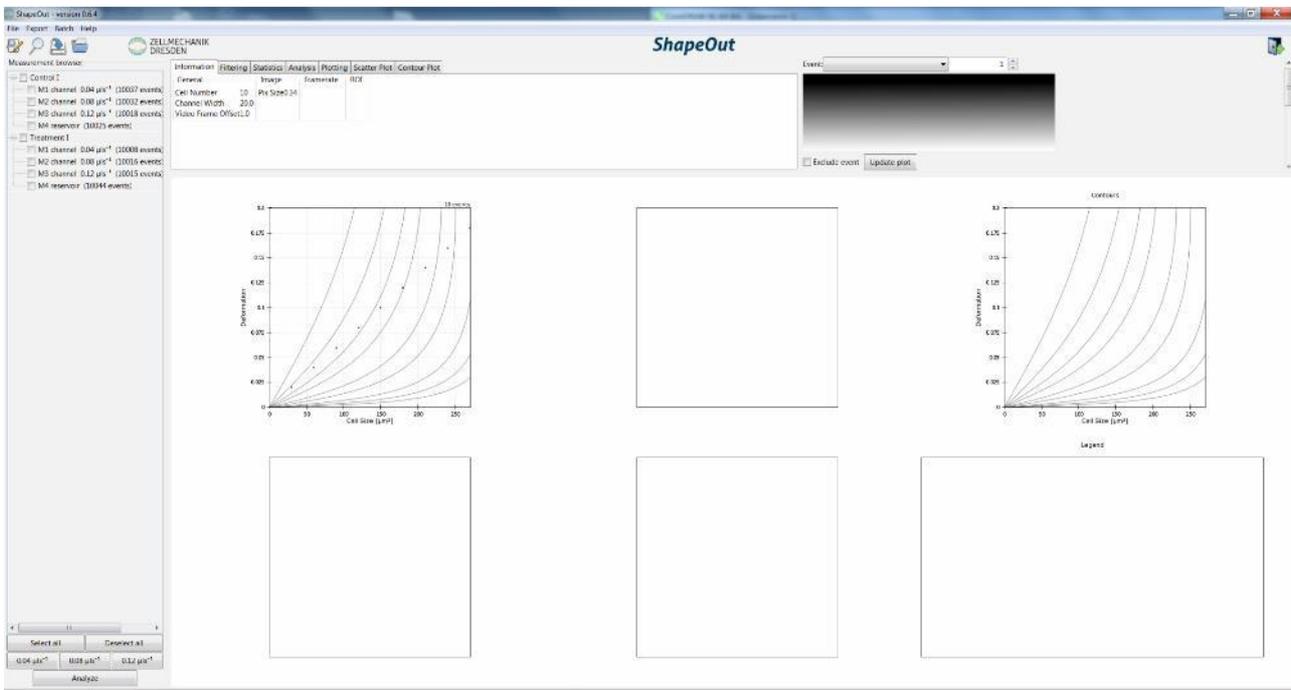
Open the menu *File* and click **Find Measurements**.



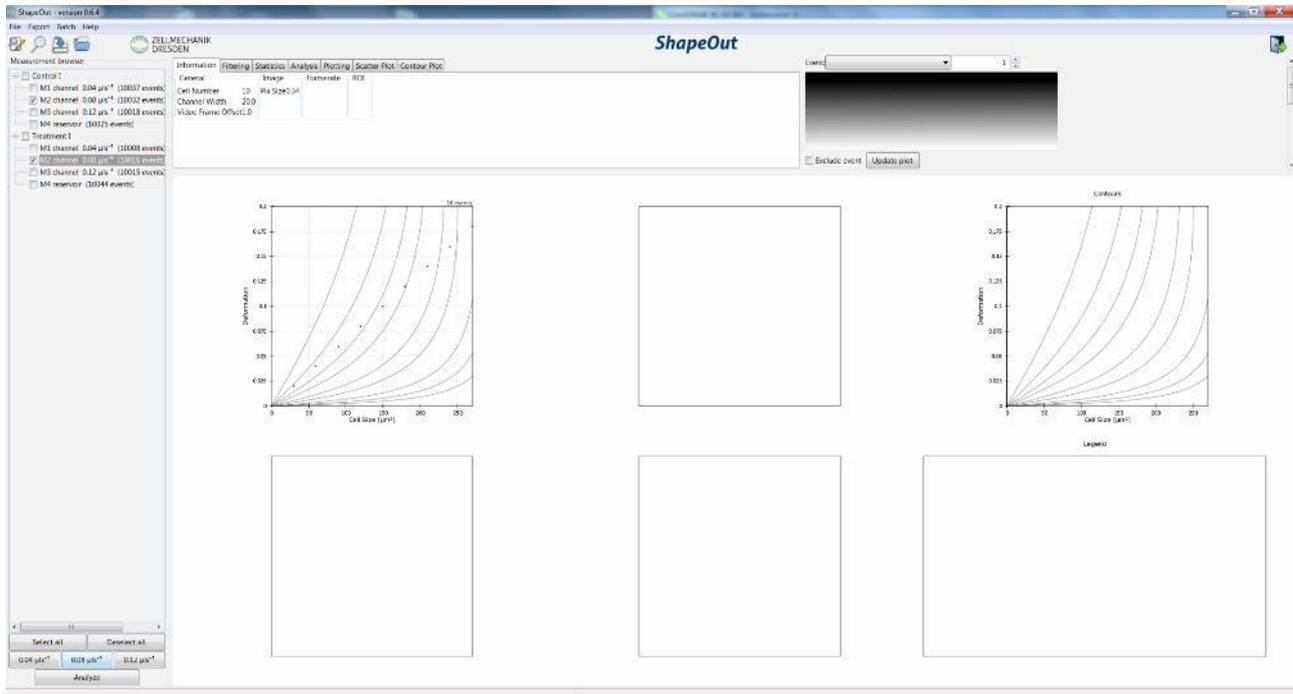
Select (and do not open) the "YYMMDD\_ProjectLeader\_ProjectName" folder (here: "161224\_User\_Treatment") and press the **select folder** button.



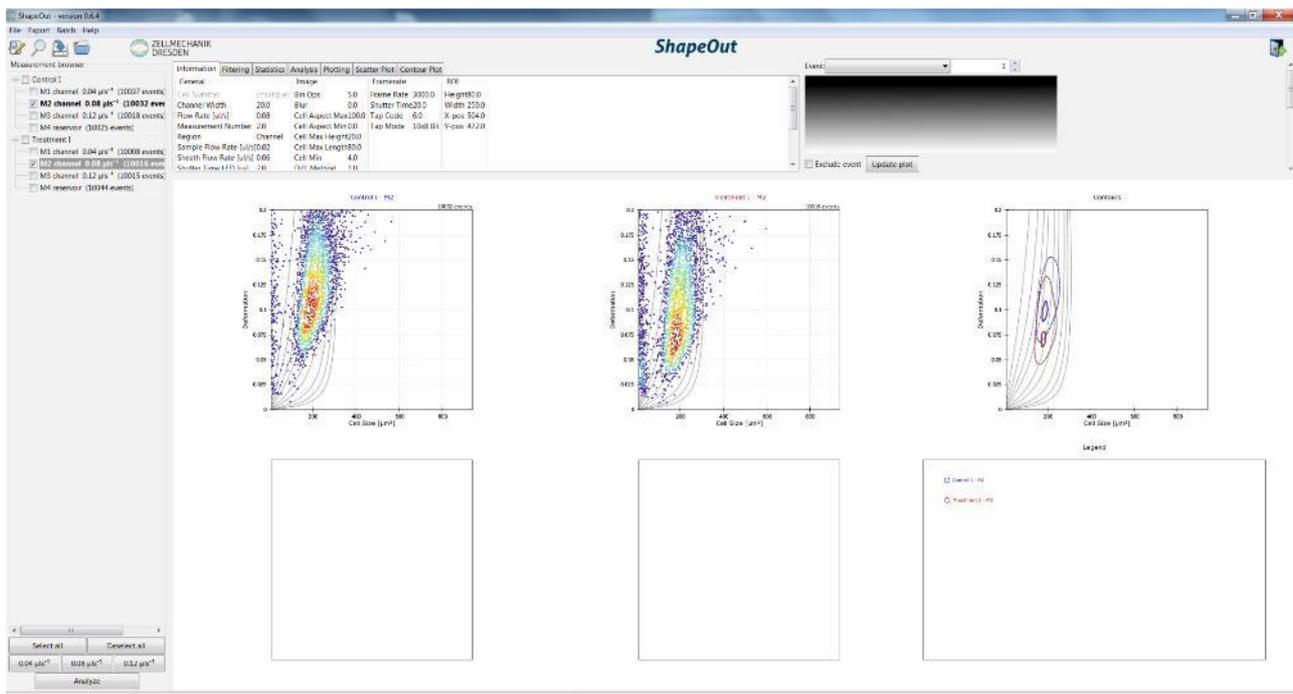
The **Measurement Browser** on the left shows all available measurements from the selected folder.



Select the datasets you want to analyze (here: all measurements at 0.08  $\mu\text{l/s}$ ) by checking the box before the measurement name or by using the selection filters which you can find in the lower left corner of the screen.

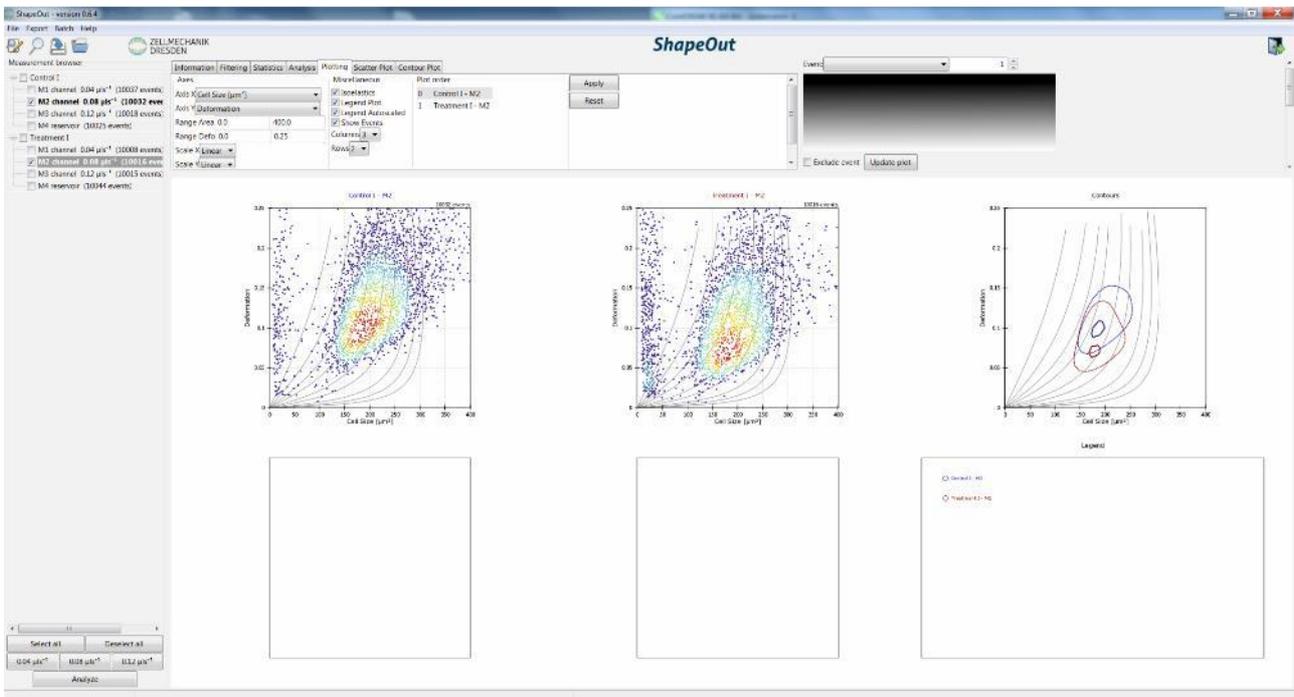


Press the **Analyze** button and wait for the datasets to be loaded. This can take up to several minutes depending on the number of events in the dataset. By default the number of events are downsampled to 2000. You can change this later.

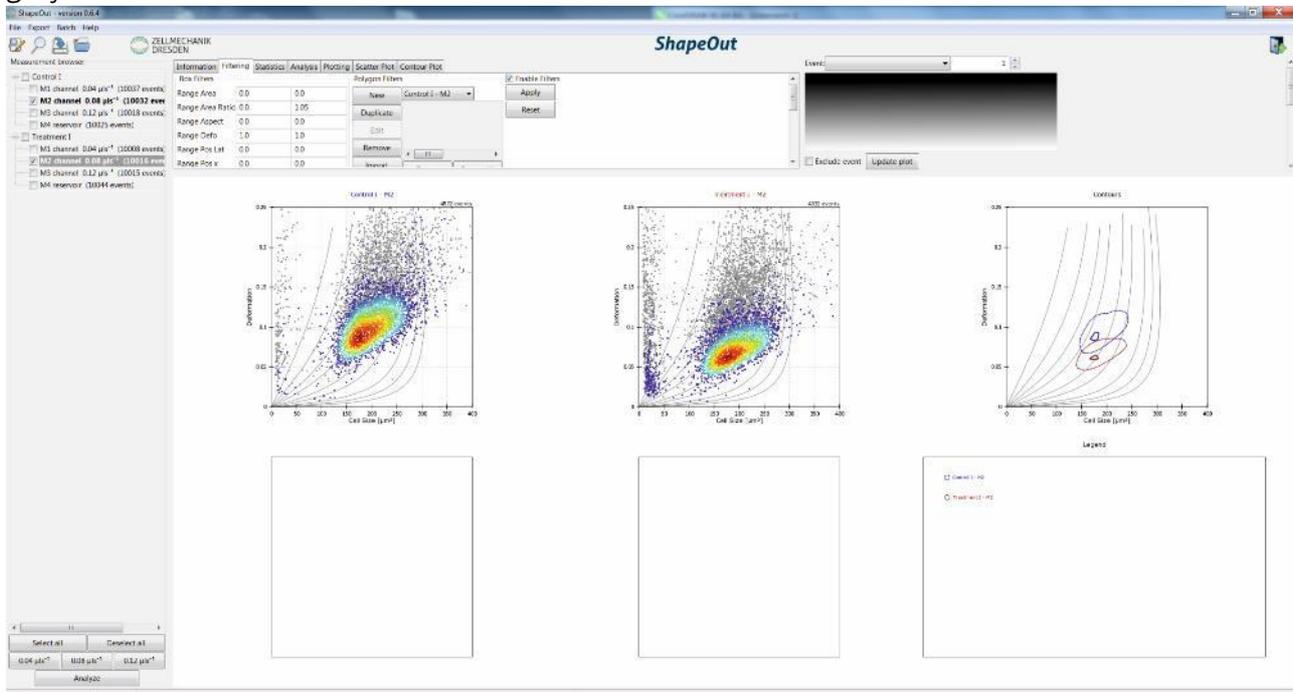


## Displaying and filtering data

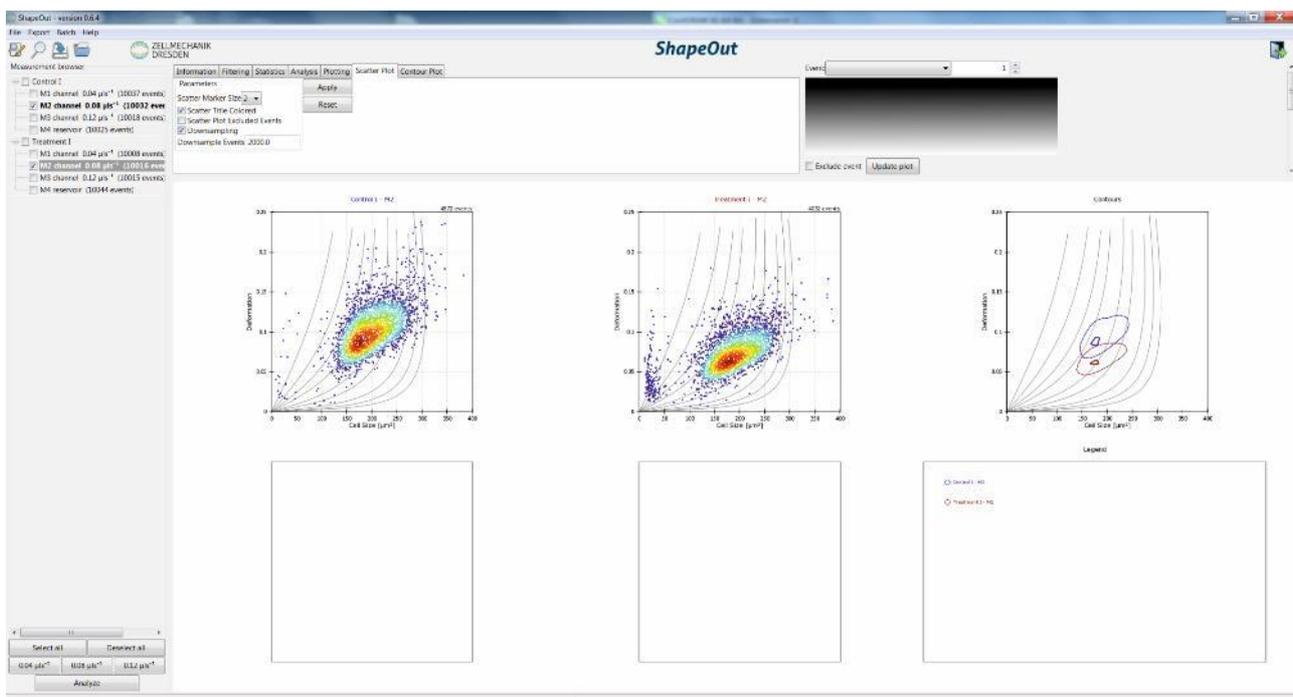
Go to the **Plotting** tab and change the **Range Area** and **Range Deformation** for best visualization of the area of interest in your plots (here: area from 0-400 and deformation from 0-0.25). Press **Apply**. The settings are applied to all plots.



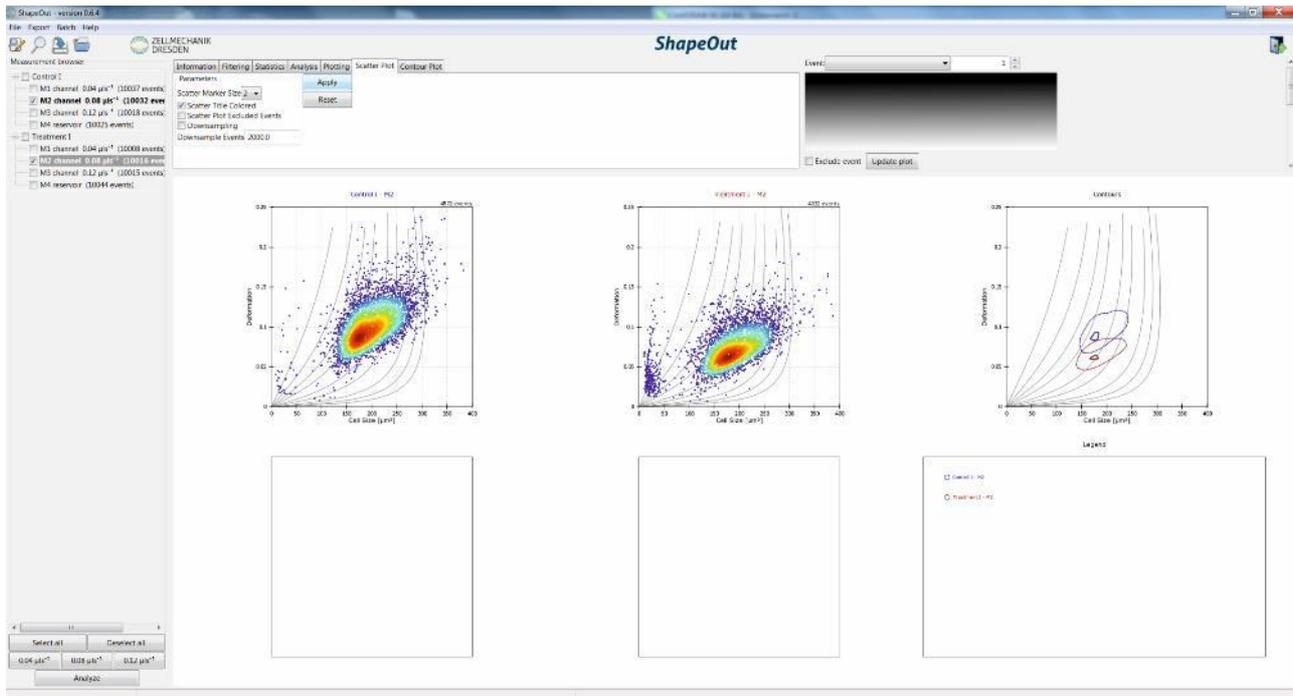
Go to the **Filtering** tab and change the **Range Area Ratio** to exclude cells with irregular shapes, like strong protrusions (here: area ratio between 1-1.05, everything with a higher deviation than 5 % of the convex hull area compared to the cell area is excluded). Excluded events are shown in grey color.



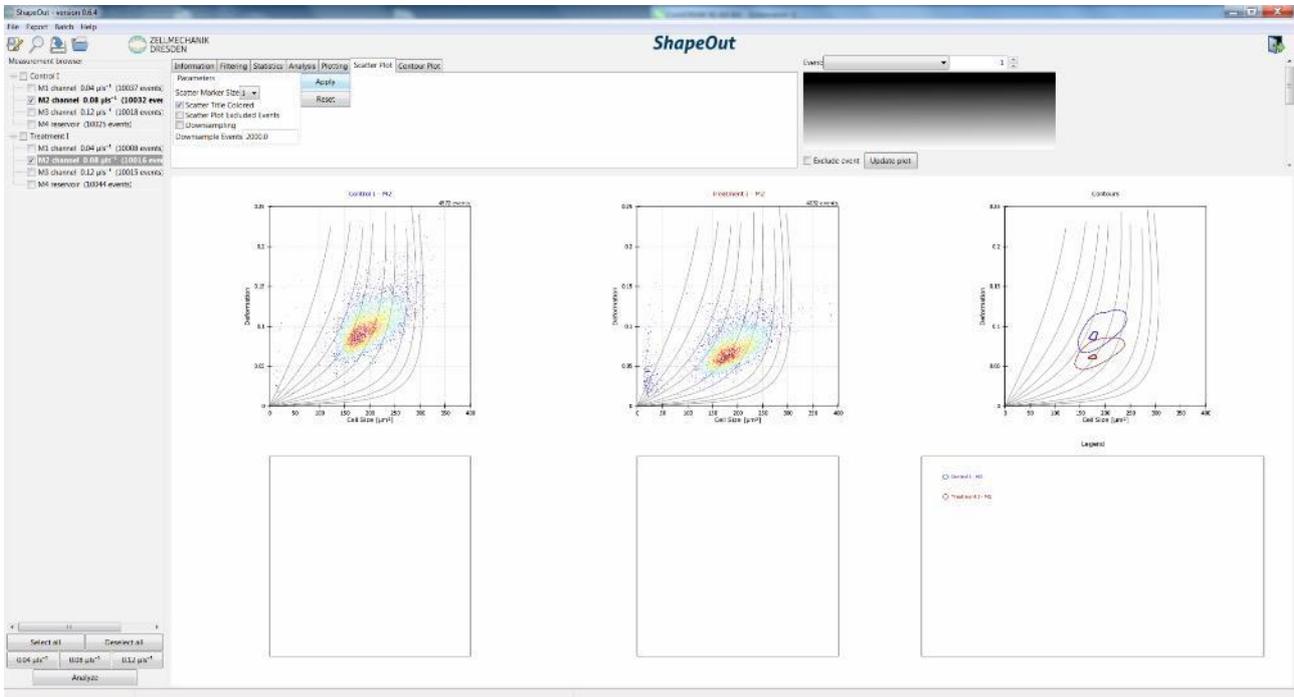
Go to the **Scatter Plot** tab and uncheck the **Scatter Plot Excluded Events** to show only the filtered data.



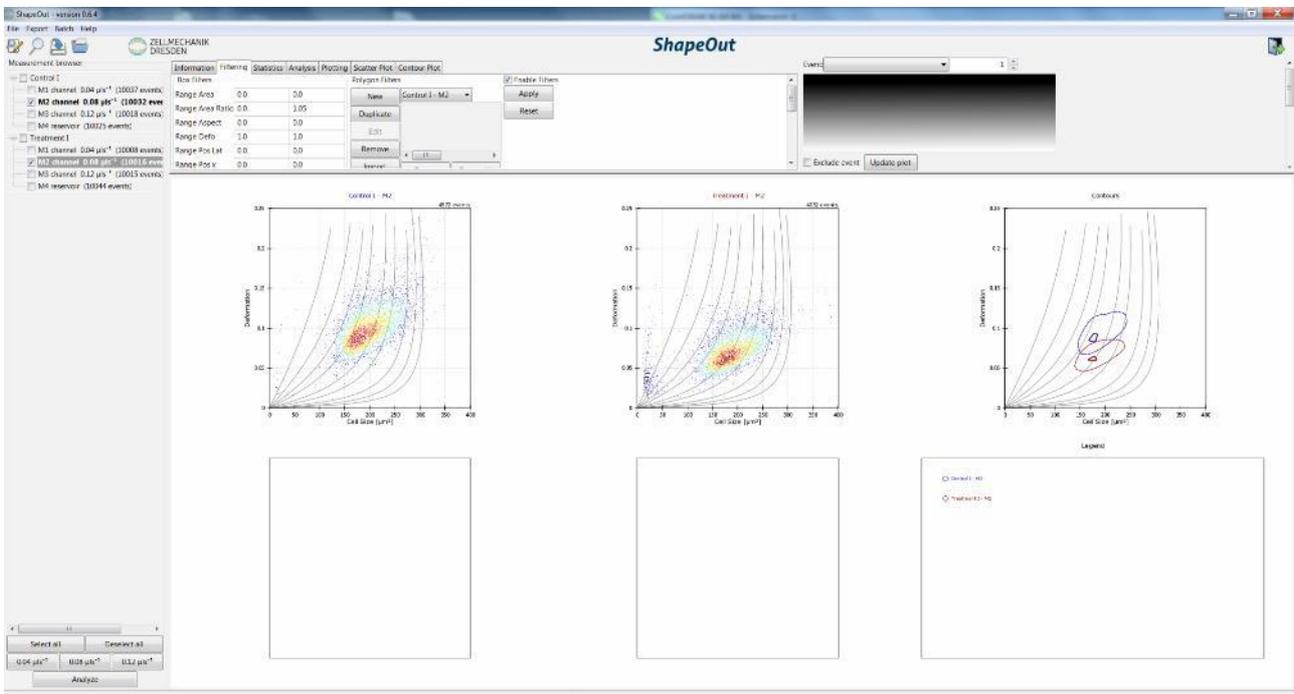
Uncheck the **Downsampling** box to include all events within the filter settings.

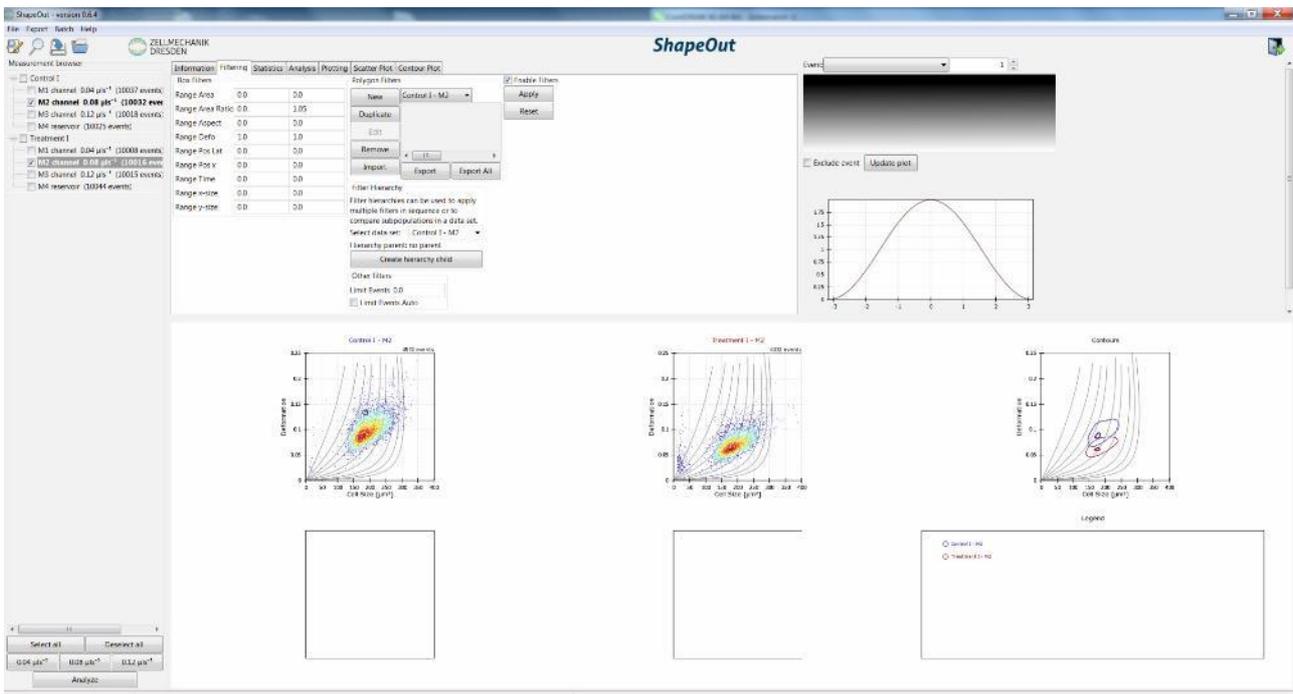
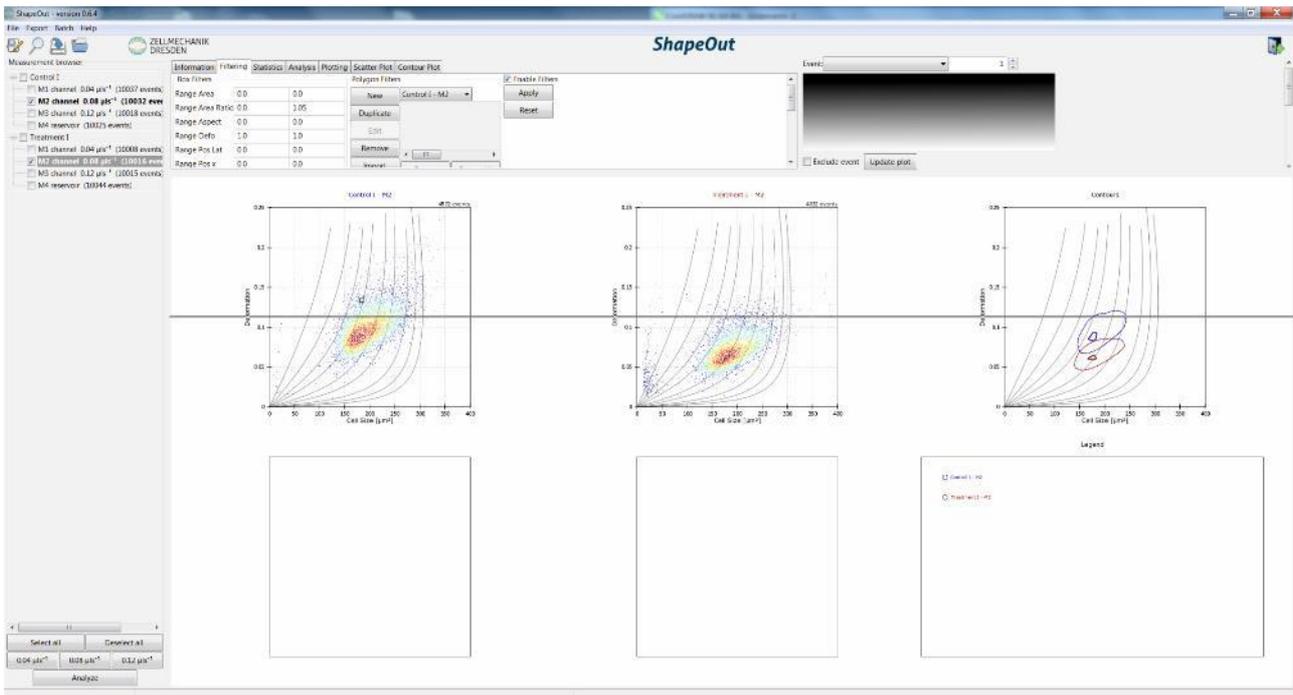


Change the **Scatter Marker Size** to adapt the plotted event size to better distinguish single events.



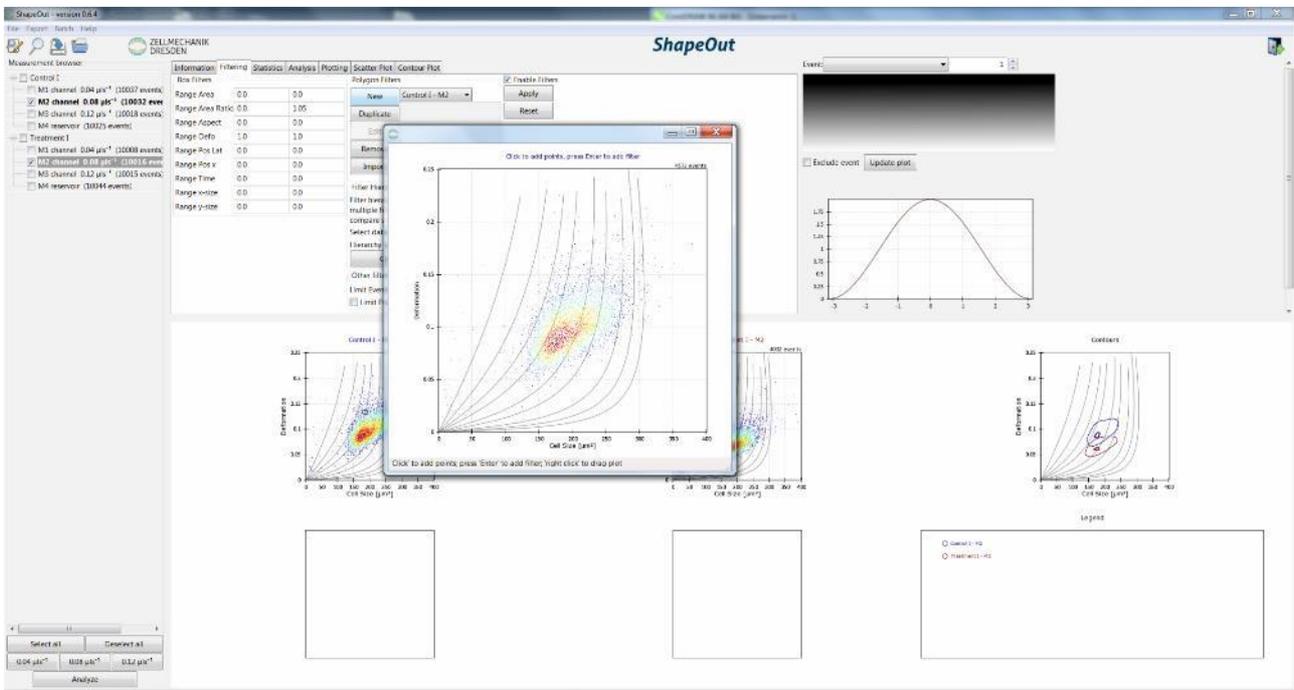
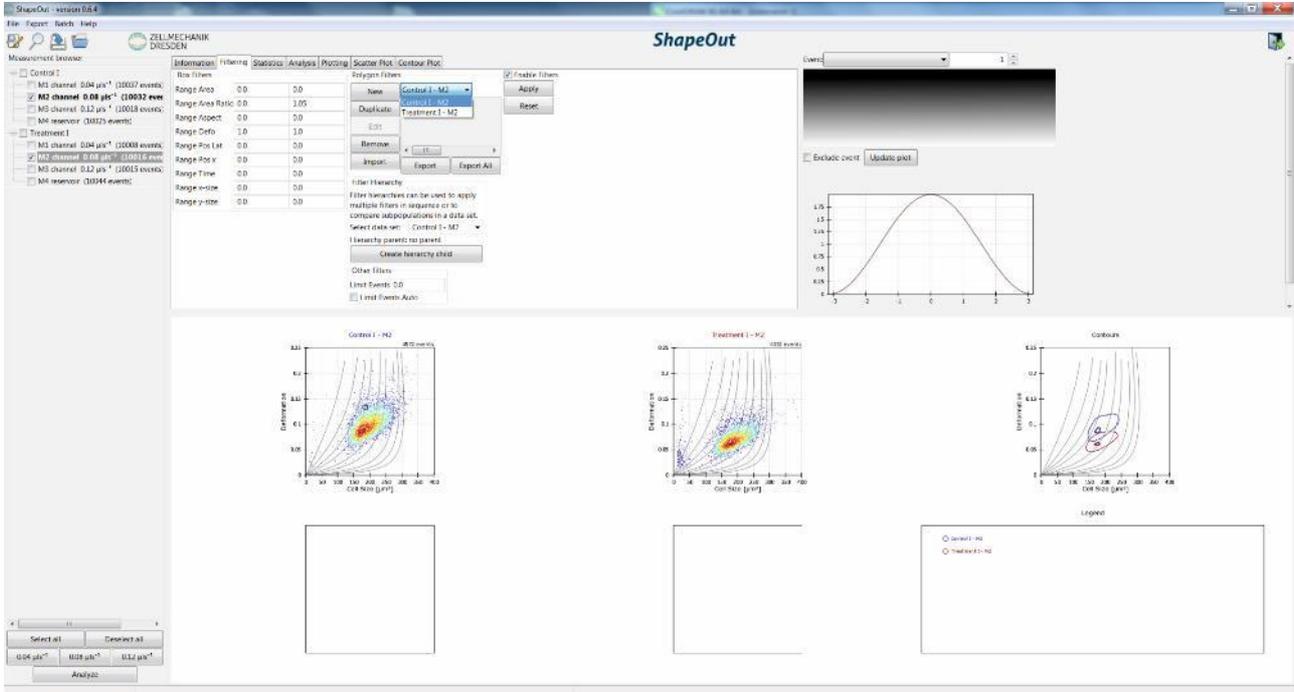
Go back to the **Filtering** tab and increase the window by drag & drop of the lower edge of the tab.



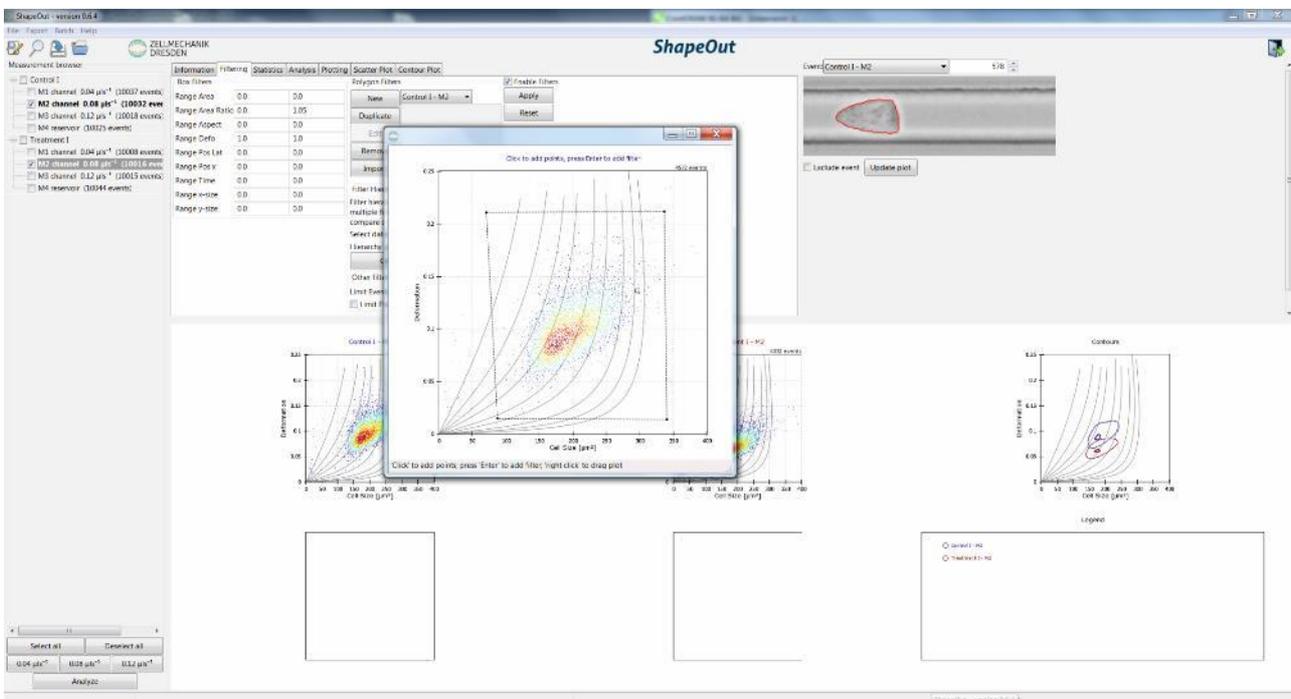
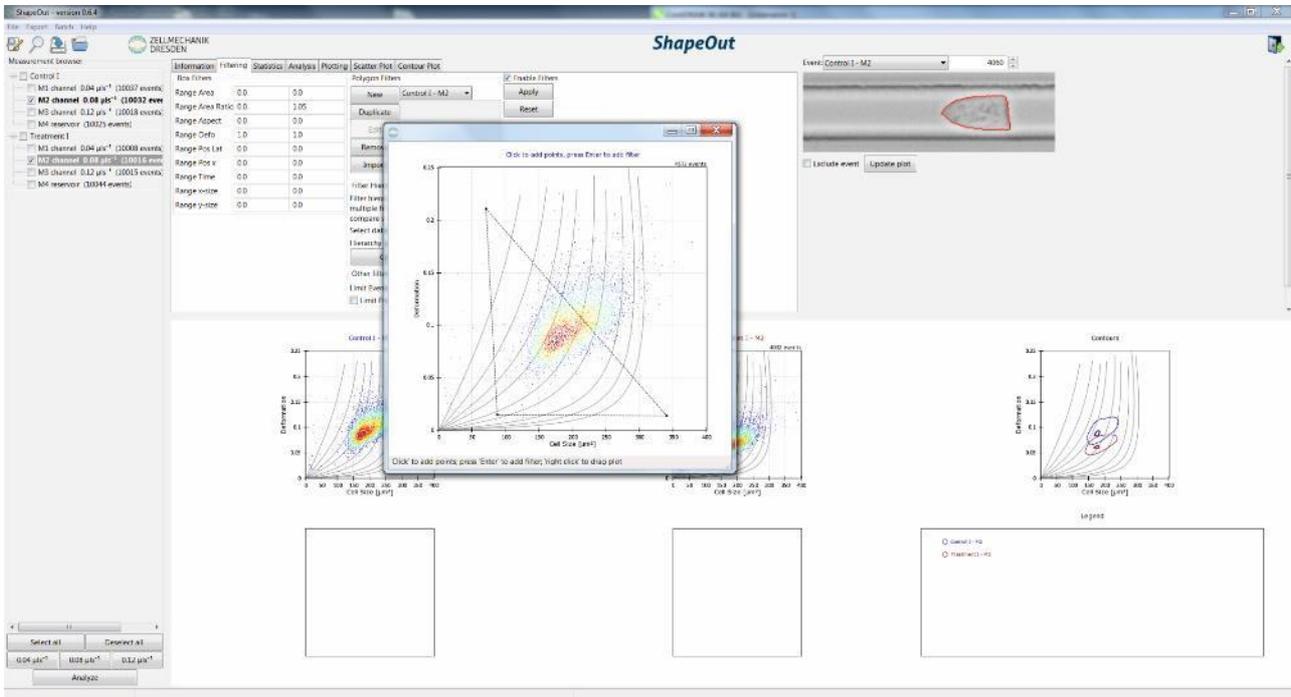


## Polygon filter

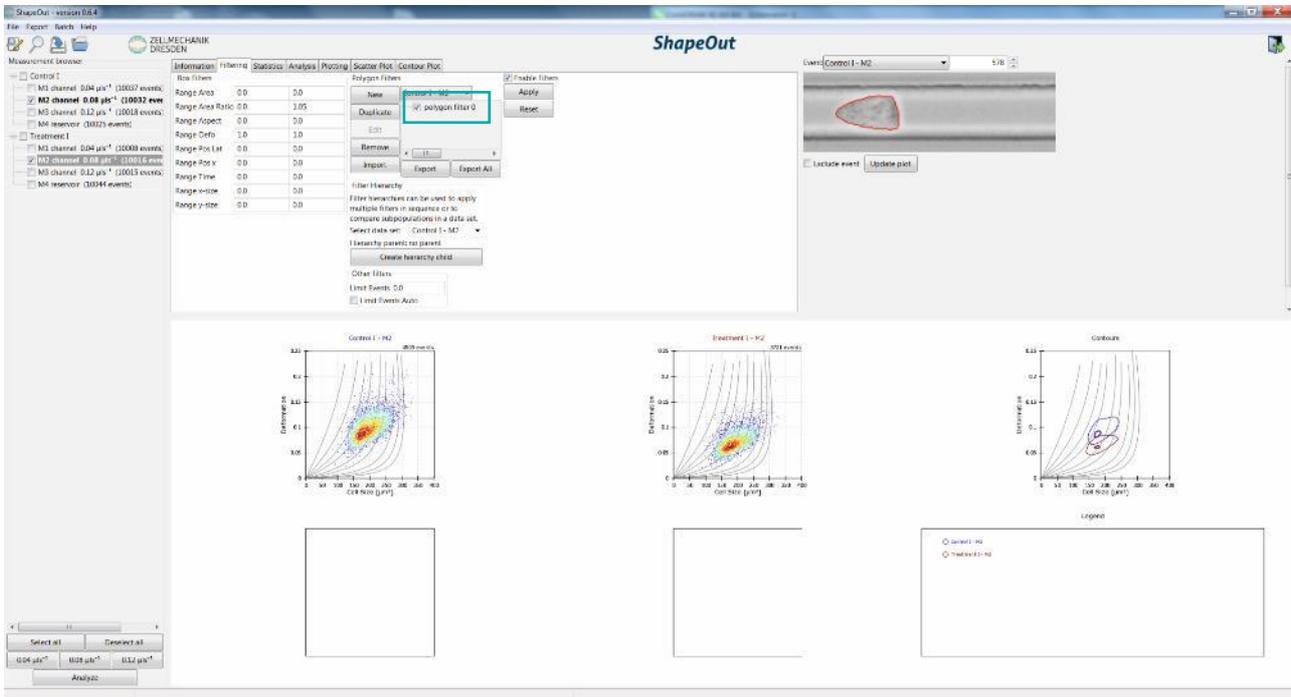
Apply a polygon filter to cut the desired population. Choose the plot where you want to draw the filter in the drop down menu under **Polygon Filters** (here: Control I – M2).



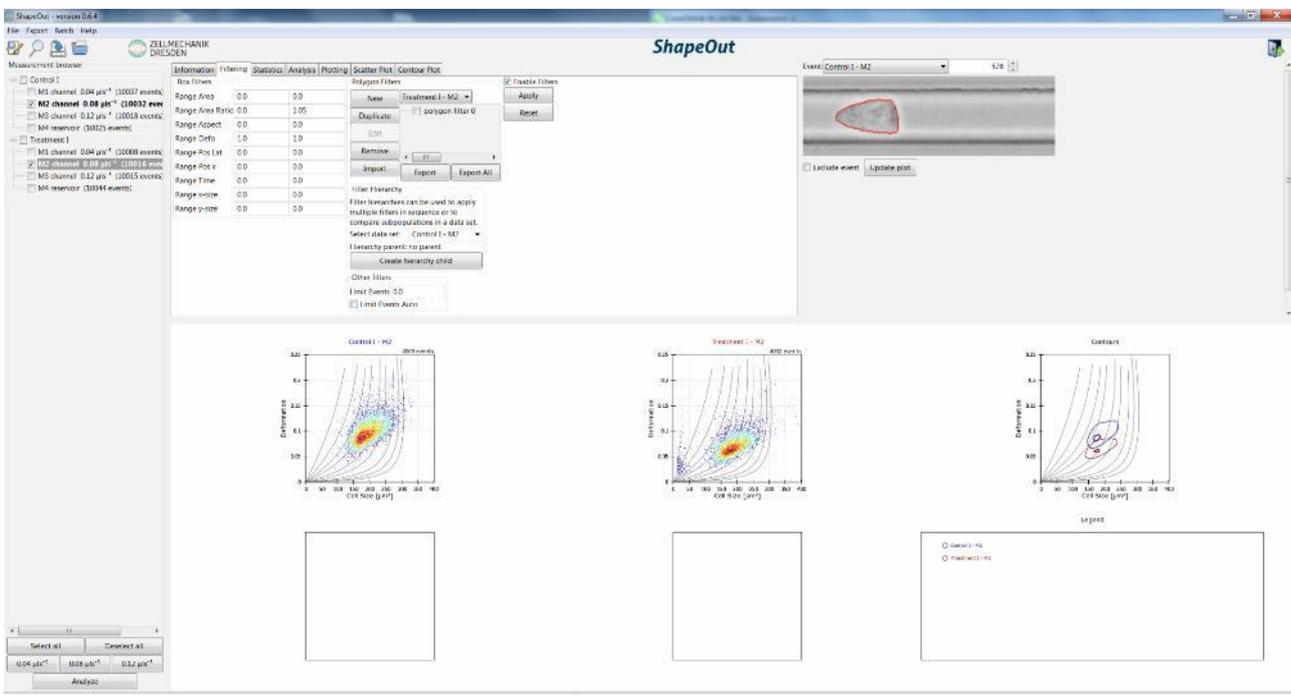
Use the mouse pointer and click where a corner-point of the polygon shall appear. The number of points is not limited. Adding point by point creates a square in this example.



Press Enter to apply the filter. The filter is labeled automatically. The name appears under the dropdown menu and is by default checked for all plot. (here: polygon filter 0)

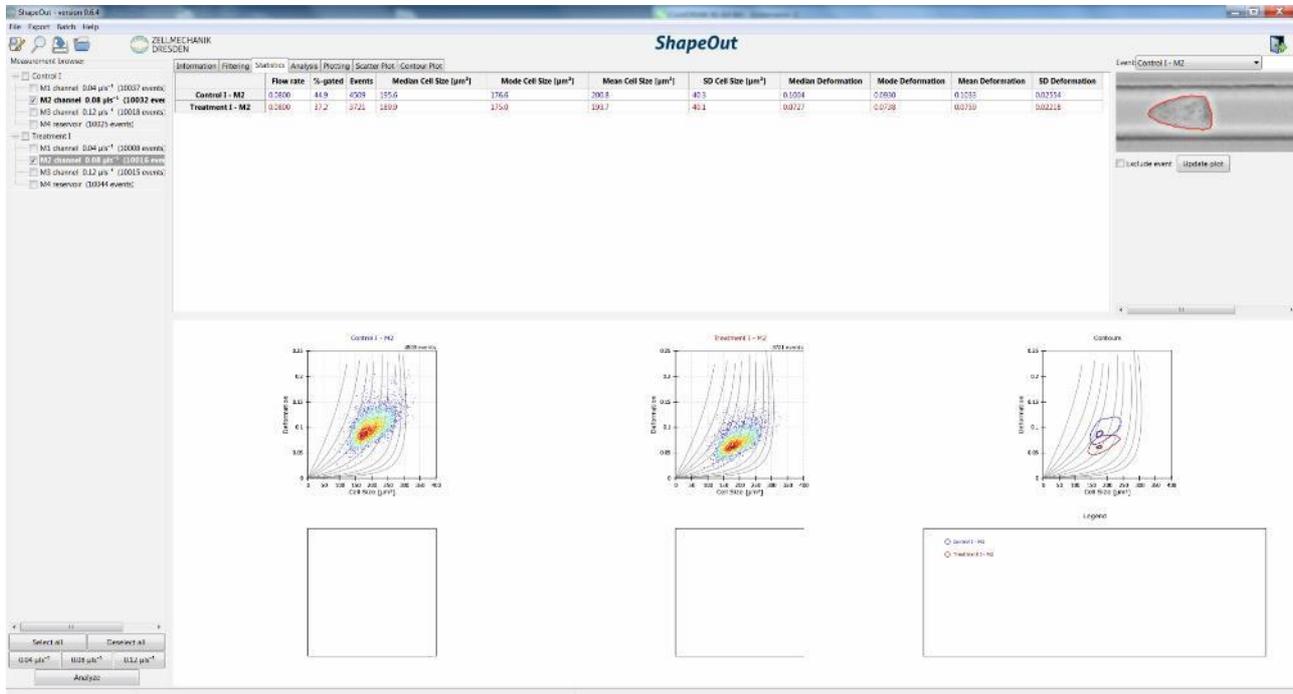


If wanted you can uncheck the polygon filter for single plots (here: for Treatment I – M2).



## Statistics

Go to the **Statistics** tab to see all calculated parameters of the filtered data of all plots.



## Properties of the Contour Plot

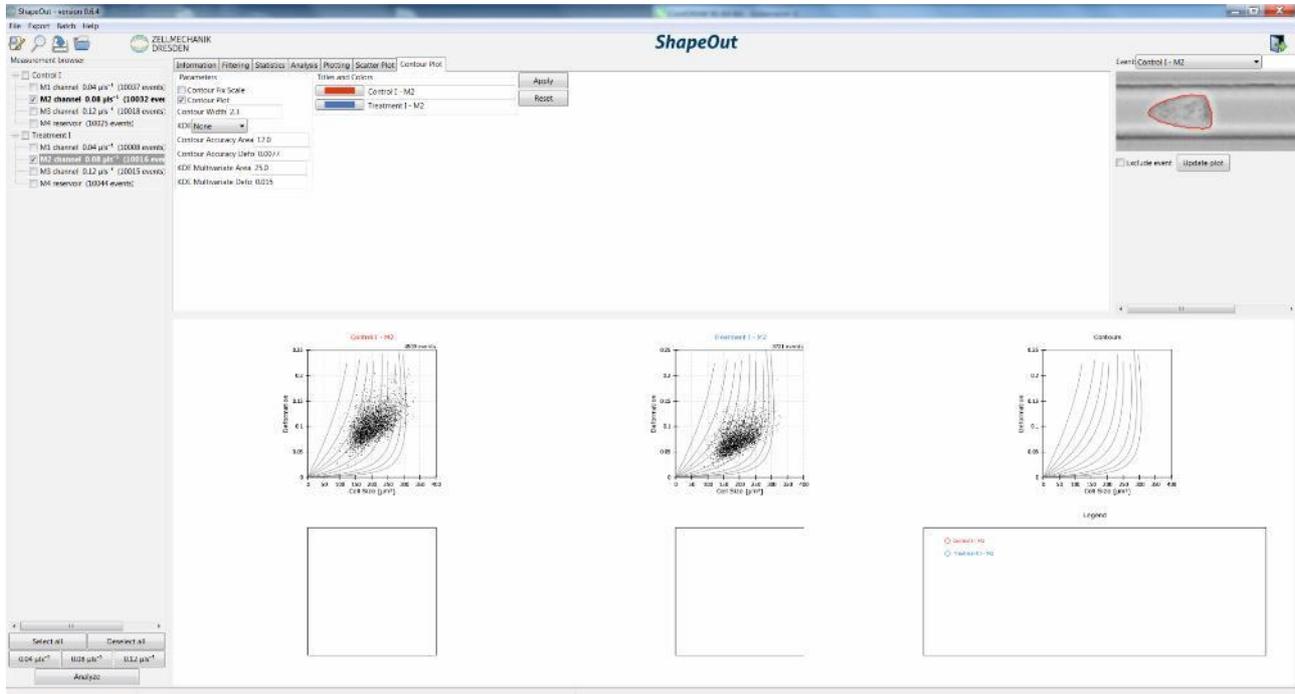
Go to the **Contour Plot** tab to...

...change the line color by clicking on the color bar, choosing a color in the color window and clicking **Apply** (here: to red and blue)

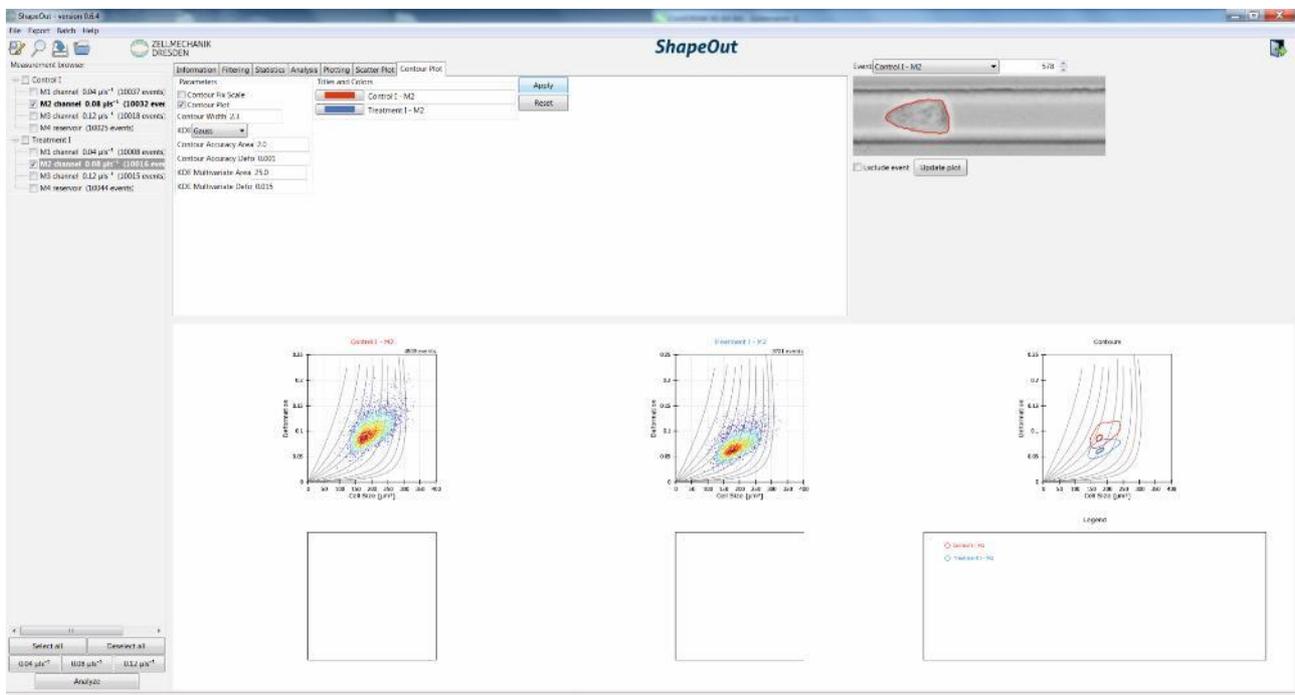


...change the way to calculate the Kernel Density, **KDE** (here: change from Gauss to no calculation)





...change the resolution of the **Contour Plot**, by changing **Contour Accuracy Area** and **Contour Accuracy Deformation**

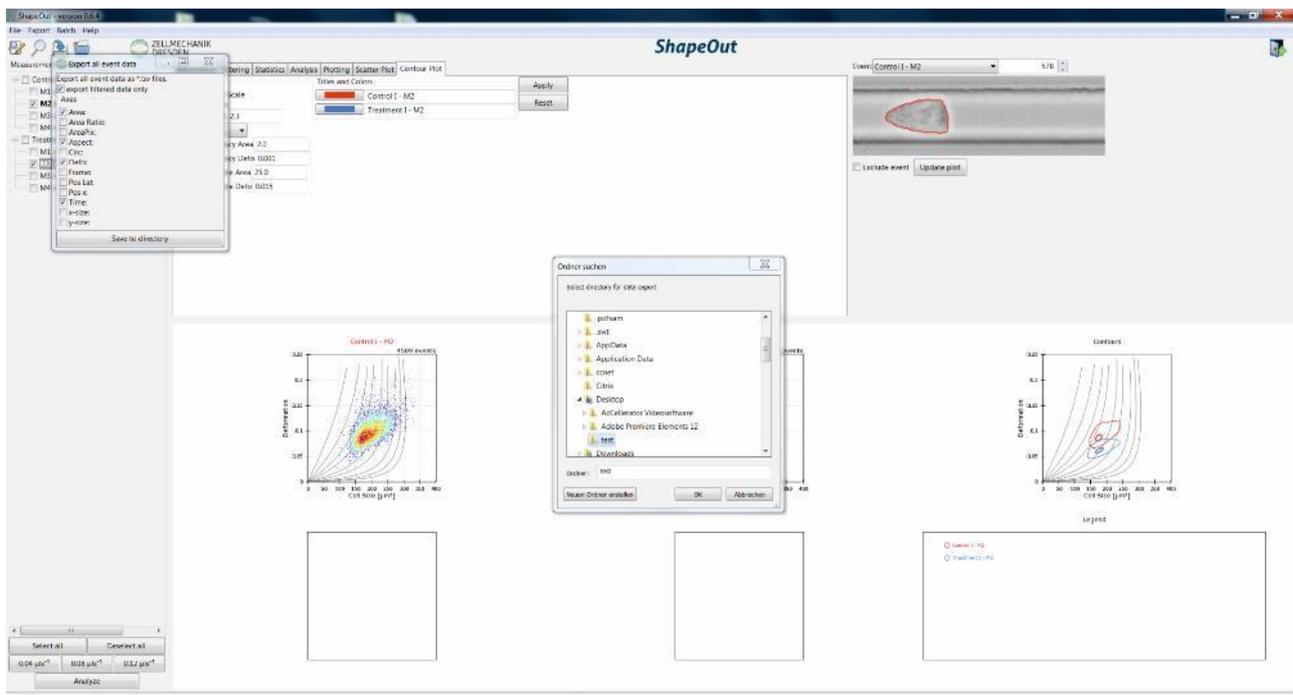


## Export data

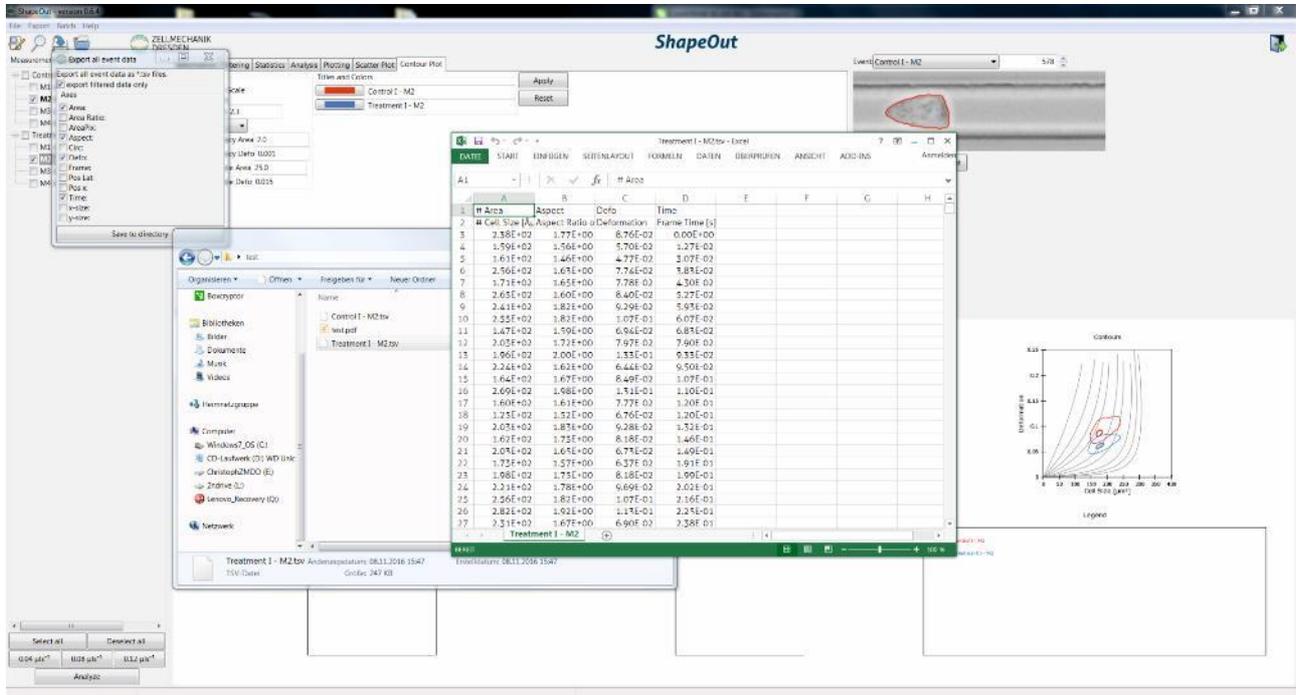
Open the *Export* menu



Click on **All event data (\*.tsv)** to open a dialog to choose the data and if you want everything or the filtered dataset only. The export function will create a datasheet. Choose a folder to store the datasheet.

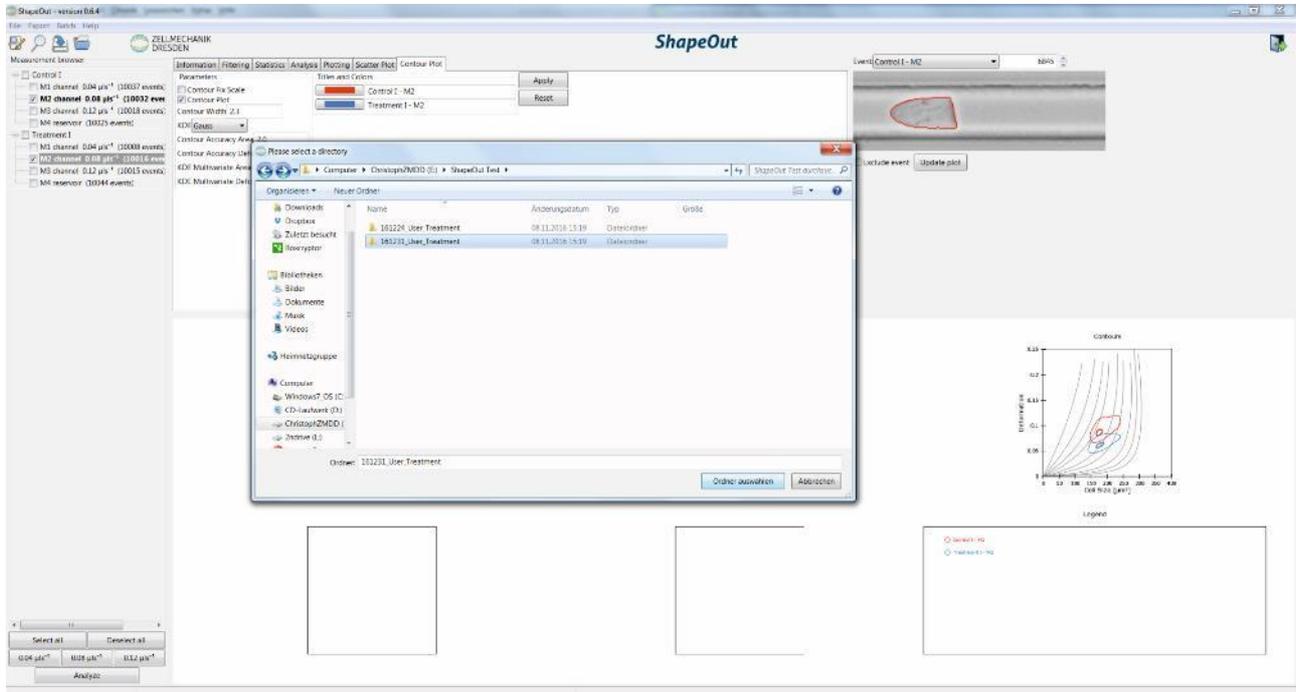


Read the data e.g. in Excel.

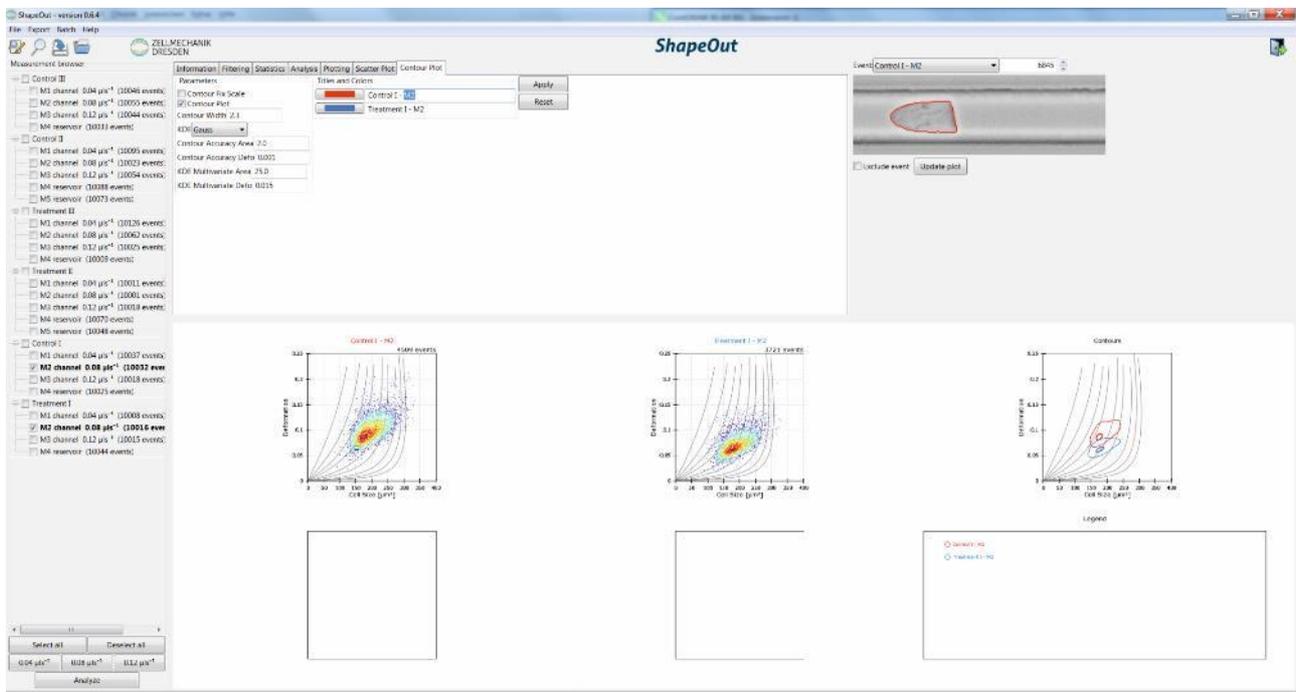


## Adding additional data

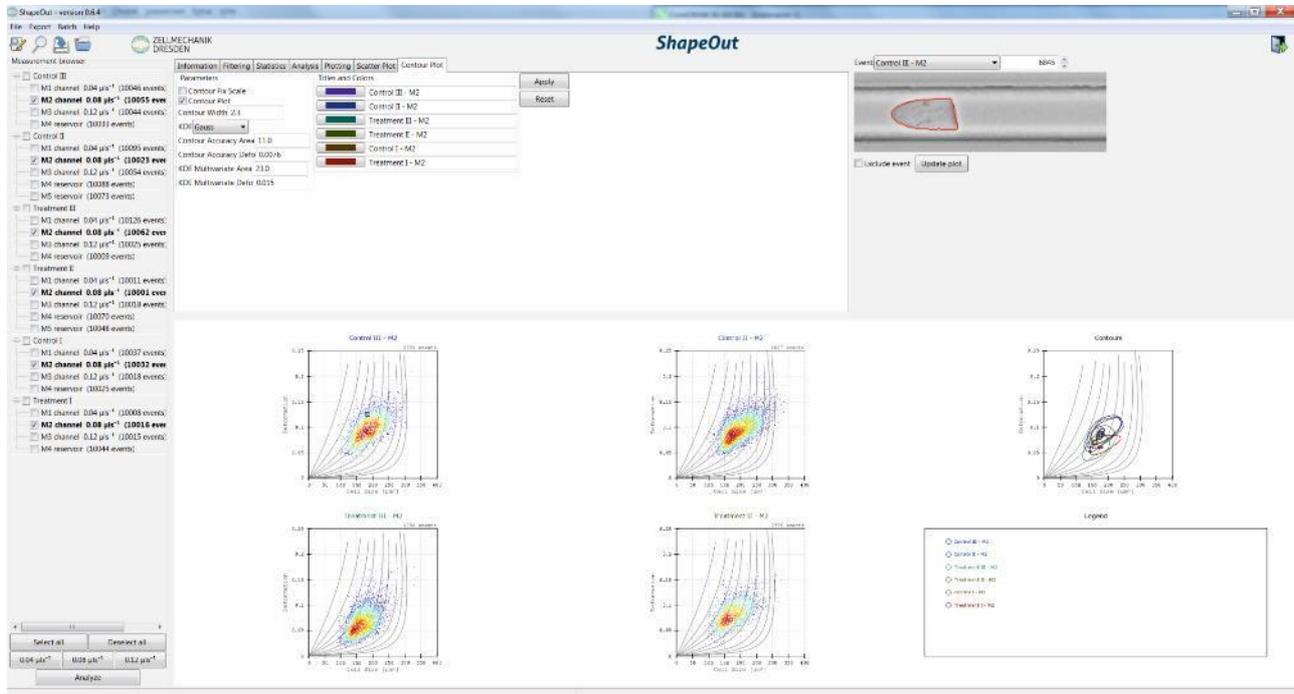
Go to the *File* menu, click **Add Measurement** and choose the folder of another experiment (here 161231\_User\_Treatment)



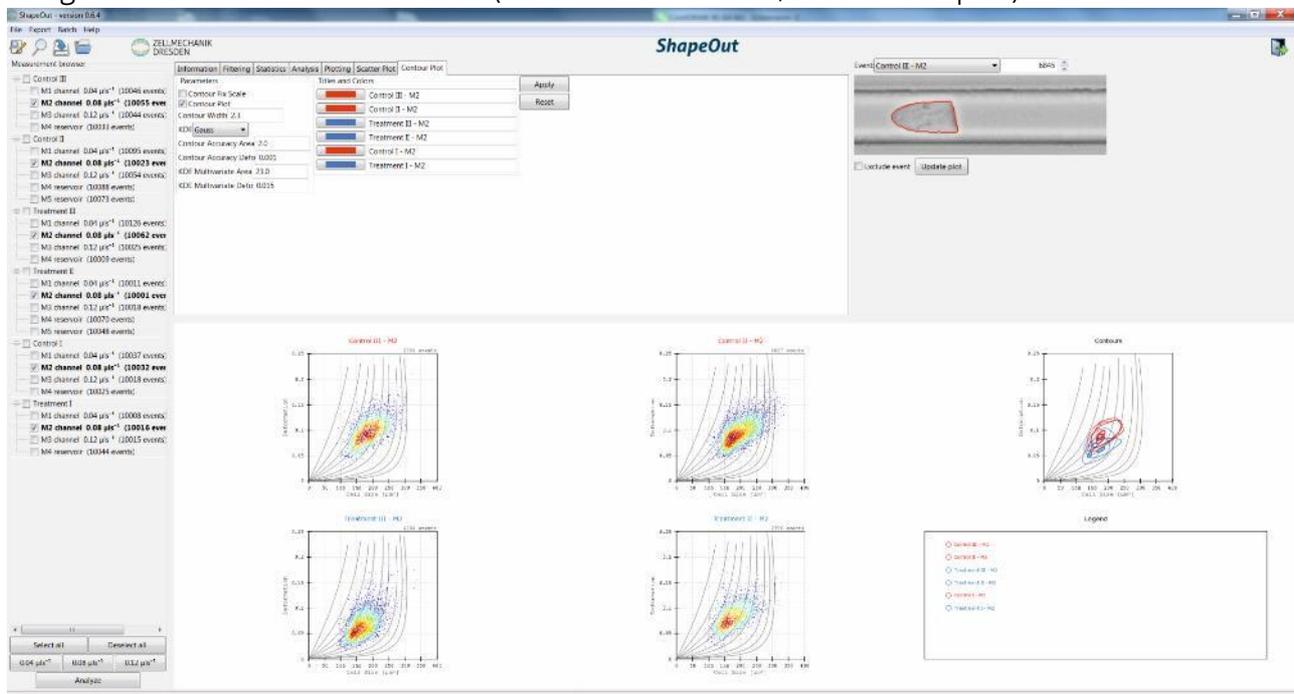
All available data is shown in the measurement browser on the left.



Select all measurements you want to compare and click **Analyze**. (here: all 0.08  $\mu\text{l/s}$  measurements are chosen). The filter settings of the previous dataset apply to the new data as well. Attention! The color-coding and naming of the contour plots are reset to default.

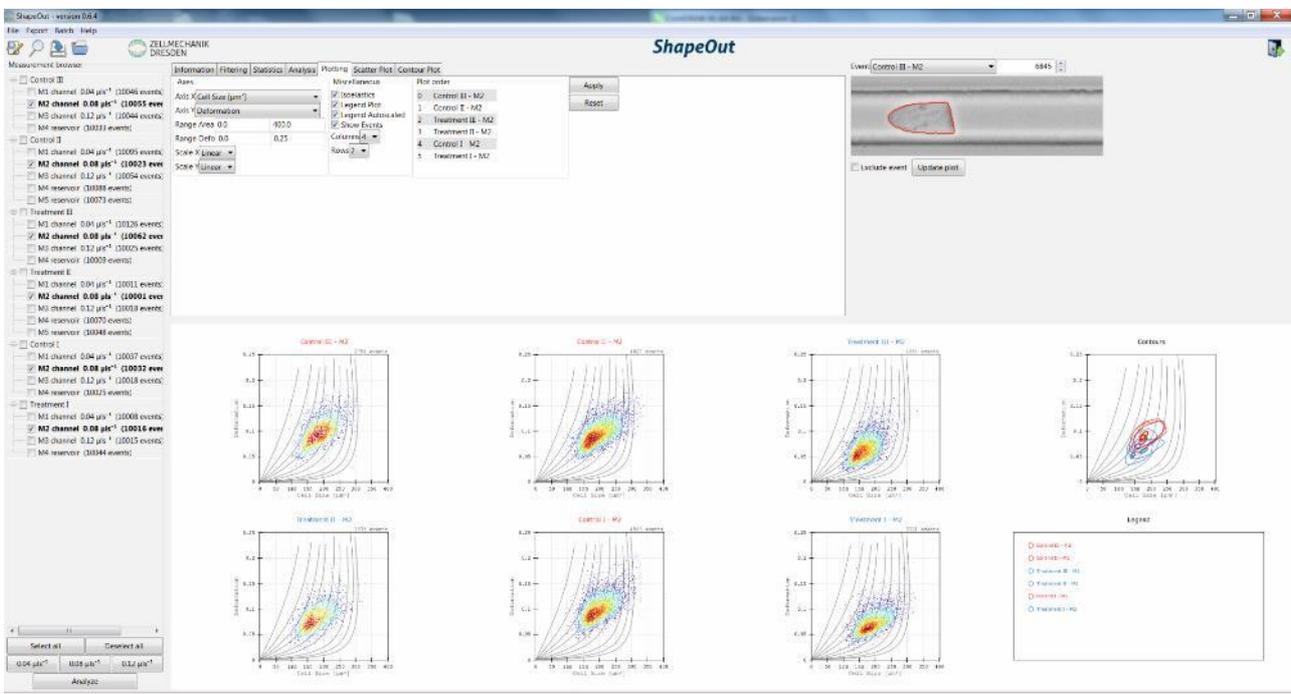
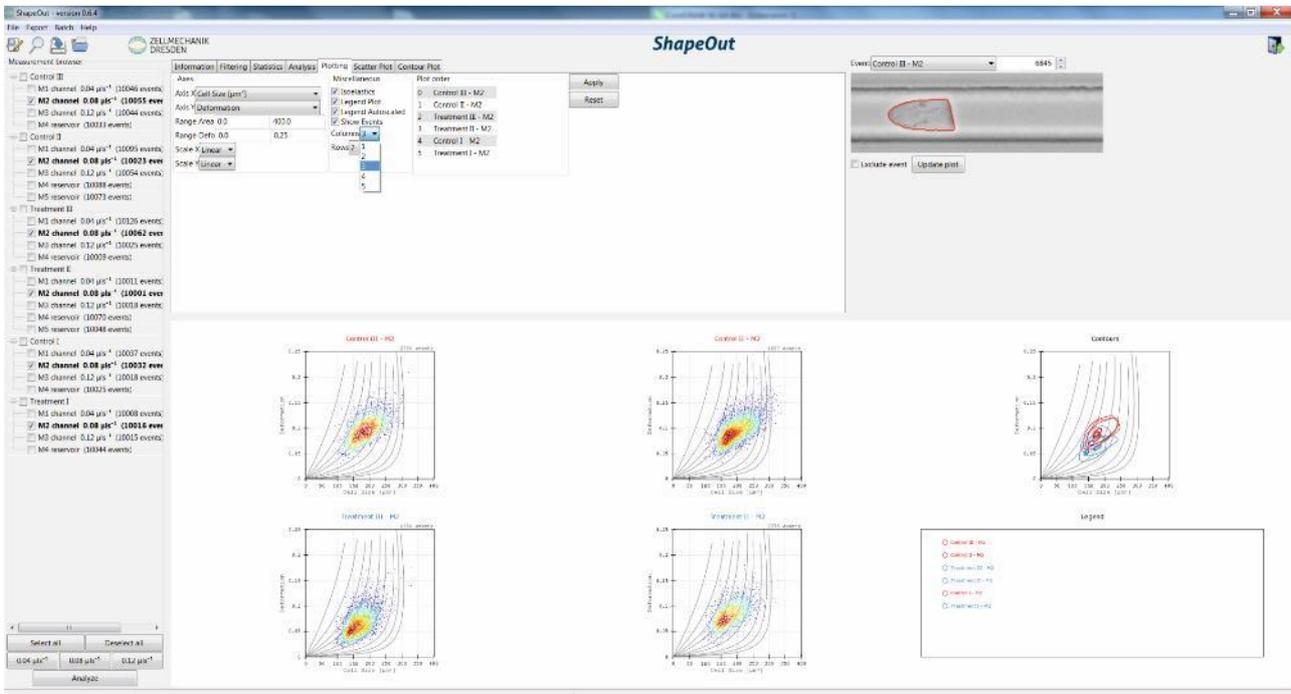


Assign colors and names to the data. (here: red for controls, blue for samples)



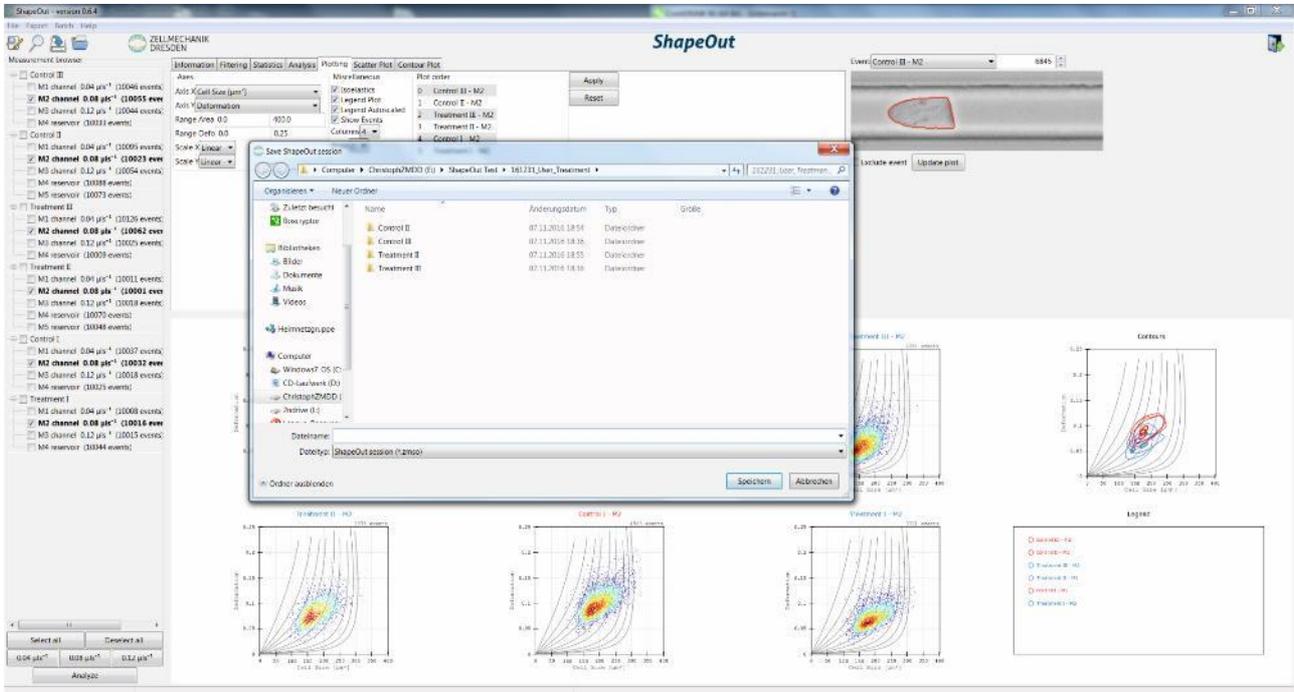
## Change the number of visible plots in the plot window

The window containing all plots, the contour plot and the legend is composed of columns and rows. The number of visible columns and rows is static and has to be changed manually to either show more or less. Go to the **Plotting** tab and change the number of rows and columns. (here: from 3 columns/ 2 rows to 4 columns/ 2 rows).



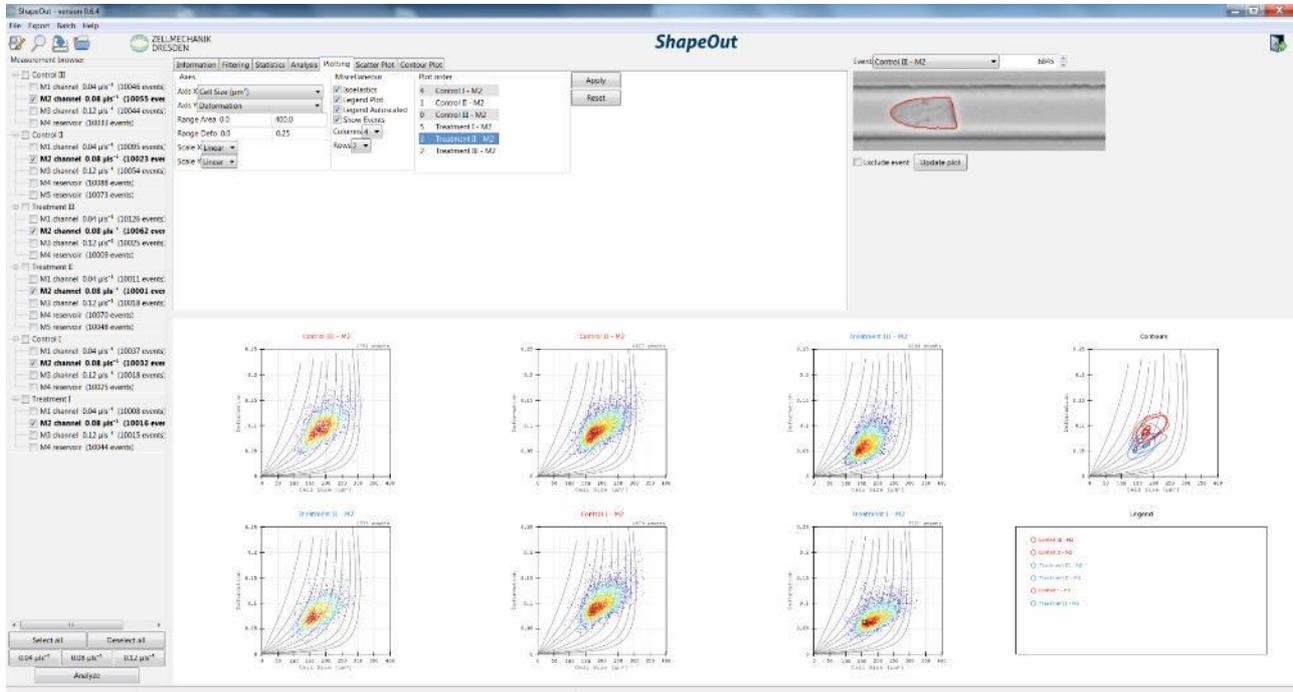
## Save the session

You can save your work and continue later. Go to the *File* menu, click **Save Session** and name the session file. The file extension is \*.zms0.

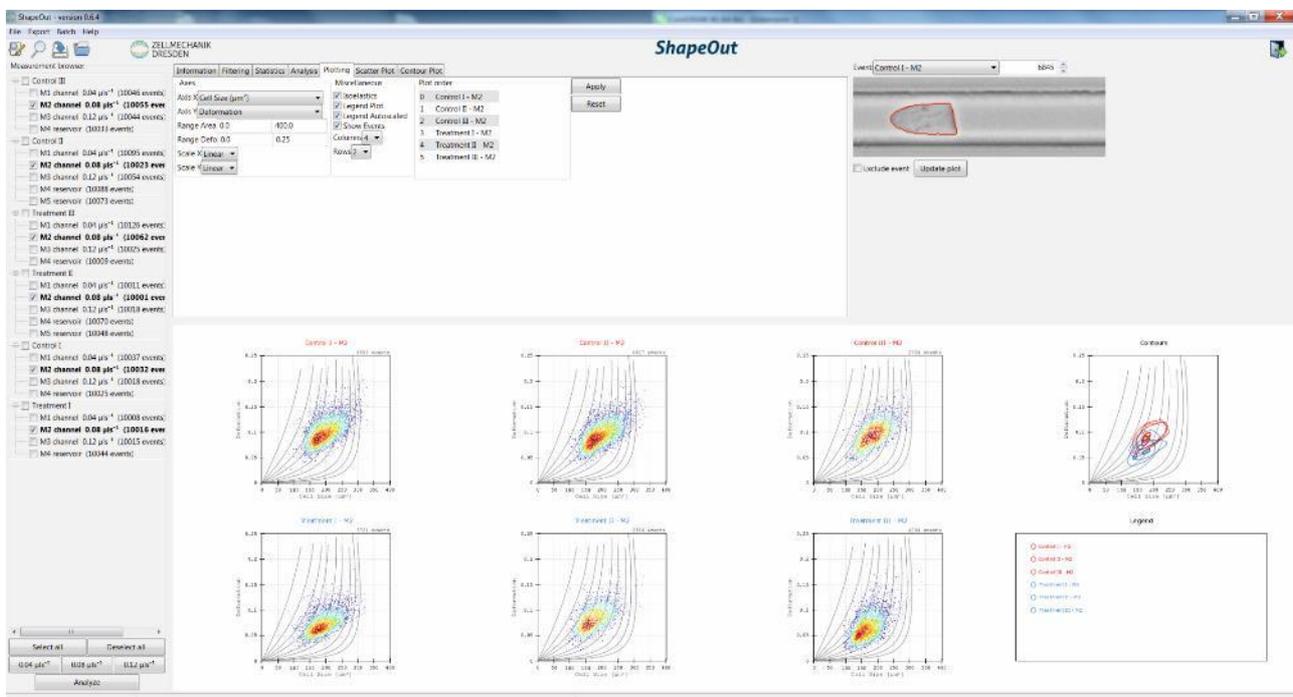


## Change the order of the displayed plots

Go to the **Plotting** tab. You can drag&drop the lines in **Plot order**. (here: all "Control" are moved to the top)

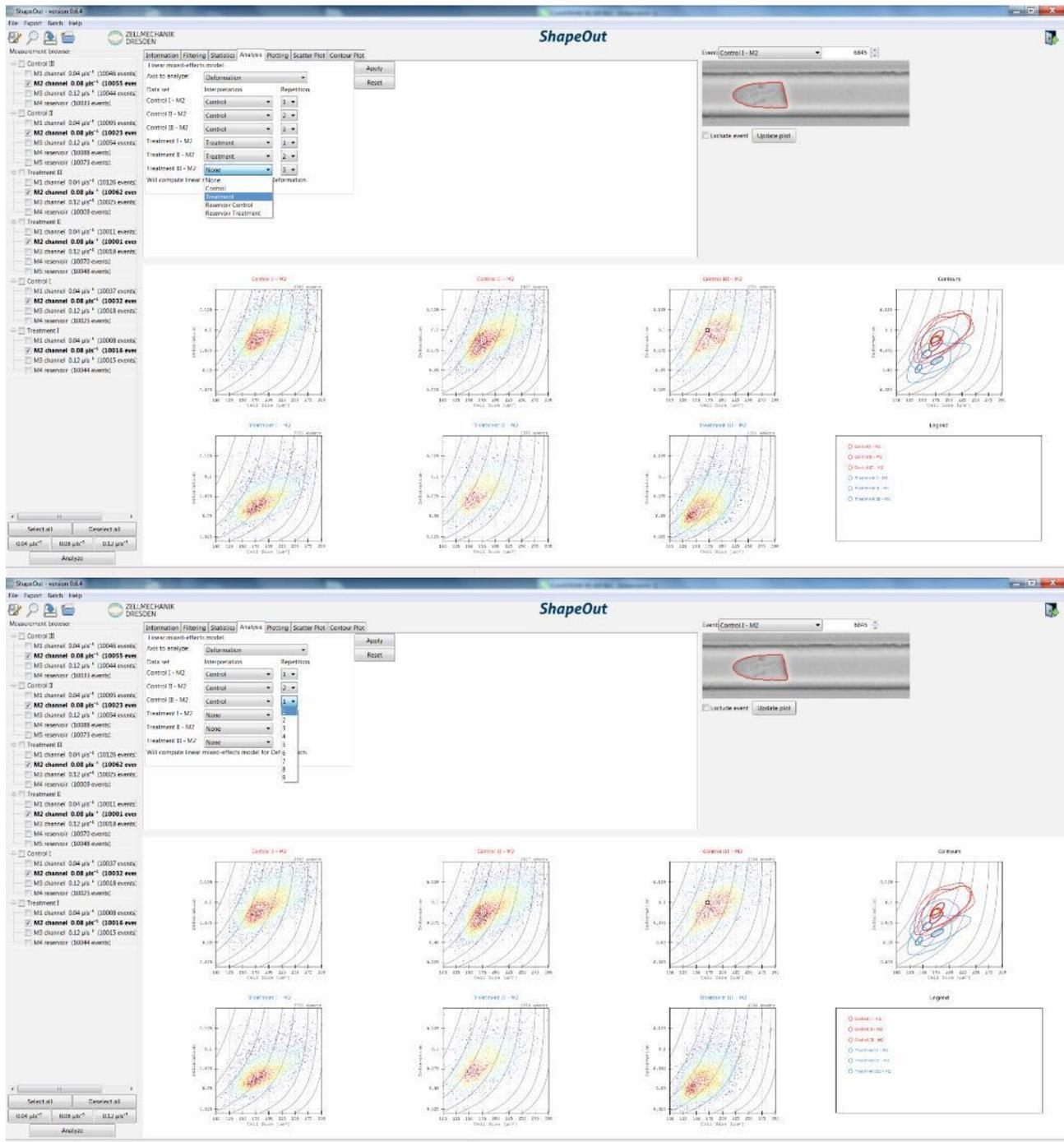


Press **Apply**. Now all "Control" are in the first row.

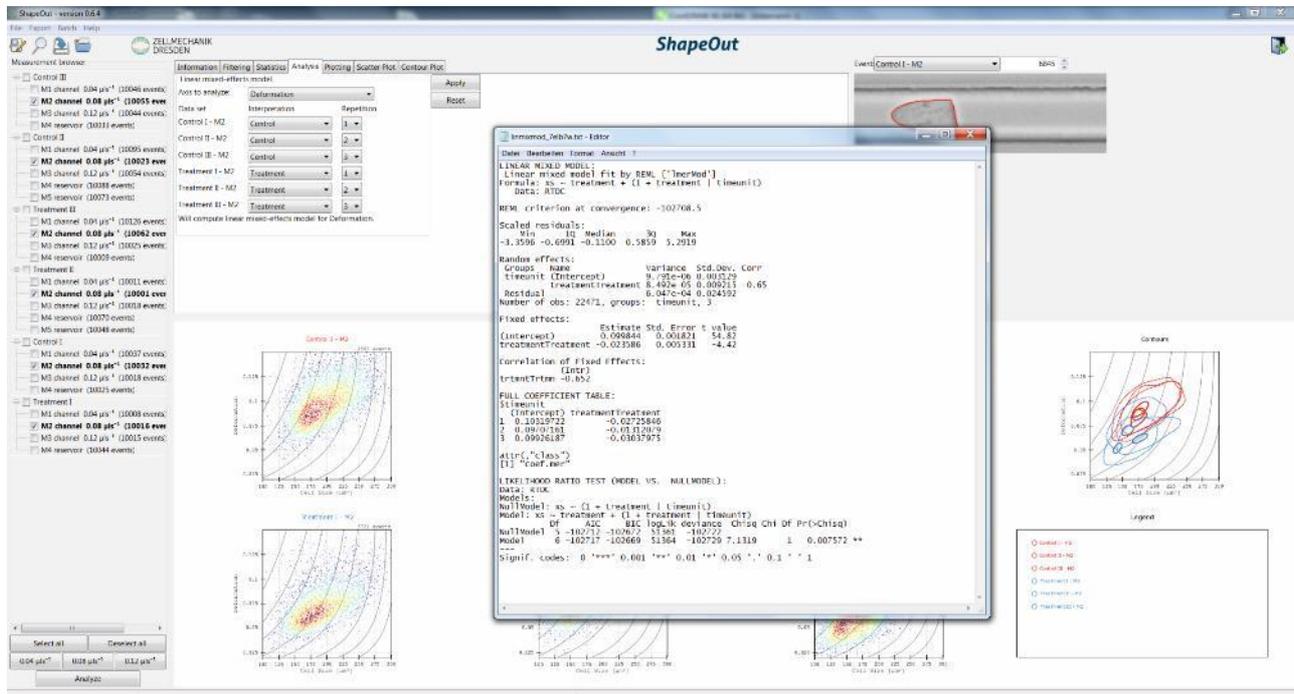


## Compare datasets

Go to the **Analysis** tab. This tool compares an experiment (control and treatment) and repetitions of the experiment. Assign which measurement is a control and which is a treatment by choosing the option in the dropdown lists under **Interpretation**. Group the pairs of control and treatment done in one experiment, by choosing an index number, called **Repetition**. (here: Treatment I and Control I are one experiment – called Repetition 1, Treatment II and Control II are a repetition of the experiment – called Repetition 2, Treatment III and Control III are another repetition of the experiment – called Repetition 3.)



Press **Apply** to start the calculations. A text file will open to show the results.



```
linmixed_7elb7w.txt - Editor
Datei Bearbeiten Format Ansicht ?
LINEAR MIXED MODEL:
Linear mixed model fit by REML ['lmerMod']
Formula: xs ~ treatment + (1 + treatment | timeunit)
Data: RTDC

REML criterion at convergence: -102708.5

Scaled residuals:
  Min      1Q  Median      3Q      Max
-3.3596 -0.6991 -0.1100  0.5859  5.2919

Random effects:
 Groups Name          Variance Std.Dev. Corr
timeunit (Intercept)  9.791e-06 0.003129
          treatment  8.492e-05 0.009215 -0.65
Residual    6.047e-04 0.024592
Number of obs: 22471, groups: timeunit, 3

Fixed effects:
              Estimate Std. Error t value
(Intercept)   0.099844   0.001821   54.82
treatment     -0.023586   0.005331   -4.42

Correlation of Fixed Effects:
      (Intr)
trtmntTrtmn -0.652

FULL COEFFICIENT TABLE:
timeunit
(Intercept) treatmentTreatment
1  0.10319722      -0.02725846
2  0.09707161      -0.01312079
3  0.09926187      -0.03037975

attr(,"class")
[1] "coef.mer"

LIKELIHOOD RATIO TEST (MODEL VS. NULLMODEL):
Data: RTDC
Models:
NullModel: xs ~ (1 + treatment | timeunit)
Model: xs ~ treatment + (1 + treatment | timeunit)
          Df    AIC    BIC loglik deviance  Chisq Chi Df Pr(>Chisq)
NullModel 5 -102712 -102672 51361 -102722
Model     6 -102717 -102669 51364 -102729 7.1319  1 0.007572 **

Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

The most important numbers are:

### Fixed effects

#### (Intercept)-Estimate

is the mean of the parameter chosen for all controls.

#### treatment-Estimate

is the effect size of the parameter chosen between the mean of all controls and the mean of all treatments.

#### Full coefficient table

shows the effect size of the parameter chosen between control and treatment for every single experiment

#### Model-Pr(>Chisq)

shows the p-value and the significance of the test

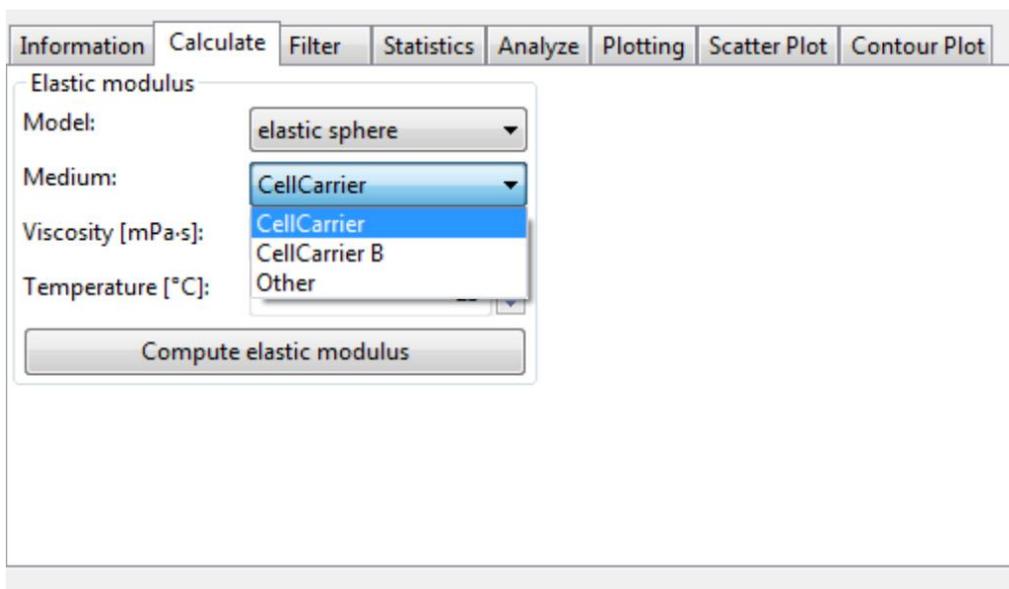


## Mapping deformation values to Young's moduli in ShapeOut (Version 0.7.3)

### Quick Guide

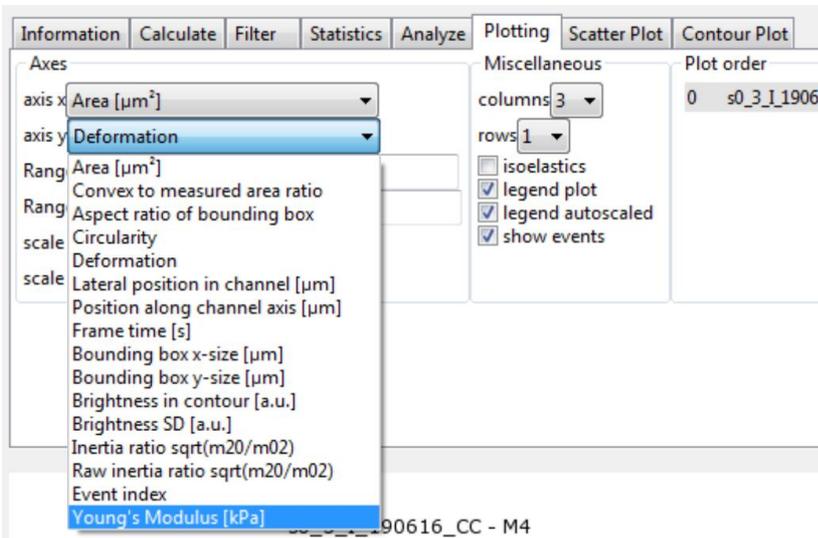
Using ShapeOut, it is now possible to convert deformation values to values of the Young's modulus based on the numerical simulation work for fully elastic spheres by Mokbel *et al.* (1).

You will find a new tab called "Calculate" that allows you to obtain the Young's modulus for the samples you selected for plotting.



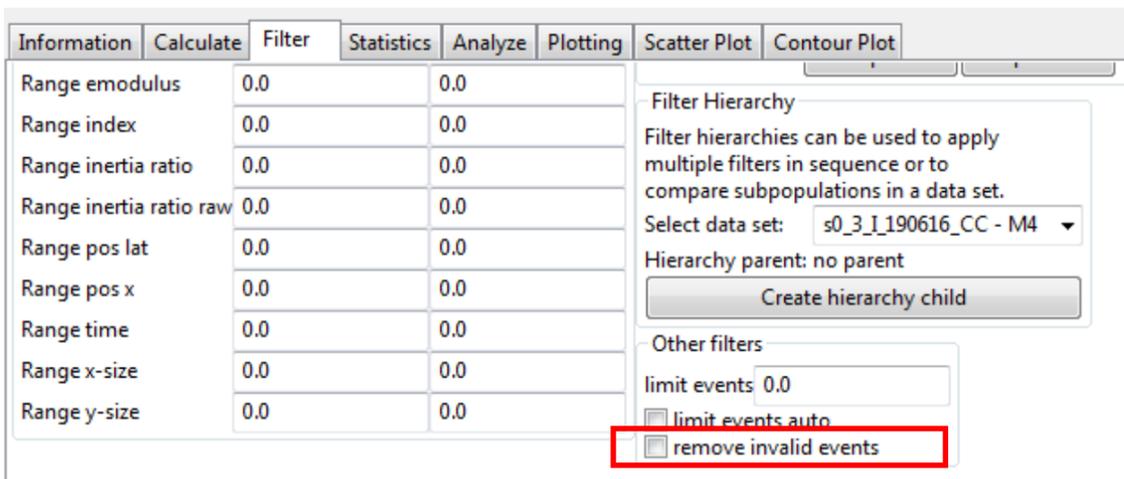
Currently, the only model available is the elastic sphere. After choosing the type of measurement medium you must set the right temperature or – in case you choose "Other" – the correct viscosity. For CellCarrier media, the correct viscosity is automatically calculated according to the shear thinning behavior as analyzed in (2).

Once "Compute elastic modulus" is clicked, a plotting option for the Young's modulus will become available.



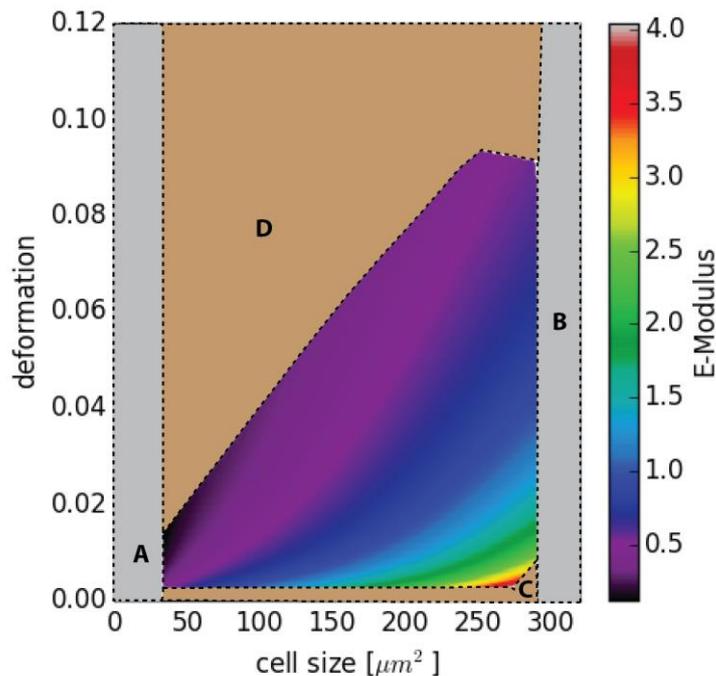
It is important to note that a conversion can only be carried out for deformation and size values in a "valid region" that will be described in more detail later in this document.

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.



## 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 (1) have yielded a valid region for the conversion in the space of deformation and cell size shown with a color gradient for a 20  $\mu\text{m}$  channel.



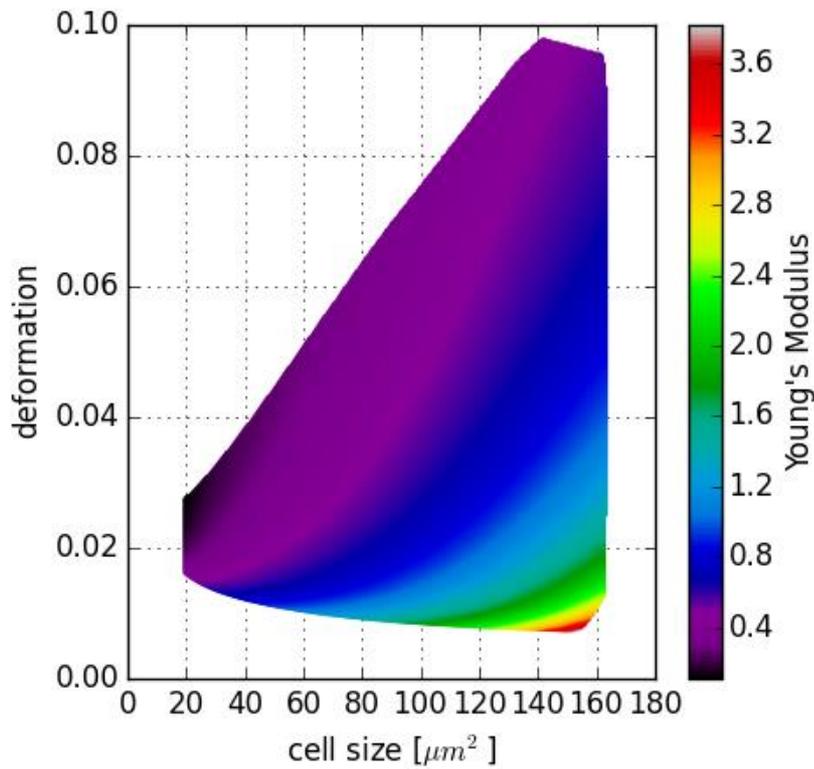
It is limited by regions A and B for objects too small and objects too large for reliable conversion. It is further limited for very small deformation values in region C. The reason for that is a very steep increase of  $E$  with little decrease in deformation that would yield potentially very large 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.

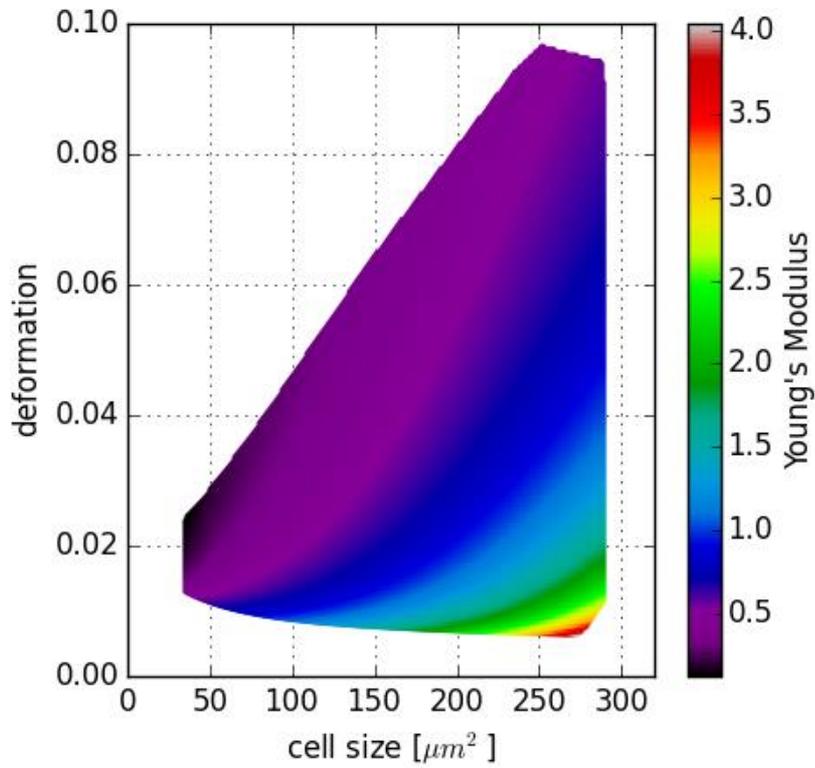
In order to make this process more comfortable, in the following, the valid regions are shown for the four standard channel sizes available. Those representations include an offset shift in deformation that would be expected in the experimental results due to the pixelation of the image as described in (2).

The values of the Young's moduli in those regions will depend on the specific flow rate and the viscosity of the medium (3). Note that in the illustrations that follow they merely represent a relative scaling and are not to be compared between illustrations.

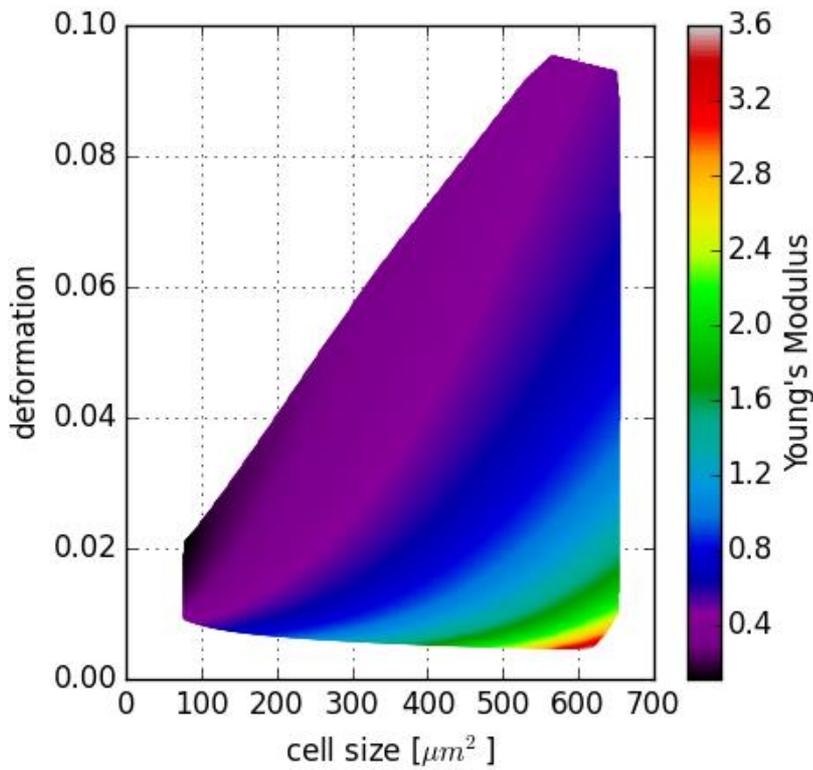
For the 15  $\mu\text{m}$  channel:



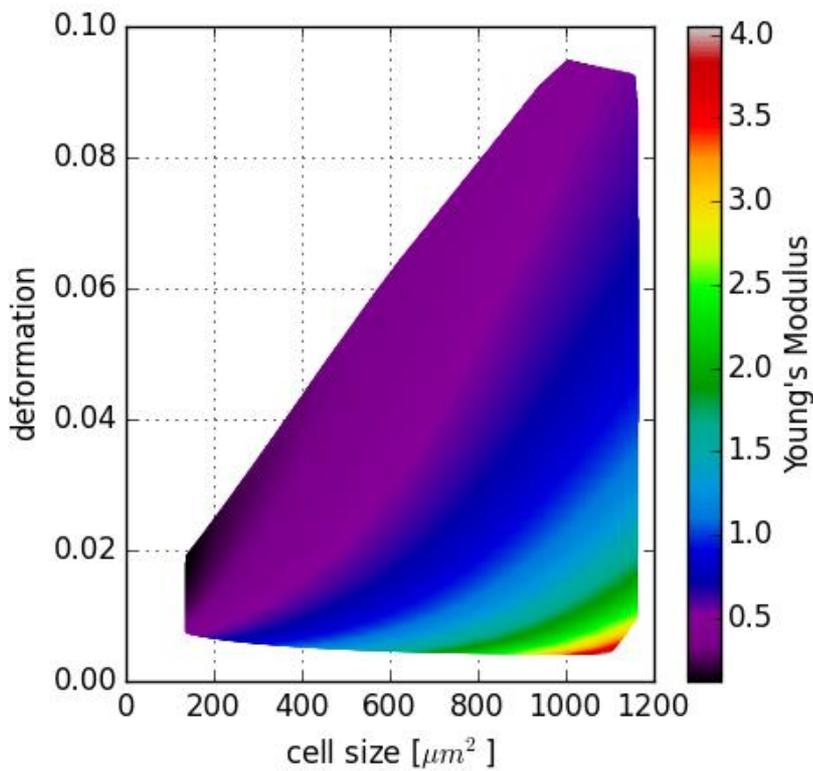
For the **20  $\mu\text{m}$**  channel:



For the **30  $\mu\text{m}$**  channel:



For the 40  $\mu\text{m}$  channel:



1. M. Mokbel *et al.*, Numerical Simulation of Real-Time Deformability Cytometry To Extract Cell Mechanical Properties. *ACS Biomater. Sci. Eng.*, acsbiomaterials.6b00558 (2017).
2. C. Herold, Mapping of Deformation to Apparent Young's Modulus in Real-Time Deformability Cytometry. *arXiv. cond-mat.soft* (2017).
3. A. Mietke *et al.*, Extracting Cell Stiffness from Real-Time Deformability Cytometry: Theory and Experiment. *Biophys J.* **109**, 2023–2036 (2015).