

# matFLUX Tutorial

## Graphical User Interface

Load main channel (\*.mat)

Shows detected Clusters (colorcoded and Loc/cluster)

DBSCAN Settings & Size Cut-Off (cluster size)

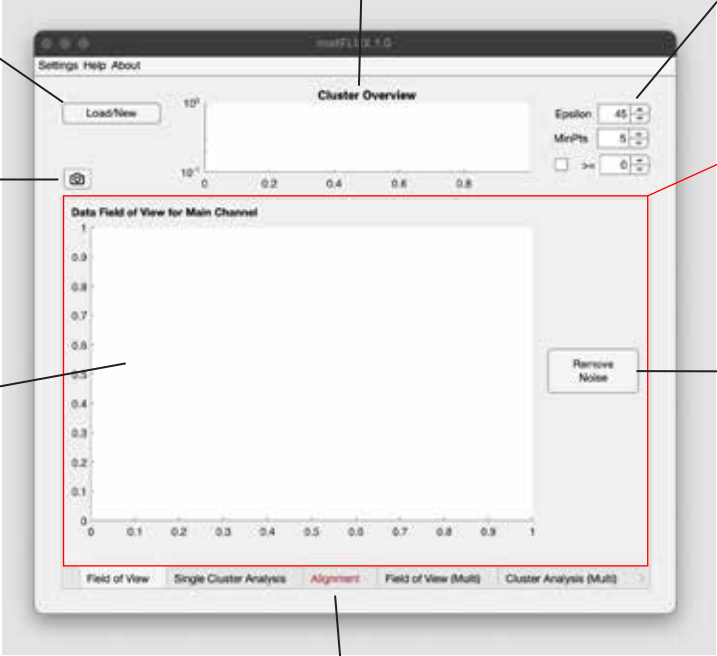
Saves Plots as PDF/PNG

Displays main data in 3D

MAIN Display

Removes Signal outside the DBSCAN clusters

Tab Navigation



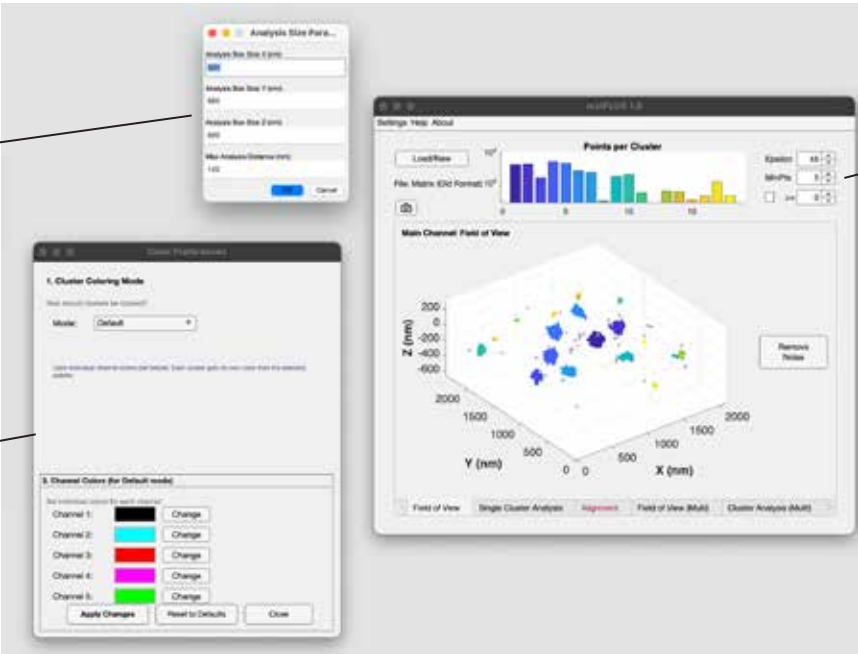
## Settings

DBSCAN parameters.  
Colormap for display of clusters.  
Channel colors.  
Box size for single Cluster Analysis, adjust to your particle's size.

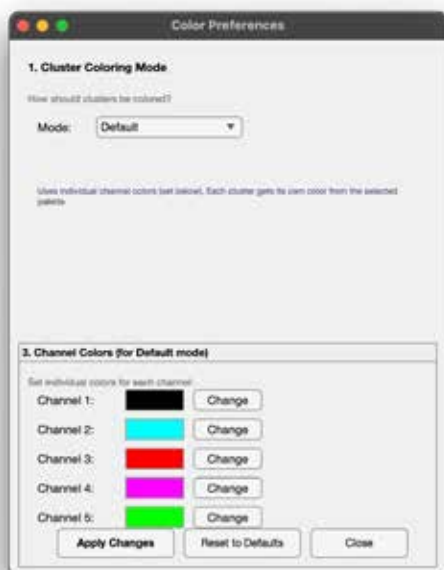
Box Size for single cluster Analysis

DBSCAN parameters and size exclusion

Colormap and Channel Colors



## Settings Modules



Color Preferences:

**Default mode** - Single Color Selection for up to 5 channels

**Color Code** by:

Z-Position

Localizations

Traces



Data Crop Settings

Select '**Enable Crop**'

**Provides** x-,y- and z- limits of loaded Dataset

**Min and Max** on each axis can be selected



Analysis Size

Select **Box Size** based on your nano-particle dimensions

**Box Size** applies to single cluster views

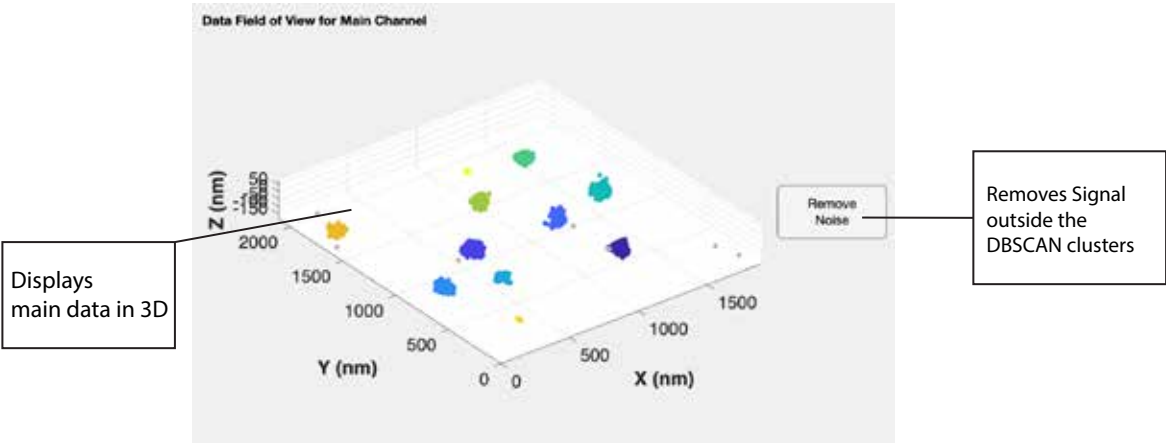
**Max Analysis Distance** applies to Polar Plots and Line Profiles

# Tabs

Every Tab is explained in this section.

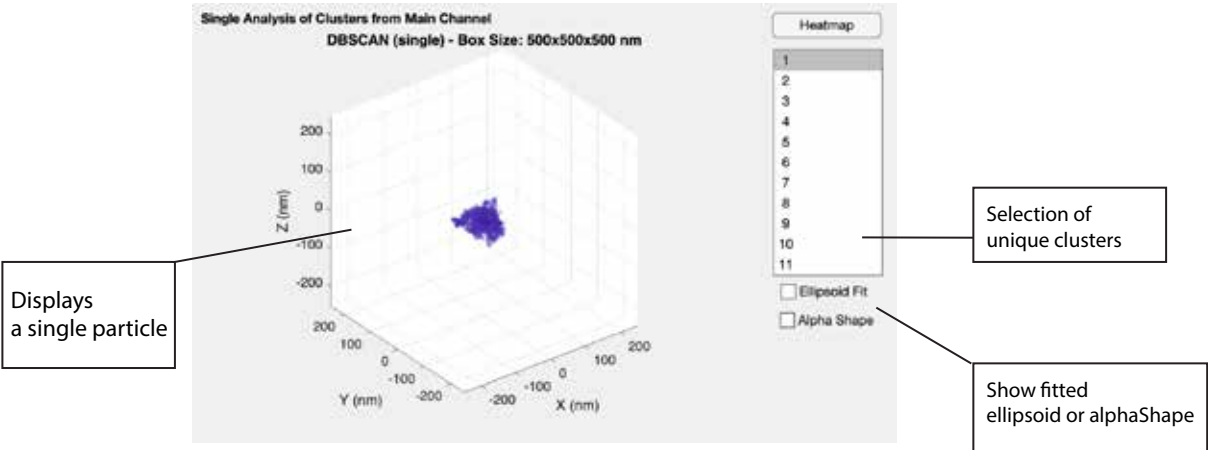
## Field of View

This tab can be used for a general overview of your data and to refine the DBSCAN parameters. Outlier localizations (noise) can be removed.



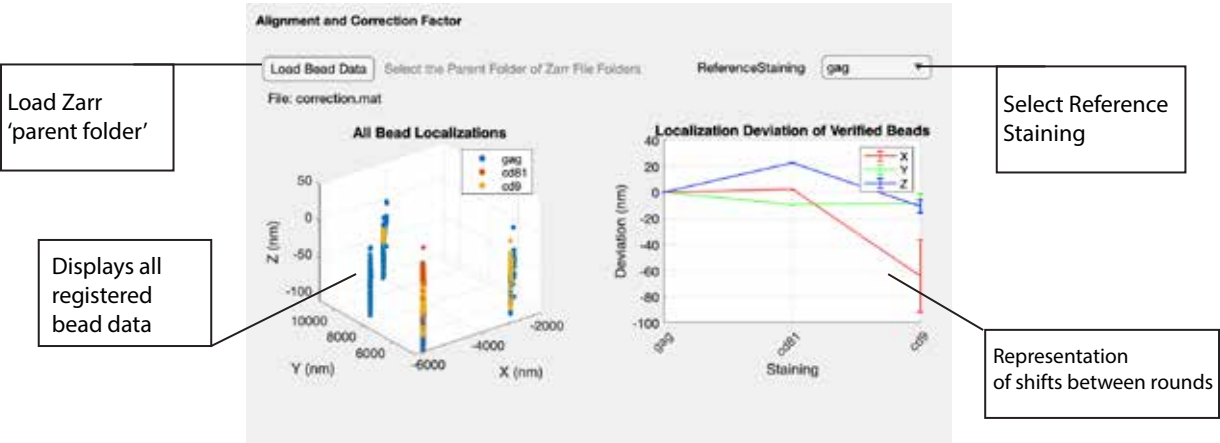
## Single Cluster Analysis

Unique Clusters defined by the DBSCAN algorithm can be displayed in this tab. Additionally, 'Ellipsoid Fit' and 'AlphaShape' provides tools for measurement and representation of clusters. Box size can be adjusted in settings.



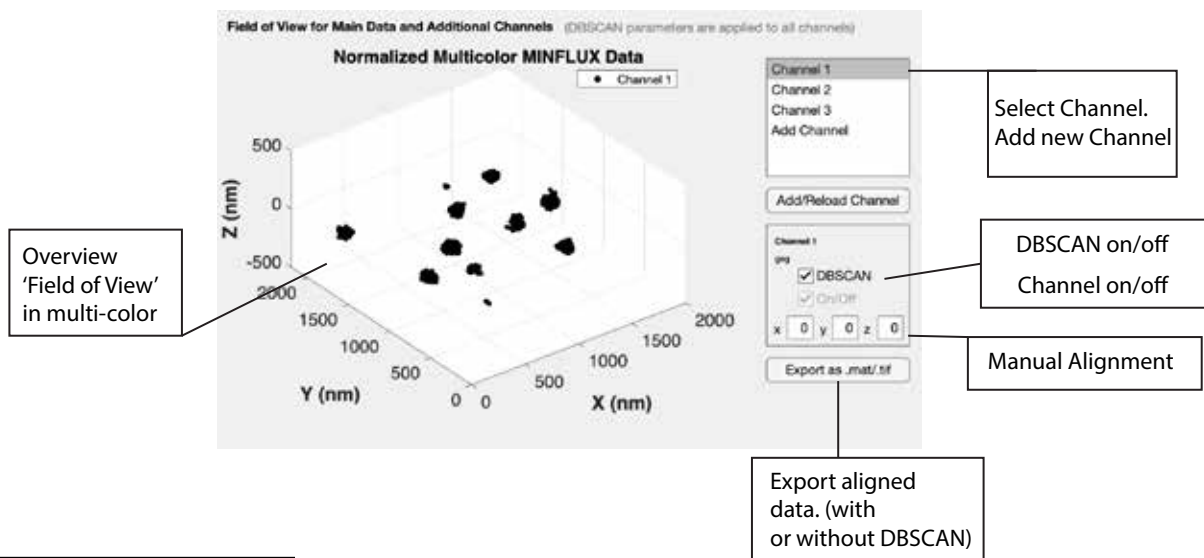
## Alignment

For automatic alignment of the loaded data, ZARR-folders, generated by the MINFLUX Software, need to be saved under the target name and stored together in one 'parent folder'. This folder can then be selected and the correction factors are calculated. Reference Staining usually is the first acquired channel.



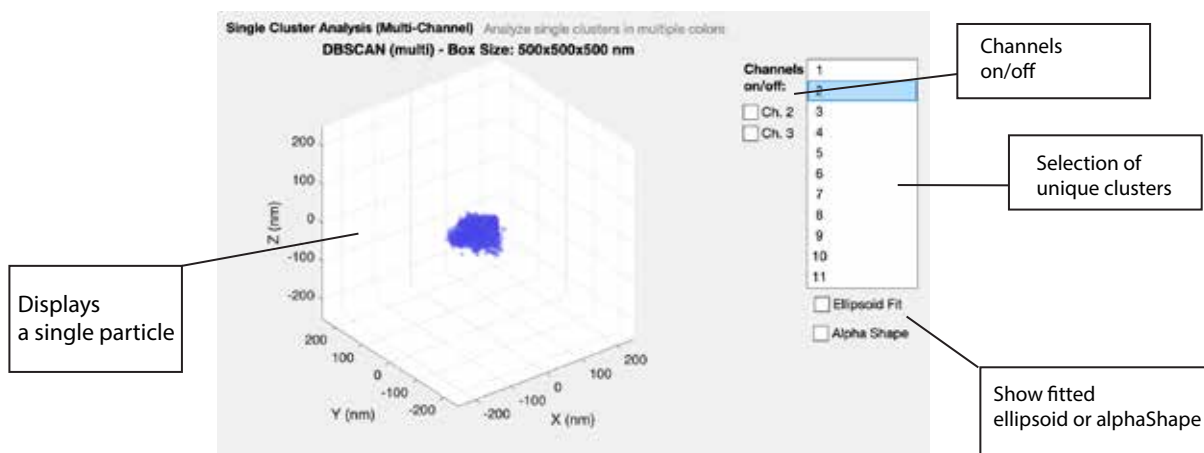
## Multi-color Field of View

Here, additional channels can be loaded and aligned. If Auto-Alignment was already used, the channel names and correction factors will be preloaded. Otherwise correction can be performed manually. For Inspection DBSCAN for the whole data set can be turned on or off. And each Channel can be individually turned on/off. Corrected multi-channel data can then be extracted into .mat or .tif files.



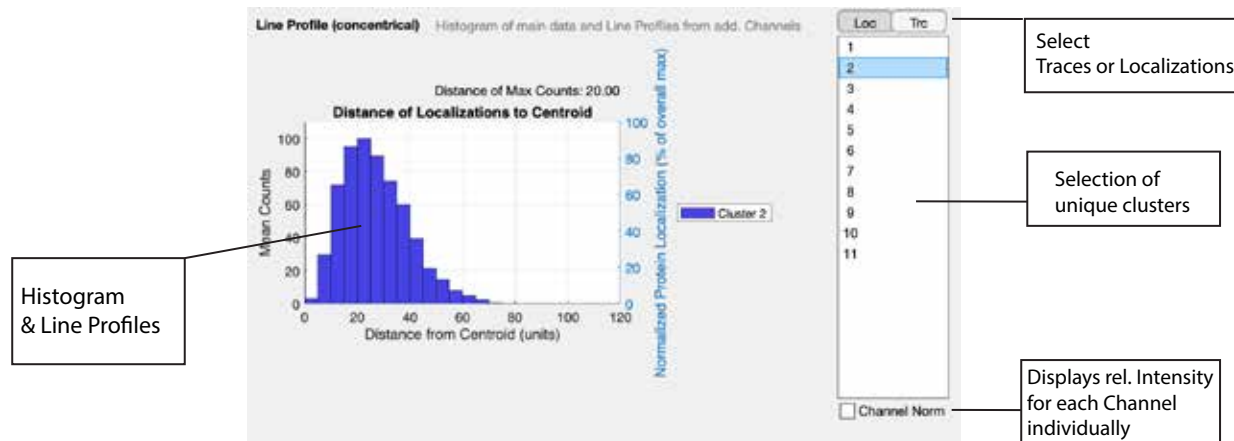
## Multi-color Cluster Analysis

Unique clusters can be displayed with additional colors. Each additional channel can be displayed individually. Box Size can be adjusted in settings.



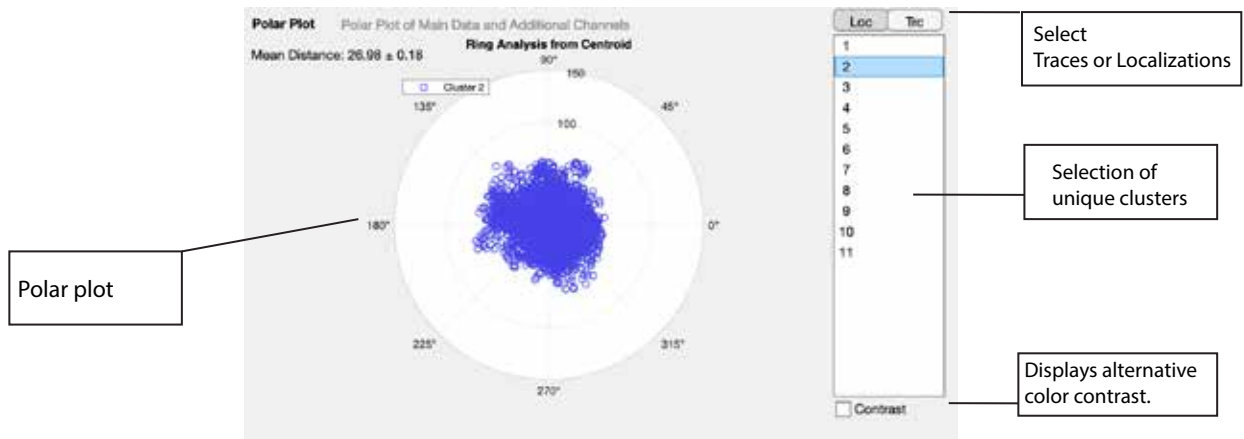
## Concentrical Analysis

Histogram (for main channel) and additional Line Plots can be displayed as concentrical intensity profiles of individual clusters. Display from Localizations or Traces.



## Polar Plot

Polar plots of main channel and additional channels of individual clusters.  
Display from Localizations or Traces.



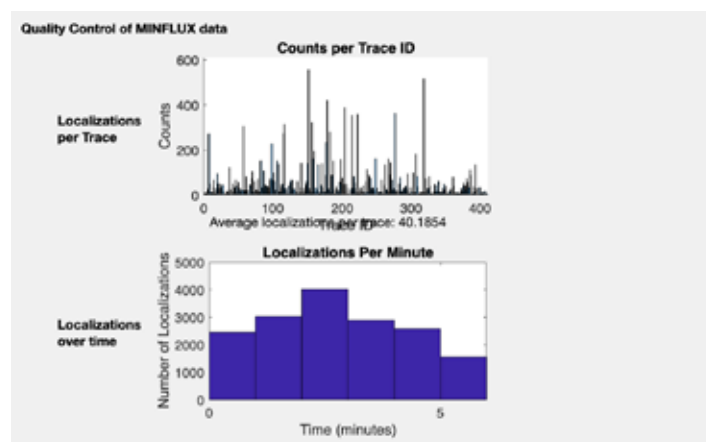
## Numbers from Single Clusters

Here, various measurements are displayed for each cluster individually:  
Count of Localizations and Traces, Volume, Surface, Average Nearest Neighbor Distance, Radius.

Cluster	Localizations	Traces	Volume (n...)	Surface (n...)	Avg NN Dist (n...)	Radius
1	1067	43	3.4751e+05	2.6985e+04	10.2178	
2	4778	90	6.1559e+05	3.8281e+04	9.2944	
3	1143	56	4.3929e+05	3.0747e+04	11.3995	
4	2459	55	3.9775e+05	2.8680e+04	9.9305	
5	424	15	1.3960e+05	1.4992e+04	14.1171	
6	1922	40	5.4963e+05	3.6577e+04	11.4675	
7	2259	31	3.4512e+05	2.7318e+04	10.4700	
8	1837	53	5.3665e+05	3.7204e+04	9.1572	
9	567	18	2.9258e+05	2.4109e+04	19.1952	
10	6	2	656.7248	615.3881	24.6324	
11	5	1	487.5043	454.0680	NaN	

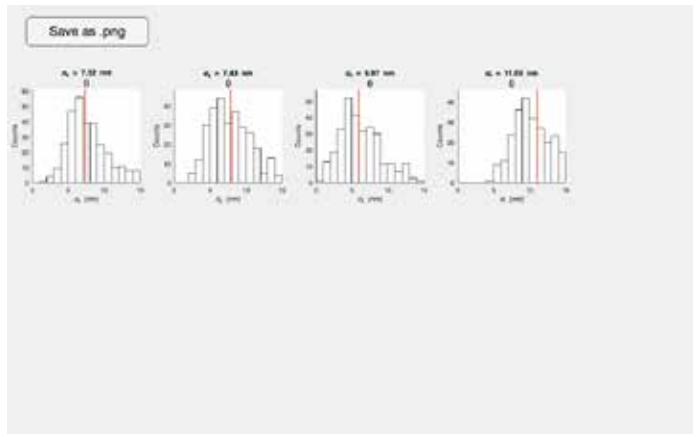
## Acquisition Parameters

Counts per Trace ID (tid), Localizations over time.



## Localization Precision

Depending on the loaded data, Localization Precision is displayed for every channel (using the algorithm from Ostersehl et al., 2022).



## WORKFLOWS

This section provides exemplary workflows for data processing and analysis.

### For one color data.

Field of View > Single Cluster Analysis >  
Concentrical Analysis / Polar Plot >  
Numbers from Single Clusters > Localization Precision

### For multi-color data.

#### auto-alignment

!! ZARR Files need to be stored in a parent folder first !!

**Field of View > Alignment >**  
**Field of View (Multi) > Multi Cluster Analysis**  
Concentrical Analysis / Polar Plot >  
Numbers from Single Clusters > Localization Precision

#### manual alignment

Field of View > **Field of View (Multi) > Multi Cluster Analysis**  
Concentrical Analysis / Polar Plot >  
Numbers from Single Clusters > Localization Precision