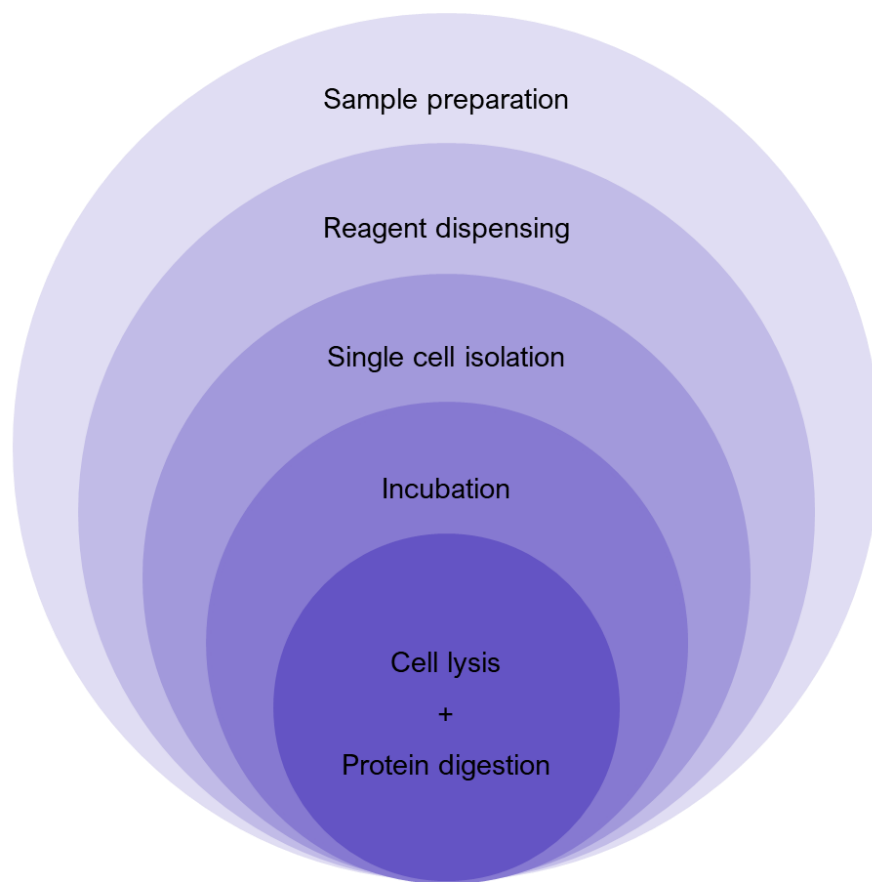


# User Manual

## Proteomics Workflows using the cellenONE®



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## Document history

Revision	Author(s)	Changes	Effective date
0.0	D. Hartlmayr	Creation	29/11/2023

# 1 Introduction

Important notes will be highlighted in green boxes.

All proteomics workflows provided by Cellenion come with dedicated quick-start manuals that contain a check-list guiding through the protocol. This User Manual for Proteomics Workflows using the cellenONE guides the user through all actions performed during the dedicated proteomics workflows (included in the specific manuals) and provides step-by-step instructions with software screenshots. However, all steps associated with liquid dispensing or cell sorting are presented in more detail in the User Manual for cellenONE software version 2.0.

**Note.** It is highly recommended to read the User Manual for cellenONE, before performing any experiment with the cellenONE.

## 2 Basic Settings

### 2.1 Nozzle and Droplet Setup

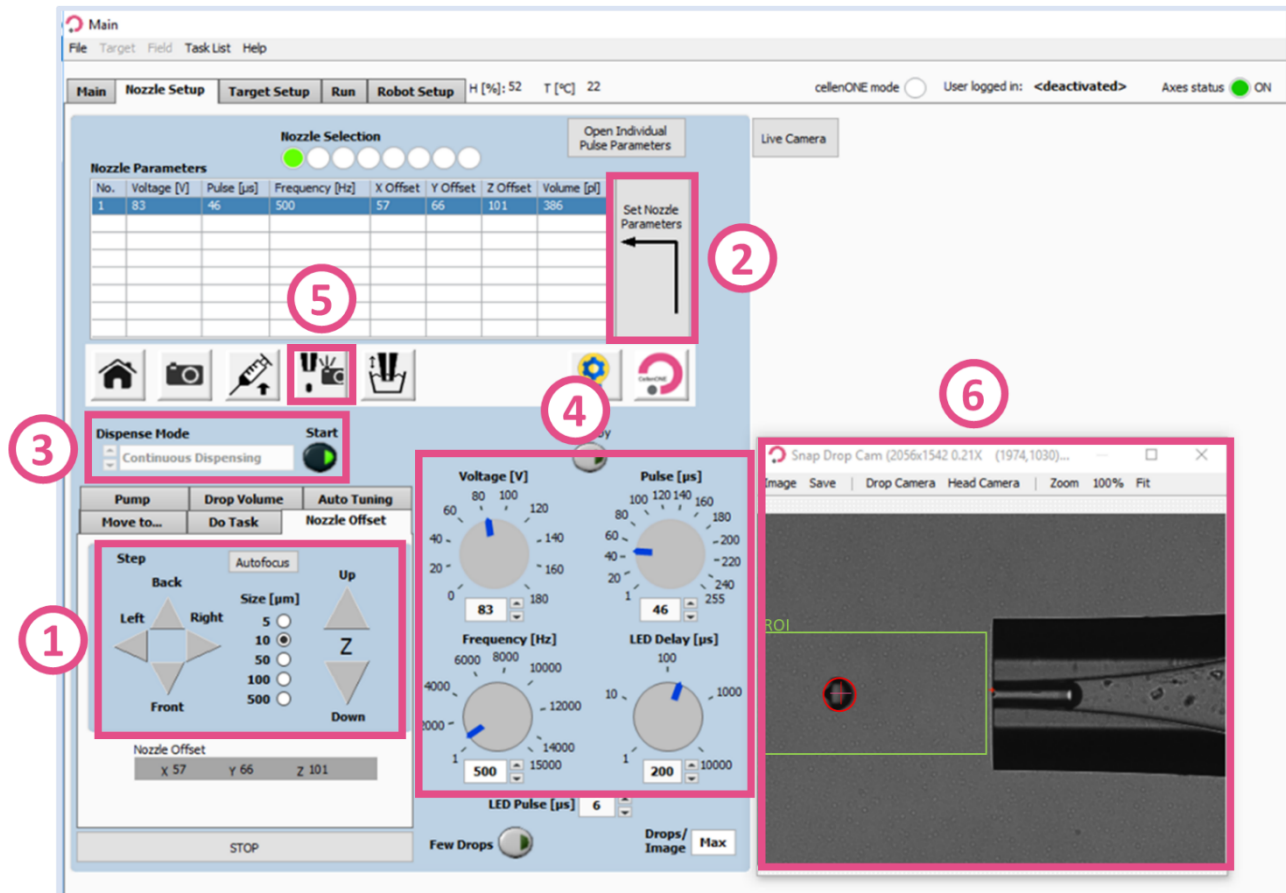


Figure 1. Various Parameters needed for Drop optimisation.

Before starting an experiment, the PDC needs to be correctly positioned and producing stable droplets.

At the beginning of the Prime Run, the PDC should be centred with the red cross. You can align your PDC using:

- ➔ **Nozzle Setup** >> **Nozzle Offset** >> **Up, Down, Back, Front, Left, Right** controls (Figure 1.1), then click on **Set Nozzle Parameters** (Figure 1.2)

Once the Priming Run is finished, enter the **Voltage** and **Pulse** parameters given on the PDC box, then click on:

- ➔ **Set Nozzle Parameters** (Figure 1.2)
- ➔ Start: **Continuous Dispensing** (Figure 1.3)

Adjust the parameters **Voltage** and **Pulse** (Figure 1.4) until the droplet is stable for at least 10 seconds, then press **Set Nozzle Parameters** (Figure 1.2)

Perform a **Drop check** before proceeding to check if the software detects the droplet correctly (Figure 1.5). This is indicated by the red circle around the drop in the **Snap Drop Cam** window (Figure 1.6).

## 2.2 Humidity and Temperature Control Setup

Throughout the workflows, the humidity and substrate temperatures are set to a specific value by the individual Runs.

**Note.** The Runs already contain specific tasks controlling humidity and substrate temperature for each run. The following describes how to change humidity and temperature “manually” if needed.

### 2.2.1 Humidity Control

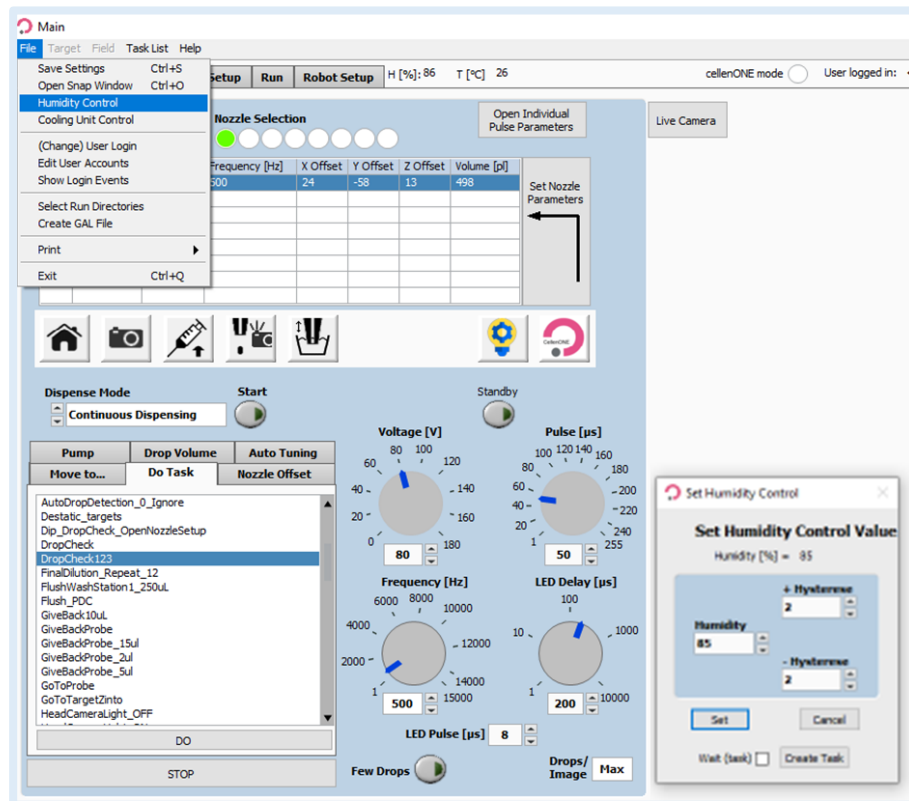


Figure 2. Humidity Control

➔ **Main** >> **File** >> **Humidity Control** >> Popup window >> Change the humidity value >> Click on “Set” (Figure 2).

### 2.2.2 Temperature Control



Figure 3. Temperature Control

**Note.** Since most proteomics Runs start at a cooling plate temperature of 10 °C, it is best to already change the value right after starting the software.

➔ **Main >> File >> Cooling Unit Control >> Pop-up window >> Change the temperature value when using *Fixed Control Temperature* or activate *Dew Point Control* and set the *Cooling Unit* to **ON**.**

## 2.3 Performing Tasks

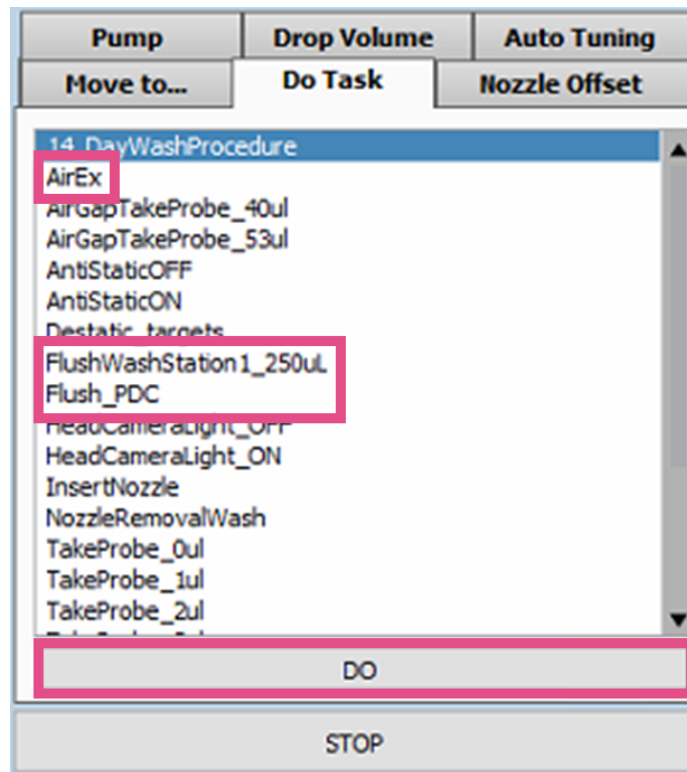


Figure 4. Do Task window.

During the workflows sometimes it will be needed to perform certain *Tasks*. Especially when droplets are unstable or when there is air trapped in the PDC, the user can perform the tasks *FlushWashStation1\_250uL* or *Flush\_PDC* to flush the PDC (thereby discarding everything inside the PDC) and *AirEx* to remove air from the system:

➔ **Nozzle Setup** >> **Do Task** >> Select the task and press on **DO** (Figure 4).

**Note.** Be aware that everything that was aspirated prior to performing the tasks *FlushWashStation1\_250uL*, *FlushPDC* and *AirEx* will be discarded as part of the selected task.

## 3 Proteomics Workflow Runs

Any of our proteomics workflows are performed by selecting individual Runs in a numerical order. Each Run of a workflow has the name of the respective Substrate in the beginning of its name (e.g., **12x16\_Run\_#**, **LF\_48\_Run\_#**, **EVO\_96\_Run\_#**) (Figure 5.2). When performing a reagent dispensing or incubation step, following the steps in Chapter 3 and the comments provided during the Runs is sufficient. For Runs that perform cell sorting, more parameters need to be specified. Please find instructions for cell isolation Runs in Chapter 4 of this manual.

### 3.1 Main Tab

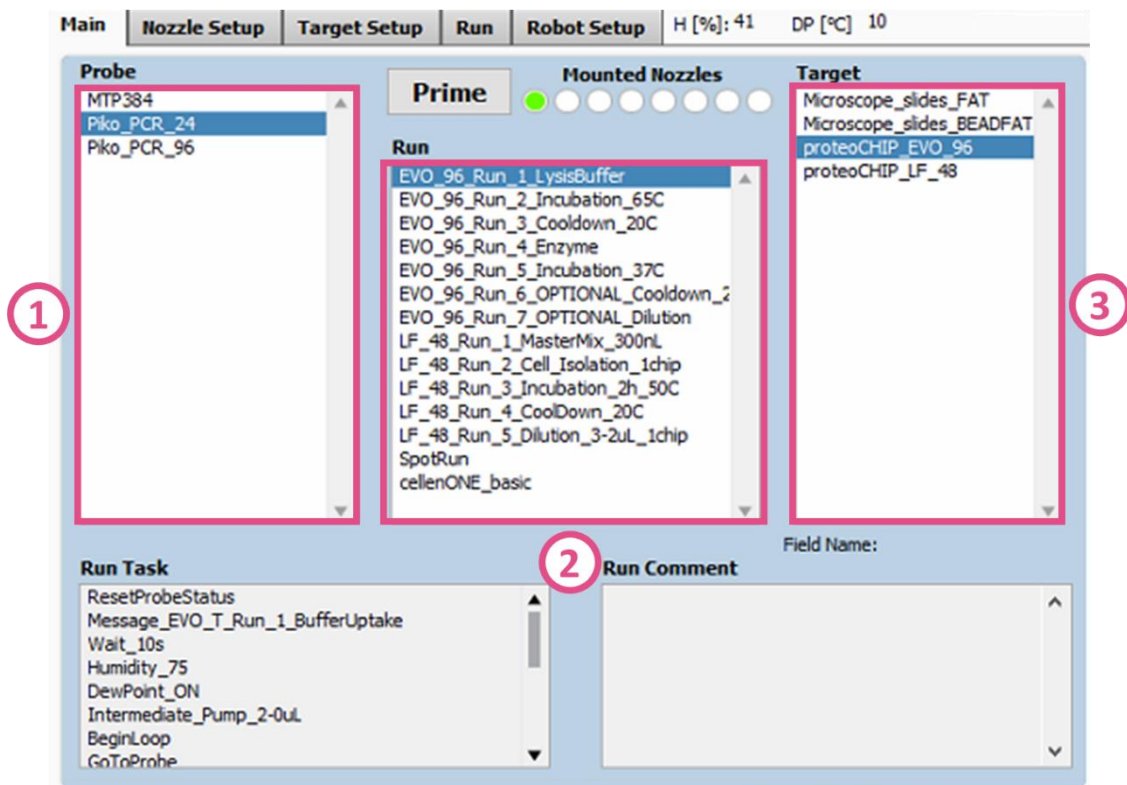


Figure 5. Main tab.

In the **Main** tab of the software, select the **Probe**, **Run** and **Target** to be used. This is specified in the respective manual of the chosen workflow (Figure 5).



### 3.2 Target Setup

#### 3.2.1 Loading a Field file

In the **Target Setup** tab, you can load the **Field file** corresponding to the Run of the chosen workflow. The name corresponds to the name of the chosen Run (e.g., **EVO\_96\_Run\_1\_LysisBuffer**):

→ **Target Setup** tab >> **Target** (Figure 6.1) >> **Load Field** (Figure 6.2) >> Select the **Field file** corresponding to the chosen Run (Figure 6.3).

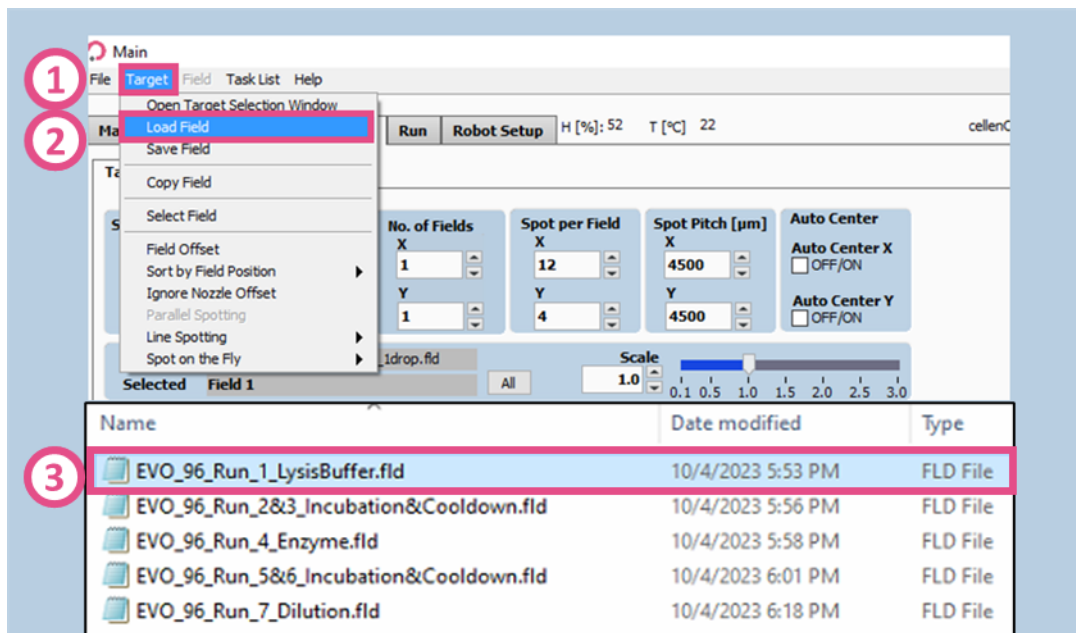


Figure 6. Load Field file.

### 3.2.2 Edit Field Table

In the **Field Table**, the exact number of Drops that will be dispensed per well is visualized in a table. Additionally, it is specified which well of a plate or which cellenWASH position or well plate will be used for sample aspiration. A Run will consider only what is written inside of the given table (Figure 7):

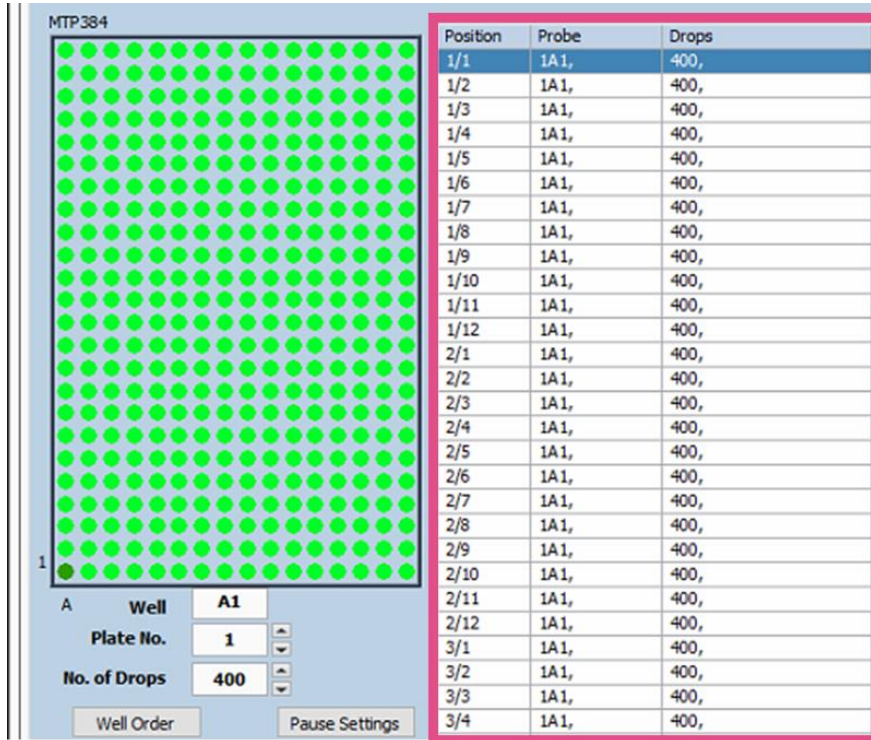


Figure 7. Field table.

The unit of dispensing can easily be changed from **Drops** to **Volume**:

➔ **Target Setup** tab >> **Field Setup** (Figure 8.1) >> **Field** (Figure 8.2) >> Check mark to **Volume Entry** (Figure 8.3).

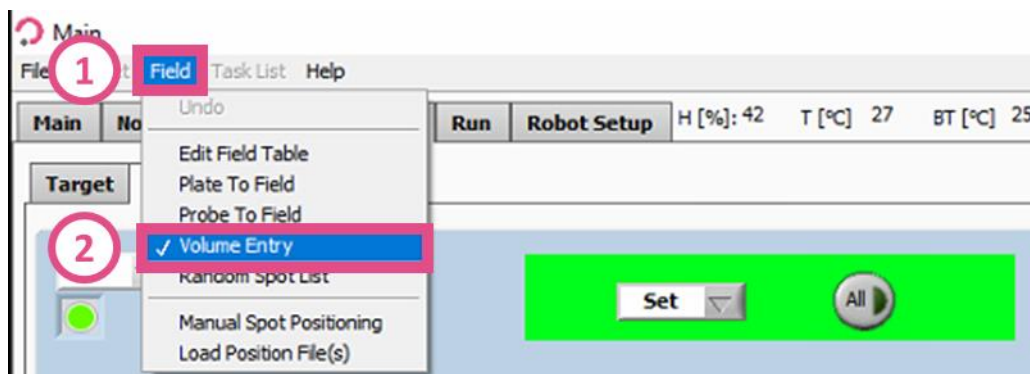


Figure 8. Changing to Volume Entry.

**Note.** Be aware that the calculation of the dispensing volume relies on the Drop Volume measurement that was done in Nozzle Setup. This must be correctly measured before starting any Run where Volume is used instead of Number of Drops.

It is essential to change the Probe well if the actual position of solution or cell suspension differs by using the **Edit Field Table** function:

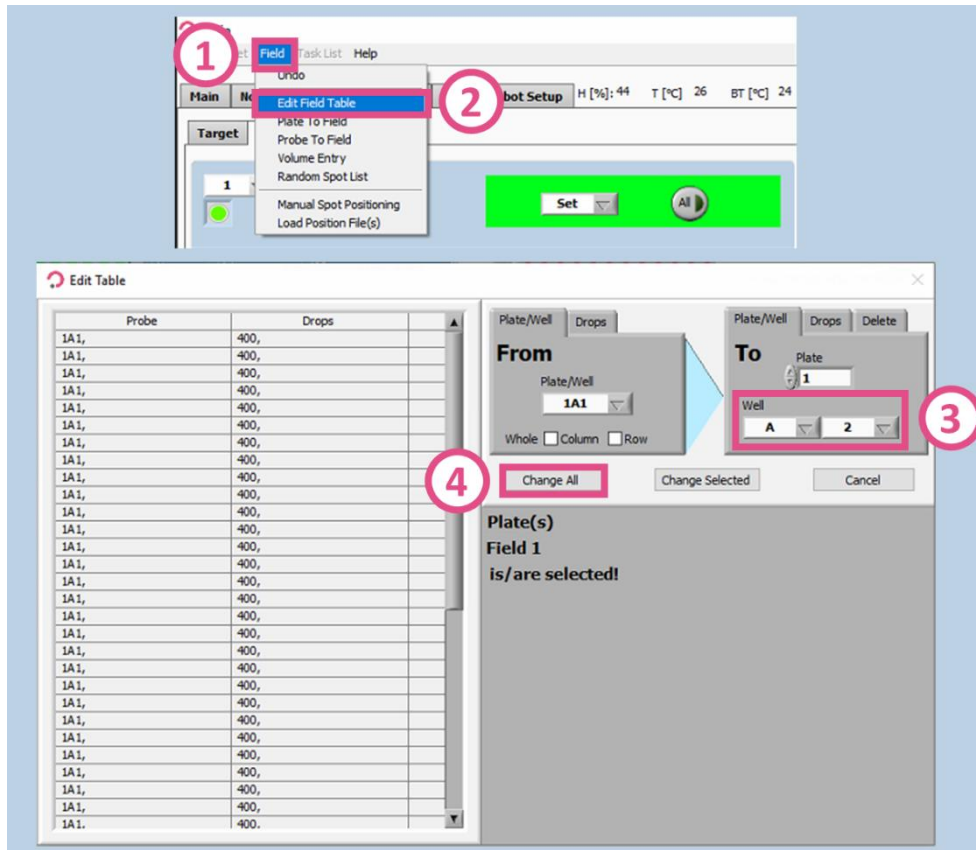


Figure 9. Edit Field Table of Plate/Well position.

→ **Target Setup** tab >> **Field Setup** >> **Field** (Figure 9.1) >> **Edit Field Table** (Figure 9.2) >> **Plate/Well** >> Change **Probe Well** (Figure 9.3) >> **Change All** (Figure 9.4).

The same can be done for the number of Drops or Volumes specified in the Field table (Figure 10).

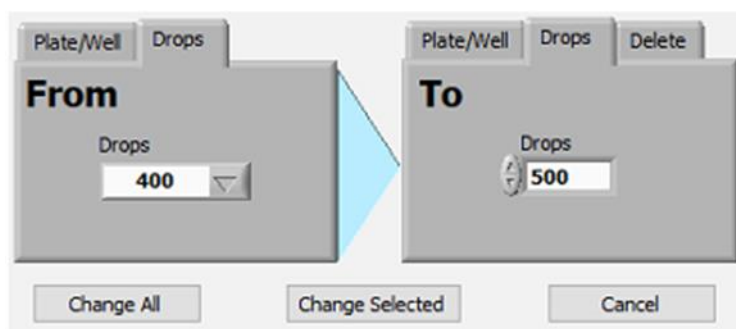


Figure 10. Edit Field Table, number of Drops.

### 3.3 Run Tab

To start the Run switch to the *Run tab*. Check if the *Probe*, *Run* and *Target* are correctly selected (Figure 11.1), then press *Start Run* (Figure 11.2). Click on the three dots to specify where the Run should be saved (Figure 11.3) and select the correct folder and click on *Current Folder* (Figure 11.4). Usually the Runs are saved at *C:\Runs\User\Folder*. Choose a Run-name at *Batch Identification* (Figure 11.5), then press OK (Figure 11.6).

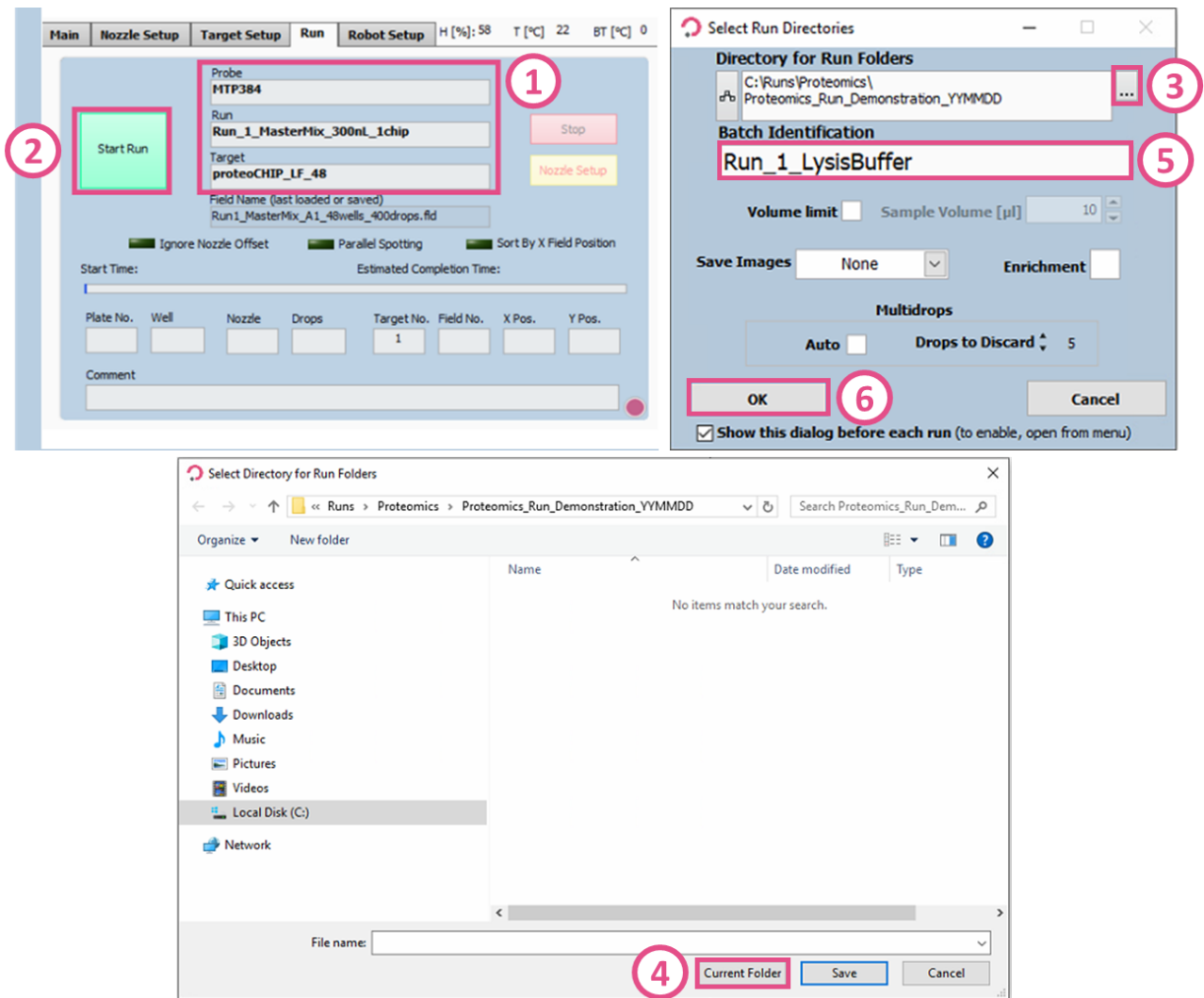


Figure 11. Start Run.

In the last window you can select individual modules (Figure 12.1), select all by pressing *All On* (Figure 12.2) or enter the module numbers directly in the white box (Figure 12.3). Start the Run by pressing *OK* (Figure 12.4).

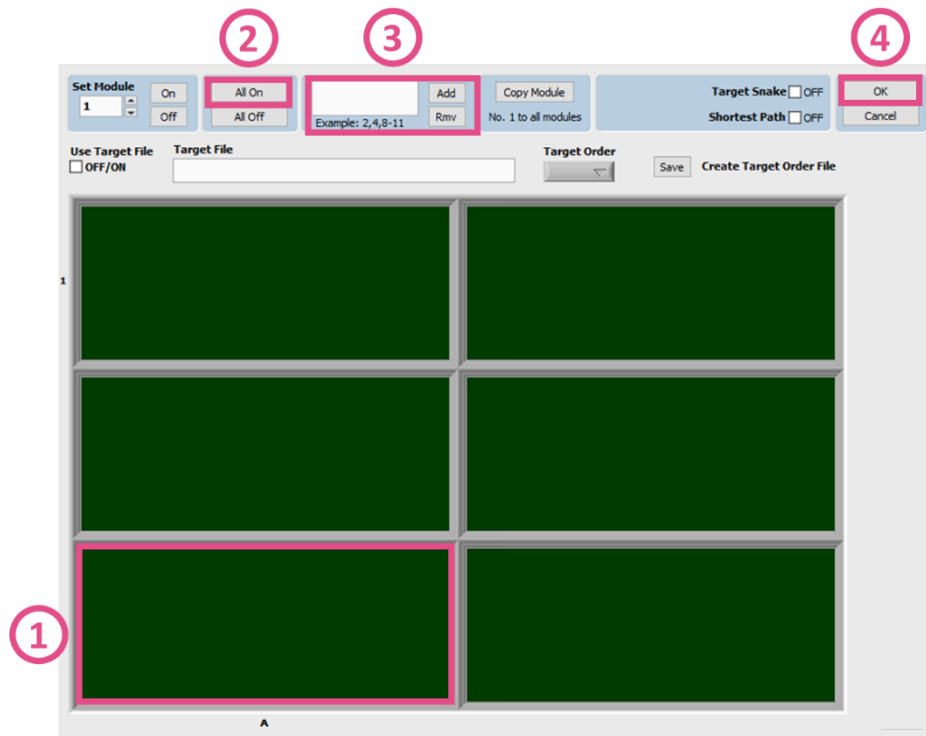


Figure 12. Start Run.

## 4 Single cell isolation

Before starting the Cell Isolation Run, load the corresponding Field file, as you would for any other Run. To isolate and dispense single cells, only 1 Drop must be defined per positions in the Field table (Figure 13).

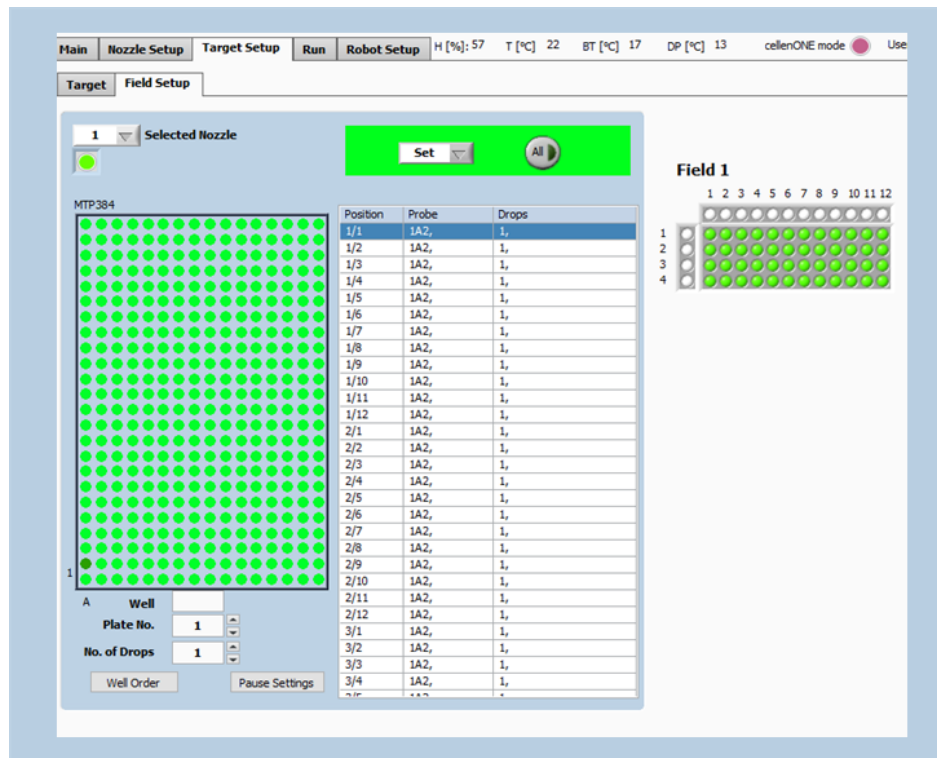


Figure 13. Field Setup for cell isolation.

**Note.** If you wish to dispense more than one single cell, the following syntax must be used: 1,1 (for isolation of two single cells); 1,1,1 (for isolation of three single cells); 1,1,1,1 (for isolation of four single cells), etc. You can do so by clicking multiple times (n) on the well where you want to have 1+n cells isolated.



### 4.1 cellenONE module

The *cellenONE module* (Figure 14.1) must be used for all Runs where single cells are to be isolated. Usually, the cellenONE module window opens automatically at the end of the previous Run, however, it can also be opened manually by clicking on the *cellenONE logo* (Figure 14.2) within the Nozzle Setup tab. Before starting the cell isolation Run, place the sample containing the cells into the specified well of the Field Setup (just as you would for Reagents in other proteomics Runs). The instrument then will aspirate the cell solution and will ask you to perform the Mapping and adapt Detection/Isolation parameters if needed.

**Note.** Close the cellenONE module window only after the cell isolation Run is finished. The module changes how other non-cell isolating Runs behave, so be aware. During cell isolation, the cellenONE module might however be minimized.

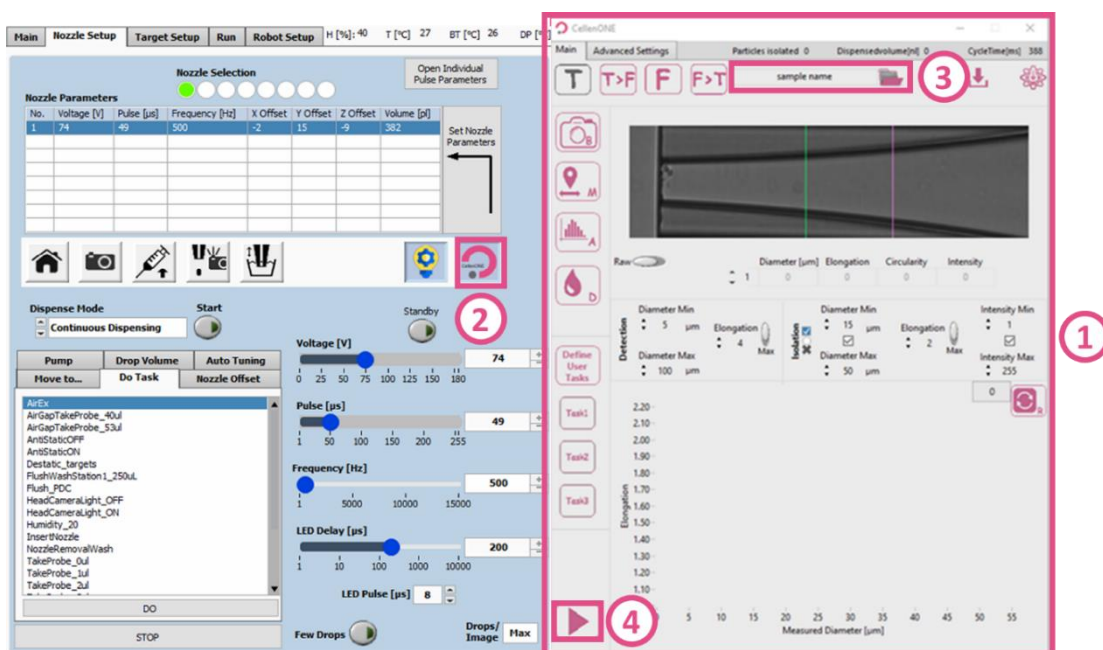


Figure 14. cellenONE module for single cell isolation (right window).

Within this Manual, only the most basic functions of the cellenONE module window will be discussed. Please refer to the Software Reference Guide Chapter 7 “Single cell isolation” to understand all the options and features for single cell dispensing. Before starting the Run, define the folder location and name of your Run by clicking on the *Folder button* (Figure 14.3). All files associated with your experiment will be saved in this location. By default, data is saved in the last selected folder and it is named “*samplename\_date*”. Load the cell solution to the defined Probe well and start the Run by clicking on the *Start button* (Figure 14.4). After cell suspension uptake, the PDC comes back to the camera, a Mapping and an Analysis can be performed and the cell isolation parameters can be defined.

**Note.** You can always pause the Run during cell sorting by clicking on the *Pause button* in the cellenONE module window to modify any cell isolation parameters if needed. However, changes made during the Run will not be considered when creating a cellenREPORT.



## 4.2 Setting the isolation parameters

The *Main* tab of the cellenONE module displays images of the region of interest of the PDC in which cells are detected. During dispensing, cells are travelling from right (upper part of the nozzle) to left (nozzle tip). The *Ejection Zone* (Figure 15, Zone 1) represents the volume of the next generated droplet containing only a single cell. The *Sedimentation Zone* (Figure 15, Zone 2) is a safety zone to assure that only one cell is dispensed to the target. Information below the image (i.e., Diameter, Elongation, Circularity, and Intensity) is given for the leftmost detected cell, i.e., the cell closest to the nozzle tip (Figure 15).



Figure 15. Main tab of the cellenONE module. Shown is the Ejection Zone (Zone 1) and the Sedimentation Zone (Zone 2). Zone 3 represents the area from which cells sediment.



The section shown in Figure 16 is used to define detection and isolation parameters for object *Diameter* and *Elongation*. These parameters are crucial to successfully isolate and dispense single cells of interest.

**Note.** The elongation factor can be used to effectively differentiate two aggregated cells from a single cell as these will have different elongation factors:  
Round single cells: <1.6; Doublets >2.5.

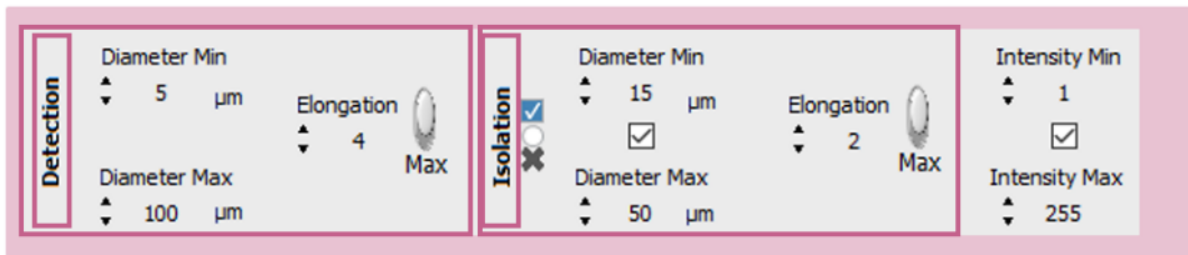


Figure 16. Detection and isolation parameters.

Detection parameters: These parameters will determine what the software will *detect* as an object (e.g., cell or particle) and later discard or *isolate* to the target. The detection parameters should be “large” enough to ensure that all cells and cell clusters are detected and only single objects are isolated.

- ➔ **Min diameter:** detected diameter of the smallest object that should be detected. An object with a diameter lower than the entered value will **not be detected**.
- ➔ **Max diameter:** detected diameter of the largest object that should be detected. Beware that cell aggregates will typically be detected as a single large object. An object with a diameter bigger than the entered value will **not be detected**.
- ➔ **Elongation:** an object with an elongation above (for MAX) or below (for MIN) the entered value will **not be detected**.

**Note 1.** The detected diameter is typically about 10% larger than real diameter.

**Note 2.** A detected object is surrounded by a green or a red circle in the respective PDC zones:

A **green circle** means that the object fulfils the isolation parameters and would be isolated.

A **red circle** means that the object does not fulfil the isolation parameters and would not be isolated.

**Note 3.** To start, set wide detection parameters and then, restrict them if needed.

- Diameter min: 5 µm
- Diameter max: 100 µm
- MAX Elongation: 4

After loading the sample, do a background:



**Background** button: Takes a reference background image. This button should only be pressed when the nozzle is free of cells and/or particles and when the PDC is in front of the camera.

Make several drops:



**Test droplet** button: Ejects a single droplet. Check visually by eye that every particle inside the PDC is detected (i.e., that the particle is circled by a red or green circle).

### 4.3 Set the ejection boundary

Once detection parameters are set, do a mapping:



**Start mapping** button: Starts the mapping process.

This process maps the position of cells inside the PDC during the dispensing of droplets in front of the camera. This will determine the size of the *Ejection zone* (Figure 15). Pressing this button during mapping will interrupt the mapping process.

At the end of the mapping process, the ejection boundary is set automatically according to the blue and green dot distribution inside the PDC (Figure 17). Cells that were detected in the location of **blue dots** were subsequently detected again inside the PDC upon next droplet generation. Whereas **green dots** correspond to the last seen location of a cells within the PDC (i.e., upon next droplet generation these cells were ejected from the PDC). Once done, click “Save & close”.

**Note.** At the end of the mapping process, the ejection boundary is set automatically according to the blue and green dot distribution inside the PDC. To adjust the ejection zone manually, move the slider by clicking and dragging the positioner on the ruler below the image, press “Set” to validate, then “Save & close” (Figure 17).

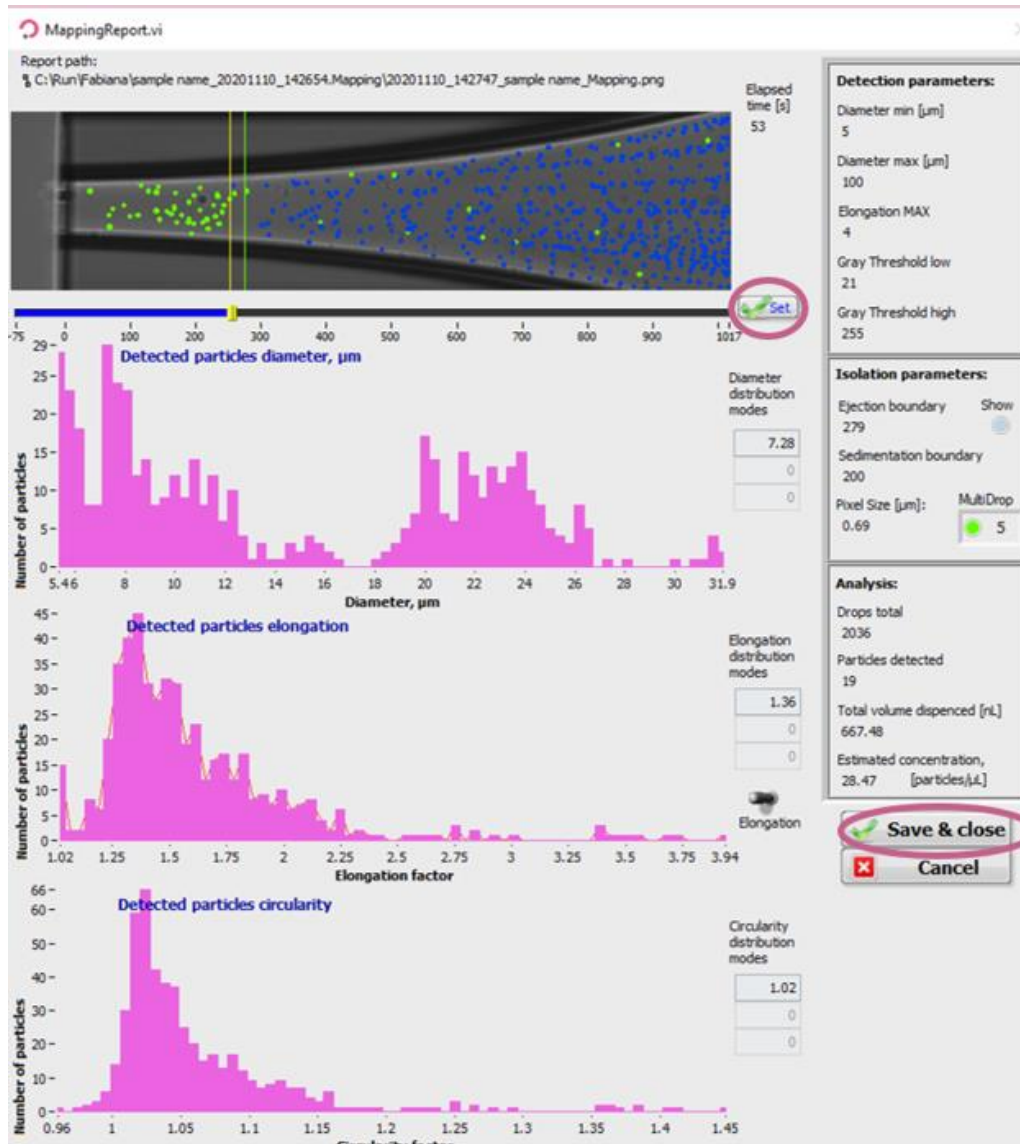


Figure 17. Mapping.

#### 4.4 Set the isolation parameters

Once Mapping is done, set up your isolation parameters.

**Isolation parameters:** These parameters can be determined from the characteristics of the cells shown in the mapping report (Figure 17) and adjusted as needed to include only cells of interest. These values are critical to ensure satisfactory single cell isolation. All objects that fulfil the isolation parameters and single cell condition will be isolated.

- **Min diameter:** detected diameter of the smallest object that should be isolated.
- **Max diameter:** detected diameter of the largest object that should be isolated.
- **Elongation:** an object with an elongation above (for MAX) or below (for MIN) the entered value will **not be isolated**.

Three options are available here to set the isolation parameters:

- Draw a gate right after the mapping from the scatter plot that was just performed or enter values manually into the isolation parameters based on the mapping.
- Open the mapping (in the folder you chose earlier, folder *name.mapping*), interpret the different scatter plots and insert the values manually.
- Perform an analysis of your sample.



**Analyze sample** button: Analyzes a number of cells from your sample.

This can be used to estimate the heterogeneity of your sample. At the end of the analysis, a window will pop up with scatter plots (Figure 18). The number of analysed cells can be set in the “Advanced Settings” tab or when the **Analyze sample** button is clicked (by default it is set to a 100). You can draw a gate on either of the generated graphs to choose new values for detection and/or isolation parameters by clicking on the I or D.

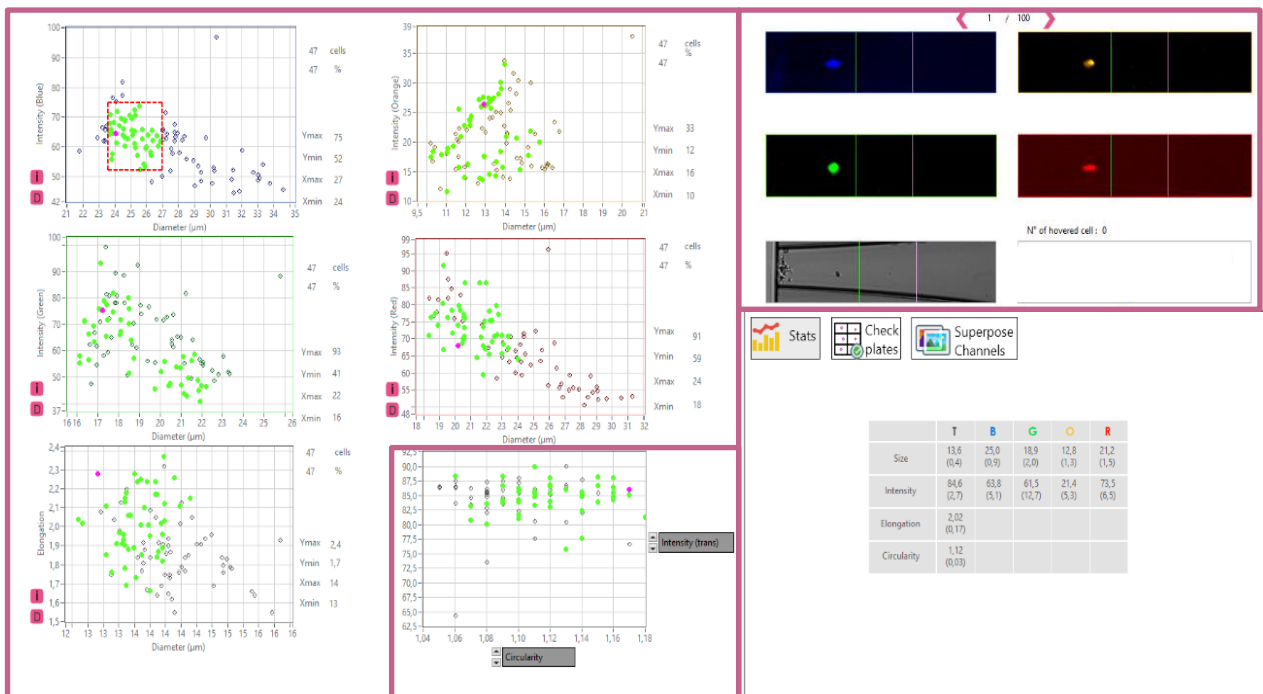


Figure 18: Scatter Plot of the sample analysis

### 4.5 Check the setup of your experiment

Check the **Ejection boundary**:



**Test droplet** button: Press it manually multiple times to verify the ejection boundary. When a particle is inside the ejection zone, it should be ejected within the next droplet (i.e., when pressing the droplet button once more).

Check the isolation parameters:

cells (or particles) of interest should be circled with a **green circle** and the other particles with a **red circle**.

**Note.** Continuous drop generation can be activated by simultaneously clicking on the left mouse button and Shift button and is interrupted by clicking once again on the test droplet button.

Once you have performed the Mapping, the Analysis and you are satisfied with your parameters you can click **Continue** to start isolating your cells. The software will let you know when the isolation is finished (Figure 19).

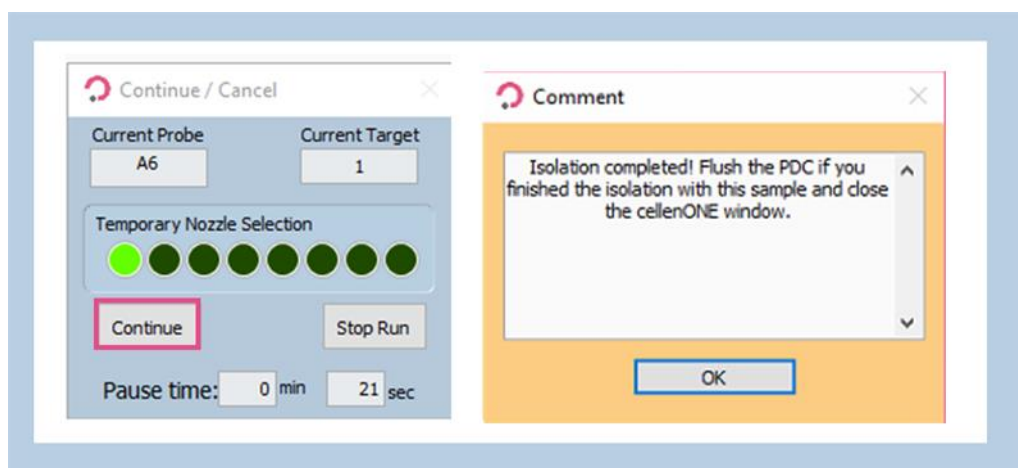


Figure 19. Starting the cell isolation Run and end message.