User Manual
proteoCHIP LF 48

for Label Free
Single Cell Proteomics workflow
using the cellenONE®

Cell isolation

Lysis & Digestion reagent dispensing

Incubation

Label free chip goes inside the cellenONE

Label free chip ready for sample transfer
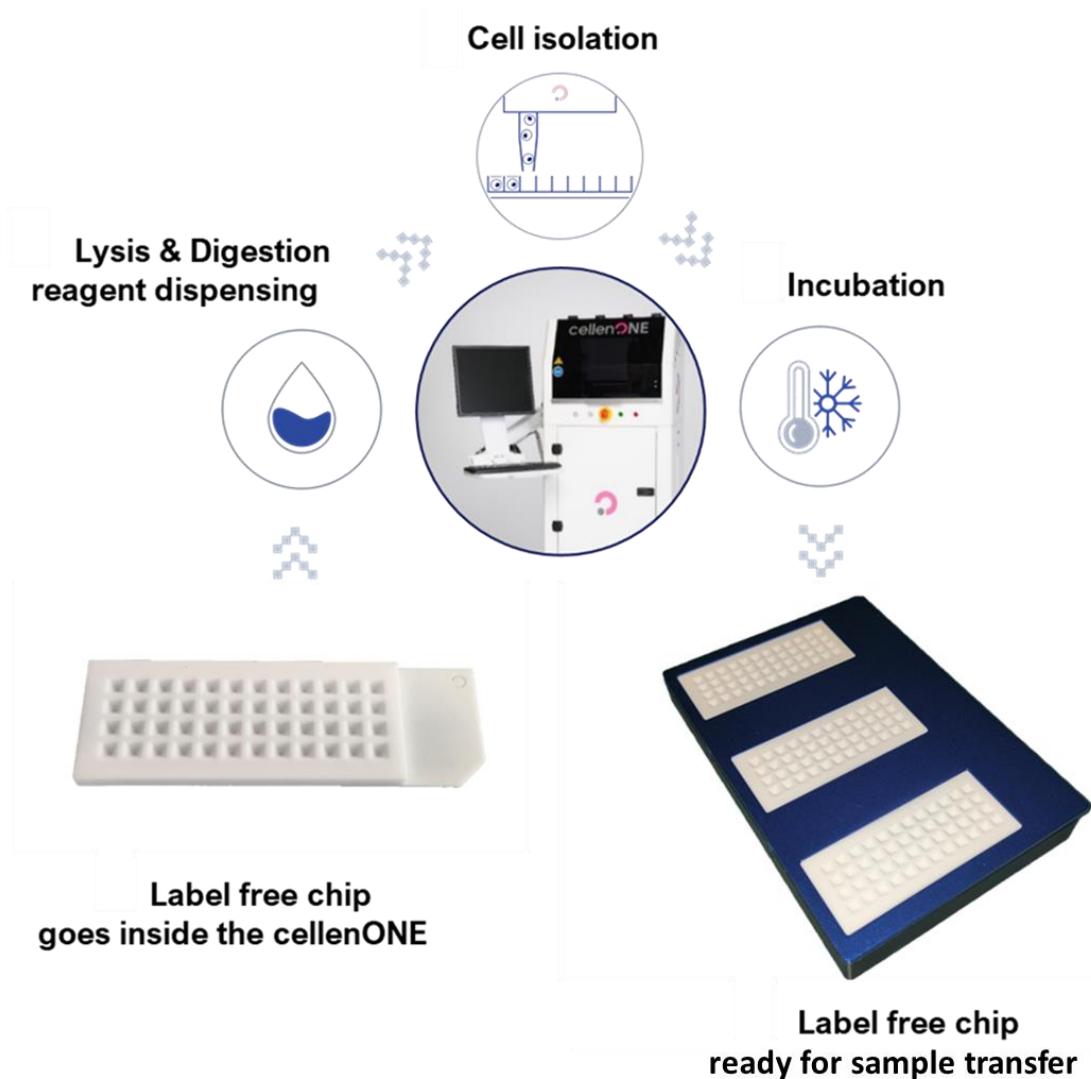
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Document history

Revision	Author(s)	Changes	Effective date
0.0	A. Demeter, D. Hartlmayr, F. Izaguirre, T. Jet	Creation	20/03/2024

Highlights layout

Warnings will be highlighted in framed boxes.

Important **notes** will be highlighted in green boxes.

Copyright notice

Disclaimers

Trademarks

Cellenion®, cellenONE®, IBSCI™, FIBSCI™

Warning

The instrument control computer comes equipped with Windows operating system and the cellenONE software along with other standard software programs related to the basic operation of the cellenONE instrument. **This computer should be dedicated to control cellenONE** robotics and is not intended for data analysis, office applications, web surfing or other non-instrument related actions. Users are warned **not to install any additional software,** including but not limited to OneDrive, Google Drive, Dropbox, other synchronization utilities, or antivirus software. Such software programs or synchronization utilities will significantly slow down the performance of any computer and may impact the rate of cell isolation.

Cellenion and its affiliates cannot be held responsible for system functionality if recommendations regarding third-party software programs like those aforementioned are not followed.

If **antivirus** software is a requirement for instrument control computers in your lab, this should be configured to have an **exception for the cellenONE** software and related folders ('PROG', 'USER', and data path i.e., 'Run'), **real-time protection** should be **switched off,** and scan/activity hours should be set to out of cellenONE operation hours. Ensure that a 'System Restore' point is created prior to the installation of any software.

The instrument control computer does not come equipped with internet connectivity and is not recommended due to antivirus incompatibilities. In the case that remote support is needed, please utilize a Wi-Fi dongle to establish a **temporary** connection. Windows updates are not recommended due to the risk of unforeseen incompatibilities and have been configured not to occur. Enabling windows updates may put your computer at risk. If required for instrument control computers in your lab, scan/download/install hours should be set to out of cellenONE operation hours. **Feature updates must be disabled.** It is highly recommended to regularly move images from the instrument control computer with the aim to secure the data and free up space for further experiments.

It is strongly recommended to perform post-run image analysis on a separate computer in order to prevent installing other third-party software programs to the instrument computer, which is dedicated to the instrument control.

WARNING

UV LEDs	VIS LEDs
UV emitted from this product. Avoid eye and skin exposure to unshielded products.	Possibly hazardous optical radiation is emitted from this product. Do not look at operating lamp. Eye injury may result.

Warnings and Safety for LED lights

Inappropriate use of the product may result in permanent eye damage. To prevent injury, use this product in accordance with the IEC 62471:2006 standard, Photobiological Safety of Lamps & Lamp Systems.

Inappropriate use of a product containing LEDs may result in permanent eye damage.

The cellenONE instrument contains an LED which falls into Risk Group RG0 – Exempt Group according to the IEC 62471:2006 standard.

The cellenONE instrument contains two LEDs which fall into Risk Group RG1 – Low Risk Group according to the IEC 62471:2006 standard.

The cellenONE instrument contains an LED which falls into Risk Group RG2 – Moderate Risk Group according to the IEC 62471:2006 standard.

UV / Visible Radiation Warning Statement: This LED emits intense UV / visible radiation during operation. **DO NOT** look directly into the UV / visible light or look through the optical system during operation, as this can be harmful to the eyes, even for brief periods of exposure, due to the high intensity of the light. If viewing the UV / visible light directly is necessary, protective glasses must be worn to avoid eye damage.

1 Introduction

The cellenONE software monitors cells inside the Piezo Dispense Capillary (PDC) before dispensing. All steps associated with single cell isolation and dispensing experiments are presented in the Software User Guide.

> **Note.** It is highly recommended to read the cellenONE User Manual before performing any experiment with the cellenONE instrument.
>
> This Manual will guide you through all the steps of the workflow dedicated to single cell proteomics for Label Free experiments using proteoCHIP LF 48.
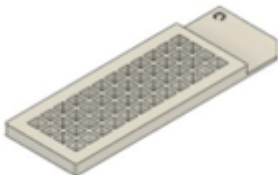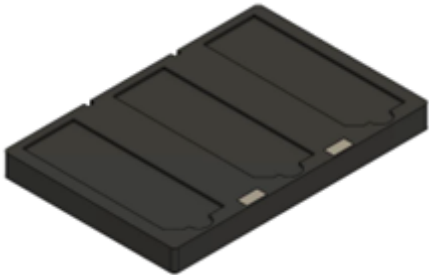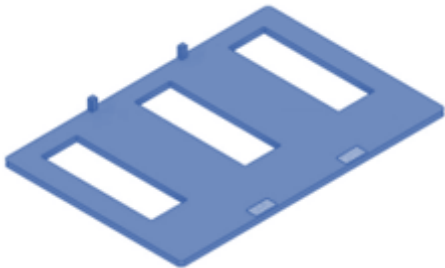
IBSCI™: Image-Based Single Cell Isolation
IBSCI^{TM} is a high resolution-based technology consisting of automated image acquisition, processing and advanced algorithms to automatically isolate single cells from a cell suspension.

FIBSCI™: Fluorescence Image-Based Single Cell Isolation
FIBSCI^{TM} utilizes multichannel fluorescence-based images to identify and isolate single cells of interest from subpopulations in mixed cell samples based on the presence, absence and intensity of fluorescent signal

2 Equipment

Part number	Description	
P-20-CL	**cellenONE PDC L Piezo Dispense Capillary** (Fixed drop volume between **450-600** pL) **4x manifold**	
P-20-CM	**cellenONE PDC M Piezo Dispense Capillary** (Fixed drop volume between **300-450** pL) **4x manifold**	
C-PLF-48-3	**proteoCHIP_LF_48** The proteoCHIP LF 48 Set containing: • 3 (three) proteoCHIP LF 48	
	The proteoCHIP LF 48 is a pyramidal-shaped bottom nanowell chip, 48 nanowells, prefilled with oil, enabling label-free single cell proteomics sample preparation.	
	proteoCHIP_LF_48 cellenONE Holder Holder to accommodate up to 3 proteoCHIP_LF_48 on the deck of the cellenONE throughout sample preparation (cell isolation, reagent dispensing and incubation steps). *(1 piece/pack)*	
C-PLF-48-CHT	**proteoCHIP_LF_48 cellenONE Holder Top** Top to maintain the proteoCHIP_LF_48 inside the cellenONE Holder on the deck of the cellenONE throughout sample preparation (cell isolation, reagent dispensing and incubation steps). *(1 piece/pack)*	

3 cellenONE setup

3.1 General remarks

- Wipe the inside of the enclosure and door with isopropanol or 70% ethanol as well as the proteoCHIP LF 48 holder.
- When manipulating inside the cellenONE, make sure that you are wearing gloves and a lab coat. No skin should be exposed inside the enclosure to avoid contamination.
- Avoid opening the door as much as possible during the experiment.
- Make sure the PDC is aligned to the red cross and produces a stable drop.
- Measure the drop volume.
- Set the temperature to 10 °C.
- Place the proteoCHIP(s) inside the cellenONE holder. If processing only one chip, insert it on the spot closest to you.
- Place the 'proteoCHIP holder top' on top of the 'holder' and insert the assembled unit on the target holder inside the instrument.
- All solutions should be prepared in a biosafety cabinet.

> **Note.** Please make sure that the 14 days wash have been performed previously (every 14 days), clean the humidifier with 70% ethanol every 14 days if possible, clean the PDC with sciClean at the end or beginning of every experiment followed by 3 flushes (Flush_PDC).

3.2 Nozzle Setup

Before starting your experiment, you will need to make sure that your PDC is correctly positioned and produces stable droplets.
At the beginning of the priming, the PDC should be centered with the red cross. You can align your PDC using **Nozzle Setup** >> **Nozzle Offset** >> Up, Down, Back, Front, Left, Right controls (Figure 1)

After the priming enter the Voltage and Pulse parameters (indicated on the PDC box), then click on:
- **Set Nozzle Parameters**
- **Start Continuous Dispensing**

If needed, adjust the Voltage and Pulse until you have a stable drop and then press:
- **Set Nozzle Parameters**

Figure 1. Drop optimization.

3.3 Humidity and Temperature Setup

Throughout the workflow, the humidity and temperature need to be set to a specific value to avoid evaporation and minimize condensation.

> **Note.** The runs already contain specific tasks controlling humidity and temperature for each run. The following describes how to change humidity/temperature "manually" if you wish to change it.

- Humidity setup:

Main >> **File** >> **Humidity Control** >> Pop-up window >> Set humidity >> Click on **Set** button (
Figure 2).
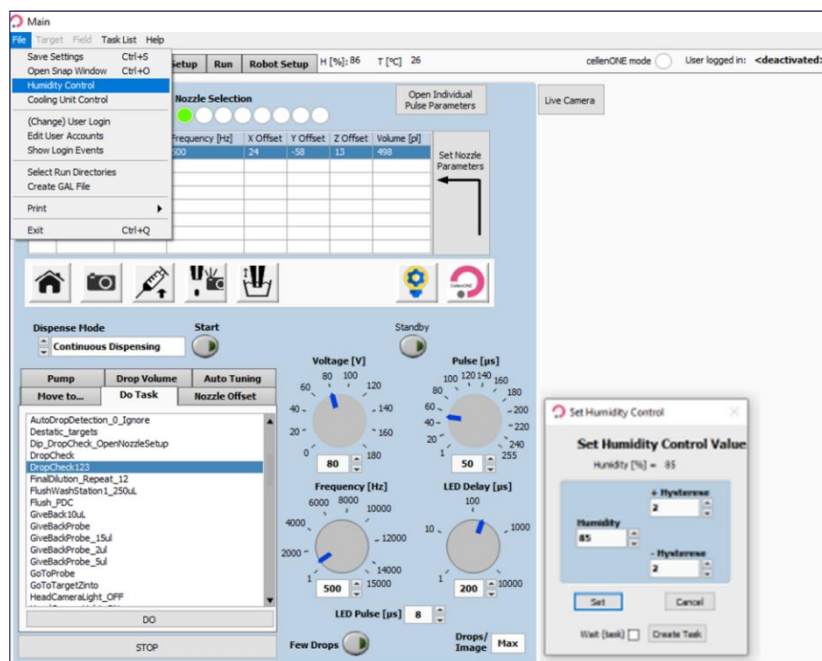
Figure 2. Humidity control.

- Temperature setup:

Main >> **File** >> **Cooling Unit Control** >> Pop-up window >> Set the temperature to fixed control and turn the cooling unit **ON** (Figure 3).

Figure 3. Temperature control.

Note 1. During a long cell isolation run, the low temperature can result in some condensation on the plate. However, this is not affecting the Master Mix efficiency nor is it leading to cross contamination.

> **Note 2.** When performing a proteoCHIP workflow, it is best to start by setting the temperature to 10 °C.

3.4 Target Setup

To display your target, go to:
- **Target Setup** >> **Target**

For the proteoCHIP LF, you will always have the following configuration in **Target** subtab (Figure 4):
- Start point: 0, 0
- Field Gap: 0,0
- Number of Fields: X=1, Y=1
- Spot per field: X=12, Y=4
- Spot pitch: X=4500 μm, Y=4500 μm

Figure 4. Target subtab.

3.5 Field Setup

The **Field Setup** subtab is used to define:
- From which well the sample will be aspirated.
    - ➔ *ONLY if the run contains a **TakeProbe** task (This is the case in the LF protocol).*
- Number of drops/volume to be dispensed in which "spot" of the Target.
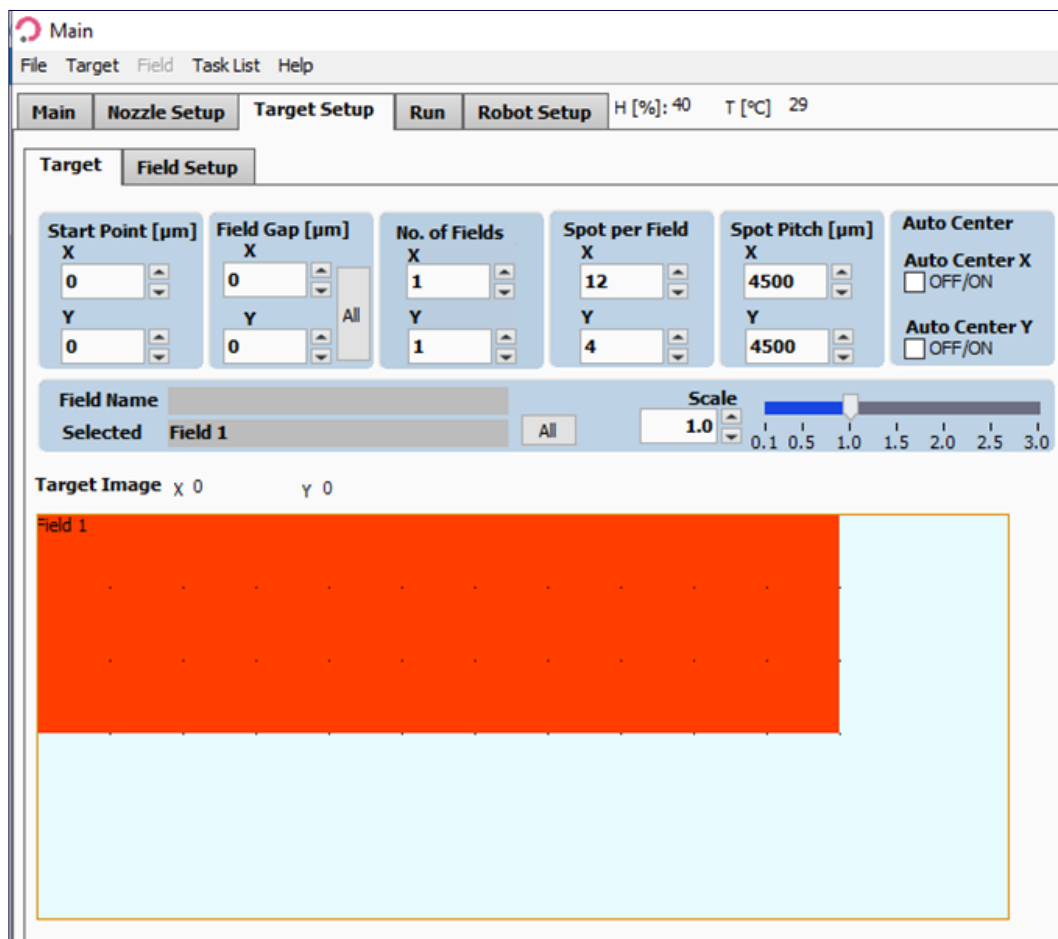
> **Note 1.** To change from Volume to drops and vice versa:
> - **Target Setup** >> **Field Setup** >> **Field** (at the top) >> **Volume entry**.
>
> **Note 2.** For every run, fields are saved in the software.
> You can edit these fields using **Edit Field Table** item to change (Figure 6):
> - From which well the sample will be aspirated.
> - Number of drops/volume to be dispensed in which "spot" of the Target.
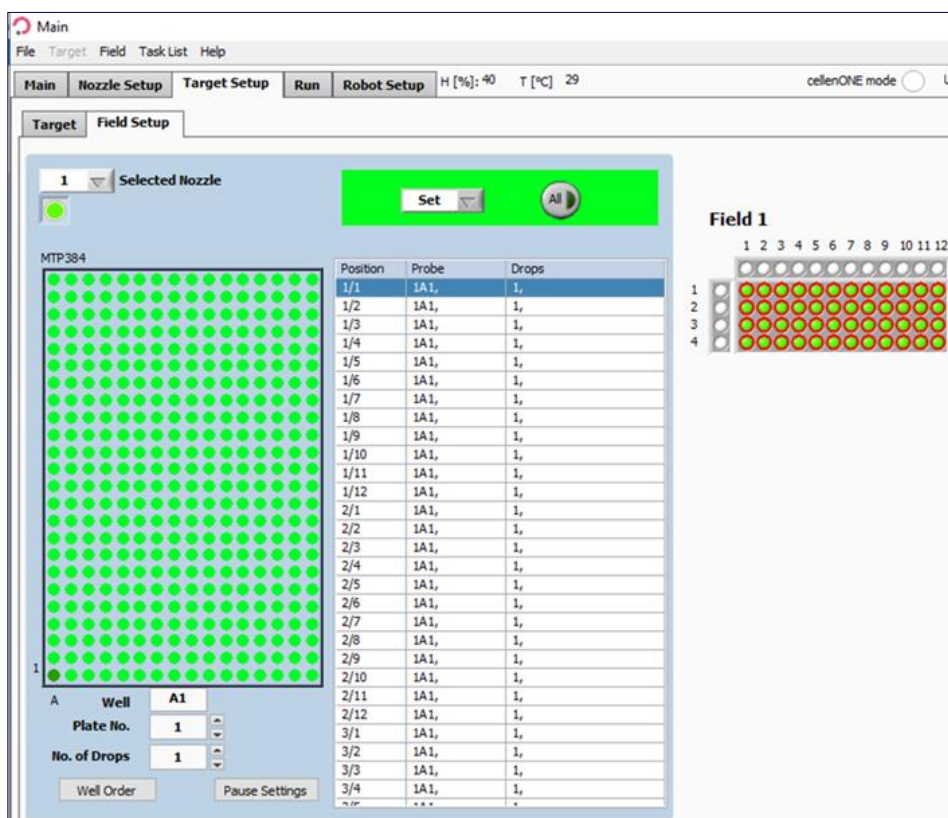
Figure 5. Field Setup subtab.

> **Note.** To assure that the exact suggested volume of each reagent is dispensed we recommend performing a Drop volume to calculate the corresponding number of drops before each liquid dispensing run.
> This should be done before dispensing the Master Mix, H_2O in the rehydration steps and 0.1% TFA/FA.
>
> To modify the number of drops/volume in the Field setup refer to Figure 6 and change the number of drops/volume by clicking on the **Drops** subtab in displays (3) and (4), then clicking on **Change all** button (Figure 6.5).

Figure 6. Edit Field Table.

3.6 Drop optimization

Each dispensing run is built with the same logic, once the run starts (Figure 7).

Several pop-up messages prompt you to load the correct volume of the desired solution in the well specified in the **Target Setup** tab.

- The sample should be loaded in a chosen well of the 384-well source plate (The well is chosen in the **Field Setup** tab, see section 3.5).
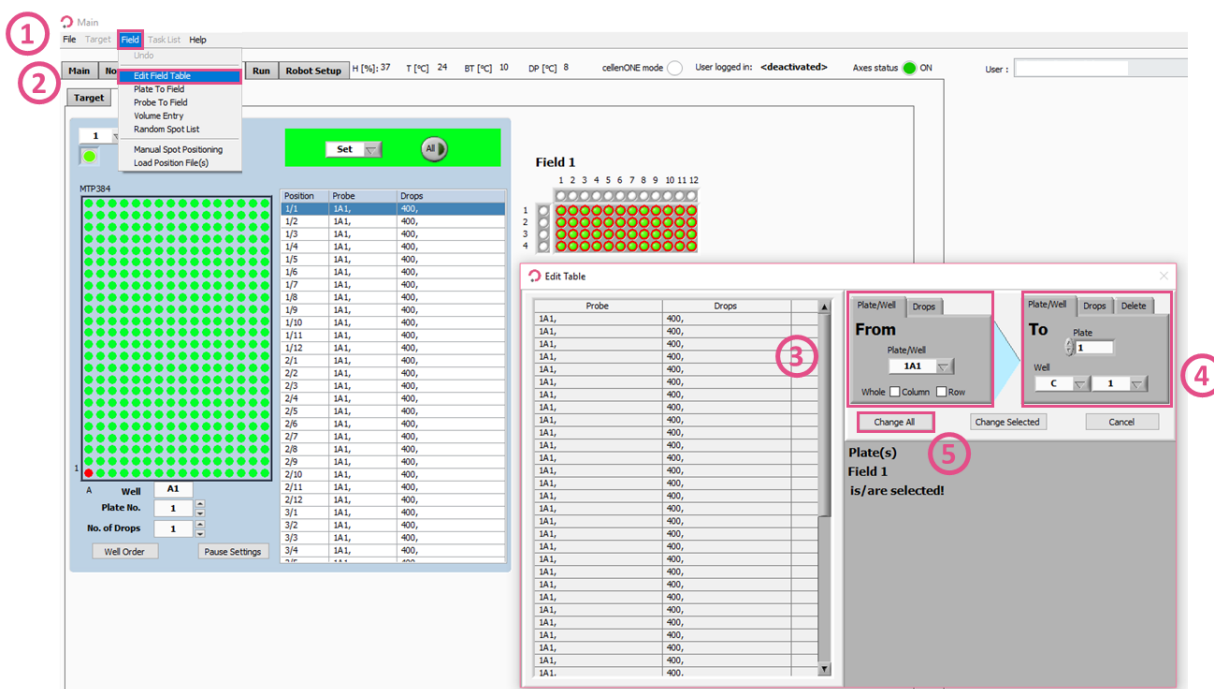- The volume to be aspirated is predefined inside the run by the **TakeProbe** task. (*Required volume is mentioned in the message that pops up at the beginning of the run*).
- The nozzle (PDC) moves to the chosen well, aspirates the chosen volume and moves back to do a wash cycle and take a picture of the drop.
- The software automatically checks that the dispensing parameters are correct by performing a **DropCheck** task. Should the system fail to detect a suitable drop, the run will pause, and the user will be able to modify the parameters to restore a stable droplet generation.
- The user must check that the dispensing parameters, Voltage and Pulse (Figure 7.2), are correct by pressing the **Continuous Dispensing** button (Figure 7.3) until a drop is stable without any satellite (if any).
- The user must save the new settings if the Voltage and Pulse were modified (Figure 7.4) and press the **Drop Check** button (Figure 7.5) and check if the drop is identified by the software, circled in red (Figure 7.6).
- If needed, you can also perform troubleshooting tasks (**AirEx**, **Flush_PDC**, etc.) and uptake more sample.
- Once the droplet generation is stabilized, click on **Continue** button to resume the run (Figure 7.7).
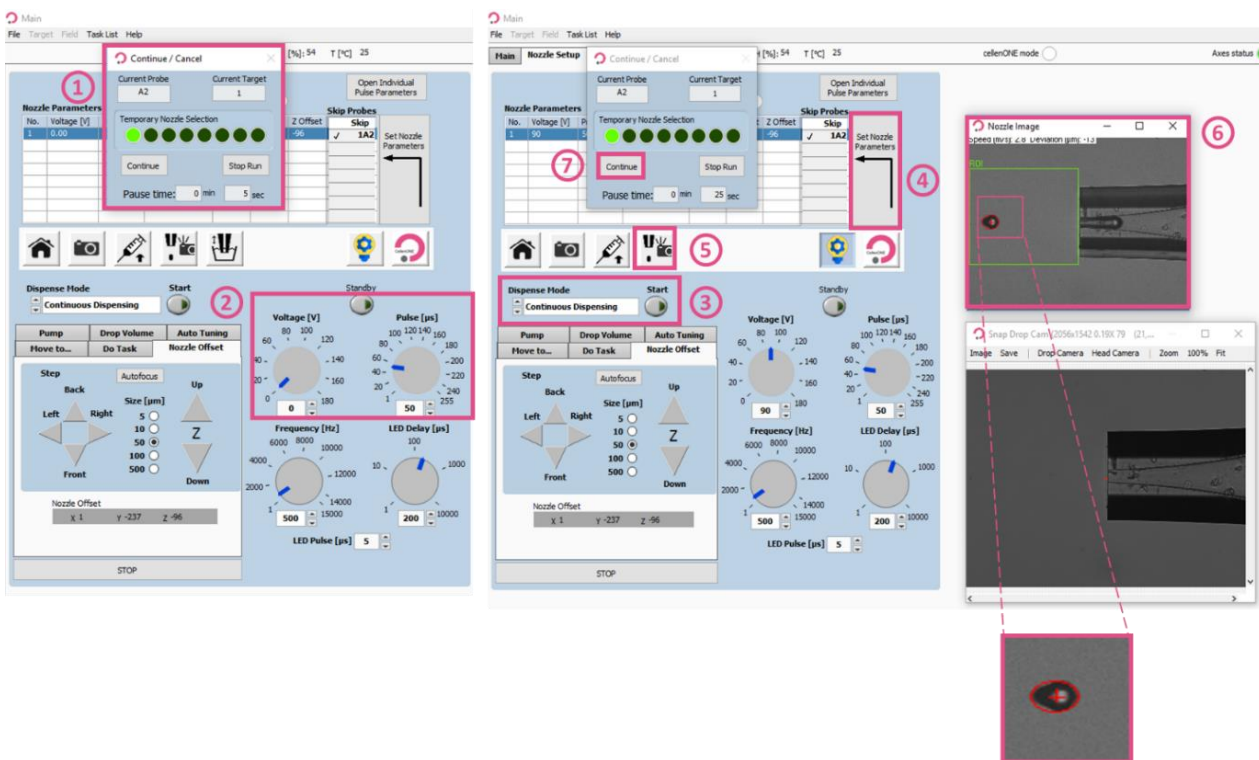
Figure 7. Drop optimization.

3.7   proteoCHIPs inside the cellenONE
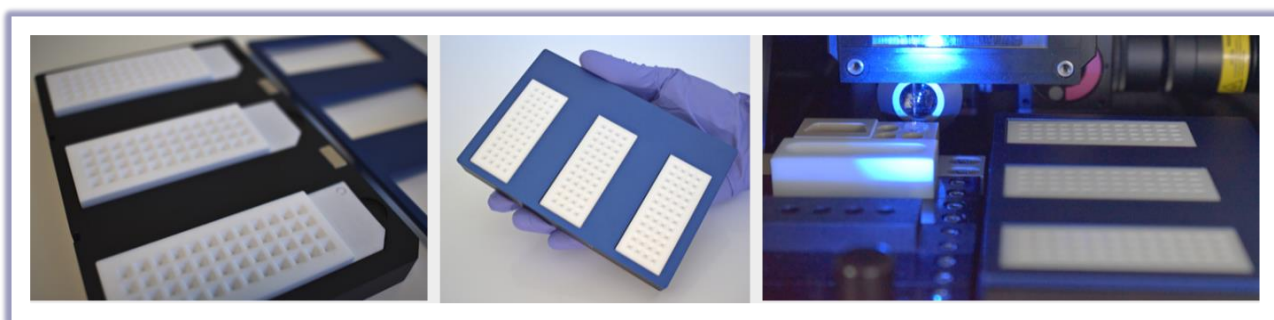
Figure 8. proteoCHIP LF inside the cellenONE holder.

- Place the proteoCHIP(s)_LF_48 inside the cellenONE holder (ref. C-PLF-48-CHB) (Figure 8).

Note. If processing only one chip, insert it on the spot closest to you.

- Place the cellenONE holder top (ref. C-PLF-48-CHT) on top of the holder and insert the assembled unit on the target holder inside the instrument.

Note. Runs 1, 2, 3 and 4 are suited for 1 to 3 chips. Run 5 can only process one chip at a time. If you are doing more than one proteoCHIP LF 48 in parallel, you will need to repeat Run 5 for each proteCHIP LF 48.

E.g., If you are running 3 proteoCHIPs you need to perform:

       1X Run 1
       1X Run 2
       1X Run 3
       1X Run 4
       3X Run 5.

4 Label free Single Cell Proteomics Workflow

4.1   Run 1 – Master Mix dispensing

> **Note 1.** We recommend using this Master Mix (Lysis + Digestion):
>
> **0.2% DDM, 10 ng/µL Enzyme(s), 100 mM TEAB**
> For each experiment 100 µL of Master Mix is needed.
>
> To prepare 100 µL of Master Mix add the reagents in the following order:
> **10 µL 1M TEAB + 60 µL H_2O + 20 µL 1% DDM + 10 µL 100 ng/µL Enzyme(s)**
>
> **Do not vortex the mixture, this will introduce air bubbles.** Mix by gently pipetting the solution up and down.
>
> **Tip:** Aliquot 20 µL 1% DDM and 10 µL 100 ng/µL Enzyme(s) at -20 °C for short term usage. For long term storage of enzymes, we recommend storage at -80 °C.
> TEAB should be stored at 4 °C.
>
> **Note 2.** We recommend using MS-grade enzymes (e.g., Trypsin Gold, Promega or Trypsin/LysC Mix, Thermo). For enzyme mixtures we recommend to also use a 10 ng/µL mixture of the enzymes.
>
> **Note 3.** For the Master Mix, you will probably need higher Voltage (+5-10%) to achieve an optimal drop formation. You can also optimize that during the run by clicking on **Nozzle Setup**.

4.1.1   Probe, Run and Target Selection
The Master Mix dispensing happens at 8 (+/- 2) °C and at humidity set to 45%.
In the **Main** tab of the software, select the probe, run and target to be used (Figure 9).
- Probe: Select **MTP384**
- Run: Select **LF_48_Run_1_MasterMix**
- Target: Select **proteoCHIP_LF_48**

This run contains a task that checks the stability of the drop after the dispensing of each row of the proteoCHIP (Figure 14). If the drop is not stable, you will have the opportunity to optimize the parameters, as explained in section 3.2.
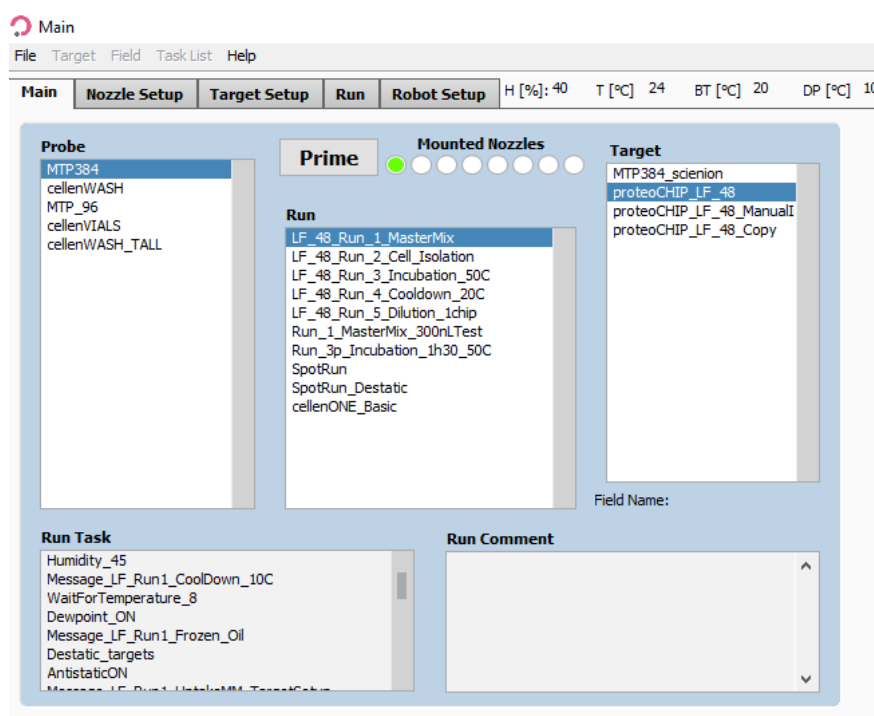
Figure 9. Probe, Run and Target Selection for Run 1 – Master mix dispensing.

4.1.2   Field Setup
Load the **LF_48_Run_1_MasterMix** field which will spot 800 drops (~300 nL in total) per well.

- To load the field:

Target Setup >> **Target** >> **Load Field** >> Select the field corresponding to the run (Figure 10) and you will have the field corresponding to Figure 11.
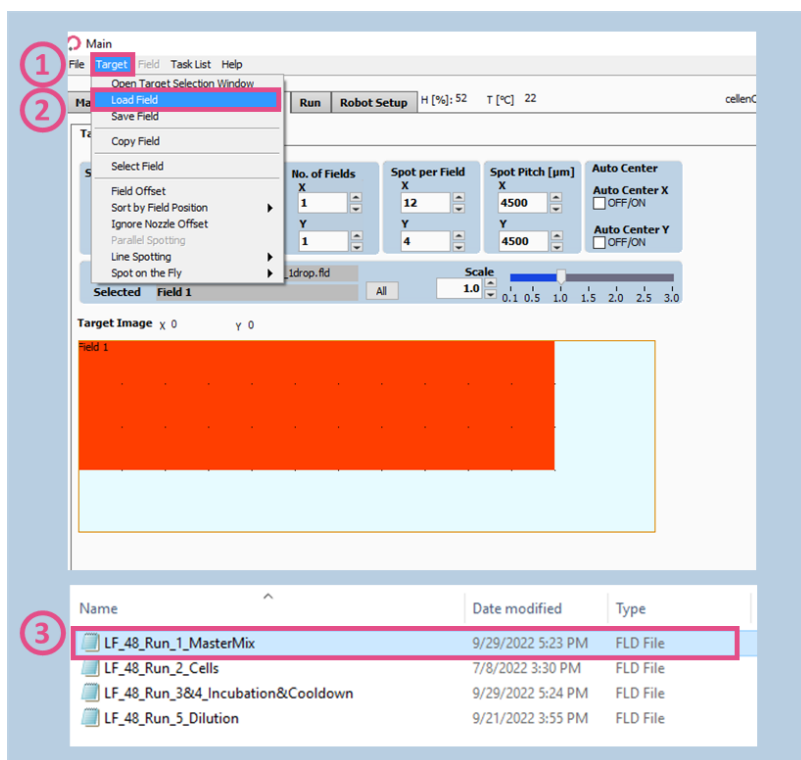
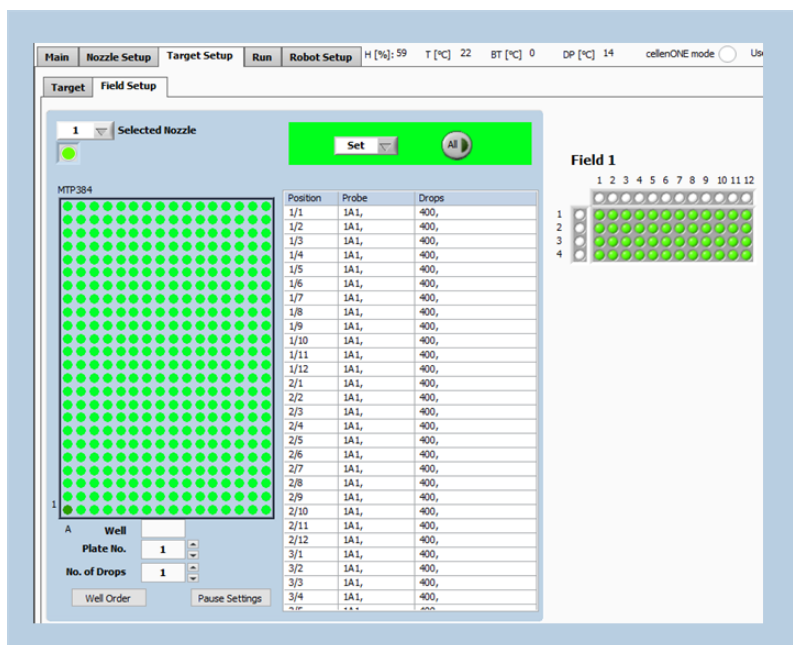*Figure 10. Field Selection for Run 1 – Master Mix dispensing.*

Figure 11. Field Setup for Run 1 – Master Mix dispensing.

4.1.3 Run Start

> **Note.** Load 60 µL of the Master Mix in the selected well.

Once the proteoCHIPs are loaded in the cellenONE as seen in Figure 8, check that you selected the right probe, run and target in the **Run** window (Figure 12.1). Start the run (Figure 12.2), name the experiment (Figure 12.3) and click on **OK** (Figure 12.4). Select the number of proteoCHIPs that you want to dispense (Figure 12.5) and click on **OK** button (Figure 12.6).
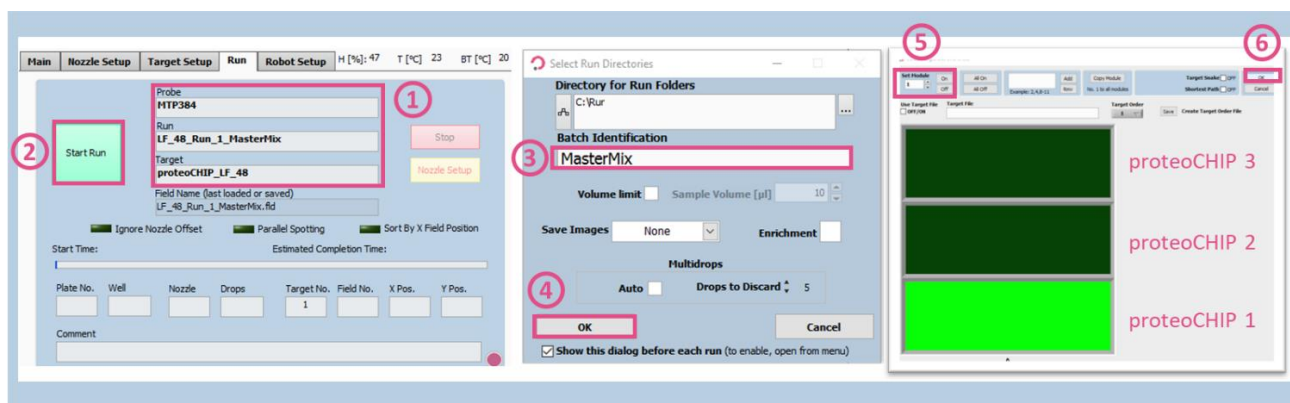A few pop-up messages will guide you before starting to spot (Figure 13).

Figure 12. Run Start for Run 1 – Master Mix dispensing.
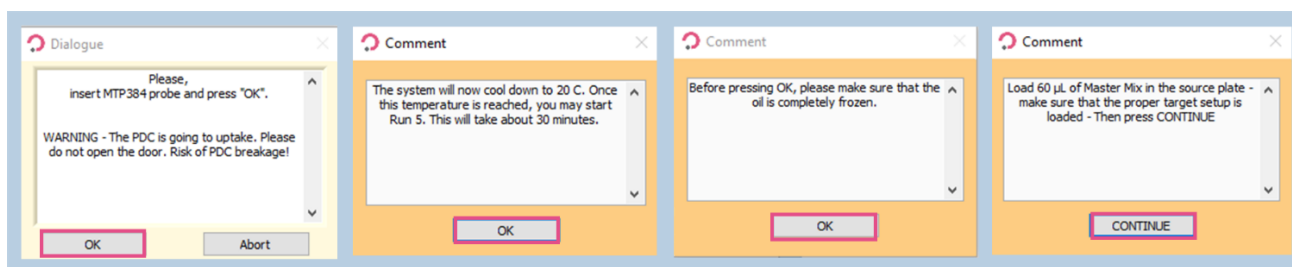

Figure 13. Pop-up messages for Run 1 – Master Mix dispensing.

> **Note.** ALWAYS use at least the target 1 so that the **AutoDropDetection** and **OpenNozzleSetup** tasks work properly (first proteoCHIP as shown above).
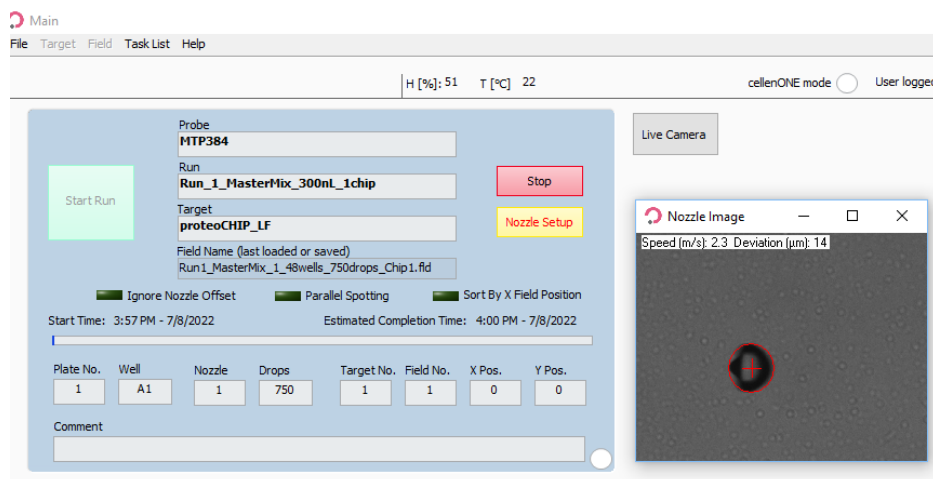
Figure 14. Autodrop check.

4.2 Run 2 – Cell isolation

Prepare your cells at a concentration of 100-200 cells/µL in degassed PBS.

4.2.1 Probe, Run and Target Selection

In the **Main** tab of the software, select the probe, run and target to be used (Figure 15).
- Probe: Select **MTP384**.
- Run: Select **LF_48_Run_2_Cell_Isolation**.
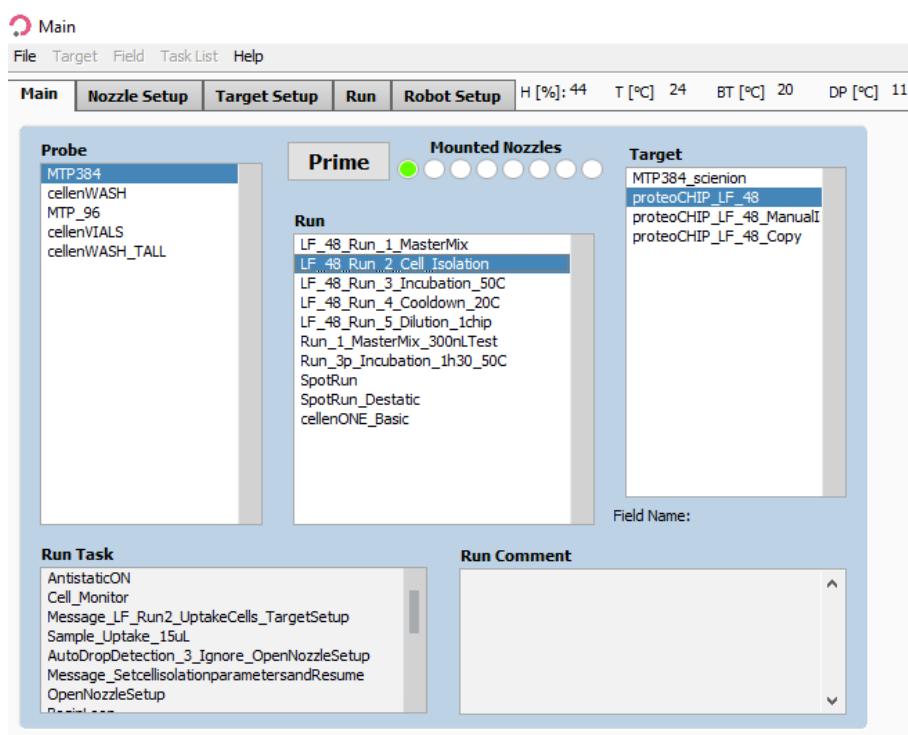- Target: Select **proteoCHIP_LF_48**.

Figure 15. Probe, Run and Target Selection for Run 2 –Cell isolation.

4.2.2 Selecting Field

Click on the 3rd tab, **Target Setup**, and the 1st subtab, **Target**. **Target** subtab is the same for the whole workflow, but the field setup needs to be changed at every step.

> **Note.** To isolate and dispense single cells, positions defined in the field setup must contain only one drop per well.

Load the **LF_48_Run_2_Cells** field which will spot 1 drop (=1 cell) per well. To load the field, click on **Target** (Figure 16.1) >> **Load Field** (Figure 16.2), select the field corresponding to the run (Figure 16.3) and you will have the field corresponding to the Figure 17.
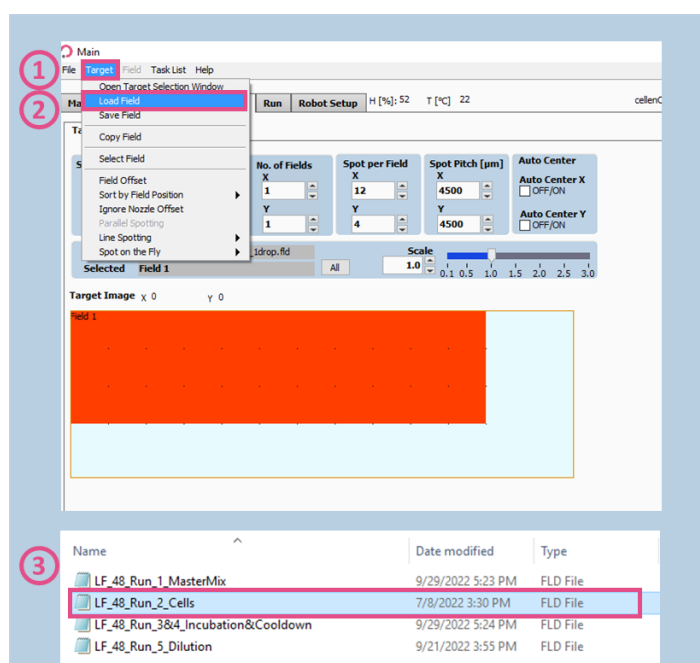

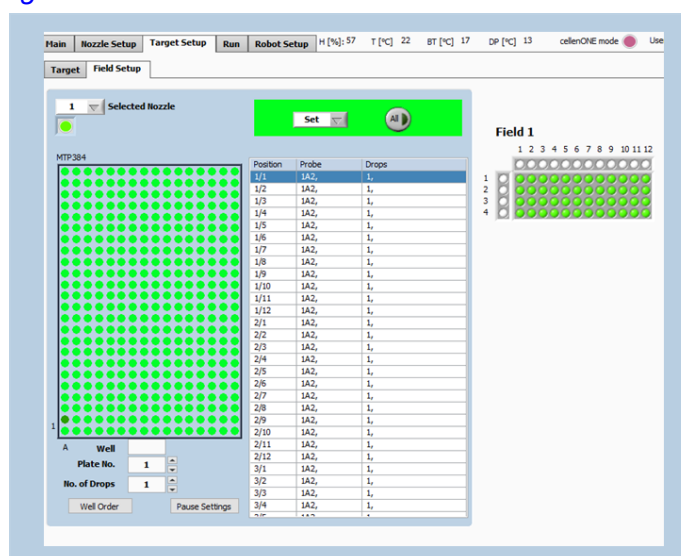*Figure 16. Field Selection for Run 2 – Cell isolation.*

Figure 17. Field Setup for Run 2 – Cell isolation.

Note. If you wish to dispense more than 1 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.

4.2.3 cellenONE module

Note. Load > 30 µL of cell suspension (100-200 cells/µL) into a chosen well of the 384-well probe plate.

For this run you need to **open the cellenONE window** and start the run in this same window. Once the **cellenONE module** is opened, it **should not be closed** before the end of the run (nevertheless the window can be minimized).
Please refer to the cellenONE User Manual, in **Single cell isolation** section, to understand all the options and features for single cell dispensing.
Select the **Nozzle Setup** tab (Figure 18.1) and press on the cellenONE logo (Figure 18) to open the cellenONE module for single cell isolation.
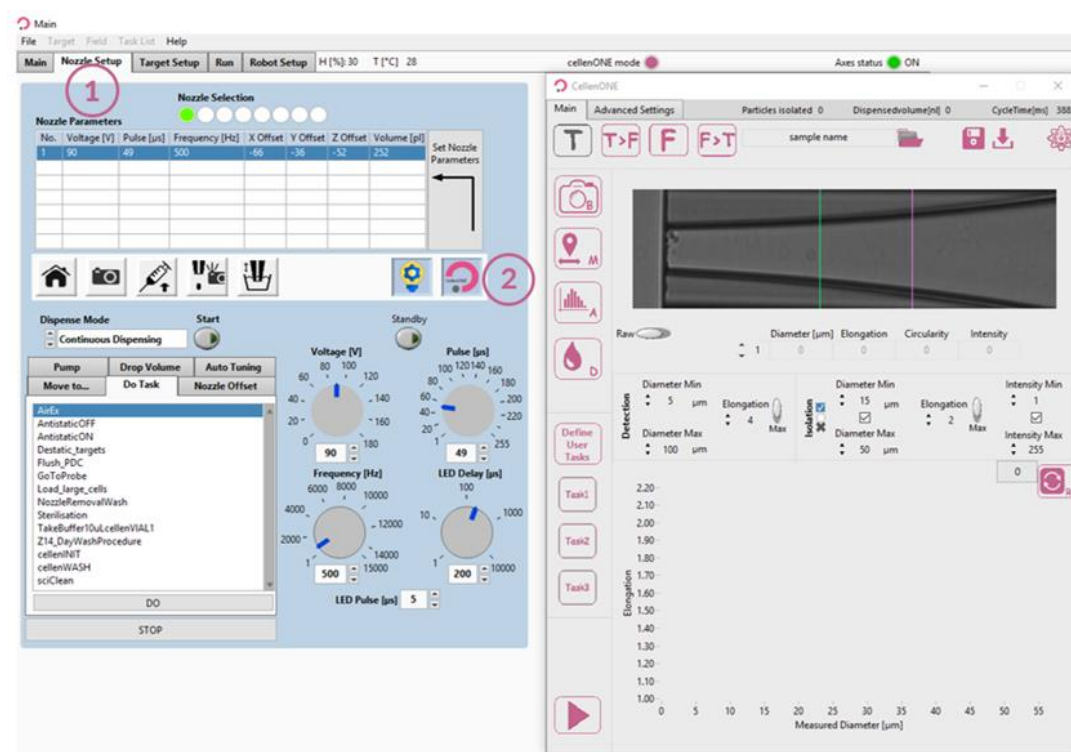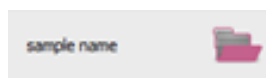The cell isolation run is still performed at 10 °C and ~45% humidity.

Figure 18. cellenONE module for single cell isolation (right window).

Before starting, define the folder location and name your experiment by pressing the following button:

All files associated with your experiment will be saved in this location. By default, the data is saved in the last folder chosen and named samplename_date.
Start the run by clicking on:

Please note that you can always pause the run with the Nozzle setup shortcut.

You will be able to modify the parameters if wanted.

Select the proteoCHIPs that you want to dispense in (Figure 19) and click on **OK** button (Figure 19.4).
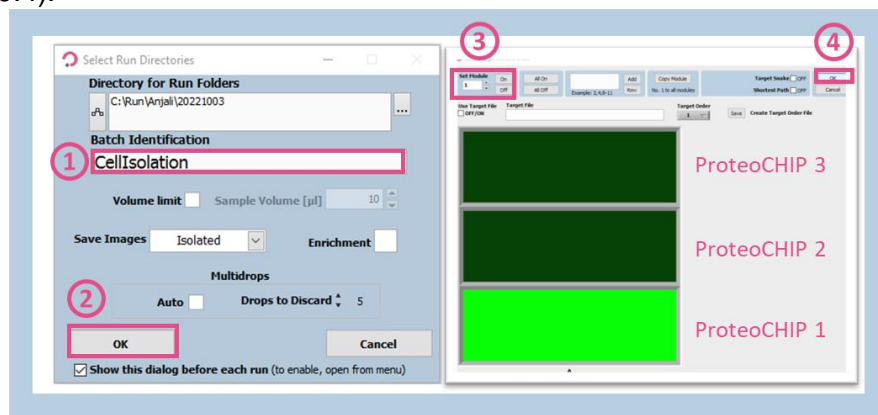
Figure 19. Starting Run 2 – Cell isolation.
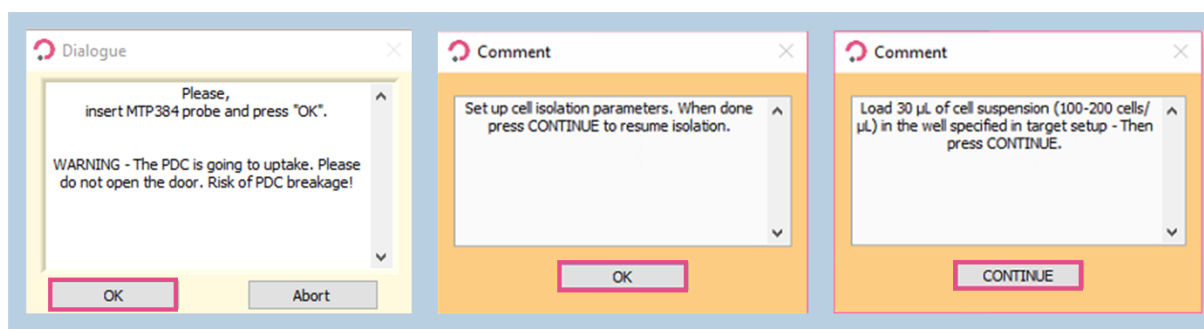
Figure 20. Pop-up messages for Run 2 – Cell isolation.

After uptaking the desired volume of the cell sample, the PDC comes back to the camera station so you can define the cell parameters used for cell isolation.

4.2.3.1 Detection parameters

The **Main** tab of cellenONE module (Figure 21) displays images of the region of interest of the PDC in which cells are detected. During dispensing, cells travel from right (upper part of the nozzle) to left (nozzle tip). Information below the image (i.e., Diameter, Elongation, Circularity, and Intensity) is given for the leftmost detected cell, i.e., the closest to the nozzle tip.

Figure 21. Main tab of cellenONE® module.

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**.
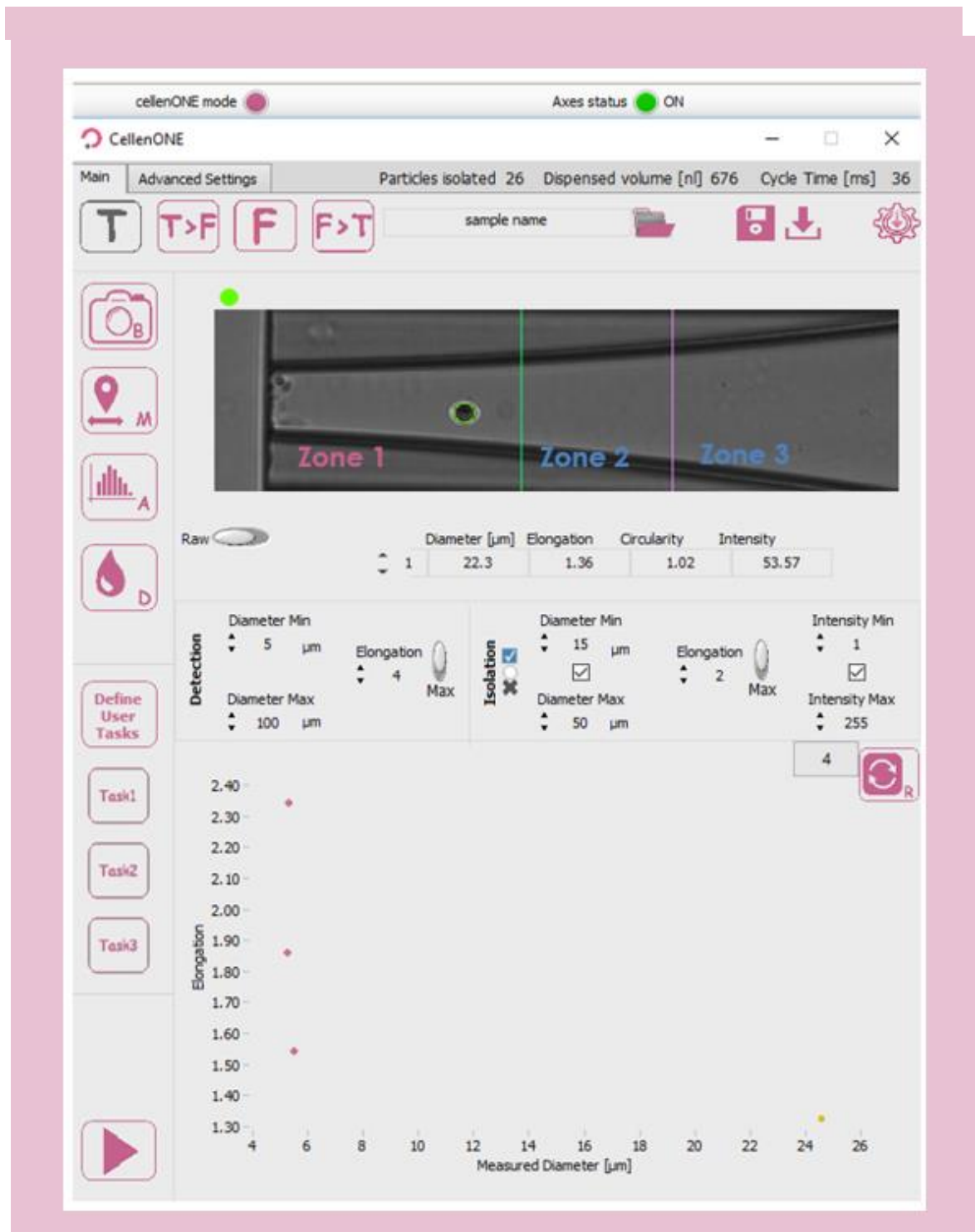
This section is used to define detection and isolation parameters (Figure 22). These parameters are crucial to successfully isolate and dispense single cells.
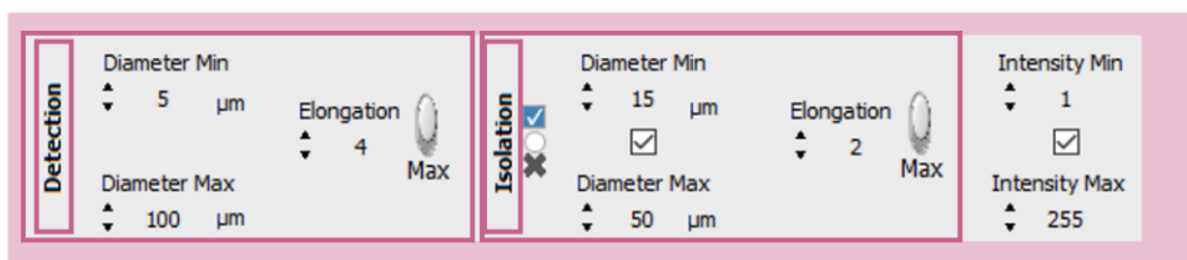

Figure 22. Detection and isolation parameters.

<u>Detection parameters</u>: These parameters will determine what the software will detect and consider as an object (e.g., cell or particle). As a result, these parameters should be "large" enough to ensure that all cells and cell clusters are detected as objects.

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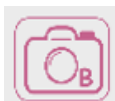
Get background button: Takes a reference background image. This button should only be pressed when the nozzle is free of cells and/or particles and when the PDC is in front of the camera.

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).

4.2.3.2   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** (Zone 1 on Figure 21). Pressing this button again 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 23). 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 on **Save & close** button.
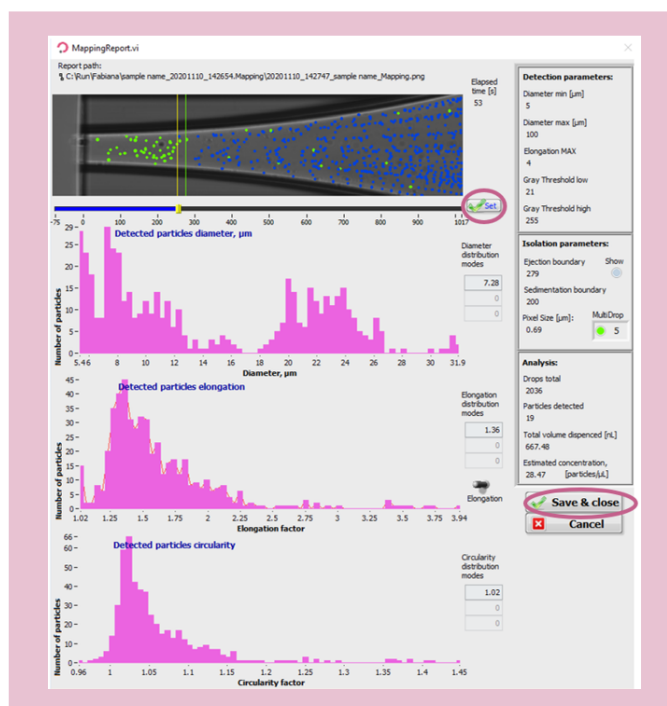

Figure 23. Mapping.

Note. At the end of the mapping process, the ejection boundary is set automatically according to the distribution of the blue and green dots inside the PDC. To **adjust the ejection zone manually**, **move the slider** by clicking and dragging the positioner on the ruler below the image and **press Set** button **to validate**.

4.2.3.3 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 23) 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:

- 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) and interpret the different scatter plots.
- Perform an analysis of your sample.

Analyze sample button: Analyzes 100 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 24). The number of analysed cells can be set in the **Advanced Settings** tab. You can do a gate to choose new values for isolation parameters.
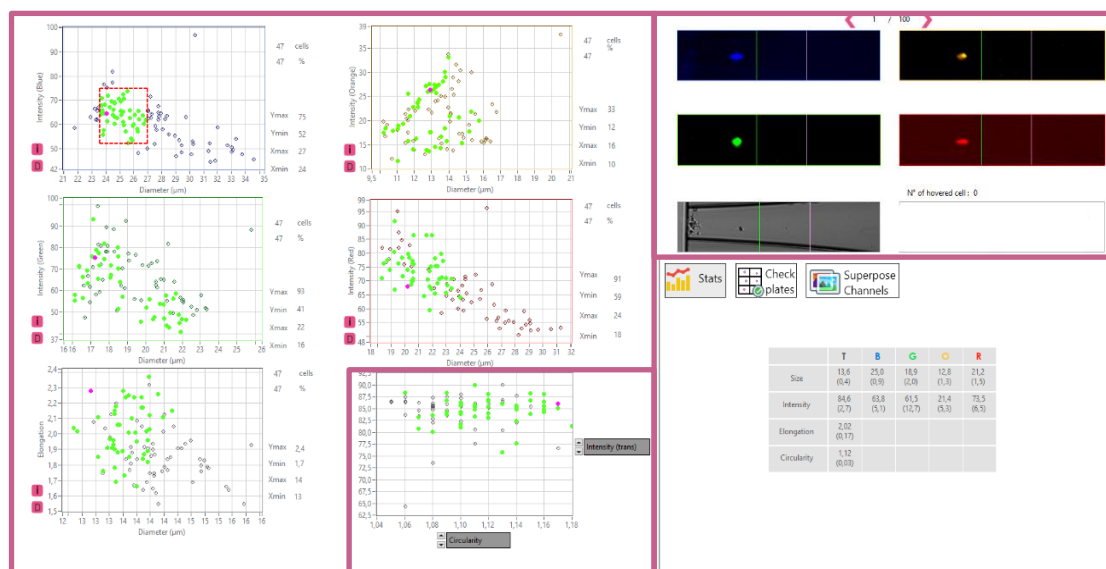

Figure 24. Scatter Plot after Analysis.

4.2.3.4   Setup check

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 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 are satisfied with your parameters you can click on **Continue** to start dispensing your cells (Figure 25).
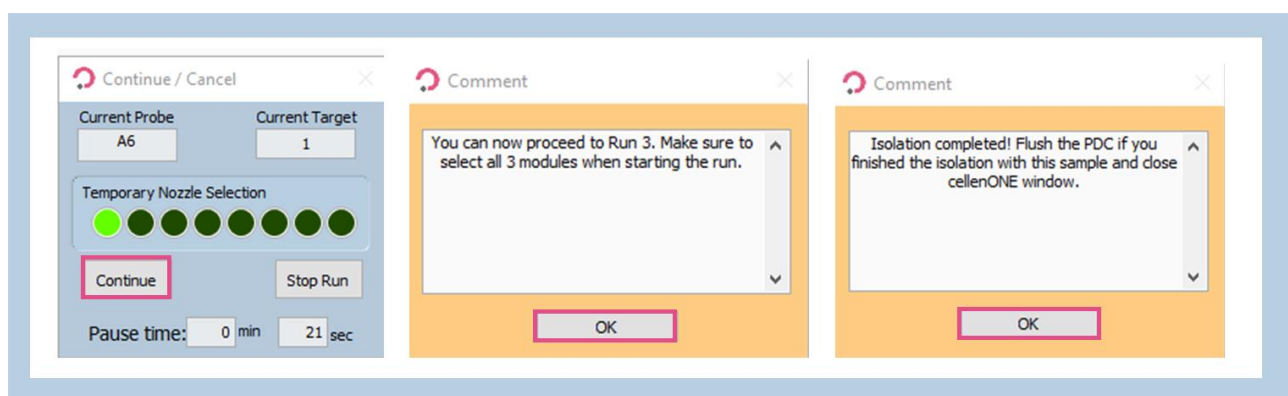

Figure 25. Run Start for Run 2 – Cell isolation.

4.3   Run 3 **–** Incubation at 50 °C

Single cells have been isolated in wells containing lysis and digestion buffer. The next step is to perform the incubation to:
- Lyse the cells,
- Digest the protein into peptide fragments.

This step can be performed directly on the deck of the cellenONE. This incubation step lasts 1h30, at a temperature of 50 °C. In addition to an increased ambient humidity, the PDC will regularly dispense deionized water in each well to maintain a constant water content and create a mechanical mixing phenomenon to help achieving homogeneous cell lysis and protein digestion.

4.3.1 Probe, Run and Target Selection

In the **Main** tab of the software, select the probe, run and target to be used (Figure 26).

- Probe: Select **MTP384**.
- Run: Select **LF_48_Run_3_Incubation_50C**.
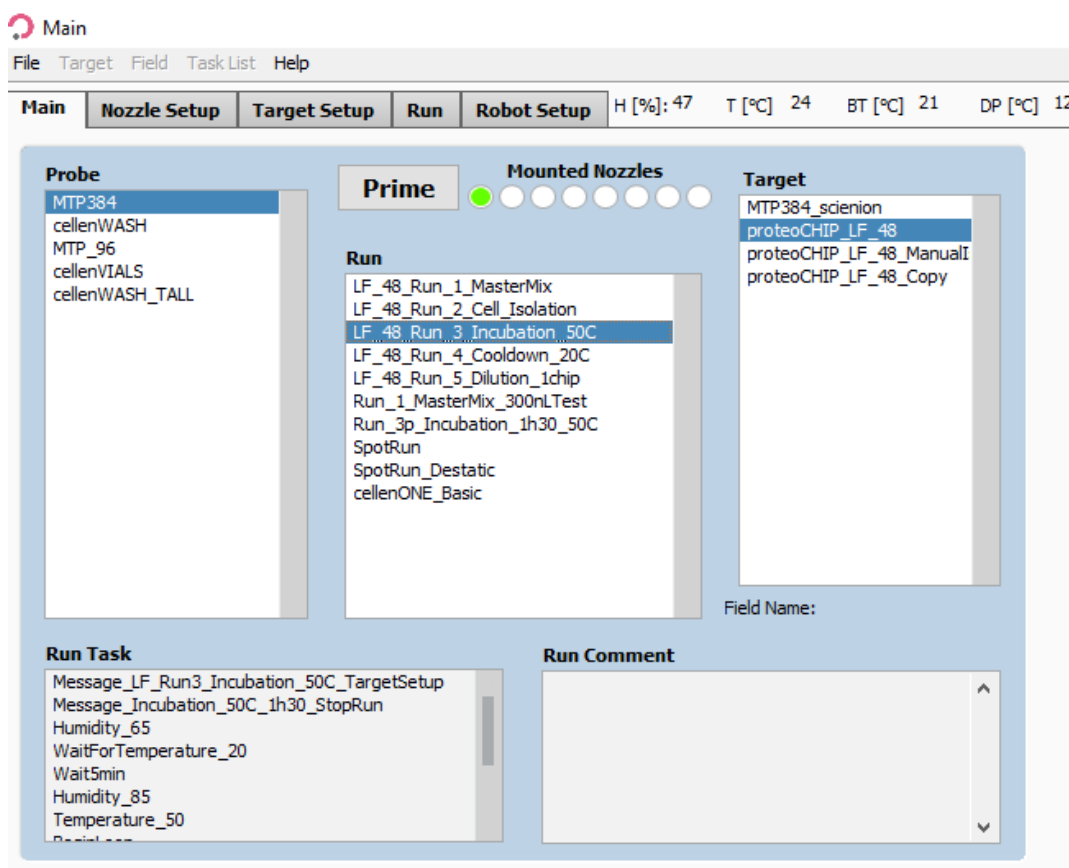- Target: Select **proteoCHIP_LF_48**.

Figure 26. Probe, Run and Target Selection for Run 3 – Incubation.

4.3.2 Field Selection

Click on the 3rd tab, **Target Setup**, and the 1st subtab, **Target**. **Target** subtab is the same for the whole workflow, but the field setup needs to be changed at every step.

Load the **LF_48_Run_3&4_Incubation&Cooldown** field which will spot 315 drops (~120 nL) per well. To load the field, click on **Target** (Figure 16.1) >> **Load Field** (Figure 16.2), select the field corresponding to the run (Figure 27) and you will have the field corresponding to the Figure 28.
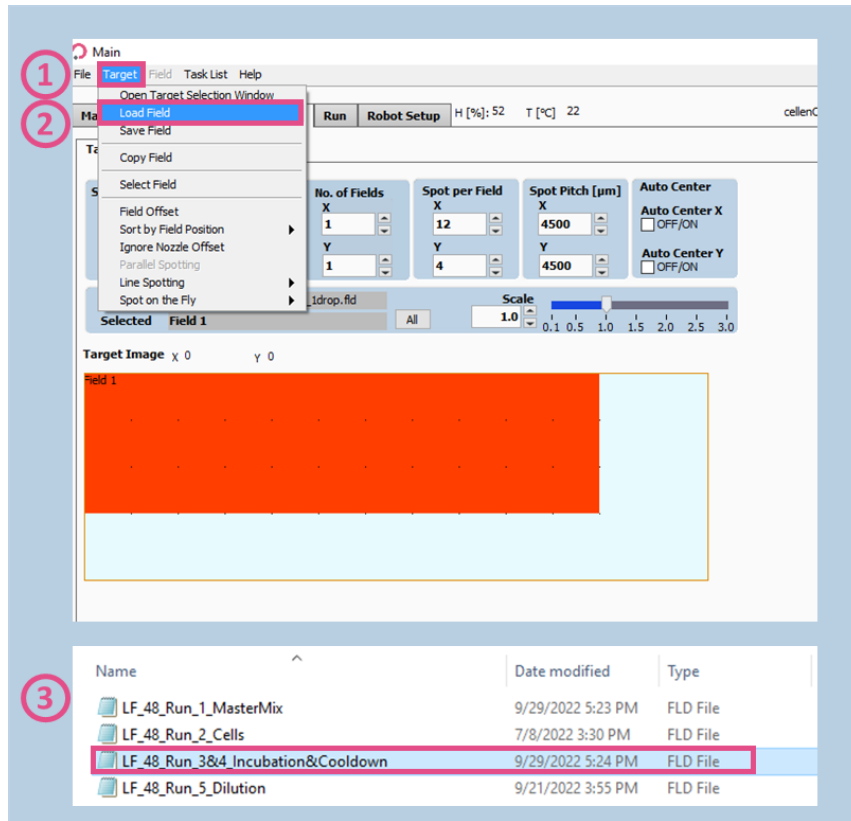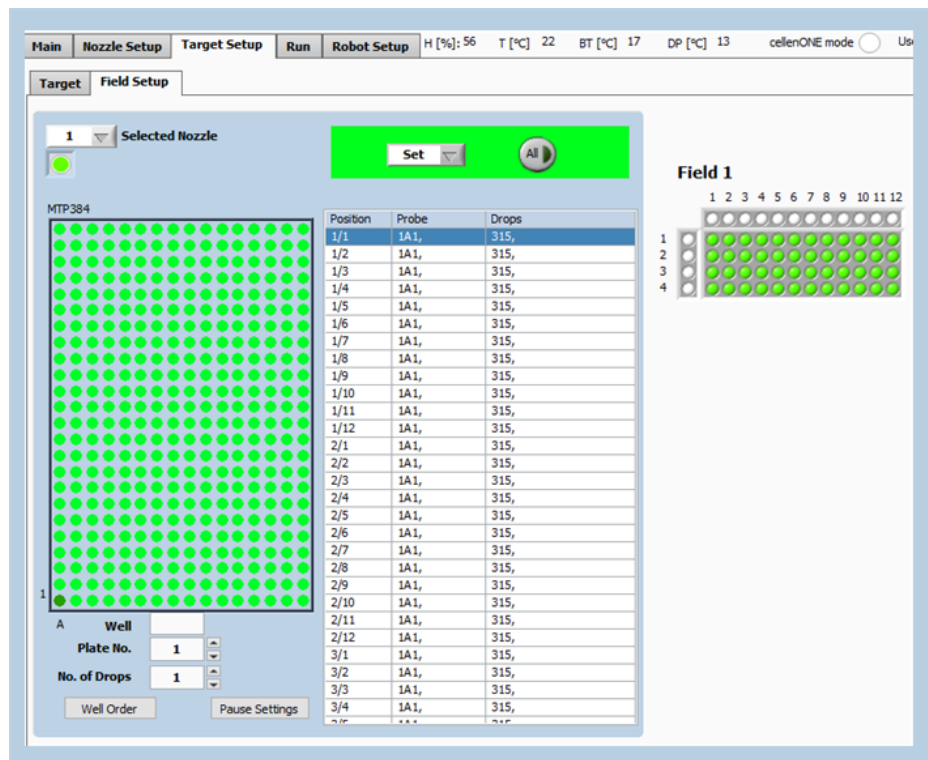
Figure 27. Field selection for Run 3 – Incubation.

Figure 28. Field setup for Run 3 – Incubation.

4.3.3 Run Start

Start the run and **select all 3 chips**, then click on **OK** (Figure 29).

> **Warning.** Please make sure that the 3 proteoCHIPs are selected regardless of the number of chips processed.
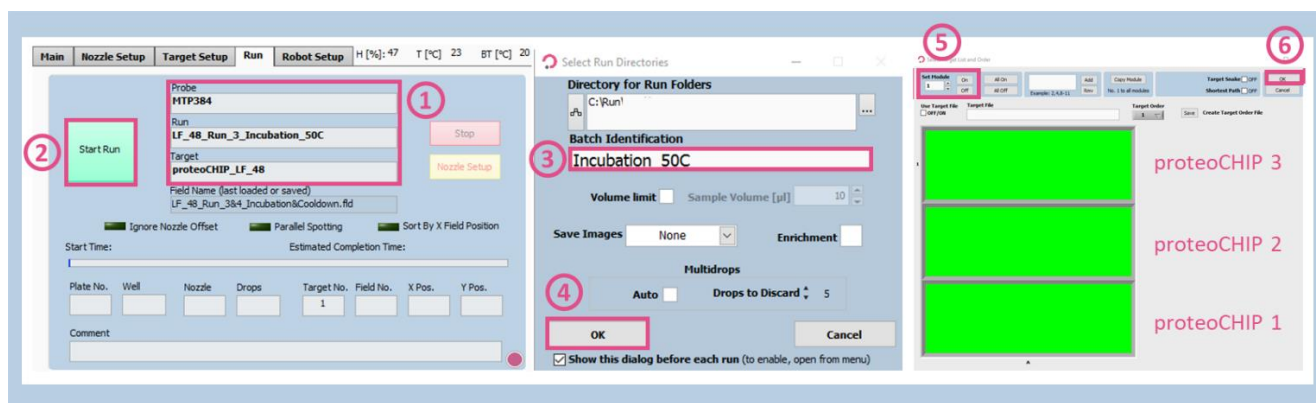

Figure 29. Run Start for Run 3 – Incubation.

Once the Run has started, humidity and temperature are automatically set. A pop-up message is displayed, click on **OK** button to start the 1h30 incubation (Figure 30).
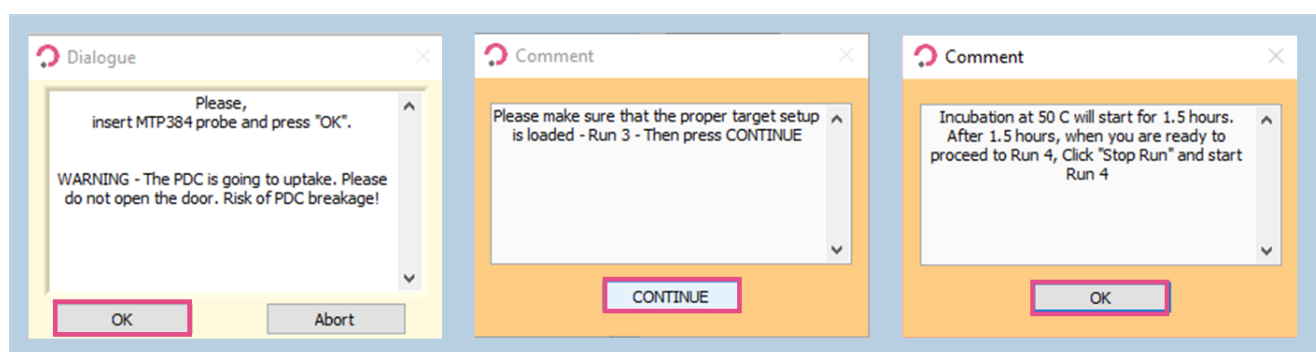

Figure 30. Pop-up messages for Run 3 – Incubation.

After 1h30, once you are ready to proceed to the next step, click on the red **Stop** button. Dismiss the pop-ups by clicking on **Exit Run** and then **Partially spotted** (Figure 31).
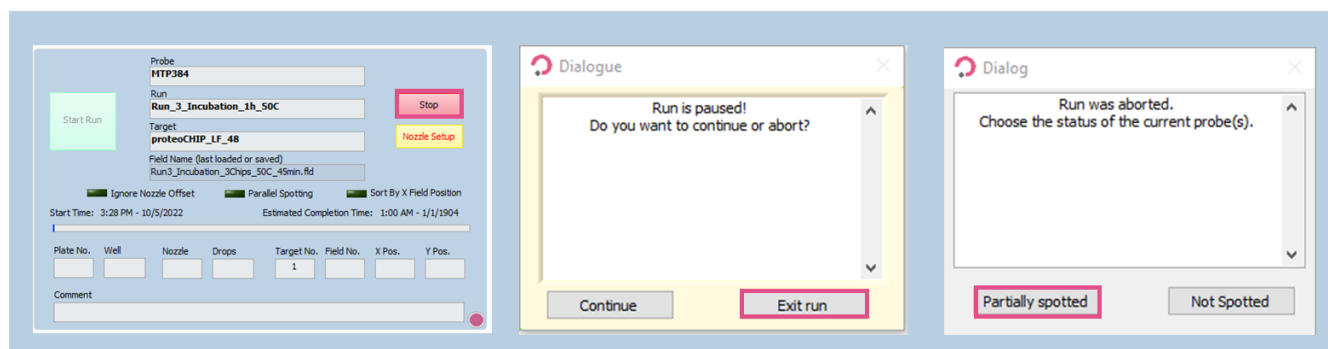

Figure 31. Exiting the run.

4.4 Run 4 – Cooldown to 20 °C

Following incubation at 50 °C, we need to bring the plate temperature back to 20 °C. During this process, the system will keep on rehydrating the wells continuously. The cooldown step takes approximately 30 minutes to complete.

This run needs to be launched **immediately** after stopping the previous run, as in the meantime rehydration cycles are interrupted, but the temperature setting is still 50 °C. **Not starting the Cooldown run immediately can result in sample evaporation.**

4.4.1 Probe, Run and Target Selection
In the **Main** tab of the software, select the probe, run and target to be used (Figure 32).
- Probe: "MTP384"
- Run: "LF_48_Run_4_Cooldown_20C"
- Target: "proteoCHIP_LF_48"

No changes need to be made in the field setup section, so you can proceed to start the run immediately.
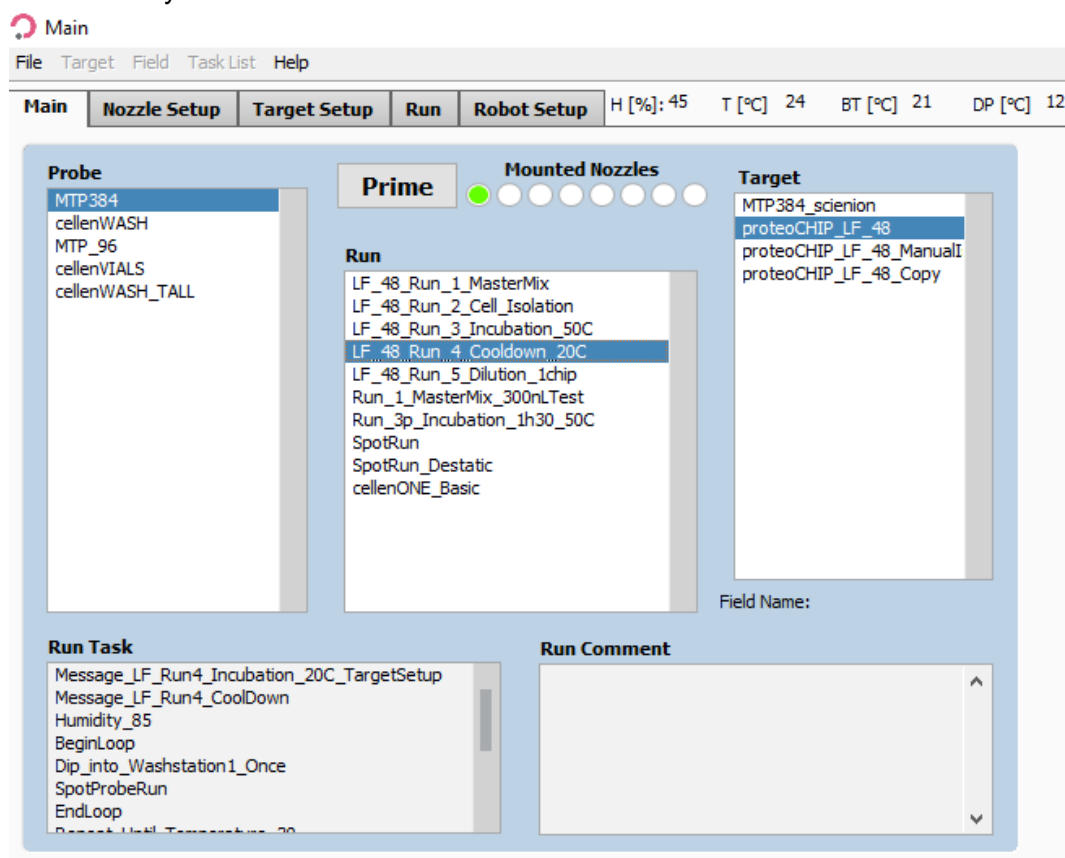

Figure 32. Probe, Run and Target Selection for Run 4 – Cooldown.

4.4.2 Run Start
Once the run has been selected, click on **Start Run** button in the **Run** tab. Select all 3 chips regardless of the number of loaded chips and click on **OK** button.

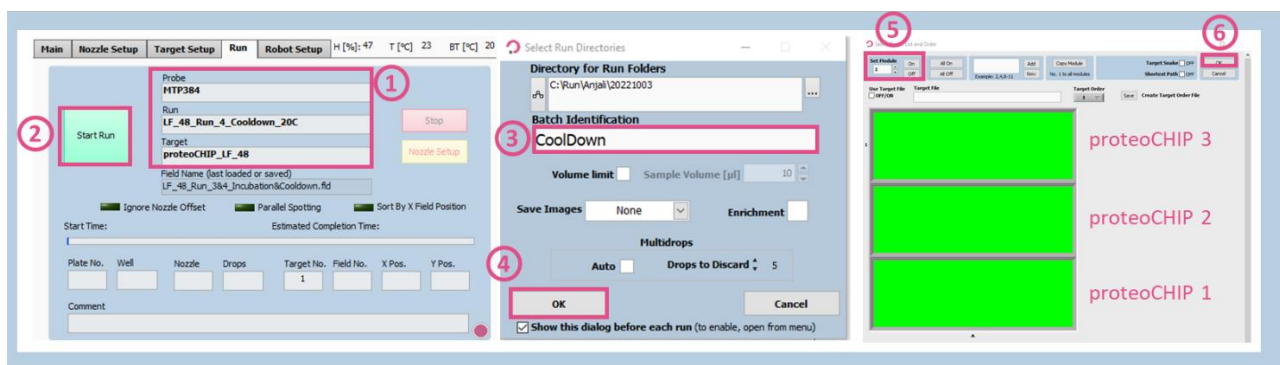The run finishes once the temperature of 20 °C is reached. This should take approximately 30 minutes.

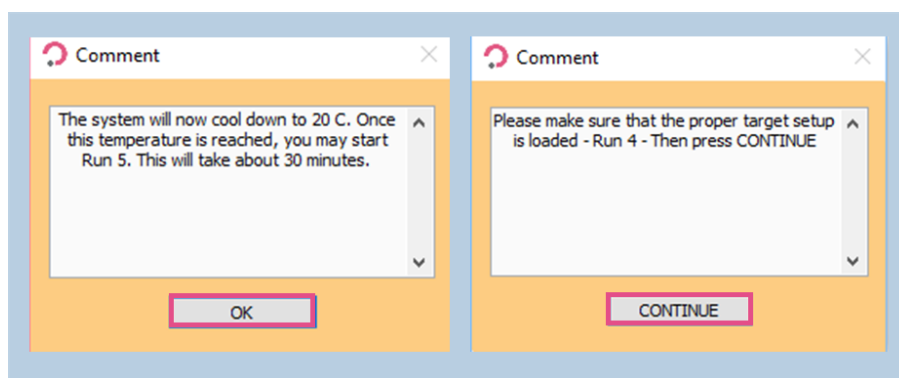*Figure 33. Run Start for Run 4 – Cooldown.*

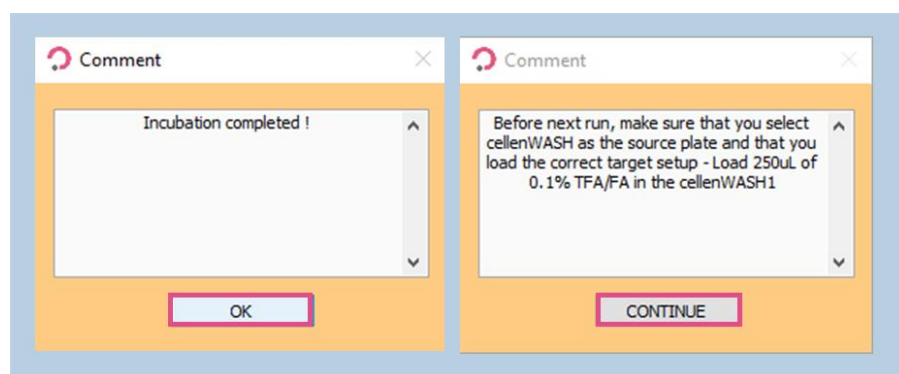*Figure 34. Pop-up messages for Run 4 – Cooldown.*

*Figure 35. Pop-up message after incubation completion.*

4.5   Run 5 – Dilution

The dilution step is used to adjust the end volume of the final sample that is going to be injected in the LC. The final volume should be around 3.5 µL to be correctly injected into the HPLC system loop before the MS analysis. To achieve that, 0.1% FA or 0.1% TFA is added to each well of the chip.

This step can either be performed manually or by an automated cellenONE run.

4.5.1   Manual dilution

Please make sure that the oil is liquid (temperature should be above 18 °C). Pipette 3.2 µL of 0.1% FA or TFA in each well of the chip. Optional: Use a multichannel pipette.
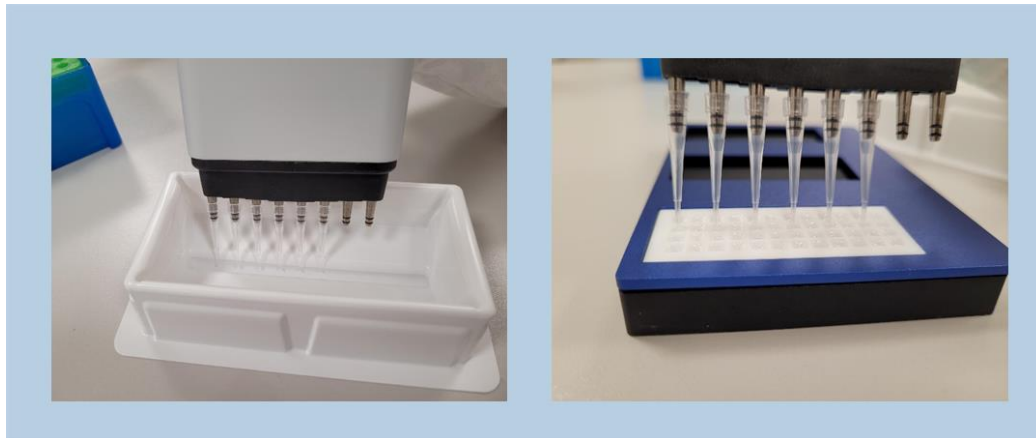
Figure 36. Manual dilution using a multichannel pipette.

4.5.2   cellenONE-automated dilution

4.5.2.1   Probe, Run and Target Selection

In the **Main** tab of the software, select the probe, run and target to be used (Figure 37).

Given the high volume to be aspirated, **the probe needs to be changed in Main.**

- Probe: Select **cellenWASH** or **cellenWASH_TALL**
- Run: Select **LF_48_Run_5_Dilution_1chip**
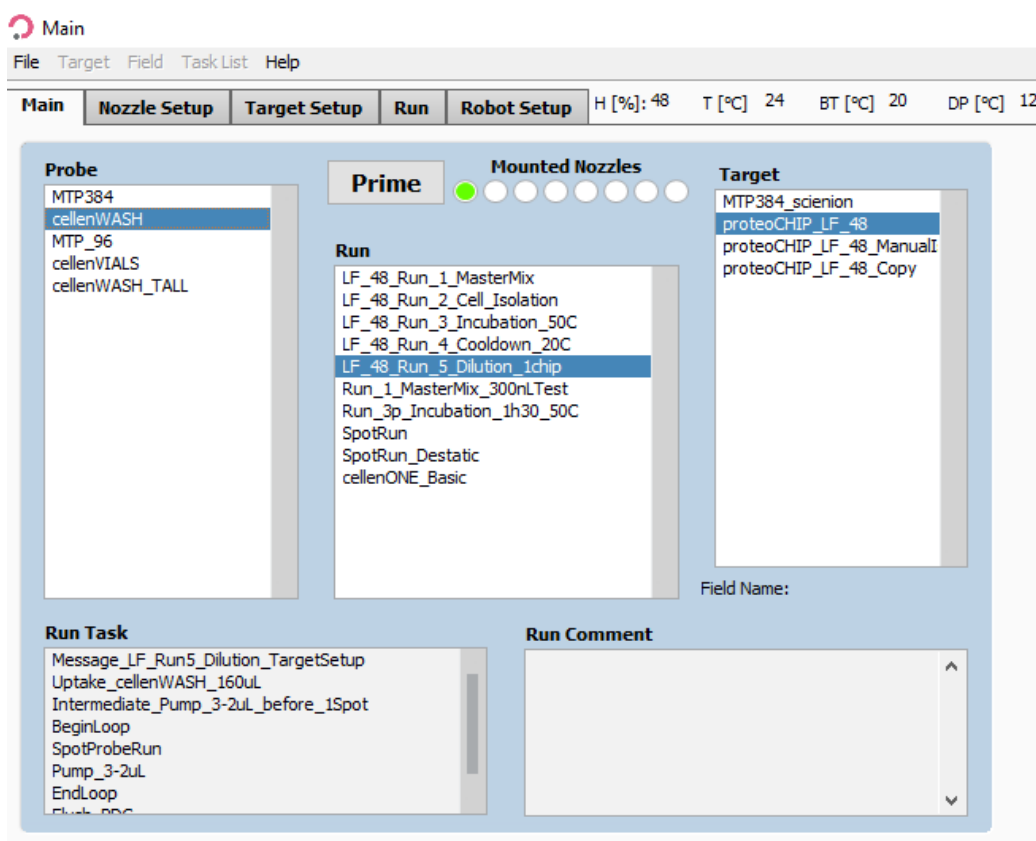- Target: Select **proteoCHIP_LF_48**

Figure 37. Probe, Run and Target Selection for Run 5 – Dilution.

4.5.2.2 Field Selection

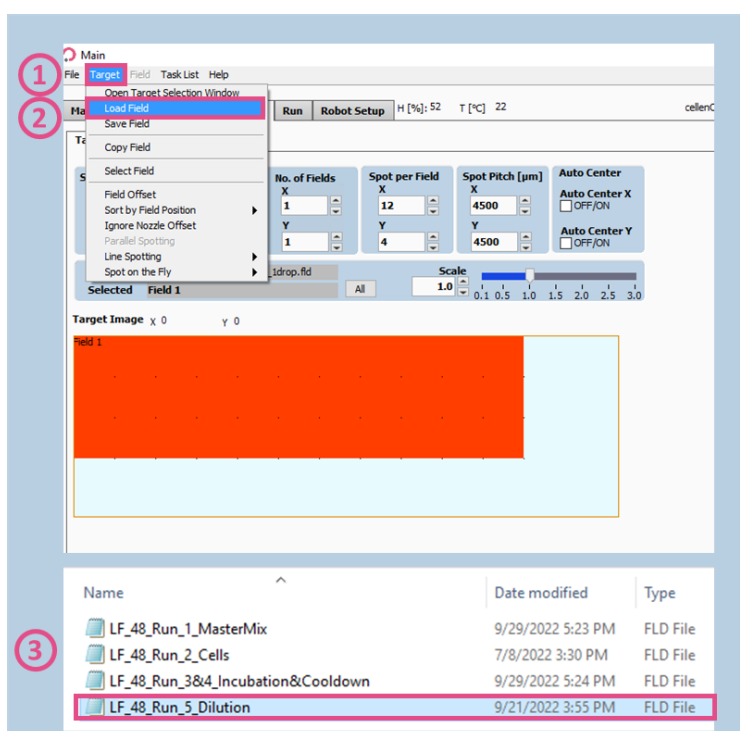In the **Target** tab, load the **LF_48_Run_5_Dilution** field (Figure 38).

Figure 38. Field Selection for Run 5 – Dilution.

The field corresponding to the dilution run is displayed in **Target Setup** >> **Field Setup** (Figure 39).
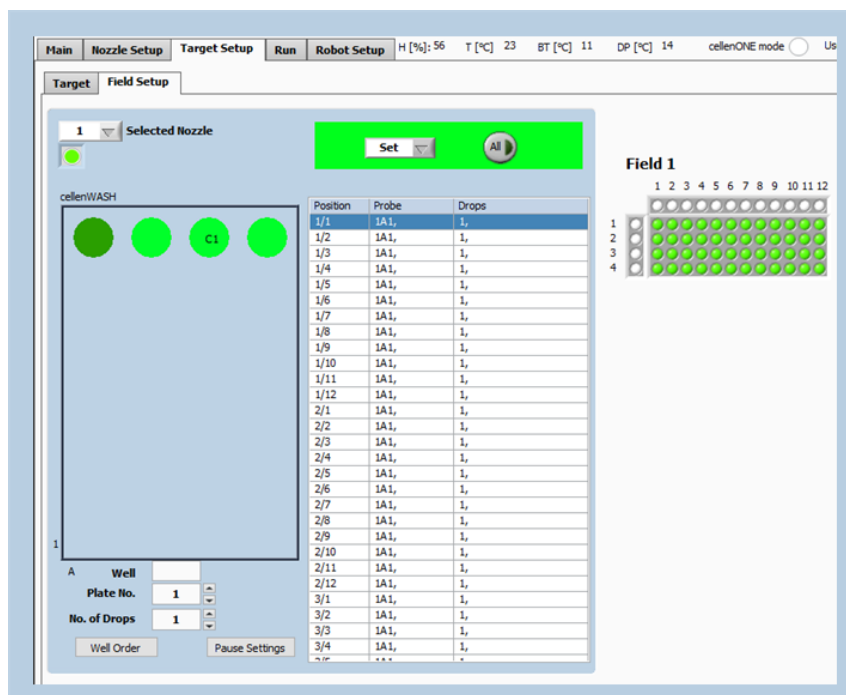

Figure 39. Target Setup for Run 5 – Dilution.

4.5.2.3 Start the Run

Once you chose the right Probe, Run, Target, and you loaded the dedicated field, click on **Start Run** button in the **Run** tab (Figure 40). A pop-up window will prompt you to insert a cellenVIAL or a pre-taught PCR tube (cellenWASH_TALL) loaded with 250 µL of 0.1% TFA or FA in the cellenWASH1 position (Figure 40). Once this is done, click on **OK** button to continue. If the cellenWASH_TALL is not taught on your machine and you would like to use a PCR tube, please contact your Field Application Specialist.
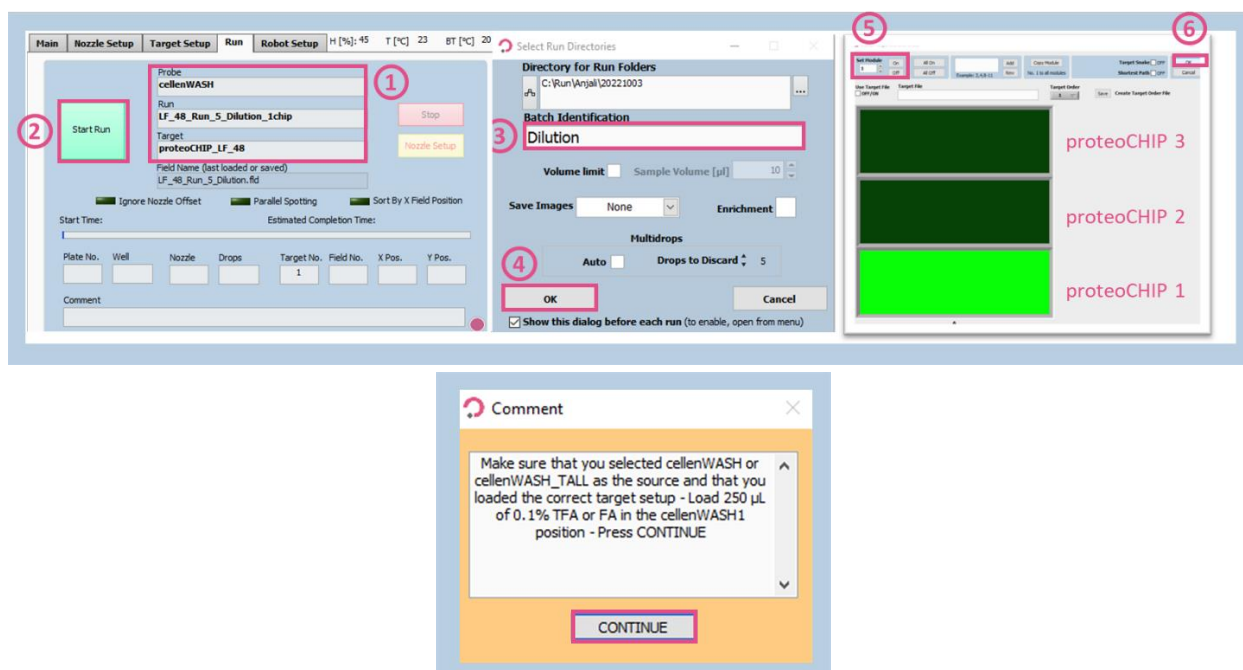

Figure 40. Run Start for Run 5 – Dilution.

4.6 Sample transfer

Place the holder containing the chip(s) on ice so the temperature decreases, and the oil becomes solid (temperature below 18 °C) (Figure 41).

Once the oil is solid (after ~5 minutes), it is easy to pipette out 3.5 µL of the sample droplet. The solidified oil will keep sticking onto the chip and will not be transferred with the sample to be analyzed.

You can now transfer your samples to the desired injection plate/vials, etc.

Figure 41. Solid oil in proteoCHIP LF 48.

Note. For the moment this step is performed manually. We are currently working on implementing direct injection from the chip to most of the common autosamplers available. As soon as this feature is available, we will let you know!

5 Service and Support

5.1 Service Contracts

A cellenONE should receive preventative maintenance (PMI) annually to replace the system fluid and wash lines and inspect / replace wear-and-tear items.

Scienion will automatically be in contact when a service contract is active, and a PMI is due. We look forward to providing your lab with outstanding service and support.

Service contracts are provided through SCIENION AG and SCIENION US to all customers worldwide with varying levels of coverage. Please contact **ticket@scienion.com** to request a quote.

5.2 Service Ticket

For service or application support, contact **ticket@scienion.com** with your request. Only certified users, those who attended basic training provided by a Scienion or Cellenion representative, may submit a service/support ticket. Training of additional users or re-training may be provided.

If your institution possesses multiple cellenONEs or sciFLEXARRAYERs please include the serial number of the instrument in your request to facilitate the support. The serial number of the cellenONE may be found on a sticker on the back panel of the system for integrated units or within the software under "Help" and the option "Info".

Tickets are tracked within our service system and dispatched to the most appropriate representative. Please do not email only your field service engineer or application specialist as a timely response is not guaranteed.

5.3 Remote support

Prior to dispatching a service engineer, we will try to solve a problem remotely. We can do this via phone call, web-conference, or remote access to your instrument control computer. In the instance that a remote connection is needed, we will ask you to establish a Wi-Fi connection via an external Wi-Fi dongle. Please remove this dongle to disconnect the Wi-Fi connection after the service is complete.

The remote connection is established through an external program pre-installed on the instrument control computer and requires the user to provide their service representative with the computer ID and password displayed within the application. Please adjust the computer sleep settings so that the screen does not power off (no screensavers) as this will disconnect the remote connection.