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Use of flow cytometry (Novocyte Advanteon) to monitor the complete life cycle of the parasite Amoebophrya ceratii infecting its dinoflagellate host

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ABSTRACT

The parasite belongs to *Amoebophrya ceratii* (Amoebophryidae or MALVII, which stands for Marine Alveolate Group II, Syndiniales, Dinoflagellata, Alveolata), a complex species that includes intracellular marine parasites infecting and ultimately killing other dinoflagellates. During its life cycle, a free-living parasitic cell (known as dinospores, measuring 2-5 µm) penetrates its host, consumes it, and eventually undergoes sporulation. In this study, we present a method for identifying and quantifying the different stages occurring during the entire infective process of this parasite, using flow cytometry (NovoCyte Advanteon, ACEA Biosciences), equipped with two lasers (405 and 488 nm). This document presents the different setup for the acquisition, analysis, and data export.

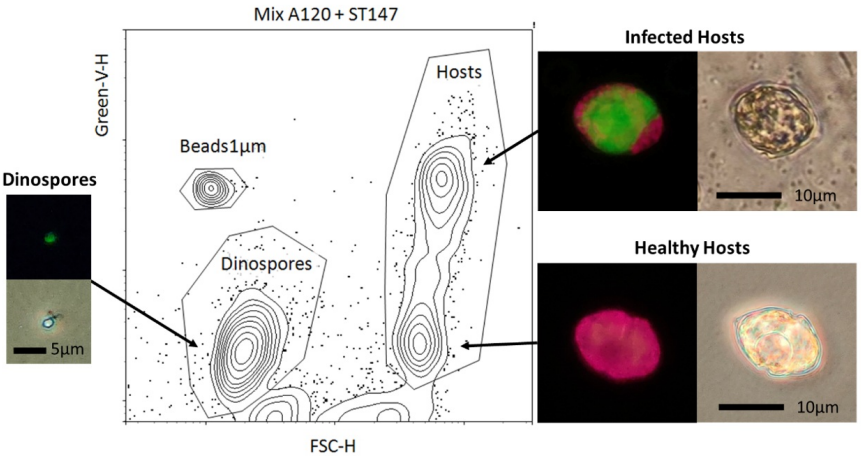

Figure 1: Cytogram displaying three distinct types of cells. The y-axis of the cytogram represents green fluorescence and the x-axis cell size, measured using forward scatter (FSC). The illustrations of the host and parasite cells on the cytogram are captured using an epi-fluorescence microscope (inverted microscope Olympus equipped with the U-MF2 Olympus cube (455/70 excitation, LP 515 emission) and a light microscope (using phase contrast). These visual representations aid in identifying and distinguishing the different cell types present on the cytogram.

Origin of strains

Strains of both the host and parasite have been isolated from the Penzé estuary, located in the north-west of France in the
English Channel (coordinates: 48°37'N; 3°56'W). The *Amoebophrya ceratii* A120 strain (RCC4398) was originally derived
from a single infected cell of *Heterocapsa triquetra* collected on June 13th, 2011, and subsequently incubated with exponentially growing *H. triquetra* (primary host HT150, RCC3596). Starting from April 23rd, 2012, A120 has also been

maintained in *Scrippsiella acuminata* (strain ST147, RCC 1627), formerly known as *Scrippsiella trochoidea* as described by Kretschmann et al. in 2015. The host strain ST147 was established from the germination of a single cyst collected from sediment in 2007. Strain A120 is a parasite belonging to MALVII-Clade 2, ribotype 4, based on the nomenclature of Guillou et al. (2008) and Cai et al. (2020).

All strains have been deposited at the Roscoff Culture Collection, https://roscoff-culture-collection.org/.

GUIDELINES

The flow cytometer:
https://www.agilent.com/en/product/research-flow-cytometry/flow-cytometers/flow-cytometer-systems/novocyte-advanteon-flow-cytometer-1270335

Novocyte Advanteon flow cytometer manual:
https://www.agilent.com/cs/library/usermanuals/public/150217-NovoCyte%20Advanteon%20Flow%20Cytometer%20Operator%20Guide.pdf

MATERIALS

Equipments:

Laminar flow cabinet (or biosafety cabinet)
Micropipette

Materials for Flow cytometry:

Cytometer tube (haemolysis tube) – Labellians – CML Group – Ref TH5-12PS

Cleaning solution: https://www.agilent.com/en/product/research-flow-cytometry/flow-cytometers/instrument-consumables-accessories/fluidics-system-solutions-for-flow-cytometry-1320867
- NovoFlow Sheath Fluid
- NovoClean Solution
- NovoRinse Solution

1µm fluorescent beads for standard: Fluoresbrite® YG Microspheres, Calibration Grade 1.00µm – Polysciences – Ref 18860-1

BEFORE START INSTRUCTIONS

Description of the flow cytometer and parameters used

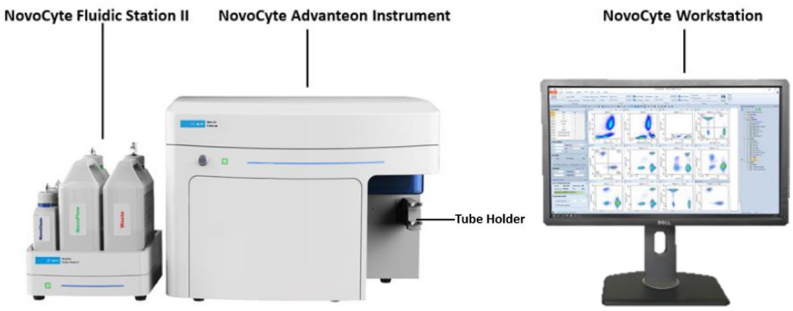

Figure 2: NovoCyte Advanteon (ACEA Biosciences)

We employed the NovoCyte Advanteon flow cytometer (ACEA Biosciences) for our study (Fig. 2), which is equipped with two lasers (488 nnm and 405nm wavelengths).

The cytometer's front panel includes the following features:

- A power switch: Steady green indicates the device is powered on, while flashing green signifies a shutdown cleaning procedure.
- An LED status indicator: Green represents normal status, orange indicates a warning (Click on the status bar in the NovoExpress software to review the warning message), and red signals errors (Click on the status bar in the NovoExpress software to review the error message).
- A front panel cover.
- The tube holder.

The NovoCyte Advanteon flow cytometer is equipped with four containers that are positioned in designated locations on the provided NovoCyte Fluidics Station (refer to Figure X). These containers serve various functions in the fluidics system:

- **"NovoFlow" sheath fluid container (green):**
  This container is used for hydrodynamically focusing the sample stream, ensuring precise analysis.
- **"NovoRinse" rinsing solution container (yellow):**
  This container contains a rinsing solution that is employed to wash away protein deposits, adherent cells, various debris, and other contaminants from the fluidics system.
- **"NovoClean" cleaning solution container (blue):**
  This container holds a cleaning solution used to thoroughly clean and maintain the fluidics system, ensuring its proper functioning.
- **Waste container (red):**
  This container is designated for the disposal of waste generated during the flow cytometry process.

The containers are continuously monitored by weight to provide real-time tracking of the remaining liquid volume. This functionality enables the system to issue a warning message when the fluid levels become critically low or when the waste container reaches its capacity.

Figure 3: Reagent containers & Tubing Connections

The host, *Scrippsiella acuminata*, could be detected by its content in chlorophyll, which autofluorescence in red under
blue light excitation (488 nm). *Amoebophrya*-like parasites infecting dinoflagellates are detected based on their natural bright green autofluorescence when illuminated under a violet light (405 nm) (Coats and Bockstahler 1994).

Based on that, cell identification by flow cytometry relied on distinctions in cell sizes and fluorescence signals, specifically in the green and red channels.

In our experimental setup, the blue laser (488 nm) serves multiple purposes. Firstly, it is used for the forward scatter measurement, which provides information about the size of cells and other particles. Additionally, the blue laser is responsible for excitation of chlorophyll, which is detected through red fluorescence (695/40). In the schematic representation (Fig. 3), the pathway from the blue laser to the photodetectors is highlighted in red. The default alias for the red fluorescence from the blue laser is "Chl," with the parameter name being B695. However, in this protocol, we modify the alias to "Red-B" to indicate "red fluorescence from the blue laser."

Furthermore, the violet laser (405 nm) is used to excite the parasite, which emits natural autofluorescence detectable in the green channel (525/45). The pathway from the violet laser to the photodetectors is indicated in green on the schematic representation (Fig. 3). The default alias for this fluorescence is "AmCyan," and the corresponding parameter name is V525. In this protocol, we opt to change the alias to "Green-V" to represent the green fluorescence resulting from violet excitation.

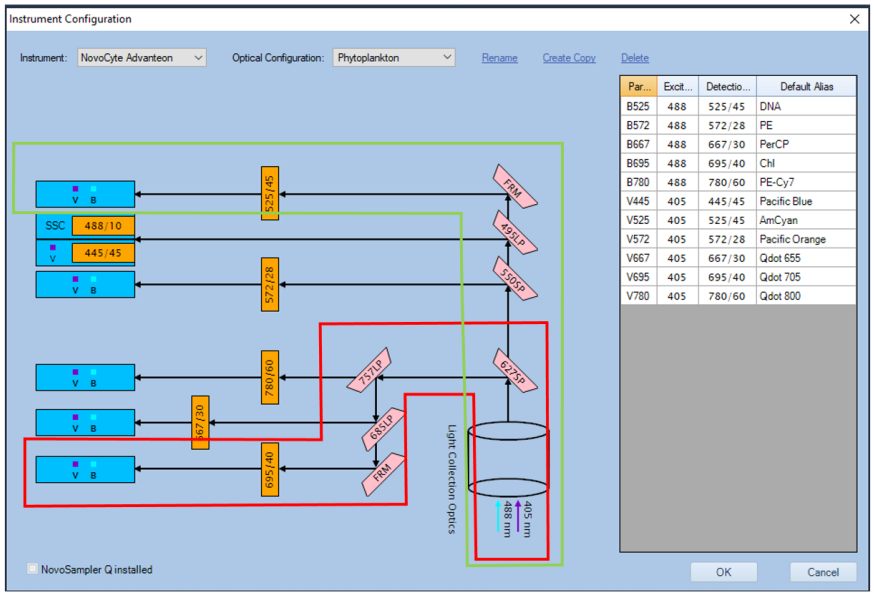

Figure 4: A screenshot of the "Instrument Configuration" in the dedicated software of the flow cytometer is

provided. The two boxed paths represent the filters specifically designed for the detection and counting of the host and parasite. The green path corresponds to the detection of green fluorescence (525/45) emitted by the violet laser (405 nm), while the red path corresponds to the detection of red fluorescence (695/40) emitted by the blue laser (488 nm).

Flow cytometer startup procedure

1

- Press the power button located on the front of the flow cytometer to switch it on. The flow cytometer will initiate a series of fluidics steps automatically, which typically takes approximately 5 minutes.
- Switch on the computer.
- Launch the cytometer software (NovoExpress).
- Log in to your session.

Comment 1 : Once the automatic fluidic startup process is completed, the status displayed at the bottom left of the NovoExpress window should indicate "Ready." This indicates that the flow cytometer is prepared for use. If any other status is displayed, such as indicating a need for maintenance, please contact the platform manager for assistance.

Comment 2 : Check cleaning solutions : If there is a warning prompts from the status bar regarding the containers, fill the sheath fluid (“NovoFlow”) and/or the cleaning solution (“NovoRince”, “NovoClean”) containers and/or empty the waste.

Flow cytometer setup for acquisition

2

2.1 Flow cytometer parameters

The NovoExpress software provides access to adjustable parameters and acquisition modes for the flow cytometer. To access these settings, open the "**Cytometer settings**" window (Fig. 4). If the window is not visible, go to the "View" tab at the top and click on "Cytometer settings" to display it.

- **"Parameters":**
  This allows you to select the parameters to be recorded during acquisition and their respective gains. By default, forward scatter (FSC) and side scatter (SSC) are selected in height. Additionally, you must select the Green-V (V525) and Red-B (B695) photodetectors in height, which correspond to the green fluorescence of the parasite and the red fluorescence of the host's chlorophyll. The default gain settings are suitable for analysis, but they can be adjusted if necessary. An underlined gain value indicates a manual change that deviates from the default value.

- **"Stop condition":**
  This allows you to specify the quantity of the sample to be analyzed. The default setting is 1 minute, but this can be adjusted as needed. It is also possible to set a stop condition based on volume, which is useful for analyzing a specific volume of the sample. In such cases, the cytometer will only collect the specified volume from the tube. If the sample volume is limited, you can define a small volume, such as 50µl, for example.

- **"Flow rate":**
  This allows you to select the volume of sample per minute to be analyzed and the core diameter size. NovoExpress provides three preselected flow rates (Slow, Medium, and Fast). For the A120 + ST147 strain combination, a Fast flow rate is suitable, such as 66µl/min with a core diameter of 16.8µm.

- **"Threshold":**
  This parameter allows you to define the value at which an event is considered for analysis and recording by the software.

Setting a threshold is important to avoid recording noise and ensure accurate results. Without a threshold, the cytometer will record all events, leading to incorrect results due to excessive noise. An optimal threshold is a balance between the number of events per second (which should be as low as possible) and the parameter value of the event of interest (such as fluorescence and FSC of a cell). The recommended threshold is based on the Green-V-H parameter with a value of 1000. In cases with a high number of events per second, the threshold can be increased up to 3000. The maximum number of events per second is 4000.

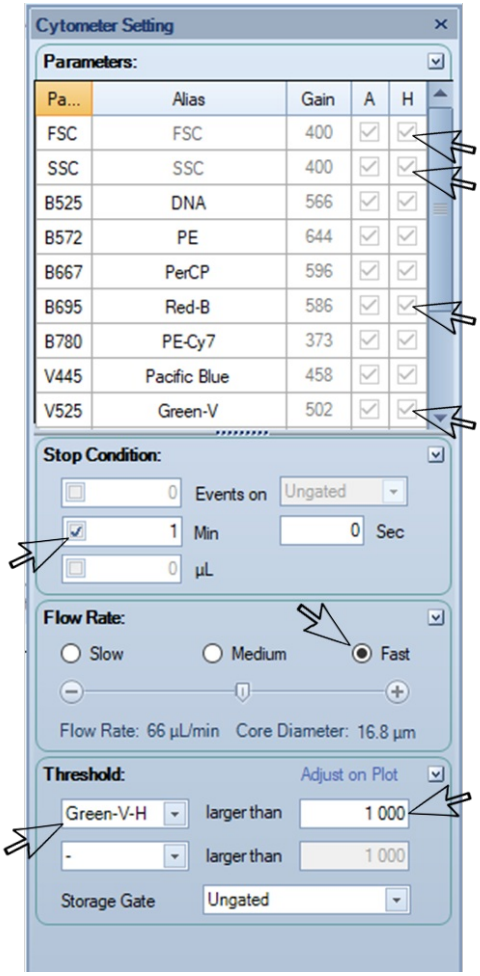

Figure 5: Screenshot from NovoExpress, the cytometer settings, arrows indicate the specific points that need to be settled.

2.2 Data management: Samples, specimens, and groups

The data generated during the analysis can be accessed and managed through the "**Experiment Manager**" window (Fig. 6). If the window is not visible, go to the "View" tab at the top and click on "Experiment Manager" to display it.

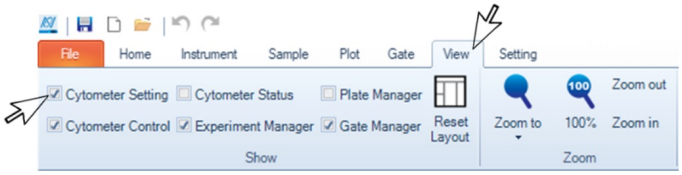

Figure 6: Screenshot of NovoExpress demonstrating how to display the "Cytometer Settings" window.

Terminology:

• A **"sample"** refers to a single acquisition from a tube, representing a measurement.
• A **"specimen"** is a group of samples. Each sample must belong to a specimen.
• A **"group"** is a collection or folder containing multiple specimens. Creating a group is optional.
• An **"experiment"** is the complete file compatible with the NovoExpress software, which includes the analysis. It contains the samples, specimens, groups, and metadata of the analysis (date, analysis template used, gates, etc.). The file is saved on the computer with the ".ncf" file extension.
• A **"population"** in flow cytometry defines a cluster or set of dots or events on a plot or cytogram.

In NovoExpress, analyses are stored in the experiment file. When the software is launched, a new experiment file is automatically created but not yet saved. If the analysis is initiated at this point, the software will prompt to save the experiment file (which will have a ".ncf" file extension) when the first sample is run. Additionally, instead of creating a new experiment file from scratch, it is possible to open an existing ".ncf" file. The "Experiment Manager" is a window within the software that allows for visualization of the list of experiments, samples, and groups, as well as the creation of new ones.

- **Creation of an "experiment"**

Click on: File / New / New Blank Experiment (Fig. 7)

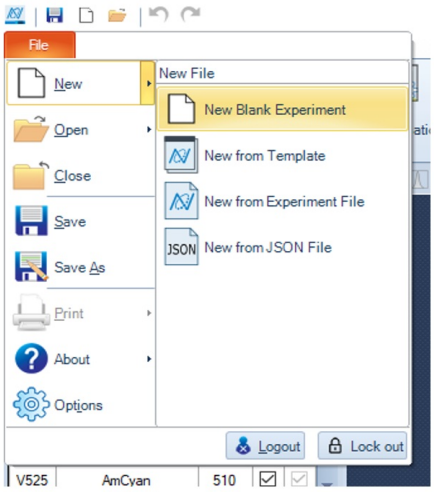

Figure 7: Creation of a new experiment

- **Open an existing "experiment"**

Click on: File / Open, then select the file with the browser.

- **Create of a new specimen**

Right-click on the first line (file_name.ncf) in the "**Experiment Manager**" window, then select **"New Specimen" (Fig. 8).** If the "**Experiment Manager"** window is not visible, go to the "View" tab at the top and click on "Experiment Manager" to display it.

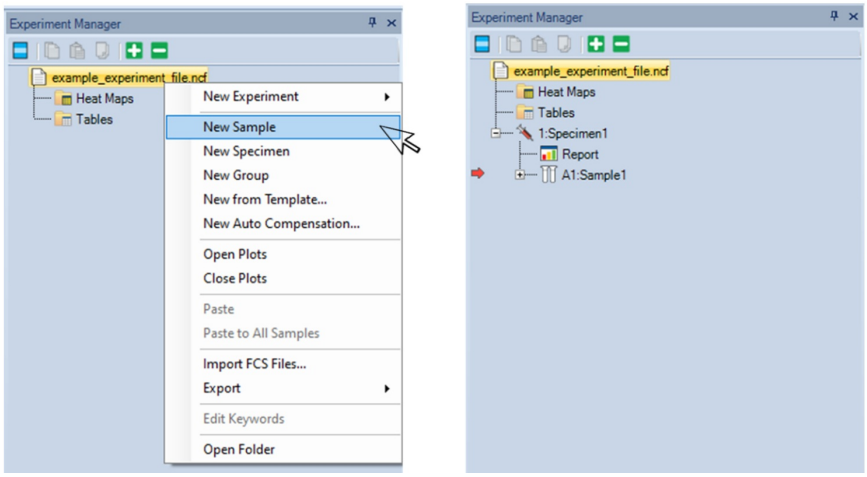

Figure 8: creation of new elements: sample, specimen or group, from the “experiment manager”.

- **Creation of a new sample**

There are different ways to perform these actions:

1. Right-click on the first line (file_name.ncf) in the "**Experiment Manager**" window, then select "New Sample" (similar to creating a new sample or group) (Fig.8).
2. If the last sample in the list is selected, click on "Next Sample" at the bottom left (Experiment Control).
3. Right-click on a sample in the list and select "Duplicate" (or after selecting a sample with a left click, press Ctrl+D on the keyboard). This is the fastest method and retains all the parameters and gates.

To move a sample from one specimen to another, right-click on the sample name, then select "Move to Specimen" and choose the desired specimen.

- **Creation of a new group**

To create a new group, right-click on the first line ("file.ncf") in the **"Experiment Manager**" window, then click on "**New Group**".

Please note that the creation of a group is optional and can be done before or after the creation of a specimen.

To move a specimen into a group, right-click on the specimen, then select "Move to Group" and choose the desired group. All the samples contained within the specimen will be moved along with their events.

- **Duplication of groups, specimens and samples**

To duplicate an item, right-click on it and select "Duplicate" (Fig.9).

Please note that when duplicating a sample, the cytometer settings (detectors, alias, stop conditions, etc.) and the analysis template (plots and gates) will be preserved. When duplicating a specimen or group, the list of samples will be copied, including their names, cytometer settings, and analysis template, but not the events. Duplicating a specimen is particularly useful when measuring the same type of samples repeatedly over time.

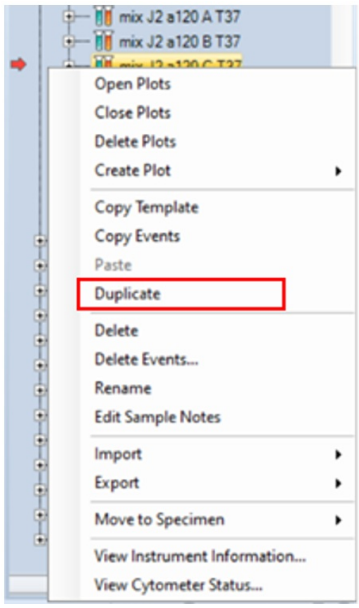

Figure 9: duplication of a sample to create an empty (without events) copy of the sample. Right-click on the item then “Duplicate”.

Sampling of the culture for the analysis

3

- Collect a sample of the culture for analysis by using a laminar flow cabinet (or biosafety cabinet) and extracting approximately 300 µl of the fresh co-culture into a specific flow cytometry tube, also known as a hemolysis tube.
- Vortex the working mix of beads (as the beads have a tendency to settle in the tube) and then add 1% (vol:vol) of 1µm fluorescent beads, which are concentrated at approximately 800,000 beads/ml and are used as a standard during the analysis.
- Mix the tube manually and place it in the tube holder of the flow cytometer.

Comment : The beads serve as stable spatial markers on the cytogram, aiding in the identification of cell populations. Another advantage of using beads is the ability to verify the proper functioning of the cytometer. In fact, the beads' population on the cytogram should appear fairly round, and any deviation from this shape could indicate a fluidic issue in the flow cytometer.

Running the sample on the cytometer

4

In NovoExpress, the "Experiment Control" window enables the initiation of the analysis (Fig. 10).

Before running the sample, select the option "**Recover remaining samples**". This allows for the retrieval of any leftover material and helps prevent clogging of certain parts of the cytometer, thereby facilitating system maintenance. Additionally, ensure that the options "**Absolute Count**" (for calculating cellular densities in number of cells per ml) and "**Rinse after sampling**" (to rinse the sampler needle after each sample) are chosen.

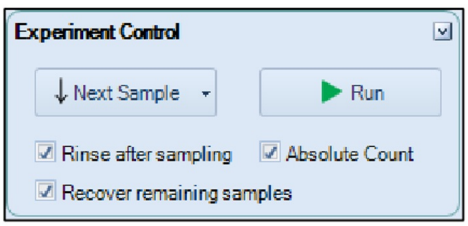

Figure 10: Experiment Control with the three option picked

Once the tube is properly positioned in the tube holder of the flow cytometer and the three options have been selected, click on "**Run**" to initiate the analysis. The sampler needle will extract the sample from the tube. It is important to keep the tube in its position throughout the analysis. The needle will then return the remaining volume back into the tube due to the selection of the "**Recover remaining samples**" option.

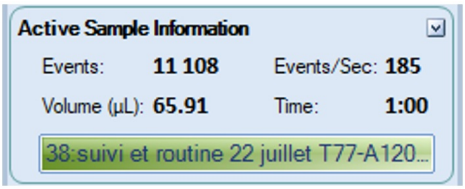

Figure 11: Active Sample Information: running information are visible during acquisition.

Data visualisation and creation of the template

5 By clicking on the icons in the toolbar (Fig. 12), you can create the corresponding plot and gate.

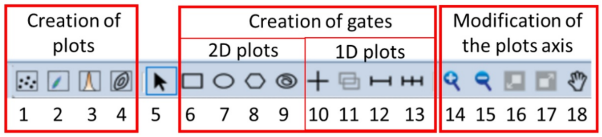

Figure 12: Screenshot of the toolbar in NovoExpress.

Description of the icons for the creation of plots, gates and adjustment of the plots (Fig. 12):
1: Dot plot (2D plot)
2: Density plot (2D plot)
3: Histogram (1D plot)
4: Contour plot (2D plot)
5: Pointer (basic tool)
6: Rectangular gate (for 2D plot)
7: Elliptical gate (for 2D plot)
8: Polygon gate (for 2D plot)
9: Freehand gate (for 2D plot)
10: Quadrant gate (for 2D plot)
11: Logic gate
12: range gate (for histogram)
13: Bi-range gate (for histogram)
14: Zoom in
15: Zoom out
16: Auto range of the axis
17: Full range of the axis
18: Move (pointer type to shift plot)

- **The template: Create density plot and histograms for the analysis of A120+ST147**

To create the template for analyzing A120+ST147, follow these steps:

5.1 1) Create a 2D plot, such as a contour plot, and two histograms using the buttons in the toolbar.

5.2 2) Define the scales for the axes (refer to Fig. 13): Set the parameters' axis scale to "log" and the counts' axis scale (y-axis of the histogram) to "linear". This will enhance the contrast between the populations and improve visibility.

5.3 3) Define the axis of the 2D plot (refer to Fig. 13): Set the y-axis as Green-V-H and the x-axis as FSC-H.

5.4 4) Draw 2D gates (e.g., polygon gate) around the events corresponding to:

a. The beads (located in the middle-left region).
b. The dinospores (below the beads, in the bottom-left region).
c. The hosts (in their respective region).

These gates will help separate and identify the different populations in the analysis.

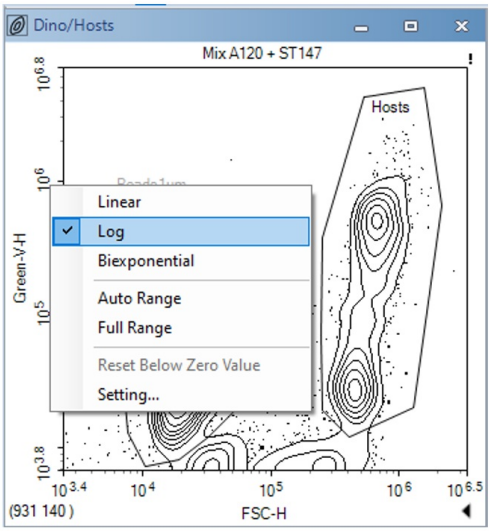

Figure 13: Selection of the scale type of the axis of a plot.

Comment: It is indeed easier to identify different populations on the cytogram when working with samples containing only one type. To facilitate this process, the following approach can be adopted:

- Run a blank sample containing only beads as a stable spatial marker. This will provide a reference for identifying the bead population on the cytogram.

- Run a sample containing only healthy hosts. This will allow you to identify the specific population corresponding to healthy hosts on the cytogram.

- Run a sample containing only dinospores. This will enable the identification of the dinospore population on the cytogram.

By conducting several trials and analyzing the resulting cytograms, you will be able to deduce the distinct dot clusters representing each population and assign specific gates accordingly. This iterative process helps in accurately identifying and separating the different populations within the analysis.

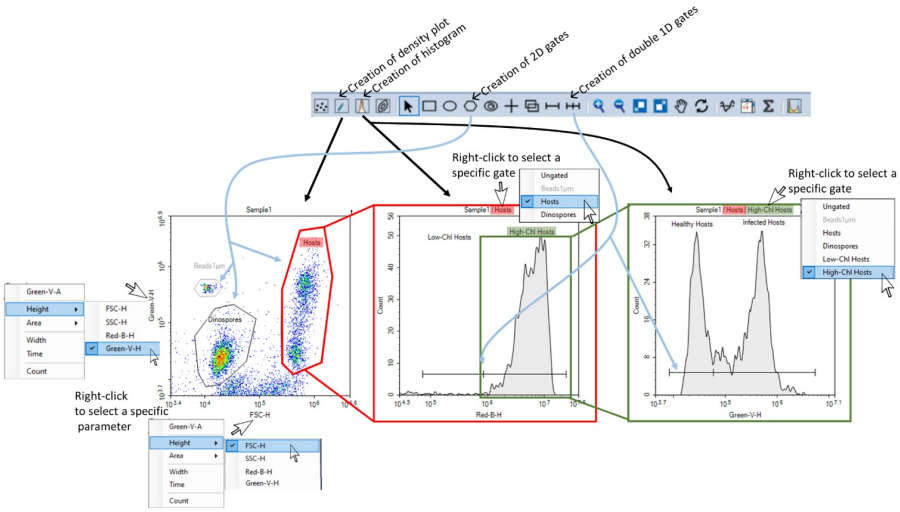

Figure 14: Creation of the template of the analysis with actual data displayed of a co-culture host and parasite with dinospores, healthy hosts and infected hosts.

5.5 5) Define the x-axis for the two histograms:

a. Set Red-B-H as the x-axis for the first histogram.
b. Set Green-V-H as the x-axis for the second histogram.

5.6 6) In the second plot (first histogram), select the "hosts" population for gating. This will allow you to focus on the host population in the analysis.

Create a 1D gate or a bi-range gate (a range gate is also suitable) to identify hosts rich in chlorophyll. This gate will help remove cell debris and dead cells from the host population. Name this gate "High-Chl Hosts." If you created a bi-range gate, you can name the other gate "Low-Chl Hosts."

In the third plot (second histogram), select the "High-Chl Hosts" population for gating. This will narrow down the analysis to the hosts with high chlorophyll content.

5.7 7) Create a bi-range gate in the third plot to determine two populations:

a. The low green hosts, which will be the "Healthy Hosts" gate.
b. The high green hosts, which will be the "Infected Hosts" gate.

By following these steps, you will be able to define gates and separate different populations, such as healthy hosts and infected hosts, in the analysis of A120+ST147.

Comment: You can adjust the upper and lower values of the axis to center the population in the plots by following these methods: Click and drag the extremities of the axis on the plots and/or use the tools in the toolbar for modifying the plot axis.

5.8 8) The template is a file that contains all the information regarding flow cytometer settings and plot visualization. This file should be in the specific format provided by ACEA Biosciences.

To create a template, follow these steps:

1. Right-click on the sample.

2. Click on 'Export,' and then select 'Export as Template...'
3. Save the template file.

To upload and use a template, use the following steps:
1. Right-click on a sample.
2. Choose 'Import,' then 'Template.'
3. Browse through the folders to select the desired template.

Alternatively, you can apply the selected template to the targeted sample(s) as followed:
1. Click on the '+' icon in front of the sample listed in the 'Experiment Manager.'
2. Drag and drop the 'Analysis' onto another sample, a specimen, or even onto the experiment (located at the top of the experiment manager).

Comment: It is good to know that the “Cytometer Setting” contains “Parameters”, the “Stop condition”, the “Flow rate” and the “Threshold” and can be dragged and dropped like the “Analysis” section but only to an empty sample because that information will define how the data of the sample will be recorded and cannot be modified after the acquisition.

Also, by double clicking on “Report section”, you can have access to information and visualization and most importantly, the date (day and time) of the record of the sample. The date of the sample is also visible in the “statistical table” (See below, “Export data”).

Save and export data

6 **Before the exportation of the data:**

To ensure accurate analysis and proper data management in NovoExpress, please follow these guidelines:

1) Ensure well-defined gates around the desired populations: It is important to carefully place the gates on the cytograms to accurately capture the desired populations. Incorrectly placed gates can result in incorrect counts in the statistical tables. If gates are moved during subsequent analyses of the cytograms, the counts will automatically update to reflect the new population definitions.

2) Set the density unit to cells per ml: To ensure the unit of density is displayed as cells per ml, navigate to "Setting" > "General" > "Absolute Count" and select "No./ml". This setting will provide cell densities ("Abs. Count") in cells per ml.

3) Manually save the experiment file: It is necessary to save the experiment file, which includes all the plots, gates, and statistical tables. To save, either click on "File" > "Save" from the menu or click on the save icon. If you attempt to close NovoExpress without saving, a reminder message will prompt you to save the data. In such cases, click "Yes" to save the data before closing.

By following these guidelines, you can ensure accurate analysis, maintain the desired data settings, and properly save your experiment data in NovoExpress.

Creation and export for data table (density of the cell population, mean value of fluorescence...)

All types of data from the analysis, such as cell densities, fluorescence values, and more, can be stored in tables that can be exported as CSV files for use in spreadsheet software.

To create a statistical table in NovoExpress, follow these steps:

- Click on the "Home" tab.

- Look for the option to create a "Statistical Table" (refer to Fig. 15).

- Click on the "Statistical Table" button to create a new table.

By following these steps, you can create a statistical table in NovoExpress to organize and store your analysis data, which can then be exported as a CSV file.

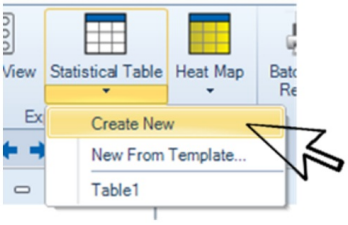

Figure 15: Creation of a data table (statistical table).

Once created, the statistical table is stored within the ".ncf" file and can be accessed from the top section of the "Experiment Manager" window (refer to Fig. 16).

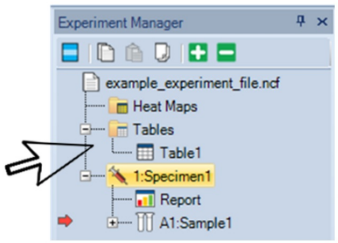

Figure 16: The “statistic table” reachable on the top in the “Experiment Manager” window.

To set up the statistical table and choose the desired variables, follow these steps:

1) Open the statistical table in NovoExpress.

2) In the statistical table window, select the metadata columns you want to include in the table. To do this:

a. Click on "Show column" or a similar option.
b. Choose the metadata variables you want to display, such as "Specimen", "Sample", and "Run time." These columns will provide metadata information about the samples.

3) Next, select the actual data columns you wish to include in the table. To do this:

a. Click on "Add column" or a similar option.
b. In the "Statistics" section, choose "Abs. Count" to include cell densities in cells per ml.
c. Select the gates for which you want to display the data. Hold the Ctrl key on your keyboard to select multiple gates.
d. Choose the gates corresponding to "Dinospores," "Healthy Hosts," and "Infected Hosts" (as shown in Fig. 17).

By following these steps, you can set up the statistical table in NovoExpress by selecting the desired metadata columns and adding data columns, including cell densities and specific gate populations, for analysis and further exploration. (Fig. 17).

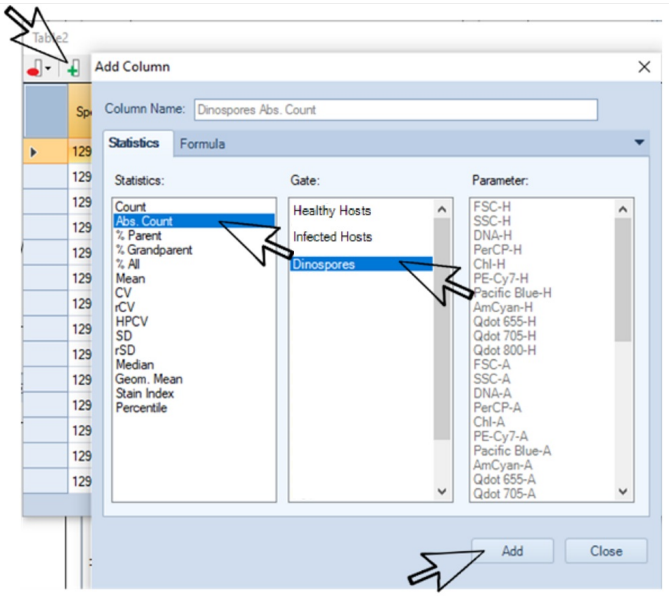

Figure 17: Selection of the types of data and gates of interest.

Comment: It is important to ensure that "Abs. Count" is selected in the statistical table, as it provides the density of cell types. This is different from the "Count" column, which simply represents the number of events and does not reflect density.

To export the statistical table as a CSV file, follow these steps:

- Open the statistical table in NovoExpress.

- Locate the "export" button, typically located in the upper right corner of the statistical table window.

- Click on the "export" button. This will initiate the export process.

- Choose a location to save the exported CSV file and provide a file name.

- Click "Save" to save the statistical table as a CSV file.

By following these steps, you can export the statistical table in CSV format, allowing you to further analyze and manipulate the data using other software or tools.

Instrument shutdown

7 To turn off the flow cytometer and initiate the cleaning process, follow these steps:

- Locate the power button on the flow cytometer device.
- Press the power button. This will start the automatic cleaning process.

Note: The computer can be either on or off during this process, as the cytometer will perform the cleaning independently.

Additionally, you have the option to clean and rinse the cytometer without shutting it down, which allows another user to use it afterward. To do this in NovoExpress, follow these steps:

- Go to the "Instrument" tab in NovoExpress.
- Click on either "Cleaning" or "Fluidics Maintenance Sequences." This will open a window where you can create a custom

cleaning sequence.
- Set up the cleaning sequence according to your requirements, such as "Rinse + Cleaning" or "Debubbling + Rinse."

At the bottom left of the window, there should be an option to specify whether or not to shut down the system after cleaning. Select the appropriate choice based on your needs.

NovoExpress will provide an estimation of the time required for the cleaning process.

By following these steps, you can turn off the flow cytometer and initiate the cleaning process automatically or create custom cleaning sequences without shutting down the system in NovoExpress.

Comment: In the main window of the software, at the bottom left, there is an indication of the ongoing cleaning process. While the cleaning is in progress, you cannot analyze new samples. However, you can still visualize the samples, access the statistical tables, view the data, and make adjustments to the gates. This allows you to continue working with previously analyzed data and make any necessary modifications during the cleaning process.

Comment: it is possible to open several ".ncf" files at the same time. It can be useful if you want to see or export data from an older file during a current analysis (or when another user does an analysis). Also, it is convenient to compare sample between different file or experiments.

Comment: You can access the flow cytometer software on the computer even when the flow cytometer itself is turned off. This allows you to manage your samples, specimens, and groups, as well as work on the data before or after analysis.