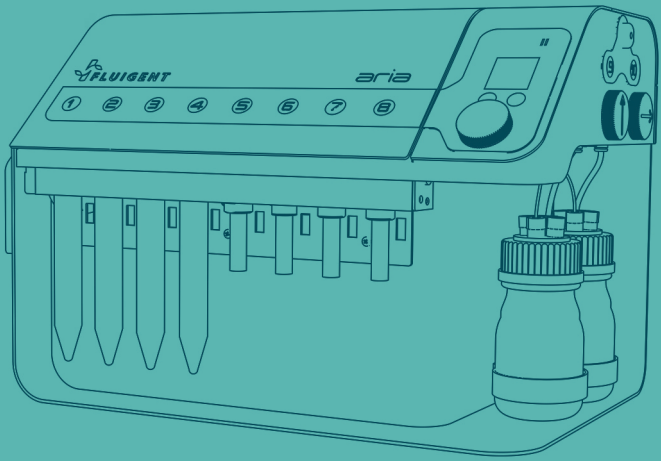
USER'S
MANUAL

ARIA



FLUIGENT

APRIL 2024

SAFETY INFORMATION

In the following cases, the intended protection of the device may be impaired. In these cases, the owner is liable for damage to property and personal injury:

- The device is not used per the user manual.
- The device is not used for its intended purpose.
- The device is used with accessories or consumables not recommended by Fluigent.
- The user has made unauthorized modifications to the device.

System use and stocking conditions

The system is intended to be used in an environment fulfilling the following conditions:

- Standard operating conditions: 20°C, with 40% relative humidity
- Altitude: up to 2000 m
- Minimum temperature: 10°C
- Maximum temperature: 40°C
- Maximum humidity: 80% relative humidity
- Indoor use
- Pollution: pollution degree 2

Caution

- **System installation:** Please manipulate the system carefully. The system must be placed on a clean, flat surface in an environment respecting the conditions listed in “System use and stocking conditions". Please do not take out the plastic film before installing the system.

- **Power supply:** The Aria is intended to be used with the 24V DC / 6.67A power supply included in the packaging. The use of any other power supply might cause security issues and will cancel the warranty.

Warnings

- **Manipulation during functioning:** Do not manipulate any part of the system during functioning.

- **Cover:** Do not open/close the system cover during system functioning

- **Ethernet port:** Only use the 2-SWITCH™ ethernet port of the Aria to connect the 2-SWITCH™ or M-SWITCH™ provided with the Aria

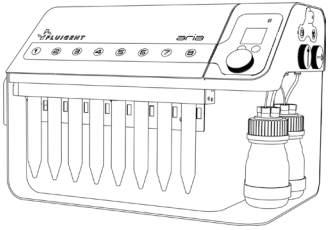
- **Humidity:** Do not use the machine in a humid environment

- **Pressure module power supply:** Do not power up the dedicated pressure supply with another cable than the one provided with the system by Fluigent.

- **Liquid hazards:** Do not put in contact with any dangerous liquids. Do not use corrosive liquids such as acids, alkalis, bases and caustic solutions, inflammable liquids, CMRs ...

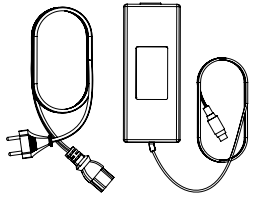
Please note that if the device is not used in accordance with the instructions described in the manual, product functioning as well as safety may be compromised.

WHAT IS INCLUDED IN ARIA'S PACKAGE

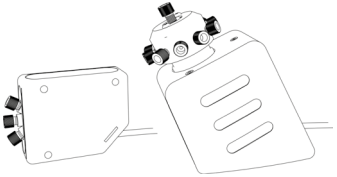


Aria unit

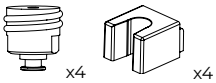
Inlet pressure tubing (2m)



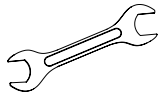
Power supply



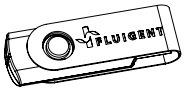
2-Switch or M-Switch



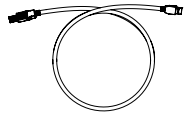
2 mL reservoir adaptor kit



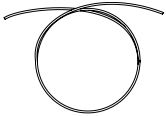
2mL adaptor wrench



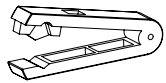
USB Key (with Aria software and its SDK)



USB cable



Fluidic outlet tubing (2m)



Tube cutter

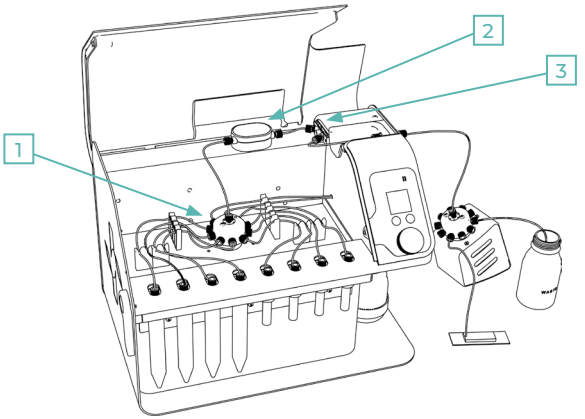
2x F-120 connector

2-Switch: 6 XP-235 et 12 ferrules
M-Switch: 15 XP-235 et 30 ferrules

ARIA USER'S MANUAL

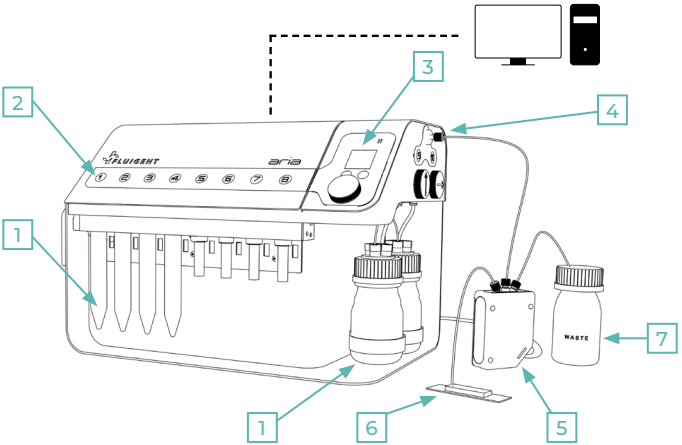
INTRODUCING ARIA

Aria is an automated perfusion system that automates perfusion or timed injection protocols destined mainly for Spatial Omics applications. It's an all-inclusive, user-friendly solution for Immunolabeling, FISH, and DNA-paint experiments but can easily be adapted to other applications where sequential controlled cellular perfusion or timed injection protocols are needed. It allows for the sequential delivery of up to 10 different solutions at the desired flow rate into a microfluidic chip or a perfusion chamber.

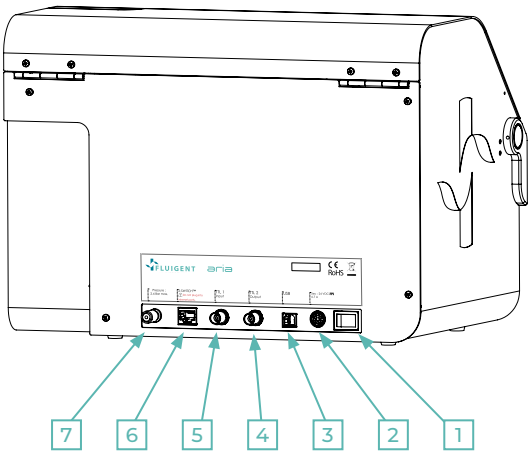


1	Rotary valve selects the solution to inject
2	Flowmeter (Flow Unit) sets injection flow rate
3	Stop flow valve can stop the flow rapidly

Aria uses a pressure pumping principle to inject solutions. During injection, Aria pressurizes all the reservoirs, and the selected solution is pushed out of its reservoir and passes through the flowmeter before exiting the instrument. The flowmeter measures the flow rate and constantly adjusts the pressure to maintain the desired flow rate.



1	Reservoirs containing the solutions to inject
2	Numbered buttons to select solution to inject
3	Injection flow rate control panel (both manual and automated control are possible)
4	Fluidic output
5	Outlet switch valve (2-Switch or M-Switch) directs flow to waste or to the application chip
6	Application chip
7	Waste reservoir



1	Main power switch
2	Power input (24VDC – 6.67A)
3	USB port for PC connection
4	TTL 2 BNC port : TTL synchronization output port (5V, 100ms pulse)
5	TTL 1 BNC port : TTL synchronization input port (5V, 100ms pulse)
6	External valve (2-Switch or M-Switch) Connection port
7	Air pressure input

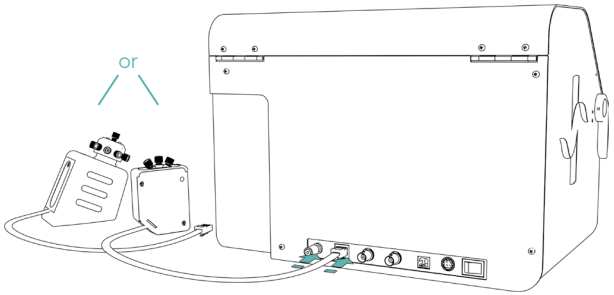
SETTING UP

Please follow the instructions listed below to ensure the proper functioning of the system. The system and all components must be positioned on a flat and stable surface. Before starting, please remove all foams from the package.

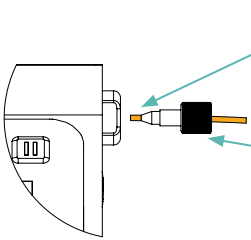
INSTALL OUTLET VALVE

In this section, the user will build up their fluidic circuit. In order to cut the tubing at the right length, Fluigent advises the user to place Aria where it will operate. It is recommended to place the external valve as close to the chip as possible. The tube cutter should be used to cut the fluidic outlet tubing at the right dimensions. It is recommended to minimize the fluidic pathway to optimize performance (reduce reagent consumption, injection responsiveness, maximum flow rate, estimated time of arrival, etc.).

Aria has an outlet switch valve (2-Switch or M-Switch). It directs the outlet solution either to the application or to waste at any moment.

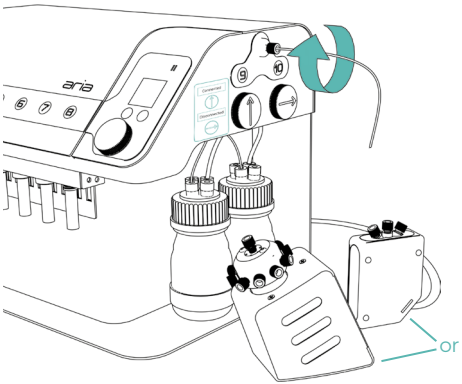


Plug the external valve to Aria



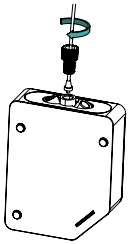
the tube cut must be flat and clean to avoid any problem.

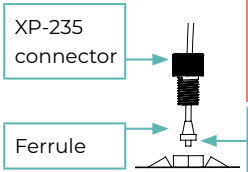
Insert the fluidic tube through the conical F-120 connector. The tube should go 1mm past the end of the connector.



Screw the connector and the tube in the fluidic output.

2-Switch valve version

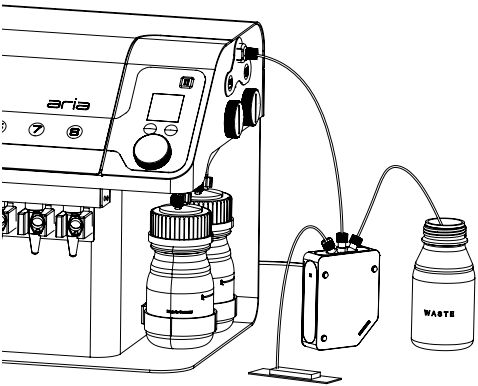


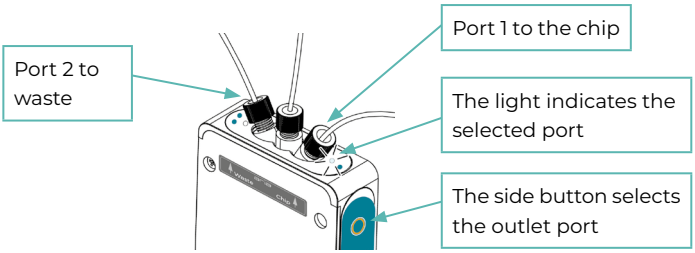


the tube cut must be flat and clean to avoid any problem.

Tube should come out of the ferrule (about 1mm)

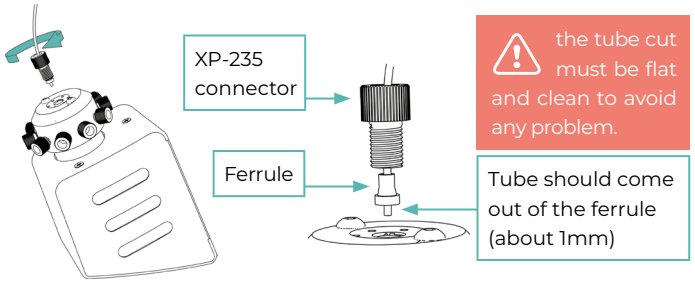
Insert the other end of the tube into the XP-235 connector and the ferrule, then screw it into the 2-Switch's central port.
Note: The tube should go 1mm past the ferrule. Please note that the 2-Switch should be placed as close as possible to the chip.



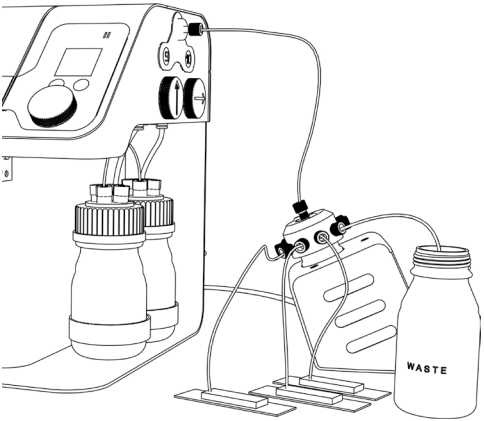


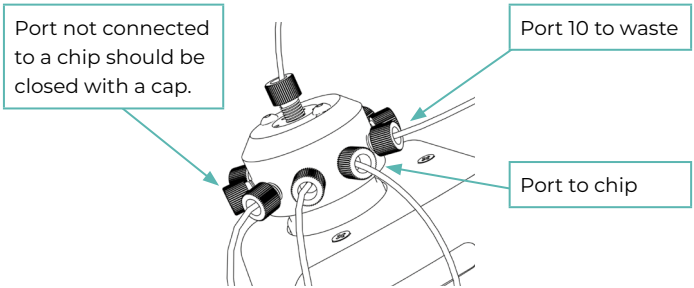
Connect port 1 (port with 1 dot) to the chip and connect port 2 to the waste. The port lighted on the 2-Switch indicates the direction of the flow.
To manually select the direction of the flow (to the waste or to the chip) press on the side button.

M-Switch valve version



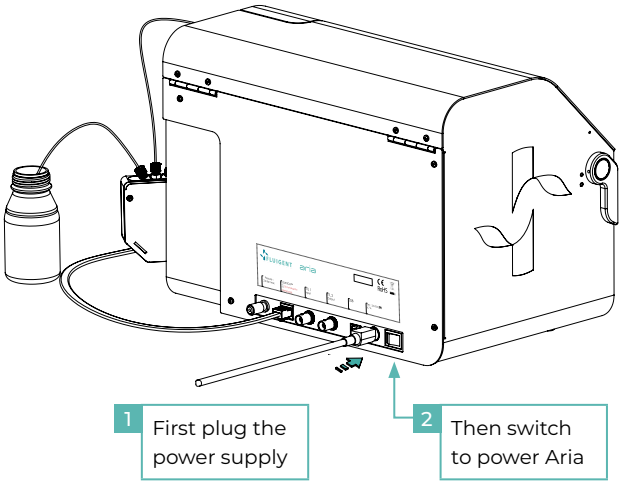
Insert the other end of the tube into the XP-235 connector and the ferrule, then screw it into the M-Switch's central port.
Note: The tube should go 1mm past the ferrule. Please note that the M-Switch should be placed as close as possible to the chip.





Connect the external ports to the chips. Port 10 must be connected to the waste. Cover the ports not connected to a chip with a cap.
Note: The M-switch cannot be used in a manual mode.

POWER ON

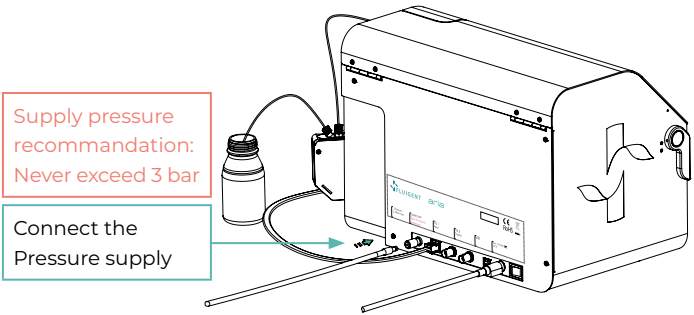


Once powered on, Aria will initialize:

- Numbered selection buttons will blink
- The internal valve will rotate and make a sound
- The control panel display will ask you to adjust the inlet pressure (see next page)

PRESSURE ON

Always wear safety protection when manipulating pressure.



Aria needs an external pressure supply to operate. This can be Fluigent's FLPG+ pressure source, a local compressed air supply, an air compressor, or any clean (filtered <10µm) dry compressed air supply.

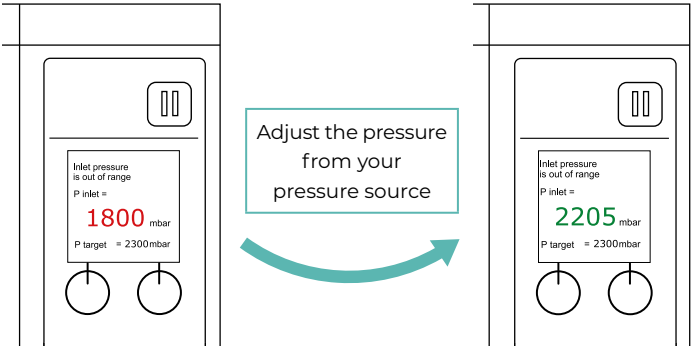
The required inlet pressure is 2.3 bar (range 2 - 2.4 bar). We advise you to first connect the air supply without pressure.

If you don't have an air pressure supply capable of supplying the required pressure, please contact Fluigent.

If you intend to use a compressed gas other than air, please contact Fluigent for further information.

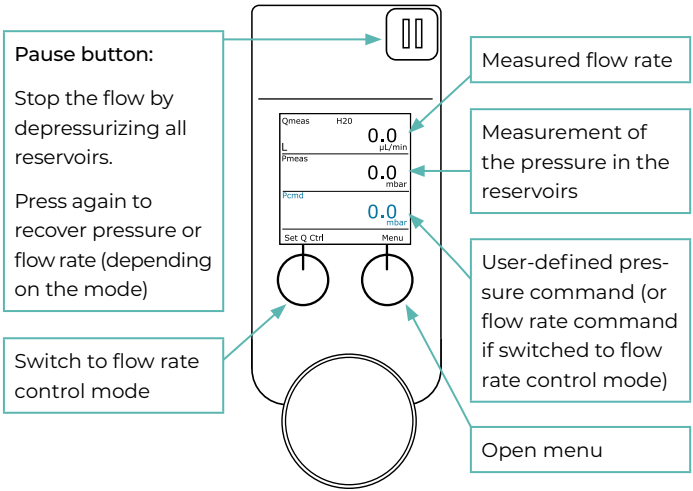
ADJUST THE SUPPLIED PRESSURE

If the inlet pressure does not meet the required pressure range (2 – 2.4 bar) the screen below will be displayed on Aria. The inlet pressure needs to be adjusted into the correct range.



When the inlet pressure is in range, the P inlet value will turn green and Aria will display the *"Operation window"*.

OPERATION WINDOW

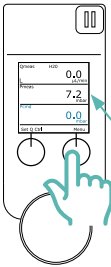


The pressure sensor inside the Aria needs to be calibrated before the first use or when necessary (see next page).

Before this calibration, the measured pressure in reservoirs (Pmeas) may not be correct.

PRESSURE SENSOR CALIBRATION

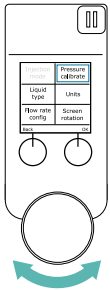
To be done before the first use or when necessary.



1

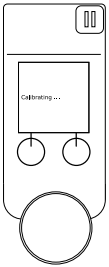
Press *“Menu”*

An example of Pmeas before calibration



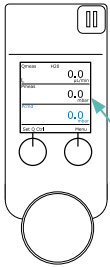
2

Use the dial (turn and press) to select *“Pressure calibrate”*



3

Wait a few seconds



4

Done!

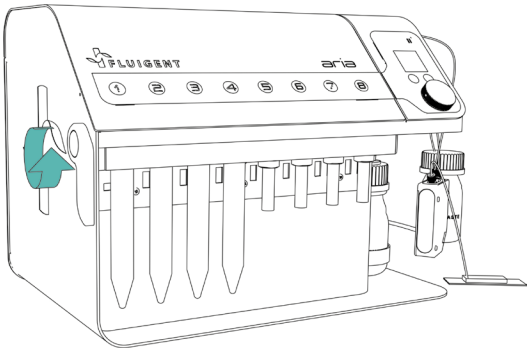
Pmeas value after calibration (Pmeas=0)

Due to the high sensitivity of the internal pressure sensor, you may observe normal fluctuations of the measured pressure (Pmeas), even after the calibration is complete.

FLUID RESERVOIRS

FRONT TUBES

Aria can remain powered on to proceed.



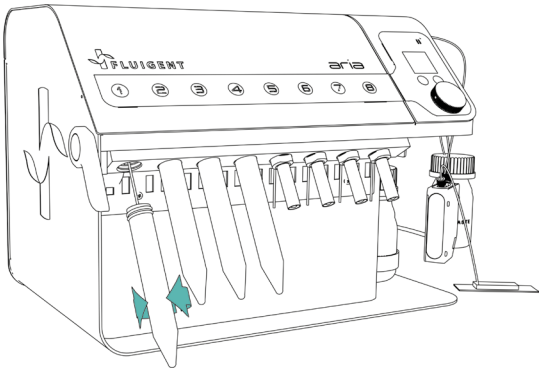
Pull up the side handle to move the tube holder to the open position (the buttons will blink).

The fluidic tubing is tilted to ease insertion into the reservoirs.

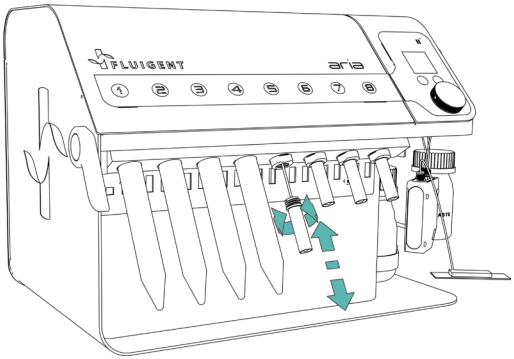
The solutions placed in the reservoirs should first be filtered through a 0.2µm diameter filter.

Reservoir compatibility: 1.5 mL and 2 mL Eppendorf, 15 mL and 50 mL Falcon

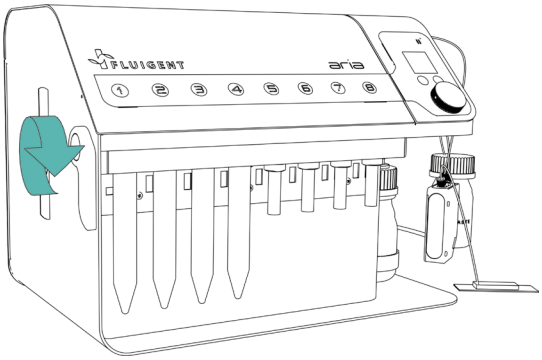
To ensure no bubbles are generated through reservoir switching, we recommend to fill all reservoirs with water or a buffer solution



For 15mL tube: unscrew the tube, refill and screw it back in.



For 2 mL tube: pull out the tube, refill and put it back.



Push down the handle to move the tube holder to the closed position (the buttons will stop blinking).

Please note that a tube needs to be placed at each position even if the tube is empty and not used during the course of the experiment. If the tubes are not securely sealed the buttons above will blink.

Please do not pull up the handle during an injection process. Doing this will cause Aria to depressurize all the reservoirs and stop the injection process.

Please note that you should press the *"Pause"* button on the control panel before refilling the tubes to depressurize all the reservoirs.

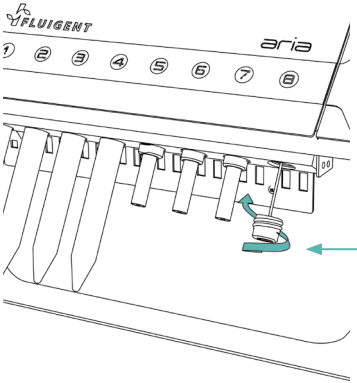
CONVERT A 15ML TUBE POSITION TO A 2ML TUBE POSITION

Aria can remain powered on to proceed.

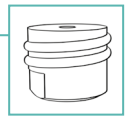
Aria's tube holder has 8 positions fitted for 15mL tubes by default.

However, with the "2mL Adaptor Kit" a 15mL position can be transformed into a 2mL position.

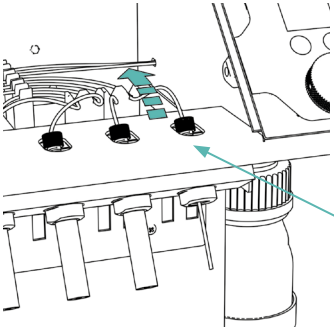
First the tube holder should be switched in its open position.



Please verify that adaptors and connectors are tightly screwed back.



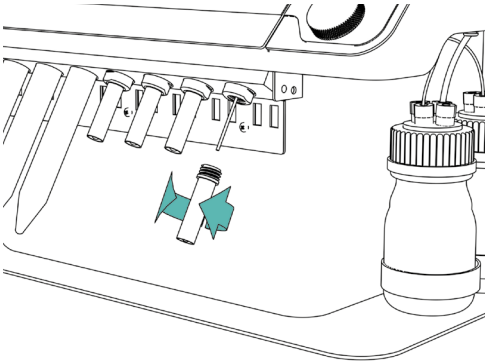
Remove the tube on a position and firmly screw in the "2mL adaptor" with the wrench included in Aria's package.



Please verify that adaptors and connectors are tightly screwed back.

Loosen the tubing connector to pull up the tube

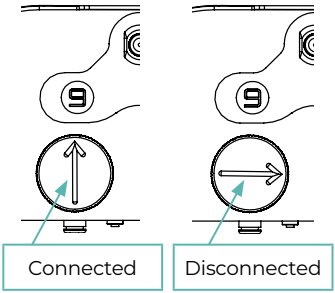
Open the lid, loosen the tubing connector. Please note that the tube holder can be placed back in its vertical position to ease the manipulation of the connector. Pull the tubing upward to leave a tubing length that will reach the bottom of the reservoir. Finally, tightly screw the tubing connector back to its initial position.



Screw the micrew tube in the tube adaptor.

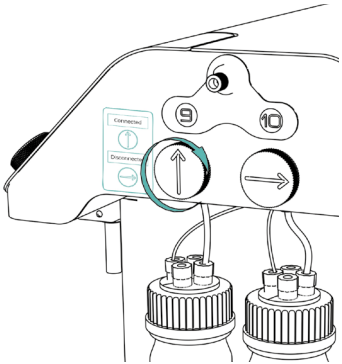
SIDE BOTTLES

In order to refill a side bottle, it should first be disconnected from the pressure by turning the side knob. This can be done during an experiment.

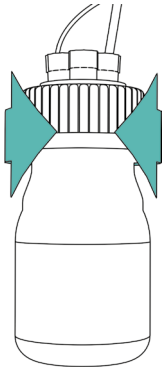


Connected:
The bottle is pressurized.

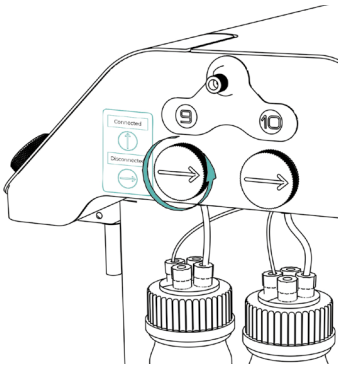
Disconnected:
The bottle is disconnected from the pressure (ready to be refilled).



Turn the knob to disconnect the bottle from the pressure source (the arrow on the knob is then horizontal).



Unscrew firmly the bottle cap to refill the bottle with solution, then screw back the cap.
Please note that the bottles should be tightly sealed to properly pressurize the system. Please note that the bottle caps have 4 entries. Two of them are not used and should be sealed with caps.



Turn the knob to reconnect the bottle to the pressure (the arrow on the knob points upward).

The ability to refill the bottle is particularly useful for long term experiments because it is possible to refill the bottles without stopping the experiment.

If the bottles are not used in your injection process, we advise you to disconnect them from the pressure. This will enhance performance (e.g. response time).

To ensure no bubbles are generated through reservoir switching, we recommend to fill all reservoirs with water or a buffer solution

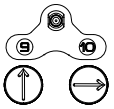
MANUAL INJECTION

Aria helps you to automate sequential injections using Aria software.

However, you can also perform manual injection depending on your needs.

Before injecting, check the position of the 2-Switch to make sure that the solution is perfused in the right direction (refer to page 11 for 2-Switch position and manipulation).

SOLUTION SELECTION

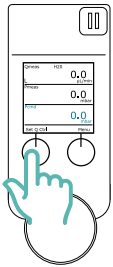


To manually select the solution to inject press on the button above its reservoir.
Once selected, the button will light up.

When a solution is selected, it will not enter the chip immediately. This solution will first push the liquid standing inside the internal tubing of Aria and in the fluidic path to the chip, before reaching the chip.

SET FLOW RATE

This page shows how you can set the injection flow rate when Aria is not controlled by the Aria software.



1

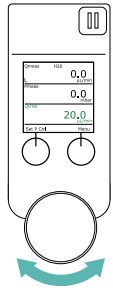
Press “Set Q Ctrl”

2

Aria is now in the flow rate control mode

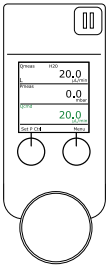
Real-time Flow rate measurement

Flow rate command (Qcmd)



3

Turn the dial to change the injection flow rate command (Qcmd)



4

Aria regulates the flow rate and “Qmeas” will meet the “Qcmd”

Although we are in flow rate control mode, the live pressure value in the reservoirs (Pmeas) is still shown in the middle, giving information on the fluidic set-up (leakage blockage, etc.).
See the FAQ page on the Fluigent website for more information.

MANUAL INJECTION SEQUENCE

Various options are available to manually inject a sequence of liquids:

A/ First set the flow rate value and then start the injection:

- Select the solution to inject
- In flow rate mode (refer to page 27 to shift from pressure mode to flow rate mode), press on the dial, select the flow rate value
- Press again the dial to start the injection

B/ Stop the flow between two consecutive solutions:

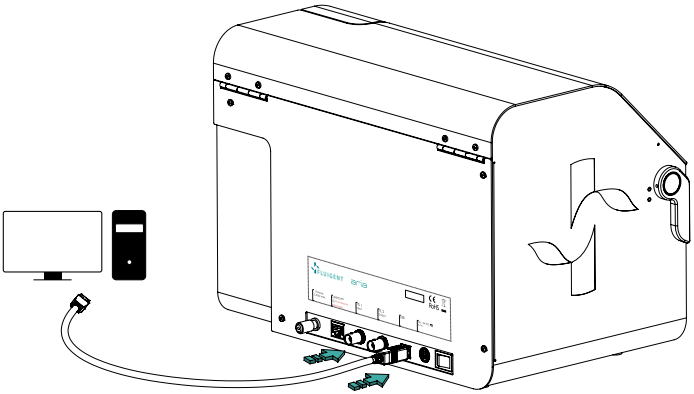
- Press on the pause button at the end of the first injection.
- Select the second solution to inject
- Switch back to flow rate mode (refer to page 26 to shift from pressure mode to flow rate mode)
- Set your flow rate

C/ Continuous injection between steps:

- Select directly the second solution to inject. Aria will maintain the flow rate during the switch

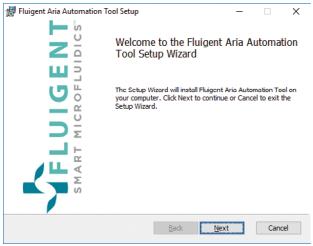
CONNECTION TO THE COMPUTER

To automate injection protocol and control Aria using the software, Aria should be connected to computer using the USB cable.



SOFTWARE & SDK INSTALLATION

Aria's software runs on Windows 7 or Windows 10 and requires .NET framework 4.7.2. The installation package comes as a single .msi file named “Aria Automation Tool Installer.msi”. To install the Aria software, double click the installer file and follow the installation dialogs.



Note: Administrator rights are needed to perform the installation

The installer will create a desktop shortcut and a windows menu shortcut to the software. The USB key is only needed to install the software and not to run the software.

INSTRUMENT DETECTION

Connect the Aria instrument to the computer using a USB cable. Launch the software by double clicking on the desktop shortcut. The software will start and detect the connected Aria instrument.
Note: if the Aria instrument is not connected to the computer, the software can still be launched and used in “Simulation” mode. Simulation mode can be used to design protocols offline.

The SDK library, which collects the software's functions, is also available in the provided USB key. It includes the following elements:

- Fluigent Aria SDK.pdf file - detailed documentation explaining global philosophy, functions, and examples
- Shared folder - header file and the native shared libraries for all supported systems
- C++ folder - C++ CMake project with console application examples
- C# folder - C# NuGet source and console application examples
- Python folder - Python package source and examples

SUPPORTED OPERATING SYSTEMS AND ARCHITECTURES

Operating system	x86 (32 bits)	x64 (64 bits)
Windows 7/8/10	X	X

SOFTWARE LAYERS

NATIVE LIBRARIES

Fluigent SDK is based on a native shared library for Windows. The libraries are sorted into folders by the operating system and processor architecture they target:

- windows/x86/fgt_SDK.dll
- windows/x64/fgt_SDK.dll

MIDDLEWARE

The SDK middleware is a set of packages that make it easier to use the SDK with various programming languages.

The middleware packages provide the following functionalities for your convenience:

- Identify, locate, and load the appropriate SDK native library according to the platform
- Convert data types to the native types used by each language to keep the user code clean
- Handle errors and display formatted error messages in the program's output

The middleware matches the conventions of each programming language while keeping the interface as similar as possible across all supported languages.

SUPPORTED PACKAGES

Language	Package
C++	Cross-platform CMake project containing middleware and examples
C#	Cross-platform .NET Core NuGet package. .NET solution containing package source and examples
Python	Cross-platform package and Windows installers. Project folder containing package source and examples

DOCUMENTATION

Refer to the user manual Fluigent Aria SDK.pdf for detailed documentation.

Wrappers and examples are also documented, depending on the environment.

CALIBRATION PROCEDURE

Since the external fluidic path between Aria, the 2-Switch or M-Switch and the chip can vary between experiments, the calibration step measures the volume of each fluidic portion. This step is necessary for the software to evaluate when the liquid will enter the chip.

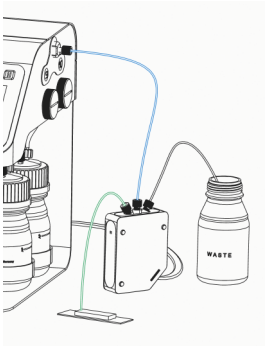
Calibration is requested on Aria first use. A new calibration will be necessary when using a new computer, or whenever changing the fluidic path.

However, since v2.3.1, it is possible to enter the fluidic path internal volumes manually. Click on “Set values manually” and edit the volumes you know. Once done, click either “Save” or “Use these calibration values” to confirm. **WARNING:** We strongly recommend performing a new calibration anytime you change the internal or external tubing.

2-Switch valve version

The calibration procedure assists the operator to complete volume calibration of the system. It consists in four screens:

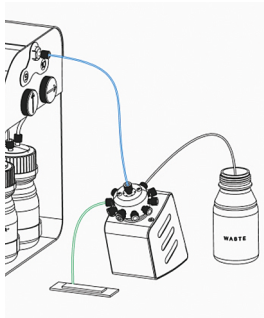
1. An operator selection of an empty reservoir and a reservoir with some buffer solution
2. An internal calibration step
3. A calibration of the tubing connecting Aria to the 2-Switch (in blue)
4. A calibration of the tubing connecting the 2-Switch to the chip (in green)



Calibration values will be recorded in the software and can be accessed from the start screen.

M -Switch valve version

When linked to an M-Switch, the procedure consists in 4 + N steps (N being the number of chip(s) connected to the M-Switch):



1. Selection of the ports to enable (at least one waste and one chip)
2. Selection of both the waste and air reservoirs
3. A calibration of the tubing connecting Aria to the M-Switch (in blue)
4. A flush of any tubing connecting the M-Swich to a chip
5. A calibration of the tubing connecting the M-Switch to the 1st chip (in green)

⋮

6. A calibration of the tubing connecting the M-Switch to the N^{th} chip (in green)

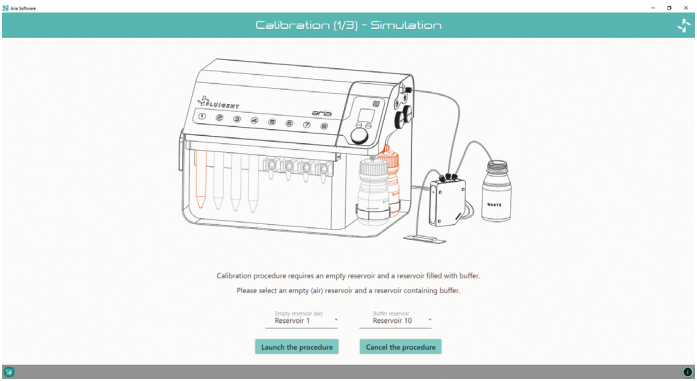
RESERVOIR SELECTION

The calibration procedure will alternatively inject air and liquid into the system towards waste.

Aria instrument should be prepared as follows:

1. One air reservoir, among 1 to 8, is empty (no solution in this reservoir)
2. One liquid reservoir, 9th or 10th, is filled with buffer (PBS, water, culture medium...)

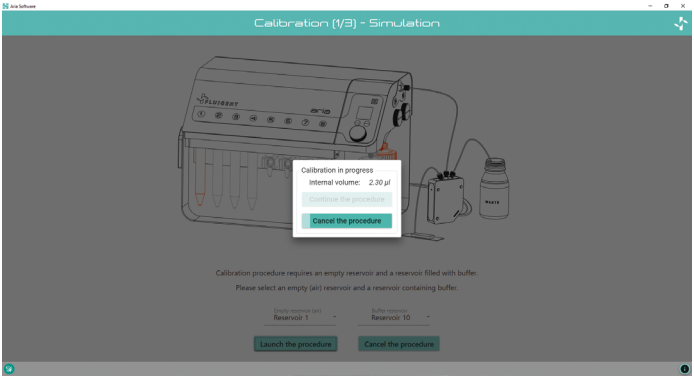
The operator must select these reservoirs in the “Calibration reservoirs selection” screen. The selected reservoirs will be highlighted on Aria’s scheme.



CALIBRATION FIRST STEP

The first calibration step evaluates internal volumes of the fluidic path inside Aria.

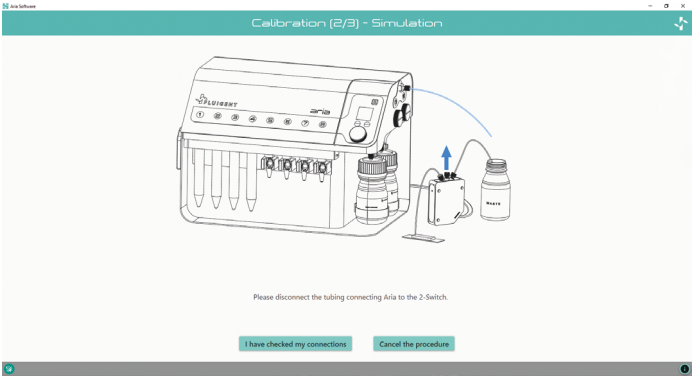
A window with a volume calculator will appear. The operator must wait until the button allowing to go forward is available – or cancel the procedure. This step shouldn't take longer than 5 minutes.



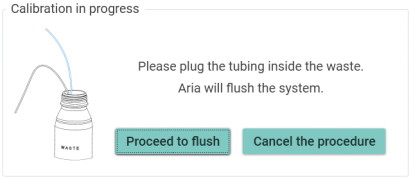
CALIBRATION SECOND STEP

Calibration step 2 evaluates volume of the tubing to the 2-Switch or M-Switch.

It requires disconnecting the tubing connecting Aria to the 2-Switch or M-Switch.

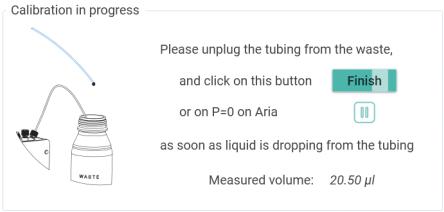


Once it is disconnected, the next screen will ask the operator to plug the tubing inside the waste in order to perform a flush operation.



Once the flush operation is complete, the dialog box will change. The buffer solution will be automatically injected in the system; the displayed volume will start increasing after a short time – typically 10 to 15 seconds).

The operator is asked to stop the injection as soon as there is liquid dropping from the tubing.



CALIBRATION THIRD STEP

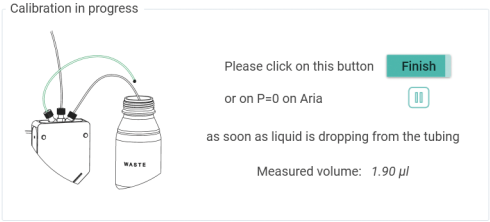
Calibration step 3 evaluates the volume of the fluidic path from the 2-Switch or M-Switch to the chip.

2-Switch valve version

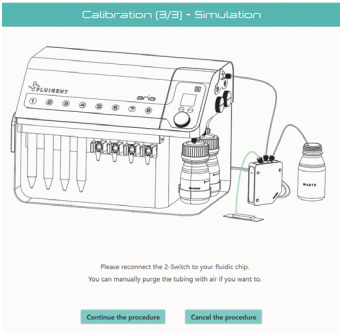
It requires disconnecting the tubing between the 2-Switch/M-Switch output and the chip(s).

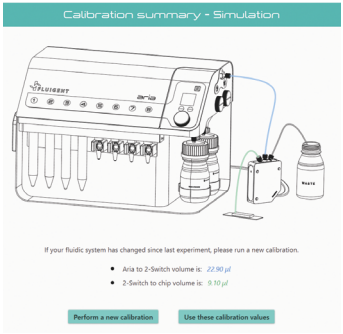
The software will then flush the system and automatically proceed to inject buffer into the system. From this point, the displayed volume will increase.

The operator is asked to wait for the system to be flushed and then stop the injection as soon as there is liquid dropping from the disconnected tubing.



Once the measured volume is validated, the tubbing should be connected back to the chip.

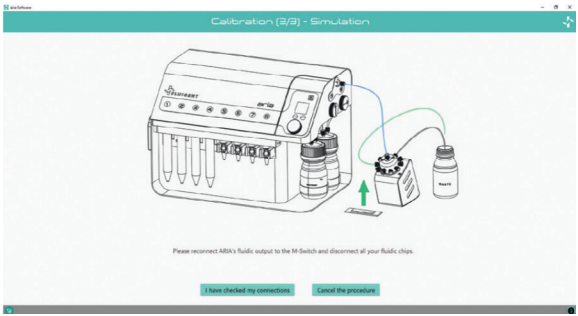




Once this step is validated, the software will display the new calibrated volumes.

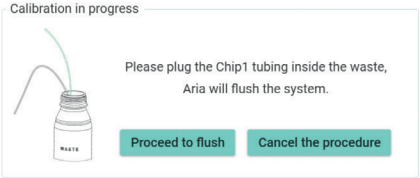
M-Switch valve version

It requires disconnecting the tubing between the M-Switch output and the chip(s).

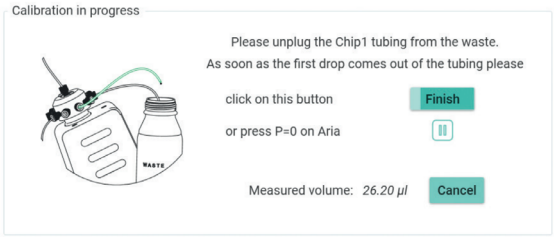


The software will then flush the system and each of the tubing connecting the M-Switch output ports to a chip.

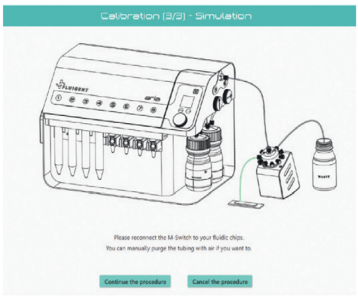
For each chip, the operator is asked to plug the tubing connecting the chip N to the waste. When proceeding, flush is done automatically and wait for another operator approval before flushing the next chip.

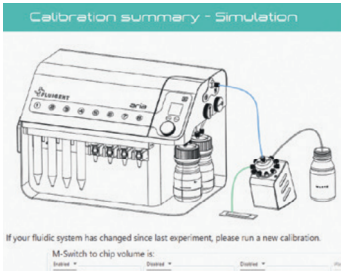


When all M-Switch -> Chips tubing have been flushed, the calibration of the first chip tubing starts right away. The operator is asked to click on “Finish” when a drop comes out of the tubing.



Once all chips tubing have been measured, the tubing should be reconnected to the chips.





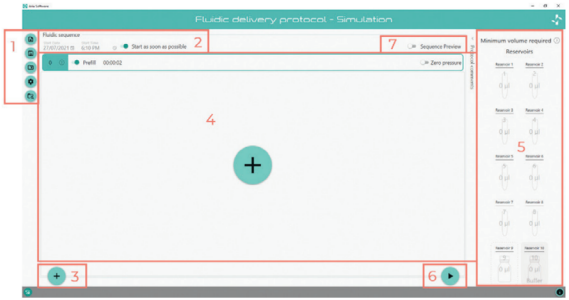
Once this step is validated, the software will display the new calibrated volumes.

INJECTION SEQUENCE, PROTOCOL DESIGN

The “Fluidic delivery protocol” is the screen dedicated to design injection protocol.

The screen layout is divided into six area which are further described in the next sections:

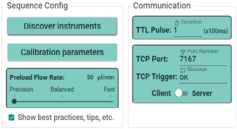
1	A menu supporting various buttons (save/load the sequence, set parameters...)
2	A “start time” area
3	A button to add new steps to the sequence
4	A main area displaying the fluidic sequence
5	A “required volume per reservoir” display
6	A “Start” button to start the injection protocol
7	Toggle button to preview the flattened list of the sequence steps



FLUIDIC INJECTION CONFIGURATION MENU

This menu consists in five buttons.

Load sequence	Loads a sequence from a file
Save sequence	Saves the current sequence in a file
New sequence	Creates a new sequence
Parameters	Opens the “Parameters” dialog box. It contains: • **Discover instruments:** Allow to re-detect any Aria hardware currently connected. • **Calibration parameters:** Go back to the calibration summary page with all calibration values • **Preload Flow Rate:** Allow to change the flow rate speed of the preload step. A low flow rate allows for a better precision at the cost of time (useful when first injected solutions must be used sparingly) while a higher flow rate will speed up the preload but might be slightly less precise to get the solution to the chip input port. • **TTL Pulse:** Set the duration of all TTL pulses • **TCP Port:** Port to be used when sending TCP messages • **TCP Trigger:** Default message to be sent and expected upon reception at the start/end of the functions (when turning ON signal notification). • **Client/Server:** Choose whether Aria must act as a TCP server or a TCP client.
Open logs folder	Opens the directory containing experiments recorded data files (flow rate, pressure, valves...)



START TIME AREA

The start time area allows the operator to choose the sequence start time. It is possible to deselect the “Start as soon as possible” option and enter the desired start date and time.

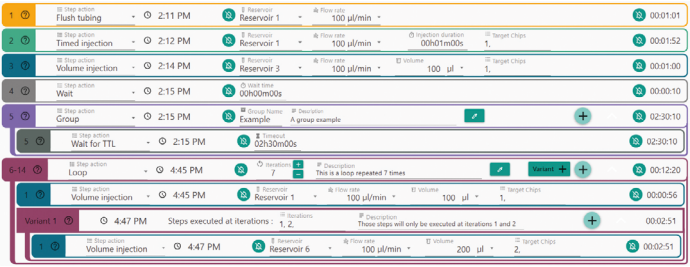
Note: The “Start as soon as possible” option ensures that the sequence starts as soon as the start button is clicked. If the prefill function (step 0) is active, the system will first preload the system (refer to prefilling section).

NEW STEP BUTTON

The new step button will add a new step at the end of the sequence. By default, this step is a “Volume inject” step. *Note: There are 10 different steps that are listed and described in the following sections.*

FLUIDIC INJECTION SEQUENCE

The fluidic injection sequence consists is an ordered list of steps that Aria will perform from top to bottom at the starting time defined by the user (see section ‘start time area’).



A sequence is composed of:

1. an optional prefill step,
2. a succession of user configurable steps.

Note: the injection sequence steps list support traditional shortcuts, such as:
- *Ctrl C, Ctrl V: copy and paste the currently selected step(s)*
- *Ctrl A: select all steps*
- *Delete: delete the currently selected step*

Multiple steps selections can also be achieved by selecting a step, maintaining Shift key and selecting another step.

The injection sequence steps list also supports natural reordering by simply dragging and dropping a step to its new position in the list.

PREFILL STEP

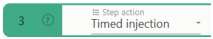
The prefill step removes any bubbles that may be trapped in the system. Aria will automatically prefill all the fluidic lines from reservoir to the external 2-switch that will be used in a given sequence. By default this step is active, it can be inactivated in case you already prefilled the system manually.
Note: If in doubt about the presence of bubbles in the system, the prefill step should be performed

The prefill step displays its duration, which mostly depends on the number of reservoirs used in the sequences.

USER CONFIGURABLE STEPS

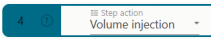
The other steps are user configurable. There are ten types of steps.

Timed injection



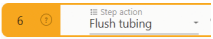
This step injects a solution into the chip over a period of time (recommended for long injection times). The operator selects the reservoir containing the solution to inject and the injection flow rate.

Volume injection



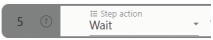
This step supports injects a precise volume of solution into the chip. The user selects the reservoir containing the solution to inject and the injection flow rate.

Clear tubing



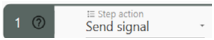
This step flushes the liquid remaining in the tubing to the waste. The operator selects the solution that will push the resting liquid to the waste. This solution will also be discarded.

Incubate



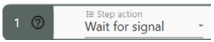
This step puts Aria on hold for a duration specified by the user. When the time is elapsed, Aria runs the next steps.

Send signal



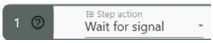
This step sends a TCP message locally to the TCP port defined in Settings (page 43).

Wait for user validation



This step puts Aria on hold. Aria waits for the operator to validate a dialog popup window to run the next steps.

Wait for external signal



This step puts Aria in a waiting state. Aria will remain idle until it receives an external signal, which can be a TTL pulse through the TTL input port (refer to page 8) or a TCP message with the trigger message configured in the Settings (see page 43). Optionally, you can enable a 'backtrack' mode where Aria begins monitoring incoming TCP messages from the point of the last signal (TTL or TCP) sent or received. This feature enables you to trace a message sent several steps earlier or manage a lengthy waiting period, for example.

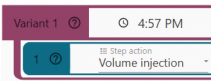
Group

Allows to gather steps within a group and to give it a name and a description. Useful to split a long experiment sequence into several phases.

Loop

Allows to execute the same set of steps several times. For instance, if one wants to execute a volume injection followed by a flush 10 times in a row, the Loop function will prevent the creation of 20 step blocks and instead makes it to 3 (Loop + Volume Injection + Flush tubing).

Variant



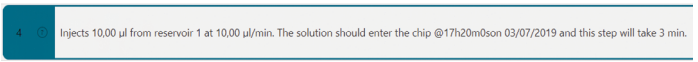
Only available within a loop, allows to define a step or a group of steps that will be executed only at certain iterations.

Steps are colored by type, i.e. injection, wait and flush, to ease the sequence readability.

Each step displays:

1	Its order within the sequence
2	A contextual help question mark
3	The step action
4	The estimated time at which the step execution begins, i.e. when the liquid starts entering the chip for injection steps
5	An external system notification at execution start button
6	The specifics parameters related to the step action
7	An external system notification at execution start button
8	Its estimated duration
9	Enable/disable this step. A disabled step will be skipped during the protocol execution

Contextual help



Hovering the mouse over the help question mark displays contextual help.

The contextual help explains to the operator the purpose of the step, according to its parameters' configuration.

External system notification

External system notification is achieved by either sending a TTL pulse through the output TTL port of the instrument (page 8) OR to send a TCP message at the software level (via an IP connection – local or remote – to another TCP server or client running in an external program).

The user can configure each step to notify an external system when the step starts executing and/or finishes its execution. To change the mode (TTL or TCP), right-click on the external notification button (buttons **5** and **7** in previous section). For injection steps, this means that the user can notify external systems while the liquid is flowing through the chip.

REQUIRED VOLUME AREA

The required volume area displays how much volume the sequence will use, for each reservoir. In order for the injection sequence to be complete, all reservoirs must contain at least the volume displayed.

Unused reservoirs are grayed out.

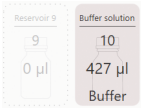
Volume consumption varies according to:

- Prefill step being performed or not
- The number of injection steps and their configured parameters

Reservoir 5 should be filled with at least 396µL of solution

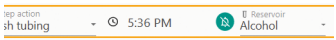
Buffer reservoir selection

The reservoir containing the 'buffer solution' is notified above the bottles (reservoir 9 or 10).
Left clicking on reservoir 9 or 10 allows to configure them as the buffer reservoir.



Reservoir naming

The reservoirs name can be customized – mouse hover the name and single click to edit. The name of solution will be automatically implemented in the display.

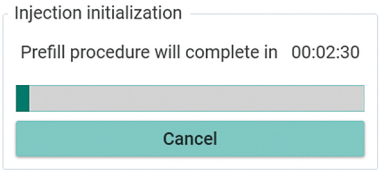


SEQUENCE PREVIEW

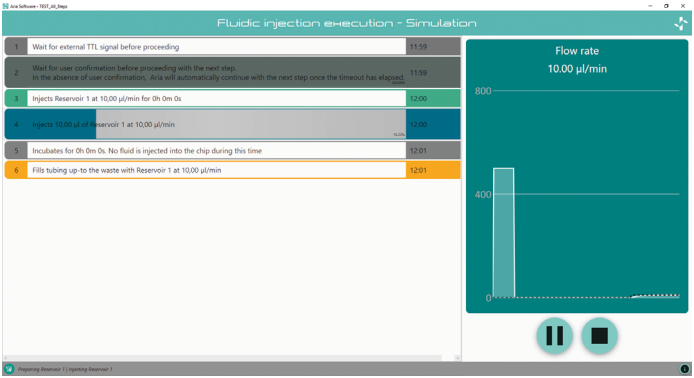
With the introduction of “Loops” in the sequence editor, one might want to have a look at a flattened list of the steps that will be executed sequentially. This can be achieved by toggling the button called “Sequence Preview” on the top right of the editor page. Note that when the preview mode is active, no edition is made possible. Disable the preview mode to edit your sequence.

SEQUENCE EXECUTION

Once the injection sequence configuration is finished, the sequence execution can be started by clicking the “Start” sequence button (page 40). The software will initialize: Aria will first be prefilled and then the system will be preloaded with the sequence to inject.



During the sequence execution, the current step progress is displayed using a progress bar. Past steps appear in grey. On the right, the current flowrate is displayed in white and the set point is displayed in black dashed lines. It takes a few seconds for the flow to reach its set point. The flow may be occasionally cut for a short duration when the following solutions are being injected into the fluidic path.



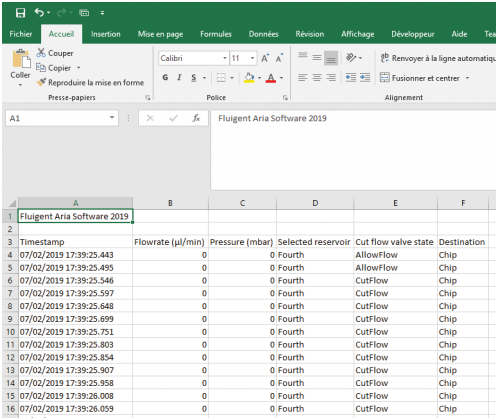
Two buttons allow the sequence to be "Paused" or "Stopped". "Pause" puts the system on hold and resume the sequence where it was stopped. This button is useful to refill reservoir 9 or 10 by disconnecting the bottles from their pressure source (page 24). "Stop" puts an end to the running sequence with no possibility to resume where the sequence was stopped.

Each time a sequence is executed, Aria's measurements data is logged in "Comma Separated Value" format (CSV) in the following directory:

C:\<current user>\AppData\Local\Fluigent\Aria\Records\Datalog.

Data log files are named after the sequence name that is executed, followed by the date and start time of the execution:

e.g. TEST_All_Steps_Data_06122019-132442.csv.



Fichier Accueil Insertion Mise en page Formules Données Révision Affichage Développeur Aide Tea

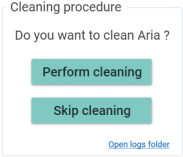
Couper
Copier
Reproduire la mise en forme
Coller
Presse-papiers
Calibri 11
Police
Renvoyer à la ligne automatiqu
Fusionner et centrer
Alignement

A1
Fluigent Aria Software 2019

	A	B	C	D	E	F
1	Fluigent Aria Software 2019					
2						
3	Timestamp	Flowrate (µl/min)	Pressure (mbar)	Selected reservoir	Cut flow valve state	Destination
4	07/02/2019 17:39:25.443	0	0	Fourth	AllowFlow	Chip
5	07/02/2019 17:39:25.495	0	0	Fourth	AllowFlow	Chip
6	07/02/2019 17:39:25.546	0	0	Fourth	CutFlow	Chip
7	07/02/2019 17:39:25.597	0	0	Fourth	CutFlow	Chip
8	07/02/2019 17:39:25.648	0	0	Fourth	CutFlow	Chip
9	07/02/2019 17:39:25.699	0	0	Fourth	CutFlow	Chip
10	07/02/2019 17:39:25.751	0	0	Fourth	CutFlow	Chip
11	07/02/2019 17:39:25.803	0	0	Fourth	CutFlow	Chip
12	07/02/2019 17:39:25.854	0	0	Fourth	CutFlow	Chip
13	07/02/2019 17:39:25.907	0	0	Fourth	CutFlow	Chip
14	07/02/2019 17:39:25.958	0	0	Fourth	CutFlow	Chip
15	07/02/2019 17:39:26.008	0	0	Fourth	CutFlow	Chip
16	07/02/2019 17:39:26.059	0	0	Fourth	CutFlow	Chip

Measurements logged include flowrate, pressure, selected reservoir, cut flow valve state (AllowFlow: no flow is permitted, or CutFlow: the flow is stopped by the internal stop flow valve) and liquid destination (Chip or Waste).
Note: If the log files size exceeds 10 Mb, the data gets logged in several consecutive csv files.

Once the sequence is finished, or if its execution is stopped by the operator, the cleaning dialog box is displayed.



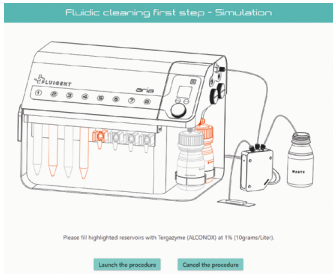
END OF EXPERIMENT AND CLEANING

FLUIDIC CLEANING PROCEDURE

The cleaning procedure must be performed before switching off Aria at the conclusion of an experiment. It ensures that Aria's internal flow sensor and switches are cleaned properly. The software assists the operator to perform such a procedure.

The procedure consists in five steps:

1. Injection of distilled water,
2. Injection of Tergazyme (ALCONOX) @ 1%, or RBD 25 diluted 10% in DI water,
3. Air Drying,
4. Injection of IPA,
5. Air drying.



The minimum quantity of liquids to fill the reservoirs with can be deduced from the following table.

Cleaning step	For each reservoir to clean	Buffer reservoir	M-switch only
Distilled water	45 µl	500 µl	30 µl * N ports
Tergazyme or RBD	45 µl	300 µl	30 µl * N ports
IPA	45 µl	500 µl	30 µl * N ports

TURNING OFF THE POWER

To turn off the system, please turn off the power supply button at the back of the system.

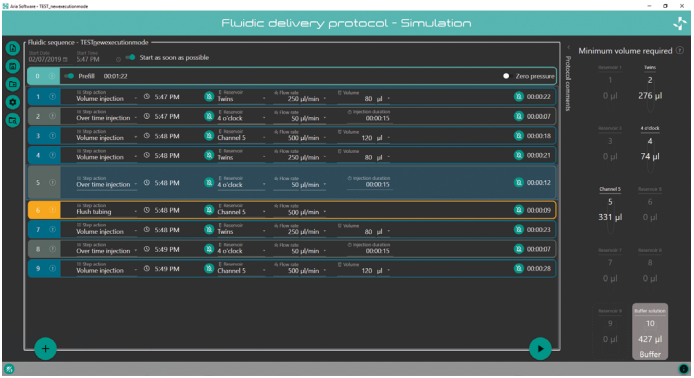
SYSTEM MAINTENANCE

When needed (dust accumulation, dirt, cosmetic matters), gently clean the surface of the system using a water-wetted non-abrasive cloth.

DARK ROOM MODE

When working in a dark room, screen brightness can be an issue. A dark mode theme has been developed for Aria software. It can be toggled on/off at any time using the button top left of the screen.

While in dark mode, all screen illumination is greatly reduced, allowing for better readability.



HARDWARE SPECIFICATIONS

Performance	
Flow Rate Control	Over the range of 40 uL/min to 1 mL/min for water
Flow Control Accuracy	+/- 5% of the measured values above 40µL/min
Flow Control Repeatability	+/- 0,5% of the measured value
Pressure Control of flow rate	to a maximum of 2.4 bar
Mechanical	
Dimensions	382 mm x 240 mm x 265 mm (W x H x D)
Weight	9 kg
Valves	Ten-position switching valve(s)
	Two-position switching valve(s)
Fluid Reservoirs (8)	15 mL standard, 2 mL available
Flushing Solution Reservoir (2)	100 mL
Tubing	FEP with OD of 1/16 inch and ID of 250 µm
Wetted Surfaces	Polypropylene, FEP, Glass, PEEK
Compressed air source	Requires non-corrosive compressed air (lab line, gas cylinder, compressor, or Fluigent FLPG)
Operating temperature	10°C - 40°C
Working fluid	Aqueous solutions only
Cleaning	With Tergazyme, Ethanol or Isopropanol, and DI water
Electrical	
Power supply voltage	24 VDC
Max Energy consumption	160 W
Max Current requirement	6,67 A
PC Specifications	Windows 7 or higher

TROUBLESHOOTING

Problem	Potential solutions
No recognition of the Aria	Check if the power supply is connected and the Control Unit is switched ON.
The system is returning Low pressure	Check that the Pressure inlet is well connected to the pressure source. Check that the pressure inlet is well connected to Aria. Check that the reservoir and all bottles are tightly screwed.
No liquid flow at the output	Check that all fluidic tubing is correctly connected and not bent. Check that the fluidic connector on the valves is not over-tightened, which may pinch and clog the tubing. Check that reservoirs are not empty.
A fluidic component is leaking	Check the fluidic connectors are sufficiently tightened. Rince the spilled liquid immediately.

ATTENTION TO QUALIFIED STAFF

- System validation: The system is provided already tested, certified, and validated with a dedicated testbench. Please do not perform any further testing that would impact the proper functioning.
- Stocking: please stock the system on a flat surface by respecting the conditions mentioned in part 4. Do not put anything on top of the systems.
- Parts replacement and support: for any support or troubleshooting that is not listed in part 11. For troubleshooting, please contact us at contact@fluigent.com. Do not intend to open the machine, or perform any parts replacement, or maintenance actions.
- Machine maintenance: for any maintenance applied to the system, the system must be returned to Fluigent.
- Machine return: when returning the machine is mandatory for repairing, please make sure a complete fluidic cleaning protocol is completed.

LABELS & MARKING

Our product is CE-certified, which means the product has been assessed to meet high safety, health, and environmental protection requirements.

RoHS

Our product is RoHS. RoHS is a directive in the European Union that stands for the Restriction of Hazardous Substances.

The product should not be discarded as unsorted waste but must be sent to separate collection facilities for recovery and recycling.

Direct current.

FLUIGENT

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www.fluigent.com

NOTES

VERSION
APRIL 2024

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