

FULLY AUTOMATED TTL SIGNAL CONNECTION FOR FLUID DELIVERY USING ARIA AND IMAGE ACQUISITION CYCLES.

TECHNICAL NOTE





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INTRODUCTION

Fluigent's Automated Sequential Injection System, named <u>Aria</u>, was designed to help biologists **simplify and automate their experiments** by programming the sequential injection of up to **10 different solutions** into a microfluidic chip. It allows one to perform a long-term experiment usually involving multiple injection steps, long incubation time and a continuous presence in the laboratory by simply pre-setting the experiment parameter and launching the program, getting rid of all the constraints aforementioned.

In this technical note, we demonstrate that not only are the fluid delivery and incubation time defined by the user in Aria's interface, but the connection between the instrument and a microscope is possible to automate the sequential imaging at several steps of the experiment. Indeed, ARIA can be seamlessly integrated with a microscope to perform multiple cycles of fluid delivery and image acquisition automatically, including the implementation of an automated immunofluorescence protocol. This simple workflow enables one to **automate and accelerate data collection**. This can be particularly useful when willing to perform multiplexed imaging. This has been nicely <u>developed and published in Nature protocols</u>.

Materials & Methods:

Materials

- ARIA: The <u>Automated Sequential Injection System</u>, known as Aria, is a microfluidic instrument designed for the automated delivery of up to 10 different solutions with a high control over flow-rates and maintaining cells in physiologically relevant conditions. The perfusion parameters can be set directly on Aria's interface.
- Widefield Microscope: compatible with most microscopes on the market. Here, we used a Zeiss Axio Observer.



Figure 1. Instruments used for the automated connection between Aria and the microscope.

Methods:

The main advantage of this procedure is to be able to program a series of solution delivery orders coupled to <u>automatic triggering of image acquisition</u>. This is achieved through a TTL signal sent by ARIA to the microscope to initiate a sequence of image acquisition after a chosen fluidic step. Once the images have been acquired, another TTL signal is sent by the microscope to the ARIA unit to restart the fluidic delivery sequence.

Hardware setting:

- On one hand, please connect a BNC cable between the TTL output port of the ARIA unit and the TTL input of the microscope (as illustrated on picture – red boxes). This will allow ARIA to send a signal to the microscope.
- On the other hand, please connect another BNC cable in between the microscope TTL output port and the TTL input port of the ARIA unit (as illustrated on picture – blue boxes).



Figure 2. Red boxes: TTL output port of the ARIA unit; TTL input of the microscope. Blue boxes: TTL output port of the microscope; TTL input port of the ARIA unit.

Here we explain how to set up the TTL signal for the automated immunofluorescence protocol when working with a Zen-controlled Zeiss microscope. The procedure will have to be adapted to your microscope (in terms of how TTL signals are received and sent by your microscope) to allow sequential imaging. However, this does not affect the way ARIA works.

To launch an image acquisition order at the end of a specific fluid delivery step:

• On the ARIA software, choose "Notify external system at end (TTL event) by ticking the "Bell" at the end of the fluidic step (on the right-hand side).

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Figure 3. Sequential injection automated protocol using Aria Automation Software

On the **Zen software**, in the Acquisition settings, check "Time Series" (even though you do not wish to perform time lapse experiments).

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Figure 4. Zen Software; Acquisition settings.

In the Time Series tab, Set Duration 1 cycle and define Start "On Trigger". Select the right TTL input port (here Digital In 7).

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Figure 5. Zen Software; Acquisition settings.

- Proceed as usual for the other acquisition settings (channels, exposure time, z-stacks...)
- To resume your fluidic sequence, trigger a TTL signal output on Zen. In the "Light Path Setting" tab, in the option "After Experiment" tick "IO Card".



Figure 6. Zen Software; Acquisition settings.

• Choose your TTL output port, here "Digital Output 7". Please set the "Pulse" to "**100ms**". This is **the parameter of the TTL signal ARIA is able to receive**.



Figure 6. Zen Software; Digital Outputs.

• On the ARIA software, please select the step action "Wait for TTL" and set an overestimated timeout to be sure that ARIA receives the signal. The signal sent by the microscope should trigger the activation of the next step action you have selected.

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Figure 8. Sequential injection automated protocol using Aria Automation Software