FLUIGENT

AUTOMATING NEURONAL CELL IMMUNOLABELING IN MICROFLUIDIC CHIPS WITH ARIA

INTRODUCTION TO IMMUNOFLUORESCENCE

Microfluidic technology has revolutionized cell biology research, offering precise control over fluid handling at the microscale. Meanwhile, one of the most powerful and widely used techniques in the field of biology and biomedical research is the immunofluorescence technique. It plays a pivotal role not only in determining protein expression, but also in providing information about the cellular or subcellular localization of the protein being studied (1). At its core, immunofluorescence relies on the principles of antigen-antibody binding, followed by visualization of the antibody either by conjugating the antibody to an enzyme catalyzing a color-producing reaction, or by conjugating the antibody to a fluorophore. When exposed to the appropriate wavelengths of light, these fluorophores emit visible light, creating a vivid and distinct fluorescence pattern that can be observed and analyzed under a fluorescence microscope (Figure 1). This technique can be used to visualize proteins in cells (both in suspension and adherent cells), tissues, and 3D culture-derived spheroids.

MICROFLUIDICS FOR IMMUNOFLUORESCENCE

Combining microfluidics with immunofluorescence allows for precise, multiplexed, and dynamic cellular analyses. However, cell handling within these microfluidic chambers presents unique challenges. For delicate neuronal cell immunolabeling, particular attention is required to preserve axons and dendrites. The geometry of the microfluidic chambers, often ranging from millimeters to micrometers, imposes significant constraints on fluid flow during medium changes or additions. Cells residing within these chambers are exposed to fluidic stresses that can lead to tearing or detachment from their substrate. Additionally, achieving optimal antibody rinsing is essential to eliminating residue that can compromise the quality of generated images. Manual handling can lead to challenges such as injecting an excessive volume too quickly, potentially damaging cells and necessitating prolonged waiting times for fluid diffusion within the chambers. To address these challenges and to enhance the quality and efficiency of cell immunostaining protocols in microfluidic chips, Fluigent's automated sequential injection system, Aria, is proposed as an alternative to manual handling.

This application note outlines how Aria, in combination with the M-Switch, facilitates parallel neuronal cell immunolabeling in up to four microfluidic chips, allowing up to three different antibodies to be used simultaneously. By automating the fixation process, users can significantly reduce the time and effort required while ensuring consistently fixed cells with minimal cell damage and no antibody residue.

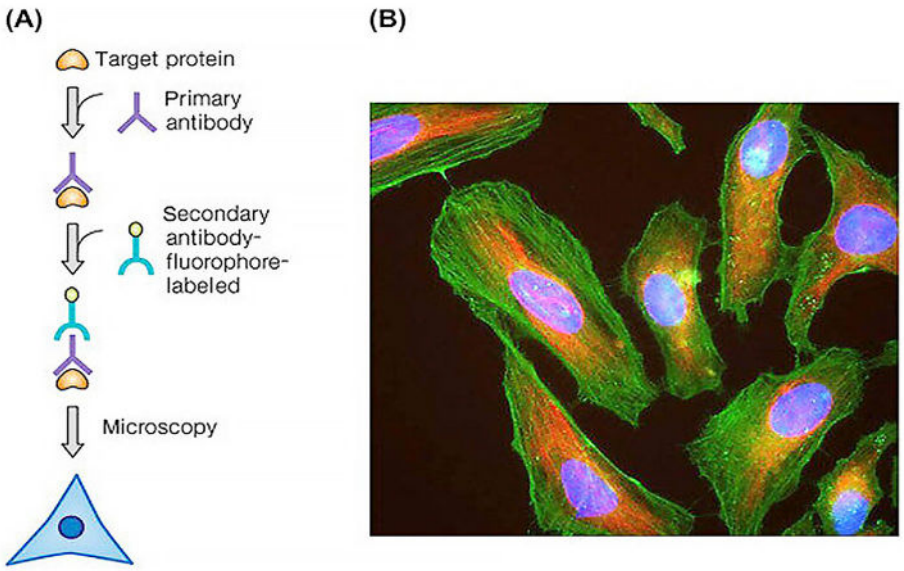

Figure 1: (A) A diagram illustrating the procedure involved in Immunofluorescence. After cells grown on cover glass are fixed and permeabilized, a primary antibody is added to detect a specific antigen, and the secondary antibody conjugated to a fluorescence tag (e.g., FITC) is sequentially added to bind to the primary antibody.
(B) Image obtained by Immunofluorescence. HeLa cells grown in tissue culture and stained with antibody to actin (green), vimentin (red), and DNA (blue) (2).

AUTOMATED NEURONAL CELL IMMUNOLABELING

Materials

Cells & reagents

Progenitor neuron cells from rat embryos at 17.5 days of gestation were used in this application note to demonstrate the convenience of using Aria for immunostaining such delicate cells while preserving axons and dendrites.
For the immunofluorescence protocol, standard reagents were used. The necessary reagents are as follows:

- Fixation solution: 4% paraformaldehyde PFA in PBS and 1% sucrose in PBS
- Permeabilization solution: 0.1% triton in PBS
- Blocking solution: 0.1% triton, 5% BSA, 2% donkey serum in PBS.

For the cell staining, the following reagents were used:

- Primary antibody: Anti-MAP2 mouse in blocking solution 1/800
- Secondary antibody: Anti mouse 488
- Hoechst 1/1000 in PBS to stain the cell nuclei.

Microfluidic chip

The microfluidic chip (Figure 2) used for this application note is a homemade chip, fabricated using PDMS (Polydimethylsiloxane) with a curing agent ratio of 1 part curing agent to 10 parts of the base silicone, specifically utilizing Sylgard 184 as the base material. This PDMS-based chip is designed to replicate two distinct cortex zones and study their projection onto remote areas of the striatum, enabling research on axonal rewiring and cortical axon distribution within the striatum. It provides a valuable platform for investigating the effects of altering axonal architectures in neuron cells.

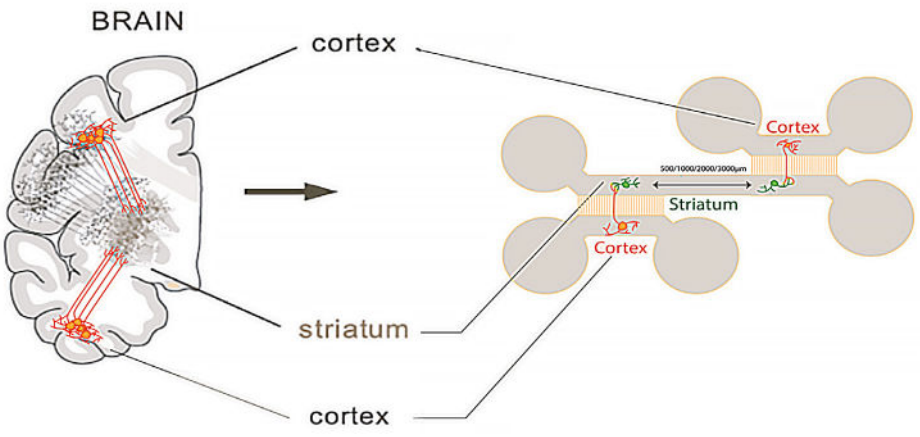

Figure 2: Illustration of the microfluidic chip used to perform progenitor neuron cell culture and cell immunostaining.

Aria serial output

Aria Serial Output is a specialized microfluidic control system that enables precise and sequential delivery of fluids in microfluidic applications. It allows for accurate control of the flow and timing of up to 10 different fluids or solutions, while also supporting user-friendly protocols. With Aria Serial Output, researchers can automate complex protocols, reducing human error and ensuring consistent results in microfluidic experiments. This system is a suitable tool for neuronal cell immunolabeling requiring very precise and gentle fluid perfusion to maintain cell integrity.

Confocal Microscope: Nikon Spinning Disk CSU-W1

The confocal microscope employed, a Nikon Spinning Disk CSU-W1 with a 10x objective, provided high-resolution imaging for neuron cell visualization. A 488 nm laser at 70% intensity with a 200 ms exposure time was used to capture precise and detailed images of the neuronal structures.

Methods

Cell culture & preparation

Prior to immunostaining, the neuron cells were cultivated in Neurobasal medium supplemented with 0.02% B27, 0.01% Glutamine, and 0.01% Penicillin-Streptomycin for a duration of 14 days. After this period, the fixation solution (PFA/sucrose) was manually added to the cells outside of the Aria to prevent contamination of tubing and system parts (flow unit, M-Switch).

Neuronal cell immunolabeling using Aria

For this application note, a typical cell immunostaining protocol was used. All steps (Figure 3) were performed within the Aria unit except for the fixation step, which was done manually to avoid contaminating the system. This step could be done inside the system, but a thorough washing step would be required to ensure that all residues are eliminated.
After cells were fixed, the microfluidic chip was loaded into Aria, and the standard steps for immunofluorescence were followed:

- Permeabilization of cells for 15 minutes, followed by several washes using PBS
- Blocking solution is then injected and allowed to incubate for 1 hour, followed by several washes using PBS
- Staining with primary antibody for 1 hour, followed by several washes using PBS
- Staining with secondary antibody for 20 minutes, followed by several washes using PBS
- Nuclei staining with Hoechst for 10 minutes, followed by several washes using PBS
- Final wash with PBS to ensure removal of any remaining reagents.

After completing the entire protocol, the microfluidic chip is disconnected from Aria and the neuron cells are ready for imaging.

This protocol allows for efficient immunostaining of up to 12 microfluidic chips in a day, with minimal user intervention. This streamlined process allows for consistent and reliable experimental results while saving time and effort.

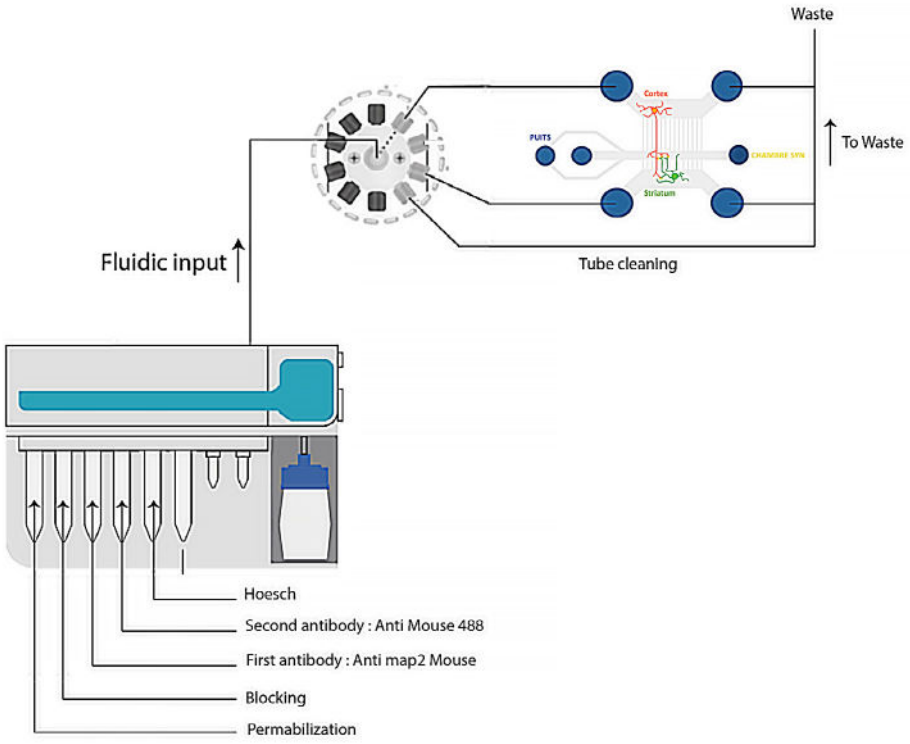

Figure 3: Illustration of the experimental setup including Aria, along with the reagents used for cell immunostaining and the microfluidic chip used for neuron cells.

Aria unit preparation

To initiate the protocol, the Aria unit must be connected to an external pressure source, which can be either an FLPG unit or a direct connection to the wall pressure source if available in the lab. The pressure must reach a minimum of 2.2 bar before powering on the Aria unit. Aria should be connected to a computer using the provided USB cable. Different reagents and solutions to be used should be placed in the specified reservoirs as indicated in the software protocol. For example, the permeabilization solution should be placed in reservoir 1, the blocking solution in reservoir 2, etc., following the requirements of the protocol.
The setup (Figure 4) is ready to launch with the Aria software, which is very user-friendly and easy to operate. It allows for straightforward calibration by following the indicated steps. Once calibration is complete, users can proceed to create a customized Aria protocol tailored to their preferred immunofluorescence protocol, including steps for permeabilization, blocking, and immunostaining, among others. Figure 5 illustrates the protocol that was used to create this application note.

Figure 4: Photo of experimental setup showing Aria, M-switch, and Aria software.

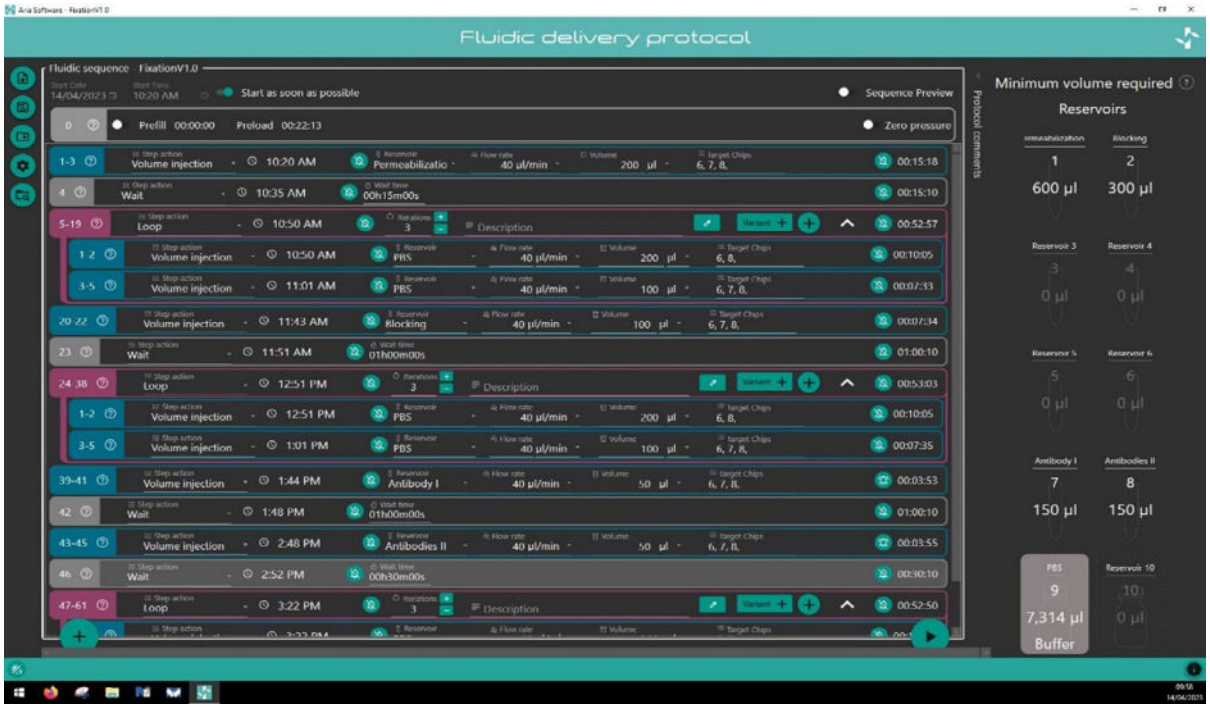

Figure 5: Example of the protocol used for a neuron cell immunostaining experiment with Aria software.

Parameters such as flow rate, volume, time of incubation, and wait steps are all available for protocol preparation, allowing users to create highly accurate protocols for the smoothest and most delicate neuronal cell immunolabeling experiments.
This approach allows for precise control and automation of your immunofluorescence experiments, ensuring accurate and consistent results while optimizing the use of Aria.

RESULTS

Figure 6 shows progenitor neuron cells inside the microfluidic chip, stained using the Aria system. Cells are stained for microtubule-associated protein 2 via the anti-MAP2 antibody (green), and for nuclei via Hoechst dye. As shown in the figure, Aria makes it possible to achieve very clean cell staining while preserving the very delicate neuronal cell structures, including axons and dendrites.

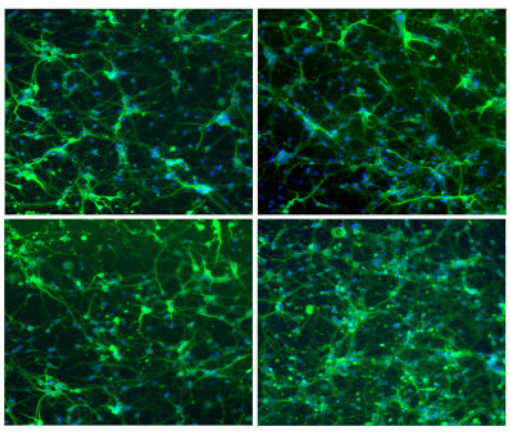

Figure 6: Immunofluorescence of neuron cells stained with anti-MAP2 for Microtubule-associated protein 2 (green) and with Hoechst for nuclei (blue). Images were acquired on a Nikon confocal microscope at 10X magnification.

CONCLUSION

Aria represents a significant leap forward in the quality of cell immunostaining and the efficiency of laboratory procedures. It addresses a crucial concern in biological research – the preservation of valuable samples, such as neuron cells. With this approach, researchers no longer need to worry about losing precious samples due to manual handling errors.

Moreover, the time-saving aspect of this method cannot be overstated. By simply loading the software and initiating the automated process, users can free up their time to focus on other crucial aspects of their research. This method not only improves the reliability and accuracy of fixation, but also streamlines laboratory workflows, making it an invaluable tool for researchers in the field of cell biology and beyond. Its ability to simultaneously enhance both data quality and research efficiency positions it as a game-changer in modern scientific endeavors.

Aria, in combination with the M-Switch, offers a transformative solution for the challenges of microfluidic neuronal cell immunolabeling.

FUTURE OUTLOOK

Looking forward, future research will likely focus on the study of axonal rewiring, requiring extensive application of immunofluorescence technique s to delve into axonal architecture within microfluidic chips. This exciting avenue promises to unlock new insights into neuronal connectivity and holds great potential for advancing our understanding of complex biological processes.

REFERENCES

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2. Wang-Shick Ryu, in Molecular Virology of Human Pathogenic Viruses, 2017.