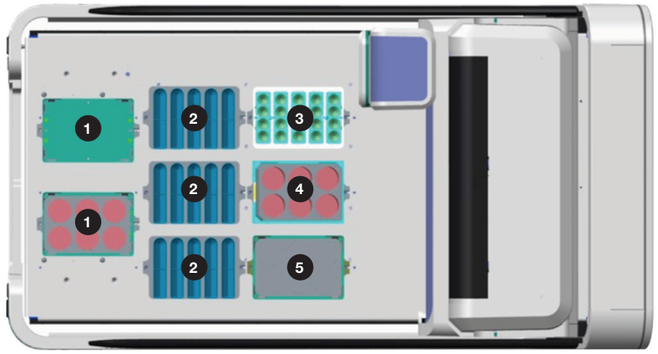
Deck Layout



1 = 6-well Plate Position

2 = 60 mL Reagent Reservoirs

3 = 5 mL Tips

4 = 6-well Plate Tilt Module

5 = 6-well Plate Lid Park Position

Table 1: System and Materials for IF Staining and Analysis

Product	Vendor	Part Number
Microlab NIMBUS4 ■ 2 Independent 5 mL Liquid Channels ■ Labware Gripper Arm	Hamilton	62797-225
Liquid Waste	Hamilton	63641-01
Deep Well Plate Pedestal	Hamilton	61053-01
Plate Tilt Module	Hamilton	188061APE
Reagent Trough Pedestal	Hamilton	61052-01
60 mL Reagent Trough, Self-Standing with Lid	Hamilton	56694-01
Framed Tip Rack Pedestal	Hamilton	61054-01
5000 uL Conductive Non-Sterile Non-Filter Tips	Hamilton	184020
CytoOne 6-well TC Plate	USA Scientific	CC7682-7506
Olympus FluoView™ Confocal Microscope	Olympus	FV1000
MDA-MB-231 Breast Adenocarcinoma	ATCC	HTB-26
RPMI 1640 Media	Corning	15-040
Vinculin	Abcam	Ab11194
GLUT1	Abcam	Ab15309
DAPI	Thermo Fisher Scientific	62248

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HAMILTON®

Immunofluorescent Staining on the Hamilton Microlab® NIMBUS®

Application: Immunofluorescent Staining; Cells & Proteins and Drug Discovery

Hamilton Product: Microlab NIMBUS

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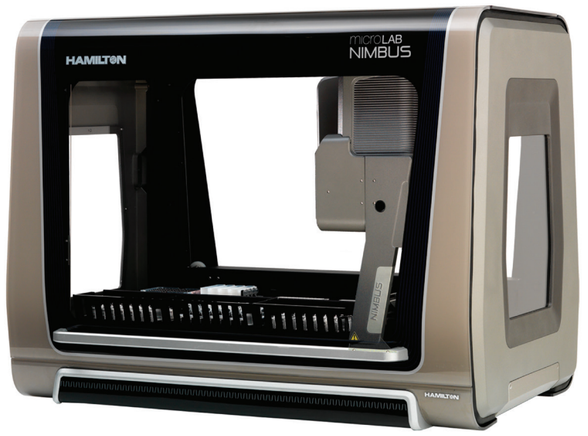
Abstract/Introduction

The use of antibodies for immunofluorescence (IF) coupled with microscopy is a powerful technique to identify subcellular protein abundance and localization. While IF technology has significantly improved in both optical resolution and sensitivity for protein identification, staining methodologies are still cumbersome and time consuming. Manual IF staining requires extensive bench time for laboratory personnel and due to the nature of detection, any variation in antibody concentration or exposure time increases the risks for artifacts or false-positive results. Increasing throughput also poses an additional problem because the process is burdened by the availability and reproducibility of the staff to process multiple plates in tandem. Thus, Hamilton Company and the University of Nevada, Reno partnered to provide an automated IF staining protocol on the Hamilton Microlab NIMBUS workstation. Here we describe an automated method that enables processing of a variety of multi-well plates per run. The results demonstrate that IF staining can be performed on an automated platform to produce quality images, improved sample reproducibility between plates, and increased staff walk-away time.

System Benefits and Key Features:

- Minimal hands-on time
- Optimized throughput up to six plates in one experiment
- Holds up to 15 reagent positions
- Flexible platform to accommodate variable IF applications and antibodies
- Maintains sample integrity



Workflow

Manual Steps

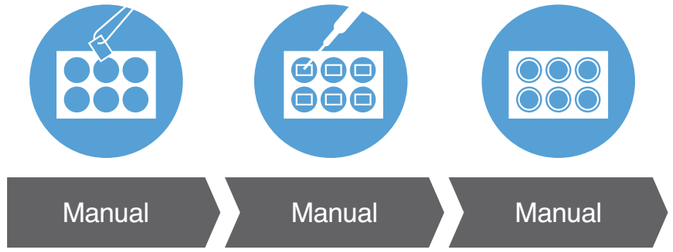


Figure 1: Manual cell culture slide preparation for immunofluorescence staining. Sterile glass coverslips were placed in the center of a 6-well plate. An even distribution of cell suspension was added to each well and incubated in a humidified 5% CO_2 atmosphere at 37 °C until optimal confluence was attained.

Automated Steps

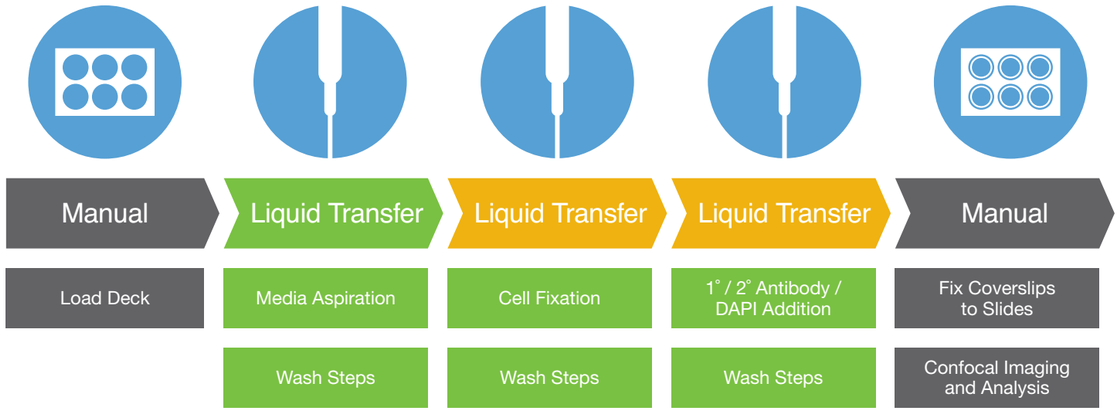


Figure 2: Simplified automated workflow on NIMBUS platform. Cells were cultured on glass coverslips fixed with 4% formaldehyde fixative solution. After washing with PBS, cells were blocked for 45 minutes at room temperature with a solution of 10% diluted donkey serum and 3% Triton X-100. Cells were stained for 24 hours at 4° with primary antibody directed against specific antibodies in dilution buffer (1X PBS, 1% BSA, 1% normal donkey serum, 0.3% Triton X-100, and 0.01% sodium azide). After washing, cells were incubated in the dark at room temperature for one hour with a fluorescent and donkey conjugated secondary antibody accordingly. After washing, the cells were stained with DAPI (4'6-diamidino-2-phenylindole) solution for five minutes. The cells were then washed and inverted onto a glass cover slide with anti-fade mounting medium. Cover slides were stored in the dark at 4° and analyzed with confocal microscopy.

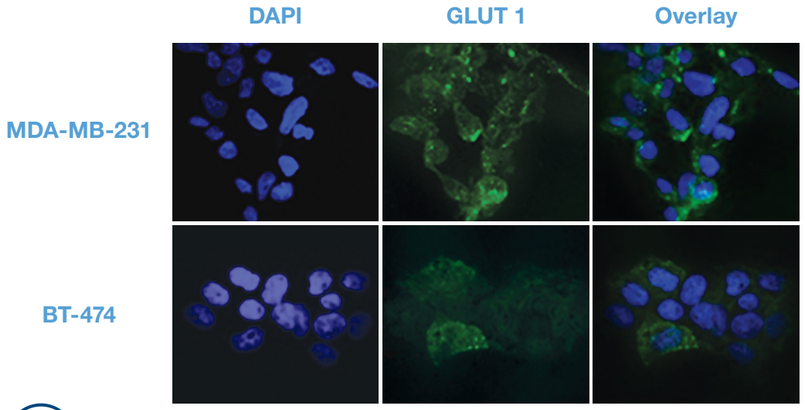


Figure 3. Assessment of GLUT 1 in Breast Cancer Cells. Automated immunofluorescence staining of BT-474 and MDA-MB-231 breast cancer cells using GLUT 1 (green) and DAPI (blue) on NIMBUS liquid handling platform. Cells were cultured on glass coverslips and fixed. Cells were then incubated for 1 hour at room temperature with primary antibody directed against GLUT 1 (1:500). Cells were incubated in the dark at room temperature for one hour with a fluorescent and donkey conjugated secondary antibody against rabbit IgG (1:500). Cells were stained with DAPI and inverted on to a glass cover slide with anti-fade mounting medium.

Results/Discussion

IF microscopy is a commonly used technique to determine specific protein localization and abundance, relying on the use of antibodies to precisely label antigens of interest with a fluorescent dye. While there are many advantages to IF, one key disadvantage is the inability to conduct live cell imaging. Once the cells are fixed and stained, intracellular dynamic processes and structural architecture may be altered, thus creating false positive signals and/or artifacts. It is therefore essential to include the proper amount of replicates and controls, which can significantly increase time and complexity of sample preparation, while trying to reduce as many manual errors as possible.

In the current study, Hamilton Company and the University of Nevada, Reno collaborated to report a proof of concept study defining the automation feasibility of IF preparation and analysis on the Microlab NIMBUS platform. Dependent on the sensitivity of the antibody, the IF processing method was separated into two parts, allowing the user to select either a one hour 1° antibody incubation at room temperature or an overnight incubation at 4 °C. While the total assay time for automated IF staining was similar to manual processing, the automated method contributed to significant hands-off time for laboratory personnel. During this time, the laboratory employee was able to fulfill other laboratory tasks during the staining process.

Based on the results, the automated IF staining method establishes compatibility for processing multiple cell lines and antibodies in a single run. Figure 3 shows GLUT 1 expression in both MDA-MB-231 and BT-474 breast cancer cell lines. Demonstrated by the intensity of the GLUT 1 expression,the results indicate that MDA-MB-231 cells have higher GLUT 1 expression in comparison to BT-474 cells. Figure 4 results also confirm excellent Vinculin and GLUT 1 fluorescent signal and sensitivity with minimal background in MDA-MB-231 cells. Clear protein localization and abundance for both Vinculin and GLUT 1 were observed with strong nuclear DAPI stain as well.

While only representative images are shown in this report, the automated method delivered a standardized, reproducible and accurate protocol for pipetting and staining, which eliminated common errors related to the manual process. Additionally, the automated method was more flexible, offering higher throughput of up to six plates per run, including inherent scheduling software to guarantee equal staining times for each cell line and antibody.

In summary, the results reported herein support an automated IF staining method on a low-cost, small footprint NIMBUS platform, allowing for accurate and precise sample processing with high scalability. Contact your local Hamilton representative today to get more information.

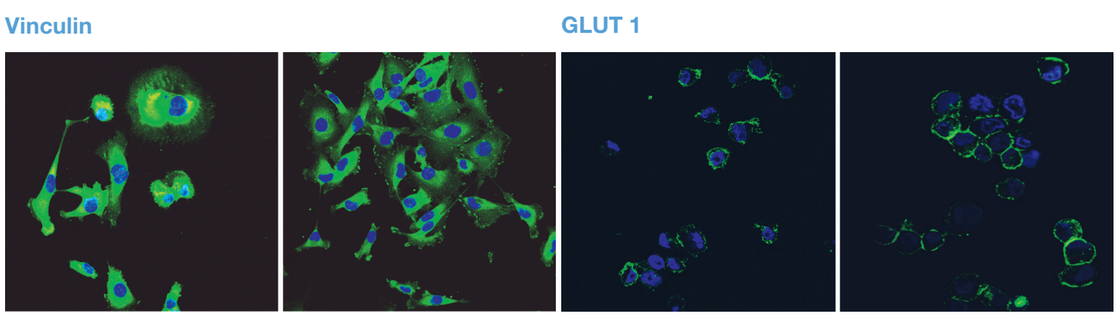


Figure 4: Protein localization assessment of Vinculin and GLUT 1. Automated immunofluorescence staining of MDA-MB-231 breast cancer cells using Vinculin or GLUT 1 (green) and DAPI (blue) on NIMBUS liquid handling platform. Cells were cultured on glass coverslips and fixed. Cells were then incubated for 1 hour at room temperature with primary antibody directed against vinculin (1:200) or GLUT 1 (1:500). Cells were incubated in the dark at room temperature for one hour with a fluorescent and donkey conjugated secondary antibody against rabbit IgG (1:500). Cells were stained with DAPI and inverted on to a glass cover slide with anti-fade mounting medium.