FluoXpert: New Upgrade of Multiplex Immunofluorescence Technology based on DNBSEQ-G400RS

mIF staining results comparable to IHC gold standard

- FluoXpert: One-machine, dual-mode (mIF staining and sequencing mode)

  DNBSEQ-G400RS FluoXpert provides users with a pleasant experience of easy switching between "sequencing mode" and "immunofluorescence staining-imaging mode".

- Efficient and high-quality sequencing data output

  DNBSEQ-G400RS, based on DNBSEQ sequencing technology, has significant features such as high accuracy, low repeat sequence rate, and low index hopping rate; meeting the sequencing demands of various sample types.

- Multiplexed immunofluorescence (mIF): Outstanding technology and User-friendly

  High-throughput ultra-multiplexed immunofluorescence, efficient and fully automated staining and imaging process, highly repeatable staining and precise spatial positioning, and low antibody consumption with mild elution.

Background

Currently, the routine clinical tumor diagnosis still mainly relies on traditional Hematoxylin-Eosin (H&E) staining and immunohistochemistry (IHC) staining[1]. However, H&E staining can only provide qualitative analysis results for pathological tissue sections[2]. Traditional IHC technology is restricted to staining a single target on a section, and serial sections staining are needed during analyzing multiple proteins, resulting in sample waste[1]. Traditional IHC is also hard to achieve quantitative analysis of multiplexed protein expression.

Multiplex immunofluorescence (mIF) technology could detect different targeted proteins on the same section while preserving spatial information. Presently, mIF has been widely used to study the cellular composition, functional phenotype, and intercellular interactions of target tissues, and is promising in clinical pathological diagnosis[3, 4]. Compared to other quantitative techniques based on tissue analysis, mIF technology eliminates the need for enzyme digestion of tissues or cell suspensions preparation. This feature facilitates mIF to avoid the destruction and transformation of original tissue sections, thus preserving information on tissues, immune systems, and tumor cells at different locations[5, 6]. The combination of mIF and analysis software can not only analyze the immune status of the complicated tumor microenvironment (TME) better, but also provide new analytical basis from the perspective of digital pathology[7, 8].

The DNBSEQ sequencing platform self-developed by MGI is increasingly favored by global customers due to its high accuracy and sensitivity, ultra-low duplication rate, low index hopping rate, etc. The flagship DNBSEQ-G400RS genetic sequencer promotes continuously sequencing throughput increasing and sequencing costs reducing through continuous technological iterations and upgrades[9]. Meanwhile, the DNBSEQ-G400RS FluoXpert integrating mIF staning and imaging, and sequencing was officially unveiled. mIF assays for different types of target proteins are achieved through multiple cycles of staining, imaging, and elution (Fig. 1). Its unique biochemical process avoids primary antibody conjugation and enables the compatibility of commercial or self-developed antibodies. Antigen-antibody precise recognition ensures accurate staining of each antigen. The imaging process have an advanced optical system and an image stitching algorithm that ensures extremely high spatial resolution and achieving precise positioning of tissue sections. The mild elution process ensures low tissue damage and a new round of antigen-antibody binding, achieving multiple cycles of staining and imaging.

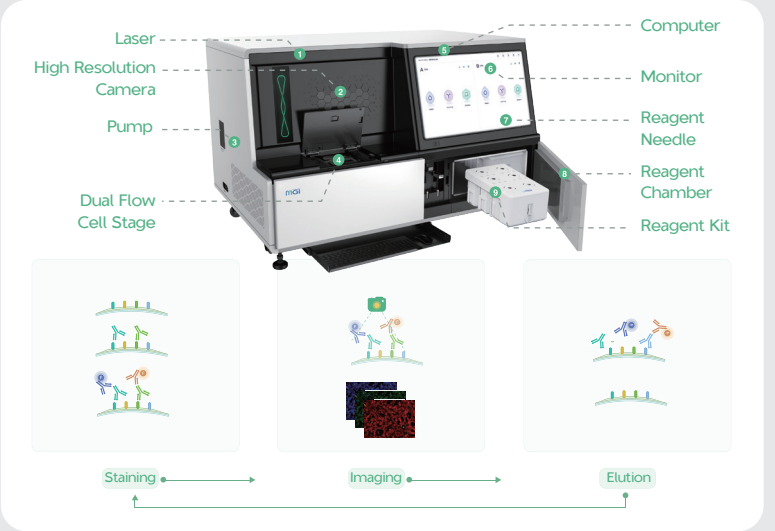

Figure 1. DNBSEQ-G400RS FluoXpert achieves mIF of different target proteins through the integration of staining, imaging, and elution. DNBSEQ-G400RS FluoXpert mainly consists of the optical section (No. 1, No. 2), chip fluid section (No. 3, No. 4 , No. 7), user interface section (No. 5, No. 6), and kit section (No. 8, No. 9). The staining kit needs to be placed in the 2-8°C module equipped within FluoXpert for later use. Reagents are drawn from different wells of the kit using the reagent needle, and flow through the chip stage containing tissue sections through a fluid pump. Different scripts of staining, imaging and elution are developed to achieve corresponding functions for tissue sections, and drive multiple cycles of staining, imaging, elution and re-staining.

This study conducted mIF and IHC simultaneously on the same pathological section for each marker protein (CD3, CD8, CD68, and PanCK), based on DNBSEQ-G400RS FluoXpert. The percentage of positive signals for corresponding protein markers was calculated separately. Each marker was compared within the same locations between mIF and IHC under different fields for different sets.

The results showed no significant difference in the average percentage of positive signals between mIF and IHC, indicating that mIF technology can be successfully implemented in DNBSEQ-G400RS FluoXpert and can be applied in basic and translational medicine research.

Materials and Methods

Sample collection and pre-treatment

Samples used in this study were provided by the collaborator and stored in a pathological section box at 4°C prior to experiment. Before mIF or IHC experiment, samples should be processed according to the routine pathological tissue sectioning procedures. Briefly: 1. Samples were deparaffinized with xylene and hydrated with a gradient of ethanol in the fume hood; 2. Antigens were retrieved with alkaline antigen retrieval buffer at 95 ℃ high-temperature oven; 3. LED light and hydrogen peroxide photobleaching.

mIF experimental procedure

Four target proteins (CD3, CD8, CD68, and PanCK) were investigated in this experiment. The details of primary antibodies are as follows: CD3 (1:1000, Rb, SinoBiological CT026-R301), CD8 (1:250, Ms, SinoBiological 10980-MM38), CD68 (1:1000, Rb, Sigma HPA048982), PanCK (1:500, Ms, Dako NBP2-29429). Before loading the sections on the machine, a mIF kit suitable for DNBSEQ-G400RS FluoXpert was prepared. The corresponding primary antibodies were added to the reagent kit and mixed well.

Assembly according to the instructional manual: The cover plate with a golden tape and the slide glass containing the tissue should be tightly aligned to each other. Staining and imaging on the machine: the automatic staining and imaging process was started after completing the staining scheme settings based on the pre-entered antibody information according to the experimental requirements. For detailed experimental procedures, please refer to the DNBSEQ-G400RS FluoXpert experimental instructional manual.

IHC experimental procedure

The IHC experiment was carried out with the traditional IHC staining procedure; including blocking, primary antibody incubation, secondary antibody incubation, DAB staining reaction, nucleus staining with hematoxylin, ethanol fixation, section sealing and preservation/imaging.

mIF/IHC image processing and analysis

The mIF automatic staining images were analyzed using the FluoXpert Vision Analysis Platform. Analysis such as image stitching and cell segmentation was gradually performed according to specific algorithms, and quantification of fluorescence intensity to each protein was also performed. After section sealing, the IHC images were observed under a microscope to find 12 sets of regions of interest (ROIs) at the same location on the mIF image for comparative study, sizing (1000-2000)* (1000-2000) pixels each ROI. Finally, the total cell count and positive cell ratio in the corresponding fields for the ROIs were counted and recorded.

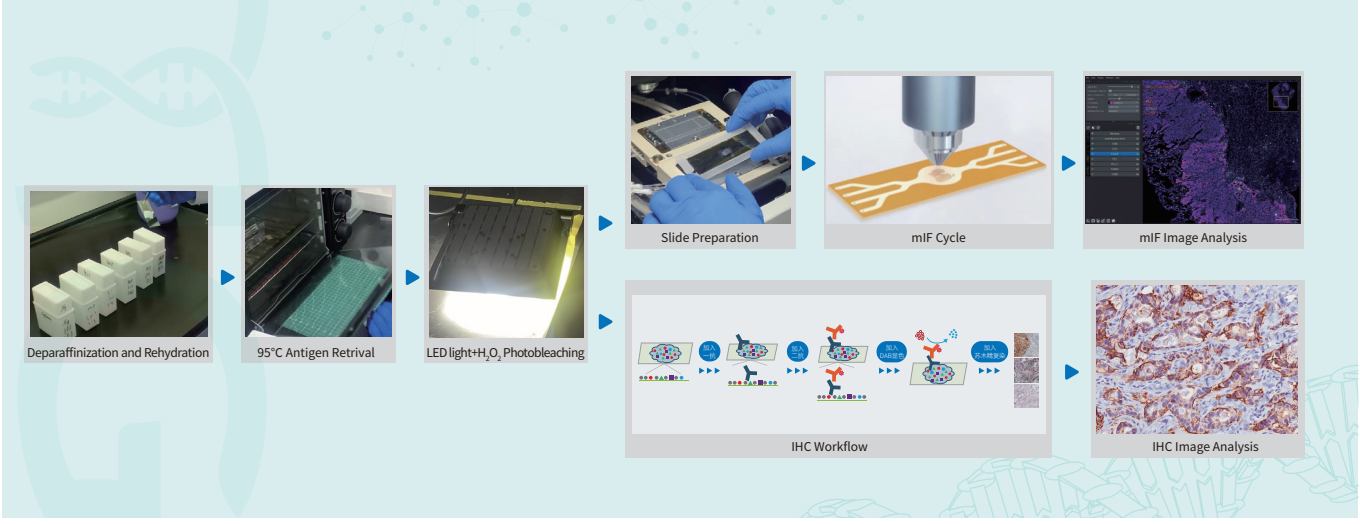

Figure 2. The schematic diagram of the workflow for tissue section samples under mIF or IHC staining. The entire process can be summarized as follows: (1) in the fume hood: deparaffinization, hydration, antigen repair, and quenching; (2) mIF workflow: preparation before loading to DNBSEQ-G400RS FluoXpert, imaging, and data analysis; (3) IHC workflow: IHC staining, image stitching and image analysis under a microscope.

Results

DNBSEQ-G400RS FluoXpert achieves accurate and efficient mIF staining and imaging of tissue sections

DNBSEQ-G400RS FluoXpert can ensure mIF staining and imaging for tissue sections. Its proprietary sequencer-level precise hardware ensures clear, fast, and accurate capture of target protein expression on tissue section. In this study, mIF images of CD3, CD8, CD68, and PanCK were obtained after a series of preprocessing operations and automatic staining and imaging processes on tissue sections (Fig. 3A, B, C, D). The results showed that the mIF staining group of positive cells were highly similar to the IHC group (Fig. 3A', B', C', D'), which could be used for the comparison of subsequent detection.

The DNBSEQ-G400RS FluoXpert based mIF is highly consistent with IHC (gold standard)

In this study, three sets of mIF vs IHC images with the same size (1024*1024 pixels) were randomly selected for each target protein for a comparative evaluation, ensuring a fair and standardized analysis. (Fig. 4A-D'). Subsequently, FluoXpert Vision was used to statistically analyze the total cell number and positive signal rate. There was no significant difference in the overall average positive signal between the IHC and the mIF groups. For most sets of ROIs, there was no significant difference in the positive rate between IHC and mIF images (Fig. 4E-H).

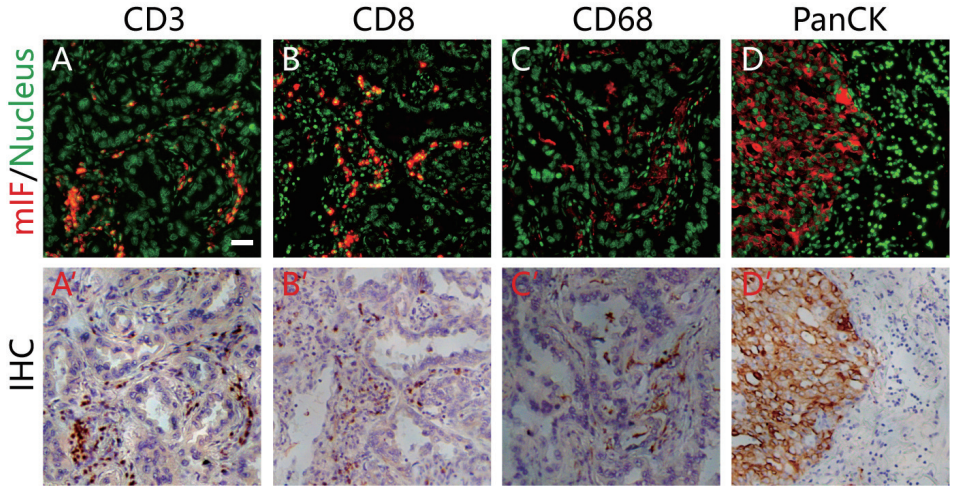

Figure 3: Representative images of target proteins in mIF and IHC. The mIF images of CD3, CD8, CD68, and PanCK generated on DNBSEQ-G400RS FluoXpert (A-D); the IHC staining images corresponded to the mIF images at the same position (A'-D'). Scale bar: 100 μm.

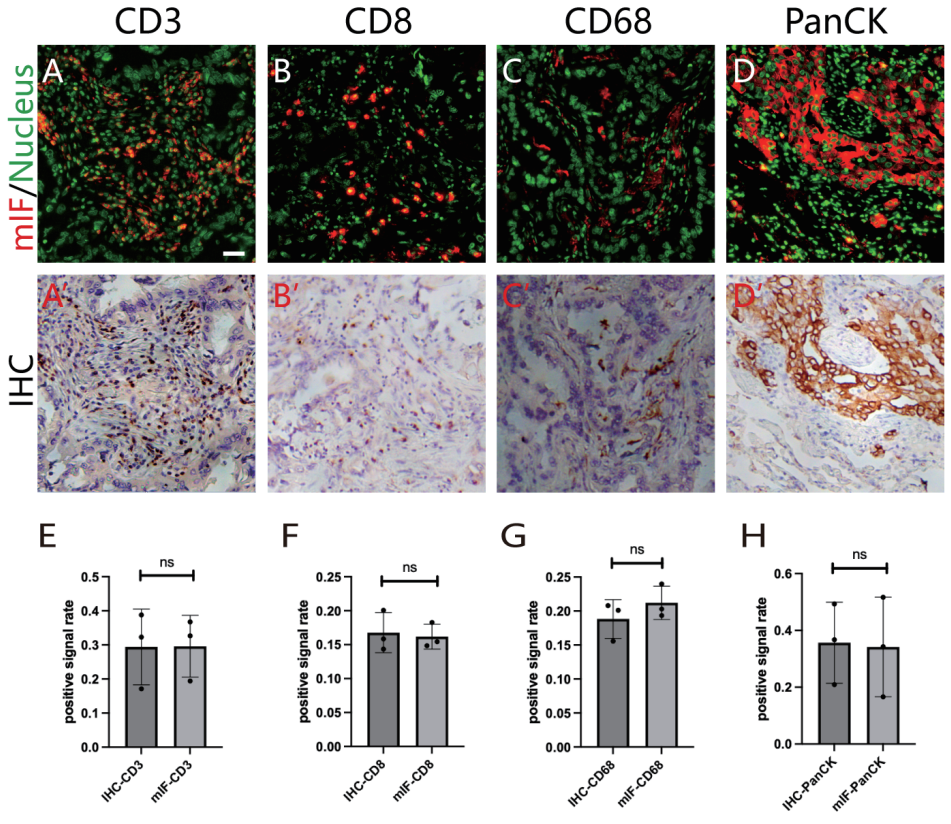

Figure 4: Statistical analysis of IHC vs mIF images under multiple fields of view for CD3, CD8, CD68, and PanCK. Corresponding representative mIF and IHC images (A-D, A'-D'); Statistical charts of positive signal ratios for different target proteins (E-H). Scale bar: 100 μm.

Conclusion

This study indicates that the mIF staining based on DNBSEQ-G400RS FluoXpert are comparable to the IHC (gold standard) staining. The statistical results showed that there was no significant difference in the average percentage of positive signals for each protein. DNBSEQ-G400RS FluoXpert can provide an effective experimental tool in the fields of pathological research, drug discovery, and translational medicine.

MultiOmics Analyzer DNBSEQ-G400RS FluoXpert (Left)
FluoXpert Vision mIF image analysis platform (Right)

References

1. Harms, P. W. *et al.*, Multiplex Immunohistochemistry and Immunofluorescence: A Practical Update for Pathologists. *MODERN PATHOL* 36 100197 (2023).
2. Dunn, C. *et al.*, Quantitative assessment of H&E staining for pathology: development and clinical evaluation of a novel system. *DIAGN PATHOL* 19 42 (2024).
3. Lu, S. *et al.*, Comparison of Biomarker Modalities for Predicting Response to PD-1/PD-L1 Checkpoint Blockade: A Systematic Review and Meta-analysis. *JAMA ONCOL* 5 1195 (2019).
4. Tan, W. *et al.*, Overview of multiplex immunohistochemistry/immunofluorescence techniques in the era of cancer immunotherapy. *CANCER COMMUN* 40 135 (2020).
5. Lee, C. W. *et al.*, Multiplex immunofluorescence staining and image analysis assay for diffuse large B cell lymphoma. *J IMMUNOL METHODS* 478 112714 (2020).
6. Parra, E. R., Methods to Determine and Analyze the Cellular Spatial Distribution Extracted From Multiplex Immunofluorescence Data to Understand the Tumor Microenvironment. *FRONT MOL BIOSCI* 8 668340 (2021).
7. Wen, Z. *et al.*, Deep Learning-Based H-Score Quantification of Immunohistochemistry-Stained Images. *MODERN PATHOL* 37 100398 (2024).
8. Ghahremani, P. *et al.*, Deep Learning-Inferred Multiplex ImmunoFluorescence for Immunohistochemical Image Quantification. *NAT MACH INTELL* 4 401 (2022).
9. Lang, J. *et al.*, Evaluation of the MGISEQ-2000 Sequencing Platform for Illumina Target Capture Sequencing Libraries. *FRONT GENET* 12 730519 (2021).

Recommended Ordering Information

Category	Product	Cat. NO.
Instrument	MultiOmics Analyzer DNBSEQ-G400RS FluoXpert	900-000938-00
mIF Reagents	FluoXpert mIF Set SS 6P containing mIF 6P kit+ SS flow cells	940-002132-00
	FluoXpert mIF Set SS 24P, containing mIF 24P kit+ SS flow cells	940-002221-00
Software and workstation	FluoXpert Vision mIF image analysis platform including analysis software (FluoXpert Vision) and workstation	900-000924-00

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