

Technical note

SEP 2026



MoxiePlex®

Multi-spectral imaging system

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1. Unmixing

1-1. Introduction

Whole Slide Imaging (WSI) of tissue sections is a technique that captures an entire tissue specimen as a high-magnification digital image. By examining the morphology and distribution of cells within the tissue, abnormalities associated with disease can be identified. This approach is widely used in applications ranging from research to clinical practice.

Various staining methods are used for tissue visualization. Hematoxylin and eosin (H&E) staining is a common technique for evaluating tissue morphology by staining nuclei and cytoplasm and observing the specimen under brightfield microscopy. In contrast, immunohistochemistry (a method in which antibodies bind to target antigens and associated labels are detected) and in situ hybridization (a method in which probes with sequences complementary to target DNA or mRNA are bound and detected through associated labels) enable visualization of the spatial distribution of specific biological targets using either brightfield or fluorescence imaging. These techniques are also used in companion diagnostics and definitive diagnostic applications.

In recent years, the development of multiplex immunofluorescence staining technologies has made it possible to stain multiple biological targets simultaneously within a single tissue section. This enables the identification of different cell types and the analysis of cell-to-cell interactions in situ at cellular and subcellular resolution, leading to expanded applications across a variety of fields.

Advances in multiplex fluorescence staining have been driven not only by improvements in reagents, but also by technological innovations throughout the entire workflow, including staining, image acquisition, image processing, and image analysis. In particular, there is increasing demand for higher throughput and improved image quality in fluorescence imaging systems.

At the same time, fluorescence imaging presents inherent challenges. Fluorophores possess characteristic excitation and emission wavelength ranges determined by their molecular structures. In conventional fluorescence microscopy, excitation light sources, excitation and emission bandpass filters, and dichroic mirrors are selected according to the fluorophores being used so that specific fluorescence wavelengths can be detected selectively.

Another important issue in fluorescence imaging of tissue sections is autofluorescence. Autofluorescence refers to fluorescence emitted by tissues or cells themselves without fluorescent labeling. Because autofluorescence overlaps with fluorophore-derived signals, it can complicate image analysis and interpretation.

Autofluorescence originates from a variety of tissue components. Examples include biological molecules such as collagen, elastin, NADH, and flavins. In addition, chemicals used during tissue fixation and processing may also contribute to autofluorescence.

This technical note introduces Unmixing as an approach for addressing these challenges and describes the benefits achieved when using the MoxiePlex multi-spectral imaging system.

1-2. Principles of spectral unmixing

In multiplex fluorescence imaging, spectral unmixing separates mixed signals into their original components. It is based on the assumption that an observed signal can be represented as a linear or nonlinear combination of multiple source signals. Methods such as linear and nonlinear spectral unmixing have been reported. This technical note focuses on linear spectral unmixing.

As an example, consider fluorescence imaging of three fluorophores (Dye1–Dye3) arranged as shown in Fig. 1-1. Fig. 1-2 shows the excitation and emission spectra of the fluorophores together with the spectral transmittance of the excitation and emission filters. The sample is assumed to be illuminated over the wavelength range transmitted by each excitation filter.

Under ideal conditions with no crosstalk between the fluorophores, excitation light, and filter sets (Figs. 1-2A and 1-2B), the signal from each fluorophore is completely separated for each excitation source (EX_B, EX_G, and EX_R), producing the corresponding fluorescence images shown in Fig. 1-3A.

In contrast, when multiple fluorophores are excited by the same excitation source and their emission spectra overlap within the detection range of the emission filters (EM_B–EM_R), crosstalk occurs, as shown in Figs. 1-2C and 1-2D. Consequently, the signals from Dye1–Dye3 are not completely separated, and each image contains signals from multiple fluorophores, as shown in Fig. 1-3B.

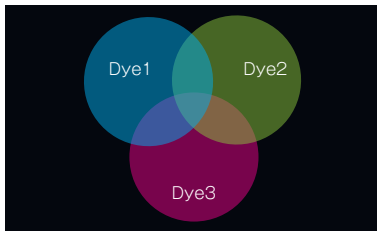


Fig. 1-1

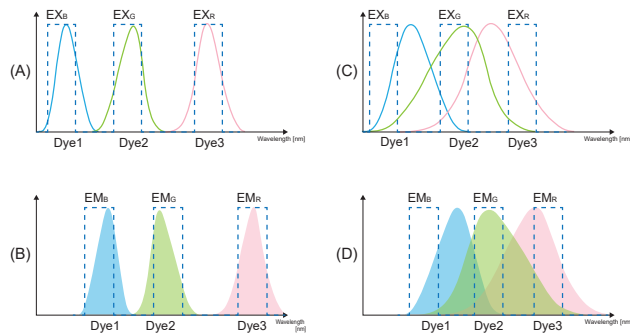


Fig. 1-2:
 (A) Excitation spectra of fluorophores and spectral transmittance of excitation filters in an ideal imaging system
 (B) Emission spectra of fluorophores and spectral transmittance of emission filters in an ideal imaging system
 (C) Excitation spectra of fluorophores and spectral transmittance of excitation filters in a system with crosstalk
 (D) Emission spectra of fluorophores and spectral transmittance of emission filters in a system with crosstalk

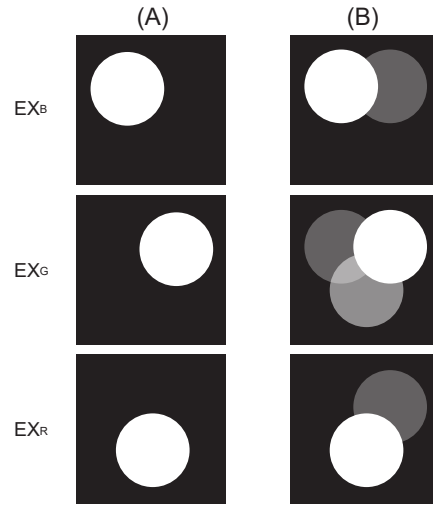


Fig. 1-3

Assuming that the mixed signals shown in Fig. 1-3B follow a linear model, the contribution of each fluorophore under each excitation condition can be expressed as a relative signal intensity measured under the imaging conditions. These relationships are defined as the spectral library matrix A , where the rows correspond to excitation conditions and the columns correspond to the relative signal intensities of the fluorophores.

$$A = \begin{pmatrix} 1 & 0.3 & 0 \\ 0.3 & 1 & 0.4 \\ 0 & 0.2 & 1 \end{pmatrix}$$

Under the ideal imaging condition shown in Fig. 1-3A, each excitation source corresponds to only one fluorophore. In this case, the spectral library matrix A becomes an identity matrix in which the diagonal elements are equal to 1 and all other elements are 0. The relationship between the true fluorophore image X and the observed image Y can be expressed using the spectral library matrix A as follows:

$$Y = AX + N$$

where X and Y are matrices whose rows correspond to excitation conditions and whose columns correspond to pixel numbers, and N represents noise.

When the observed image Y and the spectral library matrix A are known, the unmixed image X' can be calculated using the following equation:

$$X' = (A^T A)^{-1} A^T Y$$

This process is referred to as Linear Spectral Unmixing. Linear Spectral Unmixing estimates X' by minimizing the difference between the observed signal and the reconstructed signal, $\|Y - AX'\|$. It should therefore be noted that the resulting image X' is an approximation of the true signal X , rather than a perfect reconstruction.

1-3. Benefits of unmixing

1-3-1. Example of linear spectral unmixing

Fluorescent tapes were arranged as shown in Fig. 1-4, and brightfield and fluorescence images were acquired using MoxiePlex under conditions that generate crosstalk. As shown on the left side of Fig. 1-6, fluorescence images acquired under crosstalk conditions contain overlapping signals from multiple fluorescent tapes.

The results after applying Linear Spectral Unmixing are shown on the right side of Fig. 1-6. In the unmixed image, the lines corresponding to each fluorescent tape are independently separated, and their arrangement is consistent with the conceptual diagram of the fluorescent tape layout shown in Fig. 1-5 (bright-field equivalent).

Next, the quantitative performance of the method was evaluated. Fig. 1-7 shows the unmixed image and line profiles of LineP. The line profiles along the dashed lines are displayed around the image using the corresponding colors.

Although the observed signal varies depending on the vertical and horizontal overlap of the fluorescent tapes, distinct changes in signal intensity can be clearly observed in both the vertical and horizontal line profiles. These results demonstrate that the fluorescence signals obtained after unmixing retain quantitative information.

Furthermore, unmixing is effective for reducing autofluorescence. Because autofluorescence exhibits characteristic broad spectral features, it can be included in the spectral library or modeled as a background component, allowing it to be separated from the signal of each fluorophore. As a result, the accuracy of target signal extraction is improved, enabling more reliable image analysis.



Fig. 1-4: Arrangement of fluorescent tapes

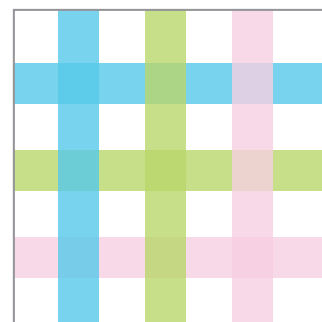


Fig. 1-5: Conceptual diagram of the fluorescent tape arrangement (bright-field equivalent)

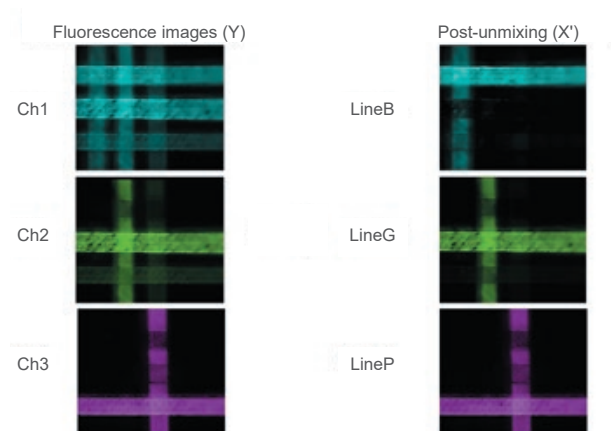


Fig. 1-6: Fluorescence images of fluorescent tapes

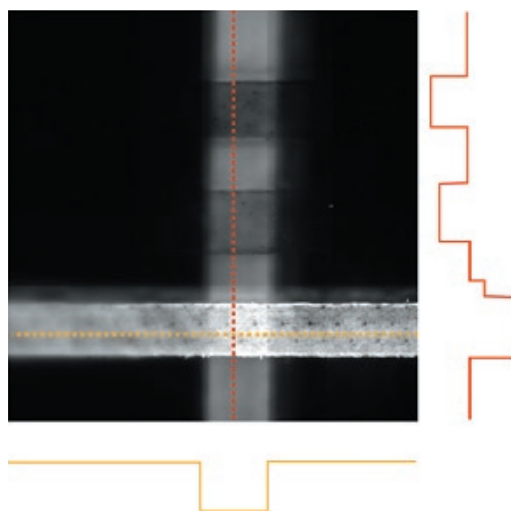


Fig. 1-7: Unmixed image and line profiles of LineP

1-3-2. Evaluation of multiplex fluorescence-stained samples

The sample information is summarized in the accompanying table. Fig. 1-8 compares multiplex fluorescence images acquired pre-unmixing and post-unmixing under imaging conditions that generate crosstalk. In the unmixed images, a spectral library that includes autofluorescence components was constructed, enabling the separation and removal of autofluorescence signals.

In the pre-unmixing images, autofluorescence contributes substantially to the background signal, making it difficult to distinguish biological target signals. In contrast, the post-unmixing images clearly separate the signals from each fluorophore while significantly reducing the influence of autofluorescence. As a result, signals originating from specific biological targets can be extracted with greater accuracy.

Fig. 1-9 provides representative examples of images acquired pre-unmixing and post-unmixing for each fluorophore. The images on the left represent the original data, while those on the right show the post-unmixing results. In shorter-wavelength channels such as CM480, CM520, and CM570, where autofluorescence is often prominent, strong background signals can be observed in pre-unmixing images. In post-unmixing images, autofluorescence is substantially reduced while preserving biomarker signal intensity.

Similarly, in the CM780 channel, crosstalk originating from the CM690/CK channel is visible in the lower-left portion of the pre-unmixing image. This crosstalk is effectively reduced after unmixing. These results demonstrate that highly accurate unmixing can effectively suppress autofluorescence and crosstalk, thereby improving biomarker identification accuracy, enhancing downstream image analysis, and reducing manual intervention during the analytical workflow.

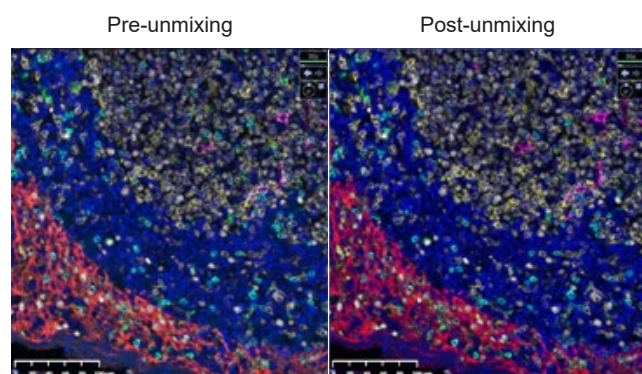


Fig. 1-8: Multiplex fluorescence images

Pseudocolor	Reagent (Celnovte)	Target	Expression
Cyan	CM480	CD8	Cytoplasm
Green	CM520	CD3	Cytoplasm
Yellow	CM570	CD4	Cytoplasm
Magenta	CM620	CD163	Cytoplasm
Red	CM690	CK	Cytoplasm
White	CM780	Ki67	Nuclear
Blue	DAPI	Nuclear	Nuclear

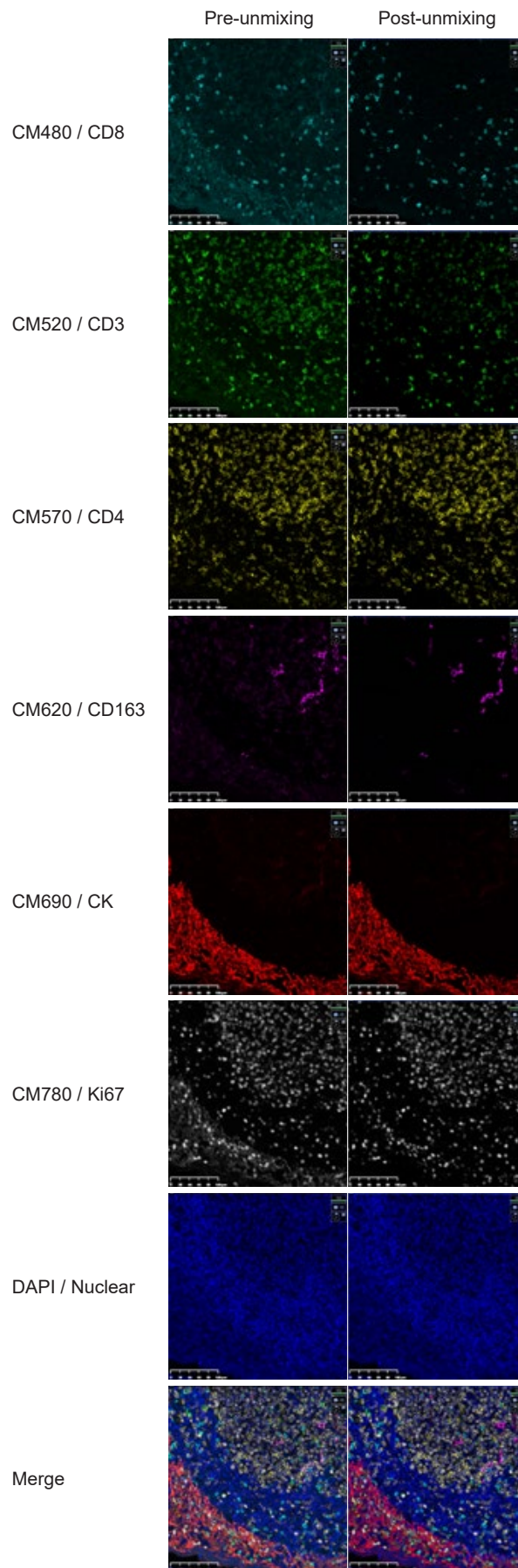


Fig. 1-9: Unmixing results for individual fluorophores

1-3-3. Detection of autofluorescence and the concept of unmixing

Autofluorescence generated by tissue-derived components such as red blood cells exhibits broad spectral characteristics and is therefore detected across multiple fluorescence channels. Consequently, autofluorescence can overlap with target fluorescence signals and interfere with accurate signal extraction.

To illustrate the impact of autofluorescence, Fig. 1-10 compares images with the autofluorescence channel displayed and hidden. The image on the left includes the autofluorescence channel, whereas the image on the right excludes it.

In the upper images, strong autofluorescence originating from red blood cells within blood vessels can be observed. Accurate detection of autofluorescence enables these signals to be distinguished from biological target signals and displayed as a separate channel.

In the middle images, FoxP3 nuclear signals are displayed in yellow. Because these signals can easily be obscured by autofluorescence, accurate nuclear detection becomes difficult when autofluorescence is not considered. By applying unmixing, however, the biological target signal can be isolated and visualized clearly.

The lower images contain staining artifacts. Such artifacts also exhibit spectral characteristics that differ from those of the intended targets and can therefore be separated based on spectral information. These examples demonstrate how accurate detection and unmixing of autofluorescence facilitate reliable extraction of target signals.

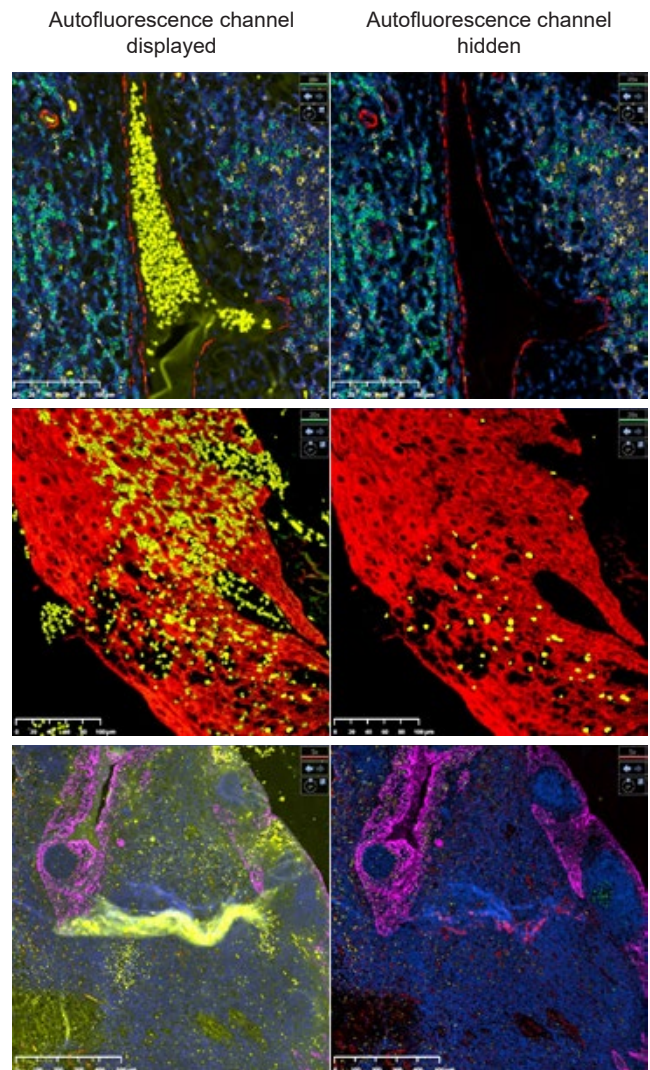


Fig. 1-10: Comparison of images with the autofluorescence channel displayed and hidden

Left: Autofluorescence channel displayed

Right: Autofluorescence channel hidden

This figure demonstrates how autofluorescence and non-specific signals, including red blood cell-derived autofluorescence in blood vessels, obscuration of FoxP3 (nuclear) signals, and staining artifacts, are observed. By correctly detecting and separating autofluorescence, extraction of target signals becomes easier and more reliable.

1-3-4. Spectral characteristics of autofluorescence and target signals

Fig. 1-11 shows the spectral characteristics of autofluorescence, the target fluorophore, and the filter configuration of MoxiePlex. Opal 480 is used as an example of a target fluorophore.

Comparison of the fluorescence spectra reveals the following characteristics:

- The fluorescence spectrum of red blood cell-derived autofluorescence is extremely broad and can be detected across multiple fluorescence channels.
- In contrast, the fluorescence signal of the target fluorophore (Opal 480) has a narrower spectral bandwidth and is concentrated within specific detection channels.

These differences in spectral characteristics provide the basis for signal separation by unmixing.

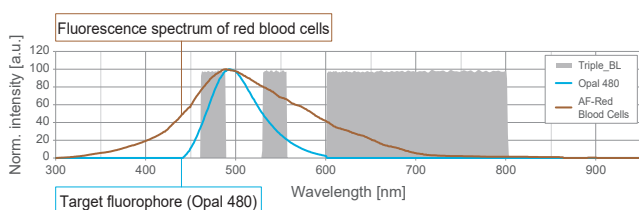


Fig. 1-11: Spectral characteristics of autofluorescence and target fluorescence

Red blood cell-derived autofluorescence exhibits a very broad fluorescence spectrum and is detected across multiple channels. In contrast, target fluorescence (Opal 480) has a narrower spectral bandwidth and is concentrated within specific channels. These differences in spectral characteristics provide the basis for signal separation by unmixing.

1-3-5. Signal differences under various excitation conditions

Fig. 1-12 shows the excitation spectra of Opal 480 and autofluorescence.

The excitation conditions generate the following responses:

1. Blue excitation: Both Opal 480 and red blood cell-derived autofluorescence are excited.
2. Green excitation: Only red blood cell-derived autofluorescence is excited.
3. Orange excitation: Only red blood cell-derived autofluorescence is excited.

These differences provide the information required to distinguish target fluorescence from autofluorescence.

When images are acquired under multiple excitation conditions:

- Blue excitation → Target fluorescence + autofluorescence are observed.
- Green / Orange excitation → Autofluorescence only.

The differences among these images can then be used for spectral separation.

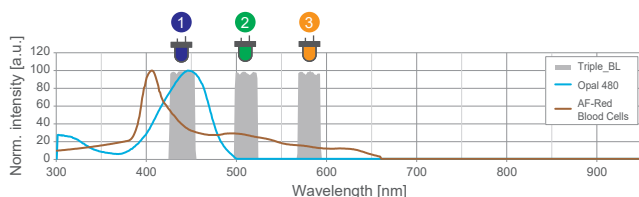


Fig. 1-12: Excitation spectra of Opal 480 and autofluorescence

Under Blue excitation, both Opal 480 and red blood cell-derived autofluorescence are excited. Under Green and Orange excitation, only red blood cell-derived autofluorescence is excited. These differences in excitation conditions provide information for separating target signals from autofluorescence.

1-3-6. Verification using actual scan images

Fig. 1-13 shows actual scan images acquired under Blue, Green, and Orange excitation conditions, together with a merged image.

Image layout:

- Upper left: Merged image
- Upper right: Blue excitation (Target fluorescence + autofluorescence)
- Lower left: Green excitation (Autofluorescence only)
- Lower right: Orange excitation (Autofluorescence only)

In the merged image:

- White regions represent signals observed under all three excitation conditions and therefore correspond to autofluorescence.
- Cyan regions represent signals detected only under Blue excitation and therefore correspond to the target fluorophore (Opal 480).

These results demonstrate that spectral information obtained using multiple excitation conditions enables discrimination between target fluorescence and autofluorescence.

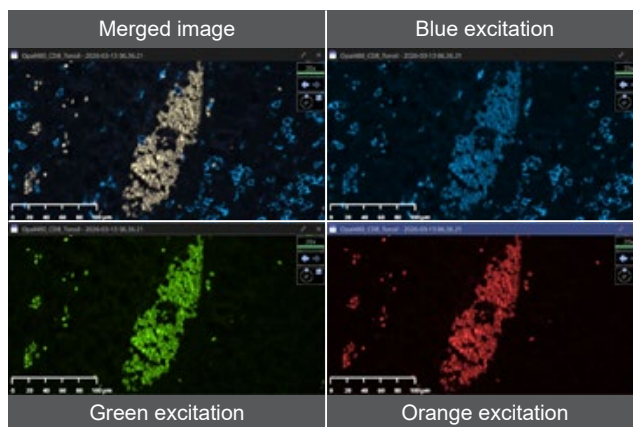


Fig. 1-13: Actual images acquired under different excitation conditions and the merged image

In the merged image, regions observed under all three conditions (white) correspond to autofluorescence, while regions observed only under Blue excitation (cyan) correspond to the target signal.

1-3-7. Extraction of autofluorescence spectra and the benefits of unmixing

Acquiring images under multiple excitation conditions enables highly accurate extraction of autofluorescence spectral information. As the number of channels containing autofluorescence information increases, the spectral characteristics of autofluorescence can be characterized more accurately, resulting in more effective autofluorescence reduction during unmixing.

Following unmixing, several important benefits can be achieved:

- Reduction of autofluorescence while preserving biological target signal intensity.
- Suppression of signal leakage from other fluorophores (crosstalk).
- Improved biological target recognition accuracy.
- Enhanced performance of downstream image analysis.
- Reduced manual intervention throughout the analytical workflow.

1-4. Unmixing workflow

The Unmixing workflow consists of the following steps:

1. Library Creation: Create a spectral library corresponding to the fluorophores and imaging conditions used.
2. Image Acquisition: Acquire fluorescence images containing signals from multiple fluorophores.
3. Unmixing Process: Process the acquired images using the spectral library to generate and save unmixed images.

Several preparatory steps are required to perform this workflow. This section describes key considerations and operational challenges associated with each step.

Library creation

Because the spectral library depends on the fluorophores used and the imaging system, it must be prepared in advance for each sample type. To create the spectral library, images of single-stained samples must be acquired under conditions equivalent to those used for imaging multiplex-stained samples. In addition, the library must be updated whenever assay conditions or the imaging system are changed, which can increase both time and cost in routine operation.

Image acquisition

For image acquisition, high-quality images with sufficient signal intensity must be obtained under conditions corresponding to the spectral library. Therefore, optimal exposure settings must be established to avoid signal saturation and low-signal conditions.

Unmixing process

Because Unmixing is typically applied to Whole Slide Imaging (WSI) data, large-scale data processing is required. Consequently, sufficient memory capacity and high-speed computational performance are necessary to ensure that processing time does not become a bottleneck.

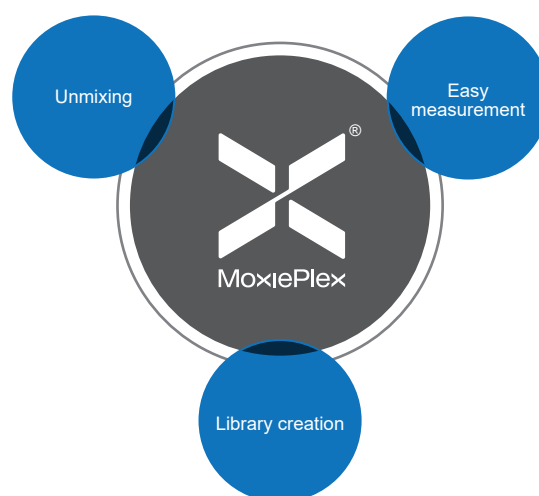
1-5. Our technology

MoxiePlex incorporates unique functions designed to address the challenges associated with Unmixing workflows.

The Library Creation function acquires spectral information from single-stained samples and generates a spectral library based on the acquired data. In addition, the system provides a library update function that allows the spectral library to be revised when assay conditions or imaging system configurations change. For samples exhibiting autofluorescence, spectral libraries that include autofluorescence components can also be generated.

The Easy Measurement function automatically applies measurement conditions (Scan Profiles) associated with the selected spectral library, reducing the complexity of parameter setup and management. During image acquisition, measurement parameters are automatically adjusted to achieve optimal image quality.

The Unmixing function performs Linear Spectral Unmixing based on the selected spectral library and can execute the process in real time during scanning. This helps reduce data processing time and data storage requirements.



Library creation

- Library update function
- Supports creation of spectral libraries including autofluorescence

Easy measurement

- Automatic linkage of spectral libraries and scan profiles
- Automatic parameter setting

Unmixing

- Linear spectral unmixing
- Real-time processing

2. Reagent compatibility

2-1. Introduction

As multiplex fluorescence staining technologies continue to evolve, the number and diversity of biomarkers analyzed within a single assay are rapidly increasing. As a result, imaging systems are required to support a wide range of fluorescent reagents and assay chemistries without dependence on a specific vendor or staining platform.

MoxiePlex is designed as a flexible imaging platform that combines broad spectral sensitivity (400–900 nm), multi-wavelength excitation, and spectral separation technologies. This enables compatibility with a wide range of fluorescent dyes, multiplex panels, and signal amplification methods, allowing researchers to select the most appropriate reagents for their applications.

Beyond simply increasing assay plexity, MoxiePlex supports the integration of diverse staining approaches—including TSA, barcode-based technologies, FISH, and other multiplex assay formats—within a unified imaging workflow. Through Unmixing-based image acquisition and analysis, fluorescence signals with overlapping spectral characteristics can be accurately separated and quantified.

This broad reagent compatibility provides both technical flexibility and future scalability, supporting applications ranging from exploratory research and translational studies to LDT development and future clinical applications. As a result, reagent compatibility represents one of the core strengths of the MoxiePlex platform.

2-2. Technical advantages of MoxiePlex

MoxiePlex provides a highly flexible imaging platform designed to support a broad range of multiplex fluorescence technologies and reagent systems. By combining multi-wavelength excitation, spectral separation, and Unmixing-based analysis, the system enables researchers to utilize fluorophores and assay chemistries that may otherwise be difficult to combine within conventional imaging workflows.

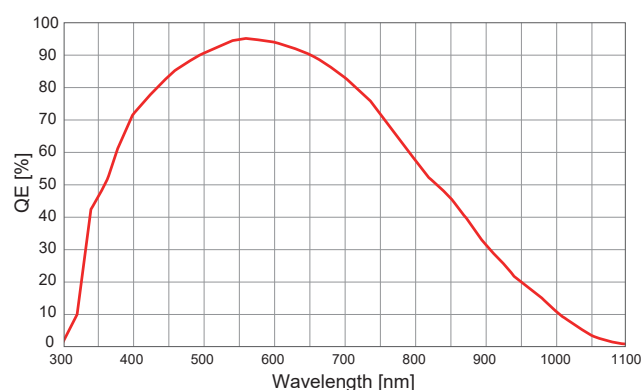


Fig. 2-1: Spectral sensitivity characteristics

2-3. Representative reagent combinations

2-3-1. Overview of representative reagent combinations

	DAPI	CFP	FITC	YFP	TRITC	mCherry		Cy5		Cy7
Akoya Opal 7-plex A	DAPI	Opal 480	Opal 520	-	Opal 570	Opal 620	-	-	Opal 690	Opal 780
Akoya Opal 7-plex B	DAPI	Opal 480	Opal 520		Opal 570	Opal 620	Opal 650	-	Opal 690	-
Akoya Opal 9-plex	DAPI	Opal 480	Opal 520	Opal 540	Opal 570	Opal 620	Opal 650	-	Opal 690	Opal 780
Vizgen In-situ Plex	DAPI	-	ATTO488	-	-	ATTO590	-	ATTO647N	-	Alexa750
Cell signal Technology Signal star	DAPI	-	Alexa488	-	-	Alexa594	-	Alexa647	-	Alexa750
Thermo Fisher Alexa	DAPI	-	Alexa488	-	Alexa555	Alexa594	-	Alexa647	-	Alexa750
Roche Discovery	DAPI	DCC	FAM	Rho6G	-	Red 610	-	Cy5	-	-

[NOTES]
Rho6G: Rhodamine 6G

2-3-2. Celnovte / TSA

Celnovte's CelMaxTSA is a multiplex immunofluorescence technology based on Tyramide Signal Amplification (TSA) using unlabeled primary antibodies. This approach enables highly multiplexed analysis without requiring pre-labeled primary antibodies and allows researchers to leverage existing antibody assets when designing panels. The reagent system is designed for multiplex immunohistochemistry (mIHC) workflows using FFPE (formalin-fixed paraffin-embedded) samples and provides high signal intensity, clear tissue localization, and low background.

Furthermore, Celnovte's proprietary MicroStacker™ Polymer Technology enables highly sensitive and specific detection, supporting accurate analysis even in complex tissue environments. When combined with MoxiePlex, the system supports spatial biology imaging with up to 9 channels.

In addition, simplified protocols are available for both manual and automated staining workflows. Validation has been performed on both Celnovte and third-party platforms, facilitating implementation within existing laboratory environments.

Reagent (Celnovte)	Pseudocolor
DAPI	Blue
CD8	Light blue
CD3	Green
CD4	Yellow
CD21	Red
CK	Pink
CD163	Orange
CD23	Light Green
CM780	White

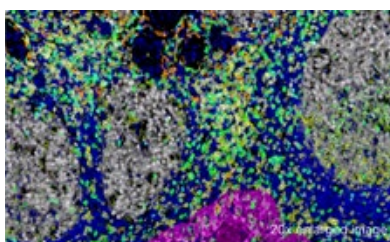


Fig. 2-2: 9-color composite image of an FFPE human tonsil sample

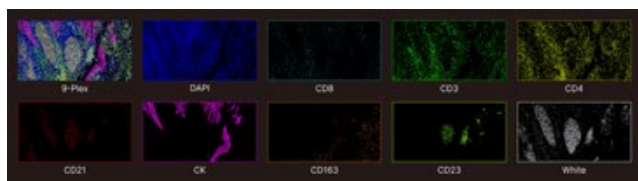


Fig. 2-3: Individual channel images

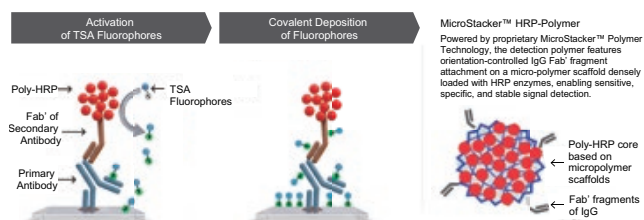


Fig. 2-4: MicroStacker™ polymer technology

MicroStacker HRP Polymer is a detection polymer featuring orientation-controlled attachment of IgG Fab' fragments on a micropolymer scaffold densely loaded with HRP enzymes. This design enables stable signal detection while maintaining high sensitivity and specificity.

2-3-3. Vizgen / In-situ Plex (OmniVUE / U-VUE)

OmniVUE and U-VUE assays use primary antibodies conjugated with DNA barcodes. These antibody conjugates are used to identify target proteins in fixed tissue or cell samples and can be applied in a single staining step.

After the sample is incubated with a mixture of antibody conjugates, the DNA barcode corresponding to each target is amplified to increase assay sensitivity. This amplification is performed simultaneously for all targets. Fluorescent probes complementary to the barcode sequences are then added to the sample, allowing targets to be sequentially bound and labeled during each imaging round.

Within a single imaging round, each target is labeled with a spectrally distinct fluorophore, enabling independent detection of each signal and Whole Slide Imaging. For high-plex assays, fluorescent probes are exchanged between imaging rounds using a gentle signal-removal (stripping) step.

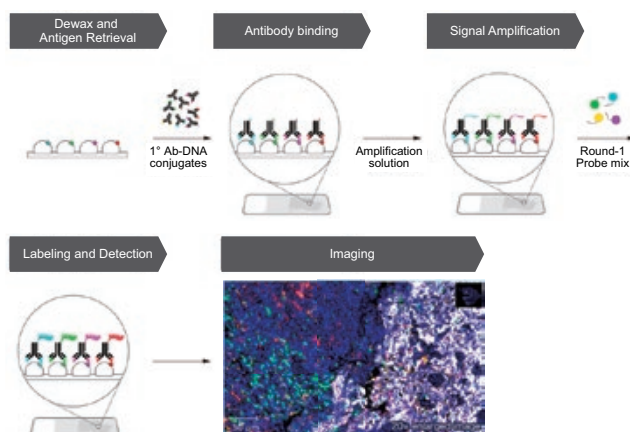


Fig. 2-5: Principle of In-situ Plex

2-3-4. Navinci / PLA

Navinci's in situ Proximity Ligation Assay (PLA) is a technology that converts molecular proximity into bright, localized DNA signals, enabling visualization of protein-protein interactions and protein modification states in cells and FFPE tissue samples.

Oligonucleotide probes conjugated to antibodies form a circular template only when they bind in close proximity to one another. This template serves as the starting point for rolling circle amplification, enabling single-molecule sensitivity. The UnFold/NaveniFlex probe design simplifies the ligation process while improving reaction efficiency, signal reproducibility, and background suppression.

The amplified products are detected using fluorescent or chromogenic probes, enabling multiplex analysis with high spatial resolution on standard imaging platforms. This amplification-based detection strategy allows quantitative mapping of protein networks and post-translational modifications in complex biological samples.

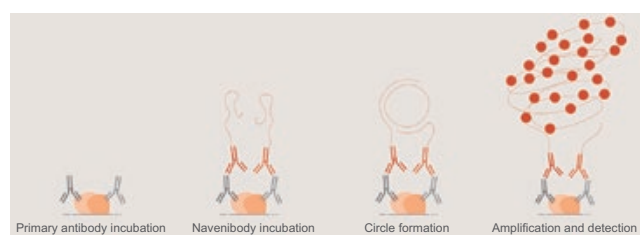


Fig. 2-6: Principle of In-situ proximity ligation

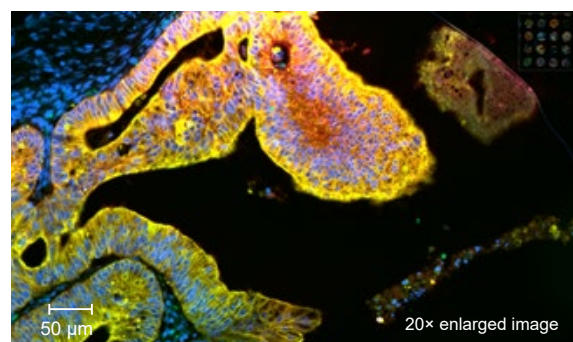


Fig. 2-7: 4-plex overlay image of an FFPE human colon sample

2-3-5. Molecular Instruments / HCR (RNA-FISH / IF)

Hybridization Chain Reaction (HCR) is an enzyme-free, isothermal signal amplification technology that provides both high sensitivity and excellent spatial resolution for in situ imaging of RNA and proteins.

In HCR, a short DNA initiator probe hybridizes to a target RNA or to an oligonucleotide conjugated to an antibody. This initiates the sequential opening and polymerization of fluorescently labeled DNA hairpins, forming an amplification polymer localized at the target site. Because the hairpin structures self-assemble only when properly initiated, HCR inherently suppresses background signals and provides excellent signal-to-noise ratios, even in tissues with high autofluorescence.

In addition, the resulting amplification polymer remains localized at the target site. This enables a wide range of quantitative imaging applications, from analog quantification of relative expression levels to single-molecule counting at subcellular resolution.

One of the key advantages of HCR for tissue imaging is its high tissue permeability, which results from the small physical size of the reagents. HCR initiator probes and hairpins consist of short DNA oligonucleotides, allowing efficient diffusion into dense or minimally processed tissues where enzymes or large amplification complexes may have limited access. This compact probe design provides reliable tissue penetration without requiring excessive permeabilization, enabling analysis of whole-mount embryos, thick brain sections, and FFPE tissues while preserving tissue morphology and spatial context.

The same small-probe principle also serves as the foundation for a common framework that integrates RNA-FISH and immunostaining. This enables simultaneous amplification of multiple RNA and protein targets in a single step, even in difficult-to-penetrate tissues, supporting highly reproducible quantitative analysis.

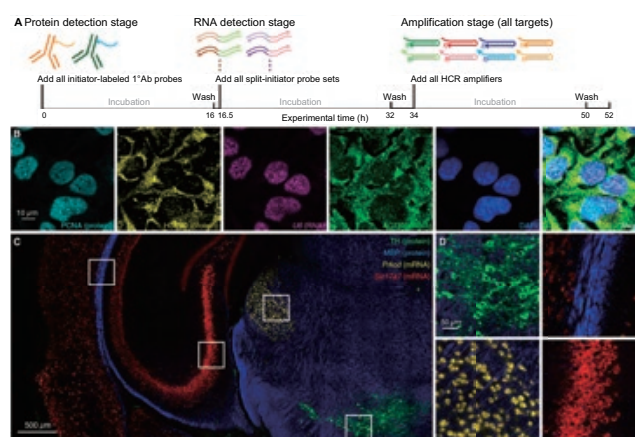


Fig. 2-8: Principle of HCR

3. Fluorescence macro imaging

3-1. Introduction

In multiplex immunofluorescence analysis, automation of sample recognition and exposure setting is essential for achieving both high reproducibility and ease of operation. However, because fluorescently stained tissue specimens are highly transparent, it has been inherently difficult to perform region detection, focus adjustment, and exposure optimization based solely on brightfield images.

The Fluorescence Macro Imaging function incorporated into MoxiePlex provides an effective solution to this challenge. This function uses low-magnification, wide-field fluorescence images acquired prior to scanning to automatically recognize tissue regions, determine optimal exposure conditions, and set focus positions. Because the analysis is based on the fluorescence signals themselves, stable control can be achieved even for samples with unclear morphological features.

This function is implemented based on patented technology. By optimizing imaging conditions for each channel, it significantly improves quantitative performance, reproducibility, and workflow efficiency in multiplex fluorescence imaging.

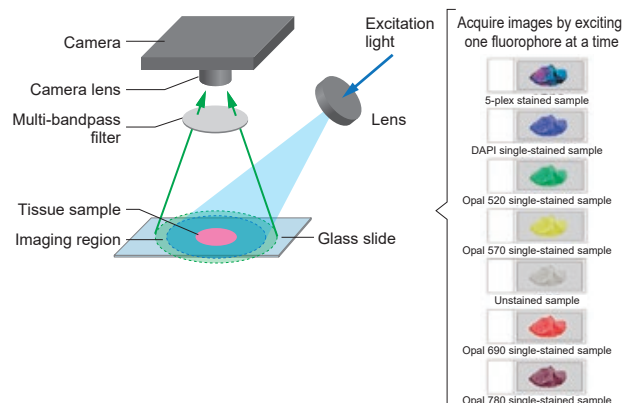
3-2. Technical advantages of MoxiePlex

For fluorescently stained tissue specimens, the high transparency of the samples makes it inherently difficult to perform region detection, focus adjustment, and exposure optimization using only brightfield images.

To address this challenge, the Fluorescence Macro Imaging function performs low-magnification, wide-field fluorescence imaging prior to image acquisition. Based on these fluorescence macro images, sample recognition, exposure adjustment, and focus positioning are carried out automatically.

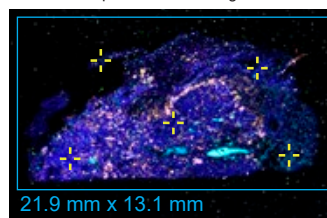
As a result, processes that traditionally required manual adjustment—including sample recognition, exposure setting, and focus control—can be automated, improving reproducibility, quantitative performance, and ease of operation in multiplex fluorescence imaging.

1. Fluorescence imaging of an entire tissue sample

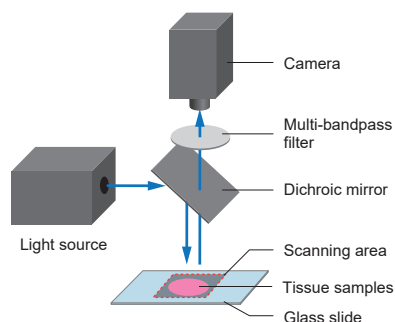


2. Detection of tissue samples and automatic calculation of exposure time

Automatic exposure time setting for each wavelength



3. Acquisition of fluorescence image



3-3. Our technology

Tissue recognition and automatic exposure adjustment in MoxiePlex are based on patented imaging control technology.

With this technology, a preliminary scan using fluorescence excitation is performed prior to the main scan. By analyzing the presence and intensity distribution of fluorescence signals, the system automatically distinguishes tissue regions from glass-slide regions. Because fluorescence signals themselves are used as the indicator, stable tissue recognition can be achieved even in samples with indistinct morphology without relying on brightfield images. Based on the identified tissue region, the scan area is automatically defined, enabling efficient scanning while excluding unnecessary background regions.

In addition, fluorescence intensity histograms obtained during the preliminary scan are used to quantitatively evaluate signal saturation, low-intensity signals, and signal-to-noise ratio (SNR). Based on these results, optimal exposure conditions are calculated automatically. Exposure optimization is performed independently for each fluorescence wavelength, or channel, ensuring high reproducibility even in multiplex immunofluorescence assays with large differences in marker expression levels.

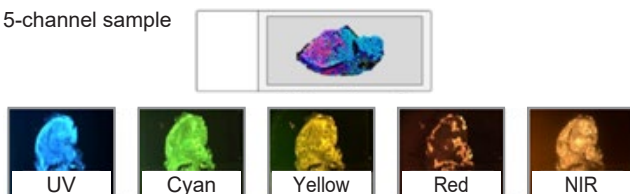
These processes are implemented within an integrated system that combines light source control, filter switching, camera acquisition, image analysis, and imaging condition control. By incorporating this patented technology, MoxiePlex achieves highly accurate and reproducible tissue recognition and automatic exposure control.

Patent: JP7640821B1 – Image acquisition apparatus and image acquisition method

3-3-1 Example of a 5-channel sample

A five-color sample was observed using the Fluorescence Macro Imaging function. The results confirmed that fluorescence images can be acquired at any excitation wavelength.

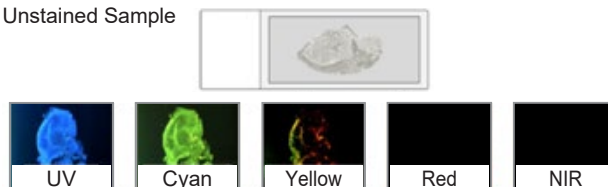
5-channel sample



3-3-2 Example of an unstained sample

An unstained sample was observed using the Fluorescence Macro Imaging function. The results confirmed that autofluorescence images can be acquired under UV, Cyan, and Yellow excitation conditions.

Unstained Sample

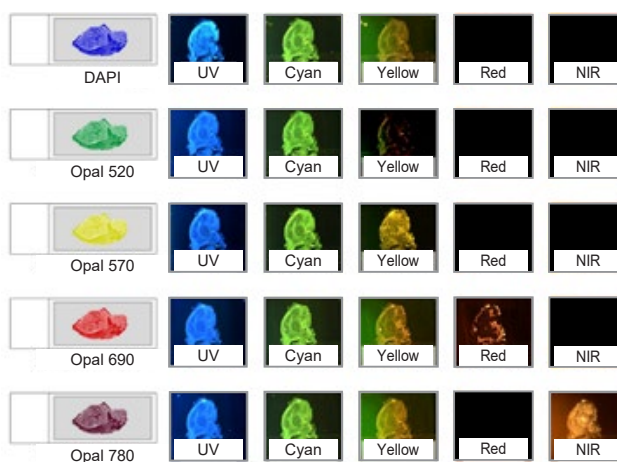


3-3-3 Example of a single-stained sample

Single-stained samples were observed using the Fluorescence Macro Imaging function. For each sample, autofluorescence images could be acquired under UV, Cyan, and Yellow excitation conditions.

Because DAPI produces signals that differ from autofluorescence under UV excitation, nuclear-derived signals may be observed. Similarly, sample-derived signals were detected under other excitation wavelengths.

MoxiePlex uses these Fluorescence Macro images to enable automatic setting of focus positions and exposure conditions.



References

Unmixing - "Spectral Imaging and Linear Unmixing in Light Microscopy" by Zimmermann T. (Adv Biochem Eng Biotechnol. 2005;95:245-65.)

Nonlinear Unmixing - "Nonlinear unmixing of hyperspectral images: models and algorithms" by Dobigeon N. et al. (IEEE Signal Processing Magazine. 2014;31 (1):82-94.)

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