BENEFITS OF AUTOMATION FOR TISSUE-BASED RESEARCH

Step-by-step guidance and top tips on implementing and validating automated staining systems

Accelerate Your Journey
Imagine The Possibilities

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INTRODUCTION

Translational research relies on human tissue samples to gain a better understanding of molecular mechanisms of health and disease, and bridges the knowledge obtained from in vitro and in vivo studies. [1-2] However, using human tissue samples for translational research can be limited by time, need, tissue availability, and budget. [2-4] Furthermore, data for biomarker discovery must be accurate and reproducible, with a need for more efficient discovery, validation, and translation to patient care. [5,6] This has created a need for more high-throughput technologies. [7] One solution to leverage laboratory resources and tissue repositories is to adopt automation. Automated techniques improve assay performance by making experiments more efficient and reproducible, as well as offering a quicker turnaround time to clinical translation. [8,9]

Immunohistochemistry (IHC) and in situ hybridization (ISH) are important research tools that detect gene and protein expression in tissue samples. [10,11] In this eBook, we look at automated approaches to IHC and ISH, and how key considerations around flexibility, consistency and processing speed can be adopted to accelerate research programs. [8] These methods take advantage of cutting-edge technology, such as the BOND RX Fully Automated Research Stainer from Leica Biosystems, to speed up discovery by integrating an automated process. With step-by-step guidance and top tips on implementing and validating automated systems, this eBook will provide key considerations to help guide you through the process.

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BENEFITS OF AUTOMATION FOR TISSUE-BASED RESEARCH

Step-by-step guidance and top tips on implementing and validating automated staining systems

E-BOOK OVERVIEW

CHAPTER 1

Research workflow automation: stain, scan and analyze

Augmenting key stages of your IHC/ISH workflow with automated systems can reduce human error, produce more consistent results and retain experiment flexibility. [8] In this chapter of the eBook, you can gain a deeper understanding of the benefits of automating your staining protocols.

CHAPTER 2

Automation to accelerate research programs

Automated staining platforms are often chosen to accelerate research while supporting optimal experimental design, consistency, and faster processing. [8] This approach also applies to digital pathology which is the acquisition, management, sharing, and interpretation of pathology information (including slides and data) in a digital environment. [12-14] Digital pathology also enables researchers to engage, evaluate, and collaborate remotely. [12]
In this section of the eBook, discover:

- Technical benefits of automated staining
- The advantages of digital pathology
- Considerations for selecting a digital pathology slide scanner
- Considerations for selecting image analysis tools
- Methodology for adoption and implementation

CHAPTER 3

Automation in practice: Webinars

In this section of the eBook, we demonstrate the power of automation using case studies from leading cancer centers and medical research institutes. Learn from industry thought leaders with live and recorded scientific presentations that are available on-demand.

CHAPTER 4

Product highlights

With advanced detection systems, probes, and a comprehensive range of antibodies across multiple pathology specialties, Leica Biosystems integrated portfolio of reagents for fully automated and manual IHC can meet your research needs. With strategic partners to offer cutting-edge technology in IHC multiplexing, explore your ideas in a variety of ways to accelerate your research programs.

CHAPTER 5

Featured products

Browse through Leica Biosystems equipment that offers an automated solution to stain, scan, and analyze IHC, ISH, and multiplex stained slides. Explore the options to determine which products can help to accelerate your research journey.

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CHAPTER 1
Research workflow automation: stain, scan and analyze

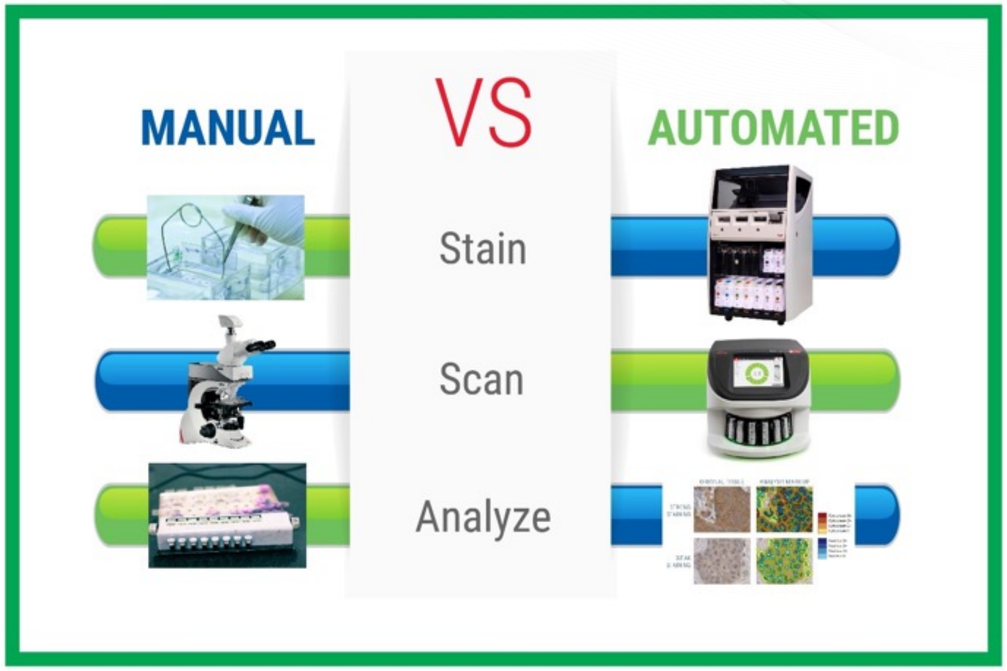
SESSION 1A: Stages of automation for tissue-based research

There are many decisions to be made in experimental/biomarker research to ensure the most suitable and robust study design is applied to gain valid and reproducible results. [15] When using IHC/ISH, the decision-making process includes the method of staining, as well as the tools for visualization and image analysis. [16-18,21] For tissue-based research there are opportunities to automate at every stage, with the possibility for implementing an entire automated workflow.

Stain: Manual staining methods can be wasteful in resources and result in slide-slide inconsistencies [19,20] however, it is still considered to offer greater flexibility for optimization. [21] Today, automated staining gives consistent and reproducible results. [8,20,22,23] Flexibility is retained by the availability of "open systems" on autostainers, [24] as well as reducing hands-on experimental time therefore allowing more efficient use of researchers' time. [8]

Scan: As an alternative to manual microscopy, stained slides can be imaged with digital scanners and can then be viewed, edited, and stored virtually on digital devices (ie computer or smartphone) at a similar resolution and magnification to conventional microscopes. [25]

Analyze: Manual image analysis is time-consuming, tedious, subject to inter-observer variability, and semi-quantitative. Digital pathology enables the application of quantitative, automated image analysis algorithms to tissue samples that can address more complex research questions with greater accuracy. [25]



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CHAPTER 1
Research workflow automation: stain, scan and analyze

SESSION 1B: Why automate?

Consistency and reproducibility are the clearest benefits of automated staining, [8,20,22,23,25] however, automated experiments may be met with resistance in some research laboratories for reasons including cost and time to implement or optimize. However, it is important to note there are many additional long-term benefits to automation.

Some of the less obvious advantages include:

- **Standardization:** Standardization of research practices between institution as well as staff training is important for translatable and reproducible research. [26] Automation provides a level of standardization in these areas by controlling variation in experiments and performance. [8]
- **Collaboration:** Particularly in the virtual space such as with digital pathology, collaboration between distant sites can become part of a laboratory's regular routine as individuals can review, annotate, and edit work without the need for travel and/or postage expenses. [12]
- **Reallocation of researchers' time:** Automation allows you to spend less time on manual tasks and more time innovating to find answers, helping you obtain the data needed to secure funding for that next breakthrough discovery. [8]
- **Return on time investment (learning curve):** The learning curve may be different when adopting automated procedures compared to manual methods with respect to effort and productivity. Automation, in the long run, reduces the hands-on time, allows you to identify and rectify errors more quickly, thus translating to improved productivity to accelerate your research. [8]
- **Speed to innovation:** Automation allows you to apply applications (eg multiplex IHC) more quickly. [8,27,28]

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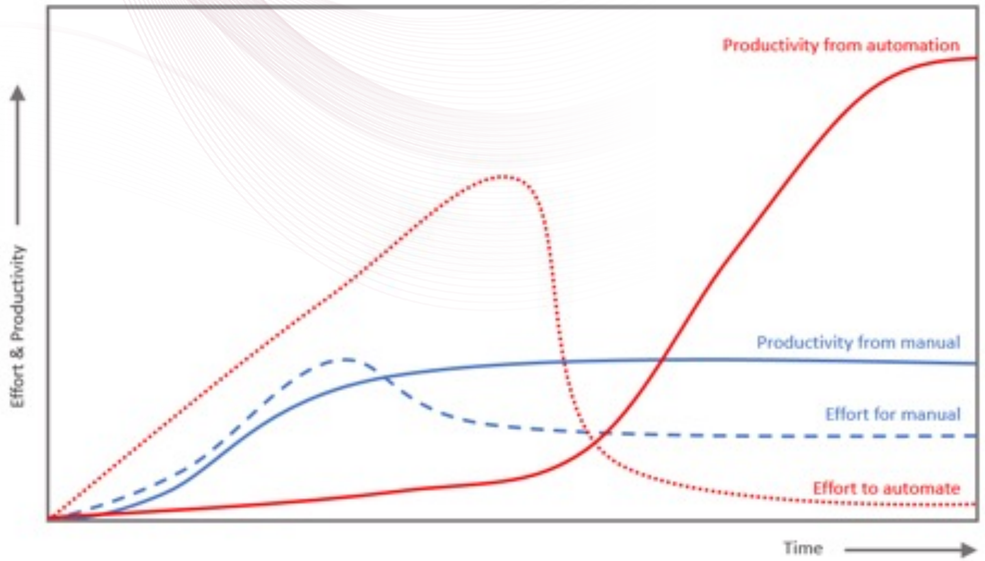
CHAPTER 1
Research workflow automation: stain, scan and analyze

SESSION 1B: Why automate?



The chart depicts how effort and productivity changes over time with respect to manual, versus automated staining. Manual laboratory methods can take time to become more efficient and easier to perform, depending on the rate of skill acquisition and improvement. However, the amount of effort over time can remain high due to the repetitive nature of assays, time required for methods, and occurrences of human error. These factors are reflected in the productivity associated with manual methods, which can remain low and will plateaus when the skill level and maximum working capacity has been reached by an individual/group. In contrast, automating the same method can involve a significantly long period requiring more effort initially due to equipment purchasing, installation, training, and initial experiment optimization. However, once set up, this effort for automated methods is greatly reduced compared to manual methods, and the productivity associated with automated methods surpasses manual methods due to the ability to speed up techniques, optimize multiple parameters at once, and reduce hands on time in the laboratory.

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CHAPTER 2
Automation to accelerate research programs

SESSION 2A: Brightfield and fluorescence tools: IHC and ISH Stainer

Automation is often chosen by researchers to accelerate research projects. [8,27,28] It provides the ability to spend time on transformative thinking, discovery, and analysis instead of the laborious creation of the data. [8] It also enables you to optimize IHC/ISH experiments and have more confidence that your work is replicable by others, [8,29] enhancing the translation to clinically relevant scenarios. [5,6] When determining if automation is the right fit for research, consider some of the lesser known technical benefits of using automated staining.

Technical benefits of automated staining

Research often requires the handling of hazardous and carcinogenic chemicals. Automation allows for a reducing of direct handling. [8,29]

A reduction in physical strain though the removal of repetitive tasks such as pipetting and leaning over a lab bench. [29]

Introducing an automated stainer can free up bench space previously utilized for manual staining, which allows utilization of space for alternative tasks and equipment. [29]

Combined benefits provide a safer and optimized work environment. [29]

Extensive documentation is required to validate research outcomes which can often be manually recorded in laboratory notebooks. Automated stainers can provide additional inventory and reporting management capabilities (eg slide tracking, reagent management, experimental design evidence). [12,25,29]

Flexibility to schedule your project work around your deadlines not around the time needed for completing the experiment. [29]

Bulk reagents allow for a reduction of costs through waste minimization and batch-to-batch variation. [29]

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CHAPTER 2
Automation to accelerate research programs

SESSION 2A: Brightfield and fluorescence tools: IHC and ISH Stainer

When you are ready to select an automated platform, there are three main pillars that should be considered. These points should be flexibility to support experimental design including supporting a product menu, consistency, and rapid processing to accelerate research. The pillars can be further refined into additional points to consider when contemplating the selection of an appropriate automated stainer.

Flexibility in design/product menu support [29]

- An intuitive and easy to use software interface.
- Allows for customization of reagent types, steps sequence, slide temperature and incubation times.
- The automation is a realistic substitute of the manual process.
- Transfer an assay to another lab.

Consistency and rapid processing [29]

- Basic bulk reagents allow for consistency and reproducibility in simple steps such as deparaffinization and antigen retrieval.
- Implement standardization across your assay steps for time, temperature, reagent dispense volume vs manual staining.
- Create confidence that another laboratory/ researcher will be able to repeat your results from peer reviewed articles and posters.

Rapid processing customer and product base [9]

- Enables rapid assay development by being able to run many protocols with alternate variables at once.
- Allows scalability of your optimized test to generate more data faster.

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CHAPTER 2
Automation to accelerate research programs

SESSION 2A: Brightfield and fluorescence tools: IHC and ISH Stainer

After deliberating on all the potential benefits, the custom needs of individual laboratories are the final considerations for anyone deliberating to embark on the journey to automation. These considerations venture beyond the main properties of the equipment and focus more on the implications the features may have in the wider context of the laboratory's overall ongoing needs and requirements. Some of these are provided below.

Coordinate and communicate to key stakeholders, comprising of a cross-functional team of research personnel, administration, facilities engineering, and IT

Confirm facility readiness

- **Pre-installation communications**
- **Pre-site evaluation**
- **Connectivity/infrastructure**

Ensure basic core competencies of manual technique you are replacing with automation

Identify compatibility and integration needs

Optimize upstream analytes and proposed protocols

- **Tissue preparation**
- **Slide preparation**

Understand maintenance requirements and cost

- **Continued technical support**
- **Service contracts**
- **Routine maintenance**

Utilize vendor-provided training for all users

- **Post-installation training**
- **Continued training for new users**
- **Access to customer support resource contacts**

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CHAPTER 2
Automation to accelerate research programs

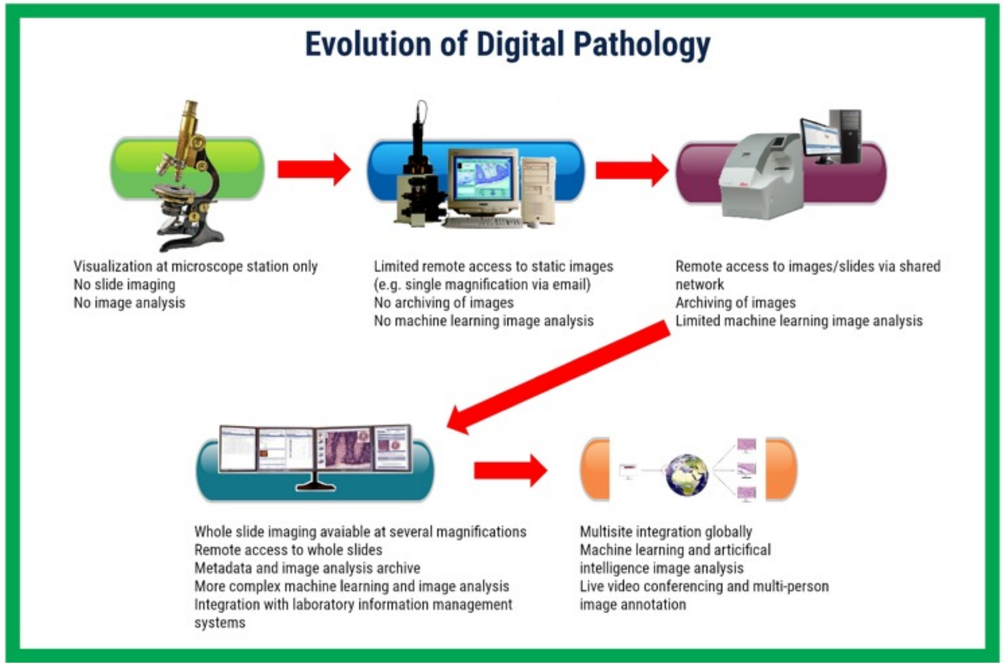
SESSION 2B: Digital scanner, image management, and image analysis – important considerations

What is digital pathology?

Digital pathology incorporates the acquisition, management, sharing, and interpretation of pathology information, including slides and data, in a digital environment. [25] It enables researchers to engage, evaluate, and collaborate rapidly and remotely with transparency and consistency, thus improving efficiency and productivity. [25]

Digital slides are created when glass slides are captured with a scanning device to provide a high-resolution digital image that can be viewed on a computer screen or mobile device. Utilizing high-throughput, automated digital pathology scanners, it is possible to capture an entire glass slide, under brightfield or fluorescent conditions, at a magnification comparable to a microscope. Digital slides can be shared over networks using specialized digital pathology software applications. [25] Automated image analysis tools can also be applied to assist in the interpretation and quantification of biomarker expression within tissue sections. [25,30-36] The rapid progress of whole slide imaging (WSI) technology, along with advances in software applications, data management software, and high-speed networking, have made it possible to fully integrate digital pathology into pathology workflows. [12]

The future of digital pathology has potential to eventually encompass enhanced translational research, [36,37] computer-aided pathology (CAP), [38] and personalized medicine. [39,40]



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CHAPTER 2
Automation to accelerate research programs

SESSION 2B: Digital scanner, image management, and image analysis – important considerations

Advantages of digital pathology

Improve quality

Enable faster access to remote specialists for discussion of novel or challenging samples, reducing turnaround time for studies, and enhancing collaboration with live slide discussion. [12,25,31,41,42,45]

Quantitative image analysis tools provide standardized, reproducible data to give researchers greater insight into novel biomarkers and therapeutic targets. [22,23,30,32-34]

Powerful image and data management platforms, allowing current and historical study data to be easily retrieved, sorted, and shared. [25,41-45]

Improve productivity

Central storage of data and images via digital pathology solutions enables easy access and improved management of study data. [12,25,41-45]

Data management systems provide all data in a single, streamlined workflow with easy retrieval, reducing time spent waiting for slide delivery, data matching, and organization. [12,25,41,42,45]

Digital pathology tools such as automated image analysis can save significant time over manual review. [46-50]

Save money

Send slides and study data internationally, without the monetary and time costs and risk of shipping fragile glass slides. [12,25,44,45]

Reduce the requirement for researchers to travel for activities such as toxicological peer review. [12,25,44,45]

Image analysis tools can be run in an automated batch-processing mode, freeing up valuable researcher time for other activities. [45-50]

Create opportunity

Novel opportunities to work with international partners in both industry and academia. [8,45,51]

Data management platforms allow you to give your partners the right level of access to study images and data. [44]

Generate new revenue streams through insourcing of research activities, without the cost, time, and hassle of shipping or travel. [25,45]

Drive innovation

Quantitative image analysis tools allow for easier investigation of increasingly complex multiplex assays, and novel biomarkers in both brightfield and fluorescence, that can be archived and stored for long term use. [30,33,34,45,52,53]

Use of novel digital pathology technologies allow research institutions to provide cutting-edge services and build brand awareness as leaders in the field. [45]

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Automation to accelerate research programs

SESSION 2B: Digital scanner, image management, and image analysis – important considerations

Considerations for selecting a digital pathology slide scanner

Determine the necessary functions for your studies

There are many digital scanners on the market today, each offering different feature sets. Decide what functionality is important to you.

- Good image quality is vital to properly assess your slides. Make sure that the scanner images are fit for purpose and clearly show the cellular features, depending on the tissue specimens you will be scanning. [12,25,46,54]
- Scanner capacity can range from one to several hundred slides. Consider how many slides you will need to scan in a typical day or batch, and what level of throughput you need. [25]
- Many companies will provide scan time in seconds, but this does not always consider time needed for the file to be compressed, sent to the image server, and then made available for viewing. Consider the total time required to get to your results. [12,25,53]
- Consider whether you will need brightfield, fluorescent, or both types of microscopic technologies, and whether you will need additional features such as multi-plane z-stacking, and software that allows live viewing. Choose a scanner that has the scan modes you need. [12,25]
- A digital pathology solution will typically include software as well as hardware, for applications such as image/data management, sharing, and image analysis. Decide what solution you need, and which vendor(s) can provide it. [12,25,46,54]

Look for device usability and flexibility

The digital pathology scanner is a new piece of equipment for users to learn. Ergonomics and ease of use are vital for user adoption. Consider the following points:

- Flexible solutions should be considered to satisfy the needs of your study today as well as your studies in the future. [46]
- Usability of the user interface to see if it is intuitive and easy to navigate. Test the ease of use to get a sense of the learning curve involved with different operations. [25,45]
- How long it will take to set up a batch of slides for scanning, and will you need different set-ups and settings for different slides and can this be incorporated by the equipment. [25,54]
- Ensure the tissue finder will capture the whole tissue sections on the slide for all tissue types that you intend to digitize for you studies. [54]
- Check if there is a “quick start” mode for scanning, so you can rapidly set up and walk away with minimal interaction. [54]
- Determine the method for accessing images after scanning for Quality Control review. [46]

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CHAPTER 2
Automation to accelerate research programs

SESSION 2B: Digital scanner, image management, and image analysis – important considerations

Consider your existing infrastructure

Minimize disruption to your laboratory workflow by ensuring that the digital pathology scanner fits as easily as possible into your existing laboratory infrastructure.

- Considerations should be made for desktop space requirements, where it will fit within the lab, the noise it will make when scanning, and the interface requirements for your specific LIS barcodes. [54]
- Digital pathology images can be up to several GB in size, with higher scan magnification producing larger image files. Consider how your IT infrastructure will handle image storage (on premise or in the cloud), as well as the network bandwidth required to view and share such large files. [12,25,43,45,46]
- Early inclusion of your IT department in the digital pathology scanner selection process is critical to ensure your selected solution contains all the necessary data security, storage requirements, deployment, and integration requirements. [12,25,46]

Consider your future needs

Digital pathology is a significant investment. Find a vendor you can rely on, and can provide peace of mind with the knowledge that your solution is future proof.

- Look for vendors that offer comprehensive service and maintenance contract support for your device, noting the hours of support and warranty periods. [54]
- You should select a vendor that offers frequent updates such as security patches and software updates. [54]
- Consider the benefits of partnering with a single solution provider, versus building your own bespoke solution with products from several vendors, [54] deployment, and integration requirements. [12,25,46]

Consider brand reputation

Research online resources and ask your peers who have experience with digital pathology, as well as which vendors have the best reputation for reliability and quality products? [54] Questions to ask as part of your research include can include:

- How long has the vendor been in the market? What is the size of their install base? [54]
- How many peer-reviewed publications reference the vendor's products? [54]
- What do industry reviews say about the quality and reliability of their solutions? [54]
- Does the vendor have a good reputation for providing service and addressing issues promptly? [54]
- Is the vendor portfolio offering broad enough? Are they continuing to add to their product offerings? [54]

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Automation to accelerate research programs

SESSION 2B: Digital scanner, image management, and image analysis – important considerations

Considerations for selecting image analysis

Consider your use case

Determine what applications you want to use image analysis for. Consider your biomarkers, tissue types, and goals. [25,30] Some examples include:

- Brightfield or fluorescence. [25,30,31]
- Immunohistochemistry and/or in situ hybridization. [25]
- Pattern recognition. [25,31,55]

Look for flexible analysis options

While there are technical limitations to image analysis, your tools should be flexible enough to meet a variety of needs. [30] Ask the following questions of the software of interest:

- Will the algorithm analyze images in the file formats you use, or is it locked to a vendor-specific format? [56-60]
- Can you customize the analysis algorithms for different stains, markers, or tissues? [30,31,57]
- Can custom algorithm parameters be saved and easily rerun as needed? [30,58]
- Will the algorithm analyze whole slide images and/or regions of interest? [30]
- Can you define your own scoring categories for the output data? [30]

Demand reproducible, accessible data

Image analysis is only useful if the results are consistent, trustworthy, and can be statistically analyzed for biological relevance. Consider the following points:

- Run the same parameters multiple times to ensure and confirm result reproducibility. [30,32-34,59]
- Will results be saved along with images, and whether there is an image/data management solution associated with the image analysis product. [31,60]
- Can images be easily exported into different formats such as CSV or XLS for analysis with statistical software packages. [31,58,60]

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Consider automating your workflow

One advantage of image analysis is the ability to automate time-consuming manual tasks, such as ISH/FISH spot counting. Depending on the volume of slides you plan to analyze, it is also worth considering additional levels of automation to decrease the amount of time you spend interacting with your image analysis solution. Factors that need to be considered include:

- Whether the user interface is easy to use, and can the algorithm settings be saved, then easily recalled and applied. [58]
- Can the algorithm(s) batch analyze whole slides/ regions of interest or each image singularly. [30,58]

Manage your resources

If you are planning to deploy an image analysis solution for use by multiple colleagues, consider how these resources will be made available to everyone.

- Local workstation image analysis solutions are typically only usable by one person at a time, while server-side or cloud solutions can be accessed by multiple users simultaneously. [12,25]
- Can saved algorithm parameters and image analysis results be selectively shares with other users in the system. [31,59]
- Determine whether there are remote users who may need to access the system. Consult with your solution provider on how best to provide them with access. [25,59,60]
- Will the image analysis solution have a built-in limit on the number of user accounts (licenses or user IDs). [31]

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CHAPTER 2
Automation to accelerate research programs

SESSION 2C: How to automate – methodology for adoption and implementation

Before implementing and utilizing automation into your facility, it is important to realize that there are different levels of automation that can be implemented into a laboratory workflow. [8,61]

A manual assay performed with the support of an automated tool to aid with the experiment such as automatic pipettes, for example. [8,61]

Integrating an automated machine as part of a workflow for a specific task, where the remaining parts of the workflow are performed manually [8,61] **(eg manual tissue processing followed by automated IHC).**

Complete transfer of all manual processes to a fully integrated, automated workflow [8,61] **(eg automated tissue processor, stainer, whole slide imaging, and automated image analysis).**

These approaches are often adopted based on many factors including budget, laboratory space, and need for automation due to workload, for example. [8] When implementing more sophisticated automated instruments, the methodology below will provide a framework to ensure a successful implementation.

Initiate the project

Create a cross-functional team representing the laboratory team, administration, facilities, and engineers, initiating the project is a crucial first step. [62] Firstly, select a group with varying levels of expertise across the research laboratory, administration, and technology. Organize for all members to participate in regular meetings that include a vote to ensure a group consensus:

For laboratory members, ensure research laboratory functions and daily life are not disregarded, [8] **and that the proper technical skills required for operation are available, while considering the complexity of the application.** [62]

Administration should guide the project team on how to make decisions and demonstrate ROI. Ensure buy-in from leadership and laboratory staff. [62]

Facilities/Engineering can support project requirements and specific needs (eg heating, plumbing, electric, etc.). [62]

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Automation to accelerate research programs

SESSION 2C: How to automate – methodology for adoption and implementation

Measure current state – Establish a "jump off point" by measuring existing practices and processes.

Create list of materials (instruments and supplies) required to perform experiments. Include all necessary equipment, experimental volumes, and staffing requirements. [63]

Observe experiments in process, and capture the following: steps required, time to complete experiments, and hands-on time vs. hands-off time. [63]

Consult with the research team to identify existing bottlenecks, future requirements (maintenance) for automation, and a "wish list". [64,65]

TOP TIPS for measuring existing practices and processes.

Lean and Six Sigma tools are helpful in capturing and documenting processes (process map, value stream map, etc.) [66]

Predict where automated instruments will be installed in the laboratory with respect to laboratory layout and the available bench space. [8,62,67]

Observe experiment from beginning to end. [65]

Determine where the bottlenecks in the process are. [65]

Calculate the time it takes to complete each stage of the experiment, as well as total time to completion. Include an analysis of hands-on, hands-off time for each step. [65]

Analyze and summarize data to better understand existing requirements and resources used to determine whether automation will have a positive impact. [65]

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Automation to accelerate research programs

SESSION 2C: How to automate – methodology for adoption and implementation

Define goals of automation

Ask yourself – what are the team's goals when implementing automation? What are the team's clear objectives? What type of experiments will automation perform? Will experiments be extended? Will automation drive to a bigger data set?

- Potential objectives include but not limited to: reduce hands-on time, increase time to perform alternate tasks, drive consistency, drive reproducibility, reduce variability, etc. [8,62,64]
- Investigate opportunities to automate tasks requiring precision in experimental designs that a manual process would preclude (eg specific or high temperatures, rapid pipetting, precise or small quantities, etc.) [8,62,64]

Decision

Work with the project team as well as the vendor to understand the impact of automation and compare your existing state with future states:

- Build anticipated future state by following the same process that was used to benchmark the experiment. [8,64,65]
- Compare total steps, hands-on time, and hands-off time. [64]
- Calculate ROI and anticipated impact. [64]

For more information, see SESSION 1A: Stages of automation for tissue-based research

Installation

- Ensure automation is installed into an acceptable location.
- Automated equipment should be installed in areas free from potential contaminants and/or environmental impacts (air conditioning vents, fans, direct sunlight, humidity, excessive heat or cold, etc.) [68]
- Automated instrumentation should be installed in areas that can be maintained and cleaned on a regular basis. [66,69]
- Ensure to follow all necessary codes and regulations for health and safety. [68]
- Ensure to place instruments in an area relevant to the requirements of an experiment, such as in a dark room for techniques requiring fluorescent antibodies. [9]
- Ensure there is adequate room for additional supplies as well as waste containment and removal. When transitioning from manual experiments to automated experiments, it is important to factor in supply storage and waste containment. [68]

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CHAPTER 2
Automation to accelerate research programs

SESSION 2C: How to automate – methodology for adoption and implementation

Implement and optimize

Optimize and eliminate inefficiency before implementing automation. Automating bad processes ensures consistently bad processing. [66]

Instrument validation and optimization:

- **Validation definition:** Verifying or validating the performance specifications stated by the instrument manufacturer. [46,69]
- **Validation plan:** Document the plan and include the validation policy and validation execution plan. This master plan specifies the instrument, scope of the validation, and process method for using validated equipment. [46,69]
- **Rationale:**
  - The goal is to replicate or validate the manufacturer's claims of performance characteristics, under the current laboratory conditions for operation (temp, humidity, water, electricity etc.) [70]
  - Identify parameters that could alter the functionality and any margin of error so it will not affect the interpretation of test results. [70]
  - Ensure proper operation metrics are occurring, especially if instrument was shipped or stored. [70]
- **Validation strategy:** Following the 3 validation principles, equipment should be validated after the following events:
  - Initial instrument installation [69]
  - New protocol or assay is implemented [71]
  - When a major part is changed [71]
  - As part of a part of QC strategy [69]
  - Relocation of instrument from one location to another [72]
- In the final stages of instrument validation, it is important to verify that it performs according to manufacturer's specifications and within the reference ranges, as well as confirming it is appropriate for its intended use under real-life conditions of use and are comparable in result and quality (sensitivity and specificity) with current practice. [73,74]
  - Achieve reproducibility from predetermined "normal" lab-based testing. To do this, run a testing assay in replicates, on different days and conduct a pilot study using a larger number of samples. [75,76]
  - Test representative samples of different types of specimens, ensuring they are normally processed, and define similar reference ranges to evaluate accuracy and/or normal cut off values. [76]
  - Perform parallel testing of the manual and automated method to compare concordance with previous laboratory results and quality of assay with new instrument. [76]

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CHAPTER 2
Automation to accelerate research programs

SESSION 2C: How to automate – methodology for adoption and implementation

Three validation principles

1. Installation qualification (IQ) [46,69]

Installation qualification verifies that the equipment and its components (eg: electrical, voltage, inner-working, and software) have been installed in accordance to manufacturer's specifications and are operational, and environmental conditions are correct. It should be performed by the instrument manufacturer expert or service engineer.

2. Operational qualification (OQ) [46]

Provide evidence that the instrument operates to specifications and confirm that installation was successful. This should be conducted by an instrument expert with trained lab staff to:

- Ensure functionality of system requirement specifications
- Provide training on instrument operations
- Test/evaluate representative samples

3. Performance qualification (PQ) [46,77]

Test the calibration and traceability of the instrument for:

- Precision (Replication)
- Analytical Measurement Range (Verify reportable range)
- Accuracy (correlation or comparison)
- Reference Ranges (Normal values)
- Analytical Sensitivity
- Analytical Specificity

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CHAPTER 3
Automation in practice: Webinars

SESSION 3: Staining automation

Webinars

Leica Biosystems provides a range of live and recorded scientific resources presented by industry thought leaders. For the latest advances in validating antibodies for research applications in IHC, multiplex Immunofluorescence and more, visit Leica Biosystems webinar resources.

Design, optimization, and validation of multiplex immunofluorescence panels

Michael J Surace
Scientist II, AstraZeneca

Multiplex immunofluorescence (mIF) combines the spatial information from immunohistochemistry (IHC) with multi-marker phenotypes. Recent advances in mIF technology have made it possible for researchers to develop novel custom panels, but additional considerations must be taken into account in order to produce a panel which performs at least as well as IHC on a marker-by-marker basis, and avoids any undesirable interactions between detection of the targets and neighboring visible light spectra.

This webinar covers: the biological and technical considerations when designing a panel; a linear process for developing, testing, and optimizing a panel; and an approach for technical validation of multiplex panels which detects and addresses known risks.

Learning Objectives

- Learn about key factors to consider when designing a new mIF panel.
- Walk through an efficient process for developing and optimizing mIF panels using TSA-linked fluorophores and iterative staining.
- Become familiar with the best use cases and advantages of most common mIF staining and imaging technologies.
- Discover an adaptable scheme for testing and validating a novel mIF panel.

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CHAPTER 3
Automation in practice: Webinars

SESSION 3: Staining automation

Morphology driven high-plex spatial analysis of tissue microenvironments

Mathias Holpert, PhD
Senior Product Application Scientist, NanoString

Traci DeGeer, BS, HT (ASCP) HTL, QIHC
Director, Advanced Staining Innovation, Leica Biosystems

Characterization of the spatial distribution and abundance of proteins and mRNAs with morphological context within tissues enables a better understanding of biological systems in many research areas, including immunology, oncology, and neuropathology. Analysis of samples across multiple tumor types and diseases has revealed novel, spatially distinct protein and mRNA candidate biomarkers. However, it has proven difficult to perform such studies in a highly multiplexed manner at a throughput scale that is required for translational research programs. To address this unmet need, we have developed a novel platform that can perform high-plex analysis of proteins or mRNAs on a single FFPE section from distinct tissue spatial regions (GeoMx™ Digital Spatial Profiler, DSP). Integrating the GeoMx DSP with either the NanoString nCounter or high-throughput sequencing, hundreds, to thousands of spatially resolved analytes can be measured. To enhance both throughput and reproducibility, we have developed automated sample processing workflows for both protein and RNA on BOND RX system from Leica Biosystems. In this webinar, we will show you how the integrated workflows of the BOND RX and the GeoMx DSP can advance your translational research.

Learning Objectives

- Demonstrate how the spatial profiling workflow can be used to gather multiplexing information.
- Describe how the spatial profiling workflow is similar and where it diverges from more traditional multiplexing methodologies.

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CHAPTER 3
Automation in practice: Webinars

SESSION 3: Staining automation

Automated multiplex immunofluorescence

Bethany Remeniuk, PhD
Global Applications Scientist, Akoya Biosciences, Inc.

Traci DeGeer, BS, HT (ASCP) HTL, QIHC
Director, Advanced Staining Innovation, Leica Biosystems

Cancer research continues to push the boundaries with new advancements in tissue analysis and biomarker detection. Now, more than ever, there is significant emphasis on understanding the underlying interaction between the immune system and the tumor microenvironment. Multiplexing immunofluorescence (mIF) has greatly increased our understanding of solid tumor biology and immunology, including tumor-infiltrating lymphocytes and cancer-induced architectural alterations, and aided in novel immunology discoveries. In this webinar, we will discuss how Akoya Biosciences Phenoptics assays support quantitative mIF to overcome the limitations imposed by conventional IHC methodologies. We will also discuss how our Opal assay kits and reagents can be integrated with the BOND RX stainer to automate your staining workflow to support consistent results for high-throughput studies.

Learning Objectives

- Understand the limitations of conventional IHC, and the benefits of transitioning to multiplex immunofluorescence.
- Learn how to design, optimize, and analyze your multiplex assay panel.
- Understand how the BOND RX enables innovations like the Opal assay.

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CHAPTER 3
Automation in practice: Webinars

SESSION 3: Staining automation

Validating antibodies for research applications in IHC

Will Howat, PhD
Director of Antibody Validation & Characterization, Abcam

Damian Cockfield
Global Product Manager - BOND, Leica Biosystems

The importance of building a validation structure within your lab cannot be overstated. Once in place, the ability to identify high quality, specific results compared to spurious, non-specific antibody staining on tissue will be easier and allow the researcher to focus on downstream applications, such as single and multiplex IHC. This webinar will focus on the steps and challenges in antibody selection and validation to enable success and reproducibility in antibody staining in IHC.

Learning Objectives

- How to select and validate antibodies for research applications in IHC.
- Understand the reagents and tools available today to support the validation of antibodies for research applications in IHC.

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CHAPTER 3
Automation in practice: Webinars

SESSION 3: Staining automation

Multiplex fluorescence immunohistochemistry using the Ultivue InSituPlex platform on the BOND RX

Traci DeGeer, BS, HT (ASCP) HTL, QIHC
Director, Advanced Staining Innovation, Leica Biosystems

Alexander Klimowicz
Principal Scientist, Boehringer Ingelheim Pharmaceuticals

Multiplex fluorescence immunohistochemistry offers a window into the biology of human disease, enabling the analysis of target protein expression in subsets of specific cells within the context of histopathological features of disease. However, the multiplexing capabilities of fluorescence IHC, using standard histology equipment, are subject to several technical challenges. This webinar will provide insight and examples of how the Ultivue InSituPlex platform may be used to address several of the current challenges associated with multiplex fluorescence immunohistochemistry. It will focus on initial user experiences using the InSituPlex platform using automated IHC on the BOND RX stainer and automated imaging with Aperio VERSA.

Learning Objectives

- Understanding technical challenges associated with tyramide-based multiplex fluorescence IHC.
- Explore what tools are available for multiplex.

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CHAPTER 3
Automation in practice: Webinars

SESSION 3: Staining automation

Multiplex immunofluorescence profiling of tumor biopsies using UltraPlex mxIF Technology

Helen Snyder, PhD
Director of Preclinical Development & Strategic Partnerships, Cell IDx

Marie-Louise Loupart, PhD
Image Analysis Specialist, Leica Biosystems Aperio ePathology

An unmet need in tissue diagnostics is the ability to simultaneously detect 3 or more markers on a single tissue specimen, such as multiplexing. Unlike other immunoassays, ie flow cytometry, ELISA and bead assays, which solely quantify the biomarker in a sample, a multiplex immunoassay quantifies the biomarker, identifies the location of the cell in the tissue, and identifies the spatial relationship of the biomarkers with respect to each other. Being able to identify the contextual relationships of immune cell phenotypes and other biomarkers in a tumor is believed to be critical to understanding the state of the immune system before and after immunotherapy. Multiplex immunostaining can also be described as image cytometry or flow cytometry in situ. Detecting multiple biomarkers on a single slide presents many challenges: correct selection of biomarkers, sourcing and qualifying antibodies against the biomarkers, detection methods, optimization of the staining-detection protocol and how to visualize, record and interpret the resulting complex staining pattern, all without generating artefact from cross talk at each step. Today, we shall demonstrate a complete solution for standardization of immunofluorescent staining and detection, digitization, and analysis for tumor biopsy material. We have demonstrated that this protocol can be carried out on the BOND RX autostainer and the stained tissue can be imaged on the Aperio system that allows the user to easily digitize the whole slide at high-resolution, manage the resulting huge image files, and provide accurate quantitative interpretation in the form of mark-up images and numerical data at the per cell level providing detailed phenotype information.

Learning Objectives

- Learn about what multiplex staining is and understand its role in digital pathology.
- Understand what tools are are available today for multiplex.

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CHAPTER 4
Product Highlights

SESSION 4A: Research detection and Novocastra antibodies

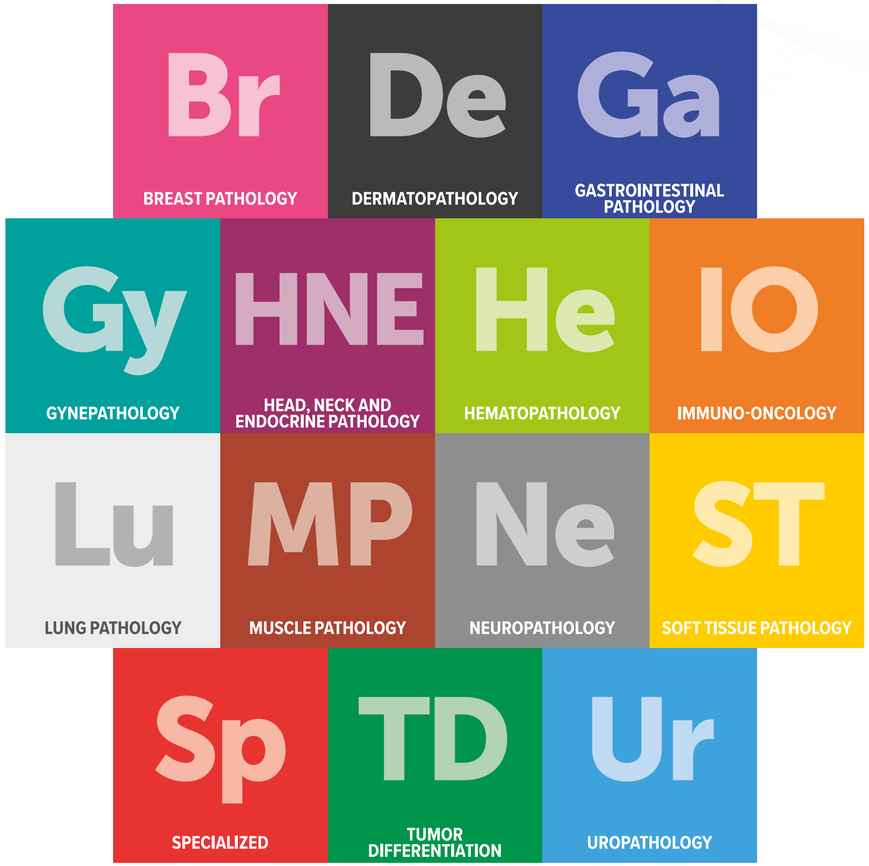
Leica Biosystems offers a comprehensive range of antibodies across multiple pathology specialties, as well as detection systems, probes, and a full range of ancillary reagents.

Each ready-to-use antibody is validated with complementary reagents required to run the test (please refer to IFUs), providing confidence that the test will perform in the customer's hands.

BOND detection systems, ready-to-use antibodies, and ancillaries have been specifically designed for use with the BOND RX and BOND RX^{m} stainers, providing customers with a fast turnaround time, reliable staining, and walk-away convenience. BOND detection kits contain all the reagents required to perform detection staining – no need to buy separate reagents.

Create high quality IHC slides with Novocastra primary antibodies. Developed in-house and backed by 20 years of IHC stain development experience, these robust antibodies have been optimized for automated and manual applications.

- Robust antibodies – in-house development ensures superior real-world performance
- Exceptional detection – Novolink™ detection with Compact Polymer™ technology for exceptional sensitivity
- A complete system – antibodies, diluent, and detection all working together



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CHAPTER 4
Product Highlights

SESSION 4B: Technology with strategic partners

Following our mission to enable researchers to accelerate their journey to transfer their scientific research to outcomes and inspire innovation, Leica Biosystems forms Strategic Partnerships with pioneering companies to offer cutting edge technology in an automated format. Each partnership is chosen to help the researcher improve their ability to stain their research slides faster with greater reproducibility and larger slide numbers than can typically be managed manually.

The choice of partners allows researchers to choose the technologies that best fits their research needs.

Make “walk-away” multiplex staining a reality

Produce up to 30 beautiful, 7-color immunofluorescence slides in about 14 hours without intervention. Use the BOND RX to get your results sooner, easier, and with less labor time.

- Simultaneously visualize up to 6 tissue biomarkers plus a nuclear counterstain
- Use the best primary antibodies, regardless of species – with no crosstalk
- Retain spatial cellular context that is lost when using other methods
- Get more information from scarce samples
- Editable protocol is easily optimized to meet your specific needs

AKOYA BIOSCIENCES®

Bringing CTC analysis to the experimental pathology lab

Automating CTC analysis: The AccuCyte CyteFinder system integrates with the fully automated BOND RX research stainer for microscopic visualization of circulating tumor cells (CTCs). The system specializes in single cell retrieval of target cells for downstream genomic analysis.

Comprehensive cell collection: AccuCyte comprehensively collects nucleated cells – including CTCs – from whole blood. CyteSealer applies a ring around the separation tube – creating a barrier between the buffycoat and red blood cells. This makes the collection of nucleated cells a seamless process.

The CyteSpreader tool ensures the precise amount of nucleated cells is spread onto a slide every time

RARECYTE

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CHAPTER 4
Product Highlights

SESSION 4B: Technology with strategic partners

DNA-based barcode technology

Powered by Ultivue's proprietary InSituPlex™ technology which combines DNA-barcoded antibodies with unique, complementary, fluorescent DNA barcodes.

- The portfolio of UltiMapper reagents enables a high level of multiplexing in a single slide, using a single antibody cocktail incubation step
- UltiMapper Kits for multiplexed immuno-profiling in FFPE tumor samples offers end-to-end application staining to analysis
- Enables high level multiplexing without damaging tissue, thus preserving sample integrity for additional analyses
- TSA Free
- Complete same day staining and whole slide imaging and analysis
- InSituPlex™ technology provides the ability to conduct cyclic multiplexing
- Complete the picture; image with Aperio VERSA and analyze with Aperio Cellular IF Algorithms

ULTIVUE

High-plex, spatial profiling of proteins and RNA from FFPE tissue sections

Combining standard immunofluorescence techniques with digital optical barcoding technology, the GeoMx™ Digital Spatial Profiler enables high plex, spatially-resolved profiling of protein and RNA. The GeoMx Assays allow for imaging and profiling from a single FFPE or fresh frozen tissue section.

- The BOND RX stainer automates time-consuming aspects of FFPE slide preparation
  - Prestaining protocols from Leica Biosystems (bake and dewax, epitope retrieval, enzyme pretreatment etc.)
- Stain for protein/RNA targets in a single step manually, overnight
- Quantify up to 96 proteins and over 1000 RNA targets with spatial context
- Focus on rare cells or compartment – specific regions of interest in tissue
- Preserve precious samples with non-destructive processing

nanoString®

A fully integrated RNA ISH solution

Automate ACD's RNAscope on the BOND RX stainer. Complete up to 30 slides in a single 11-hour run for single-plex (and a 14-hour run for duplex) with high-throughput staining. This solution is:

- Highly specific – Amplifies signals using double Z probe strategy and simultaneous background suppression strategy to ensure target-specific binding
- Sensitive & Quantitative – Detects single RNA molecules at single cell resolution
- Morphological – Provides cell-specific expression in information intact tissue architecture
- Universal – Use for virtually any gene, species, or tissue

ACD a bio-techne® brand

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CHAPTER 4
Product Highlights

SESSION 4C: Image management and analysis tools

Manage – Digital pathology software

Aperio eSlide Manager – complete digital pathology management software:
Aperio eSlide Manager provides full scalability and optimal performance from single-site installations to multi-site global hub and spoke networks. With dedicated workflows for research and biopharma coupled with an intuitive interface, Aperio eSlide Manager is the ideal solution to meet the diverse needs of both entry-level and enterprise digital pathology users.

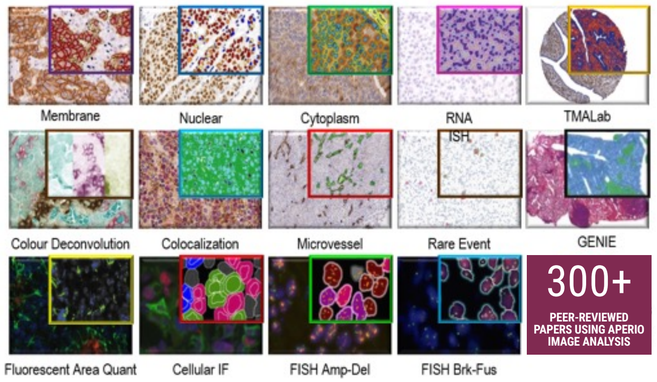
Aperio ImageScope – pathology slide viewing software:
Experience rapid access to crisp, true-color digital slide images to which you can adjust magnification, pan and zoom, compare different stains, annotate areas of interest, perform image analysis, and more.

Analyze – Image analysis for research needs

Aperio Image Analysis – Accurate. Reproducible. Quantitative:
Aperio Image Analysis provides easy-to-use solutions for the automated quantitative evaluation of brightfield and fluorescent slides. Powerful image analysis solutions combined with an intuitive interface enables users to easily tailor algorithms to their own specific needs.

Aperio RUO (Research Use Only) Image Analysis Algorithms have been validated by Leica Biosystems for use with .svs images from Aperio AT2, Aperio CS2, and Aperio VERSA RUO scanners.

Use of Aperio RUO Algorithms with other available scanners has not been validated, and Leica Biosystems cannot train or support customers in use of Aperio RUO Algorithms with images from these scanners.



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CHAPTER 5
Featured Products

CHAPTER 5A: BOND RX Stainer

BOND RX – fully automated research stainer

- Speed up your next big discovery. Use the BOND RX to confidently complete IHC, ISH, FISH, CTC, multiplexing, and other tests. Explore your ideas in a variety of ways via open reagents, open detection kits, and customizable protocols. The BOND RX provides an easy way for labs to fully automate tests and accelerate research programs.
- Push the boundaries of what is possible – The BOND RX fully automated research stainer from Leica Biosystems provides superior quality and flexibility while enabling the automation of IHC, ISH, and emerging tests.
- Accelerate test programs – through speed, efficiency, and consistent automation, the BOND RX stainer reduces manual work so that researchers can spend less time at the bench and more time innovating.
- Preserve morphology and integrity of precious tissue with the unique Covertile system that allows you to dispense reagents in a highly controlled and more consistent manner.
- Commercialize your discovery – with a clear path from research to clinical applications and leverage greater access to innovative emerging technologies via partnerships.

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CHAPTER 5
Featured Products

CHAPTER 5B: APERIO GT 450

Aperio GT 450 – automated, high capacity digital pathology slide scanner

- The Aperio GT 450 enables histotechnicians to complete scanning tasks quickly and with confidence leveraging a 32-second scan speed*. Output 81 slides/ hr at 40x* delivering high quality images with Leica optics and with an IT architecture that is secure and scalable. From the pathology lab to the IT room, the Aperio GT 450 is designed to scale up digital pathology operations.
- Improve case turnaround with no touch during scanning and continuous loading of the racks directly from the HistoCore Workstation (Stainer & Coverslipper).
- Help increase IT security and control with a dedicated SAM (Scanner Admin Manager) server and software that allows you to set up and monitor multiple Aperio GT 450s at a time. No more single workstations for each scanner.
- Ensure excellent image quality with Leica optics.

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CHAPTER 5
Featured Products

CHAPTER 5C: APERIO AT2

Aperio AT2 – high volume, digital whole slide scanner

- Easily load up to 400 slides into the Aperio AT2. The digital scanner has a sustained high throughput rate of 50 slides/hr at 20x, and z-stacking capability for up to 25 layers, the Aperio AT2 is fast, flexible, and reliable.
- Aperio AT2 consistently delivers precise, whole slide images with an unparalleled, low rescan rate and >98% first time scan success rate, eliminating unnecessary time spend rescanning slides. Slides are available for remote viewing in less than a minute. With an easy-to-use Researcher's cockpit, coupled with easy integration into laboratory information systems (LIS), the Aperio AT2 provides an ideal platform for research institutions.

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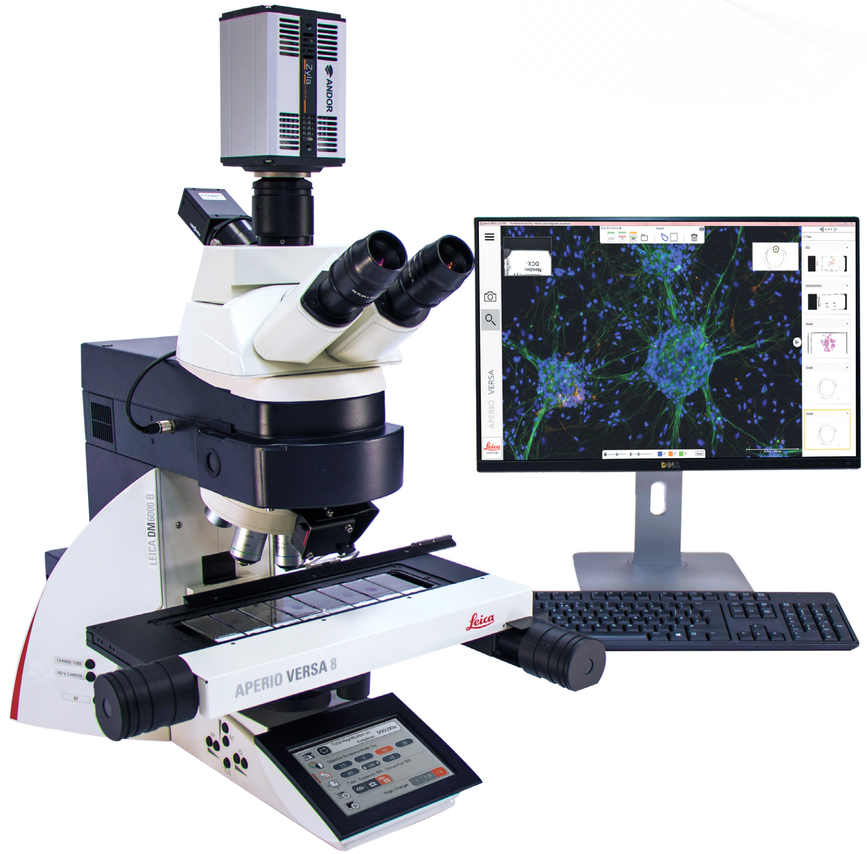
CHAPTER 5
Featured Products

CHAPTER 5D: APERIO VERSA

Aperio VERSA – brightfield, fluorescence, and FISH digital pathology scanner

- Delivering excellence in IHC, ISH, and fluorescent tissue-based research including multiplex whole slide scanning.
- The Aperio VERSA is a comprehensive digital pathology scanner, designed and developed to support the diverse imaging needs of cutting-edge research facilities. The combination scanner is optimized for precision scanning of brightfield and fluorescent samples, with the accuracy and resolution required for FISH.
- From tissue-based and proteomic markers, to subcellular, molecular, and insitu hybridization probes, the Aperio VERSA delivers high-resolution, reliable imaging. Users can create a permanent record of their research – even faint fluorescent samples, which are often subject to fading.
- The Aperio VERSA is ideal for scanning multiplex slides at any magnification from 5x to 63x for the whole slide without the need for spectral imaging.



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