HistoMapr™ Computational Pathology Platform

An Unbiased Spatial Analytics and Explainable AI (xAI) Platform Generating Data, Extracting Information, and Creating Knowledge from Transmitted Light Image Datasets that Addresses the Unmet Pathology Needs of Improving Efficiency and Accuracy of Clinical Workflows, Biopharma Discovery and Development Programs, and Precision Medicine Including Clinical Trial Support and Companion Diagnostics that will Result in Dramatically Improved Patient Outcomes.

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Current computational pathology tools for traditional transmitted light imaging data based on hematoxylin and eosin (H&E) and immunohistochemistry (IHC) labeling, do not optimally address critical challenges in real world pathology for efficiency and accuracy of clinical workflows, biopharma discovery and development programs, and precision medicine including clinical trial support and companion diagnostics. Successful computational workflows must leverage AI for efficiency and accuracy gains, and they must also optimize data generation, information extraction and deep knowledge creation for increasingly complex datasets. The revolutionary HistoMapr™ platform provides actionable solutions to these challenges, harnessing proprietary, unbiased spatial analytics and explainable AI (xAI) for transmitted light applications. SpIntellx delivers HistoMapr products and services, as well as TumorMapr™ products and services for multi- and hyperplexed image data, to ultimately deliver end-to-end support for both clinical and biopharma computational and systems pathology.

I. Background

The field of digital pathology was initially developed based on the projected value of acquiring digitized images of clinical specimens to allow pathologists to "read" hematoxylin and eosin stained (H&E) and immunohistochemistry (IHC) pathology slides on a high-resolution monitor in order to reduce eyestrain and to efficiently share images for consults and telemedicine. Generating digital image data further opened the way for the field of computational pathology, following tremendous interest shown by the major pathology organizations (e.g., DPA: Digital Pathology Association, and USCAP: United States and Canadian Academy of Pathologists). Computational pathology started with basic digital image analysis, such as detecting nuclei, counting cells, and identifying cellular structures, on traditional transmitted light datasets with H&E and IHC labeling. However, these early tools were of limited clinical utility, due to factors such as a lack of a digital pathology infrastructure and insufficient gains in efficiency.

Digital pathology is now growing rapidly with the advent of clinical-grade imaging systems approved by FDA as class II medical devices and with increased utilization during the COVID pandemic. Accelerating this trend is the emergence of more powerful computational

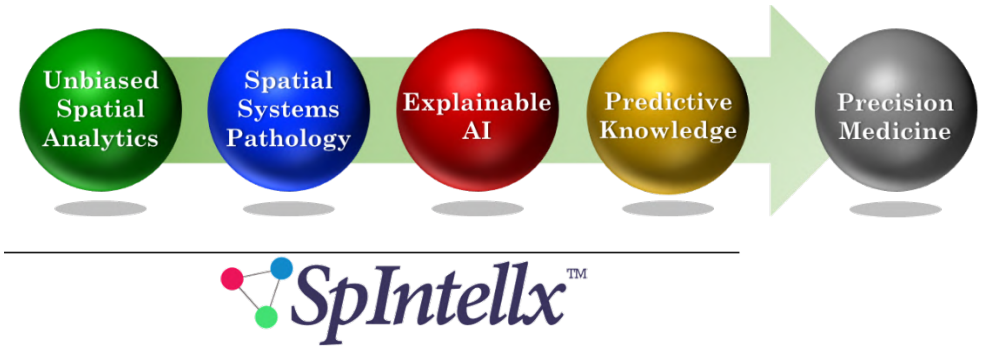

Figure 1 - Spatial Intelligence and Explainable Artificial Intelligence (xAI) Revolutionizes Computational and Systems Pathology for Precision Medicine. SpIntellx holds a very strong intellectual property position in unbiased spatial analytics, spatial systems pathology, and xAI that allows the creation of deep knowledge and powerful applications in precision medicine.

pathology machine learning algorithms using artificial intelligence (AI). Pathologists at medical centers, private pathology practices, and pharmaceutical companies are beginning to recognize machine learning as an important new aid in their workflows. Despite the great potential, the current approach of computational pathology with transmitted light datasets usually involves simple quantitation and "black box" artificial intelligence (AI) that does not explain why the algorithms made a particular recommendation. This whitepaper discusses the importance of advanced spatial analytics and explainable AI (xAI) for computational pathology applied to transmitted light image datasets in order to address the unmet pathology needs of improving efficiency and accuracy of clinical workflows, biopharma discovery and development programs, and precision medicine, including clinical trial support.

SpIntellx came to the market at this critical time with HistoMapr™ and TumorMapr™ platforms, a continuum solution for the multiple challenges facing computational, and now computational and systems pathology, applied to transmitted light, as well as multi to hyperplexed fluorescence/mass spec imaging of tissue samples (see the separately released whitepaper on TumorMapr[1]). (**Figure 1**). These platforms can be used individually or in combination.

SpIntellx's HistoMapr platform offers complete spatial analytics and xAI application guides on top of standard analytics. HistoMapr applies unbiased spatial analytics to quantify the spatial relationships between cells and histological structures in whole slide images (WSIs) of whole tissue sections and/or large bore tumor microarrays (TMAs) from surgical pathology specimens (e.g., cores, biopsies, resections). This approach generates data that accurately reflects the true complexity and heterogeneity of the clinical specimens. HistoMapr is the first xAI solution available to clinical and biopharma pathologists for building trust and transparency with end-users for recommendations made by HistoMapr's xAI algorithms. This whitepaper highlights the transformative potential of the HistoMapr platform, including why xAI is a critical feature for advancing computational pathology.

[1] TumorMapr Whitepaper: www.spintellx.com/tumormapr

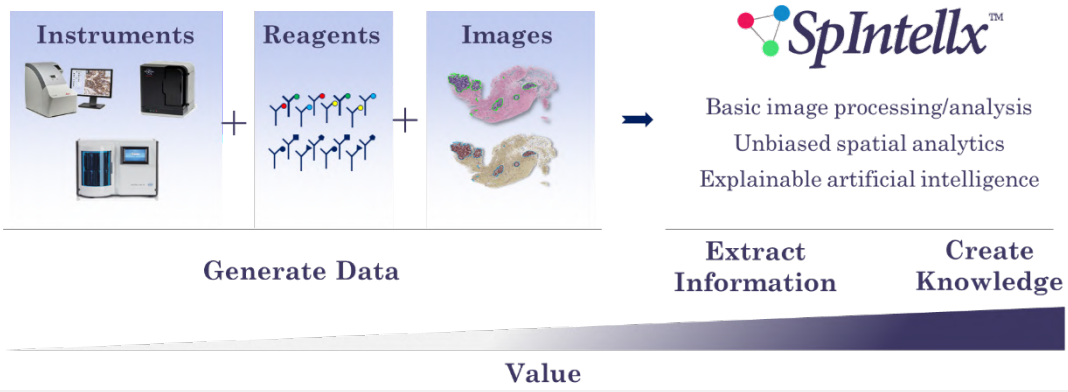

Figure 2 - The SpIntellx HistoMapr™ Computational Pathology Platform Focuses on the High Value Information and Knowledge Portion of the Workflow. There are now multiple imaging and reagent platforms that can generate huge, transmitted light image datasets, but they have very limited analytical software tools. SpIntellx offers HistoMapr to perform not only basic image processing and analyses on raw image datasets imaged on any transmitted light imaging platform, but also to harness the computational power of proprietary, **unbiased spatial analytics** and **explainable artificial intelligence (xAI)** to extract information from patient primary disease pathology samples and to create predictive knowledge that will improve patient outcomes, as well as the efficiency and accuracy of pathology.

HistoMapr goes beyond simply generating data and extracting information from data by creating the highest-value predictive knowledge from transmitted light image datasets that addresses the unmet pathology needs of improving efficiency and accuracy of clinical workflows, biopharma discovery and development programs and precision medicine including clinical trial support and companion diagnostics (**Figure 2**).

Today, clinical pathology faces stark challenges related to efficiency and accuracy gains, i.e., balancing cost with quality coupled to the added pressure of increasing demand with a declining number of pathologists. There are fewer resources, less time, and fewer people to get the pathology work completed with high efficiency and accuracy. Recent tools provided by digital and computational pathology vendors still rely on complete WSI review, where pathologists need to assess the entire WSI by manually navigating on a screen, as they review glass slides. Although these digital tools display heat maps and side-by-side WSI views for convenience, they are used as standalone sideline tools and do not address inefficiencies of slide examination. Additionally, the current solutions are limited in their ability to address the critically important issue of working with difficult, borderline and rare cases that consume the most amount of a pathologists' time. There is also no high-level, work-task based approach and it shows in the current slate of computational and digital pathology tools.

There are additional pathology challenges, since computational pathology can find novel patterns in image data that may elude or prove too subtle for pathologists to report routinely. In addition to traditional morphology and IHC biomarkers, these analytics also include advanced analyses, possibly identifying novel tissue-based biomarkers that were previously only available to basic science studies.

Biopharma companies also faces many of the same basic workflow challenges faced by clinical pathology applications. There is a need to reduce the drug discovery and development time and cost, including the optimization of selecting patient cohorts for clinical trials to get the right patients the right drug at the right time. Given the resources required to conduct

biopharma studies, it is imperative for the biopharma pathologists to maximize data generation, information extraction and knowledge creation from increasingly complex clinical trial datasets. There is a need for both computational pathology and clinical data management tools to support the interpretation and evaluation of clinical trial results, and further streamline and standardize clinical deployment, including companion diagnostic tests. SpIntellx is evaluating the opportunity to partner with one or more of the best clinical data management companies to offer a complete solution.

Just as clinical colleagues potentially can combine H&E, IHC, and other information sources together, biopharma pathologists are constrained by the limitations of existing tools that provide basic analyses or that do not integrate results from multiple labels into a coherent whole. This is increasingly important as more complex patterns are required in order to comprehend a disease microenvironment. Although some of this work can take place in specialized hyperplexed environments[1], there is also a need for this type of workflow to occur with transmitted light H&E staining and a few IHC labels or multiplexed chromogenically labeled antibodies.

These challenges present major opportunities for SpIntellx to provide actionable solutions by harnessing proprietary, unbiased spatial analytics and xAI (**Figure 3**). As a pioneering company in this area, SpIntellx has made significant contributions to advance the application of spatial intelligence and xAI in computational and systems pathology. SpIntellx also holds a leading intellectual property position in unbiased spatial analytics, spatial systems pathology, and xAI for transmitted light and fluorescence/mass spectrometry imaging applications that are creating powerful predictive knowledge for precision medicine.

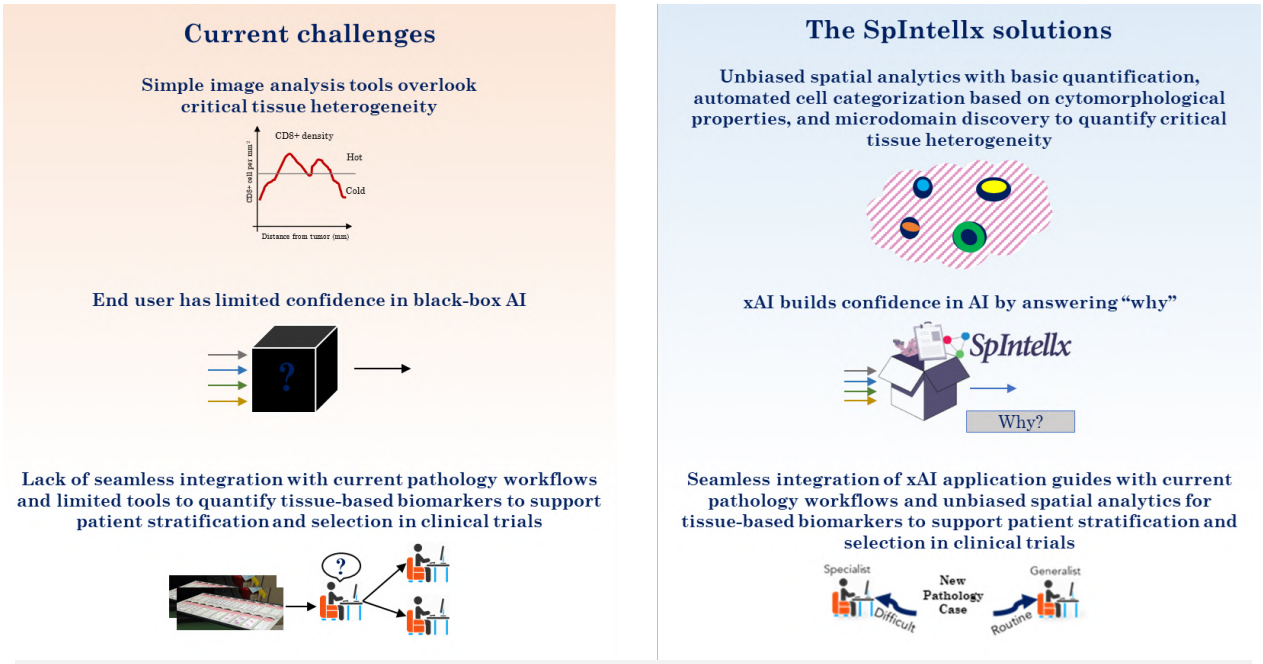

Figure 3 - The SpIntellx Solutions to the Current Challenges in Improving Efficiency and Accuracy of Clinical Workflows, Biopharma, and Precision Medicine Including Clinical Trial Support.

II. The HistoMapr Platform with Unbiased Spatial Analytics and Explainable AI (xAI) is Revolutionizing Computational Pathology for Precision Medicine

Despite the progress in computational pathology applied to traditional transmitted light imaging, serious challenges remain in several major areas: 1) simple image analysis tools overlook critical tissue heterogeneity; 2) lack of trust by the experts in non-explainable, black-box AI; 3) lack of seamless integration with current pathology workflows; and 4) limited tools to quantify tissue-based biomarkers and support patient stratification in clinical trials.

Current, simple computational pathology software applied to transmitted light imaging present a series of challenges that hinder efficiency and accuracy gains of clinical workflows, biopharma and precision medicine including clinical trial support (**Figure 3, Left Panel)**. The application of unbiased spatial analytics (automated cytomorphological cell categorization and microdomain discovery which define spatial relationships and identify critical tissue heterogeneity), and xAI (building confidence in AI algorithms by answering "why") provide a series of critical solutions to these challenges (**Figure 3, Right Panel**).

The SpIntellx unbiased spatial analytics approach combines basic quantification, automated cell categorization based on cytomorphological properties, and microdomain discovery to define spatial relationships of various cell types to characterize tissue heterogeneity. The number and the type of microdomains determine the extent of heterogeneity of a clinical specimen. A thorough and accurate characterization of tissue heterogeneity based on unbiased microdomain discovery is necessary for the subsequent pathology analyses.

Next, the xAI supported interface allows seamless interactions between end-users (pathologists/other clinicians) and the software, to provide explanations for recommendations made by the algorithms and to build confidence in the platform. Finally, seamless integration of computational guides delivers end-to-end support for both clinical and biopharma computational pathology (**Figure 3, right panel**). These powerful technologies are revolutionizing the impact of pathology in precision medicine (**Figure 4**).

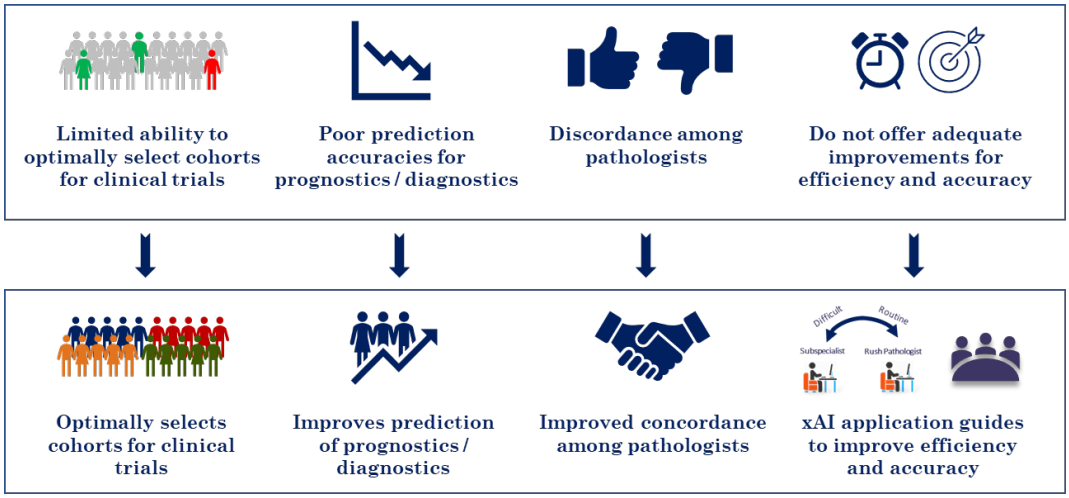

Figure 4 - Performance Comparison – (Top) Competition vs (Bottom) SpIntellx. The SpIntellx approach results in improved efficiency and accuracy of clinical workflows, biopharma discovery and development programs including clinical trial support and diagnostics.

Analytics	Competitors/Limitations	SpIntellx
Basic image processing, image analyses, and annotation tools	• Most platforms and software providers have this capability • Annotation tool functionality is limited and slow	• HistoMapr provides basic image processing, simple analyses, and rapid annotation tools • Virtual multiplexing of consecutive H&E and IHC WSIs
Spatial identification of cell types and histological structures	• Cellular identification based on positive and negative signals from IHC labels • Supervised cellular categorization and histological structure identification from H&E images requires extensive ground truth data	• Unbiased and automated approach to categorize cell types and segment histological structures with complete spatial context using both H&E and IHC images • Extract diagnostically relevant cellular categories and histological structures to build the xAI engine
Identification of microdomains	• Quantify the density of cellular types using a grid-based analysis of the WSI to define microdomains • Microdomains identified without accounting for spatial relationships between cell types • Limited ability to identify critical tissue heterogeneity associated with disease outcomes	• Based on spatial network analysis of cell-cell interactions (e.g., pointwise mutual information) • Unbiased and automated discovery of diagnostically relevant spatial configurations of distinct cell types as microdomains • Microdomains drive xAI interpretations
Addressing difficult / borderline / rare cases	• Limited ability to identify and resolve difficult / borderline / rare cases	• Pre-diagnostic case triaging and more effective QA tools to handle with difficult / borderline / rare cases
Use of AI to create knowledge	• "Black box AI" with no explanation of how the recommendations were made • End-users must blindly trust the algorithms • Summary "reports" only yield data and information with limited interpretable and predictive knowledge	• Explainable AI presents results produced by algorithms and explains why a particular recommendation has been made • End-user can intervene the results which builds trust and confidence in the algorithms • Summary "guides" yield actionable knowledge in clinically recognized language

Table 1 - Computational Pathology Analytics from SpIntellx vs Competitors.

The SpIntellx HistoMapr™ platform offers complete spatial analytics and xAI application guides on top of standard analytics for creating the knowledge that ultimately provides high confidence recommendations to clinical experts. The advantages of these elements are described below in detail and are summarized in **Table 1**.

Why Spatial Analytics Matters?

The pathophysiology of human disease is highly complex. However, recent advances in our understanding may not be available to routine surgical pathologists. For example, in-depth evaluation of the distinct cell composition, spatial interactions between cancer cells and stromal cells are critical to generate accurate predictions of disease progression and outcomes. Capturing spatial tissue heterogeneity is a prerequisite for determining the pathological basis of a disease such as elucidating spatial interactions between histological structures in disease microenvironments relative to specific cell types (e.g., tumor infiltrating lymphocytes).

The features of spatial tissue heterogeneity are often under-utilized by pathologists as they are often subtle and difficult to recognize. An example might be the limitations in cross examining H&E and IHC slides to examine specific sub-areas or to see subtle patterns. Visually this is equivalent to "co-registration" or overlaying adjacently cut slides over one another so that one can simultaneously consider a focus in multiple dimensions including the H&E and individual IHC labels (**Figure 5**).

SpIntellx has developed unsupervised machine learning algorithms, in conjunction with pointwise mutual information (PMI), to capture spatial tissue heterogeneity and to discover microdomains from disease tissue samples (**Figure 5**). Microdomains are characterized by distinct compositions and spatial configurations of distinct cell types. Thus, a thorough understanding and evaluation of the heterogenous biology of a disease by generating and analyzing spatially imaged data are essential for the applications described here.

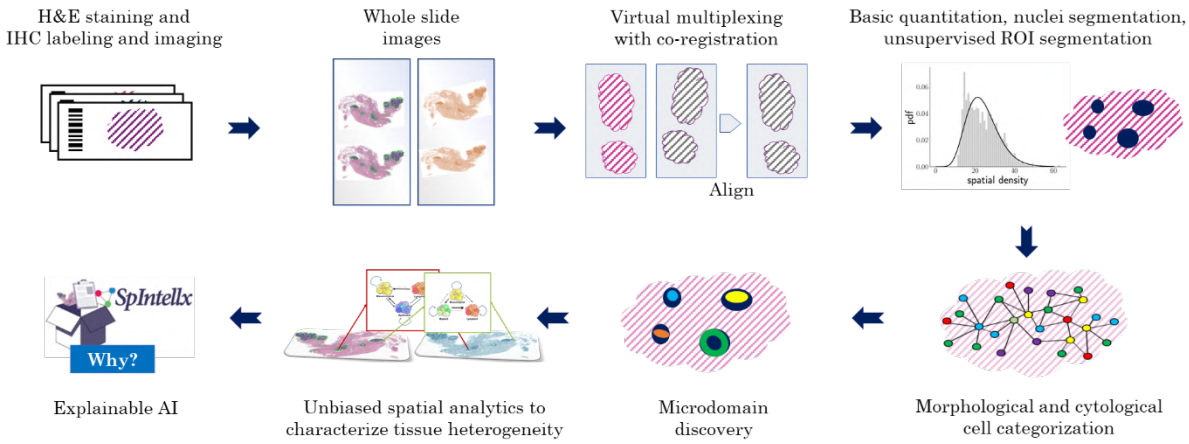

Figure 5 - HistoMapr Analytical Pipeline for Quantifying Complex Tissue Heterogeneity from Consecutive H&E and IHC Labeled Slides. Virtual multiplexing of consecutive H&E and IHC slides, basic quantitation, morphological and cytological characterization of cells, microdomain discovery, spatial analytics within microdomains, and xAI allow the HistoMapr platform to identify and to characterize complex tissue heterogeneity, which can reveal patterns that are of clinical significance (e.g., high risk for metastasis or recurrence, high likelihood of response to a targeted therapy, etc.).

Why Explainable Artificial Intelligence (xAI) Matters?

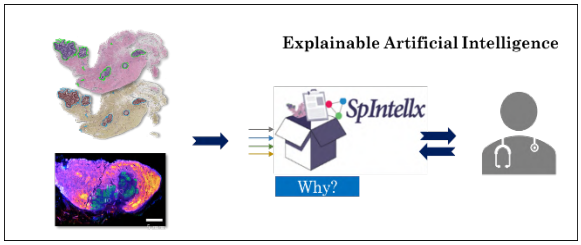

Figure 6 - Why Explainable AI (xAI) Matters? Pathologists and disease experts require transparency in applying computational and systems pathology algorithms to their data. The HistoMapr platform offered by SpIntellx using xAI provides confidence in decisions recommended by the algorithms by answering "Why?".

Pathologists and disease experts require transparency regarding why a certain decision or recommendation is made by the AI software while applying computational and systems pathology. However, without the ability to show how and/or why a certain decision or recommendation is made by the algorithms, the standard "black box" nature of AI algorithms makes it very difficult to establish confidence with clinical experts. The lack of confidence in "black box" AI decisions has been a major roadblock to the adoption of computational pathology, since additional studies may be required to verify an AI decision and may introduce inefficiency involving extra time and resources.

HistoMapr utilizes a user interface based on explainable artificial intelligence (xAI), thus, it allows critical interactions with pathologists/clinicians and it is capable of providing explanations for the decisions recommended by its AI algorithms **(Figure 6).** In addition, novel findings and supporting evidence provided by xAI can be studied to further advance our understanding of diseases. The application of xAI builds confidence in the algorithms and extends its utility in clinical and biomedical research. Therefore, xAI plays a critical role in facilitating wider adoption of computational and systems pathology**.**

III.HistoMapr xAI Platform

How does HistoMapr work?

The HistoMapr Platform offers complete spatial analytics and xAI application guides on top of standard analytics. HistoMapr takes a set of WSIs, TMA cores, or cropped images of tissue regions as input (**Figure 5 and Figure 7**). The stain colors are robustly normalized to minimize errors due to imaging or staining artifacts. Proprietary advanced AI tools are applied to recognize histological structures in an unsupervised manner, automatically segmenting nuclei and other cytological components such as ducts, cells, etc. Next, the histological structures and cells are computationally categorized based on their cytomorphological properties. HistoMapr then builds a spatial network around each computationally classified histological component, and automatically extracts distinctive spatial network patterns, or microdomains (**Figure 5 and Figure 7**). The spatial communication properties within and between cells are critical for performing predictive analytics and correlating with outcomes.

Additional immunohistochemistry (IHC) labels (either individual labels on consecutive tissue sections or chromogenic multiplexed IHC on a single section) can further enhance HistoMapr's unbiased spatial analytics and xAI capabilities. HistoMapr can co-register WSIs of successive disease tissue sections to build and analyze virtually multiplexed datasets. HistoMapr findings in the form of key diagnostic areas are presented to the clinician in an interactive and explainable fashion to build trust and transparency with the end user.

HistoMapr's methodology enables building parametric models of histological patterns from any tissue, hence creating a visual pattern dictionary that traditionally defines the standards on disease classification/nomenclature for pathologists worldwide. This dictionary allows HistoMapr to build an xAI engine, which can be applied to any tumor or organ structure and enhance the interpretability of the HistoMapr graphical user interface. This capability of HistoMapr addresses the limitations of standard AI in building trust with pathologists because it is transparent and self-explainable.

As an example, we designed and implemented an automated, efficient, and pathologist-friendly Java-based annotation software, HistoMapr-Annotator™, for collecting ground-truth labels of diagnostic ROIs. Pathologists do not have to hand-draw regions on screen or

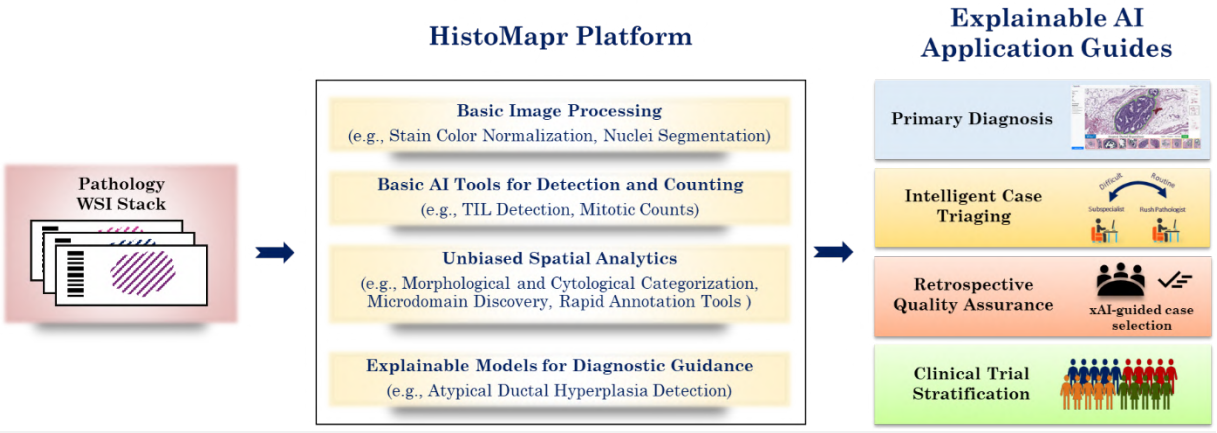

Figure 7 - HistoMapr™ Platform Offers Complete Spatial Analytics and xAI Application Guides on Top of Standard Analytics.

type in the ground-truth labels. Instead, HistoMapr Platform's automated unsupervised ROI detection enables pathologists to annotate >700 ROIs/hour by simply using numerical keyboard buttons to annotate the diagnostic areas with predefined labels.

HistoMapr xAI User Interface

The user-interface in HistoMapr is both a visualization tool and a functional tool (**Figure 8**). Spatial image datasets originating from any transmitted light imaging platform can be retrieved and observed by pathologists/clinicians. The SpIntellx HistoMapr platform presents to the pathologists and other end-users, critical knowledge created from the extracted information through an xAI enabled interface.

HistoMapr effectively changes the pathologists' view of a case from one or more WSIs into a guided series of diagnostically ranked regions of interest (ROIs). HistoMapr previews entire WSIs to discover relevant structure/features. For example, HistoMapr-Breast™, which is HistoMapr applied to breast core biopsies, finds ducts, vascular structures, and stromal features using proprietary spatial statistics based on pairwise mutual information (PMI). By analyzing PMI maps, HistoMapr can find ROIs that contain these structures. Once ROIs are identified, HistoMapr then analyzes them for certain features. An initial version of HistoMapr-Breast used 18 features to classify ROIs as atypical or not atypical. In this analysis, HistoMapr finds and quantitates features present in a ROI. The ROI analyses leads to patterns that can be matched to HistoMapr's library of diagnostic labels. The combination of diagnostic labeling and ROI quantitation is then used to prioritize the ROIs.

In an interactive work session (**Figure 8A**), the pathologist reviews the entire case, ROI by ROI, in a diagnostically ranked fashion. This approach is highly efficient because HistoMapr presents the most clinically impactful ROIs to the pathologist first, if present. This guidance enables the pathologist to focus on the hardest decisions first. If necessary, HistoMapr also keeps track of ROIs that may need further workup with additional labels, or ROIs that may require consultation with another pathologist or panel review. Critically, the pathologist is always fully in control and may take manual control of the WSI viewer software at any time if they need to review all or part of the WSIs manually.

xAI manifests as a "Why?" button that provides one or more panels of supplementary information (**Figure 8B**). In particular, the pathologist can question the software with a click of the "Why?" button. HistoMapr presents the key findings in pathologist-friendly terminology, that is equivalent to how pathologists describe the features that they see (e.g., "cobblestone nuclear arrangement" or "usual ductal hyperplasia nuclear arrangement"), to justify each recommendation. This allows collaborations and conversations between pathologists and other clinical experts, particularly in atypical cases, and allows the pathologists/clinicians to remain in full control during the entire process. The pathologists/clinicians make the final decision and the potential of a high discordancy rate between pathologists can be reduced or eliminated. The pathologist thus has complete situational awareness and is able to make the very best diagnostic decisions.

HistoMapr also facilitates the pathologist's work by managing diagnostic information and tracking the pathologist's agreement or disagreement with the provided diagnostic labels; the pathologist may also indicate uncertainty and HistoMapr collects this information for possible additional work-up or consultation. When the pathologist is ready to finalize the

case, HistoMapr automatically constructs a results report using the pathologist's interpretations of the ROIs review, and also using suggested/standardized terminology.

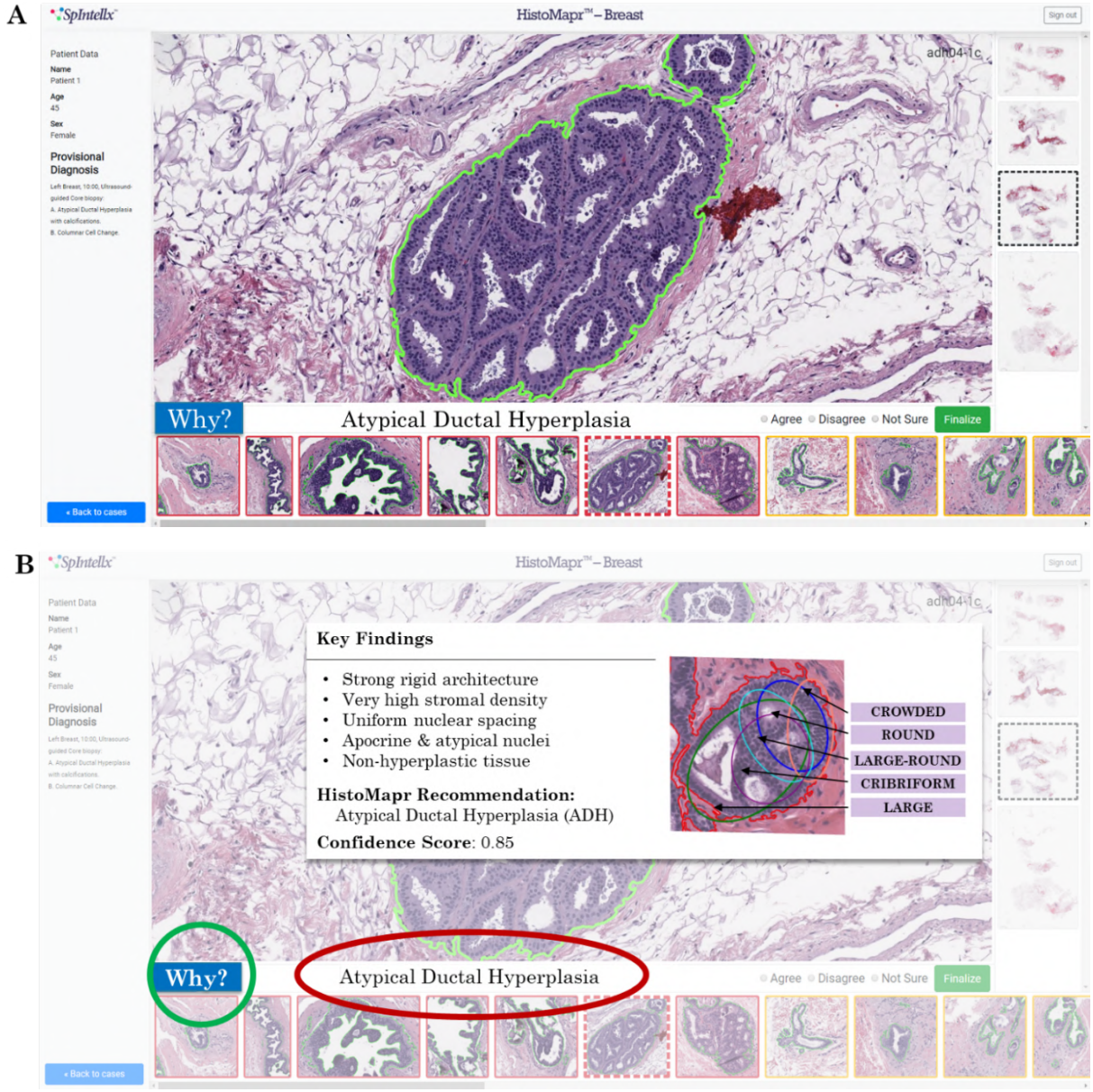

Figure 8 – HistoMapr-Breast User Interface and the Transformative Impact of the "Why" Button. (A) Web browser-based, pathologist-friendly, interactive, image viewer and analytical engine for transmitted light breast core biopsy image datasets. Left panel shows patient information and provisional diagnosis, and the right panel has thumbnail images of the patient slides. ROIs are automatically detected and presented in the bottom panel, triaged based on diagnostic significance from left to right. In this example, HistoMapr-Breast pre-analyzed the slides and recommended the diagnosis of atypical ductal hyperplasia, which is a challenging call. **(B)** Pathologist can select the "Why?" button to display transparent explanations as Key Findings that led to this recommendation.

IV. Example Applications of HistoMapr

The revolutionary HistoMapr™ platform provides actionable solutions to transmitted light applications. The HistoMapr platform brings efficiency and accuracy gains and maximizes information and knowledge for increasingly complex datasets. All the applications and advantages can be extended not only to solid tumors, but also to other conditions like autoimmune diseases, organ transplantation, organ-specific and infectious diseases.

1. HistoMapr-Breast: Primary Diagnosis of Breast Lesions Including Hard Cases

SpIntellx is currently completing the validation of HistoMapr-Breast™, which is HistoMapr applied to transmitted light image datasets of breast core biopsies, in collaboration with CellNetix Pathology and Laboratories. HistoMapr-Breast previews WSIs of breast cores to differentiate and rank a broad spectrum of breast pathologies from benign to atypia to ductal carcinoma in situ (DCIS) to invasive/malignant with high accuracy (**Figure 9**).

In an initial validation study that we previously reported[2], 4,865 WSIs of breast core biopsies were collected from which ~201,000 regions of interest (ROIs) were extracted using HistoMapr's proprietary spatial analytics. HistoMapr-Annotator™, our rapid pathologist-centered annotation tool, was harnessed for efficiently collecting ground-truth annotations (~700 ROIs per hour per pathologist) from 3 expert pathologists to collect ~5,600 ROI annotations. The spatial statistics and xAI algorithms to differentiate and rank the entire spectrum of diagnostic categories in breast pathology were tested: benign, atypia, ductal carcinoma in-situ, and invasive carcinoma. HistoMapr-Breast's precision is uniformly high compared to the state-of-the-art deep learning black box algorithms especially in detecting atypical ductal hyperplasia, a lesion which is reported to elicit high discordance among expert pathologists (**Table 2**). Our tests demonstrated dramatic improvements in concordance amongst expert pathologists; as their agreement scores significantly improved from κ=0.63 to κ=0.75. Moreover, training fellows' accuracy significantly boosted with the assistance of HistoMapr-Breast's xAI from 67% to 91%.

Figure 9 - HistoMapr-Breast for Primary Diagnosis of Breast Lesions Including Hard Borderline Cases. HistoMapr differentiates and ranks the entire spectrum of diagnostic categories in breast pathology: invasive carcinoma, ductal carcinoma in situ (DCIS), high-risk and low-risk benign lesions. The xAI interface (Figure 8B) presents the key findings to justify each recommendation and the pathologist remains in full control of the entire process and makes the final decision.

Detection Sensitivity	Deep Learning AlexNet (Convolutional Neural Network)	Deep Learning (Graph Neural Network)	HistoMapr-Breast
Atypical Ductal Hyperplasia	40%	56%	80%

Table 2 - Diagnostic Results from ADH Classification.

[2] Explainable Artificial Intelligence (xAI) for Safe Breast Core Biopsy Diagnosis Support, USCAP 2020 (p. 1495)

2. HistoMapr-Triage™: A Pre-signout Application for Intelligent Case Triaging

A pathologists' diagnostic efficiency is greatly affected by the difficulty of the case they are viewing. For example, general pathologists can spend an inordinate amount of time on difficult / borderline diagnoses, reducing efficiency and accuracy. In our customer discovery interviews, we found that ~90% of a pathologists' time is spent on borderline cases. Subspecialty experts may be better equipped to diagnose these cases and thus prevent the need for extensive consultation or to prevent misdiagnoses. Likewise, subspecialty experts can be overwhelmed with straightforward cases that could also be readily diagnosed by general pathologists. HistoMapr triages the patient samples to make sure that the borderline cases are sent to the sub-specialty pathologists. This greatly increases the accuracy and efficiency of the diagnostic process.

One of the main benefits of HistoMapr xAI capabilities is the use of x AI features to stratify all the slides belonging to a patient (**Figure 10**). HistoMapr analyzes the WSIs of a patient's biopsy by automatically segmenting ROIs. These ROIs are further ranked based on the classification results of their diagnostics importance (i.e., from malignant, then DCIS, then atypical and benign). Cases are triaged and labeled by HistoMapr to be distributed to appropriate pathologists according to their subspecialties and workload. This helps maintain a case load balance and subspecialists will save time on easy cases, improving turn-around-time and allowing better utilization of scarce pathologist resource.

Practically, WSIs are processed chronologically by running the HistoMapr xAI platform algorithms to detect critical ROIs and flag them based on the xAI outcomes and confidence scores. Then, case data are forwarded to the HistoMapr-Triage application which also retrieves xAI feedback from HistoMapr. While doing so, the HistoMapr case triaging helps to distribute cases in a balanced way while putting extra care to assigning critical cases to subspecialists (**Figure 10**). HistoMapr-Triage does not alter the original medical image and

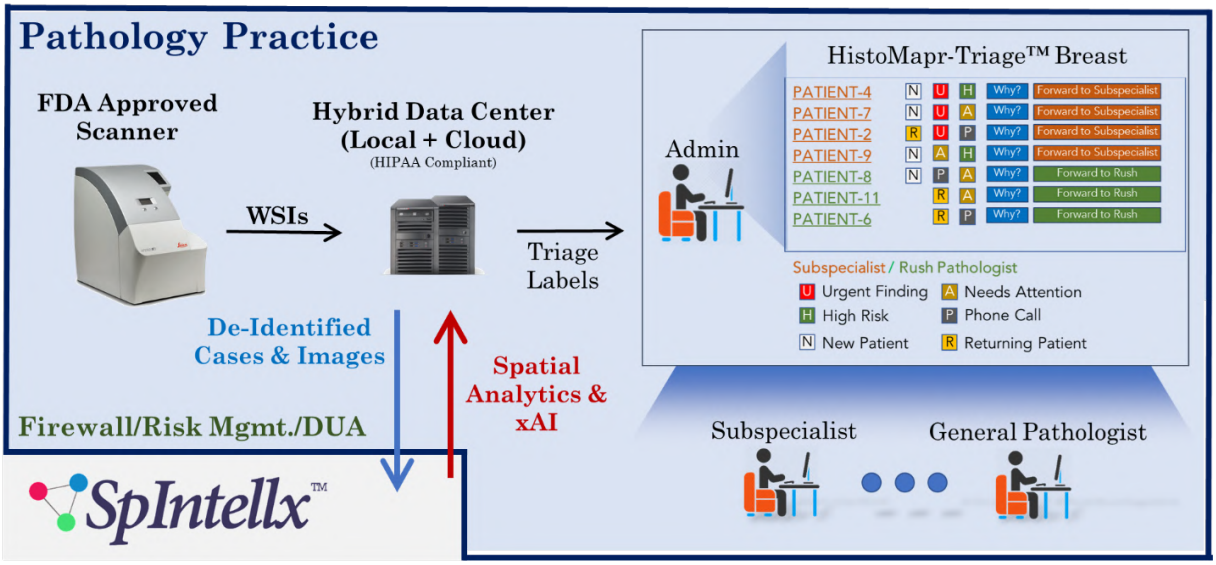

Figure 10 - HistoMapr-Triage: A Pre-signout Application for Intelligent Case Triaging. HistoMapr-Triage is designed to pre-read the pathology cases and forward the right case to the right pathologist rapidly and accurately, hence improving the efficiency in a pathology practice with better resource allocation.

is not intended to be used as a diagnostic device in this setting, it will only provide a recommendation for who to assign the case based on xAI outputs. Initially, a supervisor/lead pathologist will be using the software as the triaging administrator. HistoMapr will optimally takeover this role after it has proven its accuracy and efficiency in triaging cases.

3. HistoMapr-QA™: A Post-signout Application for Quality Control

One of the most urgent unmet clinical needs for pathology practices are tools that reduce discordance among pathologists. For tough cases like atypia, if the pathologist is not a subspecialist, diagnostic accuracies drop significantly. These inaccuracies can lead to unnecessary surgical resections or to missed diagnosis of malignancy, resulting in increased probability of poor patient outcomes and medical malpractice litigation. There is a significant unmet clinical need for methods to allow pathology practices to evaluate how concordant their pathologists are on calls and to be able to learn from poor concordance. Current QA mechanisms are not definitive and automated, as most of them are crude and semi quantitative. In our discussions with pathologists, we found that pathology practices currently execute QA for randomly selected 5-15% of cases. Cases are either selected retrospectively for second review or are automatically selected randomly such that pathologists are directed to take the case to a second pathologist for manual re-review prior to final signout (**Figure 11**). Since the selection procedure is random, there is still significant risk to miss cases that are misdiagnosed, which is especially critical for cases involving atypia in breast lesions, for example. HistoMapr-QA aims to improve this selection procedure by using xAI findings.

HistoMapr-QA reviews all previously finalized cases as ongoing post-diagnostic surveillance. The xAI features are used to help identify potential pathology result discrepancies or errors. This important QA activity reduces risk, improves clinician confidence, and helps pathologists monitor their diagnostic work in a timely fashion (**Figure 11**). In our validation study involving HistoMapr-Breast[3], it was observed that the sensitivity of non-specialist

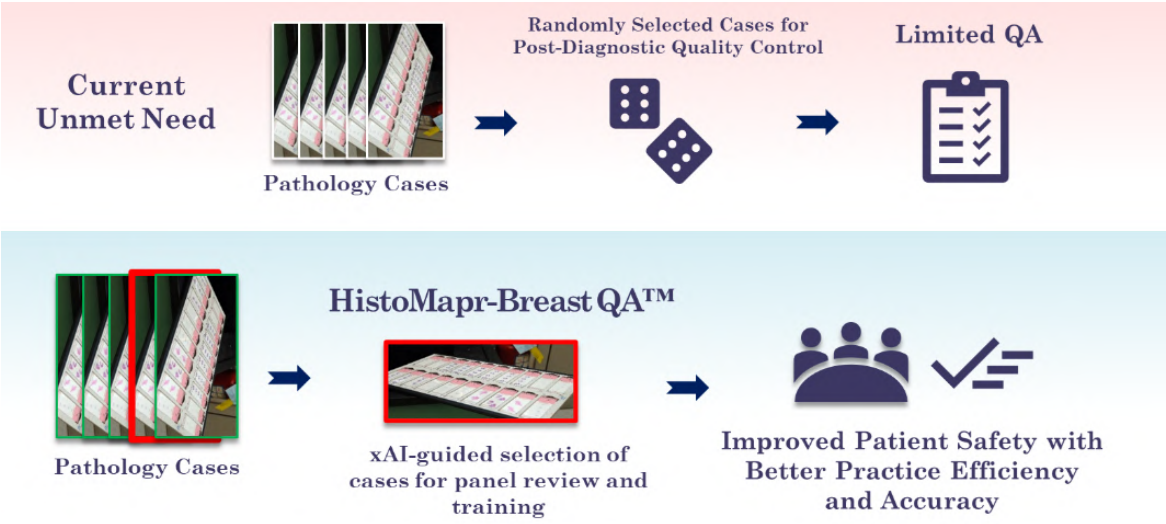

Figure 11 - HistoMapr-QA: A Post-signout Application for Quality Control Designed to Prevent Diagnostic Errors and Improve Patient Safety.

[3] Explainable Artificial Intelligence (xAI) for Safe Breast Core Biopsy Diagnosis Support, USCAP 2020 (p. 1495)

training pathologists in detecting high-risk benign lesions and ADH improved more than 50% against the ground-truth. It is predicted that the SpIntellx QA approach will augment/replace existing manual QA procedures, further improving efficiency and accuracy, as well as decreasing the pathologist's professional time for these important activities.

4. HistoMapr-Stratify™: An Advanced IHC-based Application for Streamlined Clinical Trial Cohort Selection

Current IHC digital pathology workflows, with a few labels either on consecutive tissue sections or on a single section with chromogenic multiplexed IHC, are generally manual and lack good integration with other digital pathology systems. This can lead to inefficient systems that fail to make pathologists more efficient and accurate, while also requiring them to manually report results. In addition, many systems have variable accuracy to the extent that the pathologist needs to manually interpret the label in order to carefully supervise the computer assisted result. This is suboptimal but acceptable for basic biomarker interpretations but becomes difficult when looking at more complex biomarker analyses such as PD-L1, which also varies depending upon tumor type or drug target. In addition, there may be novel utility in existing biomarkers that can only be derived from sophisticated analysis of the biomarker patterns or co-localization with specific H&E features and/or other biomarkers (e.g., tumor edge vs. center pattern, marker presence in lymphocytes rather than tumor cells, etc.).

HistoMapr-Stratify addresses these challenges by IHC workflows including: (i) Internal and external positive control management to check if the labels worked as expected; (ii) Basic pattern detection of positive labels (nuclear, membranous, cytoplasmic, and combinations). For example, lobular breast tumor pattern (e-cadherin negative, p120 cytoplasmic positive) vs ductal pattern (e-cadherin membrane positive, p120 membrane positive); (iii) Advanced pattern detection to pave the way for new discoveries (tumor edge vs center assessments, tumor heterogeneity, combining H&E features like "tumor budding" in GI with IHC); and (iv) sophisticated spatial analysis to perform advanced diagnostics or analytics or to act as novel tumor "meta" biomarker.

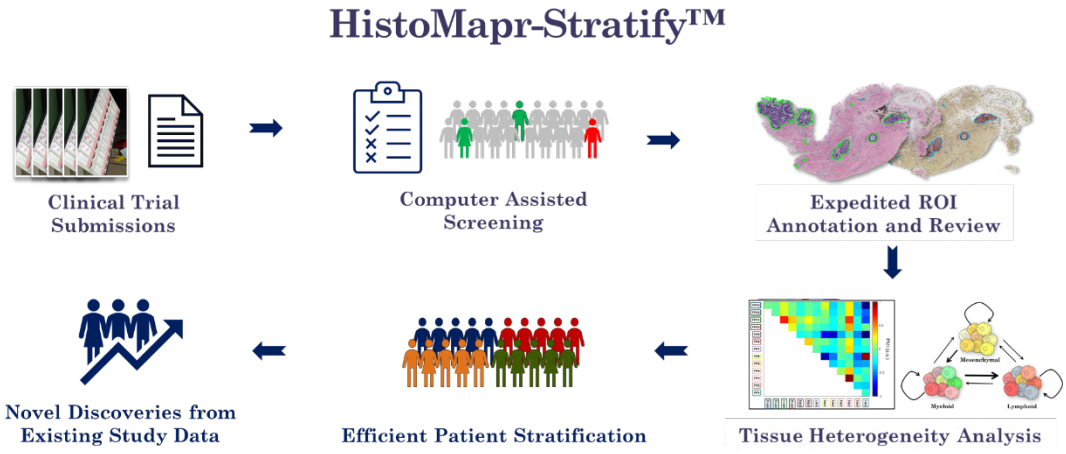

Figure 12 – HistoMapr-Stratify Pre-analyzes the Case Slides and Extracts Critical Case Attributions (e.g., Histological Components, Uncommon Structures, etc.), to Enable Streamlined Patient Stratification Through Basic Queries.

Using these workflows, HistoMapr-Stratify enables better patient stratification for clinical trials with enhanced and guided screening of a wider number of patients (**Figure 12**). It discovers spatially derived microdomains and assigns explainable features to describe the patterns found (**Figure 5**). These features build the basic streamlined reporting of pathological cases and enable more structured historical search to find the ideal candidates for clinical trials.

V. Integration of HistoMapr and TumorMapr for Selected Applications

Despite great potential and increased utilization of digital pathology, the current approaches involve mostly simple quantitation and "black box" AI, thus limiting the efficiency and accuracy gains of clinical workflows, biopharma discovery and development programs and precision medicine including clinical trial support and diagnostics. Moreover, sophisticated computational analyses of the images, including correlation of transmitted light features with multi-hyperplexed image features are not available from the competition. The SpIntellx approach to computer-assisted pathology decisions is novel and powerful in its ability to address these needs, offering a continuum of solutions to maximize the extraction of information from spatial data and to create predictive knowledge (**Figure 13**).

The HistoMapr™ platform analyzes datasets from traditional staining and labeling of transmitted light images (H&E and IHC) and it is a much more advanced solution than those currently used for disease diagnosis. HistoMapr™ software analyzes the transmitted light images, presents the key findings, and makes diagnostic recommendations to the pathologist, drives intelligent case triaging and quality assurance, and enables better patient stratification for clinical trials. Besides the application of unbiased spatial analytics, the transformative impact of HistoMapr™ resides in the "Why?" button: The pathologist can select the "Why?" button and question the software about each recommendation and the platform provides its key findings to justify each decision. The pathologist thus remains in full control of the entire process and decision making and can override the software. SpIntellx first tested HistoMapr™-Breast, the initial/prototype xAI enabled software, to evaluate

Figure 13 - HistoMapr and TumorMapr Serve a Continuum of Solutions Needed in Precision Medicine. SpIntellx has developed powerful applications to analyze image data sets from any imaging platform and reagent type based on transmitted light images such as traditional H&E and IHC (HistoMapr) or from fluorescent or mass spectrometry-based labeling (TumorMapr). SpIntellx products are backed by a very strong intellectual property position in unbiased spatial analytics, unbiased and automated functional cell phenotyping, unbiased and automated microdomain discovery, spatial systems pathology, and xAI.

breast core biopsies and, in 2020, published a review describing the successful first application of xAI for anatomic pathology to analyze breast biopsies and assist pathologists[4].

TumorMapr represents a continuum with the HistoMapr™ system and, through a rich, interactive, and user-friendly interface, expands the platform beyond analyzing information obtained from transmitted light images. TumorMapr is capable of analyzing images from multi to hyperplexed fluorescence and/or mass spec biomarker datasets (scalable to any number of biomarkers). See the separate white paper on TumorMapr[1]. The HistoMapr and TumorMapr platforms can be used individually or in combination and are applicable to any solid tumor or tissue disease pathology. Thus, HistoMapr/TumorMapr can handle both current and archival datasets which are a combination of transmitted light datasets and multiplexed fluorescence/mass spec images. As a consequence, HistoMapr/TumorMapr represent an invaluable tool for clinical experts and an essential resource for health care providers. Adoption of HistoMapr/TumorMapr will greatly boost the efficiency of clinical experts to achieve better care of patients.

VI. HistoMapr Summary

HistoMapr is able to process and analyze images acquired by any existing traditional transmitted light imaging platform to generate extensive spatial data. HistoMapr takes a set of WSIs, TMA cores, or cropped images of tissue regions as input. Then, it robustly preprocesses the stain colors to minimize imaging or staining errors, recognizes the histological structures in an unsupervised manner, and automatically segments nuclei and other cytological components. Next, HistoMapr categorizes these components based on their cytomorphological properties, applies unbiased spatial analytics to discover spatially distinct configurations of cytological components as microdomains, and associates them with diagnostic recommendations. Finally, the xAI-enabled user interface supports interactions between the software and clinical experts, increases confidence on the recommendations proposed by the software and facilitates communication between experts in the field. Implementation of HistoMapr in both clinical and biopharma settings will bring innovations needed to improve the efficiency and accuracy of clinical workflows, biopharma discovery and development programs, and precision medicine including clinical trial support and diagnostics.

VII. HistoMapr Services and Contacts

HistoMapr is presently offered as a software as a service (SaaS), where users can access our interface online to upload their data and interact with the results. Raw transmitted light image datasets from any imaging platform can serve as the starting point for HistoMapr. Data are securely shared in the cloud and the processed and analyzed images are returned to the customer along with an xAI guide customized to the project.

Our initial product is designed for breast core biopsies, but the platform is readily applicable to other cancers. For example, melanoma and lung projects are underway, as well as additional diseases (e.g., autoimmune diseases, transplantation medicine, infectious diseases, organ specific diseases, etc.). SpIntellx is also entertaining requests to license the basic capabilities and annotation tools of HistoMapr to include on imaging platforms.

[4] Explainable AI (xAI) for Anatomic Pathology, Adv Anat Pathol: July 2020 - Vol 27(4) - p 241-250

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Glossary (in alphabetical order)

Black-Box AI– Any artificial intelligence system whose inputs and operations are not visible or explained to the user. Deep learning modeling is typically conducted through black box development and is difficult for data scientists and users to interpret. It requires blind trust in the algorithms.

Computational Pathology– Application of computing algorithms and AI, to pathology data to extract information. This includes both image data such as whole slide images, and non-image data such as patient demographics, clinical information, or pathologists' observations.

Chromogenic Multiplexed IHC– Relies on chemical reactions triggered by enzymes conjugated with antibodies. Liquid substrate reacts with these enzymes and forms a solid, color marking at the target site. It can be viewed through standard microscopy and imaged with transmitted light scanners.

Digital Pathology– A sub-field of pathology that focuses on data management based on information generated from digitized specimen slides. Using computer-based technology, digital pathology utilizes virtual microscopy. Glass slides are converted into digital slides that can be viewed, managed, shared, and analyzed on a monitor.

Explainable Artificial Intelligence (xAI)– xAI algorithms are programmed to describe its purpose, rationale and decision-making process in a way that can be understood by the end user. xAI plays an important role in the fairness, accountability, and transparency in machine learning.

Microdomains– A microdomain is a localized niche or microenvironment with distinct composition and spatial configuration of multiple cell populations within a tissue sample.

Multiplex to Hyperplex Labeling and Imaging– Use of either fluorescence or mass spectrometry-based biomarker labeling and imaging to detect from a few to several dozen (multiplex < 9 and hyperplex >= 9) targeted proteins and nucleic acids in tissue sections and/or tumor microarrays at subcellular resolution.

Pointwise Mutual Information (PMI)– Two-dimensional maps for relative co-occurrences and anti-associations of spatially distributed cell types in a tissue sample. A PMI map with strong diagonal entries and weak off-diagonal entries describes a tumor sample that is locally homogeneous but globally heterogeneous. A PMI map with strong off-diagonal entries describe a tumor with many localized interactions between different cell types, thus signifying a tumor exhibiting strong local heterogeneity. The ensemble of associations and anti-associations of varying intensities along or off the diagonal represents the true complexity of tumor images in a format that can be summarized and interrogated.

Spatial Analytics/ Spatial Intelligence– Refers to quantifying the spatial relationships between cells and tissue structures in whole slide images and /or tumor microarrays in an unbiased manner.

Tumor Microarray (TMA)– Tumor microarrays are core samples of tumors arrayed on a slide to allow the investigation of multiple samples per slide.

Whole Slide Imaging (WSI)– Refers to scanning of conventional glass slides in order to produce digital slides, is the most recent imaging modality being employed by pathology departments worldwide.