PathAI MASH H&E and PathAI MASH Trichrome Algorithms

Version 1.0

REF 2012400002

User Manual

RUO

NOTE: PathAI MASH H&E and PathAI MASH Trichrome Algorithms on the **navify®** Digital Pathology software are intended for Research Use Only. Not for use in diagnostic procedures. Access is intended for authorized users only, contact your institution for access approval.

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Supported Software

The PathAI MASH H&E and PathAI MASH Trichrome Algorithms run on **navify®** Digital Pathology software, Version 2.4.x. This PathAI user manual applies to PathAI MASH H&E and PathAI MASH Trichrome Algorithms for AI-Based Measurement of MASH Histology Version 1.0.x and later.

Cybersecurity Disclaimer

The maintenance and security of the local infrastructure — including the site's network, systems access, physical and other logical responsibility -- are all outside the scope of PathAI Inc.'s responsibility and are the responsibility of the site.

Contents

Chapter 1: Important Information

This chapter includes:

About This User Manual

This user manual provides details about the slide and scanner requirements, image analysis workflow, and the image analysis results generated by the PathAI MASH H&E and PathAI MASH Trichrome Algorithms. For instructions on scanning slides using the VENTANA DP 200 Slide Scanner or VENTANA DP 600 Slide Scanner, or using the features and supported workflows in the **navify®** Digital Pathology software, see the respective user manuals.

Symbol Definitions

The following table defines symbols that appear in this user manual:

Symbol	Definition
	Indicates the product is "For Research Use Only. Not for use in diagnostic procedures."
	Indicates the manufacturer's catalog number so the product can be identified.
	Indicates the need for the user to consult the Instructions for Use.
	Indicates the manufacturer of the product.

List of Abbreviations

The following table defines abbreviations used in the software or user manual.

Abbreviation	Definition
AI	Artificial Intelligence
CRN	Clinical Research Network
FFPE	Formalin-Fixed Paraffin-Embedded
H&E	Hematoxylin and Eosin
ID	Identifier
MPP	Microns Per-Pixel
MT	Masson's Trichrome Blue
MASLD	Metabolic Dysfunction-Associated Steatotic Liver Disease
MAS	MASLD Activity Score
MASH	Metabolic Dysfunction-Associated Steatohepatitis
RUO	Research Use Only
WSI	Whole Slide Image

List of Terms

The following table defines terms used in the product or user manual:

Term	Definition
Algorithm	The algorithm comprises the specific combination of AI models that produce the outputs the user(s) interact with on the platform.
Platform	The platform comprises the infrastructure that houses the algorithm, and enables various configurations of algorithm outputs per user preference.
Product	The product comprises the specific combination of the algorithm and the platform.
Pathologist	The Pathologist reviews the PathAI MASH H&E and PathAI MASH Trichrome Algorithms-derived outputs.

Determining Software Version

Within the platform you can go to **About** in the left navigation menu to access platform and algorithm related information, including software version (algorithm and platform) and current terms of use.

Contacting Roche Support

If you have questions pertaining to the appropriate scanning of images (VENTANA DP 200 Slide Scanner or VENTANA DP 600 Slide Scanner), or effective utilization of **navify®** Digital Pathology software, contact your local affiliate or Roche Service Representative and reference the following product information:

Product Names

The following product names are referenced throughout this user manual:

Product Names	Manufacturer
PathAI MASH H&E and PathAI MASH Trichrome Algorithms	PathAI, Inc.
navify® Digital Pathology software	Roche Diagnostics
VENTANA DP 200 Slide Scanner and VENTANA DP 600 Slide Scanner	Roche Diagnostics

Chapter 2: Introduction

This chapter includes:

Product Description

This product is intended for Research Use Only. Not for use in diagnostic procedures.

The PathAI MASH H&E and PathAI MASH Trichrome Algorithms, are software tools intended for computation of artificial intelligence (AI)-derived ordinal and continuous MASH Clinical Research Network (CRN) Grades and Stages of whole slide images (WSI) of formalin-fixed, paraffin-embedded (FFPE) liver biopsy tissue sections stained with H&E and Masson's Trichrome Blue and scanned on a VENTANA DP 200 Slide Scanner or VENTANA DP 600 Slide Scanner. PathAI MASH H&E and PathAI MASH Trichrome Algorithms results must be reviewed by a qualified pathologist before final reporting and are intended for research use only. Not for use in diagnostics procedures.

Algorithm	Description
PathAI MASH H&E	PathAI MASH H&E is a software tool intended for computation of artificial intelligence (AI)-derived ordinal and continuous MASH Clinical Research Network (CRN) MASLD Activity Score (MAS), Steatosis Grade, Hepatocellular Ballooning Grade, and Lobular Inflammation Grade of formalin-fixed, paraffin-embedded (FFPE) liver biopsy tissue sections stained with H&E and scanned on a VENTANA DP 200 Slide Scanner or VENTANA DP 600 Slide Scanner. PathAI MASH H&E results must be reviewed by a qualified pathologist before final reporting and are intended for research use only. Not for use in diagnostics procedures.
PathAI MASH Trichrome	PathAI MASH Trichrome is a software tool intended for computation of artificial intelligence (AI)-derived ordinal and continuous MASH Clinical Research Network (CRN) Fibrosis Stage of formalin-fixed, paraffin-embedded (FFPE) liver biopsy tissue sections stained with Masson’s Trichrome Blue and scanned on a VENTANA DP 200 Slide Scanner or VENTANA DP 600 Slide Scanner. PathAI MASH Trichrome results must be reviewed by a qualified pathologist before final reporting and are intended for research use only. Not for use in diagnostics procedures.

Algorithm Workflows

WARNING: Always inspect the quality of the WSI generated by the appropriate scanner to ensure adequate image quality in accordance with your local laboratory procedures.

Step	Description
1	Create a case in **navify®** Digital Pathology software either manually in Data Management or automatically through communication with the laboratory information system. For instructions, see the Roche **navify®** Digital Pathology software user manual. **NOTE:** The platform processes the WSIs for viewing in the slide viewer and the PathAI MASH Algorithms selected for each slide (PathAI MASH H&E for H&E WSIs and PathAI MASH Trichrome for Masson's Trichrome Blue WSIs) are triggered to run. A. Prepare the H&E-stained and Masson's Trichrome Blue-stained slides and scan the slides. Review the slide quality in accordance with standard laboratory procedures. B. Scan the slide with the VENTANA DP 200 Slide Scanner or VENTANA DP 600 Slide Scanner in .bif file format at 20x or 40x magnification and at one z-plane. Review the slide quality in accordance with the scanner manufacturer's Instructions for Use and standard laboratory procedures. The VENTANA DP 200 Slide Scanner and VENTANA DP 600 Slide Scanner transfers the slide image to the image management system.
2	Review WSIs of H&E and Trichrome to identify any areas of non-liver tissue that may be present in the WSI. If there are regions to be excluded, perform the following steps: A. Select Re-Analyze with ROIs. B. Manually draw regions on the WSI to exclude from the analysis. C. Submit for re-scoring.
3	Review the test results after the algorithms run: A. Open the SLIDE PANEL in the Viewer to review the algorithm MASH CRN grade or stage results. B. Review the test results (overlays and scores).
4	Click **Preview** and **Sign-Out**.

About Algorithm Limitations

PathAI MASH H&E and PathAI MASH Trichrome Algorithms analyze WSIs of liver biopsy tissue stained with H&E or Masson's Trichrome Blue, respectively.

Guidelines for Ensuring Optimal Stain Quality

To optimize stain quality, use the following guidelines:

- For each staining run, follow the manufacturer’s recommendations for each applicable assay using all the positive and negative quality control materials.
- Validate the applicable assay staining run by reviewing the control slides manually using conventional light microscopy to ensure the stain quality of the slide before generating a whole slide image with the slide scanner.
- If you determine the quality of the control slides is not acceptable during your manual examination, re-stain the slide and review the quality again using conventional light microscopy. For recommendations, see the manufacturer’s assay documentation.

Guidelines for Understanding Algorithm Limitations

When reviewing algorithm results, note the following algorithm limitations:

- The algorithm results are only as good as the quality of the stain and the quality of the subsequent WSI that is analyzed.
- The algorithm may generate incorrect scores if the slide was improperly scanned or has:
  - Significant artifacts obscuring relevant tissue areas; or
  - Abnormal staining.

These factors may result in the algorithm misidentifying liver tissue features that are relevant to MASH CRN grading and staging.

Criteria for Images and Scanner

PathAI MASH H&E requires one H&E-stained WSI and PathAI MASH Trichrome requires one MT-stained WSI as input. The algorithms accept WSIs in .bif format created by a VENTANA DP 200 Slide Scanner or VENTANA DP 600 Slide Scanner at 20x or 40x magnification.

Quality Assurance Checks for Images and Scanner

WARNING: Always inspect the quality of the WSI generated by the appropriate scanner to ensure adequate image quality in accordance with your local laboratory procedures.

NOTE: Results have been tested for slides scanned on the VENTANA DP 200 Slide Scanner and the VENTANA DP 600 Slide Scanner.

NOTE: The PathAI MASH H&E and PathAI MASH Trichrome Algorithms have only been verified on WSIs generated from 40x magnification scans.

For quality assurance, the PathAI MASH H&E and PathAI MASH Trichrome Algorithms verify that each WSI within a sample meets specific criteria for scanner type, file type, and magnification. If the software detects incompatibilities, it fails to run.

Chapter 3: Algorithm Results Overview

This chapter includes:

Viewing Algorithm Overlays

After the software completes an algorithm run, it produces overlays on the WSIs that appear in the OVERLAY PANEL of the VIEWER. These overlays highlight specific tissue regions detected by the algorithm. Each overlay is represented by a different color. You can view the definitions of each color and toggle them on and off from the OVERLAY PANEL (Figure 1).

Figure 1 – Overlay Panel

Color Legend

The overlays that appear on analyzed WSIs highlight specific tissue regions detected by the algorithms. The following tables define each color:

PathAI MASH H&E and PathAI MASH Trichrome Algorithms Artifact Overlay

Color		Definition
	Grey	Artifact Region

PathAI MASH H&E Tissue Overlays

Color		Definition
	Yellow	Steatosis
	Light blue	Hepatocellular Ballooning
	Green	Lobular Inflammation

PathAI MASH Trichrome Tissue Overlays

Color		Definition
	Orange	Fibrosis

Viewing Algorithm Results

After the PathAI MASH H&E and PathAI MASH Trichrome Algorithms run, you can view the quantitative scoring results in the VIEWER's SLIDE PANEL. To open this panel, click VIEWER and select SLIDE PANEL and click on the hamburger menu (**1**) to see additional SLIDE SCORE results (**2**) (Figure 2).

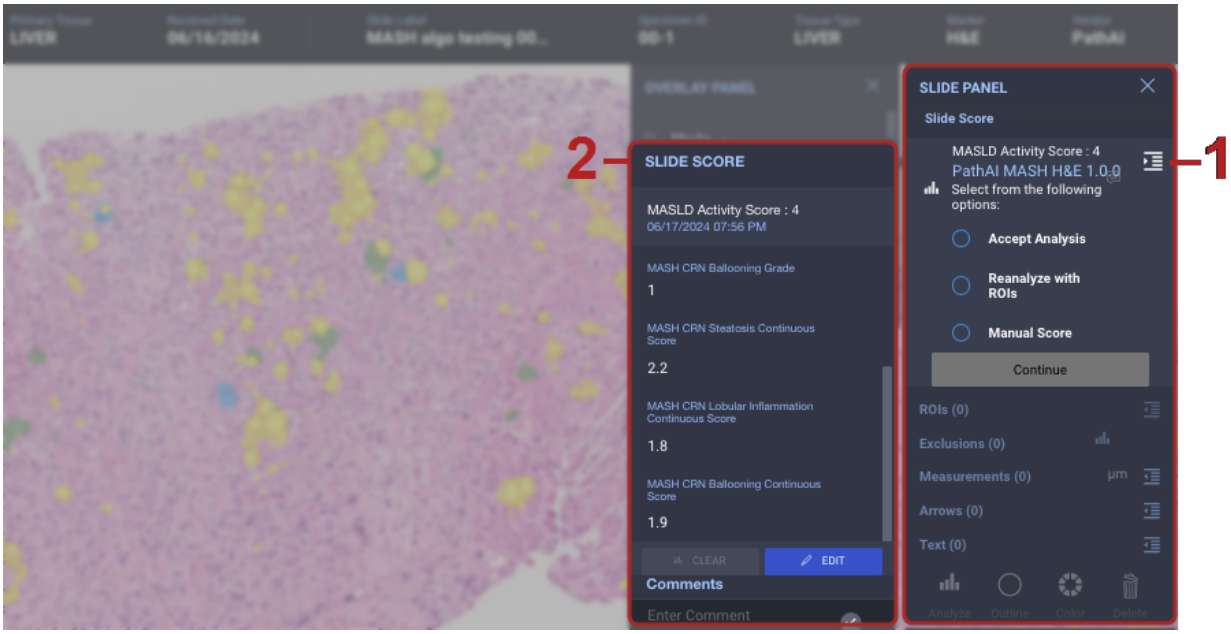

Figure 2 – Slide Panel and Slide Scores

PathAI MASH H&E Results

Results	Description
MASLD Activity Score	The MASLD Activity Score is the unweighted sum of the AI-derived ordinal grades for steatosis, lobular inflammation, and ballooning. The value will be a whole number ranging from 0 to 8. **NOTE:** If the pathologist edits any of the below Grades, they must also recalculate and update the MASLD Activity Score.
MASH CRN Steatosis Grade	The AI-derived MASH CRN Steatosis Grade computed from tissue in non-Artifact regions of the WSI. The value will be a whole number ranging from 0 to 3.
MASH CRN Lobular Inflammation Grade	The AI-derived MASH CRN Inflammation Grade computed from tissue in non-Artifact regions of the WSI. The value will be a whole number ranging from 0 to 3.
MASH CRN Ballooning Grade	The AI-derived MASH CRN Hepatocellular Ballooning Grade computed from tissue in non-Artifact regions of the WSI. The value will be a whole number ranging from 0 to 2.

Results	Description
MASH CRN Steatosis Continuous Score	The AI-derived MASH CRN Steatosis Continuous Score computed from tissue in non-Artifact regions of the WSI. The value will be a number ranging from 0 to 4.
MASH CRN Lobular Inflammation Continuous Score	The AI-derived MASH CRN Lobular Inflammation Continuous Score computed from tissue in non-Artifact regions of the WSI. The value will be a number ranging from 0 to 4.
MASH CRN Ballooning Continuous Score	The AI-derived MASH CRN Ballooning Continuous Score computed from tissue in non-Artifact regions of the WSI. The value will be a number ranging from 0 to 3.

Trichrome Results

Results	Description
MASH CRN Fibrosis Stage	The AI-derived MASH CRN Fibrosis Stage computed from tissue in non-Artifact regions of the WSI. The value will be a whole number ranging from 0 to 4.
MASH CRN Fibrosis Continuous Score	The AI-derived MASH CRN Fibrosis Continuous Score computed from tissue in non-Artifact regions of the WSI. The value will be a number ranging from from 0 to 5.

About MASH CRN Scores

The MASH CRN recommends assessing MASH disease activity and fibrosis through grading of Steatosis, Ballooning, and Lobular Inflammation, and staging of Fibrosis. The MASH CRN designed these scales[1] to serve as semi-quantitative systems that could be used to assess MASH disease severity at a single time point. The grading/staging system they proposed is described in the table below.

Assessment	Definition	Score
Steatosis	< 5%	0
	5%-33%	1
	> 33%-66%	2
	>66%	3
Lobular Inflammation	No foci	0
	< 2 foci per 200 x field	1
	2-4 foci per 200 x field	2
	> 4 foci per 200 x field	3

Assessment	Definition	Score
Hepatocytic Ballooning	None	0
	Few balloon cells	1
	Many cells / prominent ballooning	2
CRN Fibrosis Stage	None	0
	Mild to Moderate, zone 3 perisinusoidal or periportal sinusoidal without accompanying zone 3	1
	Zone 3 perisinusoidal and portal/periportal	2
	Bridging fibrosis	3
	Cirrhosis	4

1· Kleiner DE, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology. 2005;41(6):1313-1321. doi:10.1002/hep1ajssessment

Modifying Results with Manual Scoring

To provide a Manual Score for any or all of the AI-derived Grades or Stage, you must first select Manual Score (**1**). This will automatically pop out the SLIDE SCORE window with ability to edit any of the ordinal grades and stage as well as the MASLD Activity Score (Figure 3).

NOTE: If you modify any of the PathAI MASH H&E outputs, you must also manually recalculate and update the MASLD Activity Score.

After modifying any Grades or Stage, you must click CONFIRM (**2**) for results to be updated and saved in the report. By entering manual ordinal Grades or Stage, the connection to the continuous score is broken. Therefore, continuous scores will disappear if any Grades or Stage is modified as these are related to the AI-derived ordinal Grades and Stage.

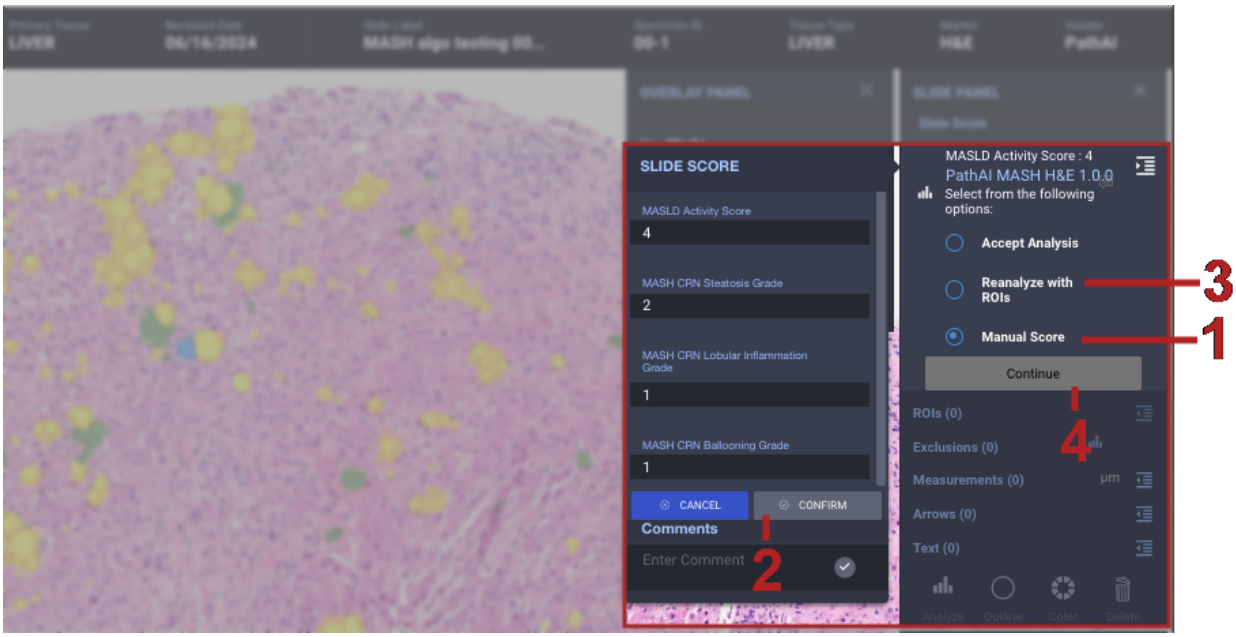

Figure 3 – SLIDE SCORE After Selecting Manual Score

To remove any control or extraneous tissue from PathAI MASH Algorithms' analyses, select Reanalyze with ROIs (**3**) and Continue (**4**) (Figure 3). After which you will prompted to confirm and will have the ability to add Exclusion regions of interest (ROIs) for secondary analysis. After drawing exclusions ROI(s) click Analyze (**1**) to submit slide for secondary analysis by the algorithm (Figure 4).

NOTE: Exclusion regions of interest need to be added separately for each algorithm and therefore on each WSI.

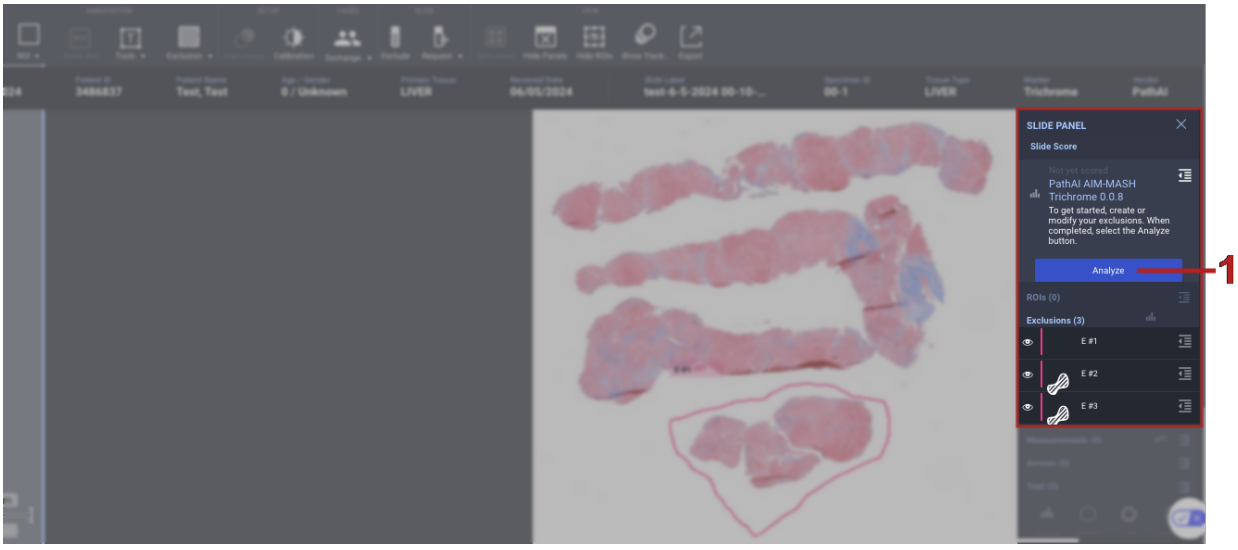

Figure 4 – Slide Score Analyze to Reanalyze with ROI

Chapter 4: Generating Reports

This chapter includes:

About Generating Reports

After an algorithm run, you can generate reports containing summary details about each case. The type of data included in the report is based on templates configured by the administrator. For details, see Producing and signing out a report in the Roche **navify®** Digital Pathology software user manual.

In instances where multiple slides are analyzed for a single case (e.g. multiple H&E slides analyzed by PathAI MASH H&E), all slides and their results are included in the report. To indicate which slide's analysis is desired as the final results, click on REPORT in the upper right corner after accepting or manually modifying results for all slides. Under the Individual Slide Section, indicate in the "Slide Notes" section for the slide whose analysis is the desired as the final results with the comment "FINAL RESULTS". The comment in the "Slide Notes" is automatically saved.

> **NOTE:** You can preset "FINAL RESULTS" to be used as a "Slide Notes" comment across cases by clicking "Preset comments" in the REPORT underneath "Slide Notes" followed by "Create a new preset comment". You can then add a preset comment to the "Slide Notes" by clicking "Preset comments" and selecting your preset comment followed by the "CHOOSE" button.

Understanding the Report

The report includes a summary of all the scoring results described in Chapter 3.

Chapter 5: Troubleshooting

This chapter includes:

Re-triggering a Failed Analysis

NOTE: You must have Administrator privileges to perform this troubleshooting task.

If an error occurs during the whole slide analysis, an error message appears under the slide. To address the failure, perform the following steps:

1. Login to **navify®** Digital Pathology software.
2. Navigate to Administer Settings and choose Job Queue.
3. Choose the **Failed** tab, all slides with failed analysis are displayed.
4. Choose the slide to be analyzed the choose the **Start Job** button. The slide moves to **Canceled/Completed** tab when done.

NOTE: Administrator settings are only available if you have Administrator privileges.

Starting an Untriggered Analysis for Reanalyze with ROI

After drawing exclusion region(s) and clicking Analyze (Figure 5), it may be necessary to click Analyze again in order to trigger secondary analysis. To determine if you need to click Analyze again. Zoom out. If you see a banner at the bottom of the slide that says **"Pending User Action**" and the option to click **Analyze** is still available, click **Analyze** again and **CONFIRM**. Repeat until the banner at the bottom of the slide updates to "**Waiting to start auto-analysis...**".

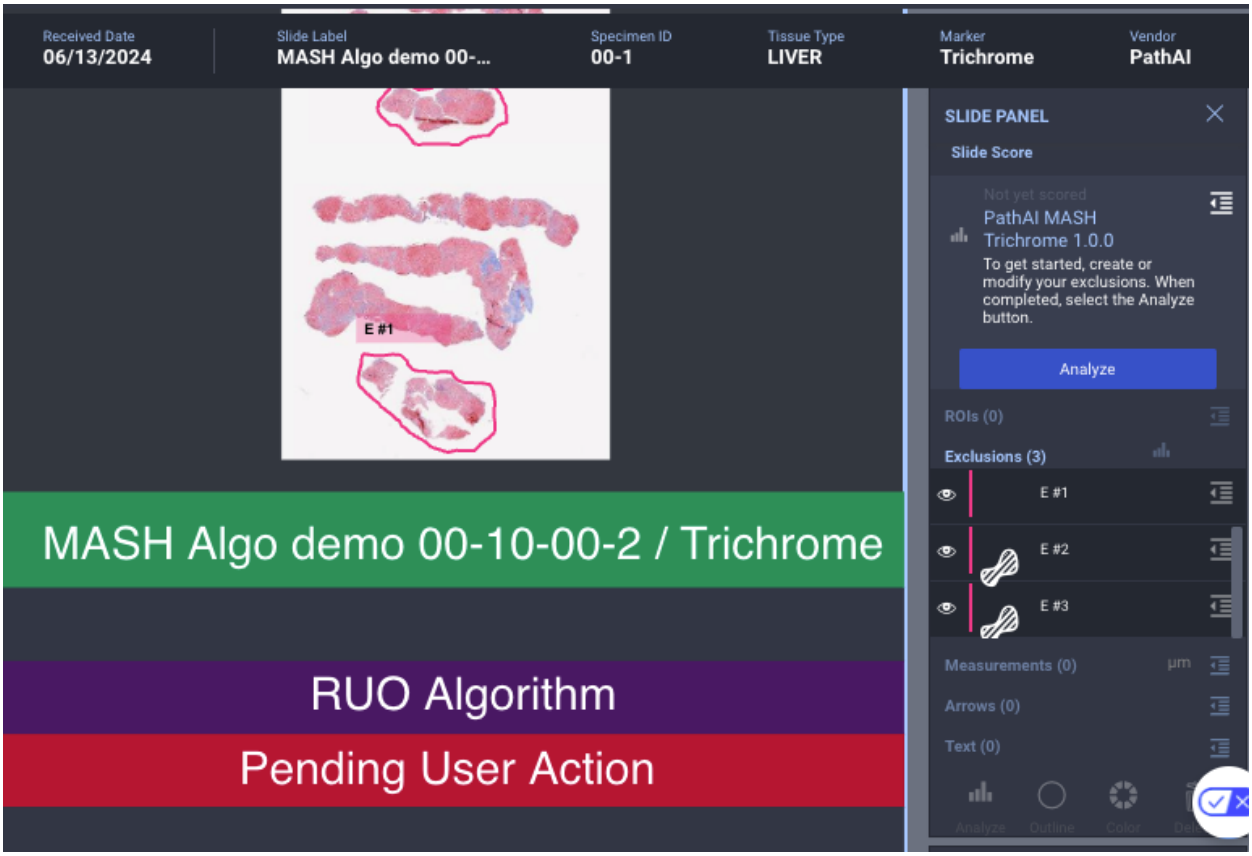

Figure 5 – Starting an Untriggered Analysis

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