Supplementary Contents

Section 1: A!magQC

With the Increasing development of Digital Pathology, a large amount of data is generated every day. There are some common quality issues (as shown in Supplementary Figure 1A) and the visual quality control of those images as become a tedious and heavy workload for researchers. The assessment of quality of an image is an active field of research; a perceptual image quality estimator calculates the no-reference image quality score for an image using a perception-based image quality evaluator (PIQE). However, this approach reveals to be more efficient on natural image compared to Histological images. Some tools have been developed for Histological images previously but restrict the "Quality evaluation" to only Hematoxylin &Eosin (H&E) images.

Therefore, A!magQC was developed to provide fully automatic quality control for any histologically relevant imaging modality (Haematoxylin and Eosin (H&E), ImmunoHistochemistry (IHC) and Multiplexed Fluorescence (MF)). The software automatically detects the image size (magnification) and type (H&E or MF) from the metadata of each image files. The interface is shown in Supplementary Figure 1B.

The First step in assessing the quality of the tissue Image is to detect the tissue and separate it from the background. To do so, an adaptive thresholding is applied. We approach the quality of the tissue at a local level using a parallel analysis of tiles of 256*256 pixels throughout the Region of Interest (i.e., tissue detected). Five relevant features are identified to differentiate local quality in whole slide images, as shown in Supplementary Figure 1C.

- Focus: We quantify the focus in an image using the Variance of the Laplacian Transform. The Laplacian operator measure the second derivative of an image. Therefore, it highlights regions of an image with sharp intensity changes. High variance of intensity change (i.e., sharp and smooth changes) is representative of a normal, in-focus image. On the contrary, Low variance indicates an image with few sharp edges, typically an out of focus image.

- Contrast: We quantify the Contrast by measuring the Difference of the Top 1% of high values positive pixels and the Bottom 1% of low values positive pixels in each tile. The range must be high enough for good separation of nuclei signal and background.
- Saturation: We measure the percentage of pixels that have an intensity max value (i.e., digital image pixels are 8 bits unsigned integer; hence, the max intensity value is 255.)
- Artifacts: The main structure of interest in Histology images is usually the nuclei. The morphological open operation is an erosion followed by a dilation, using the same structuring element for both operations. We perform an image opening using a structuring element or kernel, bigger than the average nuclei size will highlights dirt and blurry objects that sometimes are contained in the images.
- Texture Uniformity: Computing the GLCM (the Gray Level Co-occurrence Matrix calculates how often a pixel with gray-level (grayscale intensity) value “i” occurs horizontally adjacent to a pixel with the value “j”). Measuring the Uniformity of the pixels allows us to highlight region with different density of nuclei. Notably visceral fat tissue surrounding organs of interest often have a very different texture.

The pipeline was designed on multiplexed fluorescence images, directly applying the algorithm to grayscale image of DAPI fluorescence signal. The H&E images are converted to optical density (OD) using a logarithmic transformation before analysis.

Section 2: A!HistoNotes

AI-driven computational pathology diagnosis is an emerging but rapidly developing field. It uses computational algorithms to classify cancer and other diseases, based on the annotated images. Annotating pathological images requires experienced pathologists with years of training. A high-quality annotated image database is the basis for developing AI-based diagnostic solutions, because most successful models are derived from supervised learning. It is important to mark and annotate specific areas/structures/features to describe a disease at the cellular level, and then build and validate the models. Currently, there is no effective "medium" to transfer a pathologist's knowledge and experience to a machine. A!HistoNotes is a cloud-based structural annotation platform designed to enable pathologists to address this gap.

The image viewer (See Supplementary Figure 2A) based on the openseadragon software library can visualize DP images with high resolution of 40x objective lens, load image blocks quickly and smoothly, without consuming a lot of device memory and Internet data The most important basic event functions, such as "zoom", "pan" and "home page", can all be customized using its application programming interface (API) as illustrated in See Supplementary Figure 2B-C.

Annotation tool is one of the basic components of A!HistoNotes. The annotation tool provides a region of interest (ROI) management system and has the ability to create an adjustable ROI on top of the image viewer. In other words, when the entire image is moved by the user, the ROI adheres to a specific area on the image. Once the ROI shape is released, it can be fine-tuned. ROI management system refers to a way to easily manipulate and manage many ROIs on the viewer.

A!HistoNotes provides three ROI drawing methods for annotating task in the viewer, which can be found in the toolbox button as illustrated in Supplementary Figure 2D. They are the freehand drawing, polygonal-dot drawing and brush drawing shown in Supplementary Figure 2E. When selecting ROI for further operation, user can click the right-click menu panel. They include labelling, copying, attribute updating and deleting operation. When user click "More label", ROI can be renamed (default label is “unknown”), and a tag window dialog box will appear for naming choices, as shown in Supplementary Figure 2F. Multiple ROI selection is one of the great features love to be used by our pathologist (See Supplementary Figure 2G). They first draw many ROIs and then label them at once, which is very helpful for them to save a lot of valuable annotation time.

When point the mouse at them, user can easily identify the ROI and related information on the ROI panel as demonstrated in Supplementary Figure 2H. In addition, selected ROI can be hided and showed easily by click on the "eye" icon at the ROI panel as shown in Supplementary Figure 2I. This is a particularly useful feature that allows pathologists to draw ROIs of tissues that may be obscured by another large ROI.

For AI-assisted diagnosis and semi-automatic annotation, the outputs generated the AI model can be converted into ROI in A!HistoNotes. Therefore, Pathologists can view and modify the ROI annotations using the A!HistoNotes image viewer, and fine-tune accordingly.

Besides, the time spent for annotation is an important measure to understand how easy it is for the pathologist to annotate the entire image slice. They are evidence showing the time spent by pathologists on fully manual annotations and time spent on some fine-tuning (semi-automatic annotations) of the ROI generated based on the AI model. Therefore, A!HistoNotes will automatically record the time spent to draw in each ROI (See Supplementary Figure 2B) for performance evaluation.

Section 3: Evaluation Metrics of AI model Performance on image dataset

On the first level of evaluation, we measured AI model performance on prostatectomy and biopsy images. A standard set of metrics to evaluate the correspondence between model predictions and ground truth, which we defined as the pathologists' annotations for representative regions. For each annotated instance, its corresponding prediction is the dominant class (i.e., the most common predicted class) within the annotated region.

We evaluated prostatectomy samples and core needle biopsy samples differently according to the applications. For Gleason grading on prostatectomy specimens, sensitivity and F1 scores of each category, including GP3, GP4, GP5, and benign (stroma and normal) were calculated for multi-class comparison to quantify the performance. For tumor detection on core needle biopsy samples, we simplified the comparison by further grouping GP3, GP4, and GP5 as "malignant" segments. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) are used for binary comparison. Sensitivity, specificity, PPV, NPV and F1 Score are defined as:

$$\text{Sensitivity (or recall)} = \frac{TP}{TP+FN} \qquad \text{(Eq.1)}$$

$$\text{Specificity} = \frac{TN}{TN+FP} \qquad \text{(Eq.2)}$$

$$\text{PPV (or precision)} = \frac{TP}{TP+FP} \qquad \text{(Eq.3)}$$

$$\text{NPV} = \frac{TN}{TN+FN} \qquad \text{(Eq.4)}$$

$$\text{F1 Score} = \frac{2 * precision * recall}{precision + recall} \qquad \text{(Eq.5)}$$

Section 4: Clinical Validation of AI-assisted diagnosis

Although the AI model performs well on the image data set, further validation is required to examine whether it can assist pathologists in real-world application. In our study, the three-phase experiment aimed at comparing microscopic examination, WSI examination with and without AI assistance, in terms of accuracy, efficiency, and consistency of Gleason Grading. At each phase, pathologists examined 19 slides from testing set separately to determine the Gleason Score and the time they spent for each slide. The WSIs were scanned by Akoya Biosciences Vectra Polaris scanners at 20× magnification (0.5 μm/pixel). In phase 3, AI-assistance were provided, which includes, pseudo annotation, tumour percentage, Gleason Pattern percentage and Gleason Score, generated by the AI model.

The diagnostic Gleason Score retrieved from hospital's database is considered as ground truth. It should be noted that this Gleason Score was calculated by summarizing multiple slides from a patient and might not reflect the actual Gleason Score of the selected slide used in our study. When calculating the accuracy and ICC, Gleason Score were converted to Gleason Grade Group, proposed by International Society of Urological Pathology in 2014, to simplify the analysis. Grade Group 1: Gleason Score$\leq$ 6, Grade Group 2: Gleason Score 7(3+4), Grade Group 3: Gleason Score 7(4+3), Grade Group 4: Gleason Score 8, Grade Group 5: Gleason Score 9 or 10.

Supplementary Figure 5 shows the details of each phase. Only three pathologists from Singapore participated in phase 1 as the glass slides can't be shipped to China. User guide and training were provided before the experiment to ensure that all the participants know how to use A!HistoNotes. There was a washout period of 20 days (at least) between each phase. In phase 2 and 3, the filename of the WSI were randomly generated, respectively.

Section 5: Pathologist-AI Interaction via A!HistoNotes

Initially, in the process of manual annotation, pathologists annotated the WSIs from scratch, and we used the annotated data to train multiple AI models and the optimal model is selected for further analysis. This model is called base model and it is the baseline for future development. We expect more data will be collected from hospitals to enlarge the annotation database and further improve the model, but manual annotation is time-consuming and tedious for pathologists. Furthermore, it is inefficient to retrain the model every time when new data is collected. Therefore, we designed Pathologist-AI interaction through semi-automatic annotations via A!HistoNotes and applying the concept of incremental learning to update the trained model.

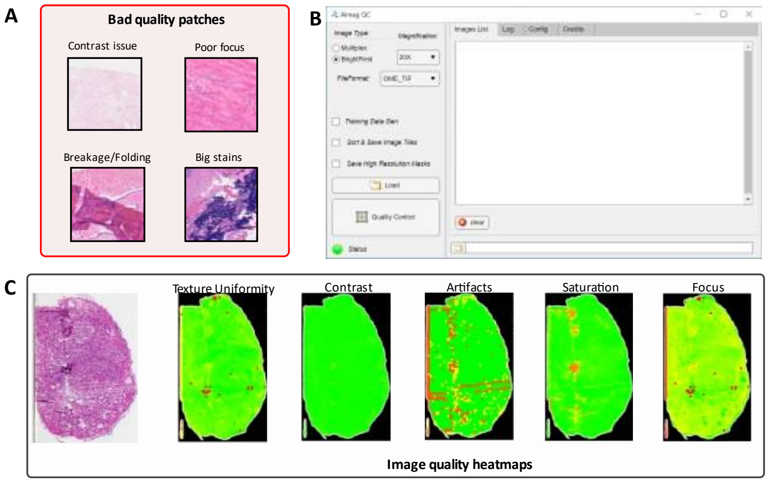
Semi-automatic annotation was performed to manually correct pseudo annotations generated by the base model as not all of them are accurate. In this study, the base model was applied to 39 slides randomly selected from the testing set to generate pseudo annotations while the rest of the 17 slides were still remain as testing images. Two pathologists from China and two from Singapore used A!HistoNotes to delete the pseudo annotations that they don't agree and applied the A!HistoNotes time-logging feature to automatically record the time they spent evaluating and correcting each image. We have recorded the time of semi-automatic annotation for 28 slides, and the fully manual annotation time of 20 among these 28 slides were also recorded.

Finally, we applied these corrected annotations to further train and update the base model using Incremental learning approach, meaning that the model learned from the new data directly based on the based model. Specifically, we froze the top layer of the model and only updated the weights of the bottom layer during training. After training, the base model and updated model were applied to the 17 test images, respectively, to compare their performances of distinguishing between malignant and benign tissues.

Supplementary Figure

Supplementary Table 1 Patient characteristic
(one patient's information is missing)

Age	Number	Percentage
45–50	1	0.5%
51–60	23	10.7%
61–70	132	61.7%
71–80	49	22.9%
81-90	9	4.2%
Gleason Score		
3+3	13	6.1%
3+4	82	38.3%
4+3	58	27.1%
4+4	4	1.9%
3+5	5	2.3%
5+3	2	0.9%
4+5	35	16.4%
5+4	8	3.7%
5+5	7	3.3%



Supplementary Figure 1 A!magQC

Supplementary Figure 2 A!HistoNotes

Supplementary Table 2 Pseudo code of train-test splitting

Algorithm 1: Stochastic Search for balanced dataset

Data:
$\mathcal{D} = \{(X_i, Y_i)\}_{i=1,\ldots,187} \leftarrow$ dataset with 187 images,
$X_i \leftarrow$ radical prostatectomy specimens image,
$Y_i \leftarrow$ collection of annotations on patch-level,
$C = \{G3, G4, G5, Stroma, Normal\} \leftarrow$ class label sets
Output:
$\mathcal{D}_{train} \leftarrow$ training dataset, 70% of $\mathcal{D}$
$\mathcal{D}_{test} \leftarrow$ testing dataset, 30% of $\mathcal{D}$

FOUND=False
while *not FOUND* **do**
    Step A:
    Random shuffle the $\mathcal{D}$, let
    $\mathcal{D}_{train} \leftarrow \{(X_j, Y_j)\}_{j=1,\ldots,132}$
    $\mathcal{D}_{test} \leftarrow \{(X_k, Y_k)\}_{k=133,\ldots,187}$
    Step B:
    Calculate the number of each class on patch-level in training dataset and test dataset respectively. i.e.,
    $N_{train}^{G3}, N_{train}^{G4}, N_{train}^{G5}, N_{train}^{Stroma}, N_{train}^{Normal}$
    $N_{test}^{G3}, N_{test}^{G4}, N_{test}^{G5}, N_{test}^{Stroma}, N_{test}^{Normal}$
    Step C:
    `// check the ratio (`$\mathcal{D}_{train}/\mathcal{D}$`) is` $70\,\% \pm 5\,\%$
    for *class in C* **do**
        $Ratio^{class} = N_{train}^{class} / (N_{train}^{class} + N_{test}^{class})$
        if $65\% \leq Ratio^{class} \leq 75\%$ **then**
            FOUND=True
        else
            FOUND=False
            Break
    end
end

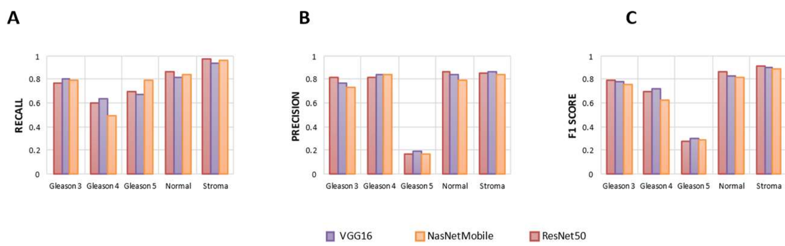
Supplementary Table 3 Pseudo code of Voting algorithm

Algorithm 1: Voting algorithm for each overlapped patch

Input: $\mathcal{D} = \{(C_i, S_i)_{i=1,\dots,4} : C_i \in \{G3, G4, G5, Stroma, Normal\},$ $S_i \in (0, 1)$, a collection of label C_i and score S_i for each overlapping patch.

Output: The label C and score S for each overlapped patch.

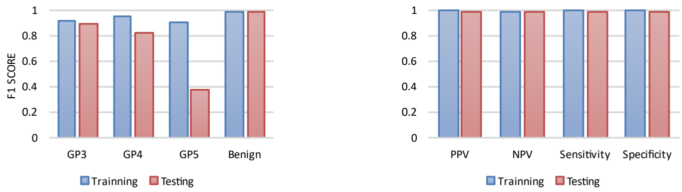
if $|Set\{C_1, C_2, C_3, C_4\}| = 4$ **then**
  Class: $C = \underset{C}{\operatorname{argmax}} \{S_i : (C_i, S_i) \in \mathcal{D}\}$
  Score: $S = S_i$
else if $most\ frequent\ class\ C \in \mathcal{D}$ **then**
  Class: $C = C$
  Score: $S = mean(S|most\ frequent\ C)$
else
  Class: $C = \underset{C}{\operatorname{argmax}} \mathbb{E}_{S \in \mathcal{D}}(S|C_i)$
  Score: $S = mean(S|C)$



Supplementary Figure 3 Model performance on image patch level (test set)

Supplementary Table 4 Configuration of color augmentation. All values are subject to ±5% variance. R, G, B values are first adjusted by addiction/subtraction, then rescaled to [0 1], followed by clipping, in which values below *Low_in* are mapped to 0 and values above *High_in* map to 1. *Low_in* and *High_in* values apply to all R, G, B channels

Configuration	**R value**	**G value**	**B value**	**Low_in**	**High_in**
1	-60	-50	-20	0.05	0.95
2	-30	-45	-60	0.05	0.95
3	+35	+70	+35	0.1	0.9



Supplementary Figure 4 Model performance (high resolution model) on training (131 WSIs) and testing set (56 WSIs).

	Hardware	Software	Samples	Participants
Step 1	routinely-used microscopes 	NA	19 glass slides 	Dr Hue Swee Shan Susa Dr Lau Kah Weng Dr Tan Char Loo
Step 2	Computers 	A!HistoNotes	19 digital slides 	Dr Hue Swee Shan Susan Dr Lau Kah Weng Dr Tan Char Loo Dr Chongchong Zhang Dr Yonghui Zhang
Step 3	Computers 	A!HistoNotes	19 digital slides, each comes with predicted annotations 	Dr Hue Swee Shan Susan Dr Lau Kah Weng Dr Tan Char Loo Dr Chongchong Zhang Dr Yonghui Zhang

Supplementary Figure 5 Setup of the 3-phase study

A!magQC

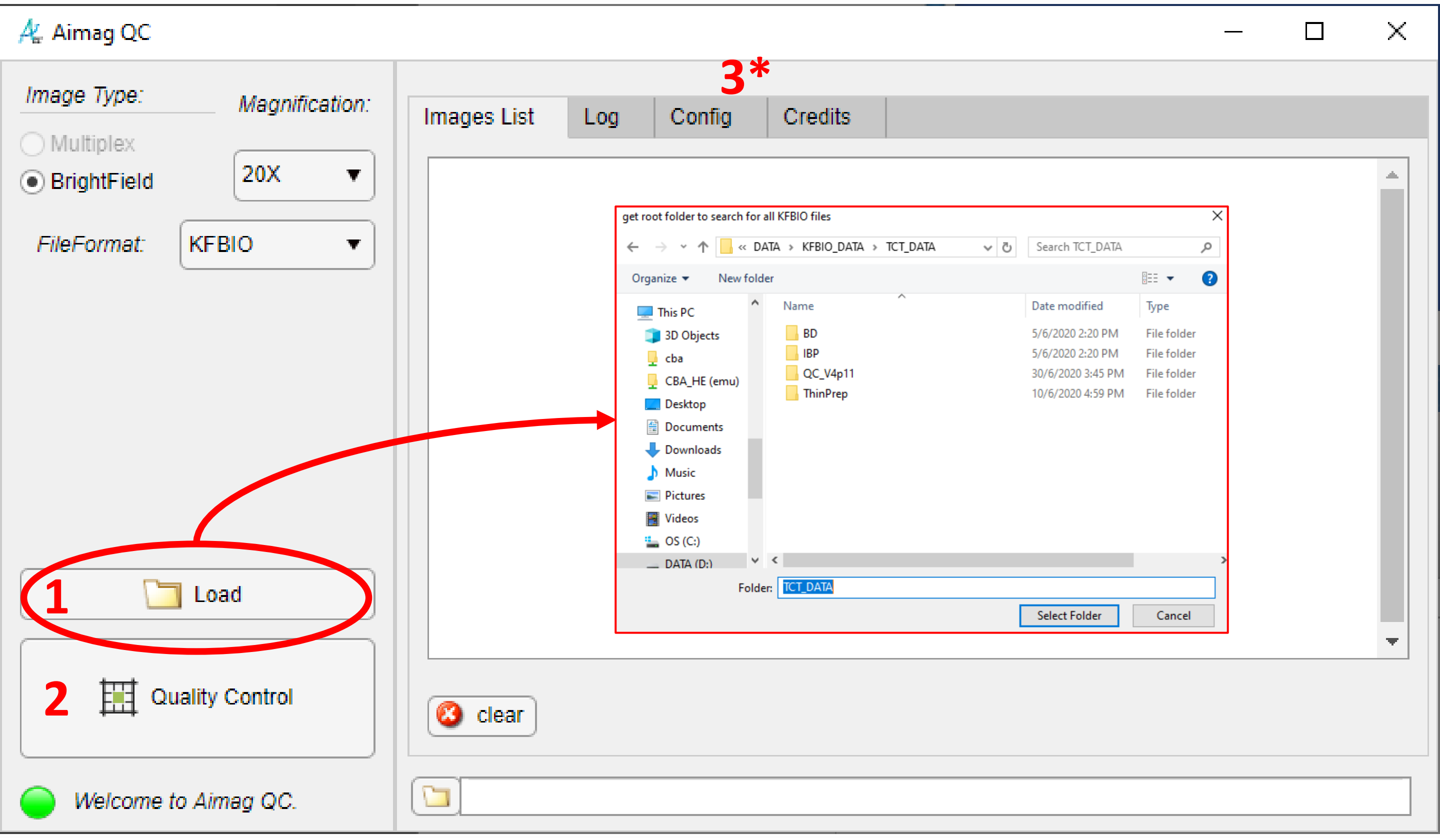
Digital Histopathology Images Quality Control.

Version 4.1.

I. Quick Start

II. Outputs

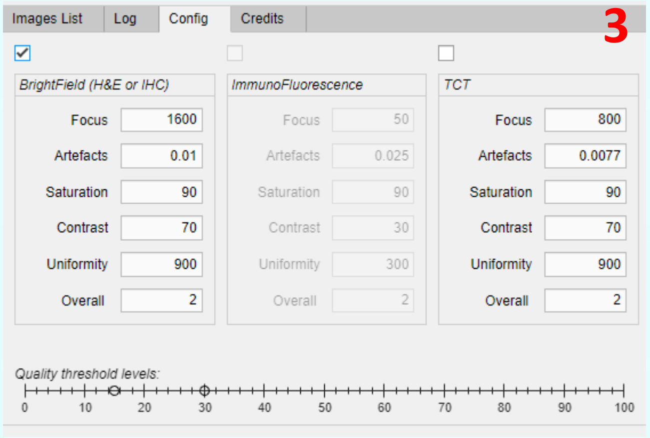
Quick Start



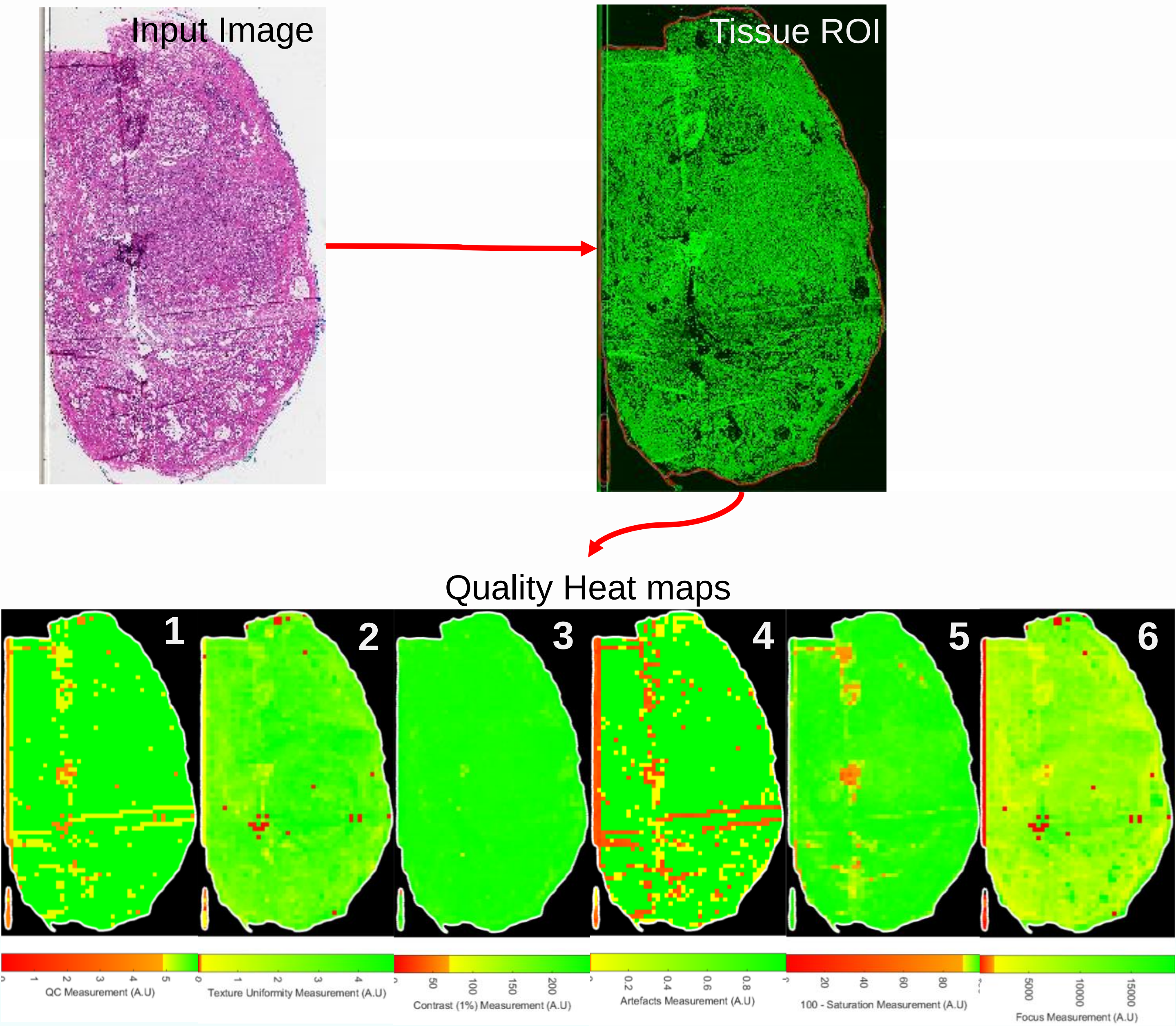
1. Press Load and Select a Root Folder Containing a List of Images or a List of Subfolders with Images. (Detects all ".kfb" files in folder and subfolders.)

2. Press Quality Control button to Start the Quality Assessment of all Files in Images List Listbox.

3. Select BrightField or TCT



Outputs



Visual representation of the Quality Assessment of the image Texture , Contrast, Artefacts, Saturation and Focus (2-6).

The QC measurement (1) is a linear combination of the 5 aforementioned image properties.

Output

Summary of quality measurements

	A	B	C	D	E	F	G
1	Image_Name	Image_Root	Image_Type	Image_Magnification	Low_Focus_Pct	Artifacts_Pct	Low_Contrast_Pct
2	26216_17_A15	10/1/2020	BrightField	20X	7.137817123	4.827637317	1.402305558
3	78822020-01-02_15_55_14	2/1/2020	BrightField	20X	3.347296323	10.64727702	1.541237303
4	81642020-01-02_15_51_19	2/1/2020	BrightField	20X	4.078844568	12.89785808	0.828570829
5	994_16_A20_correct2020-01-10_13_45_03	10/1/2020	BrightField	20X	3.354540448	12.25763029	0.127684641
6	A1_1_2020-02-18_11_09_26	SBIC_HE_20X_20200218_KFB	BrightField	20X	0	3.058589909	0.122626122
7	A1_2_2020-02-18_11_08_31	SBIC_HE_20X_20200218_KFB	BrightField	20X	0	4.32496957	0
8	A1_3_2020-02-18_11_07_24	SBIC_HE_20X_20200218_KFB	BrightField	20X	0	3.941122176	0.036573298
9	A1_4_2020-02-18_11_06_40	SBIC_HE_20X_20200218_KFB	BrightField	20X	1.080984126	3.97349304	0
10	B1_1_2020-02-18_11_05_53	SBIC_HE_20X_20200218_KFB	BrightField	20X	0	0	0
11	B1_2_2020-02-18_11_18_51	SBIC_HE_20X_20200218_KFB	BrightField	20X	0	4.745082907	0
12	B1_3_2020-02-18_11_18_03	SBIC_HE_20X_20200218_KFB	BrightField	20X	0	14.24845758	0.089016437
13	B1_4_2020-02-18_11_16_56	SBIC_HE_20X_20200218_KFB	BrightField	20X	9.079850302	4.486184913	0
14	demo1	KFB_FORMAT_TEST	BrightField	40X	1.803199221	0.486162889	0.162054296
15	demo2	KFB_FORMAT_TEST	BrightField	40X	6.887583851	0.285193901	0.998178652
16	demo3	KFB_FORMAT_TEST	BrightField	40X	1.21184475	0	3.977138159

	H	I	J	K	L	M	N
1	Saturation_Pct	High_Uniformity_Pct	Global_Low_Quality_Pct	Focus_Area_Pct	Tissue_Area_Pct	Number_of_Tiles	Status
2	0.893812967	6.495509844	7.915372862	99.70482242	77.86734375	3722.34922	Valid
3	10.50803594	2.989021885	6.987682592	100.4039872	75.38997396	3213.21836	Valid
4	3.821191648	2.253695413	6.117862933	99.39383105	74.03908235	2842.15292	Valid
5	2.708879368	1.387794287	4.582686431	100.0592014	75.5146983	2770.42717	Valid
6	81.71567196	0.349819844	0.122626122	109.109202	13.68695055	121.05088	Valid
7	92.24706535	0	1.624599225	102.1325614	35.59807219	299.24488	Valid
8	85.53027303	0.171681313	1.6448225	102.6679719	30.23592759	277.68893	Valid
9	99.83334743	0.044760057	5.028041106	100.3271906	47.11655792	250.49119	Valid
10	0.707320946	0	0	101.1603837	32.16685268	218.64332	Valid
11	48.85638861	0.403418397	3.767204013	104.5520194	14.91930084	125.99822	Valid
12	60.20747649	0.547713779	2.540198988	108.3279408	18.93868046	134.1887	Valid
13	22.46896846	0.150135377	2.401594281	103.7865171	21.77007568	243.11392	Valid
14	33.11422512	1.803199221	2.127307813	105.2119179	72.28625	617.07713	Valid
15	25.23695656	6.887583851	6.887583851	101.9538917	71.97334835	701.27727	Valid
16	0	1.23539737	1.23539737	106.3264368	71.19486325	494.97677	Valid

Low_Focus Pct	Artifacts Pct	LowContrast Pct	Saturatio Pct	High Uniformity Pct	Global Low Quality Pct	Tissue_Area Pct	Number of Tiles	Status
3.35	12.25	0.12	2.70	1.38	4.58	75.51	2770.42	Valid

Percentage of "Tiles" (256*256 contiguous blocks of the Tissue Region of Interest) Below Quality Threshold.

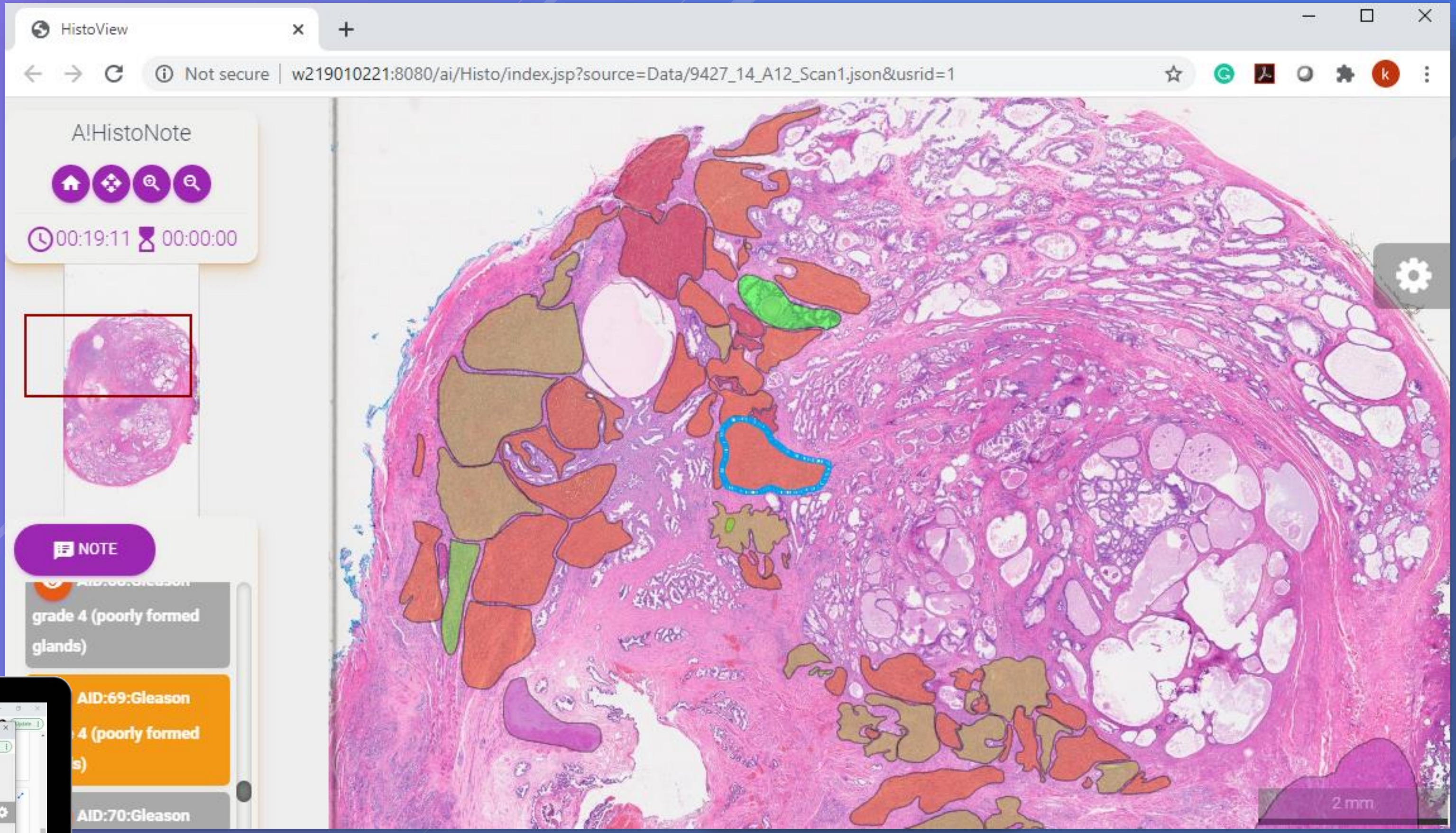
- Low Focus : % of tiles with a low focus
- Artifacts : % of tiles with Artefacts (dust ,folding, cluster, …)
- Low Contrast : % of tiles with a low contrast (faded staining, empty regions, …)
- Saturation : % of tiles with saturated colors (strong stain, clusters, …)
- Uniformity : % of tiles with a smooth Texture (faded stain, slight out of focus, …)
- Global Low quality : Linear combination of 5 aforementioned measurements.

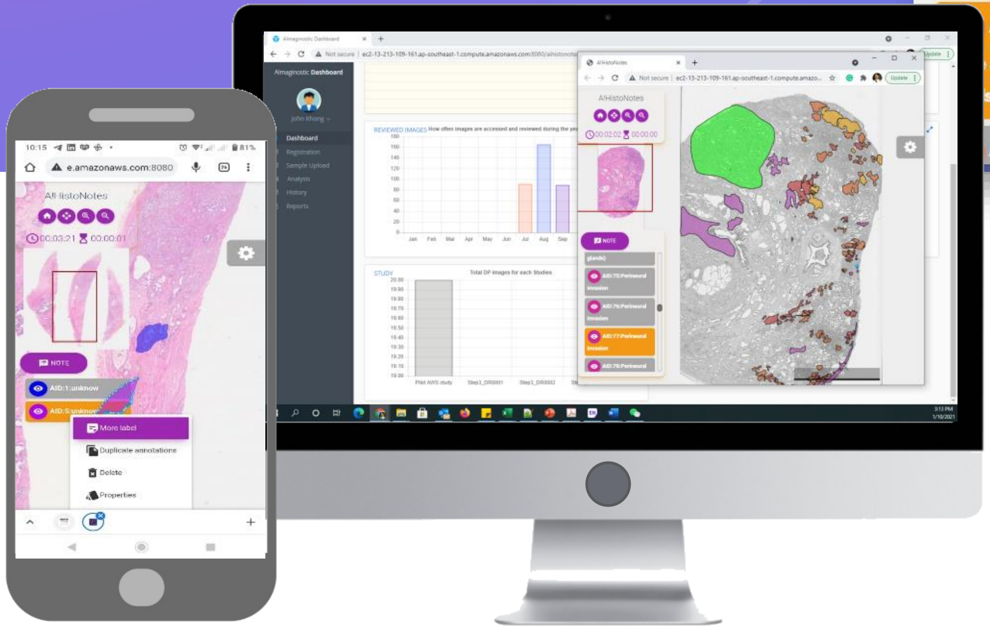
- Tissue Area : % of Image Area that contains Tissue.
- Number of Tiles : Weighted Number of Tiles with in Tissue Area. (Tiles on Tissue edges have less weights since cropped by Tissue Region boundary.)
- Status : Aimag QC estimated image Quality:
  - *Valid*: image can be used for diagnosis/ further processing
  - *To Check*: image may be used for diagnosis if reviewed by specialist
  - *Rejected*: image quality is too low for diagnosis/ further processing

A!HISTONOTE VERSION 2.0

Perfect tools for pathologists

Diagnosing & Prognosing Your Patient Can Be Faster, Which Was Previously Impossible.





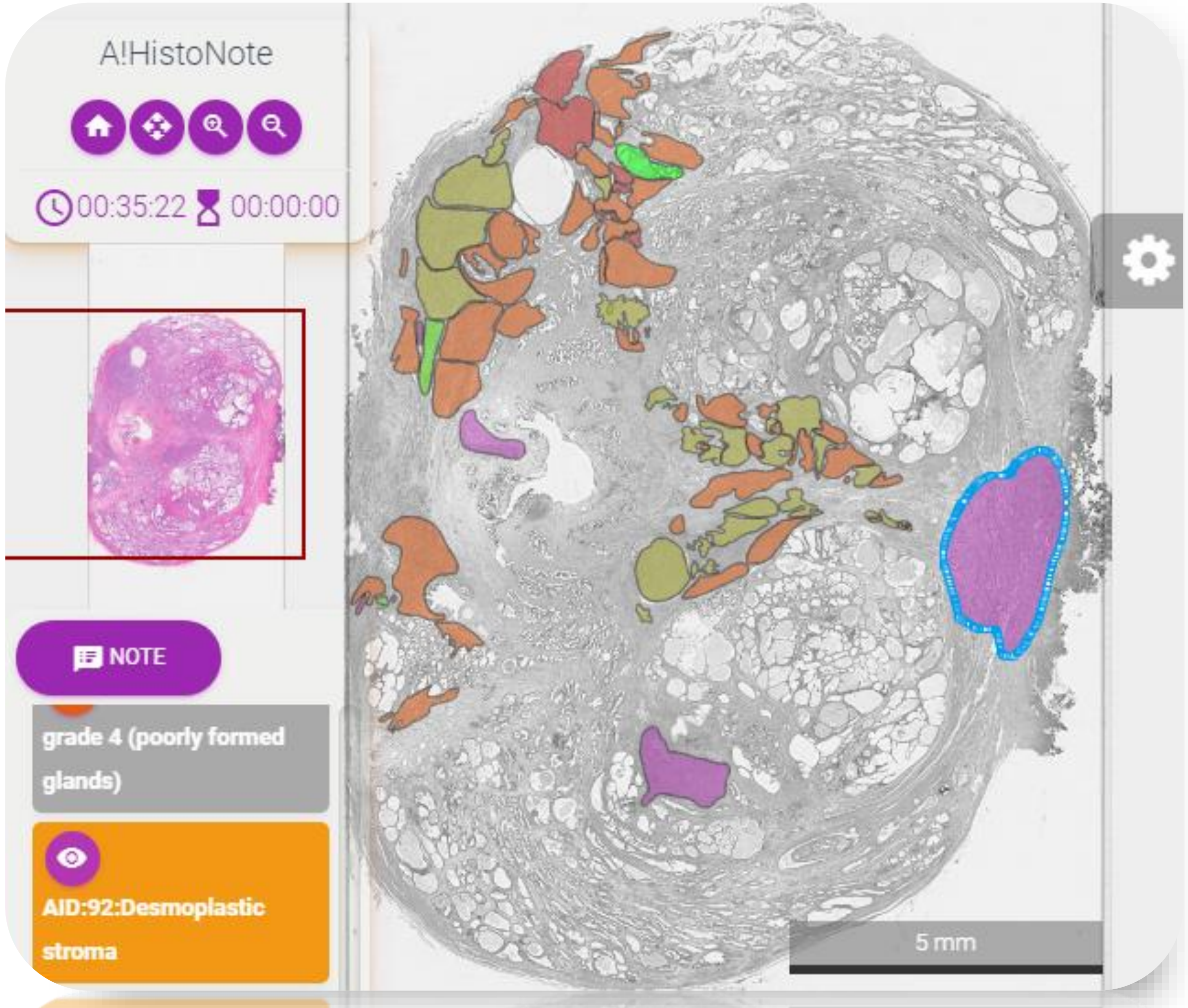
Supported Browsers

Bioinformatics Institute
BII

Institute of Molecular and Cell Biology
IMCB

About A!HistoNote

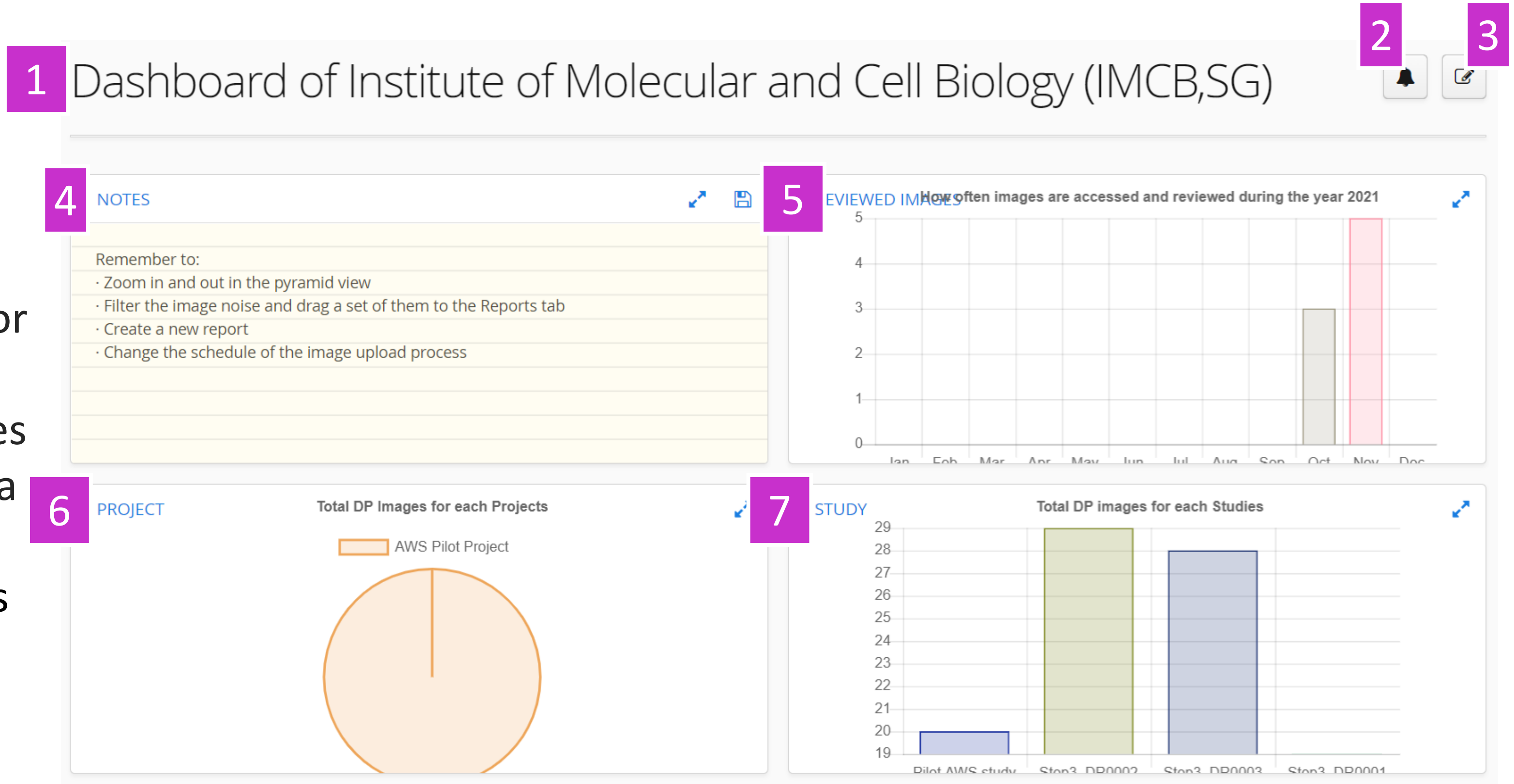
View proprietary bright field and fluorescence virtual slide formats, annotations, and analysis results directly in ANY browser from ALL your devices (desktops, laptops, tablets, and mobile phones).



A!HistoNote Homepage

Dashboard

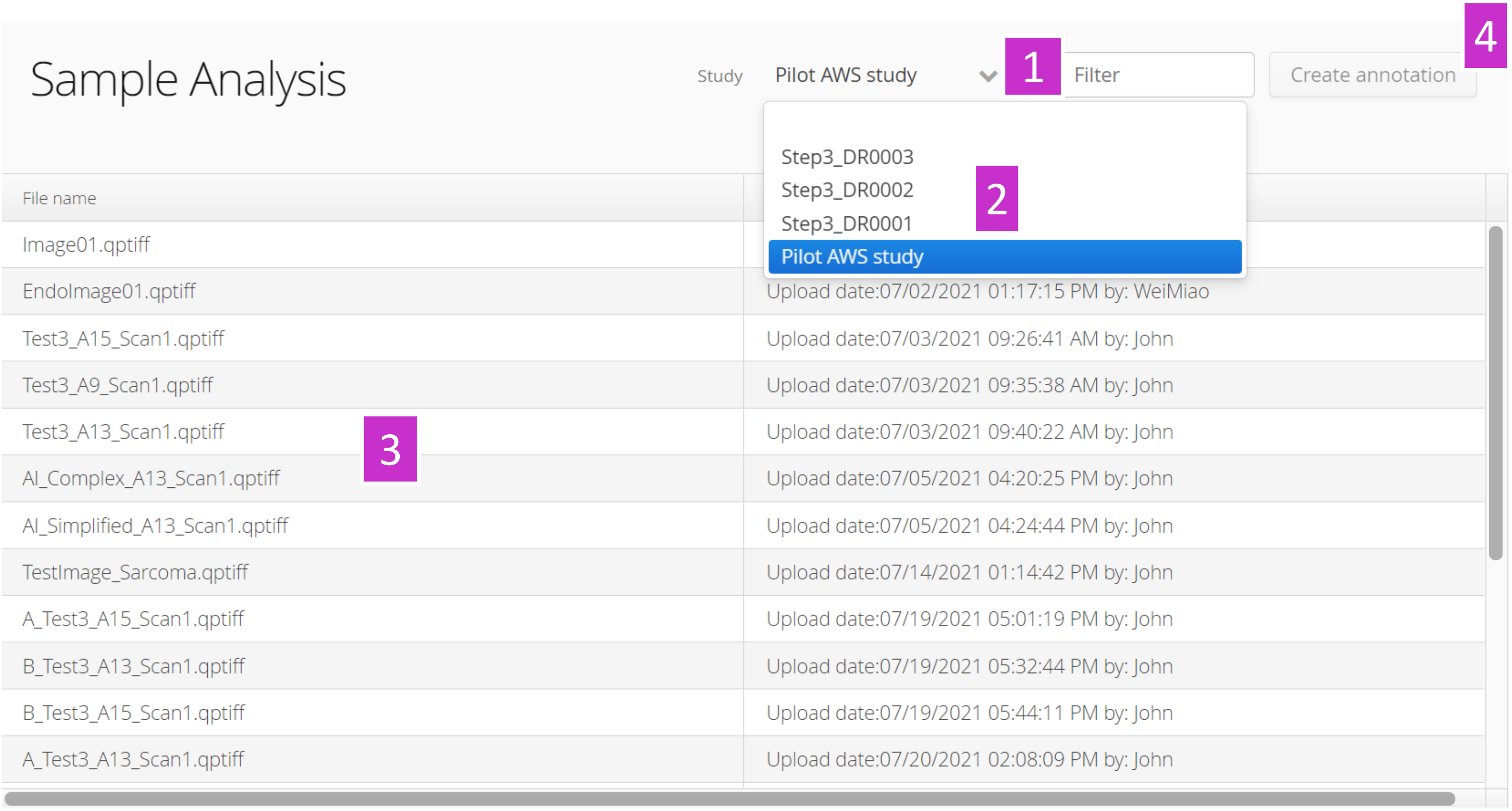
1. Title of dashboard
2. View notifications
3. Edit title of dashboard
4. Create comments and reminders for analyses
5. Bar chart to show how often images are accessed and reviewed during a calendar year
6. Total Digital Pathologic (DP) images for each project
7. Total DP images for each study



A!HistoNote Homepage

Analysis

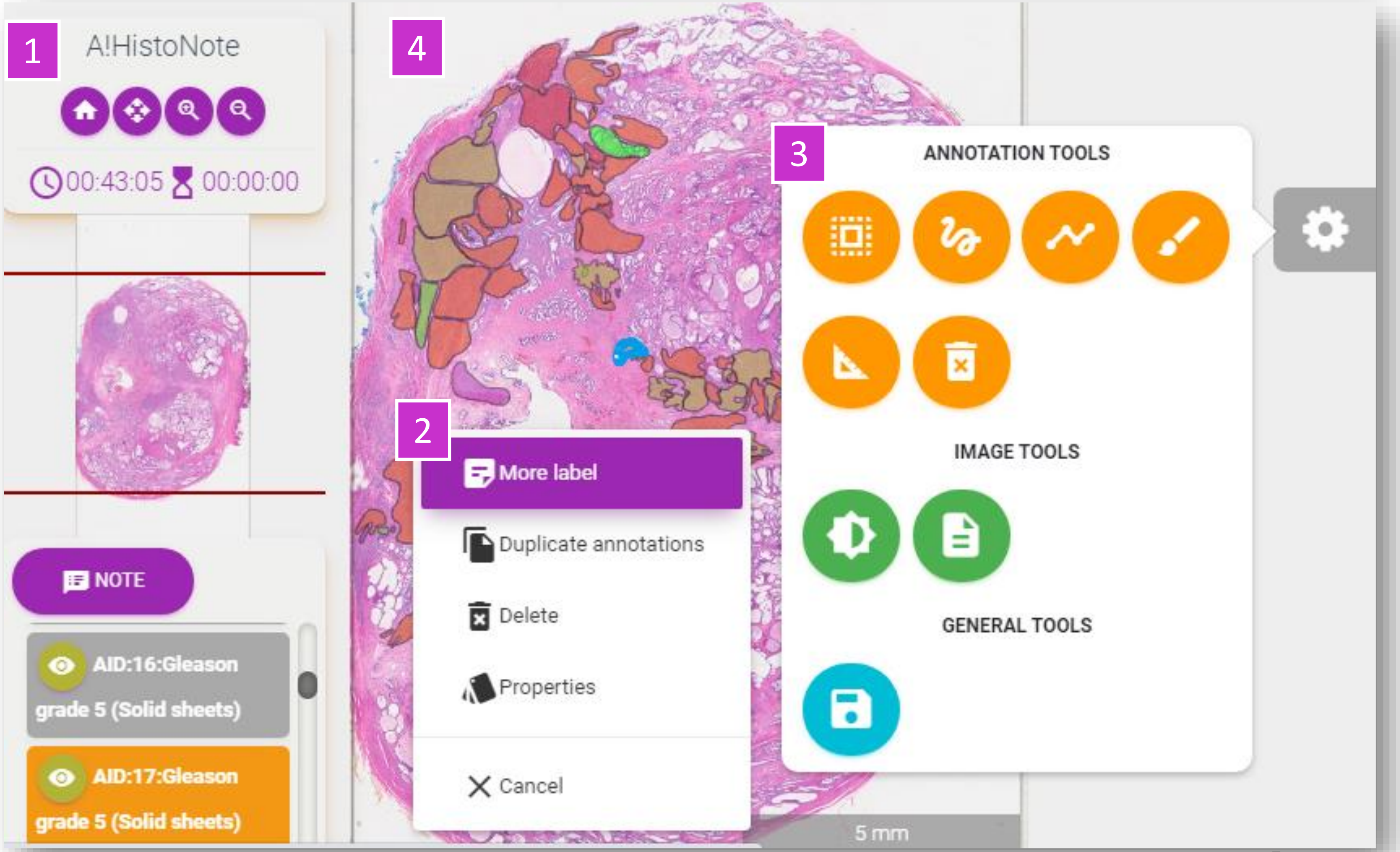
1. View list of studies
2. Select study
3. List of slide/image samples in selected study
4. Create or modify annotations on selected slide/image sample



A!HistoNote Version 2.0

Overview

1. Panel
2. Dropdown menu
3. Region of Interest (ROI) tools
4. Workspace



1 A!HistoNote Panel

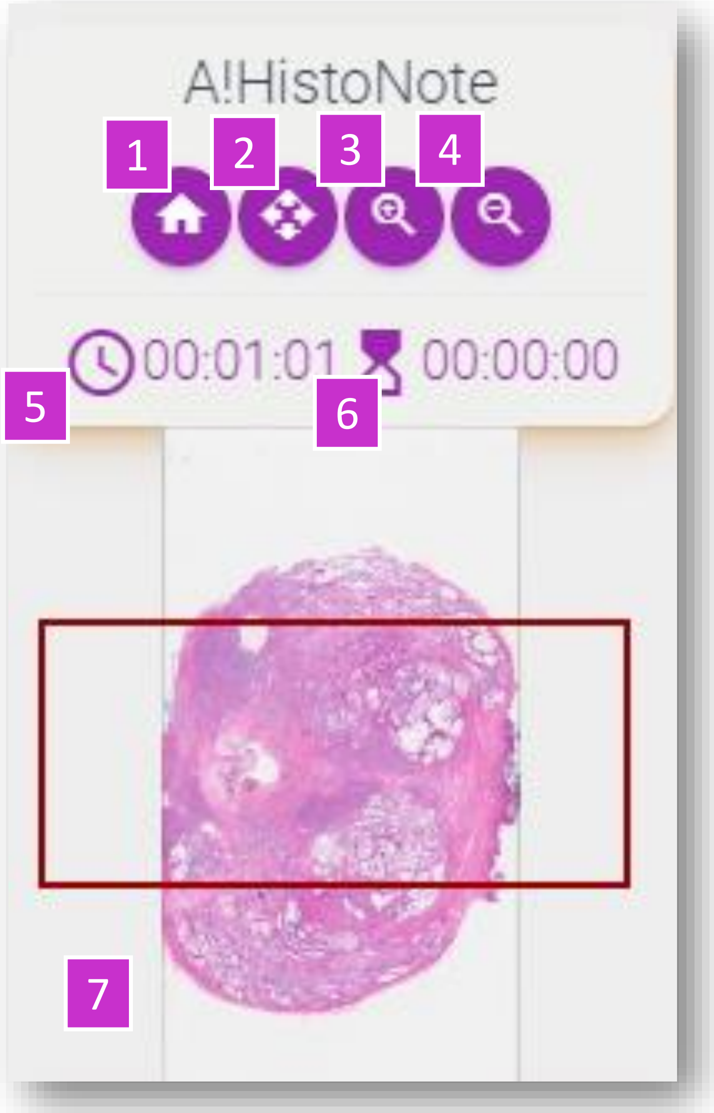


Image Navigation Panel

1. Reset image to 1:1 ratio
2. Move image position
3. Zoom in
4. Zoom out

Stopwatch Panel

5. Time spent viewing each image
6. Time recorder for each ROI

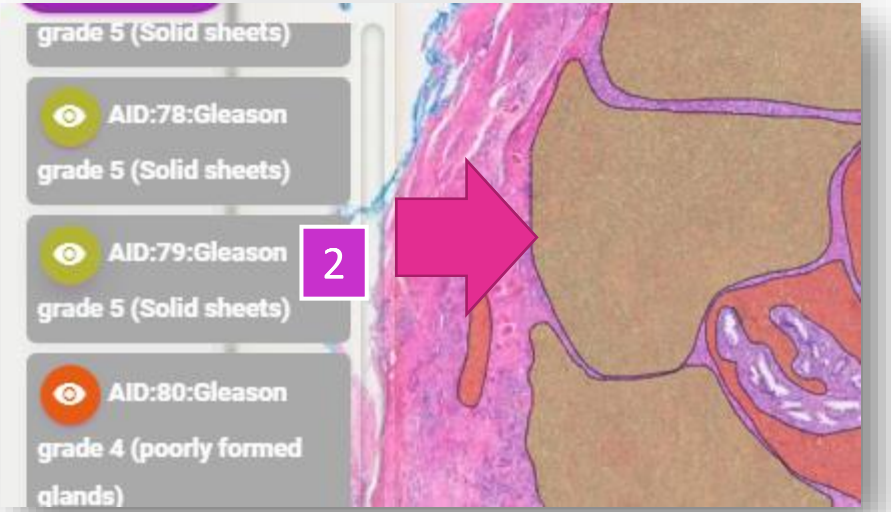
Viewport Navigator Panel

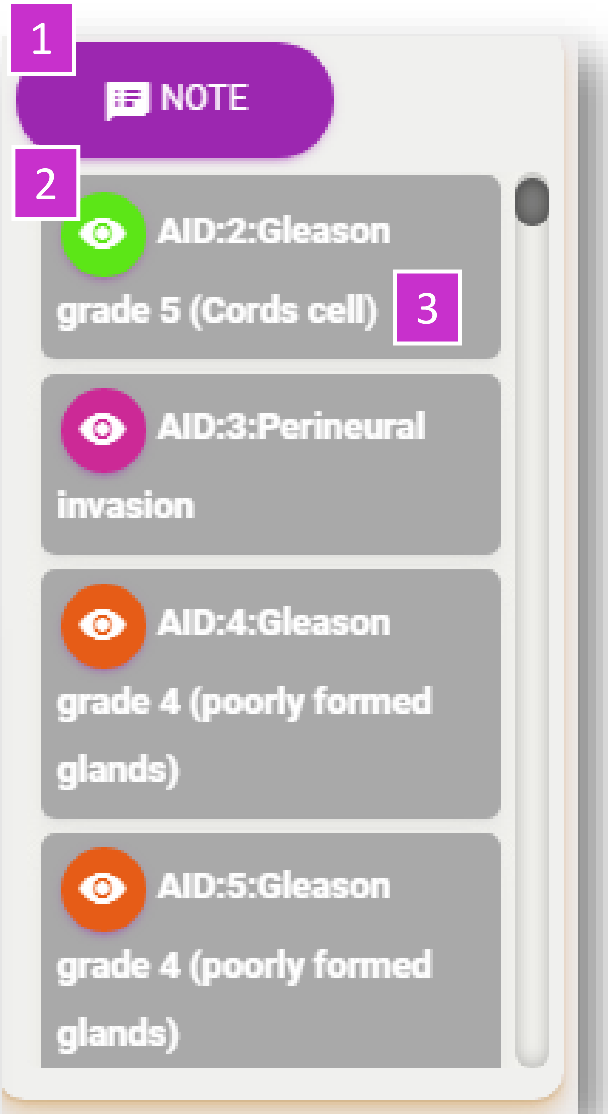
7. Display the source image as a reference, and the highlighted area shows the part of the image the user is currently viewing on the workspace.

1 A!HistoNote Panel

Annotation Label Management Panel

1. Toggle button to show or close the panel
2. Toggle buttons to show or close the annotation ROI
3. Label of the created annotation ROI





1 A!HistoNote Panel

Annotation Label Management Panel

1. Automatic label highlight and detection when ROI is selected
2. Pathologist's annotated ROI and detailed information of the annotation will be displayed on the region-tip

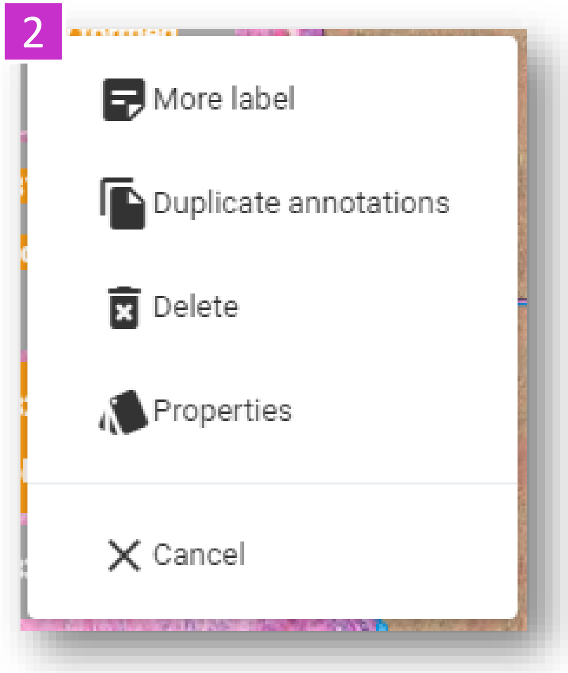
Region-tip

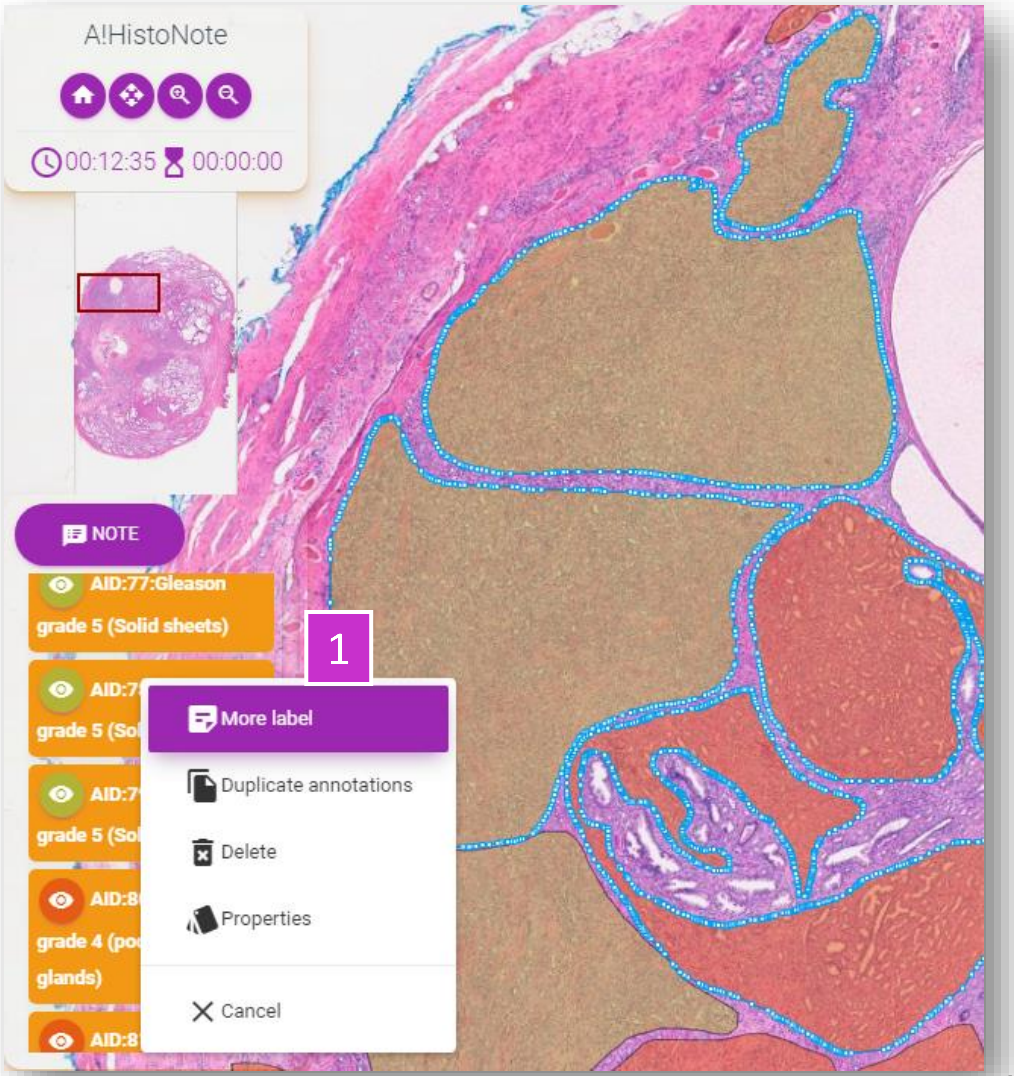
3. Tag ID: Area label
4. Area in um^2
5. Regional notes

1 A!HistoNote Panel

Annotation Label Management Panel

1. Multiple ROIs can be selected to perform operations in the drop-down menu.
2. Actions available in dropdown menu





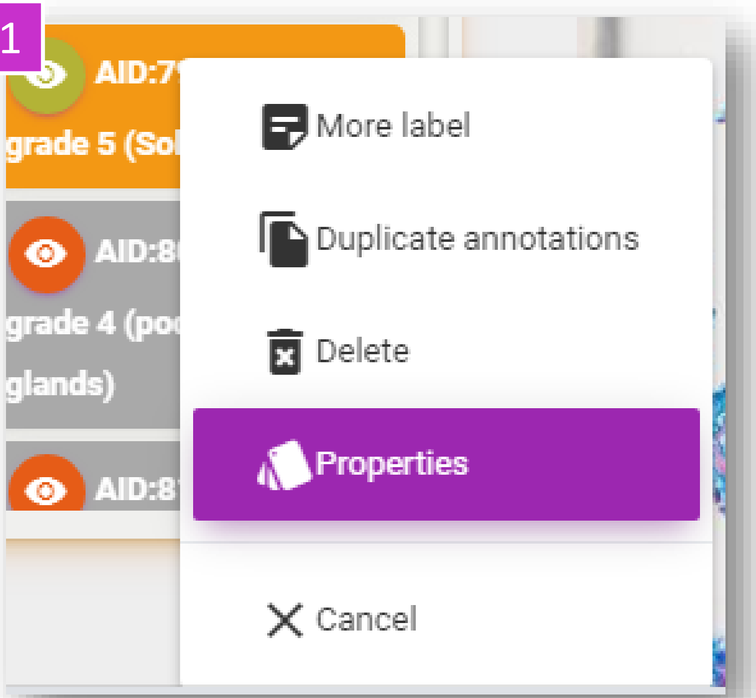
2 A!HistoNote Dropdown menu

Panels to access Label and ROI dropdown menu

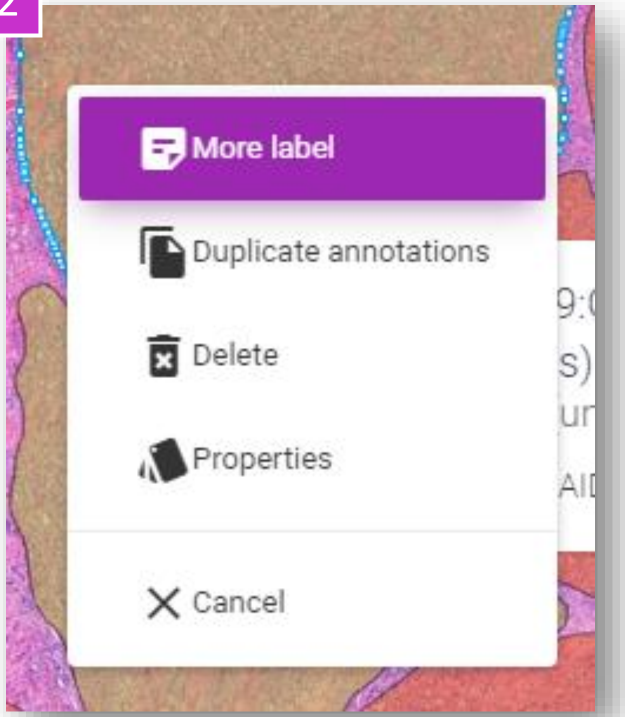
Access by right clicking the mouse

1. Annotation Label Management Panel
2. Annotation ROI Drawing Area

1



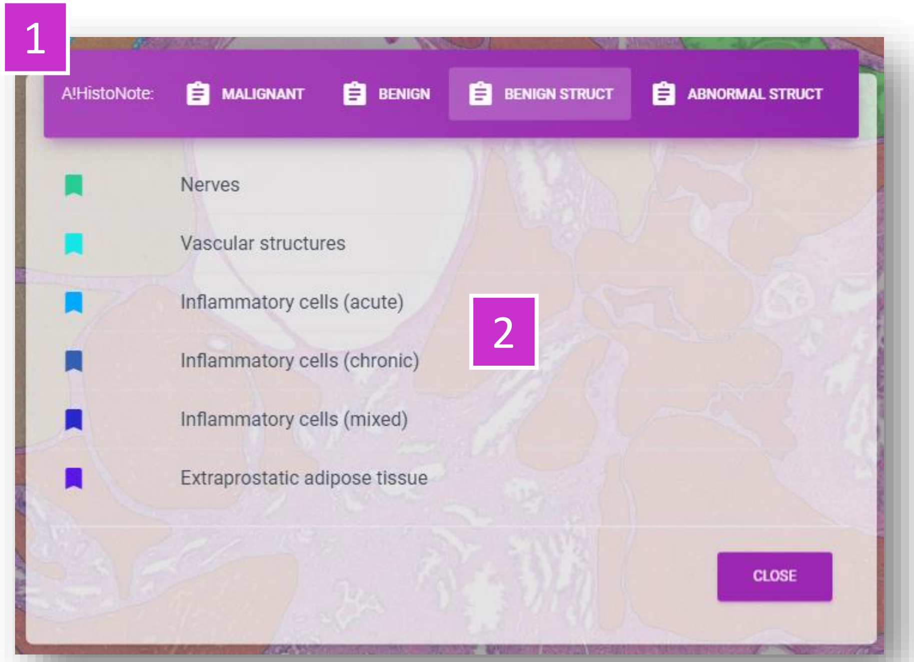
2

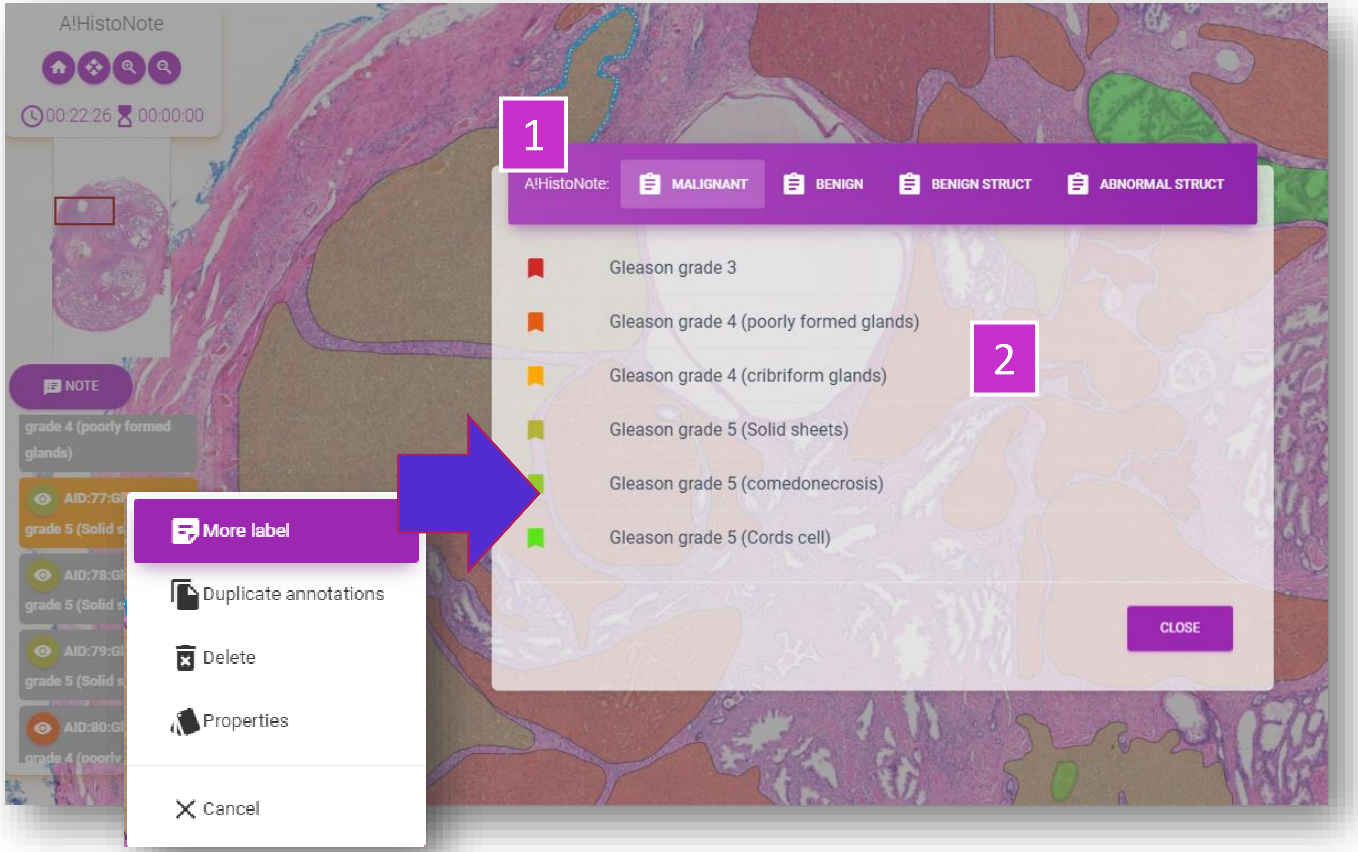


2 A!HistoNote Dropdown menu

[More Label] in the Dropdown Menu

1. Category of tissue cell type
2. Click the label of tissue cell type to assign to the ROI



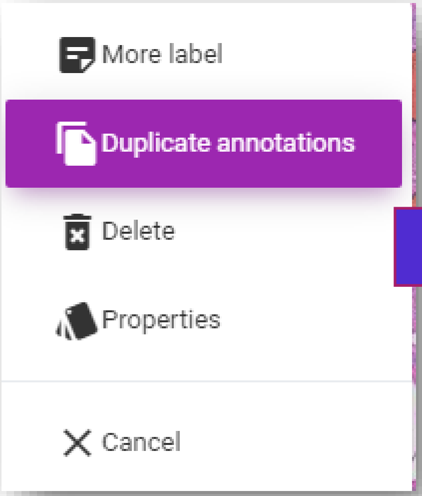


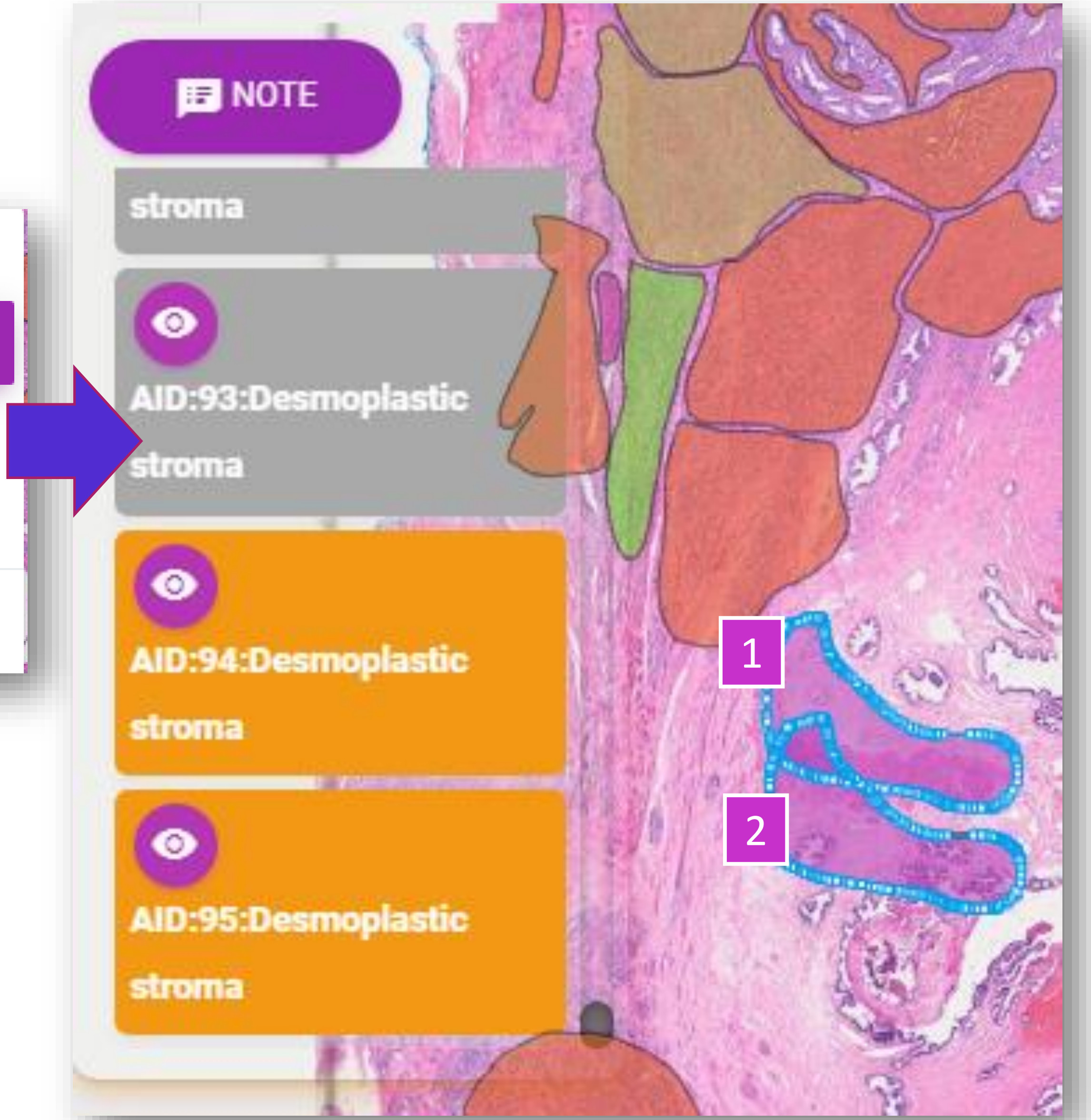
2 A!HistoNote Dropdown menu

[Duplicate annotations] in the Dropdown Menu

1. Original ROI
2. Duplicated ROI

PS: Except ROI-ID, all information about ROI labels and remarks will be copied



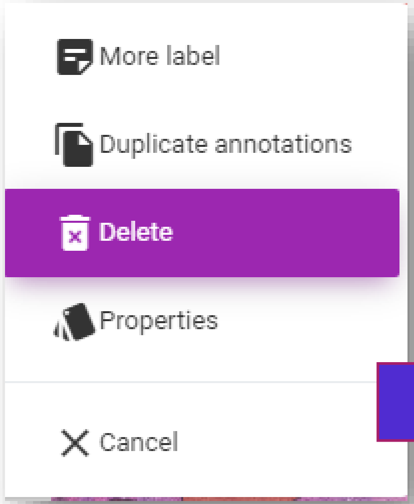


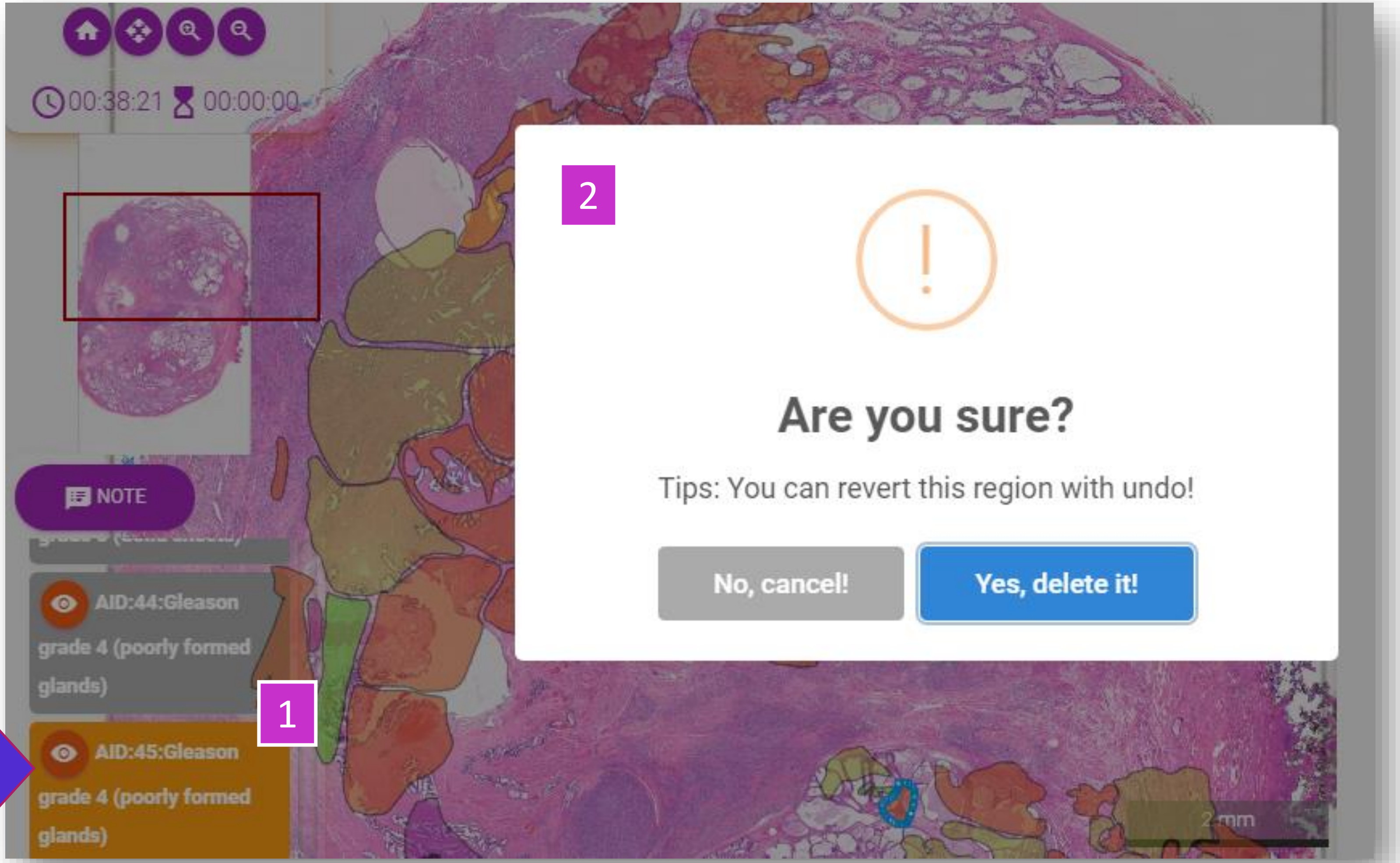
2 A!HistoNote Dropdown menu

[Delete] in the drop-down menu

Original ROI

1. Select the ROI you want to delete
2. Delete confirmation dialogue

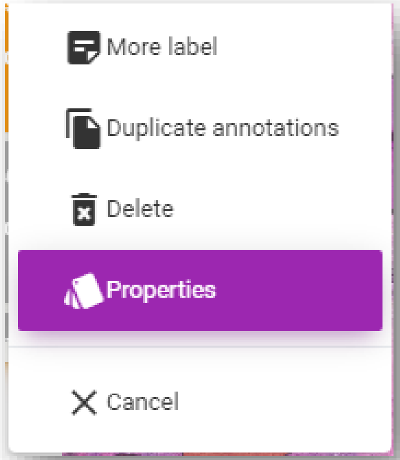


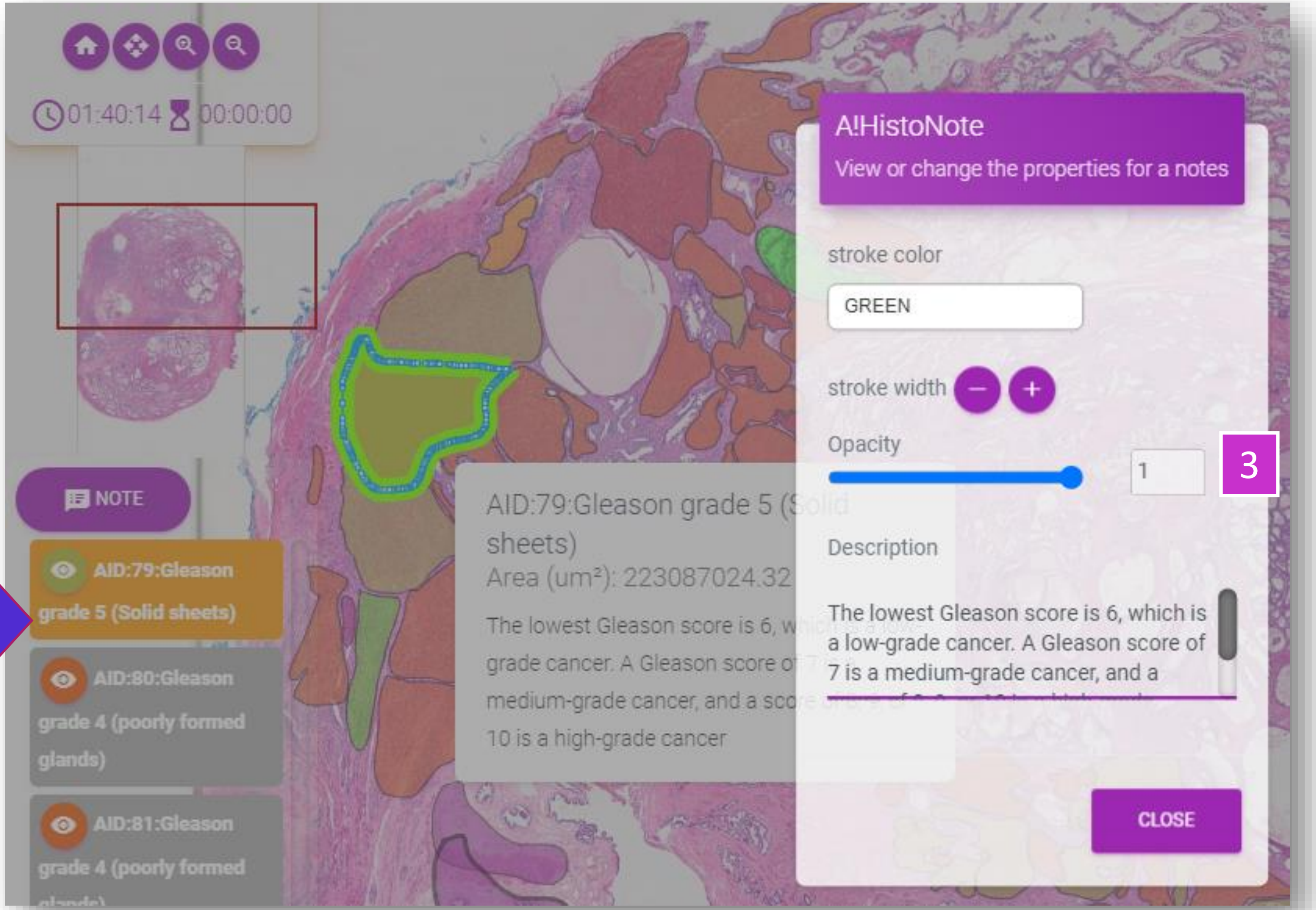


2 A!HistoNote Dropdown menu

[Properties] in the Dropdown Menu

1. Change the colour of the ROI border
2. Change the stroke size of the ROI border.
3. Change the transparency of the ROI body.
4. Record the remark/description



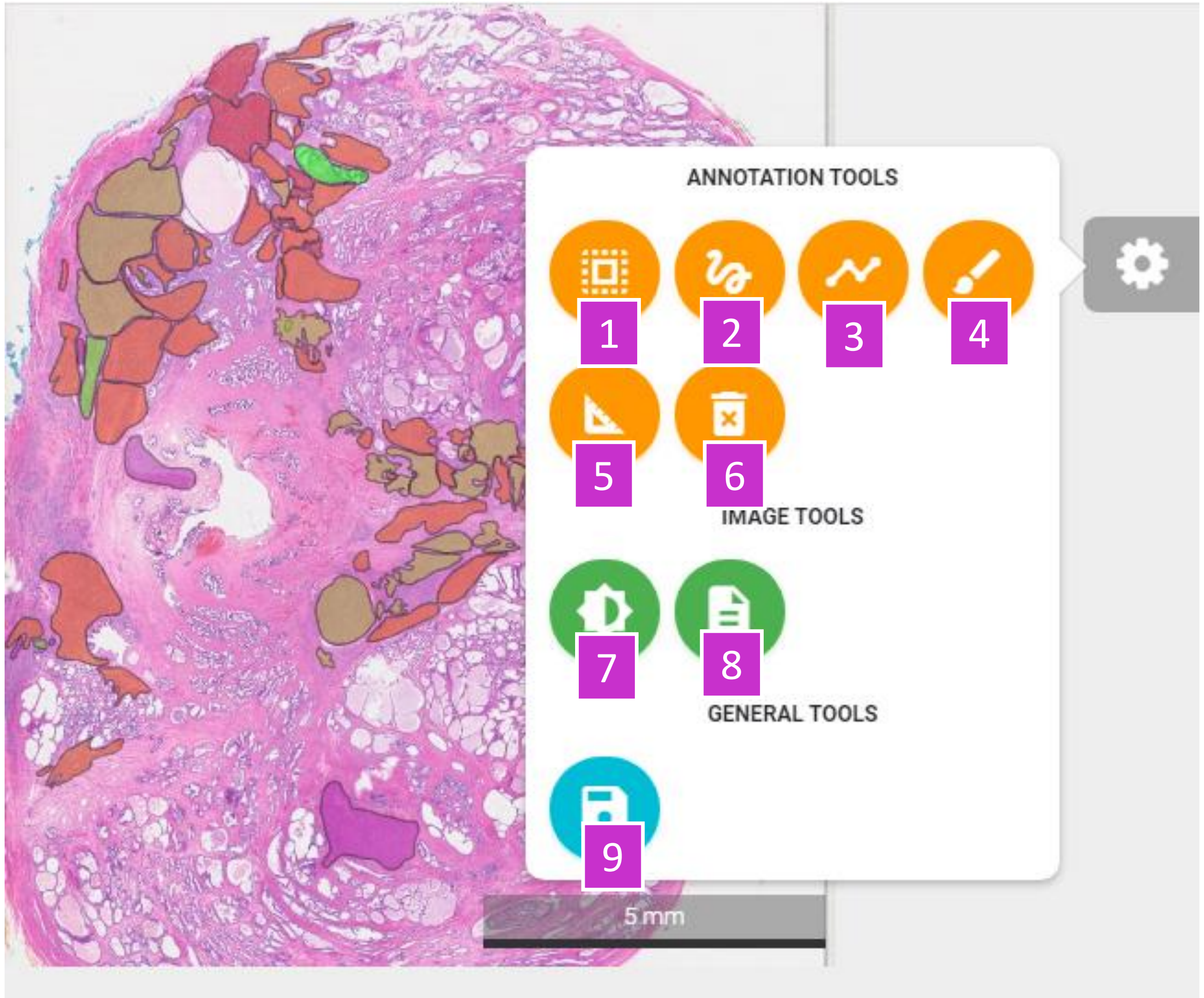


3 A!HistoNote ROI Tools

Annotation tools
1. ROI picker
2. Free-hand draw tool
3. Polygon draw tool
4. Brush draw tool
5. Measurement tool
6. Delete ROI

Image adjustment tools
7. Image filtering tool
8. Image metadata viewer

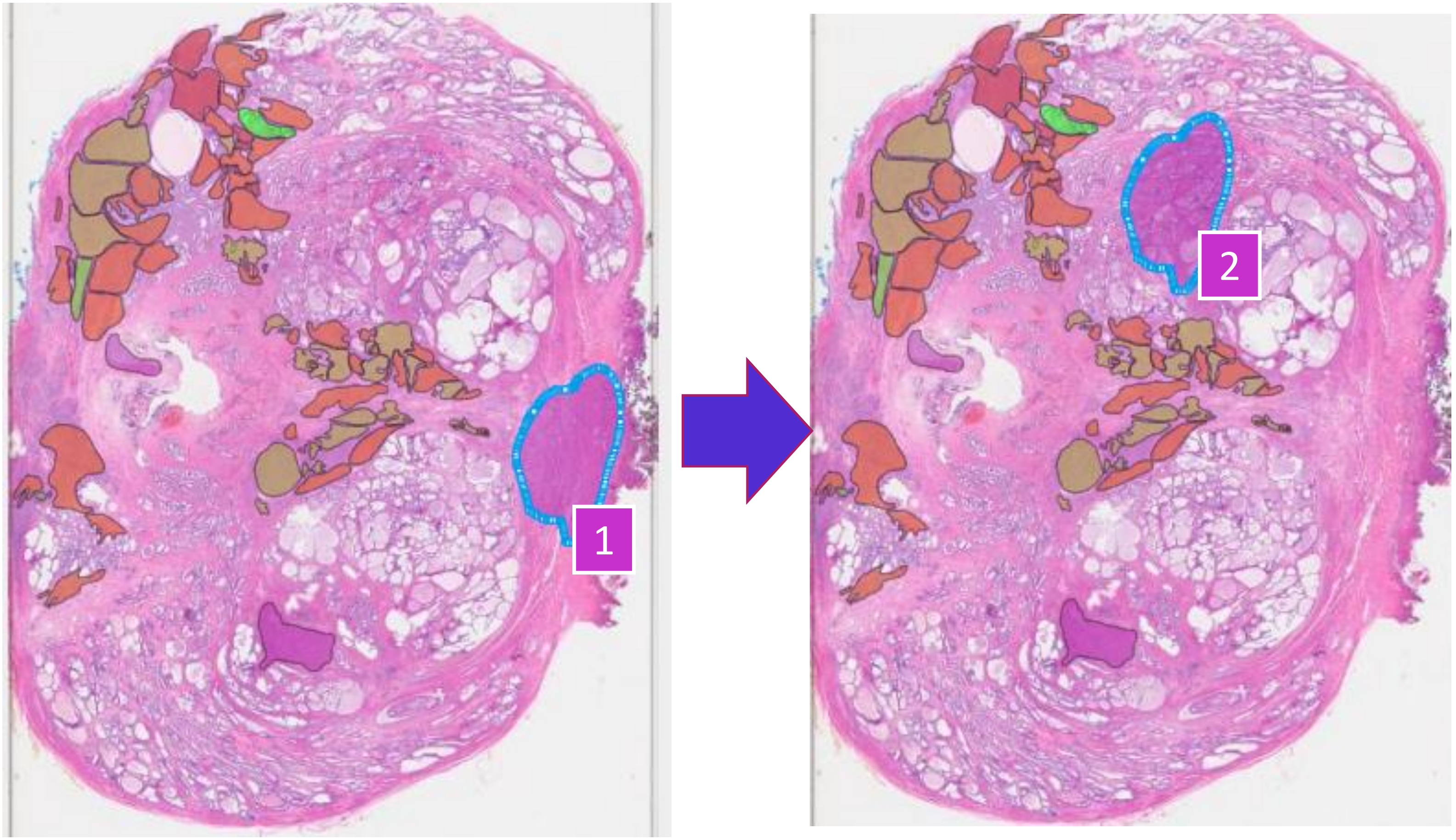
General tools
9. Save ROI



3.1 A!HistoNote ROI Tools

ROI Picker

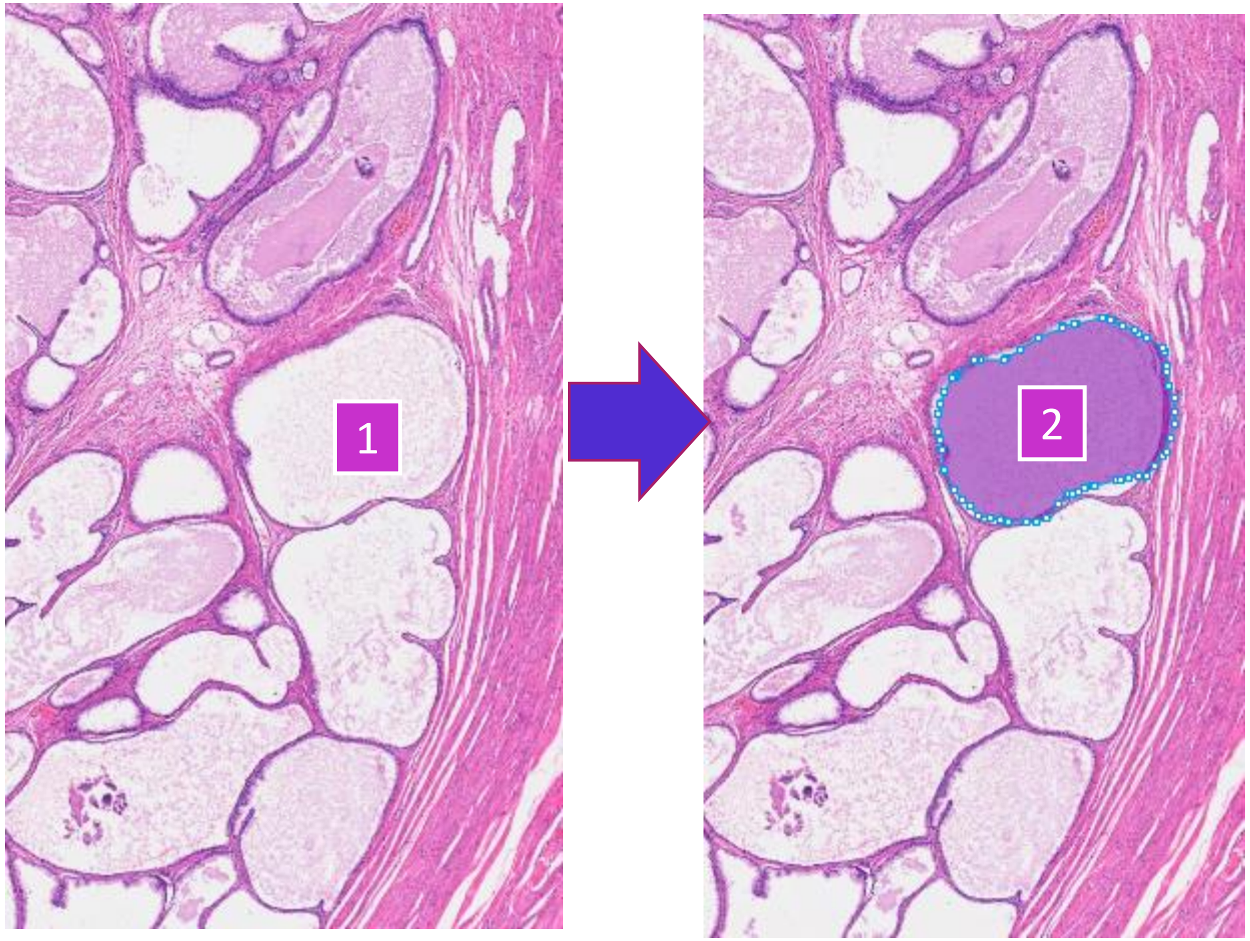
1. The location of the original target ROI.
2. Click the mouse to drag and move to the desired position on the target ROI.



3.1 A!HistoNote ROI Tools

Free-hand draw tool

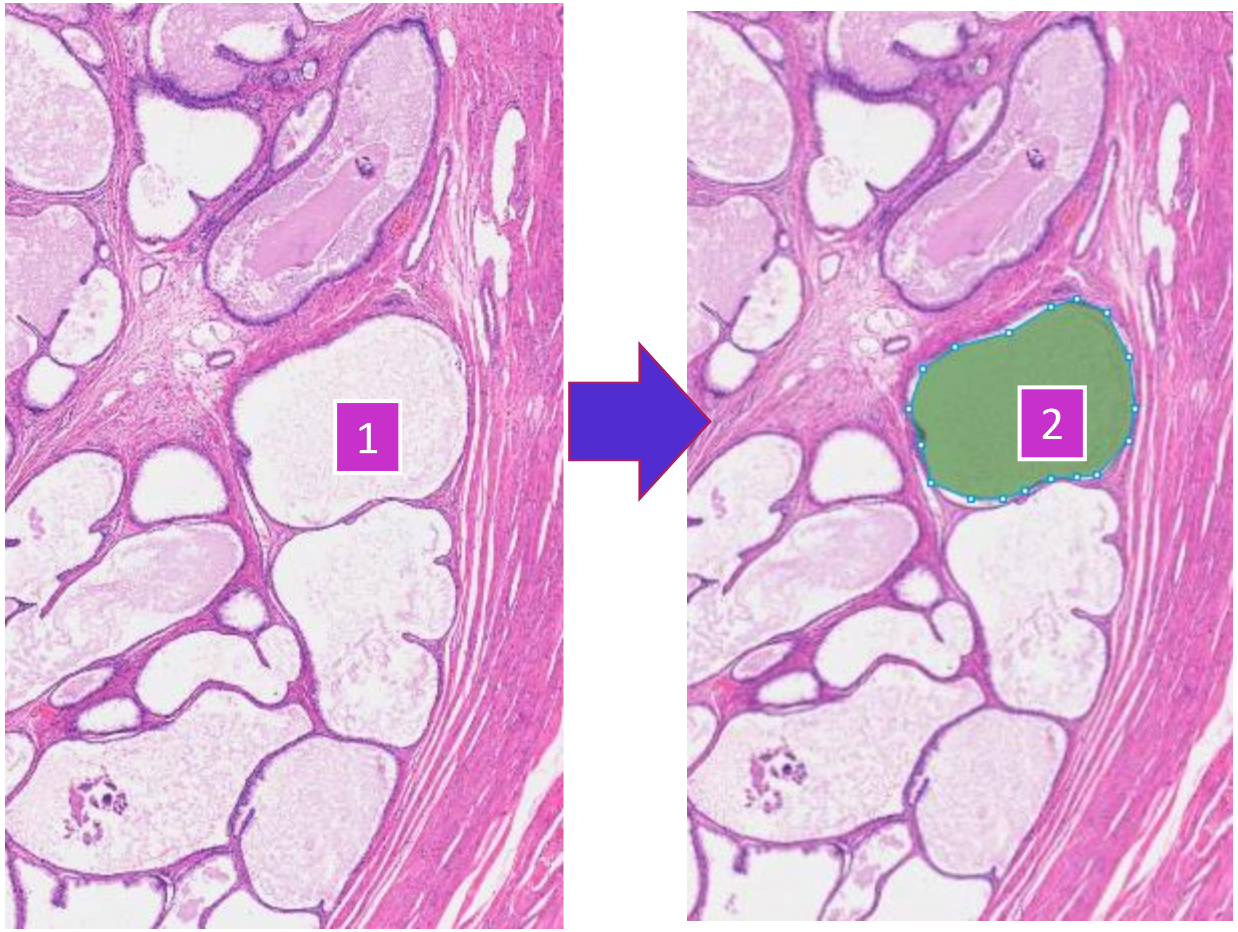
1. The location of the original target ROI.
2. Click the mouse to drag and move on the edge according to the shape of the entire targeted region.
3. Release mouse click to obtain the ROI.



3.1 A!HistoNote ROI Tools

Polygon draw tool

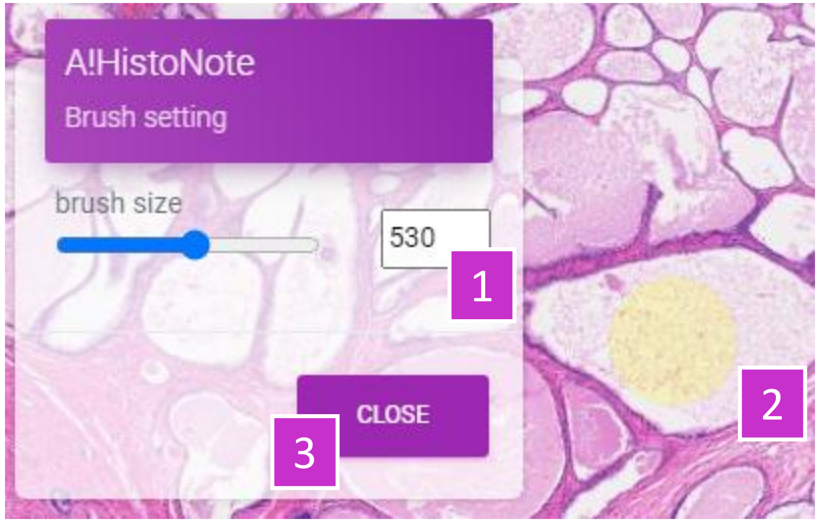
1. The location of the original target ROI.
2. Click the mouse on the edge according to the shape of the entire target area.
3. Double mouse click to obtain the ROI.

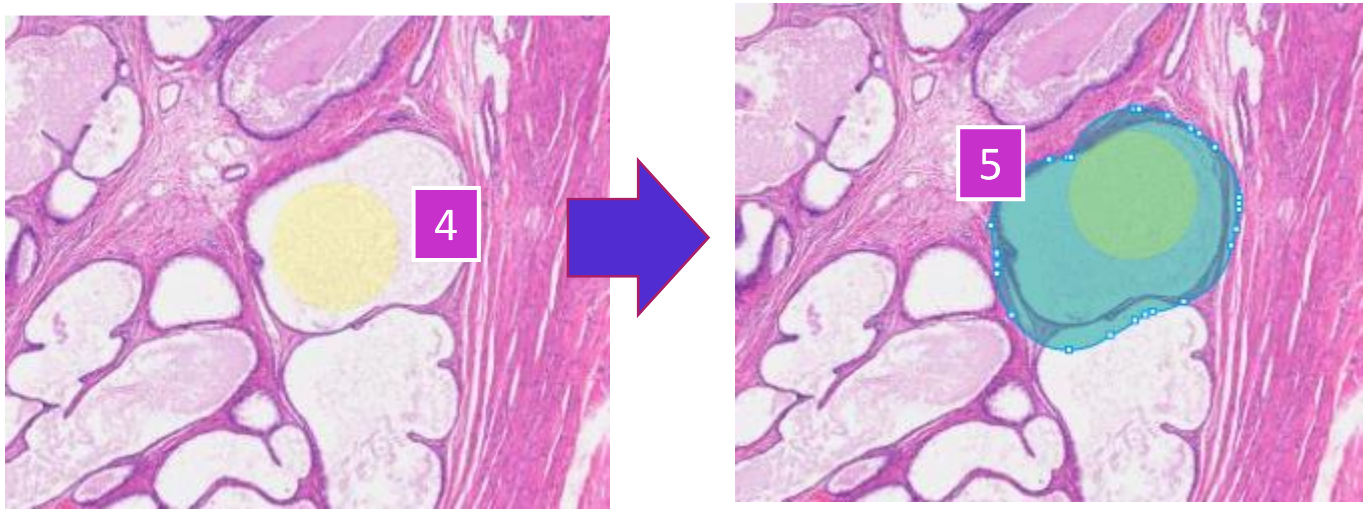


3.1 A!HistoNote ROI Tools

Brush draw tool

1. Set brush size according to the approximate and less than target size.
2. The round yellow highlight shows the estimated size.
3. Close the window or leave it alone.
4. The location of the target ROI.
5. Drag and move the mouse carefully (yellow highlighter) within the shape of the entire target area.
6. Release mouse click to obtain the ROI.





3.1 A!HistoNote ROI Tools

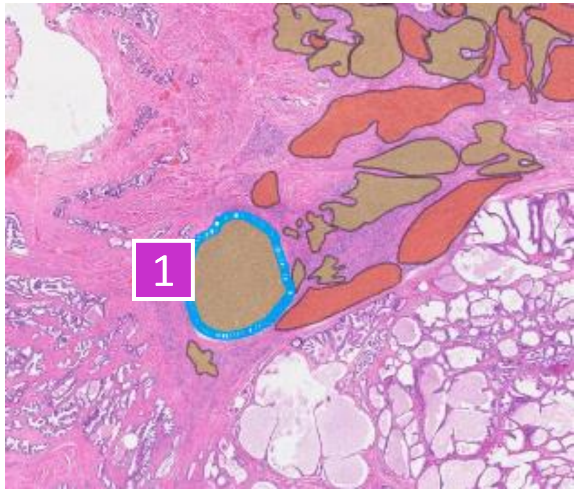
Measurement tools

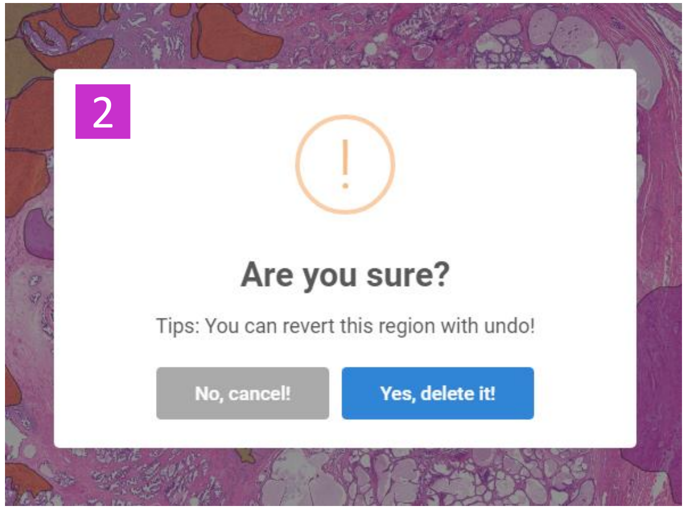
1. A circle (radius area) is displayed when the ruler is measuring.
2. An angle value is displayed when the ruler is measuring.
3. During the ruler measurement, the X (horizontal) and Y (vertical) lengths from the starting point to the target point are displayed.
4. Display text in either black or white font.

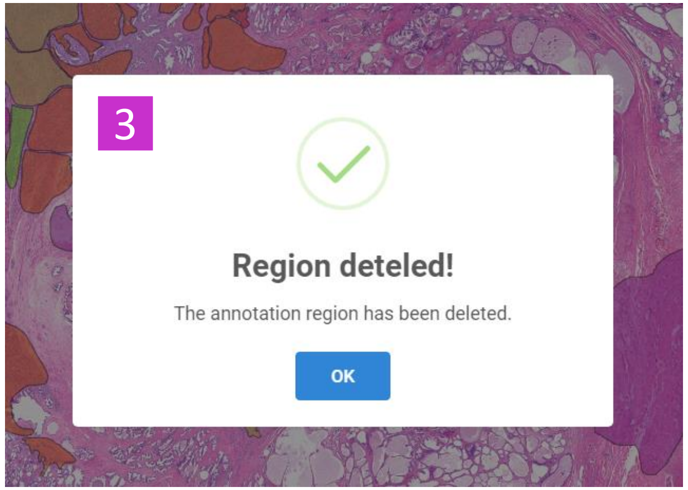
3.1 A!HistoNote ROI Tools

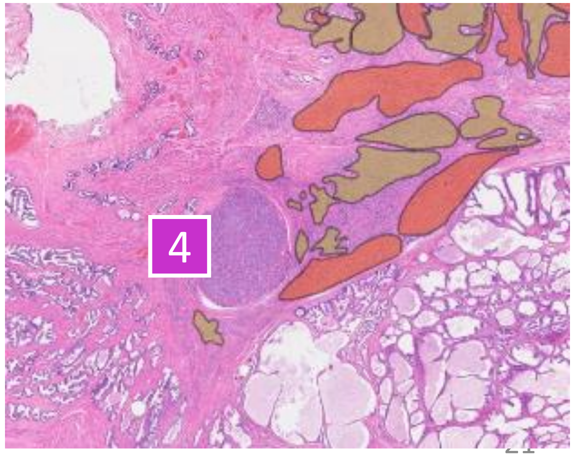
Delete tools

1. Select target ROI and click
2. Confirmation window
3. Deleting status
4. Target ROI deleted





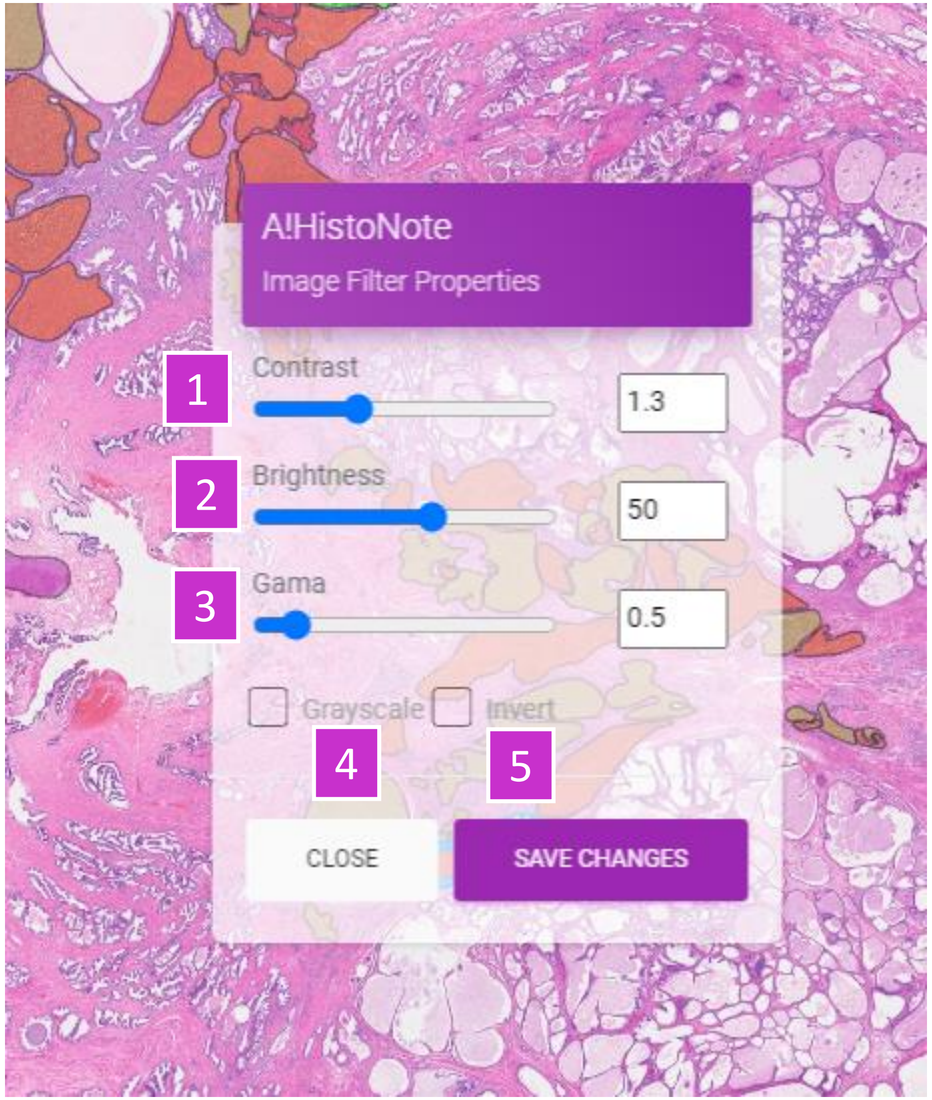




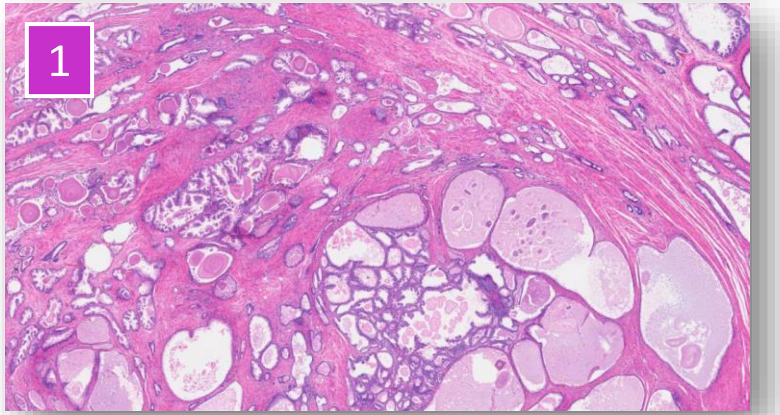
3.2 A!HistoNote Image Tools

Image Filter tools

1. Contrast
2. Brightness
3. Gama
4. Grayscale
5. Invert

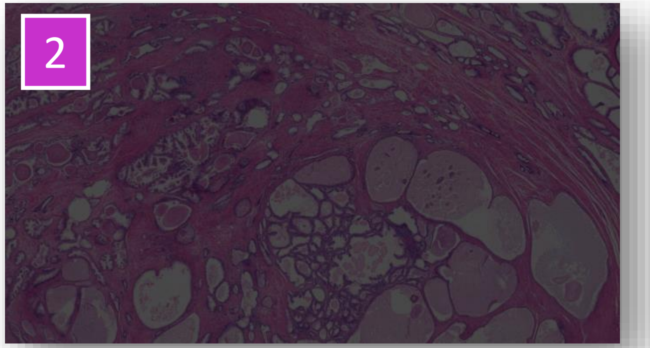


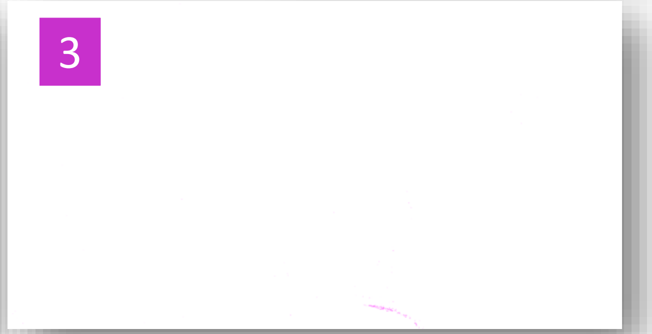
3.2 A!HistoNote Image Tools

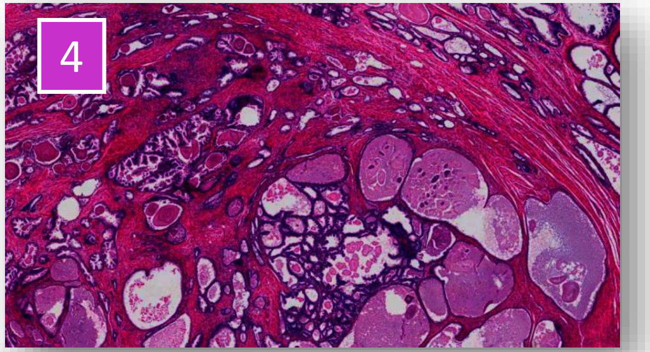


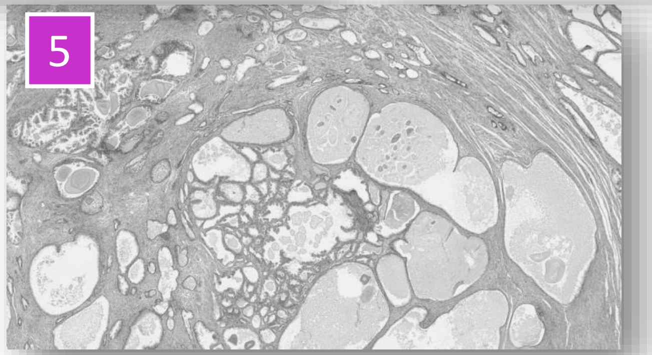
Samples of Filter Tools

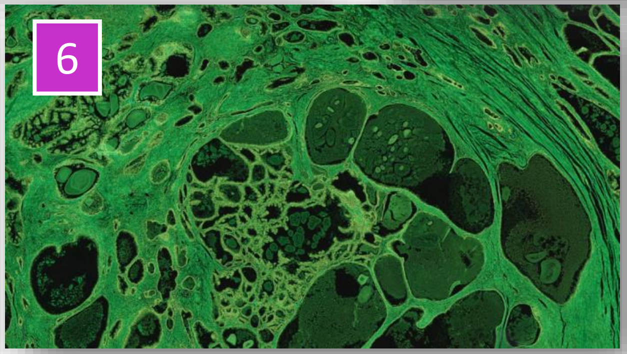
1. Contrast
2. Brightness
3. Gama
4. Grayscale
5. Invert







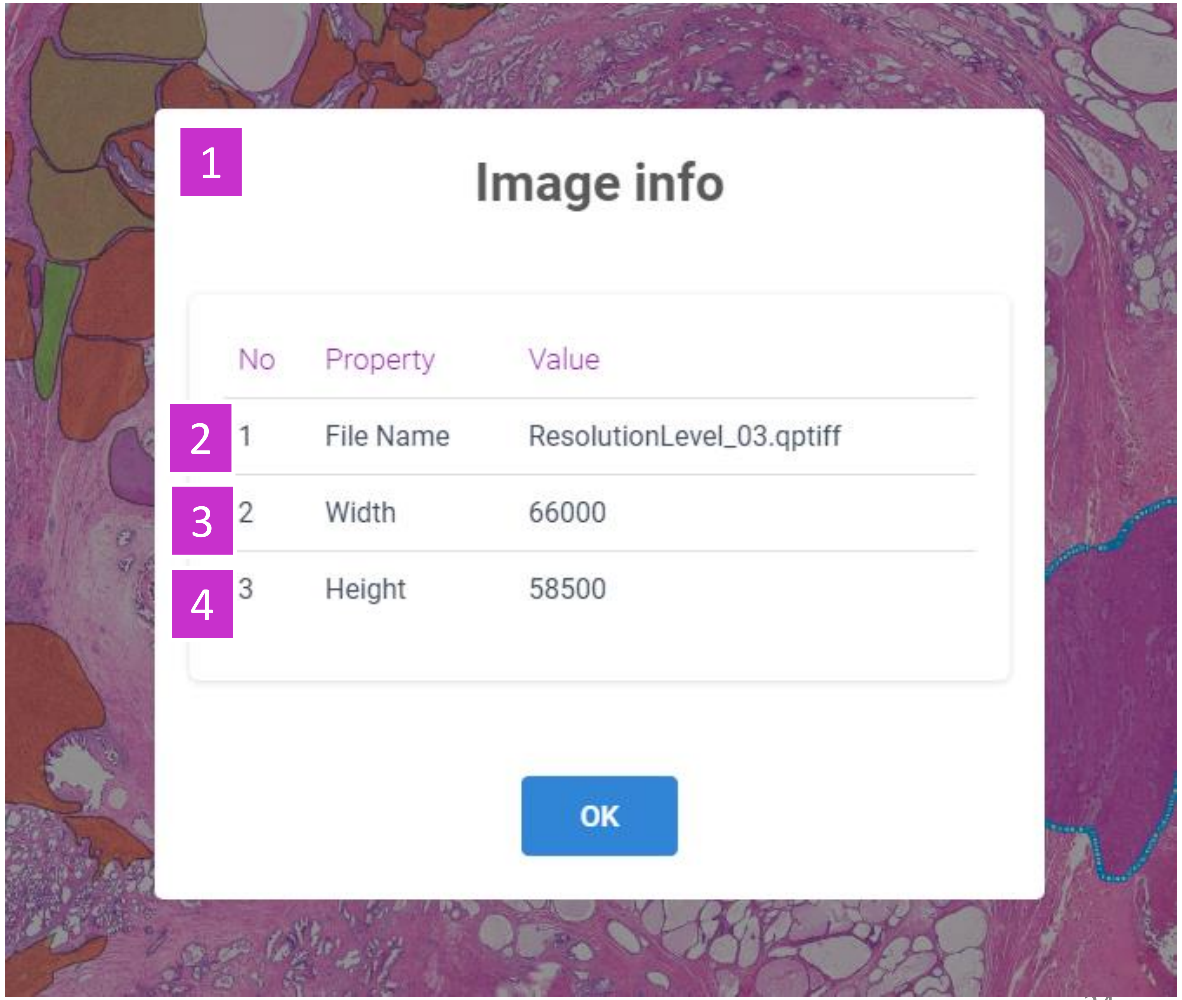




3.2 A!HistoNote Image Tools

Meta-data tools

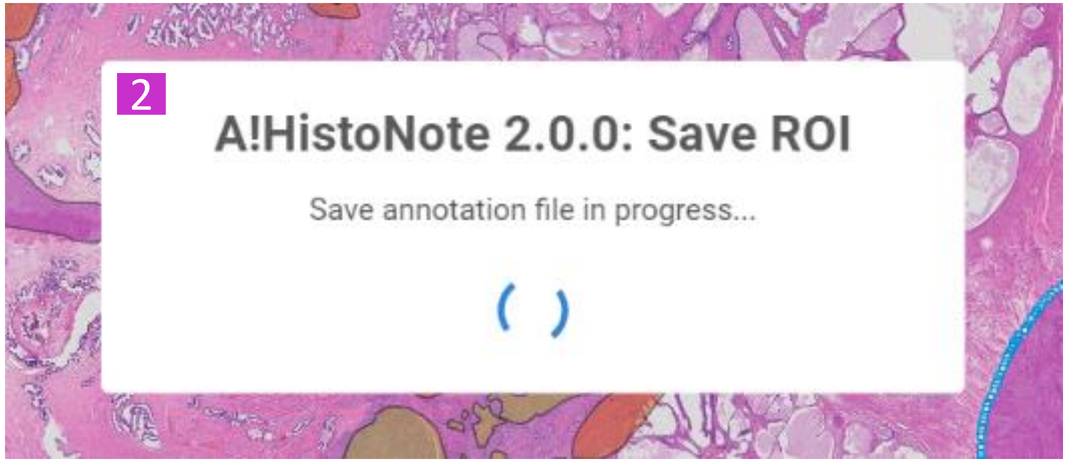
1. Image information window
2. Full file name
3. Width of image in pixels
4. Height of image in pixels

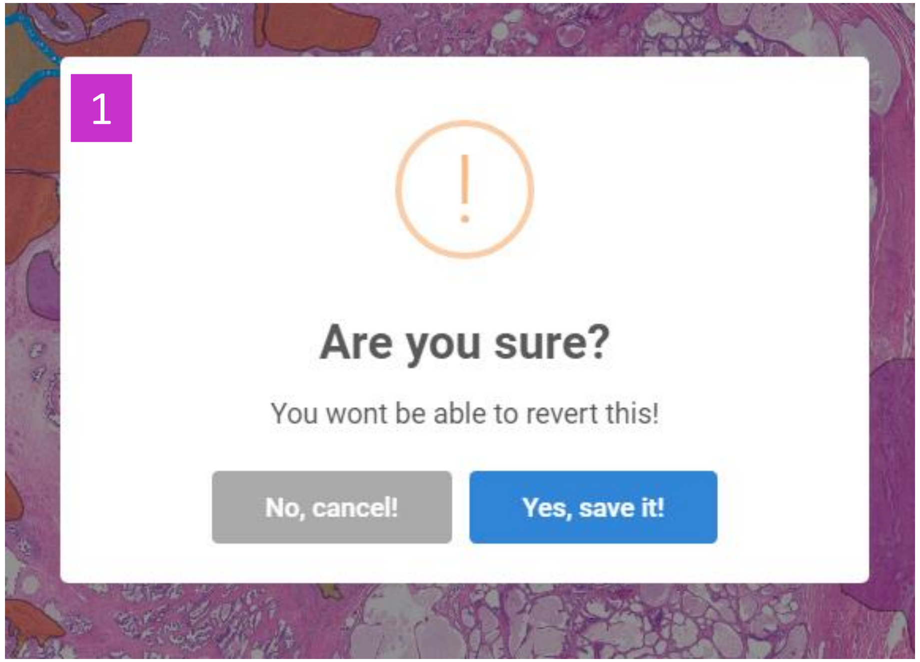


3.3 A!HistoNote General Tools

Save ROI tools

1. Save ROI confirmation dialogue
2. Communicate with server
3. ROI successfully saved





Bioinformatics Institute
BII

Institute of Molecular and Cell Biology
IMCB

Contact Us

30 Biopolis Street,
#07-46 Matrix,
Singapore 138671

Thank you

https://www.a-star.edu.sg/imcb/
https://www.a-star.edu.sg/bii/

+65 6586 9912

yu_weimiao@bii.a-star.edu.sg/ wmyu@imcb.a-star.edu.sg

kh.ong@aimaginostic.com/ong_kok_haur@bii.a-star.edu.sg