

eeDAP

Evaluation Environment for Digital & Analog Pathology

User Manual

2015

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I. Introduction

eeDAP is used design and execute correlated optical and digital pathology reader studies. This application supports two modes of reading, Digital and MicroRT. Digital mode uses a set of regions of interest (ROIs) identified and extracted from digital whole slide images (WSIs). MicroRT mode offers the reader a real-time view of the identical set of ROIs from a glass slide, through the optics of a microscope. In other words, the two modes are registered to one another. eeDAP allows for the administrator of the study to have a high level of control over the format of the tasks presented to the readers through specifications in an input file. Several simple default tasks are available to the administrator: multiple choice, continuous sliders, or binary responses. The administrator can also specify whether the reader is allowed to pan or zoom while confirming in real time the registration of MicroRT ROIs and Digital ROIs.

The ability to either present the readers with an analog image (MicroRT) or a digital image of the same specimen ROI allows for the reduction or elimination of a large source of variability in comparing these modalities: the cells being viewed. The registration of areas strengthens the evaluation of digital pathology and its comparison to the microscope.

Abbreviations

WSI, WSIs	Whole Slide Image(s)
ROI, ROIs	Region(s) of Interest
MicroRT	Microscope Real-Time Viewing
RT	Real Time
FOV	Field of View
Administrator	The (task) administrator is responsible for task set up and monitoring
Reader	Person evaluation the ROI's
Scan Scale	WSI scanning
IOP	Intermediate Ocular Plane
FN	Field Number

II. Getting Started

Information on how to download, install, and run eeDAP. eeDAP is available for download for Windows and Linux users.

Download eeDAP

Download the eeDAP.zip file from the [GitHub Release Page](#) and extract it. There are two versions inside. One is for windows system the other is for linux system.

Install eeDAP

Follow these instructions to install eeDAP according to what system you have.

Windows:

1. Double click the eeDAP_windows.exe file. It will install the eeDAP in current directory.
2. Double click eeDAP.exe file to launch eeDAP

Linux:

1. Extract the eeDAP_linux.zip file
2. Open a terminal and visit the extracted folder
3. In terminal input "./run_eeDAP.sh" + " " + "matlab runtime libraries directory"

Running eeDAP

How to run a sample eeDAP input file:

- Download Sample WSI Image
 1. Go to [Download page](#)
 2. Save image to the same folder as eeDAP application
- Load input file
 1. From the [eeDAP Administrator Input Screen](#) page, click the button "Click to browse for .dapsi input file."
 2. Navigate into the folder "**sample_inputfiles**"
- Select one of the input files according to the WSI Image and microscope system
 3. Extract the chosen sample file.
 4. Select viewing mode, **Digital** If Digital is chosen, or **MicroRT** (If MircoRT is chosen, continue to [Serial Port configuration](#) or MicroRT Registration)
 5. Click **Start** button to begin
- MicroRT Registration
 - [Low Resolution Registration](#)
 1. Place selected tissue glass onto the stage and change lens according to the file
 2. Move stage manually to a unique location on the tissue and click **Take Stage Poisiton**
 3. Click the same unique location on the thumb WSI and click **Take WSI Position**
 4. Check results
 5. If results are accurate, repeat steps 2,3, and 4 with 2 other unique points
 6. Select **Load last calibration** (Full image will appear)
 - [High Resolution Registration](#)
 1. Change to lens according to file
 2. Click on **Go To Stage Position**, the stage will move automatically to previously taken Stage Position (Low Resolution)
 3. Adjust and center the stage manually to a unique location and click **Take Stage Position**
 4. Click the same unique location on the thumb WSI and click on **Take WSI Position**
 5. Check results
 6. Repeat steps 2,3,4, and 5 with the 2 other unique points
* To skip High Resolution Registration and use only Low Resolution Registration, click on **Finish Registration** (Not recommended)
 - Camera and Eyepiece Registration
 1. Remember unique position between cells and reticle in the camera image
 2. Click **Feature Centered in Camera**
 3. Look through the eyepiece and move the stage to the center of the fracture, the relevant position
 4. Click **Feature Centered in Eyepiece**

* If this is the 1st time, do not skip. If done before, press **Skip Offset**
- eeDAP Study
 1. Input user ID and click Next (*Micro RT Model continue to 2, Digital Model bypass to 5*)
 2. Mircoscope stage will automatically move to last know tissue location and do "Fast Registration"
 3. Focus microscope and check registration result, registration may fail due to unfocused tissue (*Passed registration bypass to 5*)
 4. If registration is not valid, click on "**Reset**"
 5. Then select either "**Fast Registration**" or "**Best Registration**" in the [control panel](#)
 6. Follow the [instructions](#) to complete each given tasks
 7. Use the [control panal](#) to manage the studies

8. When you reached the end "Thank You" will appear, click **Next** to save your data and exit

- eeDAP Results

1. From "**sample_inputfiles**" folder click on "**Output_Files**"
2. Output file name is formated by, "Study Model, User ID, Time, & Input File Name .dapso"
3. Output tissue images folder name is formated same as the output file
4. Output task results are formated by, "Task Name, Task ID, Task Order, Slot (Where the glass was placed)"

**If you are still unable to run eeDAP or want more information, we recommend you go to the [Workflow page](#).*

Tasks Instructions

"select_region" (Select Region)

1. Click on image in the region of the cell that is specified in the description
2. Test will automatically give you the correct X-Y coordinates on the ROI and WSI

"count"

1. Enter the number of cells that appear to accommodate the description

"radio1of4"

1. Select the one of the options that accommodates the description

"slider"

1. On a scale 0-100, rate the tissue using the slider or typing in the number

"mitotic_train"

1. Check all the boxes that apply to the image
2. Using the slider or by typing the number, give the tissue a score
3. Click on "**expert results**" (Boxes highlighted are incorrect, Highlighted number is correct)

"mitotic_expert"

1. Write a discription of what you observe in the image above
2. Click "**Next Part**"
3. Check all the boxes that apply to the image above
4. Using the slider or by typing the number, give the tissue a score

III. Hardware and Software Requirement

Directions on how to set up the hardware and all the equipment to complete the experiment

Stage

Different Microscopes work well with certain stages. Different stages have different resolutions which will cause different results. Below are stage recommendations that will allow end users to obtain the best results as they conduct this experiment.

Lude Stage:

Now, FDA uses Lude MAC5000 and MAC6000 stage

Set up

The stage should connect with computer by RS232 serial to USB cable then continue on to the directions that correspond with the system you are using (Windows or Linux).

Windows 7

In Windows 7 system, user could find the stage by Start -> Control Panel -> Device Manager -> Ports(COM & LPT).

The name of the Port should be something like Prolific USB-to-Serial Comm Port (COM*).

User should choose COM* in eeDAP to connect with stage.

Linux

In Linux use could find the stage by command: "ls -l /dev/tty*".

The port of stage name could be "ttyUSB0". As the default setting of matlab only automatically recognizes serial port names of the form /dev/ttyS[0-255]. User should create a symbolic by command "ln -s /dev/ttyPS0 /dev/ttyS101" under root account. The S101 could be any integer port between S101 to S109. And please choose the same port in eeDAP software. The following link provide detail information about build link: [Building link](#)

If the users don't run the eeDAP under root account, users need provide permission to the account they want to use. They could log in to root account and use following command to provide permissions.

1. Serial port permission:

"chmod 777 /dev/ttyXXX", where ttyXXX is the port name like ttyUSB0

2. Lock group permission:

- a. Command "nano /etc/group"
- b. Add linux account name to lock group
- c. logout and relogin linux account.

Test

When the user selects the mode to be "MicroRT", Microscope Real Time, the program will force the user to set the communication port for the stage. The stage must be plugged into one of the communication ports, and the correct communications port must be selected.

Camera

Different cameras result in different end results. Here you are given directions on what cameras are compatible with this experiment and how to install them

Requirements

The code is written to work with a DCAM compatible camera.

eeDAP requires camera images to be RGB24 (8 bit for each channel), have a width > 640, and height > 480.

FDA uses 3 Point Grey cameras for this eeDAP software

Setup

For **Point Grey Flea2 Color (FL2G-50S5C-C)** camera, users need install special diver to run it under eeDAP software.

Windows 7

After plugging in the camera, you should disable the default Windows driver and update the driver for the attached device to the industry standard DCAM compatible firewire (IEEE 1394 interface) driver. Carnegie Melon (CMU) has a general DCAM driver (1394camera646.exe) at: <http://www.cs.cmu.edu/~iwan/1394/index.html>

Download and Install:

1. In Download tab, click "I agree to the Terms of the LGPL".
2. Download 1394camera646.exe
3. Click Installation tab and install the driver following the instruction

Test:

In the driver installed folder, there are several demo applications (1394CameraDemo32.exe). They behave similarly to the Matlab "imaqtool" mentioned above. You could use it to test whether the camera is installed correctly.

Linux

In Linux, users should install two packages libdc1394-22 and libraw1394-11, as mentioned in the documentation link below, <http://www.mathworks.com/help/imaq/linux-dcam-ieee-1394-hardware.html>

If the users don't run the eeDAP under a root account, users need provide permission to the account they want to use. They could log in to root account and use following command to provide permissions.

- a. Command "nano /etc/group"
- b. Add linux account name to video group
- c. logout and relogin linux account.

Test

When the user selects the mode to be "*MicroRT*", Microscope Real Time, there is a button that launches a Matlab utility called "imaqtool". This utility is an interactive GUI to allow you to explore, configure, and acquire data from your installed and supported image acquisition devices. If this doesn't work, Matlab can't find your camera.

Whenever you plug-and-play a camera, Windows enables the default Windows driver, which may actually be the camera manufacturer's driver. If the default Windows driver isn't DCAM compatible, Matlab can't find your camera.

Alignment

Depending on hardware, you may have the ability to completely align the camera with the eyepiece by rotating and shifting the camera. This process is made possible by loosening and tightening **one - three screws on the camera mount**. This should be done while setting up the equipment and before beginning the experiment. The alignment should persist over time and need to none little adjustments unless the screws are ineffective or the system is bumped. A **calibration slide, reticle, and virtual reticle** are very useful for this alignment. A good camera mount also has a focus screw that can be adjusted so that the camera and the eyepiece are nearly in focus at the same time.

1. Place the grid slide on the stage and change to the highest magnification optics (Figure 1)
 2. Look through the eyepiece and focus the microscope.
 3. Still looking through the eyepiece move stage to let the reticle just touch the boundary of the grid corner.
 4. Depending on your camera:
 - a. If you are **able** to focus your camera, **don't focus** camera image using your microscope
 - b. If you are **unable** to focus your camera, **focus** camera image using your microscope
 5. Loosen the screws around camera. (Figure 3)
 6. Use your hand to adjust the camera to make the reticle in camera image just touch the same boundary of the grid corner.(Figure 2)
 7. Hold the camera and while looking at the camera image, start tightening the screws until it is aligned.
- *Note: 1. Small shift could be solved by eyepiece and camera register processing.
 2. Large shift might influence the fast registration during task. Because our fast registration use a small center area of camera image, larger shift between eyepiece and camera will lead outstanding feature goes out of the camera registration area. Best registration is another method to solve the large shift problem.

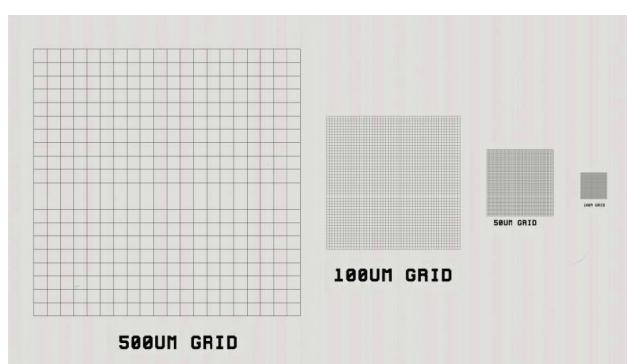


Figure 1

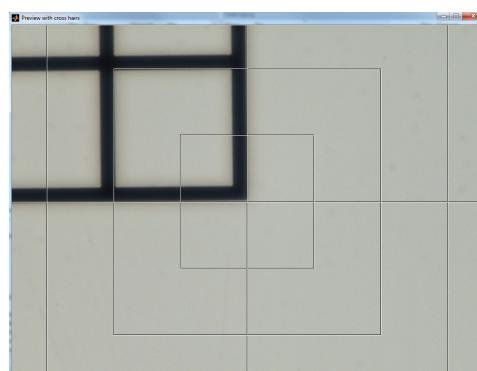


Figure 2

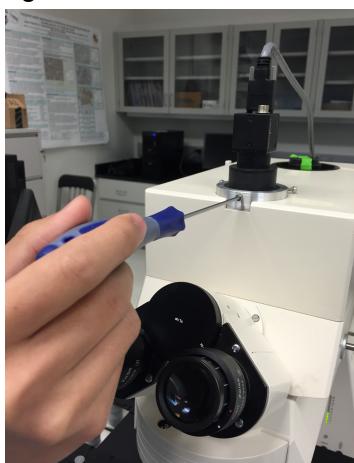


Figure 3

Microscopes

The page that opened was Field of View Diameter at microscopyU. (<http://www.microscopyu.com/tutorials/java/fielddiameter/index.html>) The first sentence is, "The diameter of the field in an optical microscope is termed the field number and represents the diameter of the field measured in millimeters at the intermediate image plane."

Microscopy from the very beginning - Carl Zeiss, Inc. <http://www.microscopy-news.com/news/carl-zeiss-microscopy-from-the-very-beginning.html>

On page 36 it says, "Eyepieces (or oculars) are the magnifiers with which you view the intermediate image in the microscope, produced by the objective and the tube lens. In the Axiolab microscope, the intermediate image has a useful diameter of 20 mm. Eyepieces are not just simple lenses, but are corrected optical systems consisting of several lenses. It would be a pity if the intermediate image produced with such sophisticated optics were to be impaired just before it reaches the eye.

Normally, the additional magnification provided by the eyepiece is 10x. The intermediate image in this example then has a diameter of 20 cm at a reading distance of 25 cm to the eye. A comparison: this diameter is about as large as the width of this page."

Axioplan 2 Imaging microscope with an Axiophot 2 head

- Field Number (FN) of the oculars (eyepieces) = 23mm
- Magnification of eyepiece (mag_e) = 10x
- Magnification of objective (mag_o) = 2.5x, 5x, 10x, 20x, 40x
- FOV at Mag_o = FNmm/mag_o
- FOV at 2.5x = 23mm/2.25 = 9.200mm
- FOV at 5x = 23mm/5 = 4.600mm
- FOV at 10x = 23mm/10 = 2.300mm
- FOV at 20x = 23mm/20 = 1.150mm
- FOV at 40x = 23mm/40 = 0.575mm
- Apparent diameter of microscope image "at 25cm" = FN*mag_e = 23cm
- Diameter for ocular micrometer (reticle) = 26mm

Olympus BX43

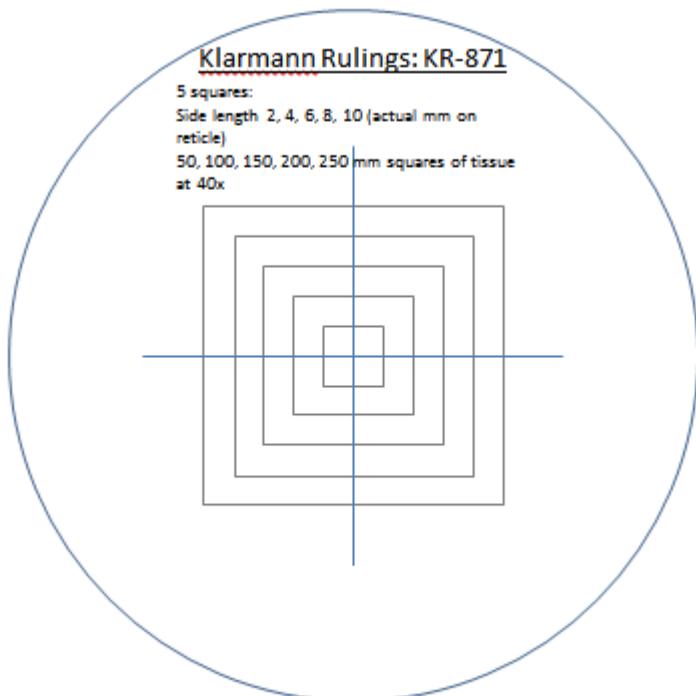
- Field Number (FN) of the oculars (eyepieces) = 22mm
- Magnification of eyepiece (mag_e) = 10x
- Magnification of objective (mag_o) = 2x, 4x, 10x, 20x, 40x
- FOV at Mag_o = FNmm/mag_o
- FOV at 2x = 22mm/2 = 11.00mm
- FOV at 4x = 22mm/4 = 5.50mm
- FOV at 10x = 22mm/10 = 2.20mm
- FOV at 20x = 22mm/20 = 1.10mm
- FOV at 40x = 22mm/40 = 0.55mm
- Apparent diameter of microscope image "at 25cm" = FN*mag_e = 22cm
- Diameter for ocular micrometer (reticle) = 24mm

Reticles

Reticle is placed in eyepiece at Intermediate Ocular Plane (IOP). It could help use define the position of the tissue under microscopes

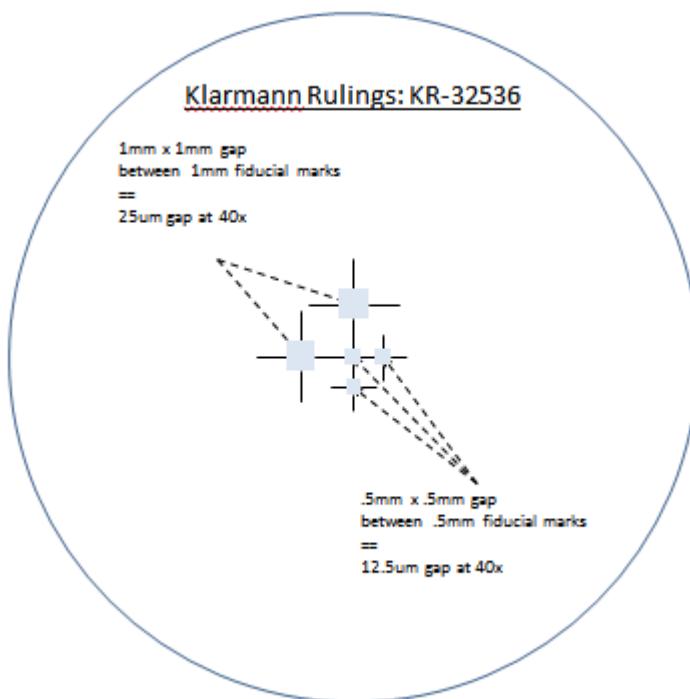
Klarmann Rulling: KR-871

This reticle contains 5 squares with actual lengths of 2, 4, 6, 8, and 10mm.



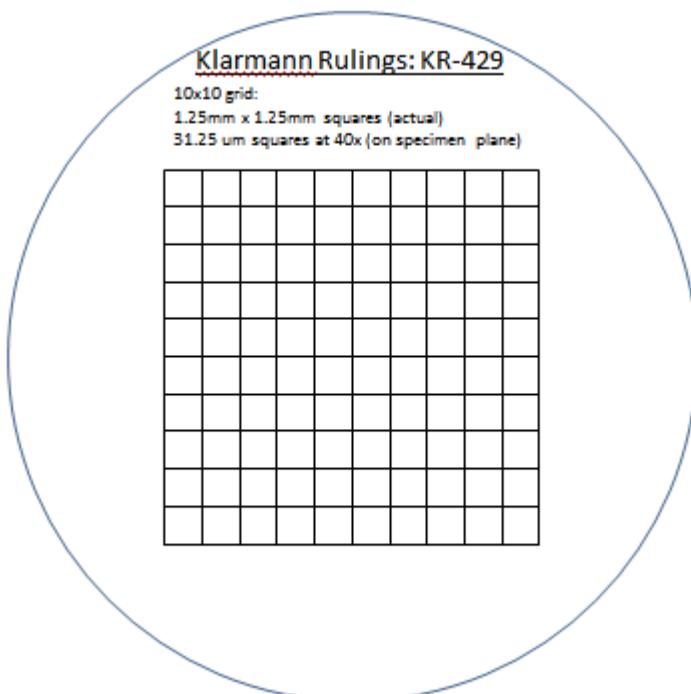
Klarmann Rulings KR-32536

This is a custom reticle with cross-hair-like fiducials pointing to gaps: 2 are 1mm x 1mm and 3 are 0.5mm x 0.5mm.



Klarmann Rulings KR-429

The width and length of 10x10 grid at IOP = 12.5mm, grid spacing "at 25cm" = 12.5mm.



Software Requirements

As you use the software there are some requirements that should be followed for the software to run smoothly. Here are some instructions on how you will be able to fix problems you may come across when using eeDAP.

Read and extract WSI image

There are two methods to extract ROI from WSI image "BIO formats" and "Image Scope".

BIO Formats

Bio-Formats is currently being used in eeDAP.

Bio-Formats is a standalone Java library for reading and writing life sciences image file formats. It is capable of parsing both pixels and metadata for a large number of formats, as well as writing to several formats.

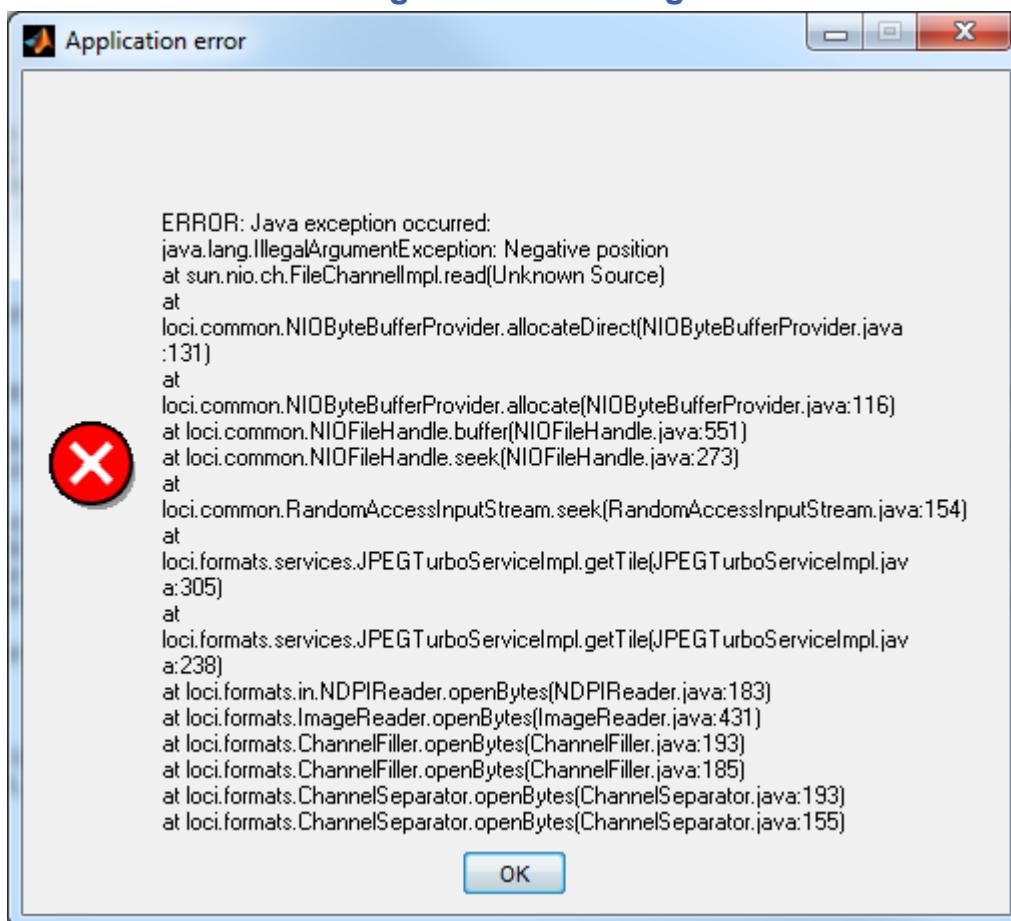
Bio-Formats is developed by the Open Microscopy Environment consortium, including development teams at LOCI at the University of Wisconsin-Madison, University of Dundee and Glencoe Software. Licensing and citing information is on the [OME licensing page](#).

The primary goal of Bio Formats is to facilitate the exchange of microscopy data between different software packages and organizations. It achieves this by converting proprietary microscopy data into an open standard called the OME data model, particularly into the OME-TIFF file format.

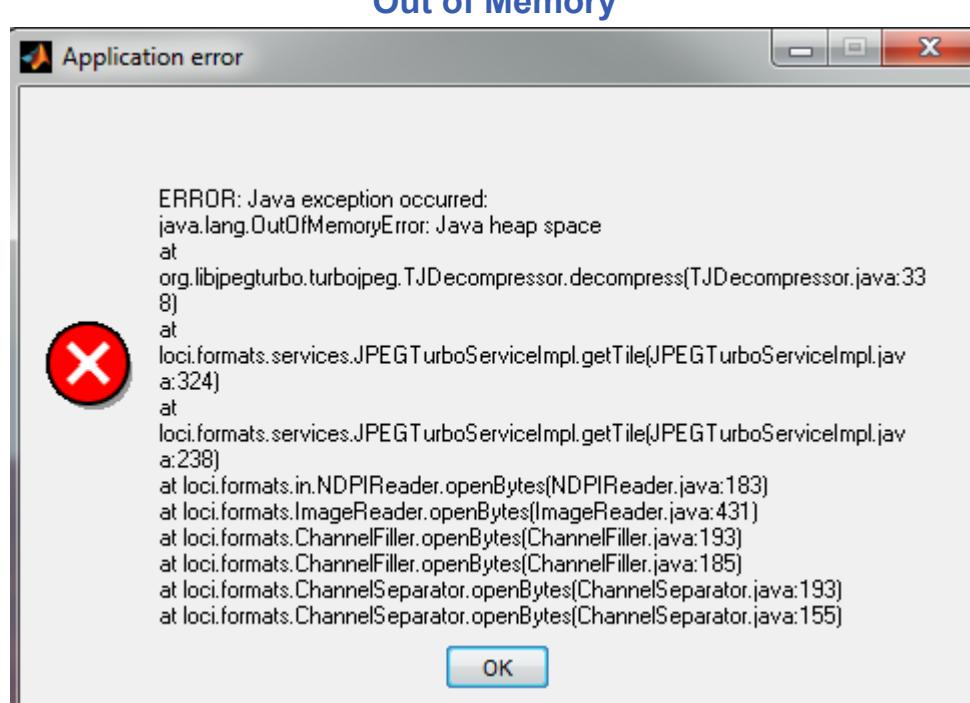
Error

The BIO_formats might have some problem when extract WSI. In this section we show 2 normally errors and the solve methods

Image File is too Large



This error is because the input WSI image file is too large. Now we are using 5.1.2 version Bio formats. It works for file smaller than 4 GB. If you want to use larger image, you might face this problem



When we process a large image, there might be a error message about out of memory. For large image a minimum of 512 MB is suggested.

Users could download the java.opts and paste it in the matlabroot directory, or follow this website : <http://www.mathworks.com/matlabcentral/answers/92813-how-do-i-increase-the-heap-space-for-the-java-vm-in-matlab-6-0-r12-and-later-versions>.

Image Scope by Aperio

Image Scanscope is our previous used method, which is a product of Aperio <http://www.aperio.com/healthcare/eslide/view>. This software contains an Active X controller name TIFFcomp which allows for manipulating of WSI images (panning, zooming, etc.). eeDAP uses TIFFcomp to extract the ROI's from the WSI images. The reason we choose new method is Image Scanscope could only works in 32 bit Matlab.

Matlab compiler runtime libraries

The precompiled stand-alone eeDAP application requires that the Matlab compiler runtime (MCR) library be installed. Which works on Windows and Linux, but it does not require any Matlab license or libraries.

The MCR library must be the same version as was used to create the stand-alone application. The current versions of the MCR library that eeDAP is using are Windows 32-bit R2013a (8.1) and Linux 64-bit R2015a(8.5)

The installer can be found at:

- possible local folder: C:\Program Files (x86)\MATLAB\R2013a\toolbox\compiler\deploy\win
- internet: <http://www.mathworks.com/products/compiler/mcr/>
- internet: <http://medviso.com/download/mcrinstaller>

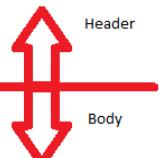
The installer will create a folder like

C:\Program Files (x86)\MATLAB\MATLAB Compiler Runtime\v81

IV. Input File

All Input files for this program have the extension .dapsi. Two sample files have been provided for instructional purposes. They can be located under the “eeDAP→Sample_Input_Files”. In the following, we use Phantom_Test.dapsi to illustrate our discussion.

An input file has two sections: Header and Body. The Header includes free text that describes the experiment and formatted text that specifies global variables. The Body is a list of ROIs and corresponding tasks.



```
tissue40x-8B-axiplan2i.dapsi - Notepad
File Edit Format View Help
Author :
Date :
Time :

For the Hamamatsu scanning at 20x
scan_scale = .4558
For the Hamamatsu scanning at 40x
scan_scale = .2279

Point Grey Grasshopper Color (GRAS-03K2C-C)
cam_format = RGB24_640x480
cam_pixel_size = 7.4um
Point Grey Flea2 Color (FL2G-50S5C-C): Full resolution, full format
cam_format = F7_RGB24_2448x2048
cam_pixel_size = 3.45
Point Grey Flea2 Color (FL2G-50S5C-C): standard format, aspect ratio = 1.33
cam_format = RGB24_1024x768
cam_pixel_size = 6.9

Reminder :
taskorder=2 user specified order
taskorder=1 listed order
taskorder=0 random order
saveimages = 1 save WSI and camera images
saveimages = 2 only save WSI image
saveimages = 3 only save camera image
saveimages = 4 don't save WSI and camera images

Input File Task Formats
task_checkMof4,TaskID,TaskOrder,Slot,ROI_X,ROI_Y,ROI_W,ROI_H,IMG_W,IMG_H,Qtext,MoveFlag,zoomFlag,Q_Op1,Q_Op2,Q_Op3,Q_Op4
task_count , TaskID,TaskOrder,Slot,ROI_X,ROI_Y,ROI_W,ROI_H,IMG_W,IMG_H,Qtext,MoveFlag,zoomFlag,Q_Op1,Q_Op2,Q_Op3,Q_Op4
task_mark1 , TaskID,TaskOrder,Slot,ROI_X,ROI_Y,ROI_W,ROI_H,IMG_W,IMG_H,Qtext,MoveFlag,zoomFlag,Q_Op1,Q_Op2,Q_Op3,Q_Op4
task_mark1_out , TaskID,TaskOrder,Slot,ROI_X,ROI_Y,ROI_W,ROI_H,IMG_W,IMG_H,Qtext,MoveFlag,zoomFlag,Q_Op1,Q_Op2,Q_Op3,Q_Op4
task_radio1of4 , TaskID,TaskOrder,Slot,ROI_X,ROI_Y,ROI_W,ROI_H,IMG_W,IMG_H,MoveFlag,zoomFlag,Q_Op1,Q_Op2,Q_Op3,Q_Op4
task_slider , TaskID,TaskOrder,Slot,ROI_X,ROI_Y,ROI_W,ROI_H,IMG_W,IMG_H,MoveFlag,zoomFlag,Q_Op1,Q_Op2,Q_Op3,Q_Op4
task_mitotic_expert , TaskID,TaskOrder,Slot,ROI_X,ROI_Y,ROI_W,ROI_H,IMG_W,IMG_H,MoveFlag,zoomFlag
task_mitotic_train , TaskID,TaskOrder,Slot,ROI_X,ROI_Y,ROI_W,ROI_H,IMG_W,IMG_H,MoveFlag,zoomFlag,ExpertCheckBoxes1-8,Expert_Score
task_mitotic_counts , TaskID,TaskOrder,Slot,MoveFlag,zoomFlag

SETTINGS
NUMBER_OF_WSI      = 1
wsi_slot_1         = C:\000_whole_slides\tissue40x-8B.ndpi
rgb_lut_slot_1     = icc_profiles\rgb_lut_gamma_invip8.txt
label_pos          = 6
reticleID          = KR-32536
cam_format          = RGB24_1024x768
cam_pixel_size     = 6.9
mag_cam             = 1.0
mag_res             = 5
mag_hres            = 40
scan_scale          = 0.2279
stage_scale         = 0.1
BG_Color_RGB        = 0.55 = 0.55 = 0.55
FG_Color_RGB        = 0.00 = 0.00 = 0.00
AxesBG_Color_RGB   = 0.10 = 0.20 = 0.10
FontSize            = 13
saveimages          = 3
taskorder           = 0

BODY
start
finish
radio1of4,1st0001,-1,1,038459,16192,300,300,300,300,Qtext_radio1of1,1,1,Q_Op1,Q_Op2,Q_Op3,Q_Op4
count,2nd0001,-1,1,027728,11381,300,300,300,300,Qtext_Count,1,1,description
slider_3rd0001,-1,1,19220,49879,300,300,300,300,Qtext_Slider,1,1,Q_Op1,Q_Op2,Q_Op3,Q_Op4
mark1_4th0001,-1,1,041163,33208,300,300,300,300,Qtext_Select_region,1,1,Q_Op1,Q_Op2,Q_Op3,Q_Op4
mark1_out_5th0001,-1,1,041163,33208,700,700,700,700,Qtext_Select_region,1,1,Q_Op1,Q_Op2,Q_Op3,Q_Op4
mitotic_train_6th0001,-1,1,041163,33208,700,700,700,700,Qtext_Select_region,1,1,Q_Op1,Q_Op2,Q_Op3,Q_Op4
mitotic_expert_7th0001,-1,1,041163,33208,700,700,700,700,Qtext_Select_region,1,1,Q_Op1,Q_Op2,Q_Op3,Q_Op4
mitotic_counts_8th0001,-1,1,1,1,1,1
```

Input File Header

The Header is all the text before the line containing “BODY”. Please refer to the figure which shows the input file being edited in a plain text editor. The first section is free text and should be used to outline the author, experimental setup, date, time, and a short description of the experiment and hardware. The figure above shows what objectives are expected on the microscope. Following the line containing “SETTINGS”, global parameters are defined. First is the number of WSI and the figure shows how to specify the corresponding WSIs (full paths and filenames). Next, "label_pos" define the direction of the glass slide in microscope stage. And microscope settings that are under control are then defined: the objective, filter, and reflector. The GUI and screen characteristics are defined: foreground color, axes color, background color, font size and screen size. Colors are defined using simple RGB coordinates scaled within the range of zero to one. Then "saveimages" controls whether save experiment image. Finally, the last line of text “taskorder” controls the order the tasks are presented to the reader.

Input File Body

The input file Body begins with the line containing “BODY” and follows with a line of column labels. Each line below the line of column labels represents a task, identifying an ROI and the task. There are 19 columns identifying the ROI and task. See “Selecting ROIs” in the “Utilities” section, and the “Tasks” section below. The 19 columns are explained here.

Default task input format

A.

Task Name: Describe name for each task

Task ID: Identification number of the task independent from the order that they are presented

Task Order: The order the slide is to be presented to the reader if the value of “taskorder” defined in the Header is set to “1”.

Slot: Specifies the stage slot for the task. This, consequently, specifies the glass slide for the task based on the header information.

B.

ROI_X: Defines center of the ROI; point of reference for the extraction process

ROI_Y : Defines center of the ROI; point of reference for the extraction process

ROI_W: Width of the area in pixels to be extracted for the ROI

ROI_H: Height of the area in pixels to be extracted for the ROI

IMG_W: This parameter defines the size of the image that will be presented to the reader.

IMG_H: This parameter defines the size of the image that will be presented to the reader.

Q_Text: This field contains the question (free text) displayed to the reader. Do not use commas here or the input file format will be corrupt.

MoveFlag: A value of 1 allows for the Reader to pan, value of 0 prohibits

ZoomFLag: A value of 1 allows for the Reader to zoom, value of 0 prohibits

Q_Op1: Displays text for this answer

Q_Op2: Displays text for this answer

Q_Op3: Displays text for this answer

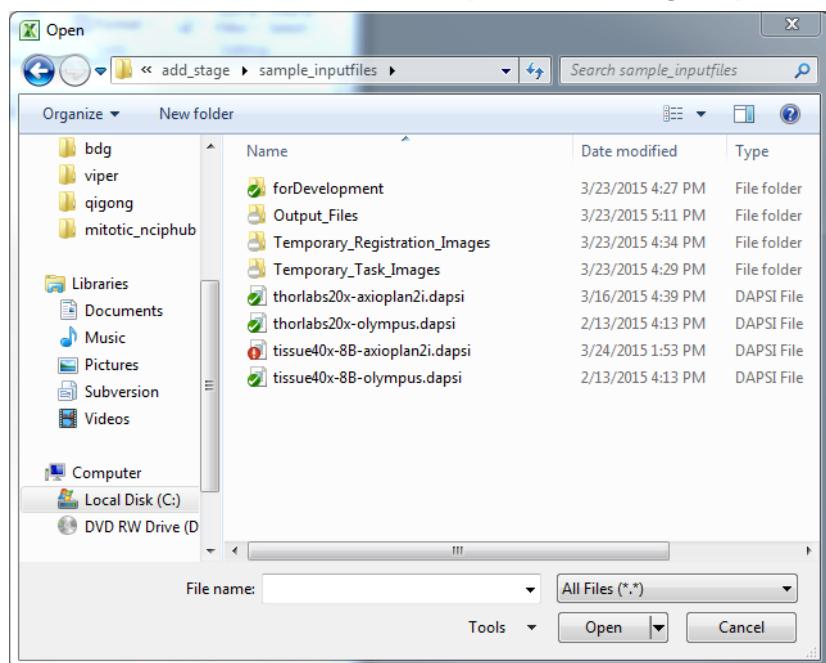
Q_Op4: Displays text for this answer

Customized Input File Body: List of ROIs and Corresponding Task

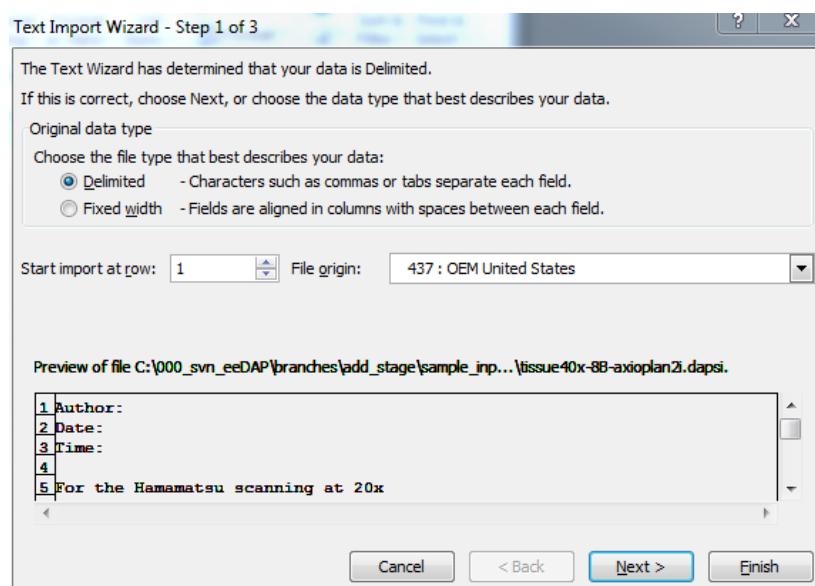
Customized Input File format should follow "Task Name, Task ID, Task Order and Slot".

The left parts could design for different task.

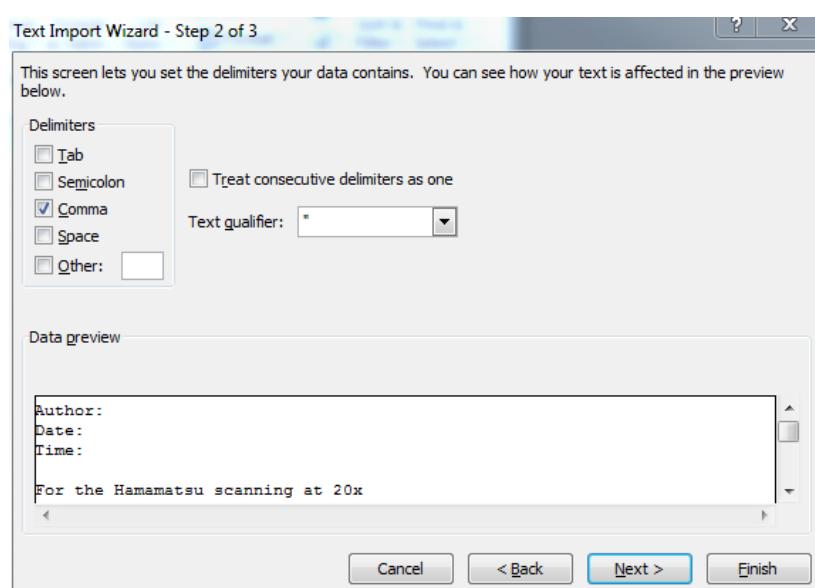
Input File Body: Opening and Editing



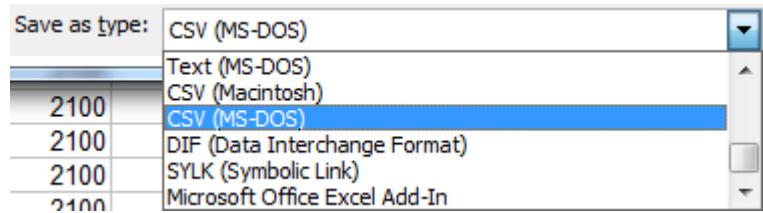
The Body of an input file is editable by a spreadsheet editor such as Excel. For example, open Excel and navigate to eeDAP→Sample_Input_Files→Phantom_Test and open Phantom_Test.dapsi. Make sure in Excel's open dialogue “all files is selected” (See Figure), otherwise you won't file eeDAP input files with the extension “.dapsi”.



After selecting Phantom_Test.dapsi, the “Text Import Wizard, Step 1 of 3” will open (See Figure). Select the “Delimited” option and click “Next”.



In the next window, “Text Import Wizard, Step 2 of 3”, select how the file is delimited, in this case highlight “Comma”.



In the final window, “Text Import Wizard, Step 2 of 3”, select “Finish”, this will open the file in Excel, aligning rows and columns, and allowing editing. Once editing is complete, “Save As” a “.CSV” file which is plain text with comma separated variables.

V. Output File

An “Output_Files” folder will be created in the folder where the input file is. eeDAP writes to this folder the experiment output files (file extension .dapso). The naming convention is “Mode.ReaderName.Date .InputFileRootName.dapso”. eeDAP also writes to a subfolder (name similar to output file) images captured by the microscope’s camera and WSI depend on the setting of "saveimages" in input file . The images captured are taken when the task is completed, and the Administrator selects the “Next” button, advancing to the next task. The captured images provide further opportunities for verification of registration and other considerations.

eeDAP Output files are very similar in appearance to input files. They are comma delimited and have a Header (mostly free-text) and Body (comma-separated variables). Please refer to the “Input File” section for basics on the content of the Output file and instructions for opening and editing the Output file. Once this file is open the header will be displayed, figure to the right. Much of this information should be repetitive from the input file’s header. The main differences between the Input file and the Output file are as follows:

- The Header begins with the Output file filename, which specifies the reading mode, reader name, date, time and the input file name -
- The Body includes additional column for task Duration (amount of time the reader spent on each task in recorded in seconds) and reader's task results.

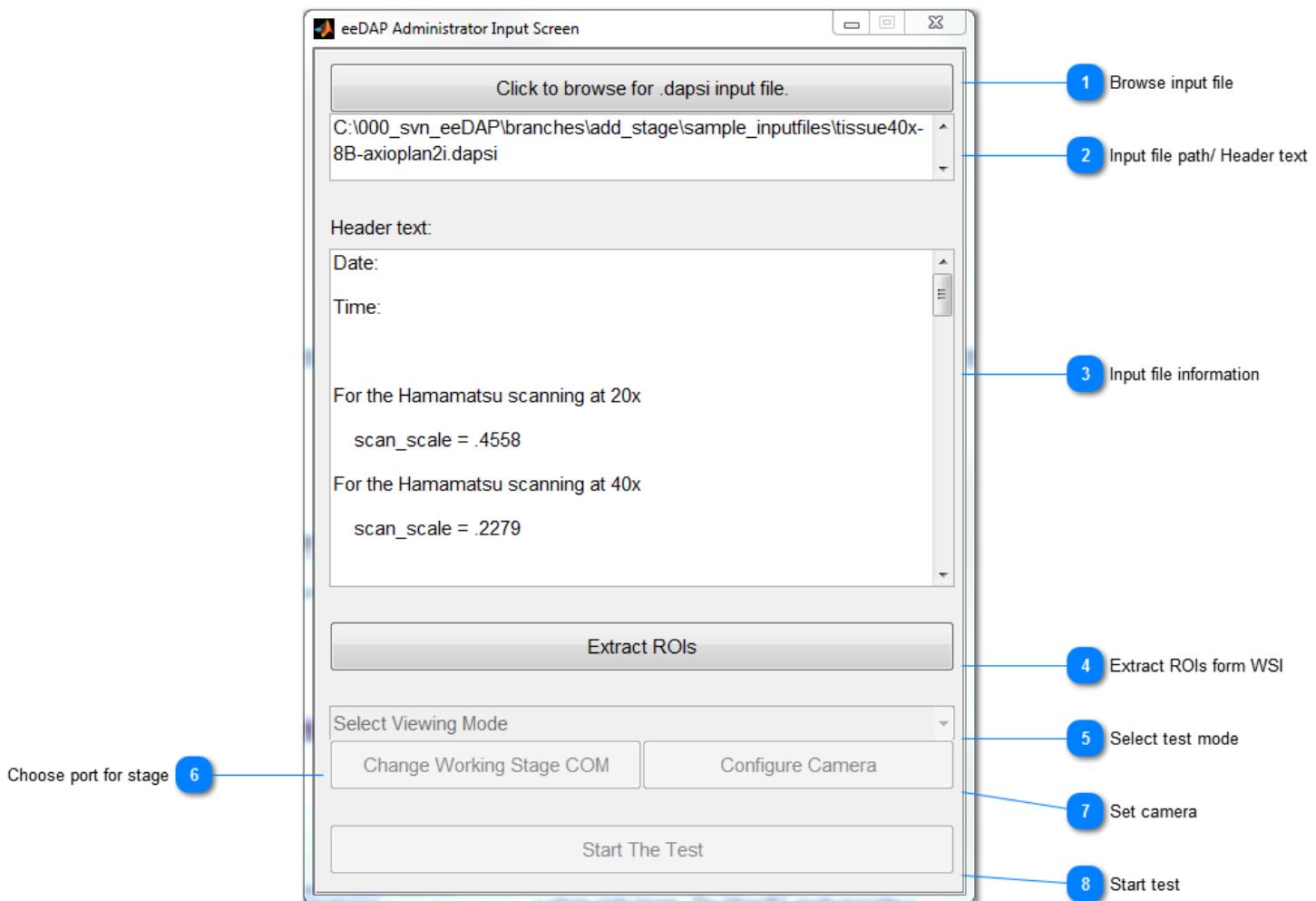
VI. WorkFlow

Guidelines of eeDAP in more detail.

eeDAP Administrator Input Screen window(c)

In the first box the admin selects the input file containing the tasks; see the “[Input File](#)” section for formatting of this file. The file is selected using the “Browse” button. Once chosen the header of the file is displayed in “Header text” box. Selecting “Extract ROIs” button will extract the experiment ROI’s from the WSI images. Extracting ROI’s may take a few minutes depending on the size of the experiment and the computer capabilities.

The drop down menu (Testing Mode) allows for the administrator to choose either the *Digital* or *MicroRT* reading mode. The Digital mode allows for the evaluation of ROI’s identified and extracted from a whole slide Image. The MicroRT mode provides a real-time (RT) view of the same ROI’s through the optics of an adjoining microscope. Furthermore, the administrator is able to track a reader’s progress during MicroRT mode; a camera provides the administrator with the reader’s field of view (FOV) and the corresponding WSI ROI for comparison.



1 Browse input file

Browse input file from computer

2 Input file path/ Header text

After browse input file, shows the path of

the file

3 Input file information

Date:

Time:

For the Hamamatsu scanning at 20x
scan_scale = .4558

For the Hamamatsu scanning at 40x
scan_scale = .2279

After browse input file, shows the detail information of the file

4 Extract ROIs form WSI

Following the input file, extract Region of

- This will create folders in the "sample_inputfiles" directory "Output_Files", "Temporary_Registration_Images", and "Temporary_Task_Images"

- This will also create .tif files in the "Temporary_Task_Images" directory.

One for each task listed in the .dapsi input file.

5 Select test mode

Choose Digital mode for just use WSI digital image in experiment. Choose microRT mode use both WSI digital and microscope live image in experiemnt

6 Choose port for stage

In microRT mode, set the stage controller connect port

7 Set camera

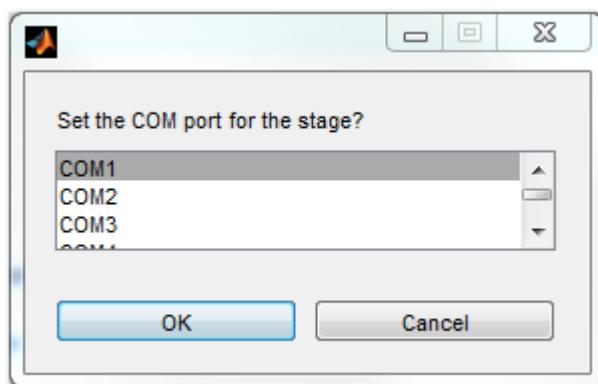
In microRT mode, set camera

8 Start test

Start test

MicroRT Registration

Before data collection in MicroRT mode, the glass slides must be registered to the corresponding WSIs. After registration, the ROIs viewed through the microscope eyepiece in MicroRT mode will be the same as those extracted from the WSI in Digital mode.

MicroRT Mode - Serial port configuration

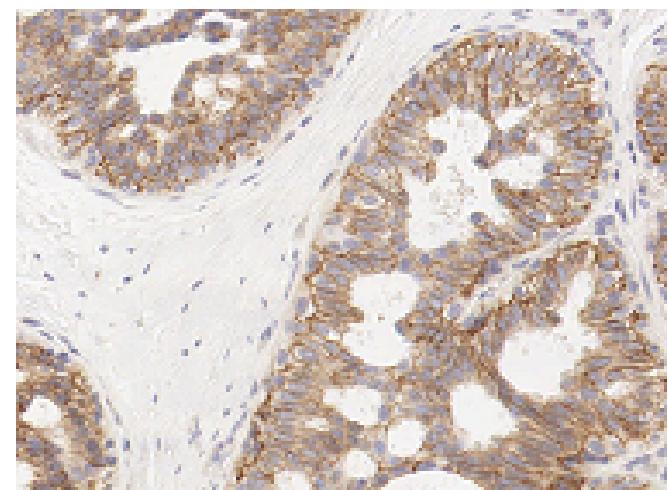
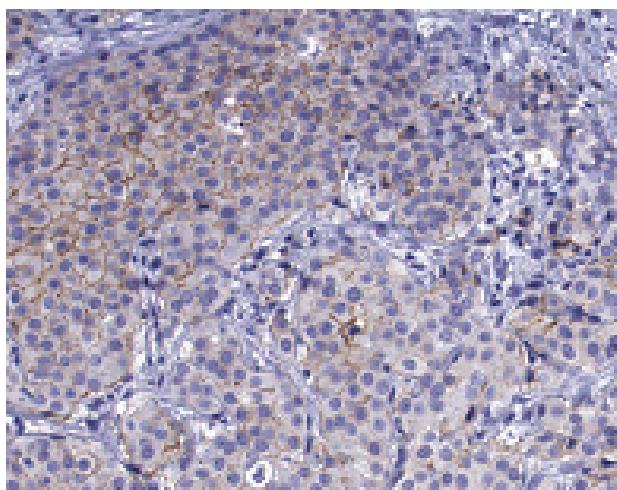
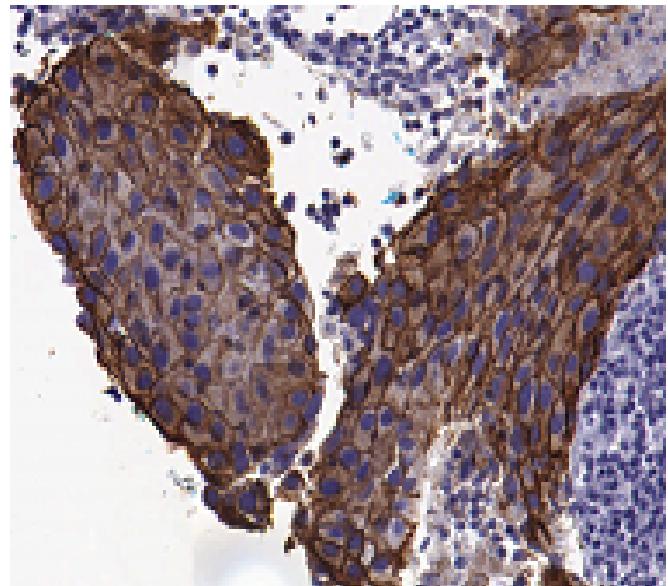
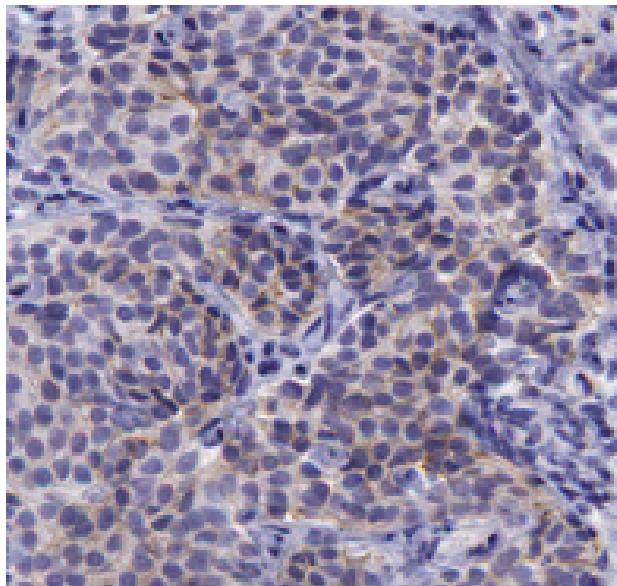
Set the *COM port* connection for the microscope stage controller.

*To specify the correct COM port, open the PC's control panel then open Device Manager and expanded the Ports.

Semi-Automated Registration

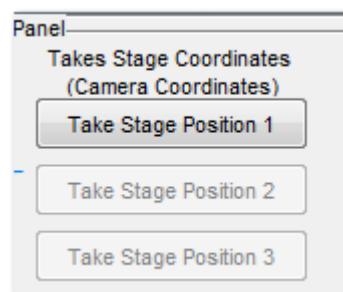
Verify the glass slide's label corresponds to the WSI presented in that slot of the motorized stage, also verify that the video preview window has been launched and lens.

Use the joystick to control the microscope stage. Navigate to a non-homogenous area of the slide, such as the end of the tissue, or a unique feature in the tissue. Good and Bad features are displayed below.

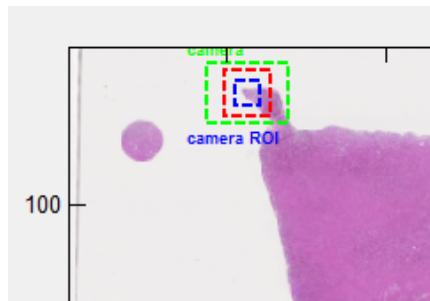


Low Resolution Registration

1. After find desire area, press "**Take Stage Position 1**"



2. Click the related position in the WSI thumb image



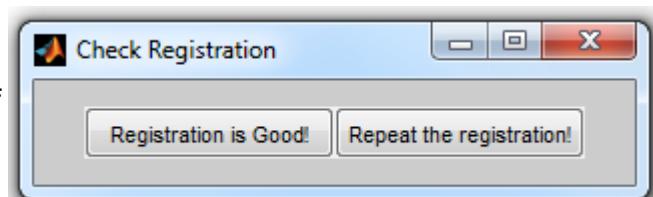
3. Press "**Take WSI Position**" to start registration



4. Check the registration result in Registration Position 1 window



5. Decide whether satisfied the registration. If yes press "**Registration is Good!**" to continue. If not, press "**Repeat the registration!**" to redo registration for this position

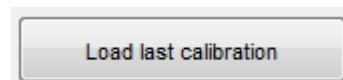


6. Repeat process twice for 2 other positions

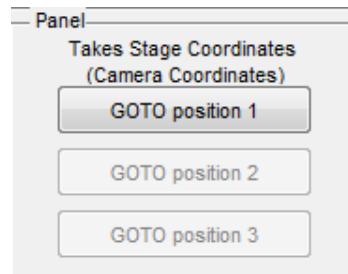
High Resolution Registration

1.Change len to high resolution

2.After finish 3 positions low resolution registration. Press "**Load last calibration**" to get into high resolution registration, we can also use this button to skip low resolution registration, If we do experiment for previous input file with glass slide put at same position on the stage.



3.Press "**GOTO position 1**" to move stage to first low resolution registration position



4.Similar as low resolution registration part, click WSI image for related area for high resolution registration

5 Repeat steps 3, 4 twice for 2 other position

*Note: User could use "**Finished Registration**" button to skip High Resolution Registration (Not Recommended)



Manual Registration

When automated registration fails for a slide, manual registration process may be necessary. This method requires the Administrator to identify three registration points and find them in the WSI and the glass slide. Remember to maximize the distance between the registration points and select points that have unique features in non-homogenous areas of the WSI. The Administrator needs to input the corresponding pairs of x,y coordinates in the Registration window: the x,y coordinates in the WSI and the x,y coordinates on the glass slide.

Panel-	WSI Position 1		
X:	<input type="text" value="3391.1"/>	Y:	<input type="text" value="79729."/>
Stage Position 1			
X:	<input type="text" value="35272"/>	Y:	<input type="text" value="10262"/>
Panel-	WSI Position 2		
X:	<input type="text" value="6321.5"/>	Y:	<input type="text" value="10172."/>
Stage Position 2			
X:	<input type="text" value="51153"/>	Y:	<input type="text" value="10735"/>
Panel-	WSI Position 3		
X:	<input type="text" value="11333"/>	Y:	<input type="text" value="10353."/>
Stage Position 3			
X:	<input type="text" value="51414"/>	Y:	<input type="text" value="35133"/>

To determine an x,y coordinate in the WSI,

1. Open the WSI file in Aperio's ImageScope

2. Select the “Extract Region”  tool in ImageScope

3. Pan over the image and note that the current x,y coordinates of the cursor are displayed at the bottom of the window.

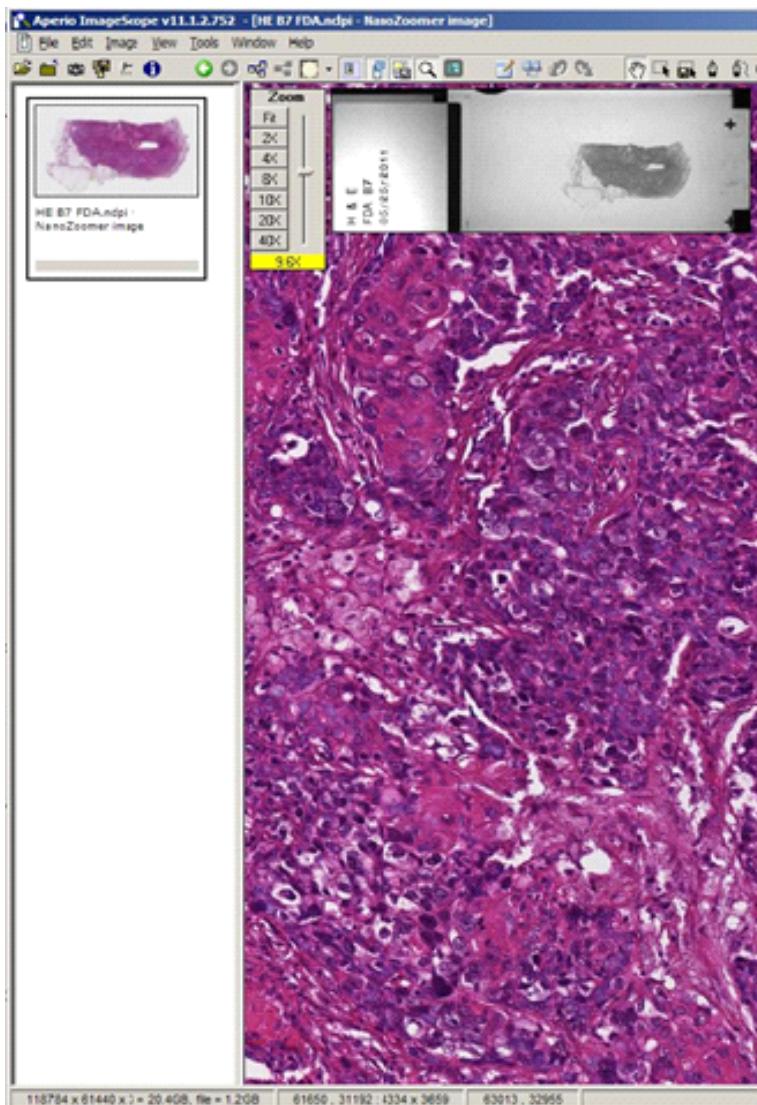
4. Identify a registration point and record the x,y coordinate in the Registration window.

To determine the corresponding x,y coordinate on the glass slide.

5. Using the joystick to control the microscope’s motorized stage, locate the same point selected in the WSI. It is useful to have a reticle with cross hairs at the center.

6. If you do not have a reticle with cross hairs, you can use the Video Preview image to center the registration point.

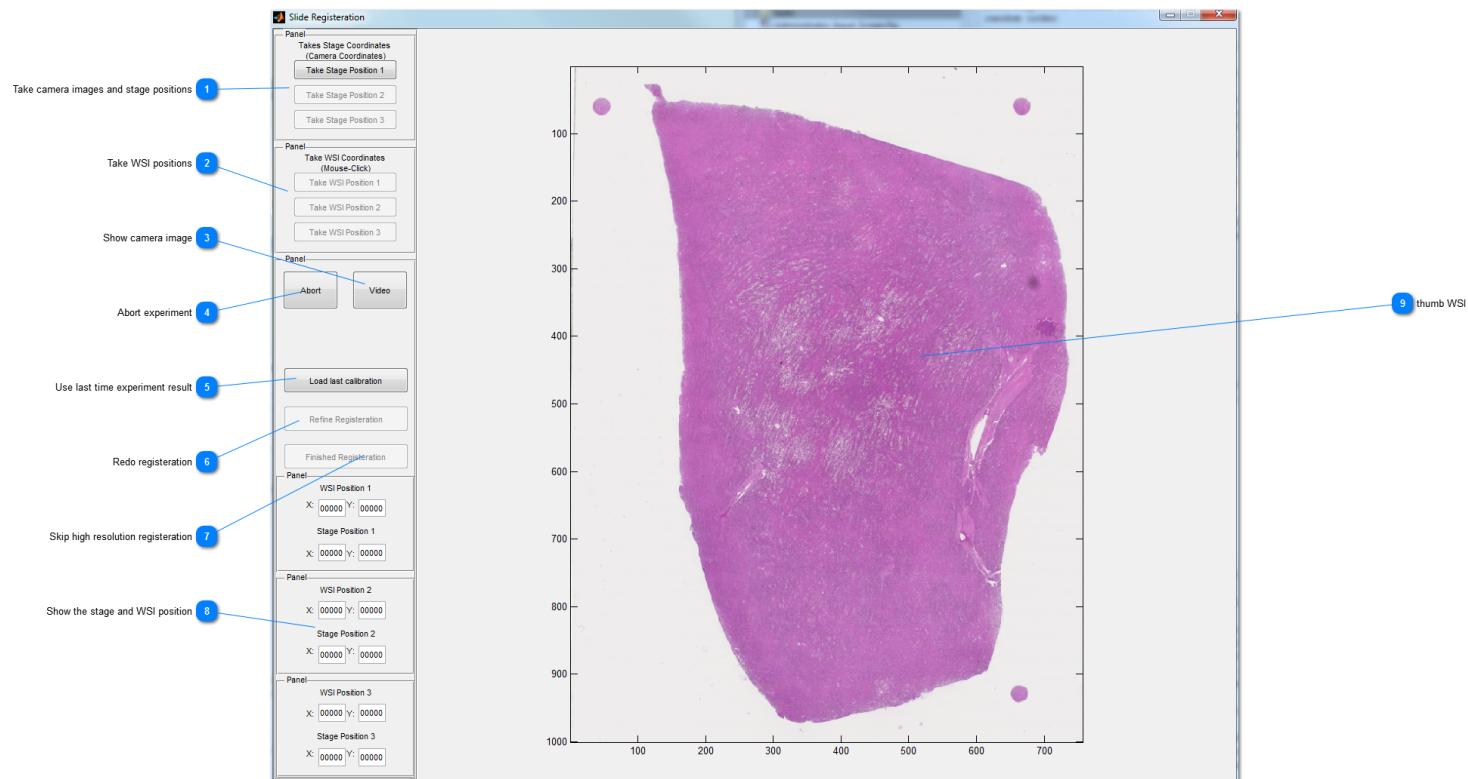
7. Press “**Take position 1**”, this fills in the glass slide coordinates.



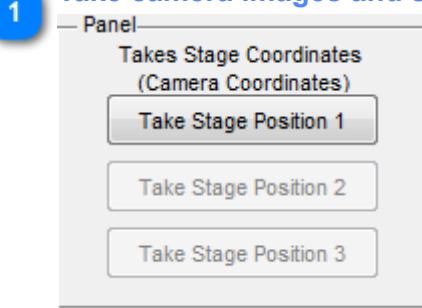
Once the x,y coordinates have been recorded for a registration points, press “**Register ROI**”. This may take a few minutes to execute. Repeat this process twice more locating and registering Points 2 and 3. Repeat this procedure for all slides loaded into slots 1-8 and hit “done” button.

Slide Registration Window

In the MicroRT mode the Slide Registration window will open. Also, the microscope camera will engage, and a video preview window will appear. In this step the Administrator verifies and registers the glass slides to the corresponding WSIs. This ensures that the reader's FOV in MicroRT will be the same as the ROI presented in the Digital mode. Refer to the "[MicroRT Registration](#)" section for details.

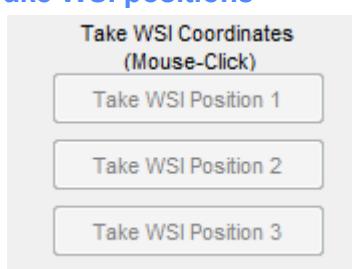


1 Take camera images and stage positions



Take the microscope live image and stage position for 3 areas

2 Take WSI positions



Take the related WSI position for 3 areas

3 Show camera image

4 Abort experiment**5 Use last time experiment result**

Load last time 3 areas WSI and stage positions

6 Redo registration

Redo registration

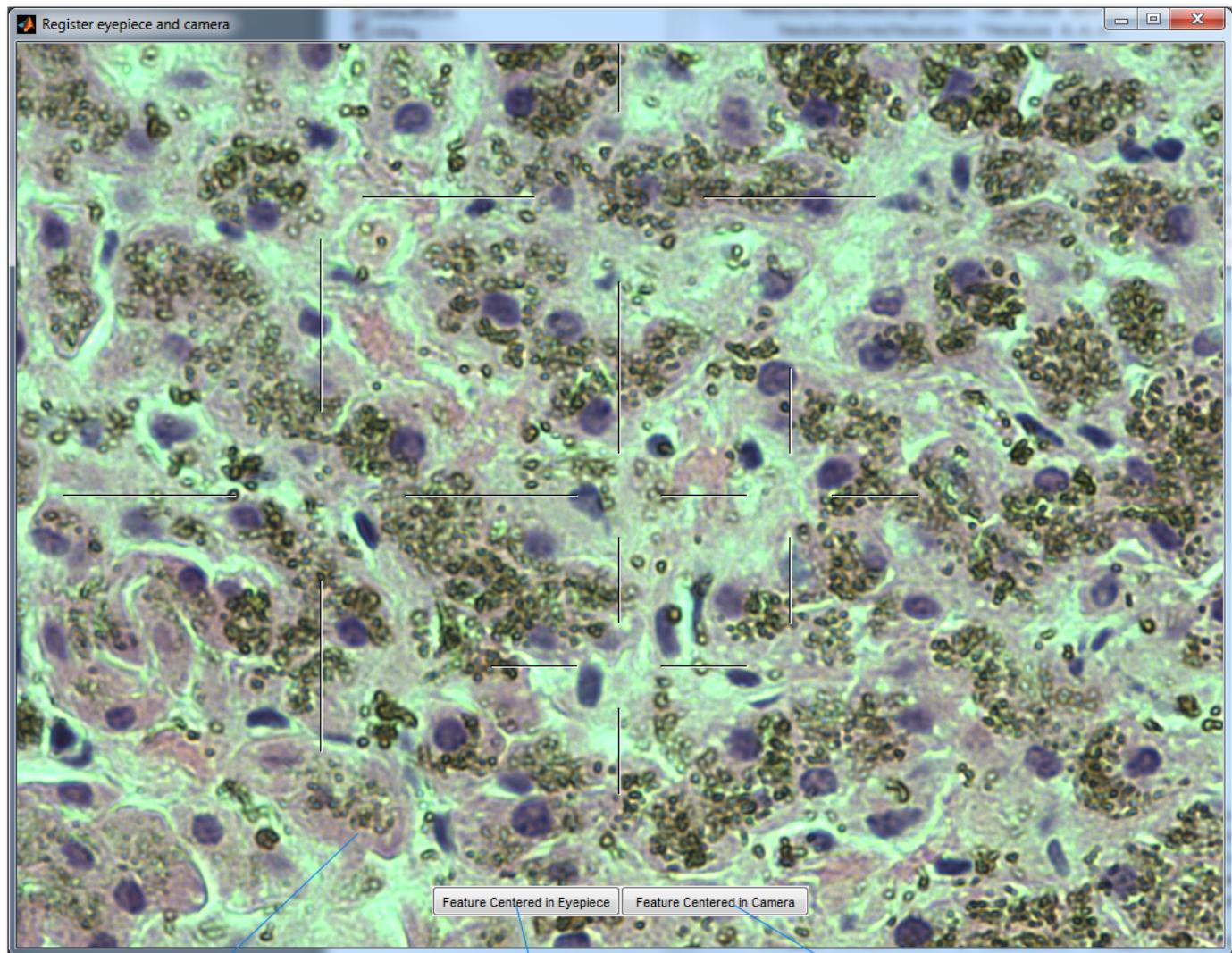
7 Skip high resolution registration

Skip high-resolution registration, directly use low-resolution or last experiment postions

8 Show the stage and WSI position**9 thumb WSI**

Register Eyepiece and Camera Window

Register the camera image with eyepiece image.



Camera image 1

Take the stage positon for eyepiece

3 Take the stage position for camera image

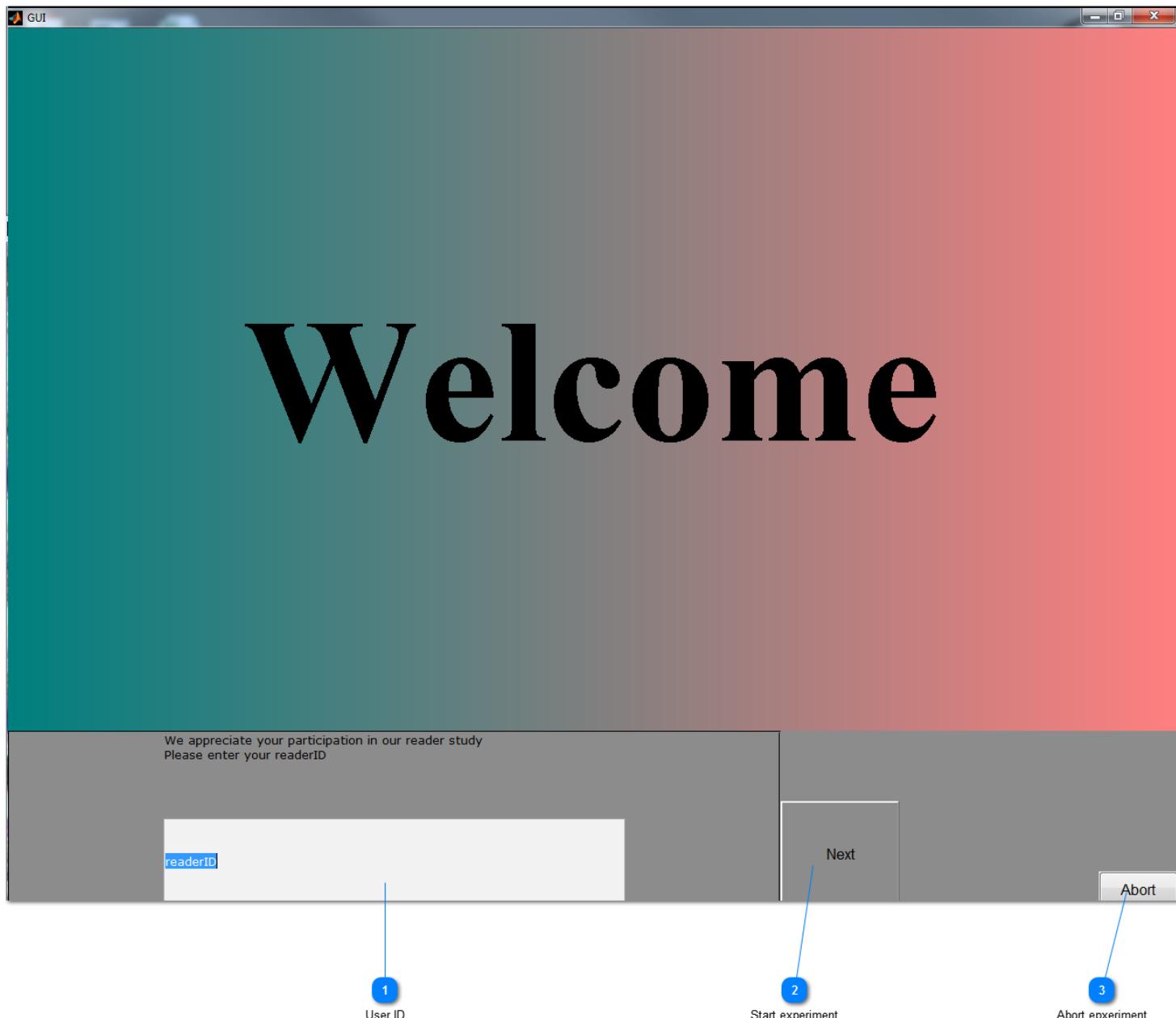
1 Camera image

2 Take the stage positon for eyepiece

3 Take the stage position for camera image

GUI Welcome Page Window

The administrator will enter the reader's name or unique identifier and then proceed by clicking on the "Next" button.



1 User ID

Input user name

2 Start experiment

After input user name start experiment

3 Abort epxperiment

Experiment Interface

Once you input the reader's ID, the evaluation tasks begin when the ROI Image Presentation Screen opens. It is presented in a fashion identical to the Digital mode experiment with the addition of the Video Preview window. The Administrator is expected to read the tasks to the reader and record the responses of the Reader so that the Reader may stay engaged with the microscope. Please refer to the section on "[Tasks Instructions](#)". The Administrator is also expected to monitor the Video Preview to confirm proper registration throughout the experiment.

WSI

1 WSI

ROI of WSI

2 Task

Shows the information and question for each task

3 Control Panel

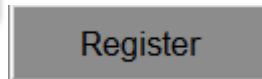


Controls for the Mirco RT experiment

4 Video button

show camera image, only available in MicroRT mode

5 Register button



Register WSI and camare image and adjust the stage position, only available in

MicroRT mode

6 Next button



go to next task

7 Reset button



Reset all the result in this task and move stage to original position

8 Pause button



Pause current task

9

Resume button

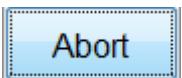
Resume task from pause status

10

Back Button

Go back to previous task

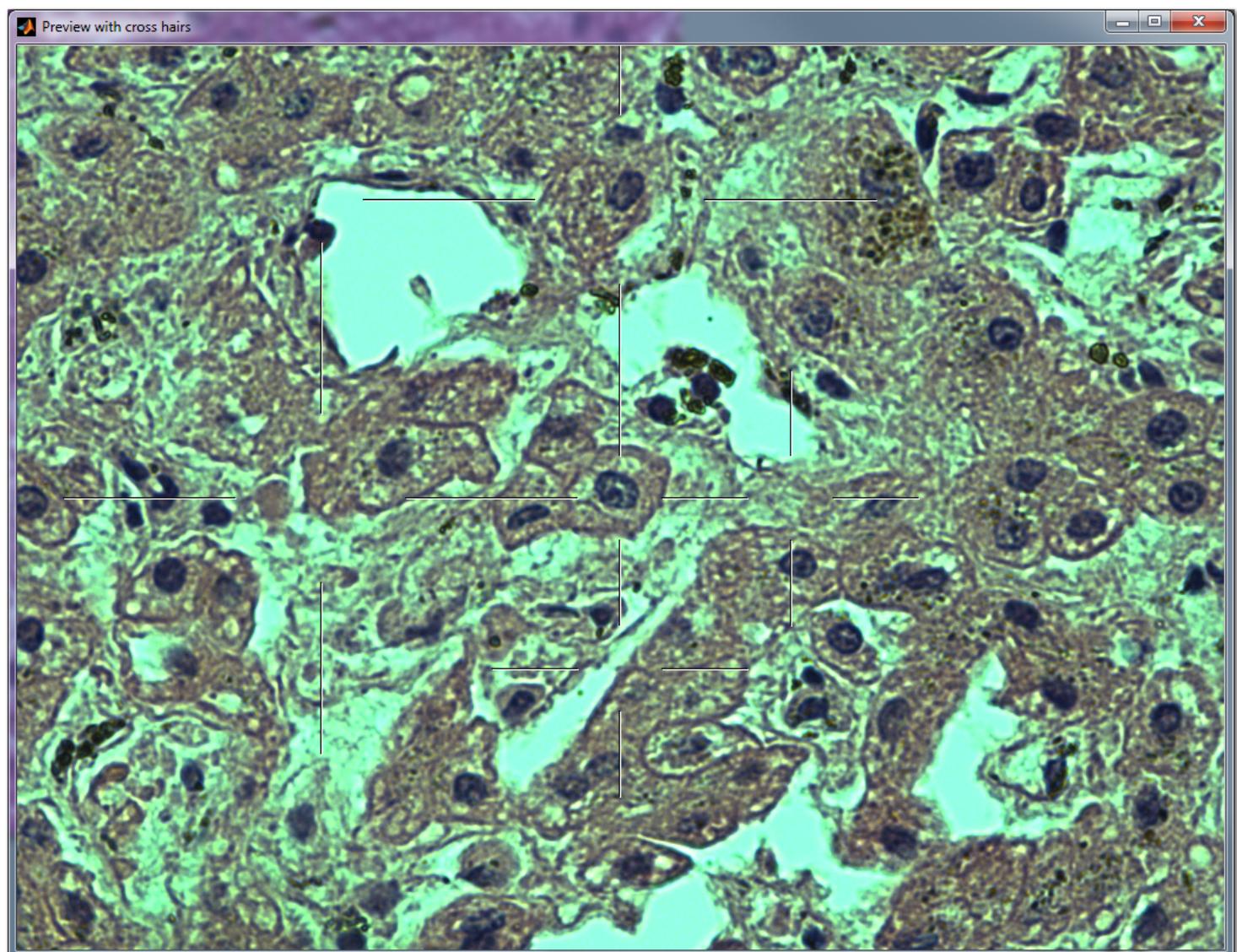
11

Abort Button

Abort/ Exit the current experiment

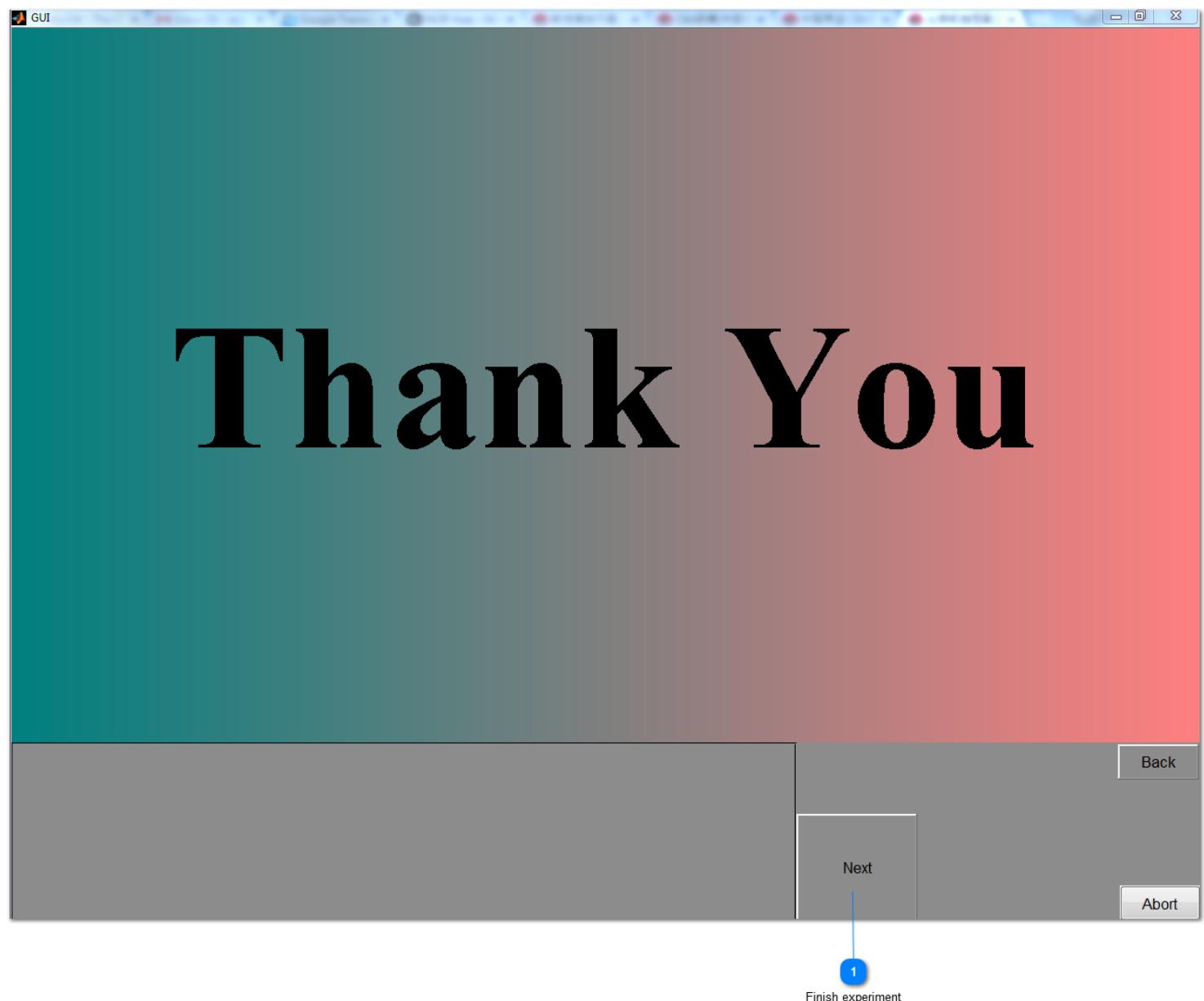
Preview with Cross Hairs Window

In MicroRT mode, preview with cross hairs window show the camera's live microscope image for the administrator view and registration between WSI and camera image.



Thank You Window

In conclusion of the last task, responses are saved in the output file. Please refer to the "[Output File](#)" section



1 [Finish experiment](#)

finish experiment and save result

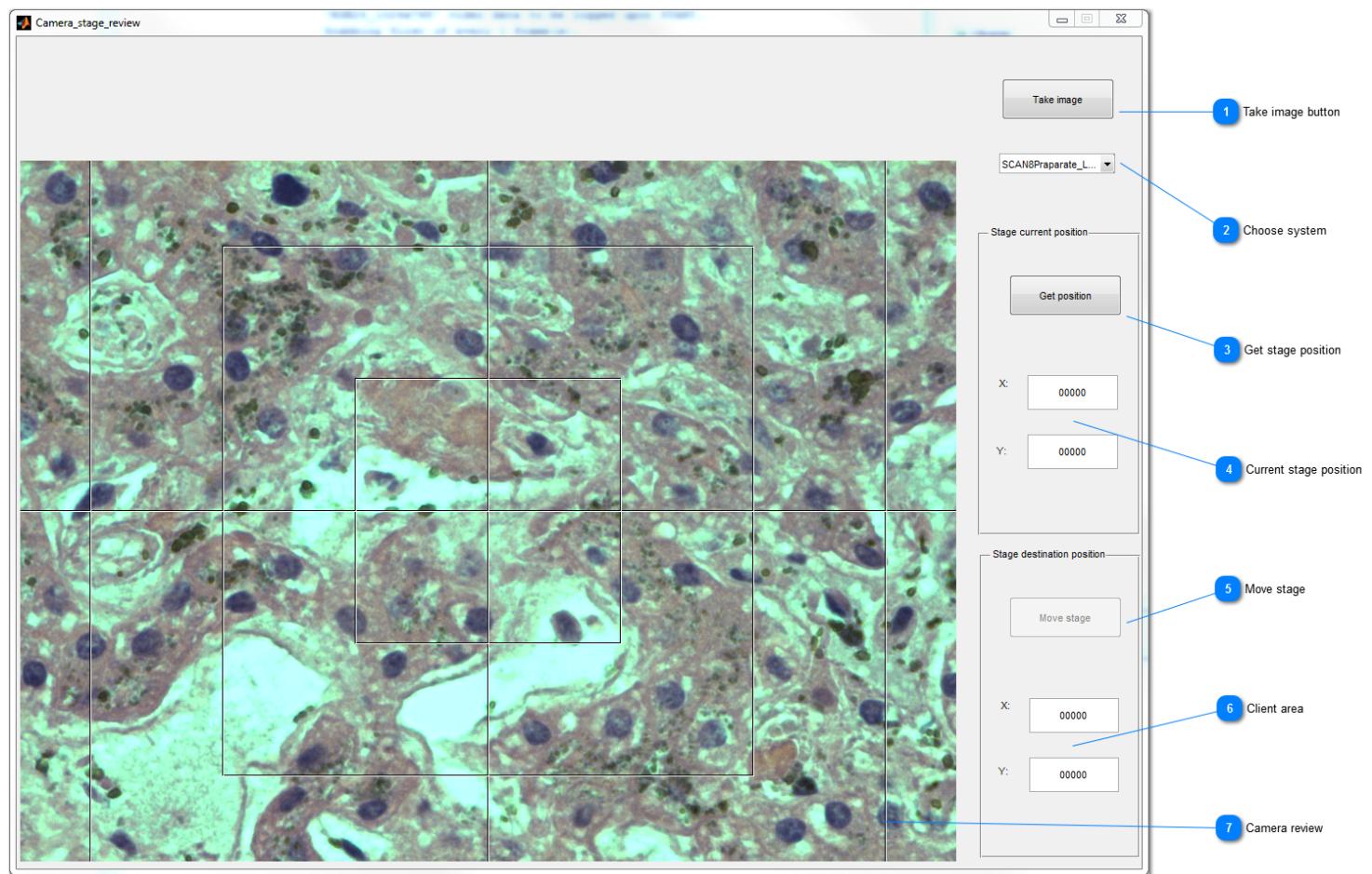
VII. Stage and Camera Control Utility Functions

There is another stand-alone application "Camera_stage_review.exe", which could run the test and control the camera and microscope stage outside of eeDAP files.

This is an individual software. It has following features

1. Taking a picture and saving it to a file.
2. Getting the stage position coordinates.
3. Moving to stage position coordinates.

Camera Stage Review window



1 Take image button

User could use this button to take image of the current camera review. If user chosen stage control system, the default image name has stage position information. Otherwise, the default name is in format "cam_+number". User could also edit the image name and saving directory.

2 Choose system

User could choose his working system at here. Without choose a system "Stage current position" and "Stage destination position" would be disabled. But user could still use Take image button to get camera image

3 Get stage position

Get current stage positions and display them in part 4

4 Current stage position

When user clicks "Get position", "Move stage" or "Take image" buttons, current stage position will update to here

5 Move stage

Move the stage to the position that user input in part 6. This button only enables when user inputs both X and Y position in part 6

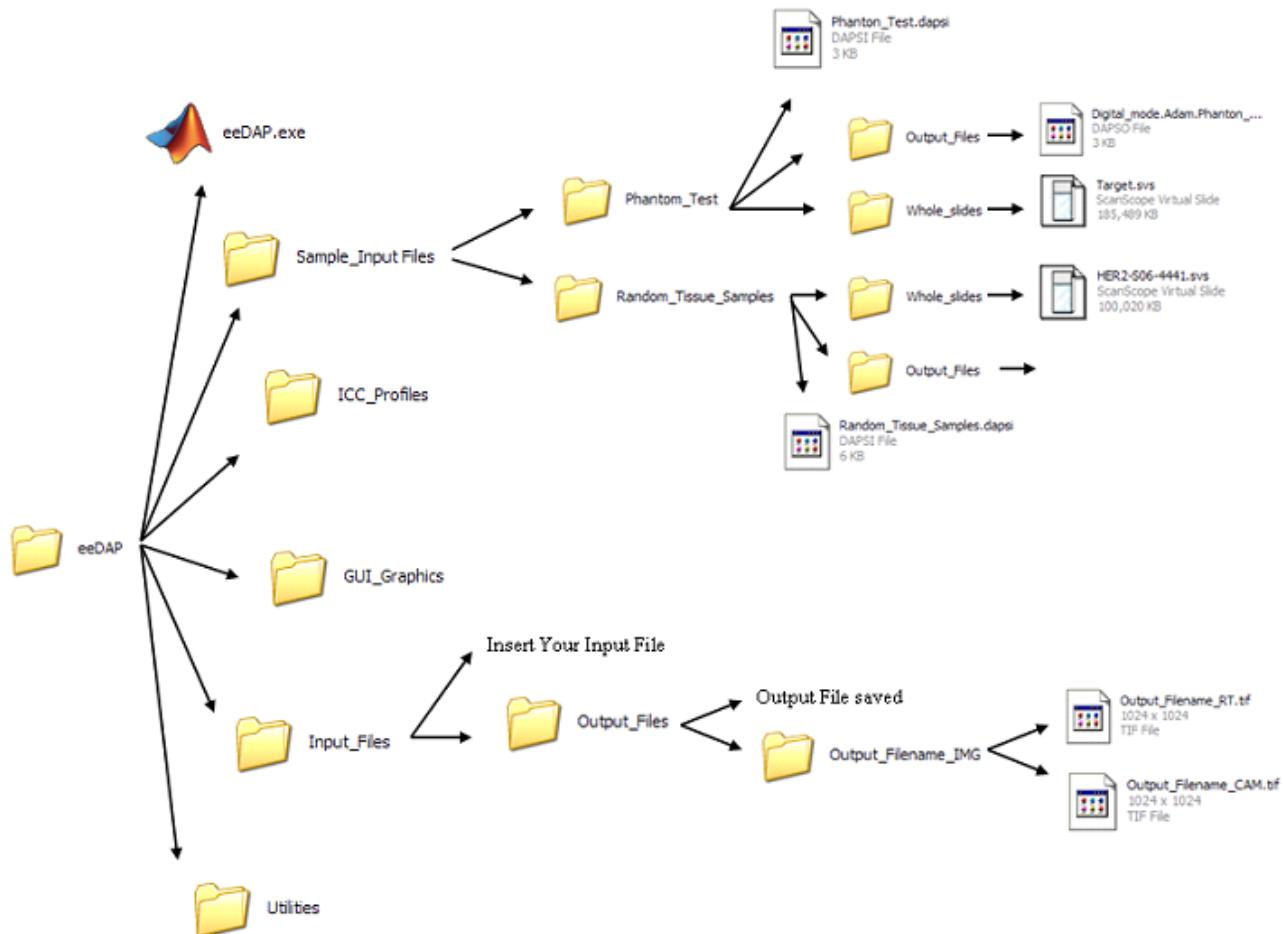
6 Client area

Input the destination positions for stage

7 Camera review

VIII. Directory Structure

A typical eeDAP installation will have the following structure.



IV. FDA Hardware Specifications

eeDAP Specifications used by FDA.

Cameras

- Point Grey Grasshopper Color (GRAS-03K2C-C)
- Point Grey Flea2 Color (FL2G-50S5C-C)
- Point Grey Grasshopper3 Color (GS3-U3-50S5C-C)

Point Grey Grasshopper Color (GRAS-03K2C-C)

Default Format:

The default Matlab format code is RGB24_640x480, which has an aspect ratio of 1.333. The pixel size and format of the default format equals that of the native sensor specs.

- sensor size = 1/3"
- sensor size = 640x480 pixels (0.3MP)
- pixel size = 7.4um

When attached to the microscope, the scale factors are equal to the pixel size divided by the camera adapter magnification (if any) and the objective magnification. When there is no camera magnification:

- 0.185um/pixel at 40x
- 0.370um/pixel at 20x
- 0.740um/pixel at 10x
- 1.480um/pixel at 5x
- 1.850um/pixel at 4x
- 2.960um/pixel at 2.5x

Point Grey Flea2 Color (FL2G-50S5C-C)

Native Pixel Format:

The Matlab format code that uses all of the native pixels is F7_RGB24_2448x2048. It is a non-standard format (Format_7) with aspect ratio 1.953 and the following specs:

- sensor size = 2/3"
- sensor size = 2448x2048 pixels (5.0MP)
- pixel size = 3.45um

Default Format:

The default Matlab format code is RGB24_1024x768, which has a standard aspect ratio of 1.333. The default pixels are 2x2 bins of the native pixels. Consequently, the pixel size is 6.9um.

When attached to the microscope, the scale factors are equal to the pixel size divided by the camera adapter magnification (if any) and the objective magnification.

When there is no camera magnification, the pixel size of the default Matlab format (6.9um) is divided by the objective magnification.

- 0.1725um/pixel at 40x
- 0.3450um/pixel at 20x
- 0.6900um/pixel at 10x
- 1.3800um/pixel at 5x
- 1.7250um/pixel at 5x
- 2.7600um/pixel at 2.5x
- 3.4500um/pixel at 2x

Alternative Format:

The Matlab format code F7_RGB24_1600x1200 uses the native pixels but a smaller standard format.

Point Grey Grasshopper3 Color (GS3-U3-50S5C-C)

<TODO>: Insert description text here... And don't forget to add keyword for this topic

Displays

HP L2335

Size: 49.6cm x 31.1cm

Size (pixels): 1920 x 1200

Pixel Pitch = 258um

Contrast: 500:1

Brightness: 250 cd/m²

Dell 1908 FPt

Size: 37.6cm x 30.1cm

Size (pixels): 1280 x 1024

Pixel Pitch = 294 um

Contrast = 800:1

Brightness = 300 cd/m²

Lenovo Thinkvision L220XWC

Size: 47.4cm x 29.6cm

Size (pixels): 1920x1200

Pixel Pitch: 247 um

Contrast = 1200:1

Brightness = 325 cd/m²

Scanners

The scale factors of the scanner were measured by Neil O Flaherty
The scale factors can also be found embedded in the image files.
Currently, we use 4 difference scanners.

Hamamatsu Nanoozoomer 2.0HT (at NIH AT)	<ul style="list-style-type: none">• scan_scale at 20x = .4558um/pixel• scan_scale at 40x = .2279um/pixel
Aperio CS (at NIH ATC and FDA White Oak)	<ul style="list-style-type: none">• scan_scale at 20x = 0.50um/pixel• scan_scale at 40x = 0.25um/pixel
Aperio T2 (at NIH AT)	<ul style="list-style-type: none">Specs are the same as Aperio CS.
Aperio Scanscope XT	<ul style="list-style-type: none">Specs are the same as Aperio CS.

V. eeDAP Developers

Creators behind eeDAP

Creating the Stand-Alone Application

<TODO>: Insert description text here... And don't forget to add keyword for this topic

Deployment Project

Start a new "Deployment Project" named eeDAP. Matlab will create a project file and folder. The location for this project is not important. This content is not archived because it duplicates content already in the archive.

- 2013: Go to the "APPS" tab on the Matlab GUI.
-OR-
- 2012: Go to File->New -> Deployment Project.

Build the Stand-Alone Application

Move the main file into the build (Administrator_Input_Screen.m). Also add the "tasks" folder to the build. Click on the "Build" button. The build takes as much as 5 minutes. The build creates the "src" folder underneath the project folder.

Presumably, the build doesn't recognize the task functions as called from the main program. This might be because they are called by a function handle and not by name.

Package the stand-alone application

Add related files and folders to the package and click on the "Package" button. This creates the "distrib" folder underneath the project folder. Packaging also creates the package to be unwrapped by the client (eeDAP-1.0_pkg.exe). This package is what you share with the client. The related folders (and their contents) we are packing in the stand-alone package are:

- gui_graphics
- icc_profiles
- sample_inputfiles (first deleting backup files and temporary files and temporary folders)
- Maybe docs some day and this user manual.

Input Variables

Variables used to save study input parameters and settings

Assigned in the Input File

NUMBER_OF_WSI (int)

The number of wsi used in the study.

wsi_slot_n

directory and file name of n'th WSI image.

rgb_lut_slot

directory and file name of WIS image RGB look up table

label_pos

direction of glass slide

reticleID (string)

The model number of the reticle.

cam_format (string)

The Matlab format code for the camera, e.g. "RGB24_1024x768"

cam_pixel_size* (float)

The size of the camera pixels [um]. This depends on possible (de)magnification of the mounting adapter.

The camera pixel size may not be equal to that given in the camera specifications. The camera format may lead to binning which changes the camera pixel size.

mag_cam (float)

Magnification of the camera and to be used in the study.

mag_lres (float)

The low resolution magnification applied to the camera to be used for registration.

cam_hres_mag (float)

The high resolution magnification applied to the camrea to be used for registration and study.

scan_scale (float)

The width in specimen units that a scanner pixel represents [um/scan_pixel].

BackgroundColor_R (float)

The red value of the background color given as a fraction between zero and one.

BackgroundColor_G (float)

The green value of the background color given as a fraction between zero and one.

BackgroundColor_B (float)

The blue value of the background color given as a fraction between zero and one.

AxesBackgroundColor_* (RGB = float, float, float)

The red value of the background color of the axes area given as a fraction between zero and one.

ForegroundColor_* (RGB = float, float, float)

The red value of the foreground color given as a fraction between zero and one.

FontSize (int)

The font size.

taskorder (int)

The place in the order that each task will be executed. (See below).

List of Variables Assigned in the Input File

Under: *myData.settings*

cam_w

int [determined from camera]
Width [pixels] of the camera image.

cam_h

int [determined from camera]
Height [pixels] of the camera image.

cam_roi_w

int [hardcoded]
used to determine width of camera patch for registration

cam_roi_h

int [hardcoded]
used to determine height of camera patch for registration

cam_scale_lres

float [derived]
The width in specimen units that a camera pixel represents at low magnification [um/cam_pixel].

cam_scale_hres

float [derived]
The width in specimen units that a camera pixel represents at high magnification [um/cam_pixel].

cam2scan_lres

float [derived]
The conversion factor to convert the width of a camera pixel at low mag to the width of a scanner pixel [scan_pixel/cam_pixel].

cam2scan_hres

float [derived]
The conversion factor to convert the width of a camera pixel at high mag to the width of a scanner pixel [scan_pixel/cam_pixel].

eye_cam_offset

(int, int) [determined]
This variable determines the offset caused by misalignment between the camera and the eyepiece. It is set in Administrator_Input_Function -> align_eye_cam.

scan2cam_lres

float [derived]
1.0/cam2scan_lres [cam_pixel/scan_pixel]

scan2cam_hres

float [derived]
1.0/cam2scan_hres [cam_pixel/scan_pixel]

Taskorder defines the place in the order that each task will be executed. After a study is executed, the place in the order that each task is executed will be saved in the field myData.tasks.order.

- 0: The order of tasks is randomized. The field myData.tasks.order is ignored on input.
- 1: The order of tasks follows the listed order. The field myData.tasks.order is ignored on input.
- 2: The order of tasks follows the order given by the field myData.tasks.order.

The field of view of the microscope (diameter of the field at the specimen) equals the field number divided by the magnification of the objective.

The apparent field of view of the microscope (diameter of the intermediate image assumed to be viewed at 25cm) equals the field number times the magnification of the eyepiece.

myData.tasks_in, myData.tasks_out

myData.tasks_in holds the tasks in the same order as they appear in the input file.
 myData.tasks_out holds the tasks in the order determined by myData.settings.taskorder

myData.tasks_in, myData.tasks_out	
Field	Type
ID	string given to the task.
order	int If taskorder==2, then this field specifies the place in the order this task will be executed. Otherwise it is ignored.
slot	int The slot number identifies the wsi file and image for the task.
roi_x	int The horizontal center of the ROI to be extracted from the wsi file.
roi_y	int The vertical center of the ROI to be extracted from the wsi file.
roi_w	int The width of the ROI to be extracted from the wsi file.
roi_h	int The height of the ROI to be extracted from the wsi file.
img_w	int The initial width of the displayed ROI. (Rotated with respect to wsi file s.t. it corresponds to the extent of the ROI).
img_h	int The initial height of the displayed ROI. (Rotated with respect to the wsi file s.t. it corresponds to the extent of the ROI).
Qtype	string A label identifying the task (See <K1.1/>).
Qtext	string The text displayed to the user providing instructions to complete the task.
MoveFlag	int Flag indicating whether or not moving is allowed (MoveFlag=1) or not (MoveFlag=0).
ZoomFlag	int Flag indicating whether or not zooming is allowed (ZoomFlag=1) or not (ZoomFlag=0).
Op1	string A label for task option #1 (See <K1.2/>).
Op2	string A label for task option #2 (See <K1.3/>).
Op3	string A label for task option #3 (See <K1.4/>).
Op4	string A label for task option #4 (See <K1.5/>).

<K1.1 ilk="SPECIALNAME" >Sect-Qtype</K1.1>

<K1.2 ilk="SPECIALNAME" >Sect-Qtype</K1.2>

<K1.3 ilk="SPECIALNAME" >Sect-Qtype</K1.3>

<K1.4 ilk="SPECIALNAME" >Sect-Qtype</K1.4>

<K1.5 ilk="SPECIALNAME" >Sect-Qtype</K1.5>