eeDAP

Evaluation Environment for Digital & Analog Pathology

User Manual

2015

Table of Contents

I. Introduction

This is a tool to 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. Furthermore this tool 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. These simple tasks can be modified through a graphical user interface in conjunction with moderate MATLAB programming skills. Furthermore, 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

Credits

- Brandon Gallas: 6/2012 - present
    - Project lead and software development

- Adam Ivansky: 6/2012 - 8/2012
    - Original Creator

- Tyler Keay: 7/2012 - 4/2013
    - Create user manual, improve eeDAP workflow and registration precision, consolidate distribution and create self-contained software package, modify hardware for simultaneous viewing of specimen (camera and eyepiece), manage first pilot study

- Neil O'Flaherty: 7/2012 - 4/2013
    - Evaluate registration precision, investigate WSI file formats and resolution (physical relationship between WSI resolution, the camera, and stage steps), organize hardware specifications and manuals

- Qi Gong: 10/2014 - Present
    - Extend eeDAP software to USB camera, Ludl 6000 and Prior stages
    - Create Linux version eeDAP
    - Create new features, debug and improve performance for eeDAP.
    - Create "Stage and Camera Control" software to test and control the camera and microscope stage outside of eeDAP files.
    - Create user manual by Dr. Explain.
    - Public code and release version to GITHUB

- A. Jessica Handoko: 10/2015- Present
    - Updating user manual, making it more simple and user friendly
    - Using GitHub to keep track of changes and updates made to the manual
    - Testing eeDAP

Other contributors:
- Wei-Chung Cheng
- Stephen Hewitt
- Catherine Conway
- Marios Gavrielides
- Adam Wunderlich.

II. Getting Started with eeDAP

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

eeDAP is used to design and complete an associated optical and digital pathology reader study. Users will have two options either Micro RT or Digital.

Download and Install

Go to https://github.com/DIDSR/eeDAP. Download the latest version from the GitHub eeDAP Release Page. There are three versions inside. Two are for windows system (with and without admin privileges). The other is for linux system.

Jump to Run Software.

Install eeDAP according to what system you have:

    <u>**Windows with admin privileges (.exe):**</u>
        1. Click the eeDAP_windows.exe file to install the eeDAP.
        2. Click eeDAP.exe file to launch eeDAP.
        Notes:
        a. The default installation directory is "C:\Program Files". But we don't recommend that you install eeDAP in this administrator directory, due to potential file saving problems. We recommend that you install eeDAP in a directory where users have read/write permissions.
        b. The eeDAP installation automatically creates a shortcut to eeDAP.exe. However, the properties of this shortcut do not define the "Start in" directory. The field is empty. User should manually add the eeDAP installation path. To access the properties, right-click the shortcut.

    <u>**Windows without admin privileges (.zip):**</u>
        1. Extract the zip file.
        2. Click eeDAP.exe file to launch eeDAP.

    <u>**Linux:**</u>
        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"

Run Software

If this is your first time using eeDAP, we recommend you use the links in this page to go to the Workflow page and view the video manuals(Digital: https://www.youtube.com/watch?v=8fOYwzjwIAc. MicroRT: https://www.youtube.com/watch?v=L81Brta9_3E) for more information on how to run eeDAP .

How to run sample eeDAP input file (sample_input_svs.dapsi):
• Download Sample WSI Image
    1. Download the WSI from CMU openslide page http://openslide.cs.cmu.edu/download/openslide-testdata/Hamamatsu/CMU-1.ndpi
    2. Save it to your computer (directory should be same as wsi_slot_x in input file)

• Setting up Hardware

When opening eeDAP, the appication does not open immediately, may take a few seconds to open.
• 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 given WSI image, camera, stage, and microscope
    3. Extract the chosen sample file by clicking **"Extract ROIs"**.
    4. Select viewing mode, **Digital** or **MicroRT**
    5. Click **Start** button to begin (If MircoRT is chosen, continue to Serial Port cofiguration or MicroRT Registration. If Digitial is chosen, continue to eeDAP study)

• In MicroRT mode, user needs to do MicroRT Registration (Both the **WSI** and corresponding **glass slide** is needed)

• eeDAP Study
    1. Input user ID and click Next *(Micro RT Model continue to 2, Digital Model bypass to 6)*
    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 6)*
    4. If registration is **not** valid, click on **"Reset"**. If the registration is valid, skip to **6**
    5. Then select either **"Fast Registration"** or **"Best Registration"** in the control panel
    6. Follow the task instructions to complete each given tasks and press next
    7. Use the control panel 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)"

Setting up Hardware

1. Install the camera on the microscope and connect it to the computer
2. Following the "Camera Setup and Test" page, download and install the camera driver
3. Test and align the camera
4. Connect the stage controller with microscope and the stage to the computer
5. Following the "Stage Setup & Test" page, download and install the stage driver
6. Check and test the connecting status between the computer and joystick

MicroRT Registration

A. Low Resolution Registration (More info on Low Resolution Page)

    1. Place selected tissue glass *(label_pos)* onto the stage and change lens *(mag_lres)* according to the input file

    2. Move stage using the joystick and center and focus a unique location *(that stands out on the thumb WSI)* on the tissue and click **Take Stage Position**

    3. Click the same unique location on the thumb WSI and click **Take WSI Position**

    4. Check results, Click on **"Registration is Good!"** if the registration is accurate

    5. If results are accurate, repeat steps 2,3, and 4 with 2 other unique points

    6. Select **Load last calibration** (Zoomed in image will appear)

    * To skip High Resolution Registration and use only Low Resolution Registration, click on **Finish Registration** (Not recommended)

B. High Resolution Registration (More info on High Resolution Page)

    1. Change lens *(mag_hres)* according to input file, the WSI will show the zoomed up area on the screen

    2. Click on **GoTo Position**, the stage will move automatically to previously taken Stage Position (Low Resolution)

    3. Adjust and center the stage using the joystick to a unique location in the same ROI of the low resolution registration *(that stands out on the thumb WSI)* and click **Take Stage Position**

    4. Click the same unique location on the thumb WSI and click on **Take WSI Position**

    5. Check results, Click on **"Registration is Good!"** if the registration is accurate

    6. Repeat steps 2,3,4, and 5 with the 2 other unique points

C. Camera and Eyepiece Registration

    *It this is the 1st time, do not skip. If done before, press **Skip Offset**

    1. In the camera image, move the center of reticle to one small cell

    2. Click **Feature Centered in Camera**

    3. Look through the eyepiece, move the center of reticle to the same small cell

    4. Click **Feature Centered in Eyepiece**

Default Tasks

There are several default tasks that come with the eeDAP installation. They can be separated by the baseline of registration:

Base on WSI:.

> • User should pre-define Region Of Intereting (ROI) in input file based on WSI (center and size of ROI).
>
> • It is available in both MicroRT and Digital Modes.
>
> 1. MicroRT: GUI window shows WSI ROI. Microscope stage automatically move to the same ROI on glass slide.
>
> 2. Digital: GUI window displays WSI ROI.

Base on Microscope:

> • User do not need to pre-define ROI in input file.
>
> • It is only available in MicroRT Mode.
>
> 1. MicroRT: User uses joystick to move microscope to a region. eeDAP extracts the same ROI on WSI and displays on GUI window

Note: Most of the text that appears in the data collection GUI can be customized in the input file. The format is showed in section IV

Base on WSI

mark1:
* **Description and purpose:** Select a reason in WSI (e.g. find a mitosis), eeDAP can save the X-Y coordinates on the ROI and WSI *(Figure 1).*
* **Procedures:**
    1. Click on image in the region of the cell that is specified in the description.

count, count_imagescope, count2_imagescope:
* **Description and purpose:** Count the number of cells that appear to accommodate the description *(Figure 2).*
* **Procedures:**
    1. Enter the number in the block.
    2. Count_imagescope, count2_imagescope allow user to use annotation in imagescope to point out the cells and save the annotation.
    Note: After make all annotations in one image, please close curret image and click save annotation. eeDAP will automatically move the annotation to output folder.

radio1of4:
* **Description and purpose:** Select the one of the options that accommodates the description (*Figure 3).*
* **Procedures:**
    1. Select one option out of four.

slider:
* **Description and purpose:** Rate the cell or tissue based on description (*Figure 4)*.
* **Procedures:**
    1. Use 0-100 slider or type the number.

mitotic_train:
* **Description and purpose:** Answer described questions (Rating and pre-defined check boxes), and show expert results.
    Pre-defined check boxes:
    1. Spindle darker (general)
    2. Ropy, hetero. texture (general)
    3. Visible cytoplasm (general)
    4. Not apoptotic: brown stain or dissolving, shrinking (general)
    5. Spindle area majority of cell, N/C~2 (prophase)
    6. Spindle aligned with cell (metaphase)
    7. Two spindles have formed (anaphase)
    8. Two cell membranes visible (telophase)
* **Procedures:**
    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:
* **Description and purpose:** Answer described questions (Free text, rating and pre-defined check boxes).
* **Procedures:**
    1. Write a discription of what you observe in the image above.
    2. Click **"Next Part"** botton.
    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.
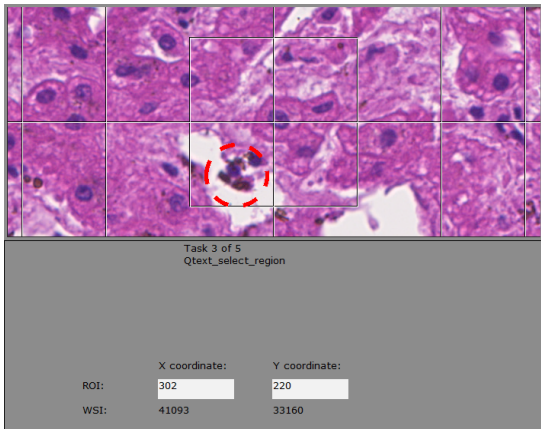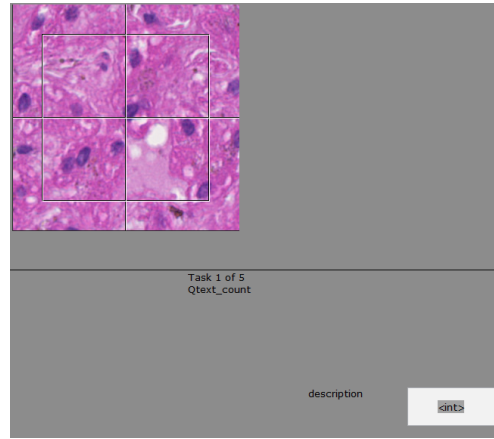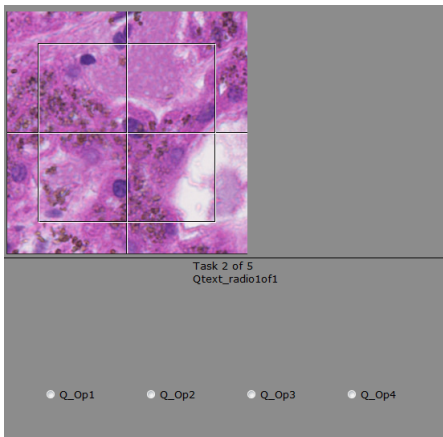
Figure 1

Figure 2

Figure 3

Figure 4
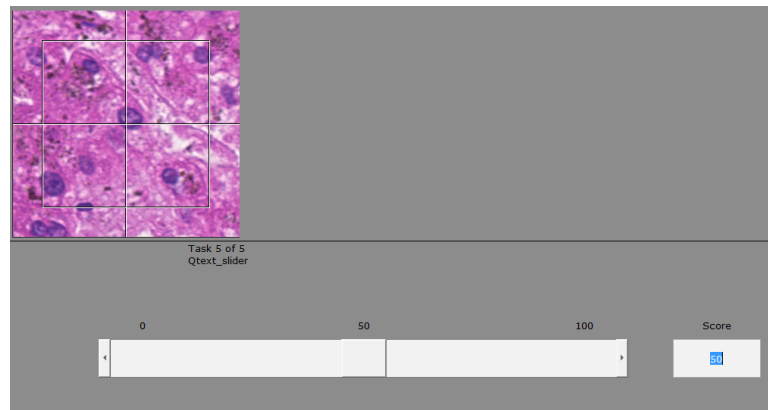
Base on Microscope

get_WSI_position:

     • **Description and purpose:** Get WSI position for an interesting ROI find in microscope.

     • **Procedures:**

         1. Uses joystick to move microscope to a region.

         2. Click **"Get WSI ROI Position"** button to extracts the same ROI on WSI and save its position.

Note: In input file, do not need to define slot ID and ROI center position.

Dynamic Stop and Start

• Dynamic Stop

eeDAP records reading result task by task continually.

If the reader only finishes part of tasks in an input file, eeDAP generates a temporary output file that records finished results. The temporary output file is saved in "Output_Files" folder.

• Dynamic Start

eeDAP can continue unfinished task.

eeDAP can load in a temporary output file and continue where left off. When a reader browses the temporary output file, the reader should set browsed file format to "All Files". eeDAP will generate a "Output_Files" folder in the temporary output file directory and save the new output file in it.

III. Hardware and Software Requirement

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

Camera

Requirements

The code is written to work with DCAM and USB compatible cameras.
eeDAP requires camera images to be RGB24 (8 bit for each channel).
eeDAP requires camera images to have width > 640 and height > 480.
FDA has used 3 Point Grey cameras for this eeDAP software

Name and model
Point Grey Grasshopper Color (GRAS-03K2C-C) **Interface**: Firewire 1394 **Specifications:** (https://github.com/DIDSR/eeDAP/blob/master/000_docs/hardware_manual/Camera/Grasshopper_Specifications.pdf) **Technical Manual** (https://github.com/DIDSR/eeDAP/blob/master/000_docs/hardware_manual/Camera/Grasshopper_Technical.pdf)
Point Grey Flea2 Color (FL2G-50S5C-C) **Interface**: Firewire 1394 **Specifications:** (https://github.com/DIDSR/eeDAP/blob/master/000_docs/hardware_manual/Camera/Grasshopper_Specifications.pdf) **Technical Manual** (https://github.com/DIDSR/eeDAP/blob/master/000_docs/hardware_manual/Camera/Grasshopper_Technical.pdf)

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

Interface:
USB
Specifications:
(https://github.com/DIDSR/eeDAP/blob/master/000_docs/hardware_manual/Camera/Grasshopper3_USB3_Specificat
Technical Manual
(https://github.com/DIDSR/eeDAP/blob/master/000_docs/hardware_manual/Camera/Grasshopper3_USB3_Technical.

User could get more Point Grey Camera document from https://www.ptgrey.com/support/downloads

Camera Setup and Test

For Point Grey camera, users need install special diver to run it under eeDAP software.

Windows:

After plugging in the camera (USB), you should disable the default Windows driver and update the driver for the attached device to the industry standard driver.

Download, Install, and Test DCAM Driver:
1. Go to Carnegie Melon (CMU) Download Driver Page
2. In the **Download** tab, click "I agree to the Terms of the LGPL".
3. Download **1394camera646.exe**
4. Click **Installation** tab and install the driver following the instruction
5. Select **"1394CameraDemo32.exe"** and run it

Download, Install, and Test Fly Capture Driver:
1. eeDAP 5.0 and high versions only support the 2.11 version of FlyCap2, while the old versions only support the 2.5 version of FlyCap2
2. Based on camera model, download driver from: https://www.ptgrey.com/support/downloads
3. Install the FlyCap2 and test camera through it
4. Restart computer

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.

Camera Test:
If "MircoRT" mode is selected:
1. Click the button "**imaqtool**".
*This launches a Matlab utility which is an interactive GUI that allows 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.)

Go back to Run Software

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 can only 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 eypiece move stage to let the reticle just touch the boundary of the gird corner.
4. Depending on your camera:
      a. If you are **able** 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 gird corner.*(Figure 2)*
7. Hold the camera and while looking at the camera image, start tighting the screws until it is aligned.
*Note: 1. Small shift could be solved by eyepiece and camera register processing.
      2. Large shift might influenct the fast registration during task. Because our fast registration use a small center area of camera image, lager shift between eyepiece and camera will lead outstanding feature goes out of the camera registration area. Best registration is another method to slove the large shift problem.
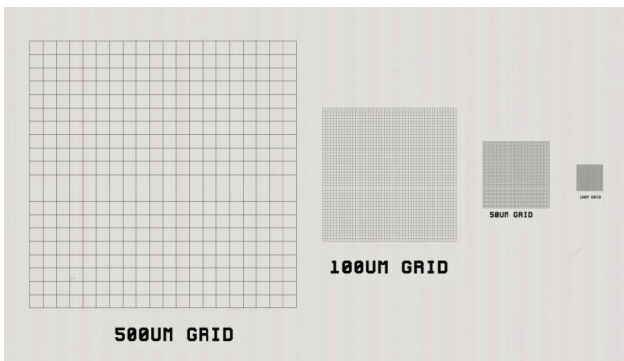
Figure 1
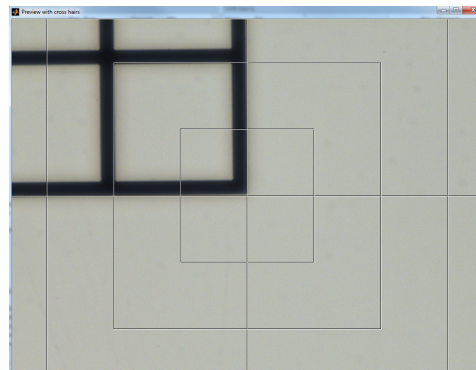
Figure 2
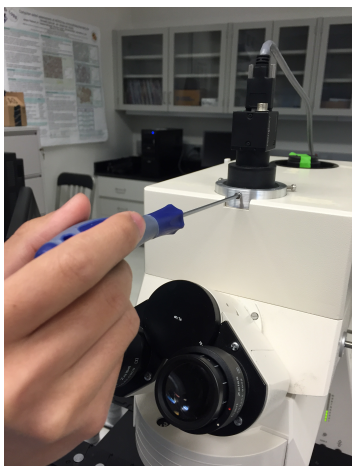
Figure 3

Go back to Run Software

Stage

We have used following 3 stage controllers.

Name and model	Interface	Document
Ludl (MAC5000)	RS232	Specifications and Manaul (http://ludl.com/downloads/?wpdmc=mac5000)
Ludl (MAC6000)	RS232	Specifications and Manual (http://ludl.com/downloads/?wpdmc=mac6000) Note: MAC6000 uses same command as MAC 5000
Prior ProScan III (H31XYZE-US/A)	USB	Specifications and Manual (http://www.well.ox.ac.uk/_asset/file/prior-proscan-iii-motorised-stage-controller-manual.pdf)

We use following 3 stages in development.

Name and model	Document	Description
Marzhauser (SCAN 8Praparate Axioplan2)	Specifications (http://www.marzhauser.com/en/pim/produktdetail-popup.html?view=details&pimid=a210&no_cache=1&m=null&p=null)	8 slots (Works with Ludl controllers)
Ludl (96S109-LE2)	Specifications (http://www.bioimagingsolutions.com/Ludl/ludl_bioprecision_upright_96S100.html)	5 slots (Works with Ludl controllers)
Prior (H101A/B)	Specifications (http://www.prior-us.com/files/H101A%20ProScan%20Stage%20Datasheet.pdf)	4 slots (Works with Prior controllers)

Stage Setup and Test

Windows system:

1. Users can 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*).
2. Choose COM* in eeDAP to connect with stage.
3. Download and install the proper driver

Prior Stage:

Downloading Stage Driver:

1. Go to Prior offical Download Center Website: http://www.prior-us.com/Customer-Support/Download-Centre/
2. Download the "ProScan III USB Driver" and software depend on operating system.

Installing Stage Driver:

1. Make sure the stage is connected before installing
2. Install driver from device manager.
Option: Try to use Prior software control the stage.

Ludl Stage:

Normally User can use default driver. User can also download driver and software from Ludl official download page: http://ludl.com/downloads/.

Linux: use 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 prot 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 prot permission:
  "chomd 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.

Stage 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.

Go back to Run Software

Microscopes

Here are a couple notes on understanding the size of the field of view and the size of the intermediate image (the apparent size of the image viewed by the pathologist).

The page that opened was Field of View Diameter at microscopy. 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

"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."

Types of Microscopes

There are currently only two microscopes that we have used with the software. The basic microscope requirements are the following:

1) It has a trinocular tube: a camera can be mounted and the camera view is the same as the eyepiece view.
2) It is compatible with one of the computer controlled stages.

Note: Kohler illumination calibration tutorial https://www.microscopyu.com/tutorials/kohler

Name and model
Axioplan 2 Imaging microscope with an Axiophot 2 head Specifications and Manaul (https://github.com/DIDSR/eeDAP/blob/master/000_docs/hardware_manual/Microscope/Axioplan2_specification_and
Olympus BX43 Specifications and Manaul (https://github.com/DIDSR/eeDAP/blob/master/000_docs/hardware_manual/Microscope/olympus_specification_and_r
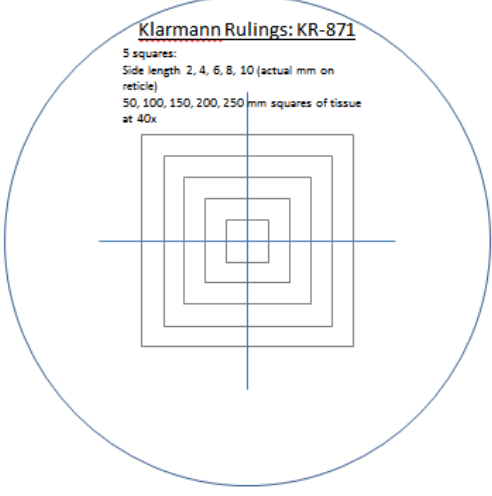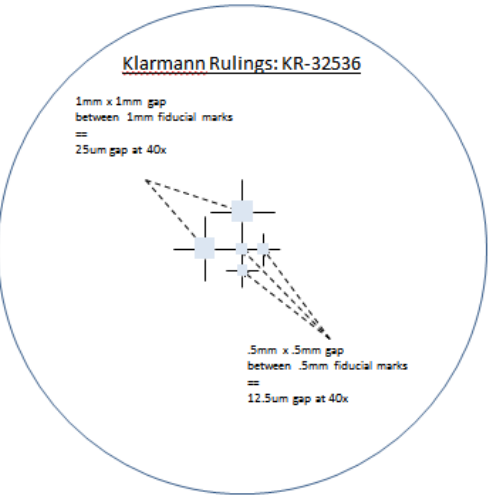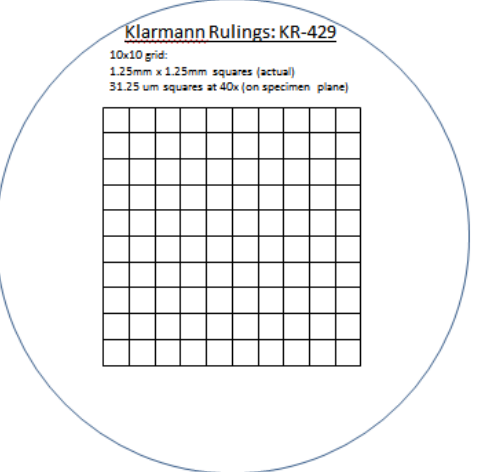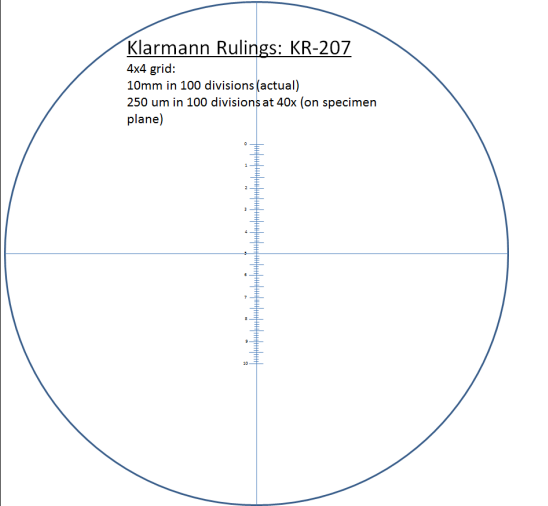
Reticles

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

Name of Reticle	Display View	Description
Klarmann Rulling: KR-871	Klarmann Rulings: KR-871 5 squares: Side length 2, 4, 6, 8, 10 (actual mm on reticle) 50, 100, 150, 200, 250 mm squares of tissue at 40x	This reticle contains 5 squares with actual lengths of 2, 4, 6, 8, and 10mm.
Klarmann Rulings KR-32536	Klarmann Rulings: KR-32536 1mm x 1mm gap between 1mm fiducial marks == 25um gap at 40x .5mm x .5mm gap between .5mm fiducial marks == 12.5um gap at 40x	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	Klarmann Rulings: KR-429 10x10 grid: 1.25mm x 1.25mm squares (actual) 31.25 um squares at 40x (on specimen plane)	This reticle has a width and length of 10x10 grid at IOP = 12.5mm, an apparent width and length of 10x10 grid "at 25cm" = 12.5cm, a grid spacing at IOP = 1.25mm, a grid spacing at stage at mag_o (5x= 1.25mm/5 = 0.25000mm, 10x= 1.25mm/10 = 0.12500mm, 20x = 1.25mm/20 = 0.06250mm, 40x = 1.25mm/40 = 0.03125mm), and apparent grid spacing "at 25cm" = 12.5mm.

Klarmann Rulings KR-207	Klarmann Rulings: KR-207 4x4 grid: 10mm in 100 divisions (actual) 250 um in 100 divisions at 40x (on specimen plane)	This reticle contains a 10mm ruler with 100 divisions.

Scanners

These are currently 4 different scanners we have tested with the eeDAP software.

The scale factors can also be found embedded in the image files.

Scanner Name	Description
Hamamatsu Nanozoomer 2.0HT (at NIH AT)	• scan_scale at 20x = .4558um/pixel • scan_scale at 40x = .2279um/pixel
Aperio CS (at NIH ATC and FDA White Oak)	• scan_scale at 20x = 0.50um/pixel • scan_scale at 40x = 0.25um/pixel
Aperio T2 (at NIH AT)	• Specs are the same as Aperio CS.
Aperio Scanscope XT	• Specs are the same as Aperio CS.

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 accross when using eeDAP.

Read and Extract WSI image

There are two methods to extract ROI from WSI image "BIO formats" and "Image Scope". The individual pages for the methods will go into more depth with the details of each of them.

BIO Formats

Bio-Formats is currently being used in eeDAP. It is a stand-alone 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. It was 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.

BIO Formats: Possible Errors

The BIO formats might have some problems when extracting the WSI. In this section we show two normally errors and the solving methods

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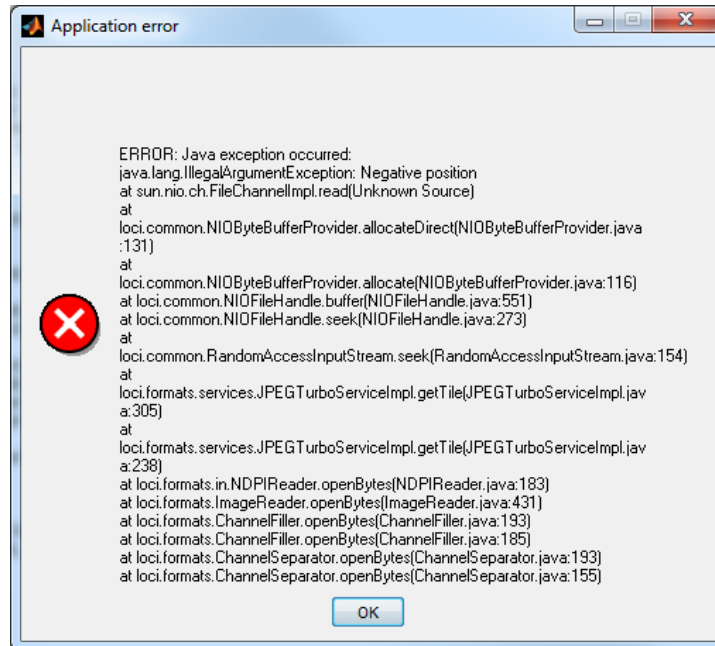
This error is because the input WSI image file is too large. Now we are using 5.1.2 version Bio formats. It works for file smaller than 4 GB. If you want to use a larger image, you might face this problem.

2.Out of Memory/Storage
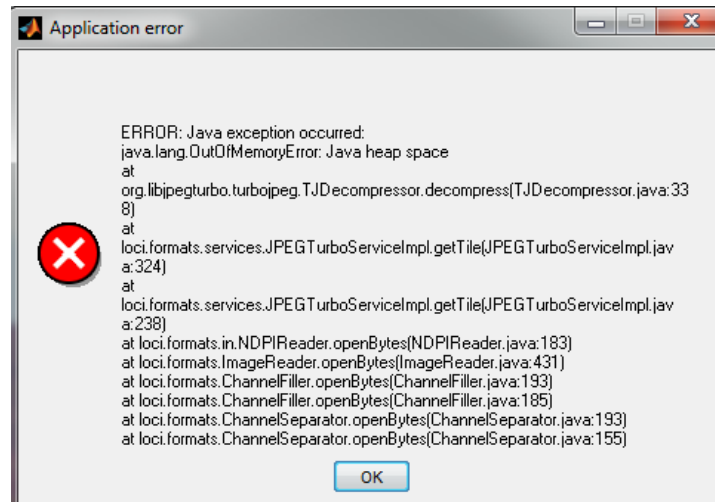
When we process a large image, there might be an error message about being out of memory. For large image a minimum of 512 MB is suggested.

Users can download the java.opts and paste it in the matlab root directory, or follow this website : Mathwork Answers

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 TIFF comp to extract the ROI's from the WSI images. The reason we chose a 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 64-bit R2016a (9.1.1) and Linux 64-bit R2015a(8.5)

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

Installer will create a folder like: C:\Program Files (x86)\MATLAB\MATLAB Compiler Runtime\v91

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 three sections: Header, Study Settings, and Body. The Header includes free text that describes the experiment and formatted text that specifies global variables. The Study Settings are just the setting for the following experiment. The Body is a list of ROIs and corresponding tasks.

Input File Header

The Header is all the text before the line containing "**SETTINGS**".

Refer to the figure on the previous page which shows the input file being edited in a plain text editor.

> **Header** outlines the author, experimental setup, date, time, description of the experiment, dareware (camera, scanner, & microscope)

Input File Study Settings

The input file Study Settings begins with "**SETTINGS**".

Study Settings include:
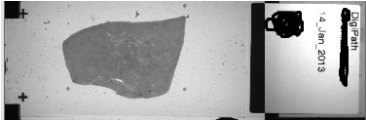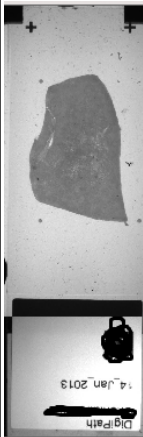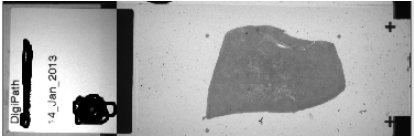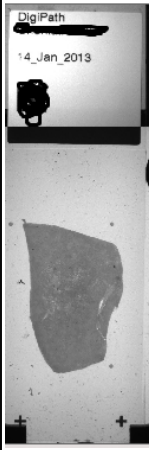
    NUMBER_OF_WSI: Number of WSI image in the study.

    wsi_slot_1: WSI image saving directory and name.

    rgb_lut_slot_1 : International Color Consortium file directory and name.

    label_pos: Direction of the glass slide in microscope stage.

        Sit in front of microscope eyepiece, the glass slide label clock position

label_pos	3	6	9	12
example				

    reticleID: Reticle used in study

    cam_kind: Kind of camera

    cam_format: Camera image format.

    cam_pixel_size: Camera pixel size

    mag_cam: Magnification between camera and eyepiece

    mag_lres: Low magnification registration lens

    mag_hres: Low magnification registration lens

    scan_scale: WSI image scan scale

    stage_label: Stage name

    BG_Color_RGB: GUI back ground color (3 color channels)

    FG_Color_RGB: GUI front ground color (3 color channels)

    AxesBG_Color_RGB: Axes color (3 color channels)

    FontSize: GUI font size

    saveimages: Study image saving options

    taskorder: Controls the order of the tasks

Input File Body: List of ROIs and Corresponding Tasks

The input file Body begins with the line containing "**Body**" and follows with a line of column labels. The first two tasks should be "**start**" and "**finish**". User should not edit them. Each line below defines one task. For most task there are 9 columns identifying the ROI and task.

Default Task Input Columns:

A. Task Instructions

Task Name (String): Name for each task, user should choose one task from task list.

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

Task Order (Int): Task appear order

1. If the value of "taskorder" defined in the Header is set to "1": User should set task order at here starting from 1 without repeat.

2. If the value of "taskorder" defined in the Header is set to "0": User should set task oredr for all tasks as -1.

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

B. Input Variables

ROI_X (Int) : WSI ROI center pixel position in X direction.

ROI_Y (Int): WSI ROI center pixel position in Y direction.

ROI_W (Int): Pixel width of the ROI.

ROI_H (Int): Pixel height of the ROI.

Q_Text (String): This field contains the question (free text) displayed to the reader.

Do not use commas here or the input file format will be corrupt.

Default Task Input format: Each line defines one task, use comma to separate input columns.

Task Name, Task ID, Task Order,Slot, ROI_X, ROI_Y, ROI_W, ROI_H,Q_Text

Other Task Input Columns: Some special tasks has different input columes

mitotic_train: padding 8 more columns for expert checkbox result (1: checked; 0: not checked), 1 more column for rating result at end of **Default Task Input Columns**.

checkMof4, radio1of4: padding 4 more columns to define 4 opitions at end of **Default Task Input Columns**.

get_WSI_position: Do not have **Slot, ROI_X, and ROI_Y** columns.

Editing Input File Body

The Body of an input file is editable by a spreadsheet editor such as Excel.

Editing and Opening an Input File in Excel:
1. Open Excel, double check Excel's open dialogue "all files is selected"
2. Navigate to **eeDAP→Sample_Input_Files→Phantom_Test**
3. Open "**Phantom_Test.dapsi"**
4. **"Text Import Wizard"** will open automatically
5. Select the "**Delimited**" option and click "**Next**".
6. Find "**Delmiters**", and select "**Comma**" in this case *(Figure 1)*
7. Select **"Finish"** not **"Next"**
8. The file will open in Excel automatically
9. Edit what is necessary for this case
10. Once Complete, click **"Save As"** a **".CSV"** file which is plain text with comma separated variables *(Figure 2)*
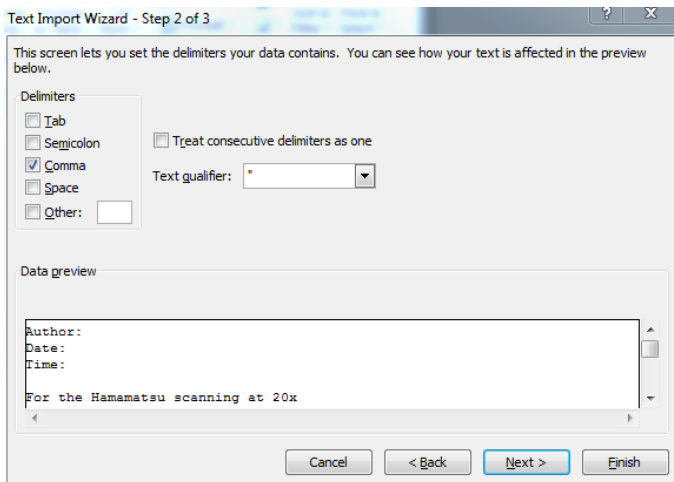
Figure 1
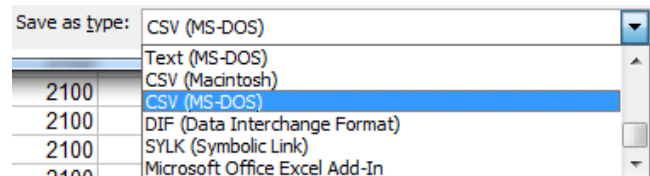
Figure 2

WSI Formats

The current version of BIO Formats can load these types of images:

- .svs
- .ndip
- .tiff

*New soon to be released version will be able to load .czi images.

V. Output File

An "Output_Files" folder will be created in the folder where the input file is located. eeDAP writes to this folder the for 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 depending 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). 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. 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** section
• "start" task: record study mode and readerID.
• "finish" task: not change.
• study tasks: keep input contains in input file and additional column for the *task duration* (amount of time the reader spent on each task in recorded in seconds) and some other columns related to the reader's answers (different for different kinds of task).

VI. WorkFlow

Guidelines of eeDAP in more detail.

*To return to Run Software

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. It 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.
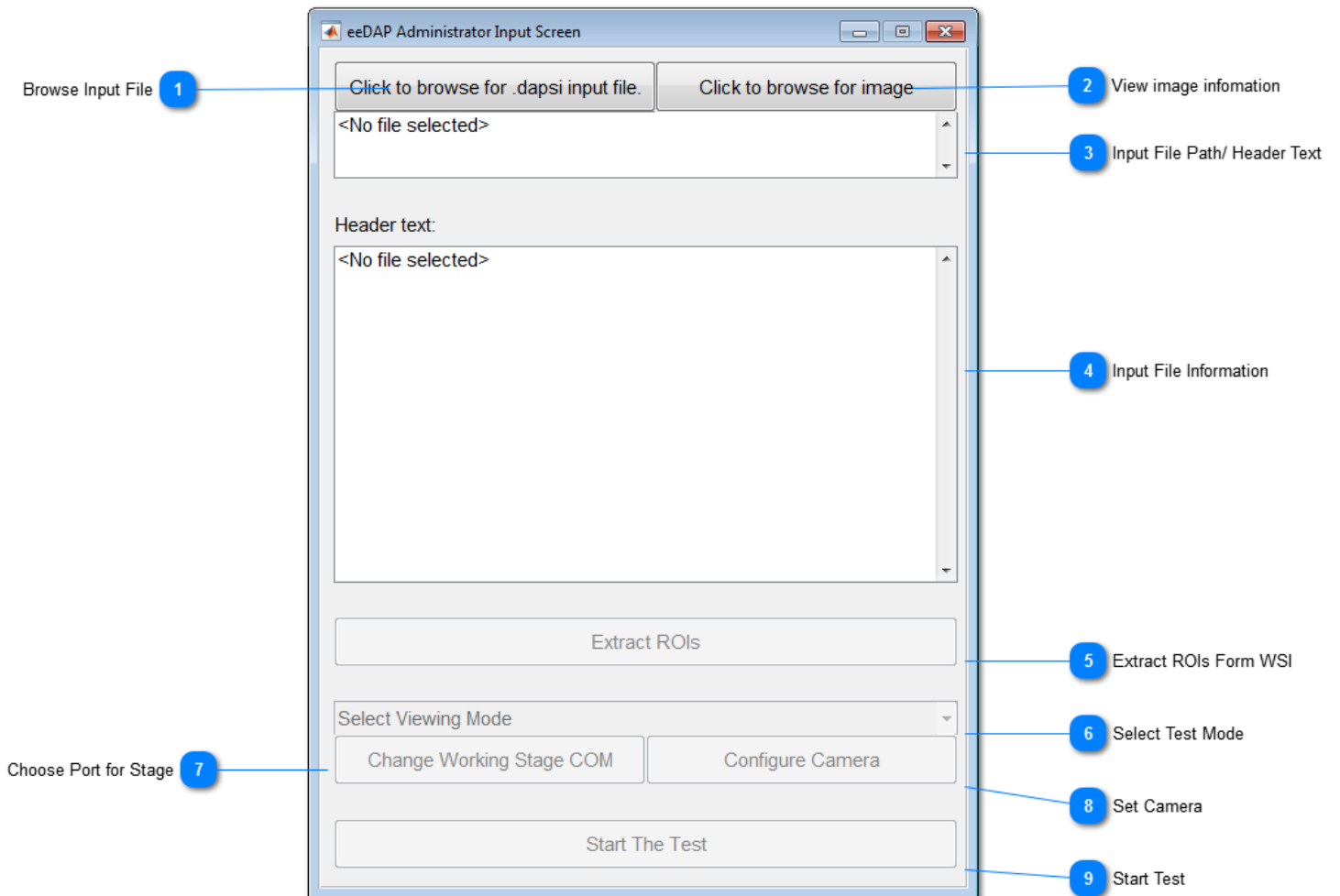
Browse Input File

Browse input file from computer

View image infomation

Browse a WSI image to view thumbnail and general information.

Input File Path/ Header Text

After browse input file, shows the path of the file

Input File Information

4

```
<No file selected>
```
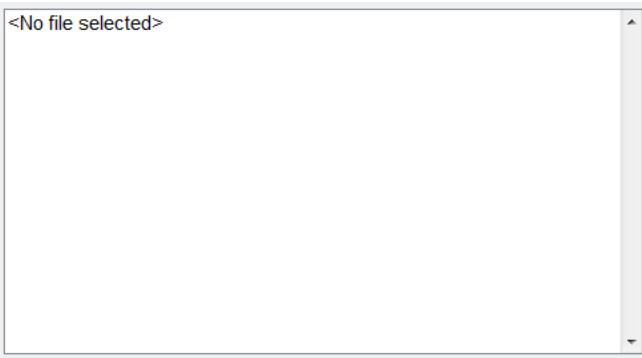
After browse input file, shows the detail information of the file

Extract ROIs Form WSI

5

```
Extract ROIs
```
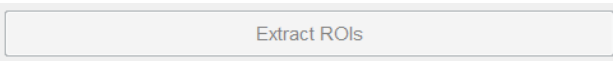
Following the input file, extract Region of interestion from WSI

- 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)

Select Test Mode

6

```
Select Viewing Mode                                    ▼
```
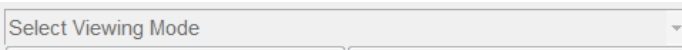
- Choose Digital mode for just use WSI digital image in experiment
- Choose microRT mode use both WSI digital and micorscope live image in experiemnt

Choose Port for Stage

7

```
Change Working Stage COM
```
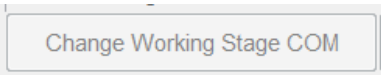
In microRT mode, set the stage controller connect port

Set Camera

8

```
Configure Camera
```
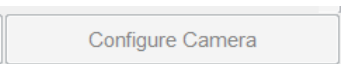
In microRT mode, set camera

Start Test

9

```
Start The Test
```
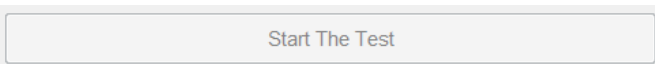
Start test

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.

Serial Port Configuration:

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

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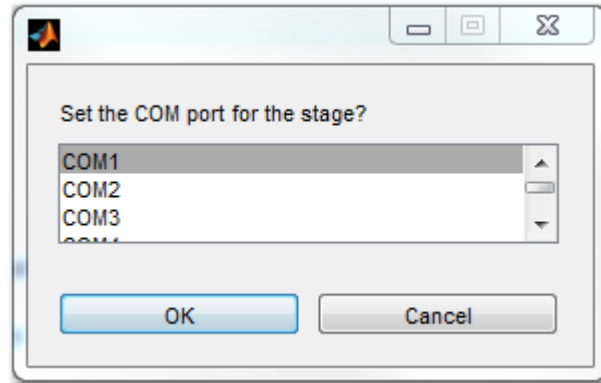
Slide Registration Window

When the MicroRT mode is selected the Slide Registration window will open. The microscope camera will also 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.
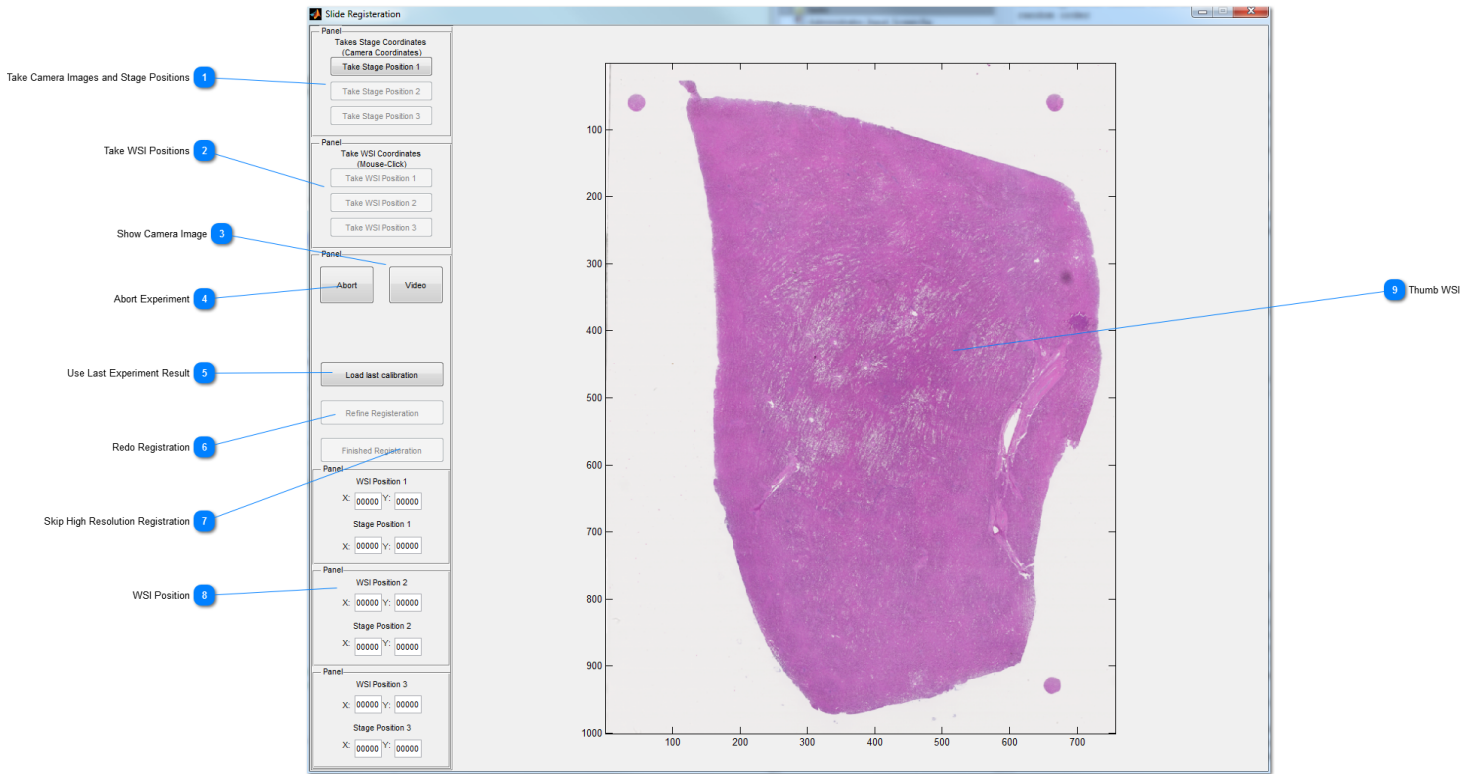
Take Camera Images and Stage Positions
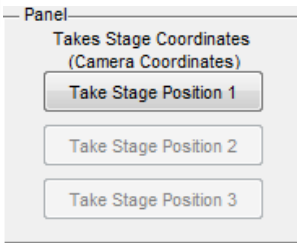
          Take the microscope live image and stage position for 3 areas

Take WSI Positions
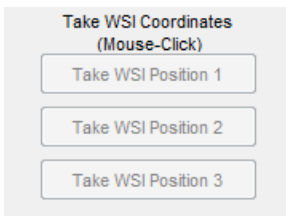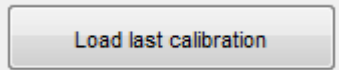
          Take the related WSI position for 3 areas
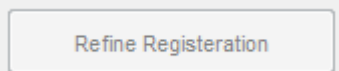
Show Camera Image
Show the real time image
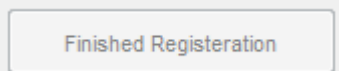
Abort Experiment
Exit and discard the experiment

Use Last Experiment Result

5

| Load last calibration |

Load the past 3 areas WSI and stage positions from the most recent experiment

Redo Registration

6

| Refine Registeration |

Redo registration

Skip High Resolution Registration

7

| Finished Registeration |

Skips high-resolution registration, directly uses low-resolution or the last experiment postions

WSI Position

8
Shows the exact position of what was chosen on the WSI image and Camera image

Thumb WSI

9
Digital image of the WSI image

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 and lens has been launched.

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
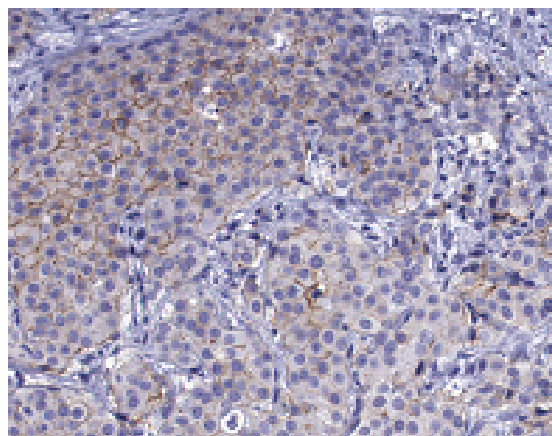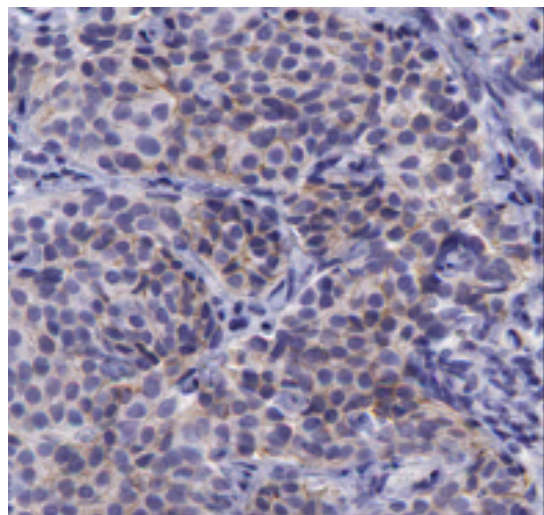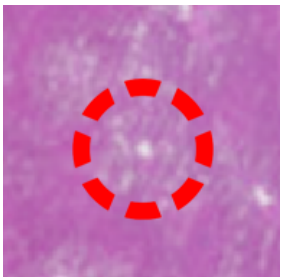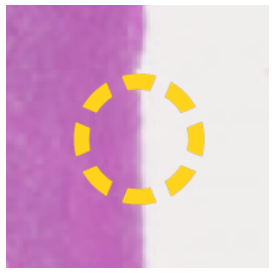
| Bad | Good |
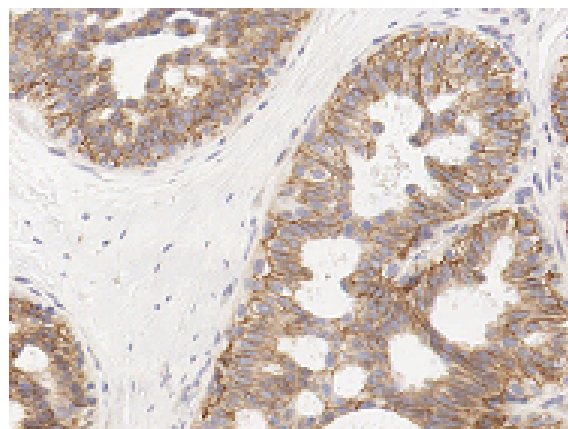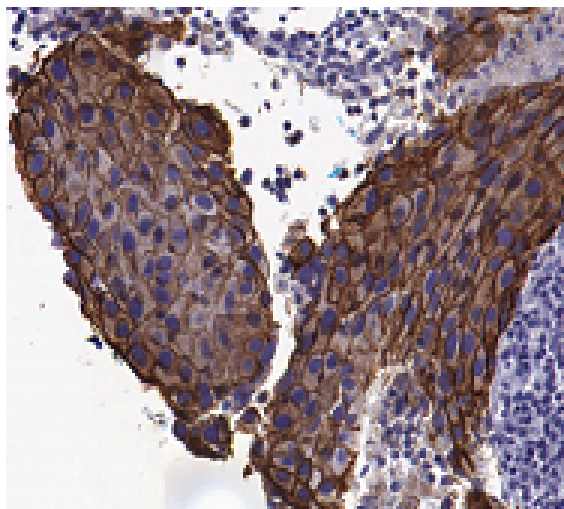
Unique Locations

Here are a few examples of what selecting a unique location means:
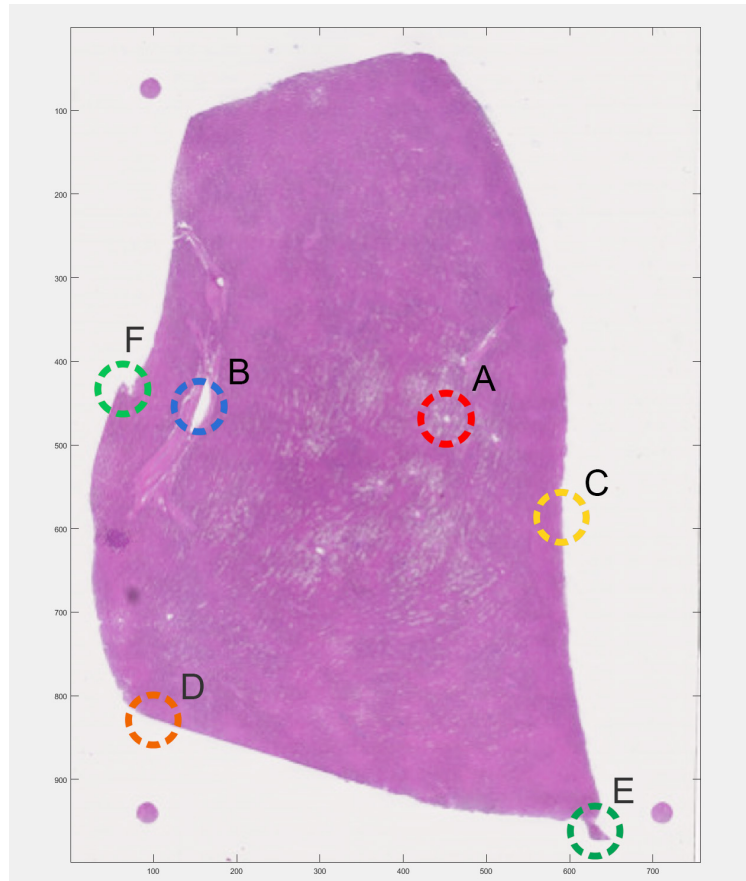
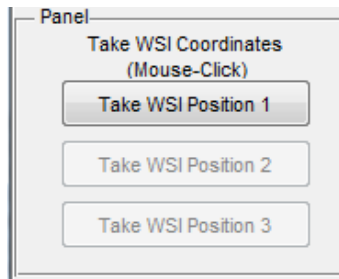|	Not Unique	Not Unique Enough or Centered	Unique and Centered
Example 1	A	C	E
Example 2	B	D	F

Low Resolution Registration

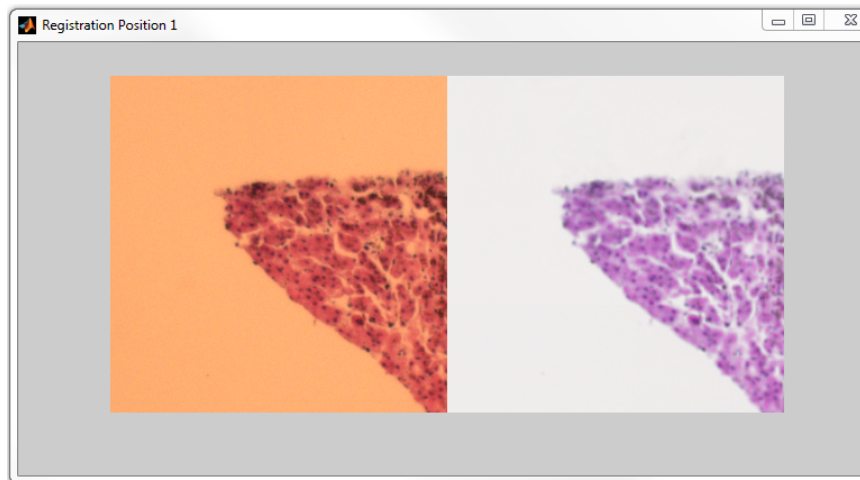1.After finding the unique desired area, press "**Take Stage Position 1**"
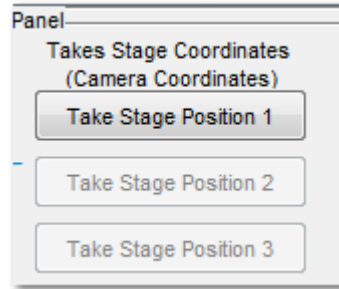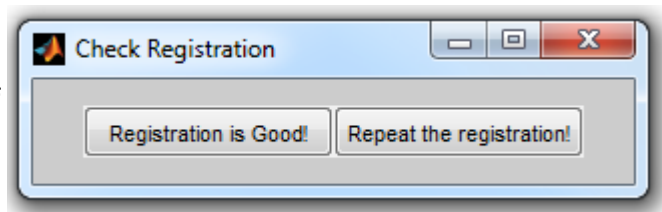

2.Click the same position in the WSI thumb image
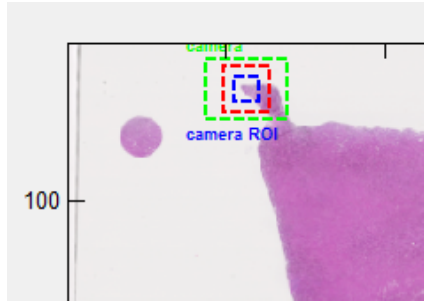
3.Press "**Take WSI Position"** to start registration

4.Check the registration result in Reigistration 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

* "Load last calibration" button can be used to skip low resolution registration

High Resolution Registration

1.Change lens to high resolution registration's setting (Digital image is a zoomed up interface of the low resolution registration and WSI image may not be focused well)

2. After completing the low resolution registration, press **"Load last calibration"** to get into high resolution registration. If users decide to use **"Load last calibration"** the stage will move the glass slide to the previous experiment's position.

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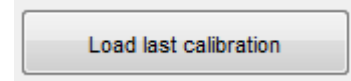
3.Press "**GOTO position 1**" to move stage to first low resolution registration position

Panel
Takes Stage Coordinates
(Camera Coordinates)
GOTO position 1
GOTO position 2
GOTO position 3
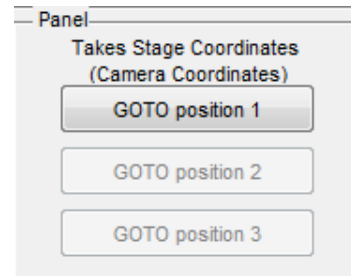
4.Similar as low resolution registration part, click WSI image for related area for high resolution registration.

5. Repeat steps 3, 4 twice for two other positions

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

Finished Registeration

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.

Selecting WSI points:

    1. Select points that have unique features in non-homogenous areas of the WSI (Remember to maximize the distance between the registration points) *(Figure 1)*

    2. Administrator will 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.

Determining 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 *(Figure 2)*

    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.

    8. Once the x,y coordinates have been recorded for a registration points, press "**Register ROI**" (This may take a few minutes to execute)

    9. Repeat this process twice more locating and registering Points 2 and 3

    10. Repeat this procedure for all slides loaded into slots 1-8 and hit "done" button.
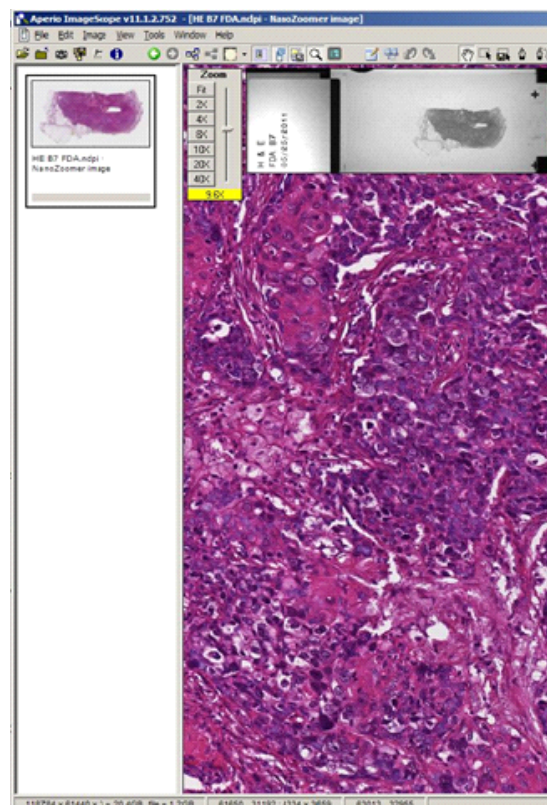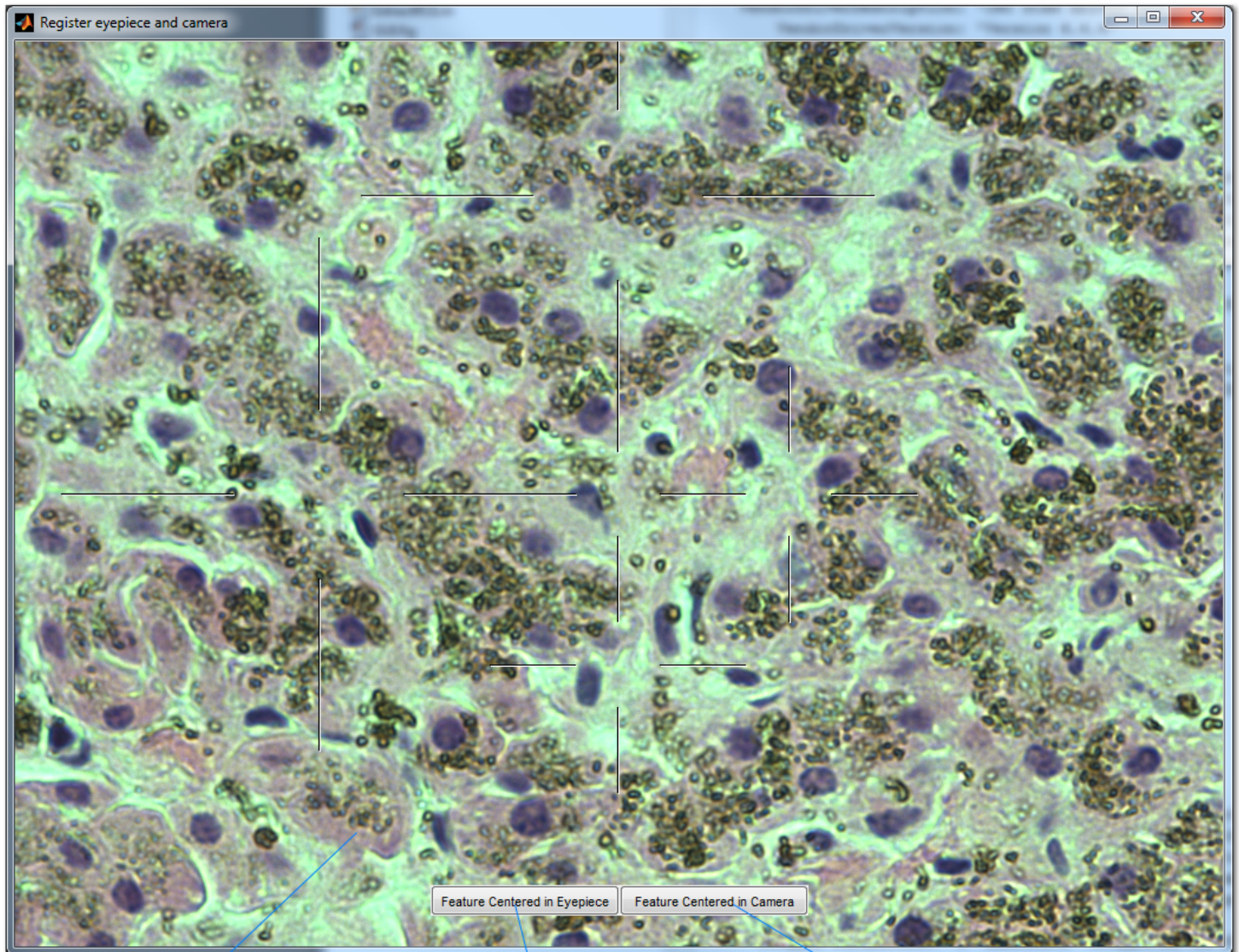

Figure 1

Figure 2

Register Eyepiece and Camera Window

In most the, there is offset between camera image and microscope eyepiece. User can do registration of the camera image with eyepiece image in this section.

1 Camera image
Real time image of the WSI image

2 Feature Centered in Eyepiece
 Take the stage position for eyepiece

3 Feature Centered in Camera
Take the stage position for camera image
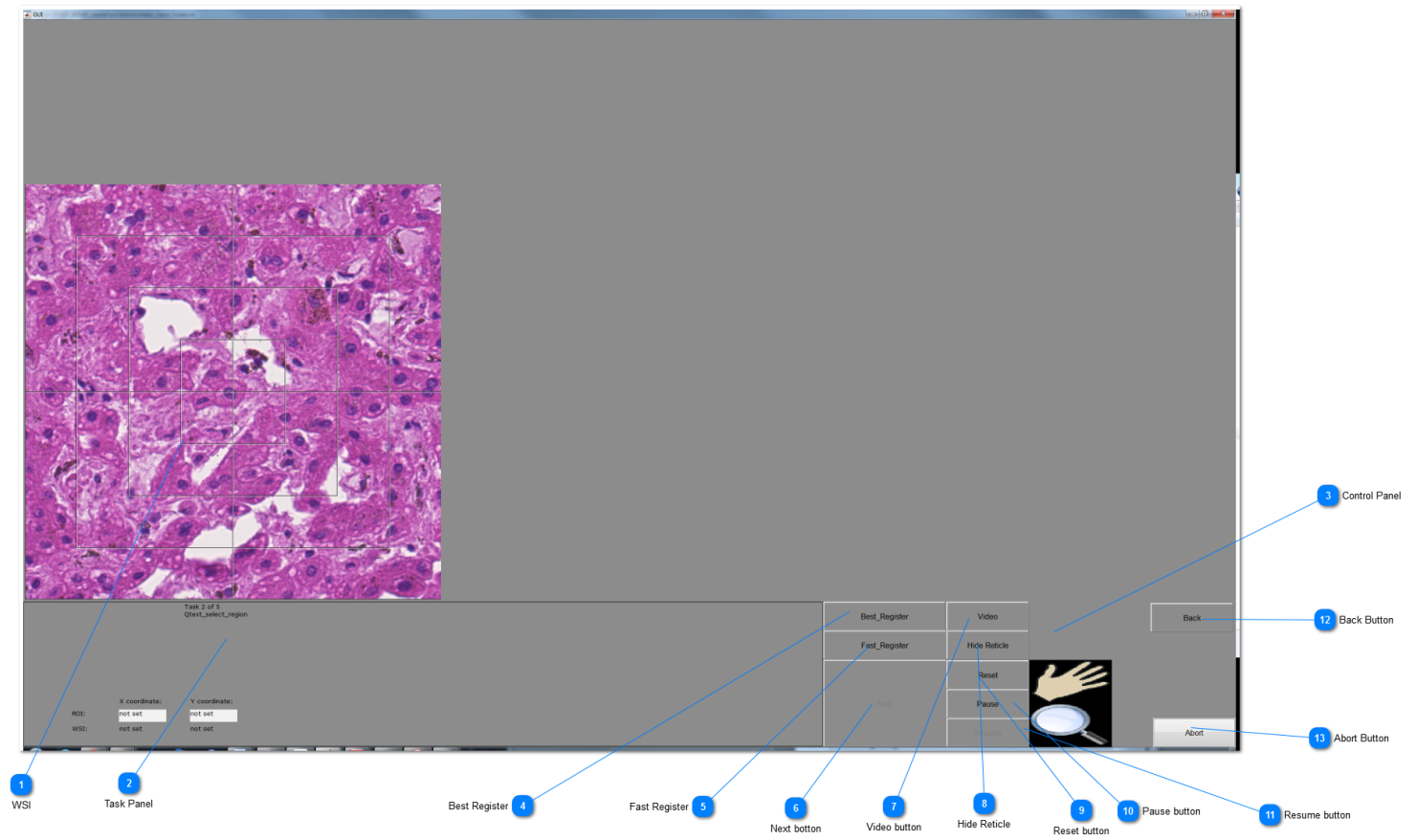
GUI Welcome Page Window

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

User ID
Input user name

Start Experiment
After input user name start experiment

Abort Experiment

Experiment Interface

Once you input the reader's ID, the evaluation tasks will begin when the ROI Image Presentation Screen opens. It is presented in a fashion identical to the Digital mode experiment. 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".
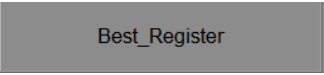

WSI
ROI of WSI

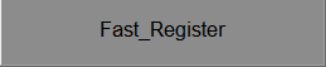### Task Panel
Shows the information and question for each task

Control Panel

Controls for the Digital experiment

Best Register

Best_Register

Do high quality registration WSI and camare image and adjust the stage position, only available in MicroRT mode

Fast Register

Fast_Register

Do fast registration WSI and camare image and adjust the stage position, only available in MicroRT mode

Next botton

Next

Go to next task

Video button

Video

Display camera image

Hide Reticle

Hide Reticle

Hides the reticle shown on the current digital WSI image
(Show Reticle: Reveals the hidden reticle on the current WSI image shown)

Reset button

Reset

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

Pause button

Pause

Pause current task

Resume button

Resume

Resume task from pause status

Back Button

Back

Go back to previous task

Abort Button

Abort

Abort/ Exit the current experiment

Preview with Cross-Hairs Window

In MicroRT mode, users can preview with Cross-Hairs window and show the camera's live microscope image for the administrator view and registration between WSI and camera image.
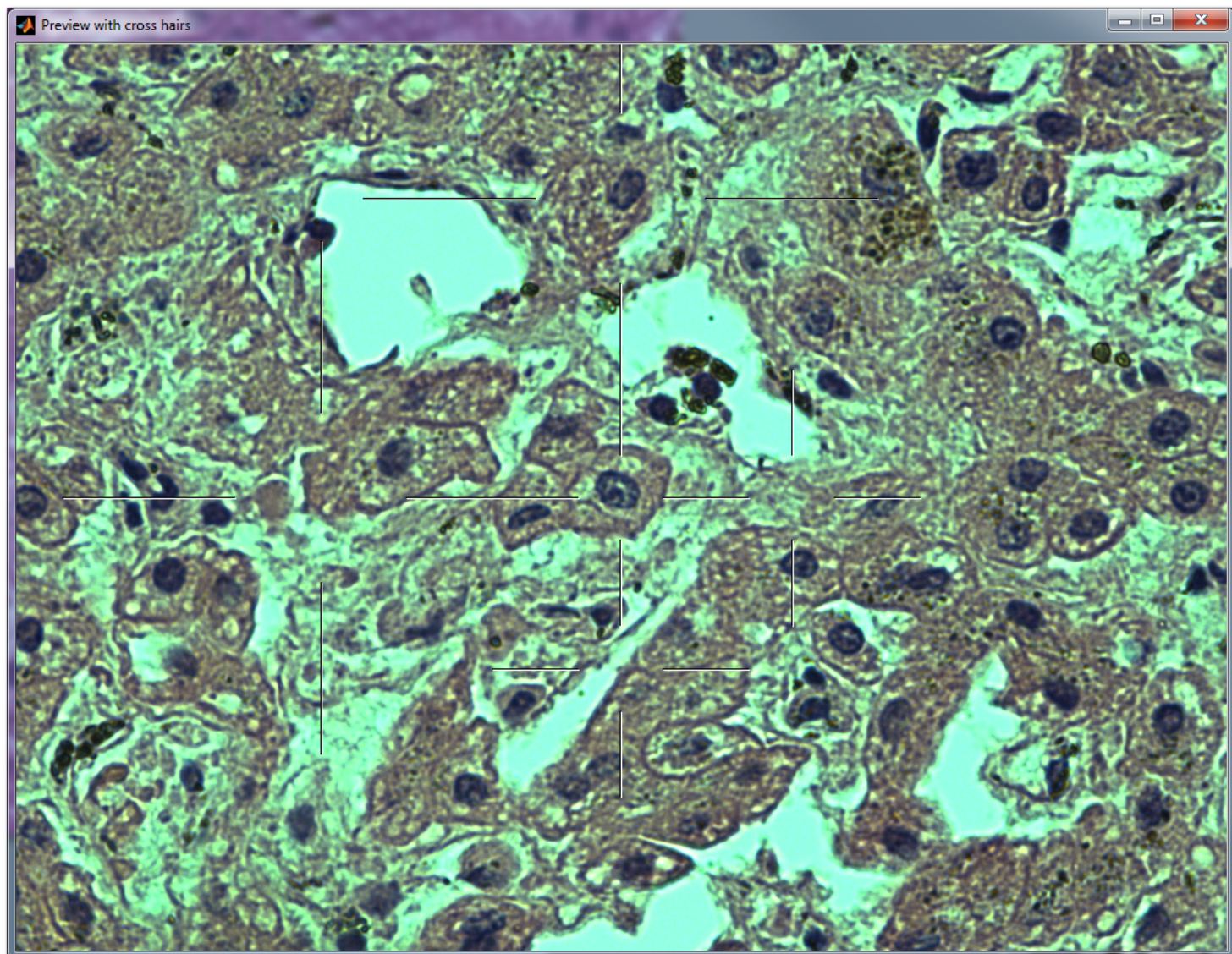
Thank You Window

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

Next
Finish experiment and save result

Directory Structure

A typical eeDAP installation will have the following structure. This is a sample structure of where your results/data will be stored.
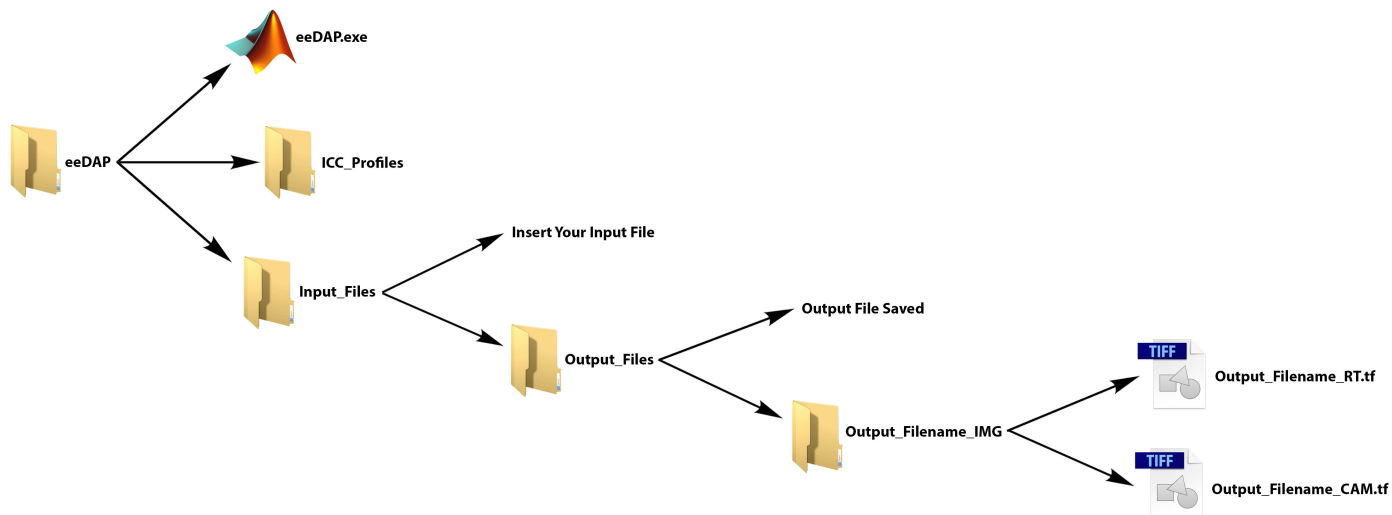
VII. Stage and Camera Control Utility Functions

There is another stand-alone application (individual software) "**Camera_stage_review.exe**", which could run the test and control the camera, microscope, and stage outside of the eeDAP file.

It has the following features:
1. Take a picture and save it to a file.
2. Get stage position coordinates.
3. Move 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 the user has 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

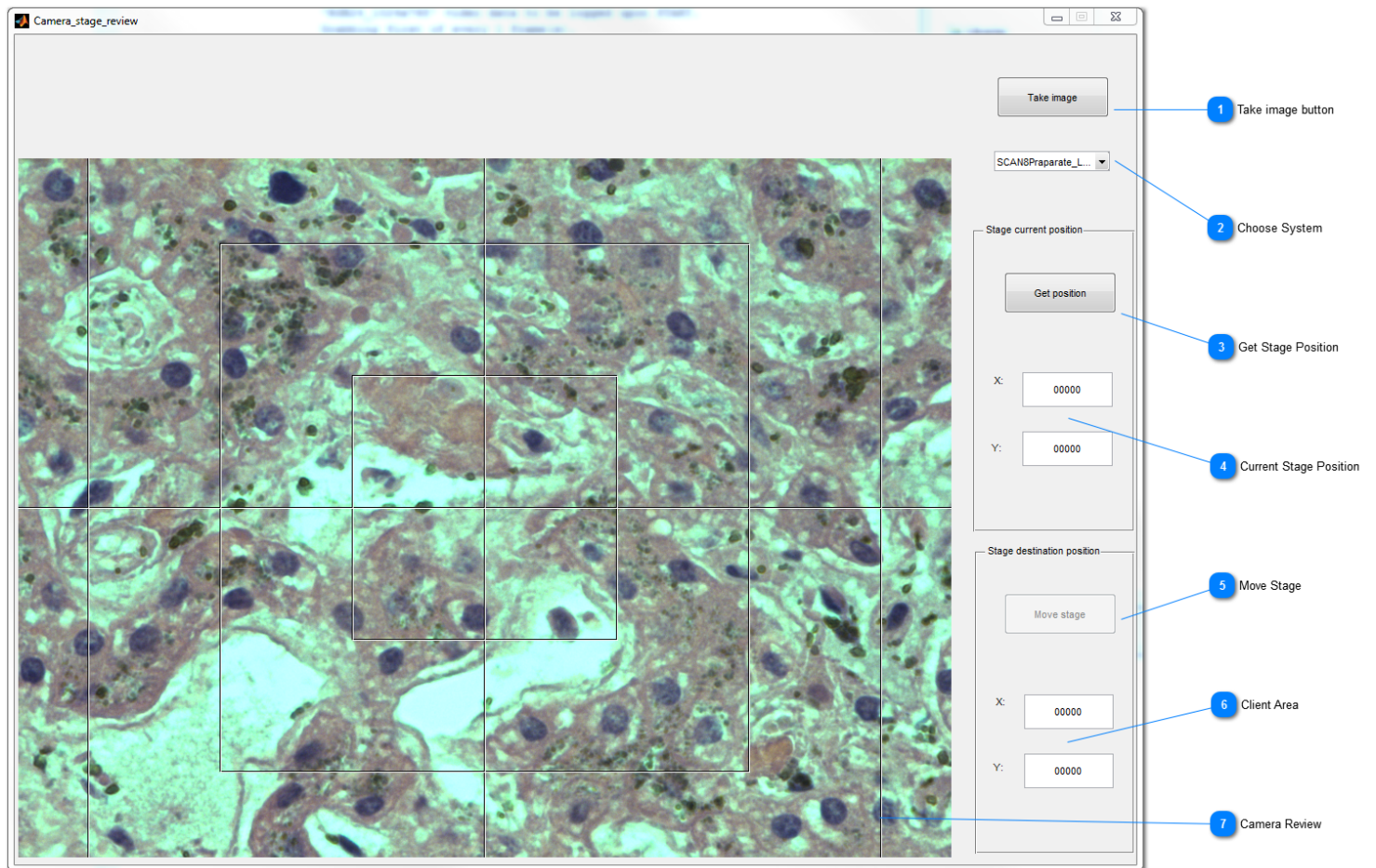User could choose the working system here. Without choosing a system "Stage current position" and "Stage destination position" will be disabled. But tge user could still use **"Take image"** button to get camera image

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**
Exact view of the WSI image

6 **Client Area**
Input the destination positions for stage

VIII. FDA Hardware Specifications

FDA specifications that are suitable for the eeDAP software.

Cameras and Displays

Current **Cameras** that are compatible with the eeDAP software.
• Point Grey Grasshopper Color (GRAS-03K2C-C)
• Point Grey Flea2 Color (FL2G-50S5C-C)
• Point Grey Grasshopper3 Color (GS3-U3-50S5C-C)

Different **Displays** and a few of their descriptions.

Name of Display	HP L2335	Dell 1908 FPt	Lenovo Thinkvision L220XWC
Size	49.6cm x 31.1cm	37.6cm x 30.1cm	47.4cm x 29.6cm
Pixal Size	1920 x 1200	1280 x 1024	1920x1200
Pixal Pitch	258um	294 um	247 um
Contrast	500:1	800:1	1200:1
Brightness	250 cd/m^2	300 cd/m2	325 cd/m2

Package and Unpackage

Package:

A. Camera
      1. Unplug USB3 cable from camera.
      2. Use allen wrench to take off camera (adapter) and install caps.
      3. Separate camera adapter(if necessary) and the camera, and put the cap on camera, adapter and microscope.
      4. Put camera and adapter in pink bubble bag and put the bag in microscope frame box (on the top of the paper frame).

B. Stage
      1. Unplug stage cable from stage and controller.
      2. Unplug power, joystick cables from stage controller.
      3. Put all cables and joystick in cable bag (bag from prior box).
      4. Use allen wrench to take off stage from Microscope.
      5. Put stage in stage box.
      6. Use bubble paper to package stage controller and stage. Then put them in the main microscope box.

C. Microscope
      1. Unplug the power cable and put in cable bag.
      2. Use allen wrench to take off the eyepiece part, and put eyepiece into eyepiece box.
      2. Put the microscope in microscope frame box (tape over hole where eyepiece attaches).
      3. Put eyepiece, frame boxes in main microscope box.
      4. Put microscope cover in main box.

D. Computer
      1. Unplug power, display and camera USB cable.
      2. Put cables, computer, mouse and working dock in computer bag.

E. Screen
      1. Unplug display cable and put in screen box.
      2. Put screen in box.

F. Other
      1. Allen wrench bag (put in main box).
      2. Power strip (put in screen box).
      3. Microscope cover (put in main box).

Un-package:

Bring all small boxes from microscope main box.

A. Microscope and camera
      1. Bring camera and adapter from frame box.
      2. Bring microscope frame out from box and remove the cover.
      3. Bring microscope eyepiece out from box.
      4. Use Allen wrench install eyepiece, adapter (remove caps).
      5. Install camera on adapter.
      6. Plug in the power.

B. Stage
      1. Bring stage and controller out.
      2. Install stage to microscope by Allen wrench.
      3. Connect controller with stage, joystick and computer.
      4. Plug in the power.

C. Screen
      1. Connect screen with computer and plug in the power.

D. Computer
      1. Plug computer on working dock.
      2. Plug in camera, controller and screen.

IX. For eeDAP Developers

Extra information for eeDAP Developers

The [GitHub Master Page](#)

Creating the Stand-Alone Application

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.

File required to run (complied):
           - btmatlab
           - stages
           - tasks folder

Fild installed (package):
           - sample_inputfiles
           - gui_graphics
           - icc-profiles