

# eeDAP

**Evaluation Environment for Digital & Analog Pathology**

---

## User Manual

2015

## Table of Contents

I. Introduction .....	3
Credits .....	4
II. Getting Started with eeDAP .....	5
Download and Install .....	6
Run Software .....	7
Tasks Instructions .....	9
III. Hardware and Software Requirement.....	11
Stage .....	12
Stage Set up.....	13
Stage Test.....	14
Camera .....	15
Requirements [EDIT] .....	16
Camera Setup and Test .....	18
Alignment .....	19
Microscopes .....	20
Types of Microscopes.....	21
Reticles .....	22
Scanners .....	23
Software Requirements .....	24
Read and extract WSI image.....	25
BIO Formats .....	26
Possible Errors .....	27
Image Scope by Aperio.....	28
Matlab Compiler Runtime Libraries .....	29
IV. Input File.....	30
Input File Header.....	31
Editing Input File Body .....	32
Input File Body: List of ROIs and Corresponding Tasks .....	33
V. Output File.....	34
VI. WorkFlow.....	35
eeDAP Administrator Input Screen Window(c) .....	36
MicroRT Registration .....	38
Slide Registration window .....	39
Semi-Automated Registration .....	41
Low Resolution Registration.....	42
High Resolution Registration .....	43
Manual Registration .....	44
Register Eyepiece and Camera Window .....	45
GUI Welcome Page Window.....	46
Experiment Interface.....	47
Preview with Cross-Hairs Window .....	49
Thank You Window.....	50
Directory Structure .....	51
VII. Stage and Camera Control Utility Functions .....	52
Camera Stage Review Window [EDIT] .....	53
VIII. FDA Hardware Specifications .....	55
Cameras.....	56
Displays.....	57
IX. For eeDAP Developers.....	58
Creating the Stand-Alone Application .....	59

## 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

<b>WSI, WSIs</b>	Whole Slide Image(s)
<b>ROI, ROIs</b>	Region(s) of Interest
<b>MicroRT</b>	Microscope Real-Time Viewing
<b>RT</b>	Real Time
<b>FOV</b>	Field of View
<b>Administrator</b>	The (task) administrator is responsible for task set up and monitoring
<b>Reader</b>	Person evaluation the ROI's
<b>Scan Scale</b>	WSI scanning
<b>IOP</b>	Intermediate Ocular Plane
<b>FN</b>	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 simpler and more user friendly
  - Using GitHub to keep track of changes and updates made to the manual

### 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 studies. Users will have two options either Micro RT or Digital.

## Download and Install



Go to [www.github.com/DIDSR/eeDAP.com](https://www.github.com/DIDSR/eeDAP.com). Download the "eeDAP3p0.zip" file from the [GitHub eeDAP Release Page](#) and extract it. There are two versions inside. One is for windows system the other is for linux system.

Jump to [Running eeDAP](#).

**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"

## Run Software

**\*If this is your first time using eeDAP, we recommend you go to the [Workflow](#) page for more information on how to run 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
- Setting up Stage and Hardware
  1. Install [the camera](#) on the microscope and connect it to the computer
  2. Install the property camera driver, [test](#) and [align](#) the camera
  3. Connect the stage controller with microscope the stage
  4. Check and [test the connecting status](#) between the computer and joystick
- 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
  3. Extract the chosen sample file.
  4. Select viewing mode, **Digital** or **MicroRT** (If MicroRT 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 Position**
    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. Microscope stage will automatically move to last known 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 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 formed by, "Study Model, User ID, Time, & Input File Name .dapso"
3. Output tissue images folder name is formed same as the output file
4. Output task results are formed by, "Task Name, Task ID, Task Order, Slot (Where the glass was placed)"

## 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 (Figure 1)

### "count"

1. Enter the number of cells that appear to accommodate the description (Figure 2)

### "radio1of4"

1. Select the one of the options that accommodates the description (Figure 3)

### "slider"

1. On a scale 0-100, rate the tissue using the slider or typing in the number (Figure 4)

### "mitotic\_train"

1. Check all the boxes that apply to the image
2. Using the slider or by typing in 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 in the number, give the tissue a score

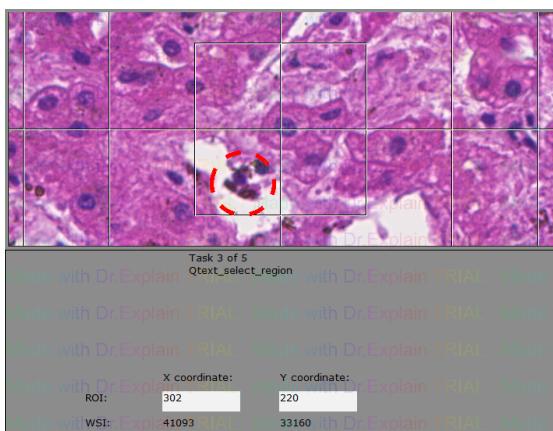


Figure 1

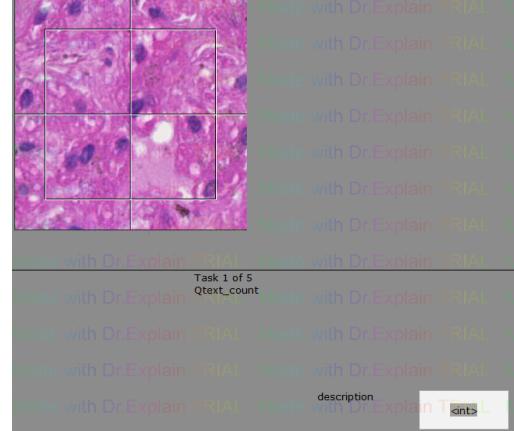


Figure 2

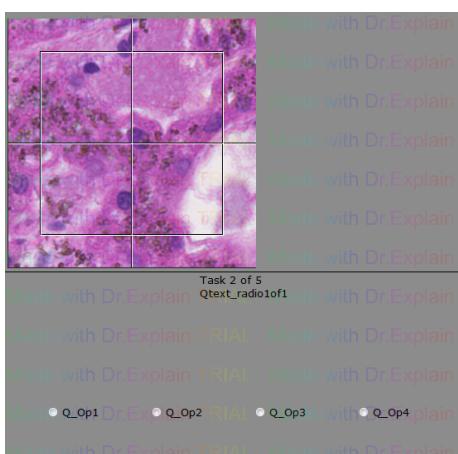


Figure 3

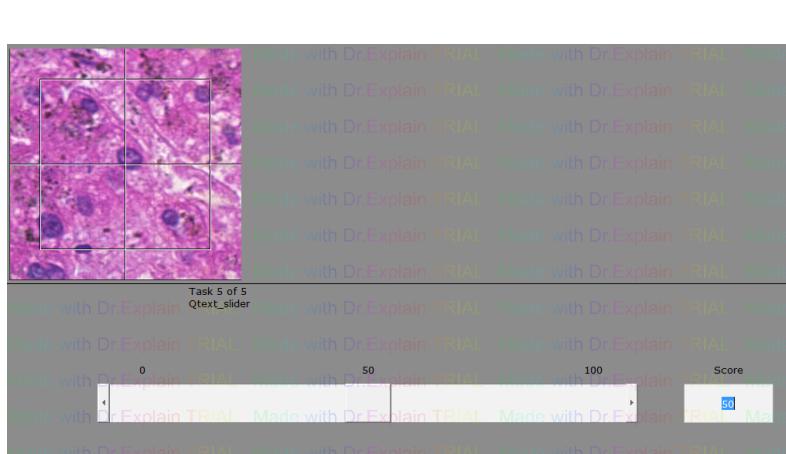


Figure 4



### 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. End users we suggest you use the stages we recommend to obtain the best results.

Different stages have different resolutions which will cause different results. Follow the directions to gain the best results as you conduct this experiment.

#### Ludl Stage:

Now, FDA uses Ludl MAC5000 and MAC6000 stage

## Stage Set up

The stage should connect with computer by RS232 serial to USB cable.

For **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.

For **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 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.

### 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.

---

---

## 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 [EDIT]

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

eeDAP requires camera images to be RGB24 (8 bit for each channel).

eeDAP requires camera images to have width > 640 and height > 480.

FDA uses 2 Point Grey cameras for this eeDAP software

Name of Camera	Description
Point Grey Grasshopper Color (GRAS-03K2C-C)	<p><b>Default Format:</b></p> <p>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.</p> <ul style="list-style-type: none"> <li>• sensor size = 1/3"</li> <li>• sensor size = 640x480 pixels (0.3MP)</li> <li>• pixel size = 7.4um</li> </ul> <p>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:</p> <ul style="list-style-type: none"> <li>• 0.185um/pixel at 40x</li> <li>• 0.370um/pixel at 20x</li> <li>• 0.740um/pixel at 10x</li> <li>• 1.480um/pixel at 5x</li> <li>• 1.850um/pixel at 4x</li> <li>• 2.960um/pixel at 2.5x</li> </ul>
Point Grey Flea2 Color (FL2G-50S5C-C)	<p><b>Native Pixel Format:</b></p> <p>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:</p> <ul style="list-style-type: none"> <li>• sensor size = 2/3"</li> <li>• sensor size = 2448x2048 pixels (5.0MP)</li> <li>• pixel size = 3.45um</li> </ul> <p><b>Default Format:</b></p> <p>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.</p> <p>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.</p> <p>When there is no camera magnification, the pixel size of the default Matlab format (6.9um) is divided by the objective magnification.</p> <ul style="list-style-type: none"> <li>• 0.1725um/pixel at 40x</li> <li>• 0.3450um/pixel at 20x</li> <li>• 0.6900um/pixel at 10x</li> <li>• 1.3800um/pixel at 5x</li> <li>• 1.7250um/pixel at 5x</li> <li>• 2.7600um/pixel at 2.5x</li> <li>• 3.4500um/pixel at 2x</li> </ul> <p><b>Alternative Format:</b></p> <p>The Matlab format code F7_RGB24_1600x1200 uses the native pixels but a smaller standard format.</p>

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

**Default Format:**

- sensor size = 2/3"
- sensor size = 1384 x 1036
- pixel = 6.45 um

## 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, 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 application (1394CameraDemo32.exe). It behaves similarly to the Matlab "imaqtool" mentioned above. You could use it to test whether camera is installed correctly. t operating modes.

### 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:

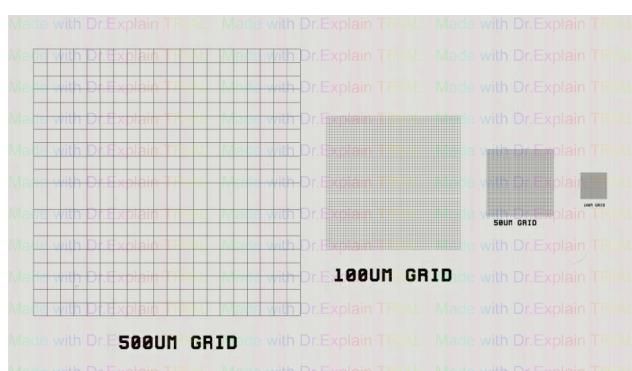
1. Click the button "**imaqtool**".

\*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.)

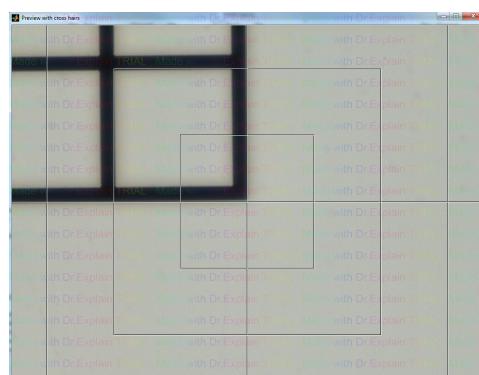
## Alignment

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 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** 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 [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 (2) microscopes fully compatible with the software. Developers are researching on how to make eeDAP compatible with any microscope at hand.

### 1. 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

### 2. 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.

Name of Reticle	Display View	Description
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		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 ( $5x = 1.25mm/5 = 0.2500mm$ , $10x = 1.25mm/10 = 0.12500mm$ , $20x = 1.25mm/20 = 0.06250mm$ , $40x = 1.25mm/40 = 0.03125mm$ ), and apparent grid spacing "at 25cm" = 12.5mm.

## Scanners

These are currently 4 different scanners are compatible with the eeDAP software in certain locations.

The scale factors of the scanner were measured by Neil O Flaherty

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

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

## 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". The individual pages for the methods will go in more depth with the details of each of them.



## BIO Formats

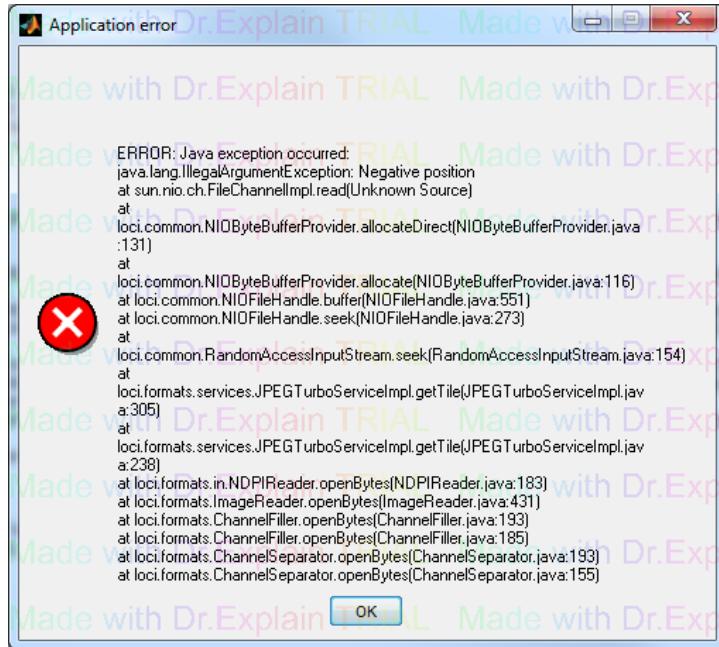
Bio-Formats is currently being used in eeDAP. It is a stand-alone Java library for reading and writing life sciences image file formats. It is capable of parsing both pixels and metadata for a large number of formats, as well as writing to several formats. It was developed by the Open Microscopy Environment 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.

## Possible Errors

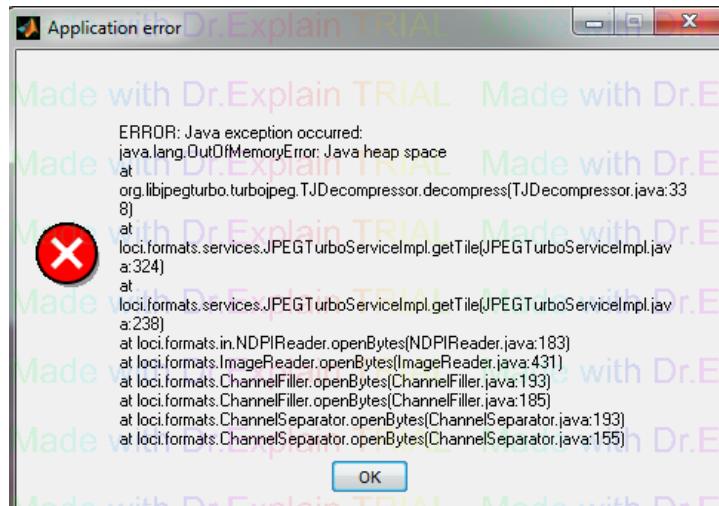
The BIO formats might have some problem when extract WSI. In this section we show two normally errors and the solve methods

### 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 larger image, you might face this problem.

### 2. Out of Memory/Storage



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 can download the java.opts and paste it in the matlabroot 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 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)

**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\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 three sections: Header, Free Text, 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. The Free Text are just the setting for the following experiment

```
tissue40x-8B-olympus - USB.dapsi - Notepad
```

**Header**

```

File Edit Format View Help
Author: Made with Dr.Explain TRIAL Made with Dr.Explain TRIAL Made with Dr.Explain TRIAL M
Date: Made with Dr.Explain TRIAL Made with Dr.Explain TRIAL Made with Dr.Explain TRIAL M
Time: Made with Dr.Explain TRIAL Made with Dr.Explain TRIAL Made with Dr.Explain TRIAL M

For the Hamamatsu scanning at 20x Made with Dr.Explain TRIAL Made with Dr.Explain TRIAL Made with Dr.Explain TRIAL M
scan_scale = .4558
For the Hamamatsu scanning at 40x Made with Dr.Explain TRIAL Made with Dr.Explain TRIAL Made with Dr.Explain TRIAL M
scan_scale = .2279

Point Grey Grashopper Color (GRAS-03K2C-C)
cam_format = RGB24_640x480
cam_pixel_size = 7.4um
Point Grey Flea2 Color (FL2G-5055C-C): Full resolution, full format
cam_format = F7_RGB24_2448x2048
cam_pixel_size = 3.45
Point Grey Flea2 Color (FL2G-5055C-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,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
```

**Study Settings**

```

SETTINGS
NUMBER_OF_WSI      = 1
wsi_slot_1         = C:\000_whole_slides\tissue40x-8B.ndpi
rgb_lut_slot_1     = icc_profiles\rgb_lut_gamma_inv1p8.txt
label_pos          = 12
reticleID          = KR-871
cam_kind           = USB
cam_format         = F7_RGB_1224x1024_Model
cam_pixel_size     = 6.9
mag_cam            = 0.5
mag_lres           = 10
mag_hres           = 40
scan_scale         = 0.2279
stage_label        = H101-Prior
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         = 1
taskorder          = 0
```

**Body**

```

BODY
start
finish
radio1of4,1st0001,-1,1,038459,16192,300,300,300,300,Qtext_Eradio1of1,1,1,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,-1,1,041163,33208,300,700,300,700,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_seTect_region,1,1,Q_Op1,Q_Op2,Q_Op3,Q_Op4
```

## Input File Header

The Header is all the text before the line containing “**BODY**”.

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)

**Study Settings** include:

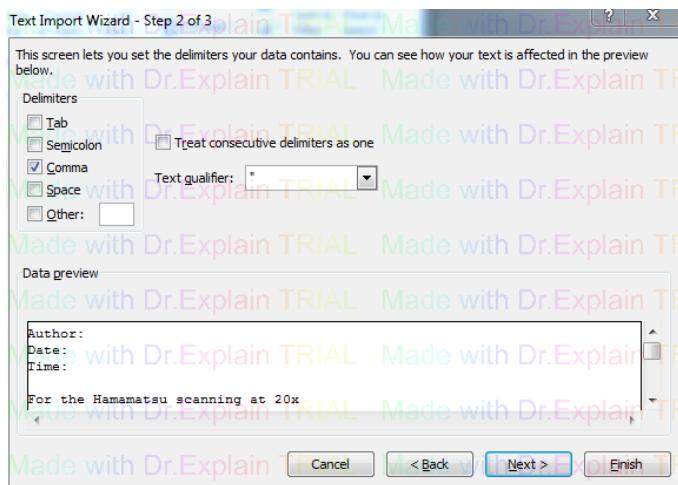
- Number of WSI & other WSI specifications
- “**label\_pos**”: Direction of the glass slide in microscope stage
- Mircoscope Settings (objective, filter, and reflector)
- GUI and Screen Characteristics (foreground color, axes color, background color, font size and screen size)
- “**saveimages**”: Save the experiment image or not
- “**taskorder**”: Controls the order of the tasks

## Editing Input File Body

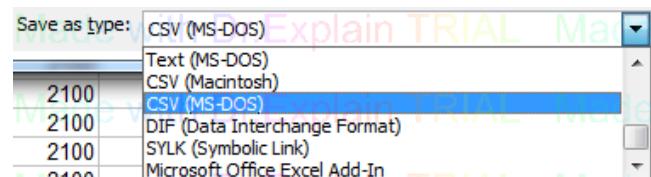
The Body of an input file is editable by a spreadsheet editor such as Excel.

### 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 "Delimiters", 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*

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.

## Default Task Input Format:

### A. Task Instructions

**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. Input Variables

**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:

Customized Input File format should follow "Task Name, Task ID, Task Order and Slot". The other sections could be different depending on the design for that specific task.

## 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** includes an 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 responses.

## VI. WorkFlow

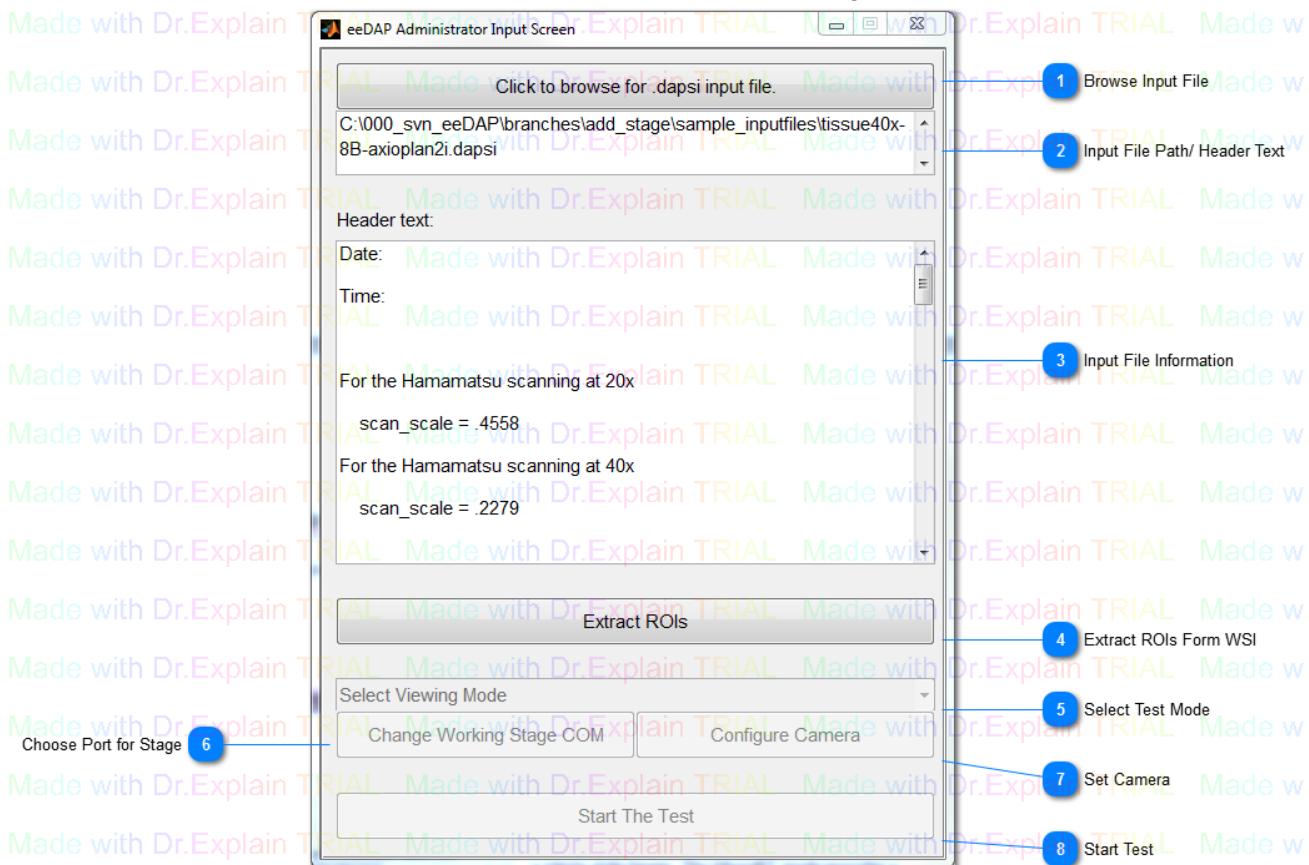
Guidelines of eeDAP in more detail.



### 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. 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.



#### 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: Made with Dr.Explain TRIAL Made with Dr.Explain TRIAL

Time: Made with Dr.Explain TRIAL Made with Dr.Explain TRIAL

Made with Dr.Explain TRIAL Made with Dr.Explain TRIAL  
For the Hamamatsu scanning at 20x

scan\_scale = .4558

Made with Dr.Explain TRIAL Made with Dr.Explain TRIAL  
For the Hamamatsu scanning at 40x

scan\_scale = .2279

Made with Dr.Explain TRIAL Made with Dr.Explain TRIAL

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

#### 4 Extract ROIs Form WSI

Made with Dr.Explain TRIAL Made with Dr.Explain TRIAL

Following the input file, extract Region of interest 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)

#### 5 Select Test Mode

Made with Dr.Explain TRIAL Made with Dr.Explain TRIAL

- 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

Made with Dr.Explain TRIAL

Change Working Stage COM

In microRT mode, set the stage controller connect port

#### 7 Set Camera

Made with Dr.Explain TRIAL

Configure Camera

In microRT mode, set camera

#### 8 Start Test

Made with Dr.Explain TRIAL Made with Dr.Explain TRIAL

Start test

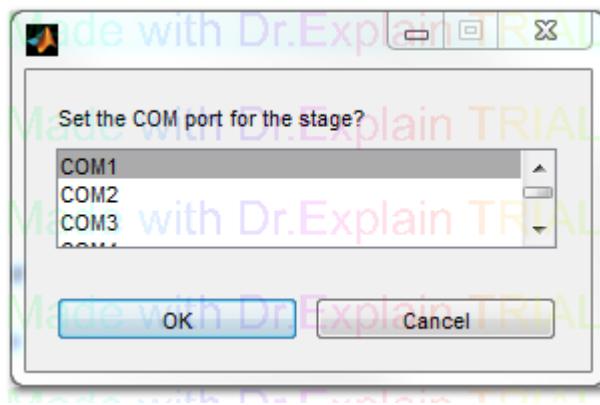
## MicroRT Registration

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:

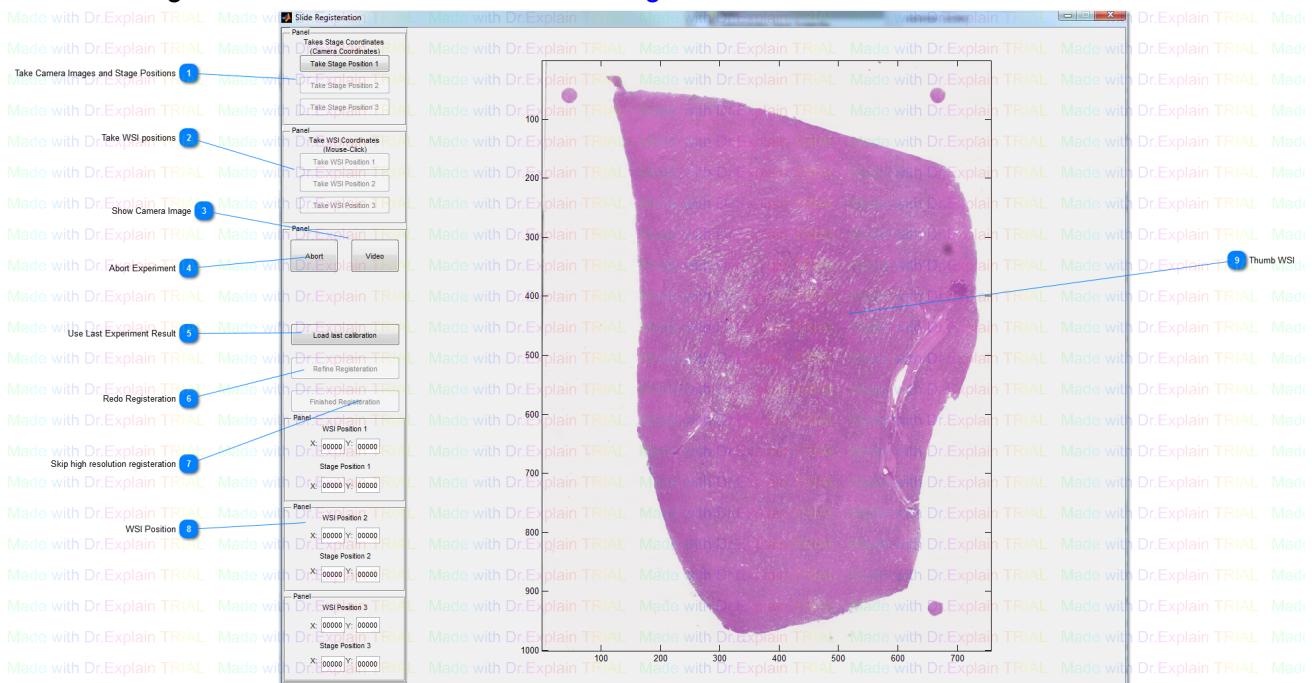
Set the *COM port* connection for the microscope stage controller.

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

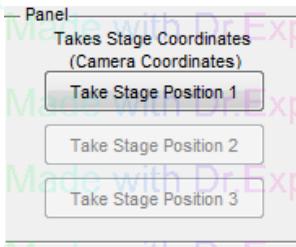


## Slide Registration window

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.

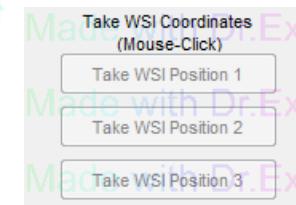


### 1 Take Camera Images and Stage Positions



Take the microscope live image and stage position for 3 areas

### 2 Take WSI positions



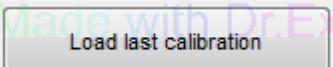
Take the related WSI position for 3 areas

### 3 Show Camera Image

Show the real time image

### 4 Abort Experiment

Exit and discard the experiment

**5 Use Last Experiment Result** Load last calibration

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

**6 Redo Registration** Refine Registration

Redo registration

**7 Skip high resolution registration** Finished Registration

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

**8 WSI Position**

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

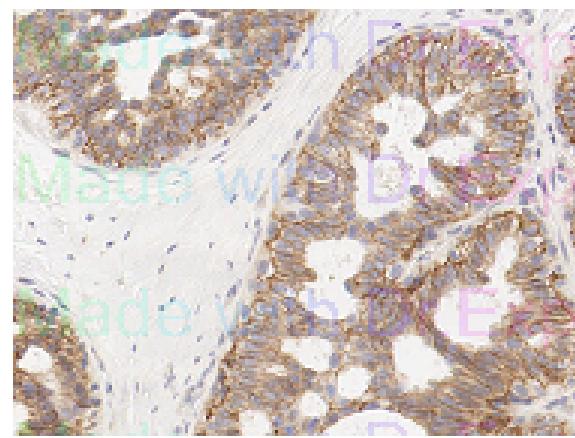
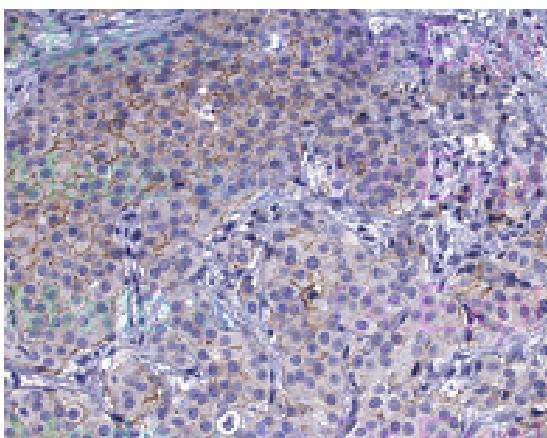
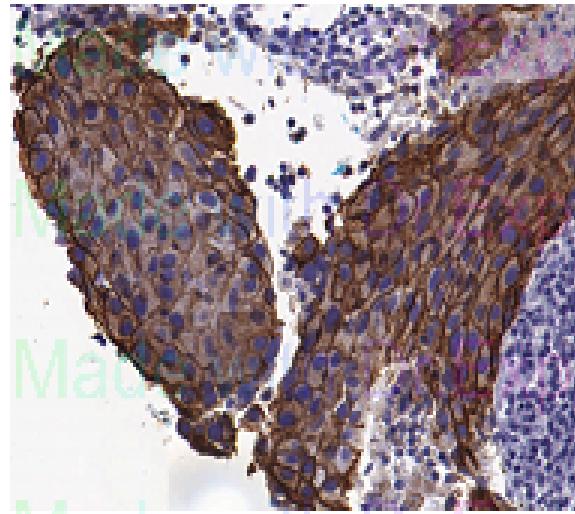
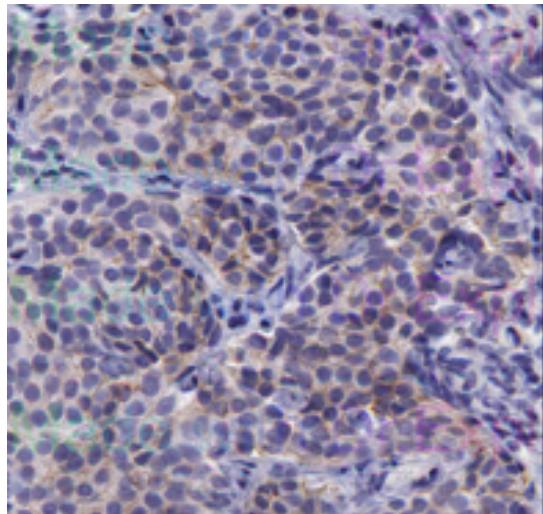
**9 Thumb WSI**

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 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.

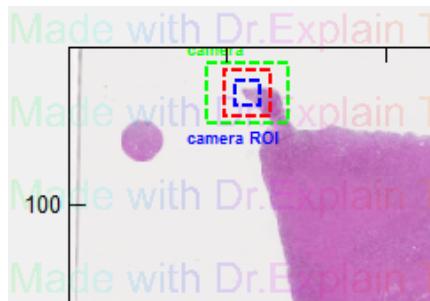


## Low Resolution Registration

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



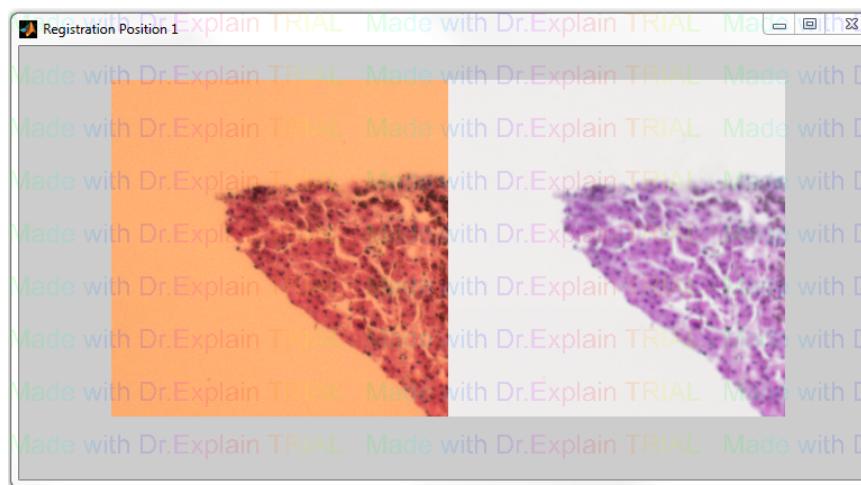
2. Click the related position in the WSI thumb image



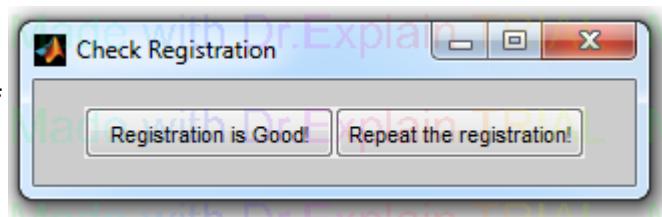
3. Press "**Take WSI Position**" to start registration



4. Check the registration result in Reigstration 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

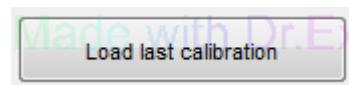


6. Repeat process twice for 2 other positions

## 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.



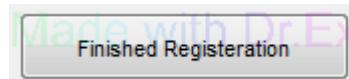
3.Press "**GOTO position 1**" to move stage to first low resolution registration position



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

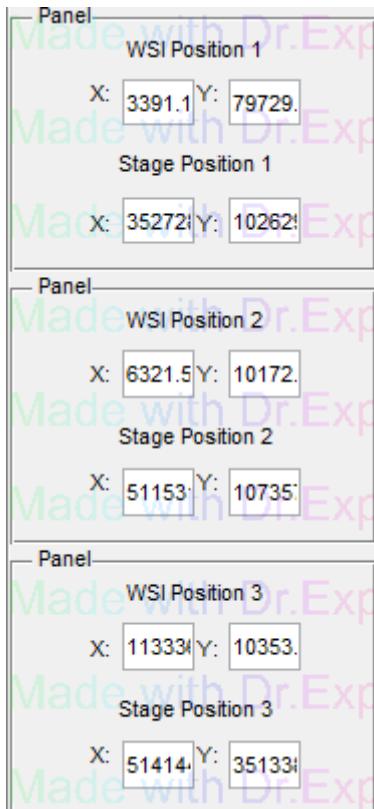
When automated registration fails for a slide, manual registration process may be necessary. This method requires the Administrator to identify three registration points and find them in the WSI and the glass slide.

### Selecting WSI points:

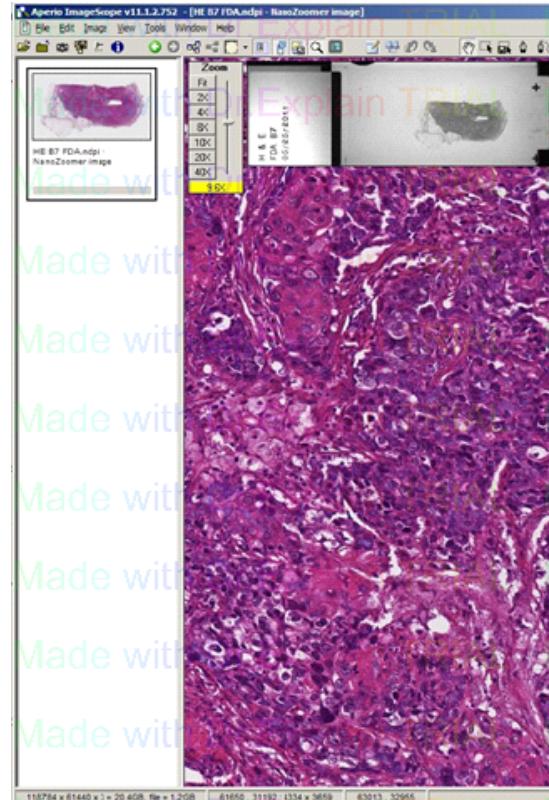
1. Select points that have unique features in non-homogenous areas of the WSI (Remember to maximize the distance between the registration points) (*Figure 1*)
2. Administrator will input the corresponding pairs of x,y coordinates in the Registration window: the x,y coordinates in the WSI and the x,y 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.



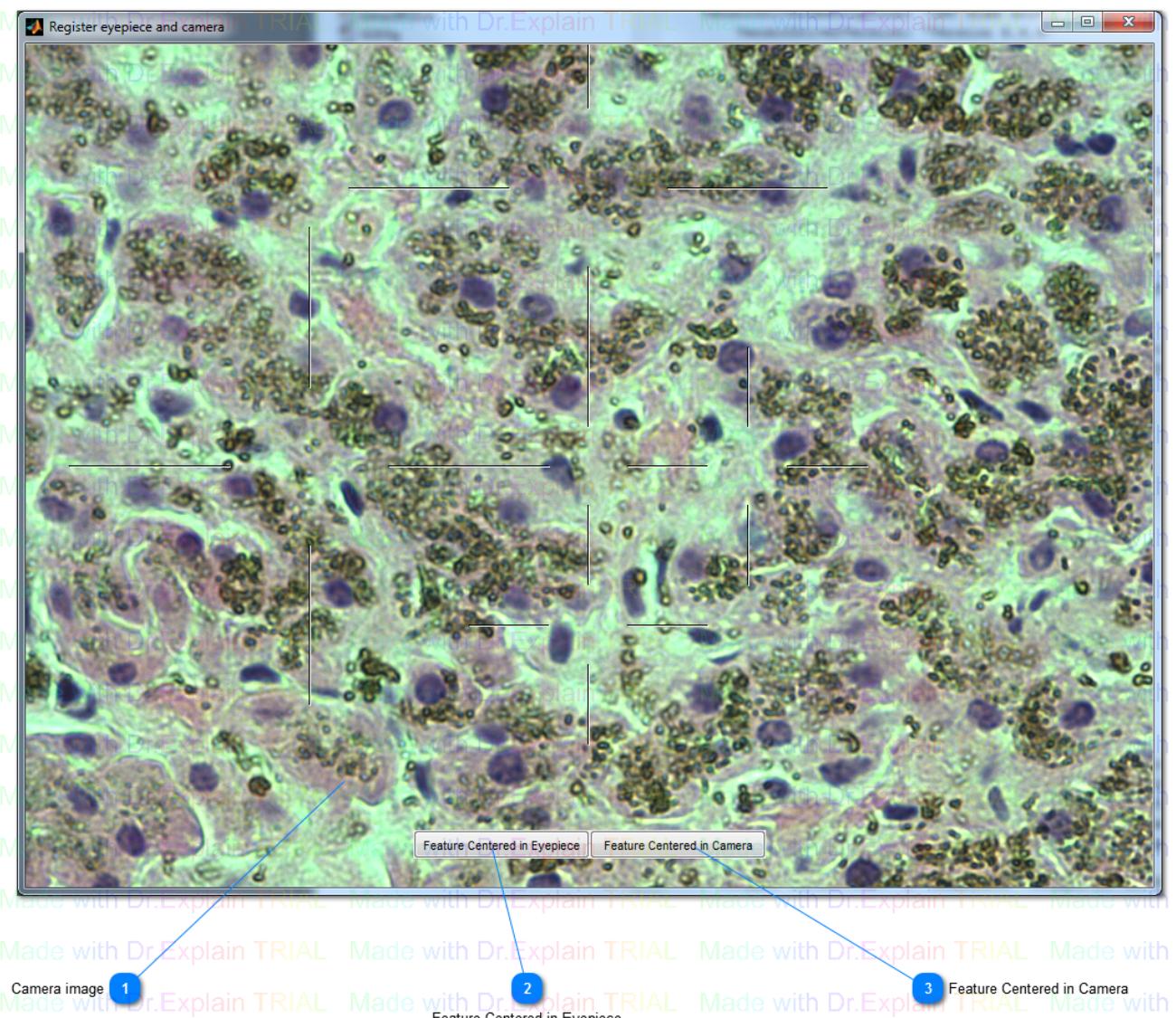
*Figure 1*



*Figure 2*

## Register Eyepiece and Camera Window

Registration of the camera image with eyepiece image.



### 1 Camera image

Real time image of the WSI image

### 2 Feature Centered in Eyepiece

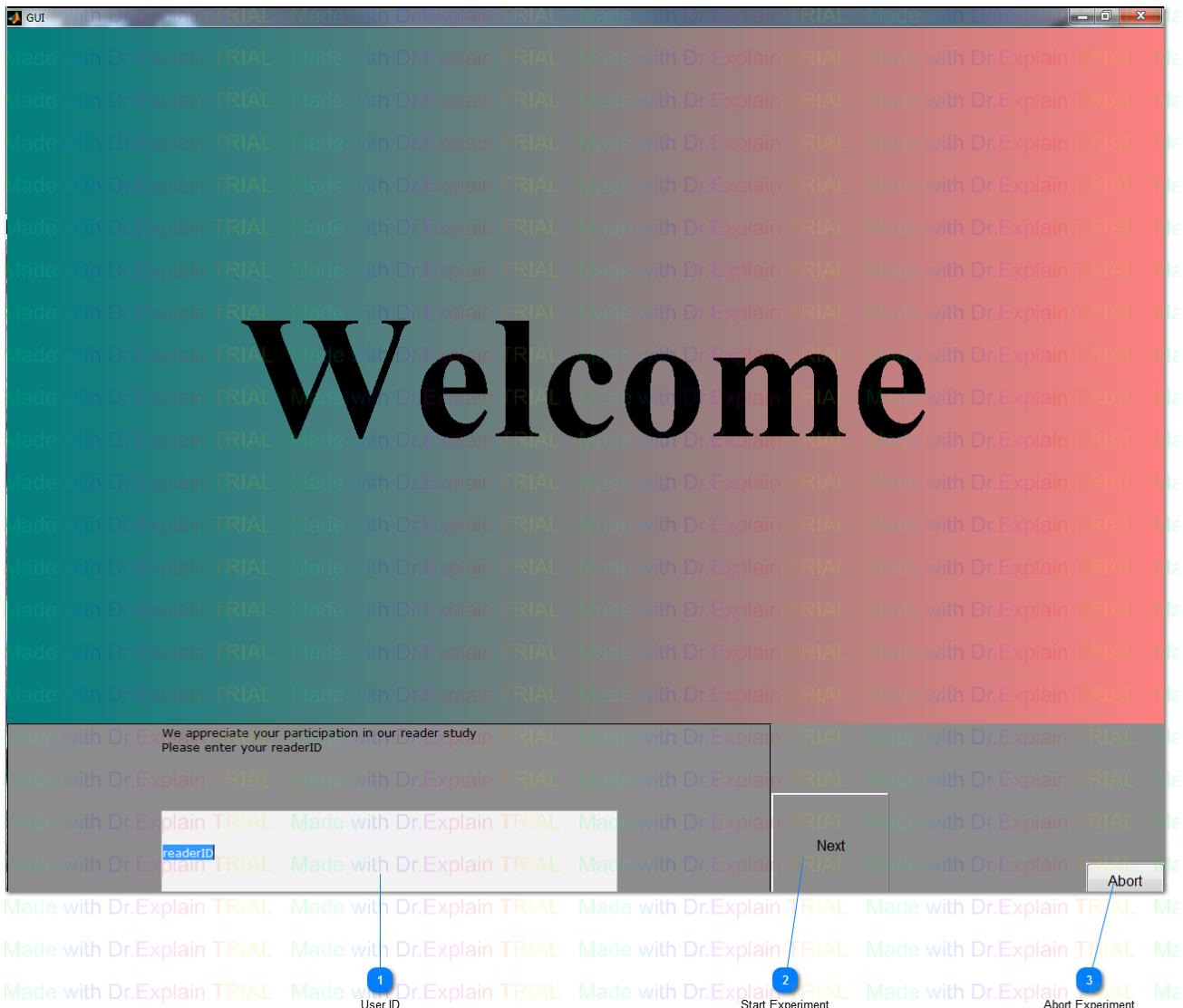
Take the stage position for eyepiece

### 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.



### 1 User ID

Input user name

### 2 Start Experiment

After input user name start experiment

### 3 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

**8 Resume button**

Resume task from pause status

**9 Back Button**

Go back to previous task

**10 Abort Button**

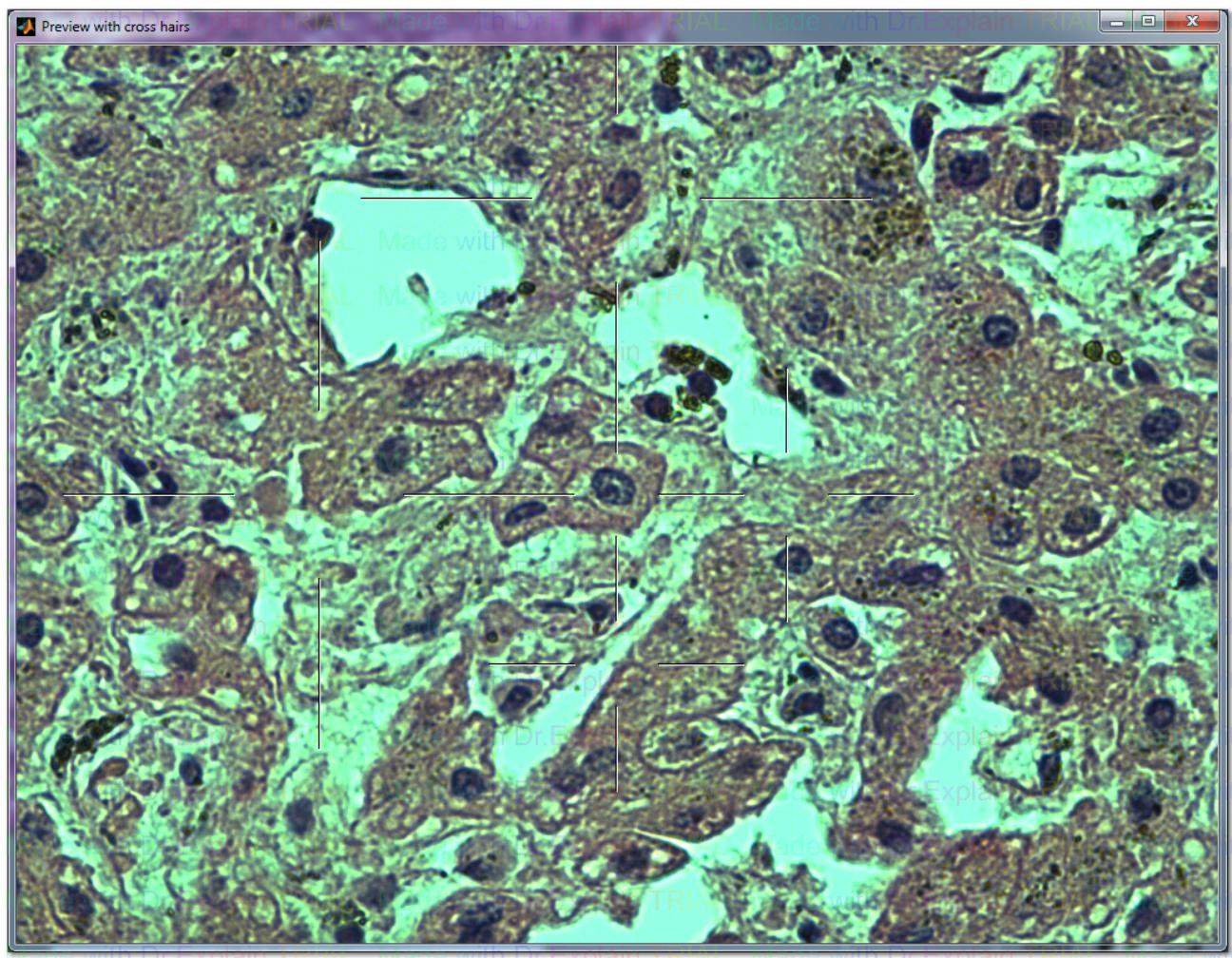
Abort/ Exit the current experiment

**11 Hide Reticle**

Hides the reticle shown on the current digital WSI image

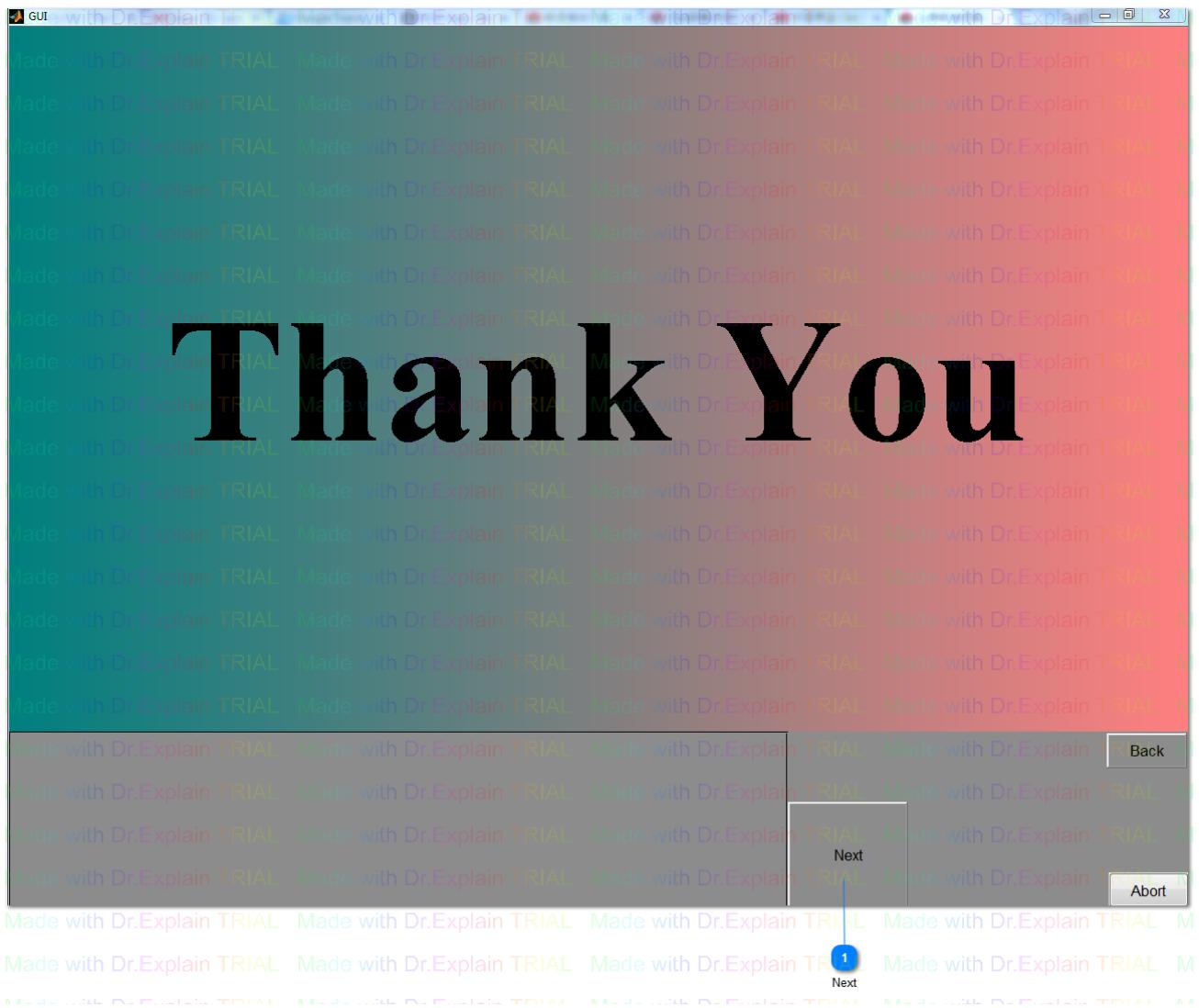
## Preview with Cross-Hairs Window

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

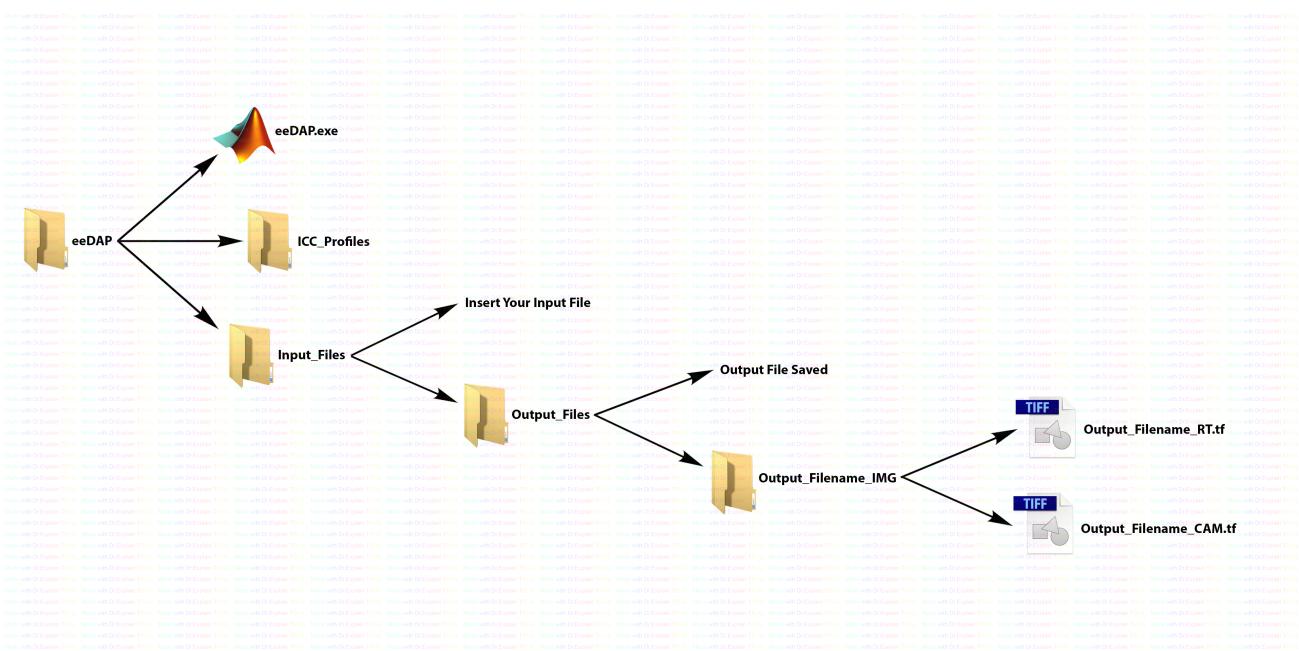


**1** **Next**

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.

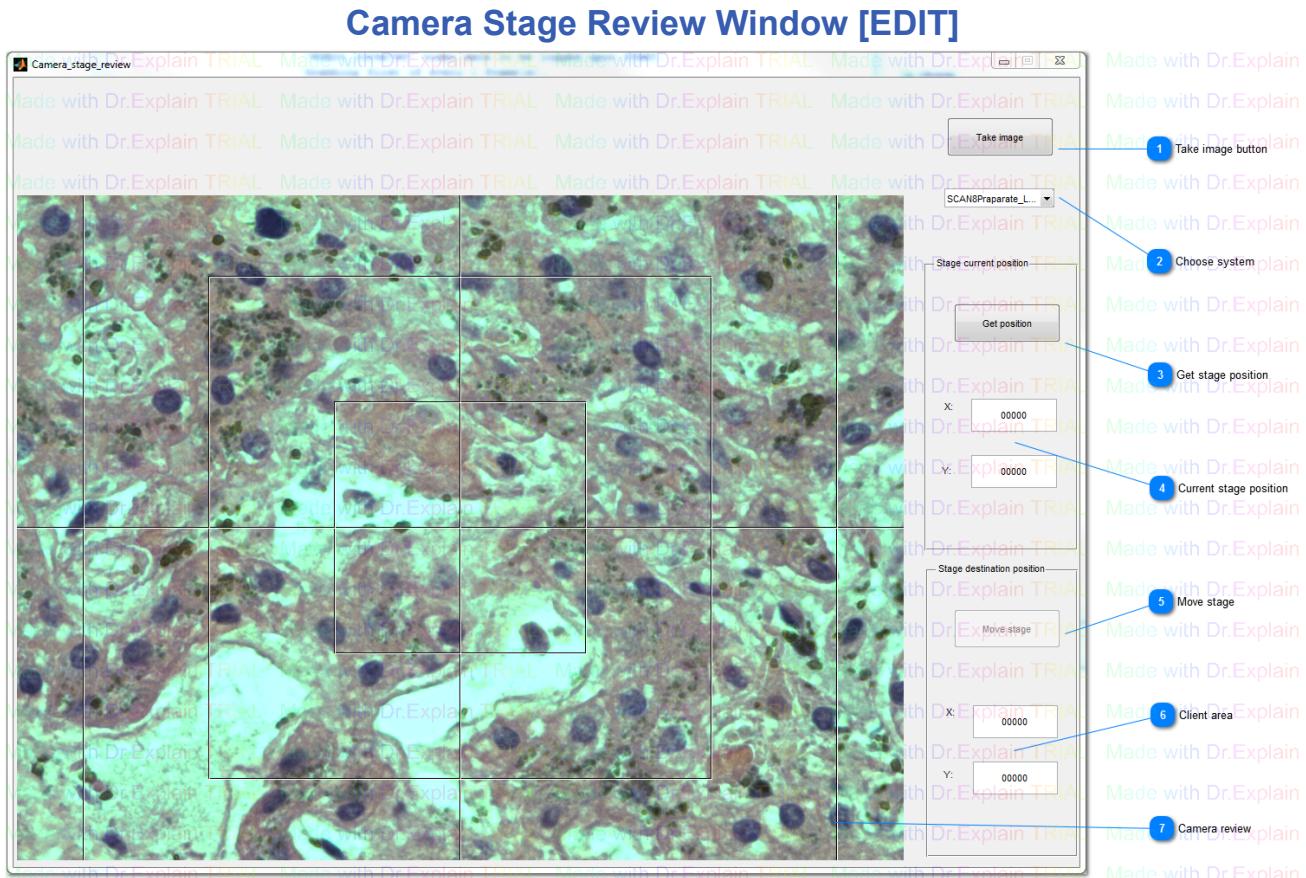


## VII. Stage and Camera Control Utility Functions

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

It has following features:

1. Take a picture and save it to a file.
2. Get stage position coordinates.
3. Move to stage position coordinates.



### 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. FDA Hardware Specifications

FDA specifications that are suitable for the eeDAP software.

## Cameras

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)

## Displays

Different displays and a few of their descriptions.

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

## IX. For eeDAP Developers

Extra information for eeDAP Developers



## Creating the Stand-Alone Application

### Deployment Project

Start a new "Deployment Project" named eeDAP. Matlab will create a project file and folder. The location for this project is insignificant. 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.