Modula's Custom-Made Whole Slide Imaging System: Design and Development

BY

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ABSTRACT

This research presents a design for a modular custom-made whole slide imaging system that is easily maintainable and adaptable for specific research purposes. The system is based on the design of a light microscope, but with 3D-printed components to enhance modularity. Additionally, the LabView application is used to control the system, allowing for flexibility in software customization.

In addition to presenting the design of the modular custom-made whole slide imaging system, this research details the various components used in its construction and the applications that enable control of the system. Specifically, the TLX4000 and ImageJ software applications are utilized for data acquisition and analysis, providing researchers with powerful and adaptable tools for a variety of applications. The implications of this research are significant, as it offers a low-cost, highly adaptable alternative to traditional whole slide imaging systems, empowering researchers to pursue a wide range of investigations with precision and ease.

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Sakwaroon Phuenphol

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF SYMBOLS/ABBREVIATIONS

Symbols/Abbreviations	Terms
α	- The half-angle of the cone of light that enters the objective lens.
CMOS	- Complementary metal oxide semiconductor
FOV	- Field of View
Ih	- Image height
Iw	- Image width
M	- Magnification
M(lens)	- Magnification of the eyepiece lens
M(Obj)	- Magnification of the object lens
MP	- Megapixels
NA	- Numerical aperture
n	- Refractive index of the medium
Sh	- Sensor height in mm
SNR	- Signal-to-Noise Ratio
Sw	- Sensor width in mm
u	- Object distance
v	- Image distance
WSI	- Whole slide imaging
µm	- Microns

CHAPTER 1

INTRODUCTION

This chapter begins with the background and significance of the research, which introduces the theory of whole slide imaging. The research objective is to make Modula's custom-made whole slide imaging both hardware and software parts.

1.1 Background and Significance of the Research

Whole slide imaging (WSI) is an advanced technology used in pathology that captures and digitizes entire glass slides containing tissue specimens at high resolution. It allows pathologists to examine tissue samples in a virtual environment, making it possible to identify and analyze minute details that may be missed with traditional microscopy techniques. This technology can significantly enhance the accuracy of diagnoses and treatment planning in pathology and other related fields.

Custom-built WSI systems have numerous advantages over commercial systems, such as optimized imaging processes for specific samples, improved image quality, and reduced experimental variability. These systems can be made more flexible and adaptable because of the modularity of the custom-made whole slide imaging which makes it easy to fix and maintain.

Custom-made WSI systems are cost-effective for small or specialized research projects, as they allow researchers to select components based on their specific requirements and budget, resulting in reduced ownership costs and increased flexibility. This approach avoids unnecessary costs associated with features that are not needed and incorporate low-cost solutions without compromising performance.

In conclusion, WSI technology has the potential to revolutionize pathology and related fields, enabling faster and more accurate diagnoses and treatment planning. Custom-built WSI systems offer researchers and clinicians the opportunity to further optimize and tailor this technology to meet their specific needs, leading to breakthrough discoveries in the future. Ongoing development and refinement of WSI

technology will undoubtedly have a significant impact on the way we analyze and understand tissue samples, paving the way for new discoveries and advancements.

1.2 Research Objectives

1.2.1 To design and build a custom-made WSI system for high-resolution imaging of tissue samples.

1.2.2 To optimize the imaging process for specific samples, leading to improved image quality and reduced experimental variability.

1.2.3 To create a modular system that can be easily maintained and upgraded over time.

1.2.4 To evaluate the cost-effectiveness of the custom-made WSI system for small or specialized research projects.

1.3 Research Hypothesis

- The aim of this research project is to build a custom-built whole slide imaging (WSI). Our hypothesis is that this system will enhance the precision and accuracy of diagnoses and treatment planning, leading to high-quality images with minimal experimental variability compared to commercially available WSI systems also for the modular custom-made both hardware and software parts, we believe that the cost of it will be low and it will be easy to fix and maintain because it uses the modular system.

1.4 Research Scope

1.4.1 Design and build a custom-made WSI system for high-resolution imaging of tissue samples.

1.4.2 Optimize the imaging process for specific samples, leading to improved image quality and reduced experimental variability.

1.4.3 Create a modular system that can be easy to fix and maintain.

1.4.4 Evaluate the cost-effectiveness of the custom-made WSI for research projects.

1.4.5 Compare the performance and image quality of the custom-made WSI system to commercially available WSI systems.

1.4.6 Assess the precision and accuracy of diagnoses and treatment planning using the custom-made WSI system.

1.4.7 Identify any potential limitations or challenges of the custom-made WSI system and suggest areas for future improvement.

CHAPTER 2
THEORY

2.1 Introduction

This chapter will cover the theoretical background of modular custom-made whole-slide imaging (WSI). It will provide an overview of the concept and technology behind whole slide imaging, modular design, and customization. The chapter will also discuss this approach's potential benefits and limitations in the context of the research study.

2.2 Imaging technology

This topic refers to the various technologies used to capture images in whole slide imaging, such as digital cameras, slide scanners, and microscopes. It involves understanding the technical specifications and capabilities of different imaging devices.

2.2.1 Microscope

The microscope is one important part of a larger system designed to capture and analyze high-resolution digital images of tissue sections for research or diagnostic purposes. The modular custom-made WSI allows for customization and flexibility in the components used, including the microscope.

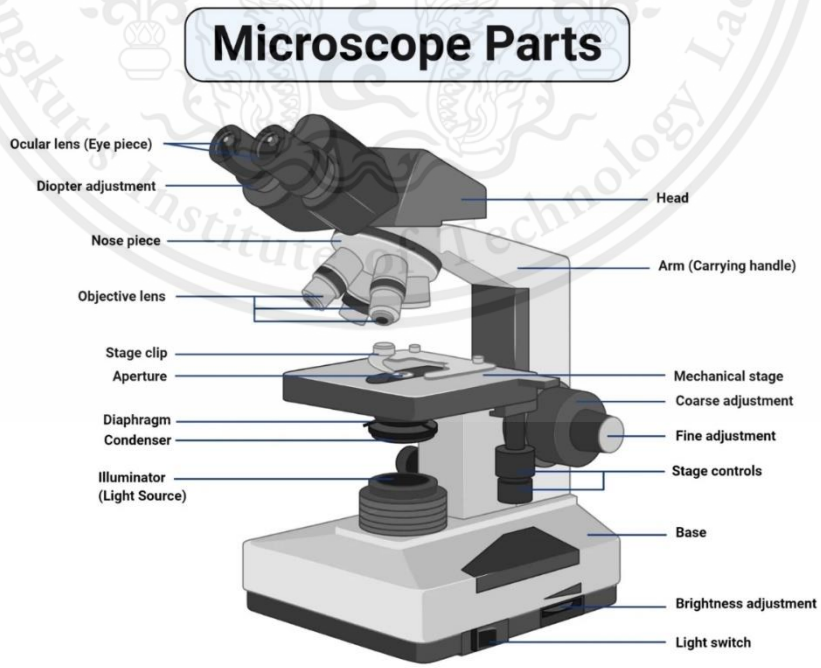

Figure 1 – shows the image of the microscope[1].

2.2.1.1 Components of the Microscope

2.2.1.1.1 Eyepiece

The lens that you look through to observe the specimen. It typically has a magnification of 10x.

Figure 2 – shows the image of the eyepiece[2].

2.2.1.1.2 Objective lenses

The lenses located on the revolving nosepiece are used to magnify the specimen. Compound microscopes typically have 3-4 objective lenses with different magnification powers.

Figure 3 – shows the image of the Objective lens[3].

2.2.1.1.3 Stage

The flat platform on which the specimen is placed for observation. The stage often has clips or other mechanisms to hold the slide in place.

Figure 4 – shows the image of the stage[4].

2.2.1.1.4 Focus knobs

The knobs are used to adjust the focus of the microscope.

Figure 5 – shows the image of the stage[5].

2.2.1.1.5 Illumination source

The light source is used to illuminate the specimen. This may be an LED or another type of bulb located in the base of the microscope.

Figure 6 – shows the image of the light source[6].

2.2.1.1.6 Condenser lens

The lens located beneath the stage concentrates the light from the illumination source onto the specimen.

Figure 7 – shows the image of the condenser [7].

2.2.1.1.7 Diaphragm

A device that controls the amount of light that reaches the specimen.

Figure 8 – shows the image of the diaphragm[8].

2.2.1.1.8 Revolving nosepiece

The part of the microscope that holds the objective lenses and allows you to switch between different magnifications.

Figure 9 – shows the image of the diaphragm[9].

2.2.1.2 Magnification

2.2.1.2.1 Meaning of Magnification and Formula

Magnification is the process of enlarging the apparent size of an object, making it appear larger than its actual size. In the microscope, magnification means 'How many times larger the image is than the object'. The magnification formula can be written as,

$$M = \frac{hj}{h0} \quad , \quad M = \frac{v}{u}$$

M= Magnification
h_j = height of the image
h_o = height of the object
v=image distance
u=object distance

The objective lens is the most important component of the microscope for achieving high magnification, and its magnification is determined by its focal length and numerical aperture.

2.2.1.2.2 Numerical Aperture (NA)

Numerical Aperture (NA) is a number that describes the ability of the lens to capture and resolve fine details in the specimen.

The numerical aperture (NA) can be determined by two key factors. The first one is the angle at which the light cone reaches the objective lens, while the second is the refractive index of the medium that place between the specimen and the lens.

NA is calculated using the following formula:
$NA = n * \sin(\alpha)$
NA is Numerical Aperture
n is the refractive index of the medium,
α is the half-angle of the cone of light that enters the objective lens.

An objective lens with a high NA can gather more light and resolve the specimen's finer details. As a result, higher NA targets are typically favored for high-resolution imaging applications. There will only ever be a small portion of the specimen in focus because the depth of field contracts increases as the NA rises.

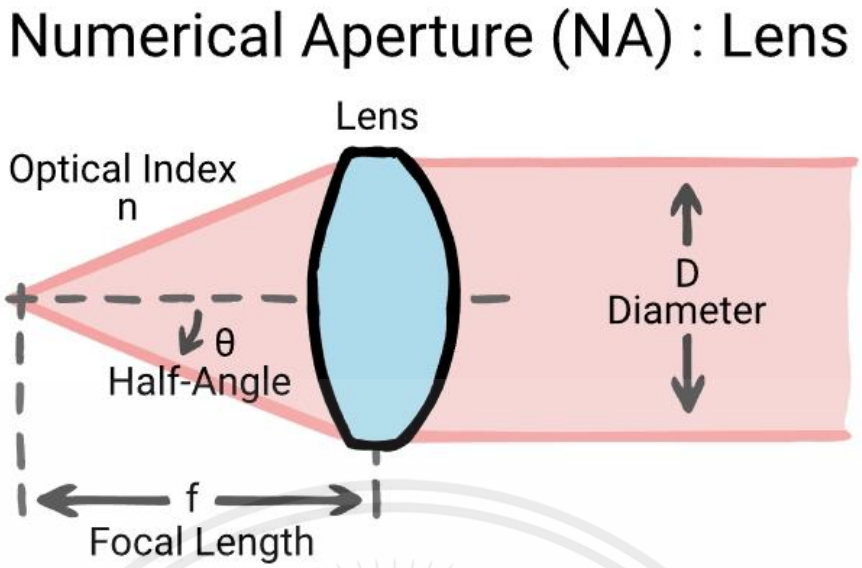

Figure 10 – shows how the picture of the Numerical aperture[10].

2.2.1.3 Autofocus

Autofocus is a feature found in many modern microscopes that helps to maintain focus on the specimen as it is moved or adjusted. For the whole slide imaging the system need to move the stage to continue to capture many images so It needs the autofocus feature to ensure that each image is in focus. There are different methods used to achieve autofocus in microscopes, including manual, semi-automatic, and fully automatic methods.

In this research, we use semi-automatic methods that use the ASI linear stage to adjust the level of the objective lens and also use the condenser holder to adapt the level of the condenser. Also, autofocus is a valuable feature in modern microscopes that enhances the ease and accuracy of specimen observation and analysis.

2.2.1.4 Illumination

The illumination theory used in microscopes is known as Köhler illumination. This theory involves adjusting the microscope's illumination system to provide uniform and bright illumination of the sample while minimizing glare and other artifacts. For our modular custom-made WSI, we use the Köhler illumination to achieve optimal imaging results. This technique helps to ensure uniform illumination across the entire sample area, providing high-quality and accurate images for analysis and diagnosis.

2.2.1.4.1 Köhler illumination

Köhler illumination is used in microscopes to ensure the sample is evenly and brightly lit up without any annoying glare or artifacts. The technique was first developed by a guy named August Köhler way back in 1893, and since then it's become pretty much the gold standard in microscopy.

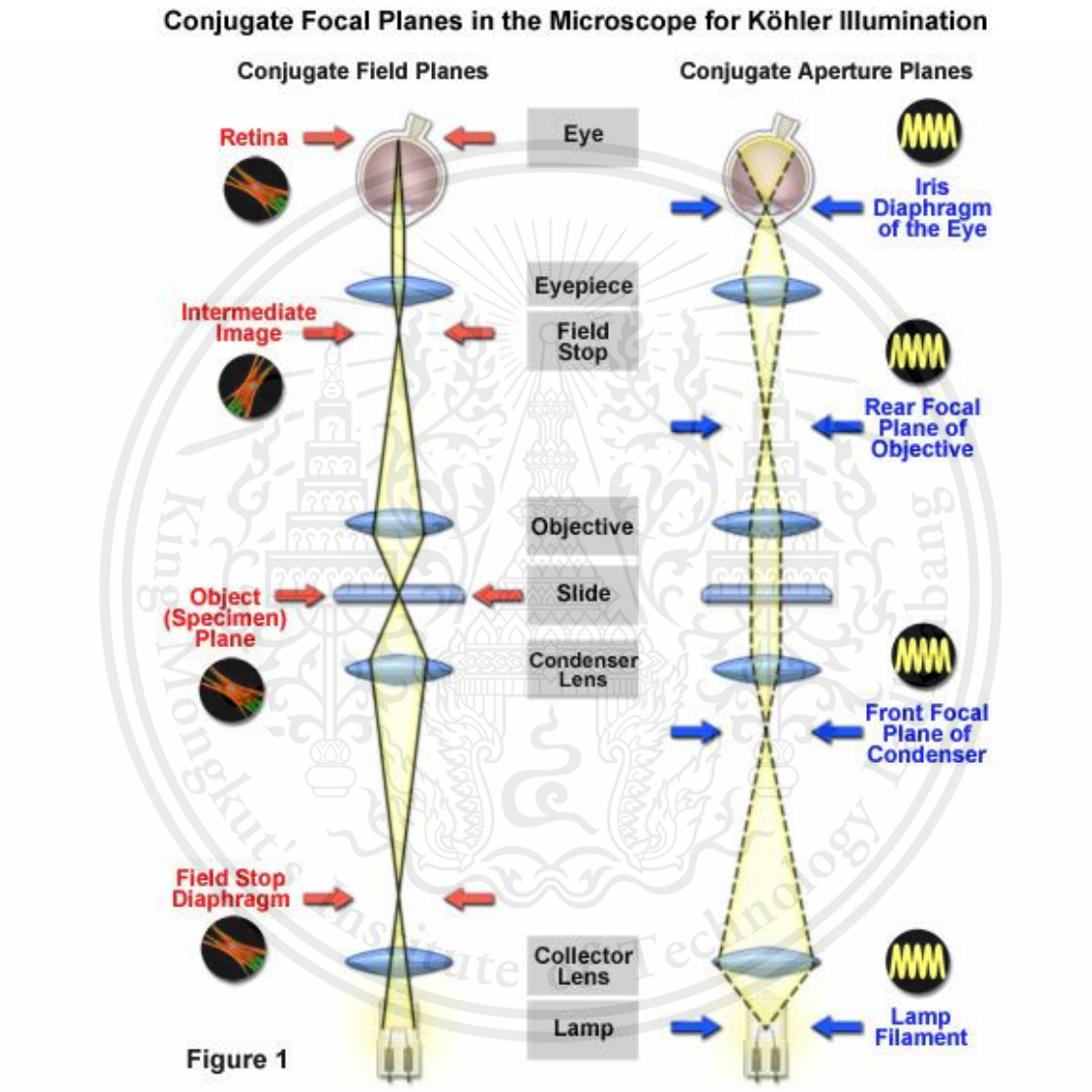

Figure 11 – Show a schematic diagram of the microscope illumination system that is used to achieve Köhler illumination[11].

Figure 11 shows how the Köhler illumination work. Starting from the light source the light was emitted by the lamp filament and focused by the collector lens then the light passed through the field diaphragm to control the size of the illuminated area on a specimen After that the light will be passed through the condenser aperture diaphragm to control the angle of the light that entering the specimen. Finally, the objective lens collects the light that has passed through the specimen and forms an image.

2.2.1.4.2 Components of Köhler Illumination

2.2.1.4.2.1 Light source
2.2.1.4.2.2 Condenser lens
2.2.1.4.2.3 Iris diaphragm
2.2.1.4.2.4 Field diaphragm
2.2.1.4.2.5 Objective lens

2.2.1.4.3 Steps for Köhler Illumination

2.2.1.4.3.1 Place the slide and bring the focus

Place the slide on the stage and adjust the illumination system to provide enough light to focus on the specimen. Centering the condenser and opening both the aperture and field diaphragm then focus the condenser.

2.2.1.4.3.2 Stop down the field diaphragm

The field diaphragm is the diaphragm in the fixed part of the microscope.

2.2.1.4.3.3 Focus on the condenser.

Turning the focus knob until the edge image of the field diaphragm is sharp. There may be a red fringe on one side and a blue on the other, so focus on the center between these.

2.2.1.4.3.4 Open the field diaphragm and centering

Open the field diaphragm to the edge of the field and center using the condenser centering controls.

2.2.1.4.3.5 Adjust the condenser aperture diaphragm,

Adjust the condenser aperture diaphragm to reduce the aperture to about 2/3 of fully open. This can be done by either stopping down the diaphragm until the image flare disappears or by removing an ocular and looking at the back of the objective and closing the CAD control.

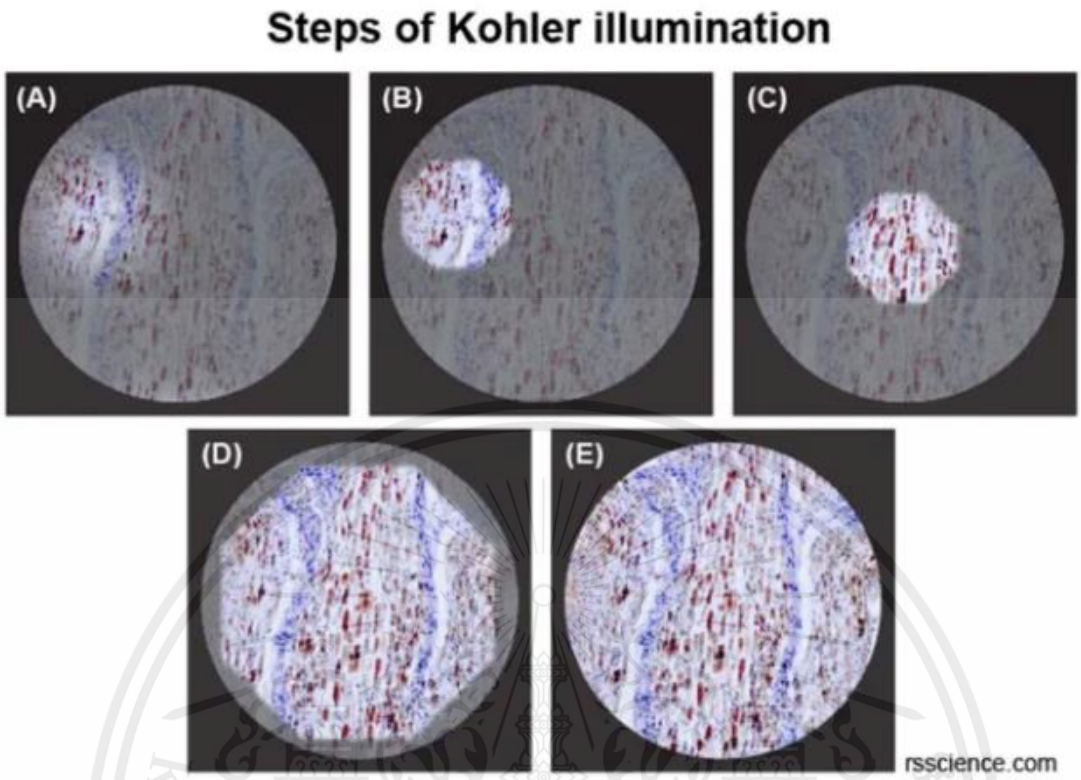

Figure 12 - Steps of the Köhler illumination[12].

2.2.1.4.4 Advantages of Köhler illumination

2.2.1.4.4.1 Even illumination

Köhler illumination provides even illumination of the sample, resulting in high-quality and accurate images for analysis and diagnosis.

2.2.1.4.4.2 Reduced artifacts

The technique minimizes glare and other artifacts, which can interfere with image analysis.

2.2.1.4.4.3 Standardization

Köhler illumination has become a standard practice in microscopy, allowing for consistent and reproducible results across different instruments and laboratories.

2.2.2 Motorize stage

The motorized stage is a motion control system used in microscopy and other applications that require accurate and repeatable positioning. In the modular custom-made WSI, we need to use it to move the stage to collect the picture in the serpentine pattern. In our project, we use ASI MS-2000 to be our Motorized stage we will be moving the slide stage and also the linear stage we use it to move the lens arm to change the level of the objective lens to keep the focus of the whole slide imaging while we moving it.

Figure 13 – ASI MS-2000 motorized stage[13].

Figure 14 – ASI linear stage[14].

2.2.2.1 Components of a motorized stage

2.2.2.1.1 Stepper motors:

These are high-precision motors that convert electrical pulses into precise angular or linear motion. They are commonly used in motorized stages because they offer precise positioning and high torque.

2.2.2.1.2 Linear actuators

These are devices that convert rotational motion into linear motion. They are used to move the stage along a linear axis.

2.2.2.1.3 Controllers

These are devices that regulate the movement of the motorized stage. They receive commands from an application and convert them into signals to drive the motors or linear actuators.

2.2.2.1.4 Encoder

These are sensors that are used to measure the position of the stage. They send the feedback to the controller to achieve precise positioning.

2.2.2.1.5 Mechanical bearings

These are used to support the stage and provide smooth and stable movement. They may include linear bearings, ball bearings, or air bearings, depending on the specific requirements of the application.

2.2.2.1.6 Drive system

This includes the mechanisms that transmit the motion from the motor to the stage. Depending on the specific application, this may include gears, belts, or direct drive mechanisms.

2.2.2.2 Type of a motorized stage

2.2.2.2.1 XY motorized stage

An XY stage is a motorized stage that moves along two perpendicular axes. That is X and Y axis. This is the type of motorized stage that we use as a slide stage in this research.

2.2.2.2.2 Z-axis motorized stage

A Z-axis stage is a motorized stage that moves along a single vertical axis used to lift and lower components.

2.2.2.2.3 Rotary motorized stage

A rotary stage is a motorized stage that rotates around a central axis

2.2.2.2.4 Multi-axis motorized stage

A multi-axis stage is a motorized stage that can move along multiple axes simultaneously, typically used for complex positioning and motion control tasks

2.2.2.2.5 Linear motorized stage

A linear motorized stage is a motorized stage that moves along a single linear axis, typically used for high-speed and high-precision positioning tasks. Linear stages are used in this research as a stage to move the level of the objective lens.

2.2.2.3 Resolution and Accuracy of the motorized stage

A motorized stage is a device used for the precision movement of objects in scientific and industrial applications. The resolution and accuracy of a motorized stage are critical factors in determining its usefulness in a particular application. The resolution of a motorized stage refers to the smallest movement that can be detected and controlled by the stage. It is also in a unit of distance example micrometer or nanometer. The resolution of a motorized stage is determined by the precision of the positioning sensors and the mechanical system that controls movement.

The required resolution of a motorized stage depends on the specific application. Also, the task that needs to use more resolution is more expensive depending on the resolution that is used in the task.

Accuracy refers to the degree to which the actual position of the stage matches the desired position. It is usually specified as a percentage of the total distance traveled by the stage. Same as the resolution if the task wants more accuracy it will more expensive.

2.2.2.4 Control Interfaces for motorized stages

To control motorized stages, there are several interfaces available such as USB, Ethernet, RS-232, Wireless, and Analog, depending on the specific stage and application requirements. For our project, we have chosen to use RS-232, which is a serial communication interface, to connect our computer to the ASI MS-2000 and linear stage.

Initially, we tested the stage using the TLX4000s software application, however, for the remainder of the project, we will be using LabView to control both the motorized stage and the digital camera. This software will allow for smooth and efficient control of the stage, providing a user-friendly interface to make the project run smoothly.

Figure 15 - The Icon of TLX4000s.

2.2.3 Digital camera

A digital camera is a type of camera that uses to capture and stores images in digital format. They offer high-quality images, customization, instant review, and editing capabilities. Digital cameras have made photography more accessible and sharing photos easier. The primary benefits of digital cameras are their ability to instantly review and edit photos and also their ability to capture high-quality images.

The digital camera that we use in this research is E3ISPM Series C-mount it is a CMOS sensor. Same as the motorized stage we will use the LabView application to make it work with a motorized stage to keep save the picture in the serpentine pattern.

Figure16 – This picture shows the picture of the E3ISPM digital camera[15].

2.2.3.1 Sensor Types in digital cameras

There are several types of sensors used in digital cameras, The type of sensor that we use in our research is CMOS (Complementary Metal-Oxide-Semiconductor) sensor also it is the most popular in many types of the sensor because of its properties such as low power consumption, and high image quality, and fast readout speeds. It has more 4 types of the sensor

There are other types of sensors available. The CCD sensor, once a widely used technology, offers superior image quality in some scenarios, though its production cost and power consumption are high. Foveon X3 sensors employ a unique design that captures color information at different depths in the sensor, primarily found in Sigma cameras.

Backside-illuminated (BSI) sensors are having circuitry at the back so more light can go to photodiodes making it better than CMOS in the low-light condition. Dual-Pixel CMOS sensors feature two photodiodes per pixel, making it better for autofocus accuracy and enhanced image quality in certain scenarios.

2.2.3.2 Pixel Size and Resolution in digital cameras

The pixel size in a digital camera is expressed in microns (µm), it determines the amount of light each pixel can capture. A larger pixel size results in higher-quality images, as it can gather more light. Conversely, smaller pixels gather less light and lead to lower-quality images.

The resolution of an image, measured in megapixels (MP), is the total number of pixels in the image. A higher resolution means it has higher pixels that result in finer detail and a shaper image. Thus, the pixel size and the resolution is relatable if your digital camera has a larger pixel it means fewer pixels then the image will have less resolution so the pixel size and the resolution in the digital camera should be balanced to achieve a high-quality image.

Figure17 - shows the quality of the with different pixel sizes and resolutions [16].

2.2.2.3 Signal-to-noise Ratio in digital cameras

Signal-to-noise Ratio (SNR) refers to the ratio of signal and noise of your captured image, it shows the value of the quality of the image, the higher in SNR it's mean higher the quality of the image. A higher SNR can be achieved by using a larger sensor, decreasing the ISO sensitivity, or increasing the exposure time. The image that gets from the whole slide
imagine should have a high ratio of SNR to make the image that we combine see more clear.

2.2.2.4 Camera control interfaces

Camera control interfaces are the means by which photographers can interact with their cameras and control various settings and functions, for the digital camera in this research we use
E3ISPM Series C-mount USB 3.0 CMOS Camera that has its own software the ImageView.

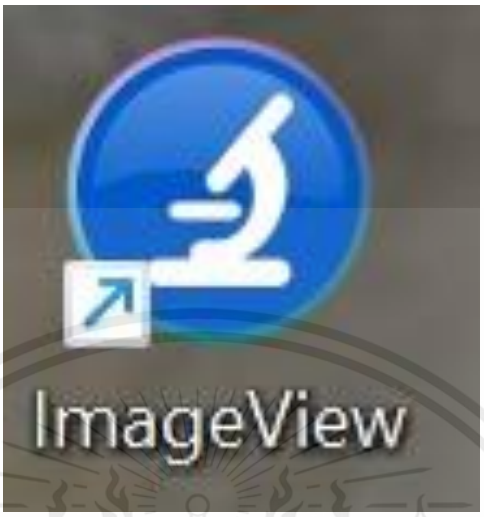

Figure 18 -The Icon of the ImageView application.

ImageView is an application that uses with E3ISPM Series C-mount USB 3.0 CMOS Camera it can use to capture the image and adjust many things of the camera examples
The frame rate, color/gray, dark field correction, and flat field correction but in this research we need to use LabView instead because we need to work with the ASI stage and the digital capture camera at the same time.

2.3 Image processing

Modular custom-made whole slide imaging systems need to make use of image processing theory to analyze and enhance the images that the system captures. There are several keys of image-processing theories that are important to the imaging system. Such as image segmentation, feature extraction, image registration, image restoration, machine learning, and deep learning.

2.3.1 Image segmentation

It is an image-processing technique that will divide the image into segments depending on the characteristics of the pixel. This can help the user to identify specific areas of interest within the specimen that they are studying. It is an important technique in whole slide imaging as it can help identify specific structures or areas of interest within the specimen such as tumor cells or blood vessels.

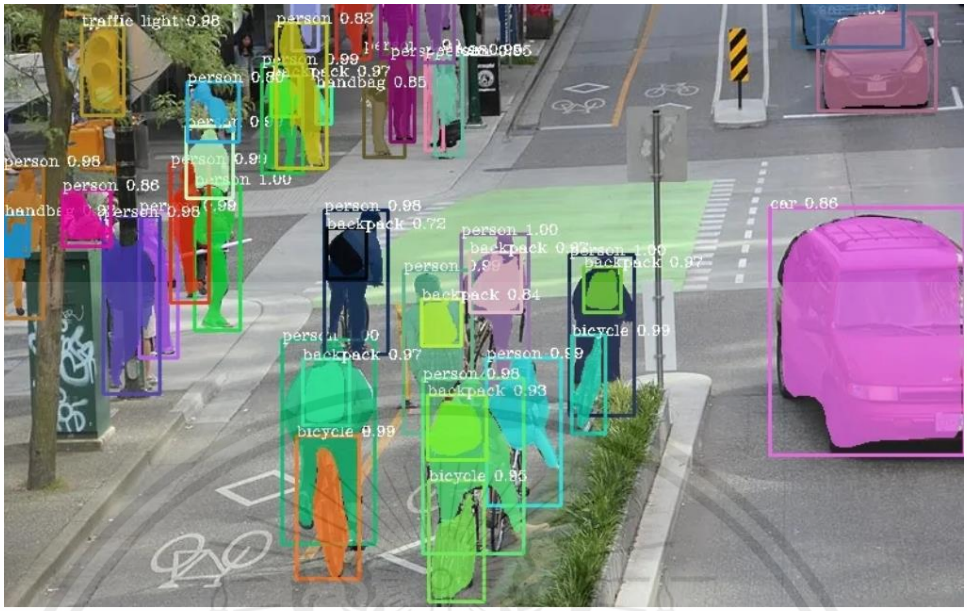

Figure 19 - shows a picture of an Image segmentation[17].

2.3.3 Image Registration

Image registration is the process of aligning two or more images of the same scene or object. It is used in whole slide imaging to stitch together multiple images into a single large image of the specimen. This allows for a better view of the entire specimen, rather than just small parts of it. Image registration is an important step in whole slide imaging as it can improve the accuracy of subsequent analysis techniques such as feature extraction and classification.

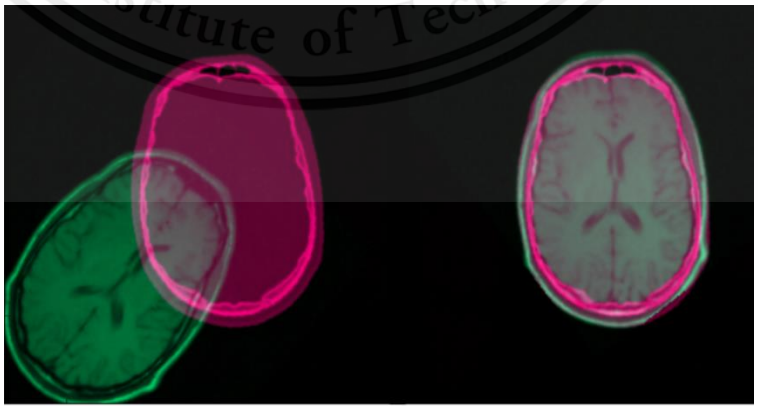

Figure 20 - shows a picture of an Image registration[18].

2.3.4 Image restoration

Image restoration is a technique to enhance the quality of images that have undergone damage or corruption. The impairment can manifest in various forms such as blurriness, noise, or distortion, rendering the image incomprehensible or unusable. Restoration techniques seek to retrieve the original image by lessening or eliminating the impacts of the damage. This is accomplished using methods such as employing filters or employing algorithms to recover lost information in the image while minimizing undesirable artifacts or noise. This process has significant applications in the medical imaging, remote sensing, and computer vision industries, where obtaining high-quality images is critical for accurate analysis and interpretation.

Figure 21 - shows a picture of a tiger after getting an image restoration (right)[19].

2.4 Optics and Lens

Optics and lenses play a critical role in whole slide imaging systems. They are used to capture high-quality images of the specimen being studied, which is necessary for accurate analysis and diagnosis. There are several key concepts in optics and lenses that are important to understand when developing modular custom-made whole-slide imaging systems.

2.4.1 Optical resolution

Optical resolution is a fundamental concept in whole slide imaging that refers to the imaging system's ability to distinguish between two closely spaced objects in an image. Having high optical resolution is essential for whole slide imaging since it enables researchers to detect and examine tiny structures within the specimen, including individual cells or blood vessels. The numerical aperture of the lens, the light wavelength used, and the size of the image sensor's pixels are only a few of the variables that affect optical resolution. Whole slide imaging systems can attain high levels of optical resolution by optimizing these parameters, allowing researchers to draw useful conclusions from the massive volumes of data these systems create.

2.4.2 Depth of Field

Depth of field is another critical concept in whole slide imaging that is equally important as optical resolution. It refers to the range of distance in an image that appears to be in sharp focus, spanning from the nearest to the farthest objects that

appear acceptably sharp in a photograph. Having a high depth of field is crucial for whole slide imaging as it enables researchers to capture sharp images of the entire specimen, including structures that are located at different depths within the sample. Whole slide imaging can optimize the depth of field by adjusting parameters such as the aperture size, the focal length of the lens, and the distance between the lens and the specimen. Optimizing the depth of field its make the researchers can capture clear and detailed images of the specimen

Figure 22 - shows an image of a flower after adjusting the Depth of Field (Right)[20].

2.4.3 Contrast

Contrast refers to the difference in brightness or color between different parts of an image. In whole-slide imaging, high contrast is important as it enables researchers to distinguish between different structures within the specimen. Contrast can be influenced by several factors, including the lighting conditions during image capture, the color balance of the camera, and the properties of the specimen being imaged.

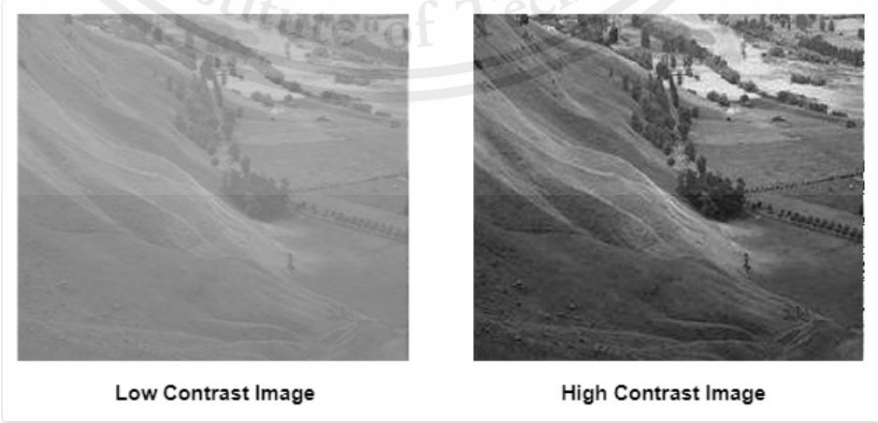

Figure 23 - shows an image of a flower after adjusting the Contrast (Right)[21].

2.4.4 Chromatic aberration

Chromatic aberration is a common optical artifact that occurs when different wavelengths of light are refracted differently by a lens, causing a loss of image sharpness and color fringing. In whole-slide imaging, chromatic aberration can be problematic as it can affect the accuracy of subsequent analysis techniques. Chromatic aberration can be corrected using specialized lenses or post-processing techniques.

Figure 24 - shows an image of a flower after adjusting the Contrast (Right)[22].

2.4.5 Lens selection

In the whole slide imaging, The correct lens must be used To produce high-quality images. The lens should be chosen based on the specimen that is being investigated, the required magnification, the field of vision, and other elements. When choosing a lens for a complete slide imaging system, other elements such as the numerical aperture, working distance, and chromatic aberration must be taken into account.

Overall, understanding the key concepts in optics and lens is essential for developing modular custom-made whole-slide imaging systems that can capture high-quality images of specimens for accurate analysis and diagnosis. By selecting the right lenses and optimizing key parameters such as optical resolution, depth of field, contrast, and chromatic aberration, researchers can improve the accuracy and efficiency of whole slide imaging systems.

2.5 Calculation Theory

2.5.1 Field of View (FOV)

The Field of view (FOV) is the area of the specimen or scene that is visible through the imaging system at a given magnification. For the research, we calculate the FOV in 3 magnifications that is 5x 10x, and 20x.

The formula that we used in this research is

$$FOV = (\text{Sw} / \text{Iw})(\text{Sh}/\text{Ih}) \text{ x M}$$

Field of View = (Sensor size / Image width in pixels) x (Sensor height in mm / Image height in pixels) x Magnification.
In addition, The sensor size of your camera is 1/1.8-inch

2.5.1.1 5x

$FOV = (7.2 \text{ mm} / 2720 \text{ pixels})(5.3 \text{ mm} / 1824 \text{ pixel}) \text{ x5}$ = 0.079 mm x 0.059 mm.

The FOV of the imaging setup at 5x magnification is approximately 0.079 mm x 0.029 mm.

2.5.1.2 10x

$FOV = (7.2 \text{ mm} / 2720 \text{ pixels})(5.3 \text{ mm} / 1824 \text{ pixel}) \text{ x10}$ = 0.079 mm x 0.059 mm.

The FOV of the imaging setup at 10x magnification is approximately 0.039 mm x 0.029 mm.

2.5.1.3 20x

$FOV = (7.2 \text{ mm}/2720 \text{ pixels})(5.3 \text{ mm}/1824 \text{ pixel}) \text{x20}$ = 0.02 mm x 0.015 mm

The FOV of the imaging setup at 20x magnification is approximately 0.020 mm x 0.015 mm.

2.5.2 Pixel size

Pixel size is the physical size of an individual pixel. The pixel size is typically measured in micrometers (μm). The smaller the pixel size, the higher the resolution of the image.

The formula of the Pixel size is Pixel size = Sensor size / Image width in pixels. Based on the specifications, the E3ISPM20000KPA camera has a 1/1.8-inch CMOS sensor with a resolution of 3840 x 5400 pixels. To calculate the pixel size of the camera, we use the following formula:
Pixel size = Sensor size / Number of pixels.
The sensor size for this camera is the 1/1.8-inch specification refers to the sensor diagonal.

The sensor size can be calculated as follows:
Sensor diagonal = 1/1.8 inches = 8.47 mm , Using the resolution of 2720 x 1824 pixels
The pixel size would be: Pixel size = 8.47 mm / 2720 pixels x 8.47 mm / 1824 pixels = 3 um
Therefore, the pixel size of the E3ISPM20000KPA camera is 3 um.

2.5.3 Image Overlap

Image overlap is the area of overlap between two adjacent images captured by a camera or any imaging system.
Its units are in pixels or in percent. To calculate the Image overlap:
Image overlap= (Image width / 2) x (Overlap percentage / 100)

2.5.3.1 5x
Overlap in width: 1824 pixels / 2 = 912 pixels
Overlap in height: 0.029 mm / 0.059 mm x 912 pixels = 450 pixels (approx.)

2.5.3.2 10x
Overlap in width: 1824 pixels / 2 = 912 pixels
Overlap in height: 0.029 mm / 0.029 mm x 912 pixels = 912 pixels

2.5.3.3 20x
Overlap in width: 1824 pixels / 2 = 912 pixels
Overlap in height: 0.015 mm / 0.029 mm x 912 pixels = 479 pixels (approx.)

Therefore, at 5x magnification, there is an approximate overlap of 912 x 450 pixels between adjacent images, at 10x magnification there is an overlap of 912 x 912 pixels, and at 20x magnification there is an approximate overlap of 912 x 479 pixels.

2.5.4 Image size

Image size refers to the dimensions of an image, typically measured in pixels. For the Image size of the stitching image :

Stitched image size = [(number of columns x image width) - (overlap width)] x [(number of rows x image height) - (overlap height)]

Stitched image size = (3 x 2720) - (1360) x (3 x 1824) - (912)
Stitched image size = 8160 - 1360 x 5472 – 912
Stitched image size = 6800 x 3975 pixels

Therefore, the stitched image was created from 9 individual images captured by the E3ISPM20000KPA camera with a 50% overlap in a zigzag serpentine pattern will have a size of 6800 x 3975 pixels.

CHAPTER 3

METHODOLOGY

3.1 Introduction

This chapter will cover all of the methodologies of modular custom-made whole-slide imaging (WSI). It will show you how to build modular custom-made whole slide imaging such as the Design, 3D Printing, and Component assembly also I will write about all the applications that we use to control the whole slide imaging both hardware and software.

3.2 Application

For this topic, it will provide all the applications that we use in this research whatever the design, control, image processing, or 3D-printing application.

3.2.1 Autodesk Inventor Professional 2022

For all designs that we make in this research, we use computer-aided design which is the Autodesk Inventor Professional 2022

Figure 25 –shows the picture of Autodesk Inventor[23].

This software allowed us to create detailed and precise designs, enabling us to visualize and test the components before manufacturing.

3.2.2 TLX4000

This is the application that use to control the motorized stage through the Rs-232 and serial port It has the joystick to move the XY-stage in the x and y direction and also control the linear stage.

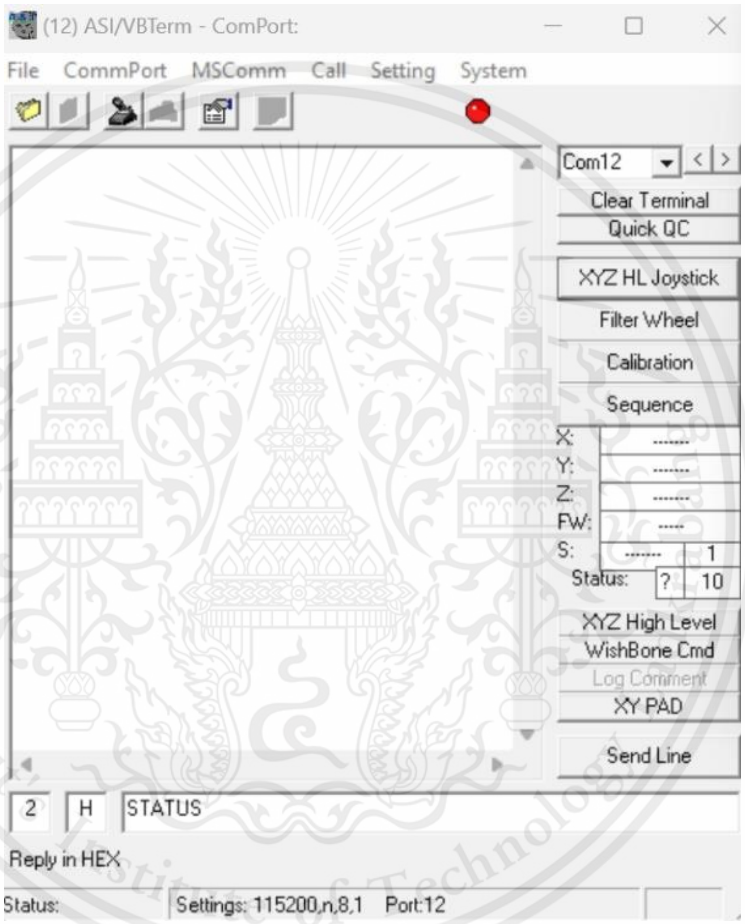

Figure 26 – shows the user interface of TLX4000.

3.2.3 ImageView

It is an application that we use to control and capture the image right now. It can use to save a batch of pictures at the same time then it can send the image to the same folder also Camera can be used to capture the image and adjust many things of the camera example frame rate, color/gray, dark field correction, and flat field correction but in this research, we need to use LabView instead because we need to work with the ASI stage and the digital capture camera at the same time.

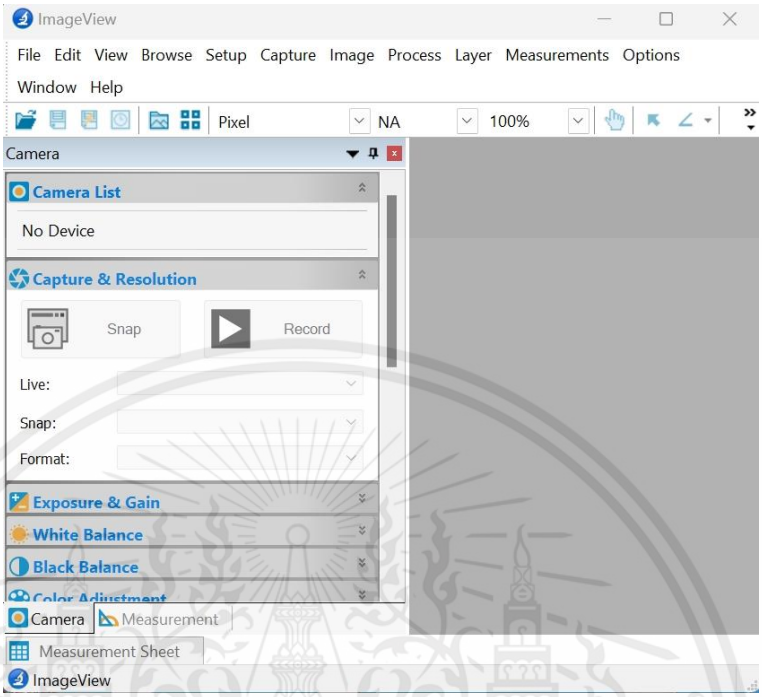

Figure 27 – shows the user interface of ImageView.

3.2.4 ImageJ

This is the image processing application that use to adjust the image. For this project, we use it as the stitching image and also it uses to find the %overlap between the 2 images. For this research, we use Fiji which is the ImageJ application with many plugins in it.

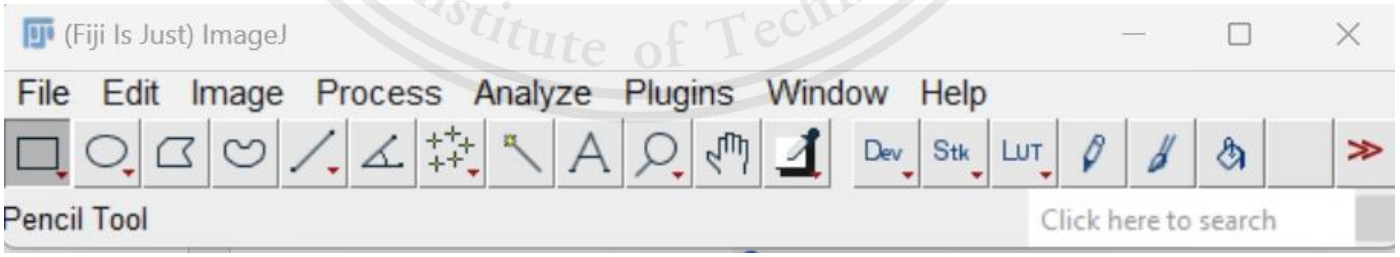

Figure 28 – shows the user interface of ImageJ.

3.2.5 Flashpoint

This is the application that we use to open the STL files after we finished the design from the Autodesk inventor. It allows users to prepare 3D models for printing by setting parameters such as the raft, support, and infill.

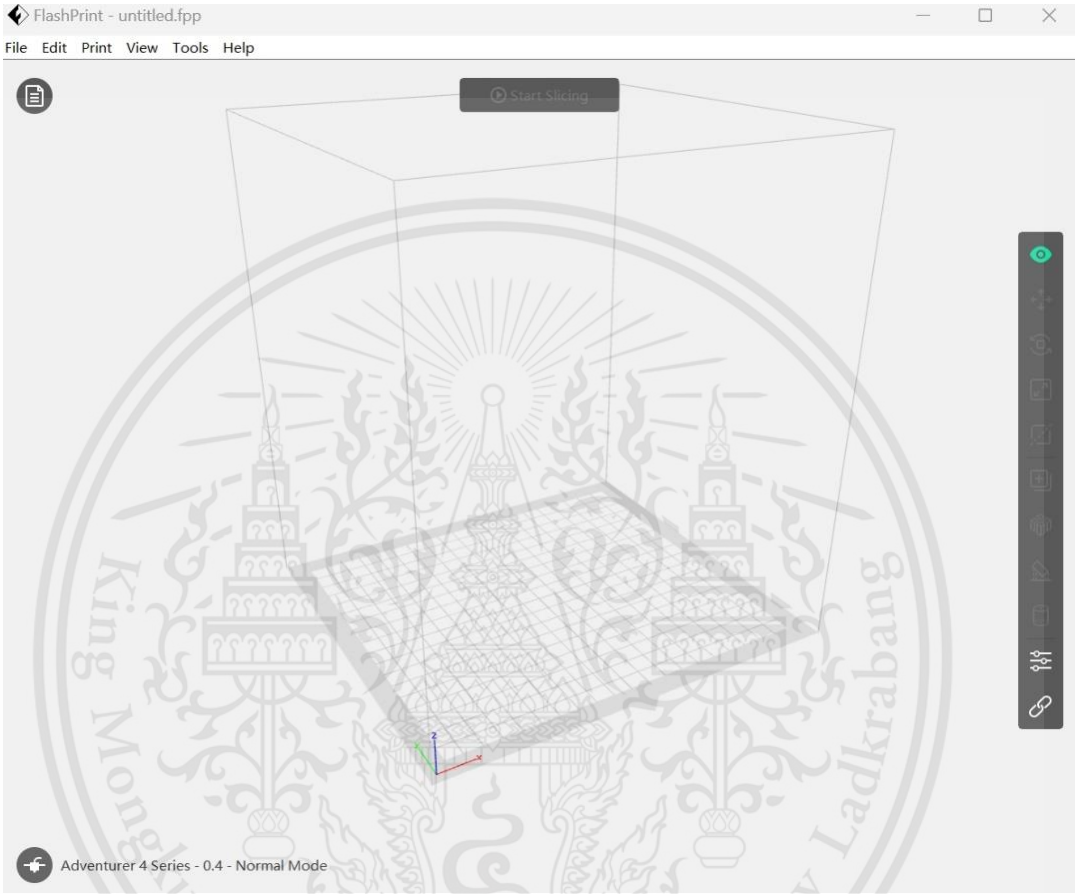

Figure 29 – shows the user interface of FlashPrint.

3.2.6 LabVIEW

LabVIEW is a graphical programming language and integrated development environment (IDE) created by National Instruments. In this project, it will use to control both the motorized stage and the digital cameras to make it work together at the same time to make this whole slide imaging can use an auto scan function that will autofocus, auto-capture, and auto-move the slide.

Figure 30 -shows the picture of LabVIEW[24].

3.3 Components

3.3.1 Optical Aluminum Breadboard

It is an aluminum stable platform that uses mounting optical components, In this research, I will use it to mount with the 3D printing that connects the components of the whole slide imaging.

Figure 31 – shows a picture of the optical breadboard[25].

3.3.2 Light source

It is a component that is used to emit the light of the whole slide imaging located at the bottom of the slide imaging.

Figure 32 - shows a picture of the light source.

3.3.3 Field diaphragm

It is the components that use to adjust the size and shape of the light beam located upper the light source.

Figure 33 - shows the picture of the field diaphragm.

3.2.4 Condenser

It is a component that is used to focus and direct the light from the whole slide imaging's light source also located on the condenser holder above the field diaphragm.

Figure 34 - shows the picture of the condenser.

3.2.5 Condenser holder

It is the components that use to hold the condenser above the field diaphragm also it can adjust the level of the condenser to make it adjust the focus.

Figure 35 - shows the picture of the condenser holder.

3.2.6 Z-stage

It is the components that use to support the condenser holder and the linear stage by connecting the z-stage to the 3D-print and the 3D-print will connect to each component also this z-stage can used to adjust the level of the condenser holder.

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Figure 36 - shows a picture of the Z-stage.

3.2.7 ASI MS-2000 XY Stage

It is a slide stage that is used to move along the x and y-axis. It is the most important component in the whole slide imaging.

Figure 37 - shows a picture of the ASI MS-2000 XY stage.

3.2.8 Linear stage

It is a component that is used to hold the camera holder to adjust the focus automatically for the whole slide imaging It is a stage that can move up and down in the z-axis it control by the Rs-232 same as the XY stage. Both of XY stage and linear stage also use the control box that connects to the computer to move it and use the specific application to move it which is TLX4000.

Figure 38 - shows a picture of the light source.

3.2.9 Microscope camera holder

It is the components that use to hold the body tube and it connects to the linear stage by the 3D printing, it uses to adjust the level of the objective lens manually.

Figure 39 - shows a picture of the camera holder.

3.2.10 Body tube

It is a component that is used to mount the digital camera and the nosepiece. It is a way of the light from the objective lens to the digital camera.

Figure 40 - shows a picture of the body tube.

3.2.11 nosepiece

It is the components that use the mouth to the objective lens 5x, 10x, 20x, and 40x also it can rotate to change the magnification of the objective lens.

Figure 41 - shows a picture of the condenser holder.

3.2.12 Objective lens

It is the lens that captures the light from the object to view and forms the image. It has variants in magnification depending on which research and detail of the specimen we want to observe.

Figure 42 - shows a picture of the condenser holder
(20x left ,10x middle, 5x left).

3.2.13 Digital Camera

It is an electronic device that use to capture and store digital images or videos. For this research, we use the E3ISPM20000KPA as our digital camera.

Picture 43 - The image of the digital camera.

3.4 Design

For all methods, we divide into 3 steps. The 3D sketch of the components, The 3D design of the assembly module, and the component assembly.

For the 3D sketch of the components we use a vernier caliper to measure the real scale and make a sketch then we use these sketches to make an assembly module after that we try to print it out and use it to connect the real components.

For the design of our modular, custom-made whole-slide imaging system, we chose to utilize a microscope as the foundation. However, instead of using a slide stage, we incorporated an XY stage to allow for movement along the x and y axes. Additionally, we mounted a digital camera to the body tube to function as an eye and capture images for stitching.

3.4.1 The 3D sketch of the components

3.4.1.1 The 3D sketch of the Light source

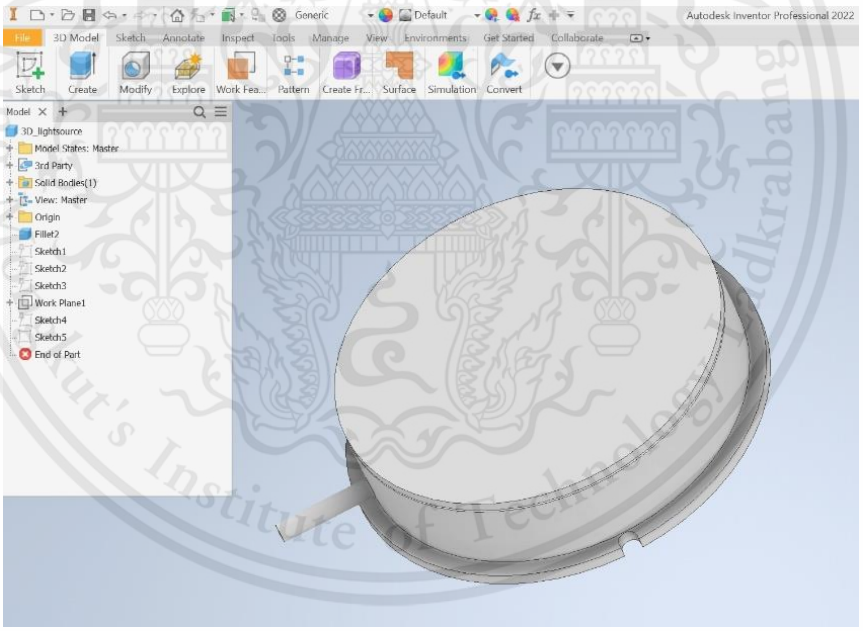

Figure 44 - shows a 3D sketch of the Light source.

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3.4.1.2 The 3D sketch of the field diaphragm

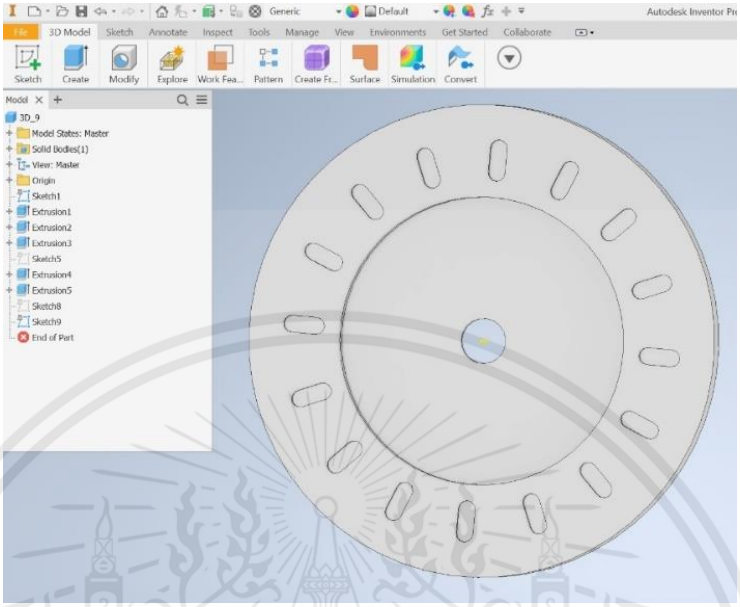


Figure 45 - shows a 3D sketch of the diaphragm.

3.4.1.3 The 3D sketch of the condenser

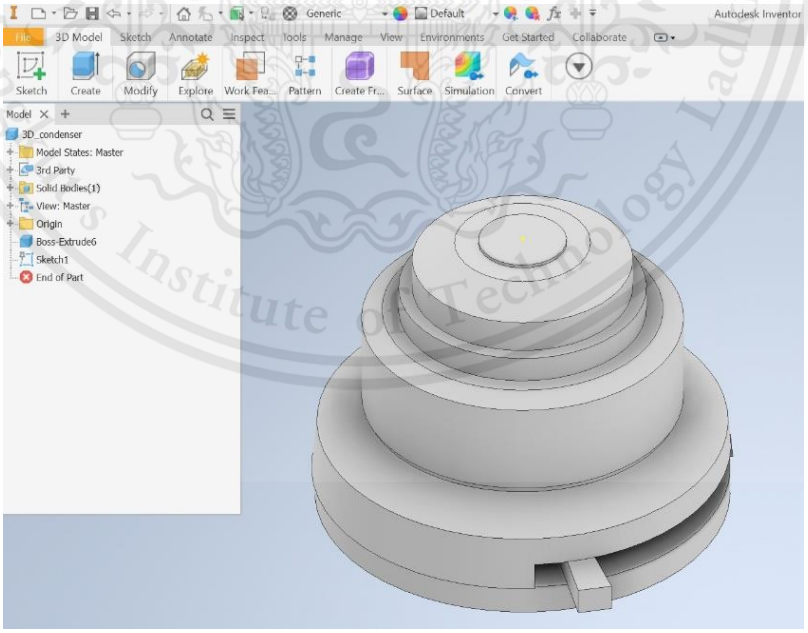
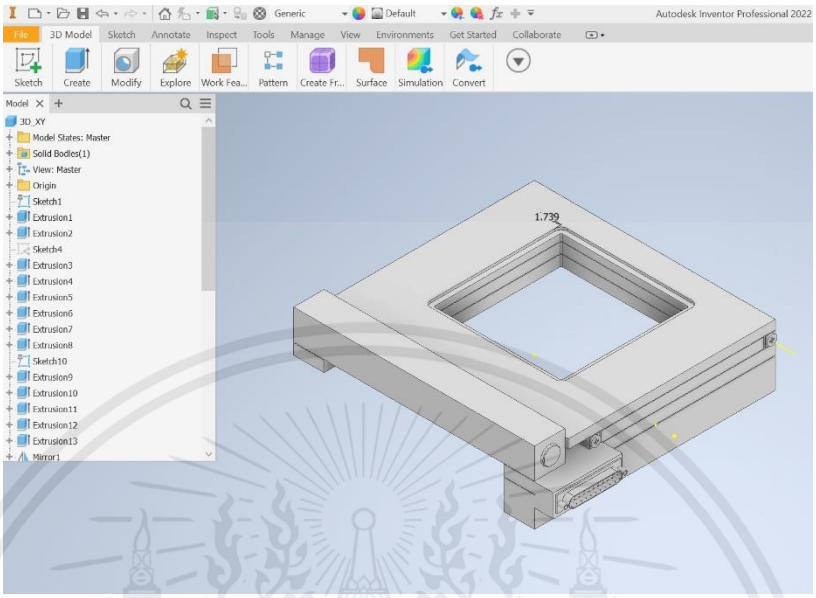


Figure 46 - shows a 3D sketch of the Condenser.

3.4.1.4 The 3D sketch of the XY stage



. Figure 47 - shows a 3D sketch of the XY stage.

3.4.1.5 The 3D Sketch of the Linear Stage

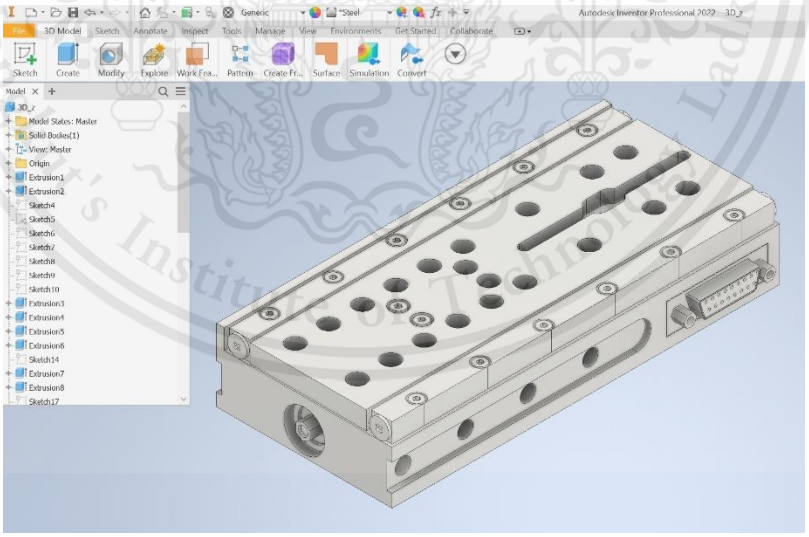


Figure 48 - shows a 3D sketch of the Linear stage.

3.4.1.6 The 3D Sketch of the Body Tube

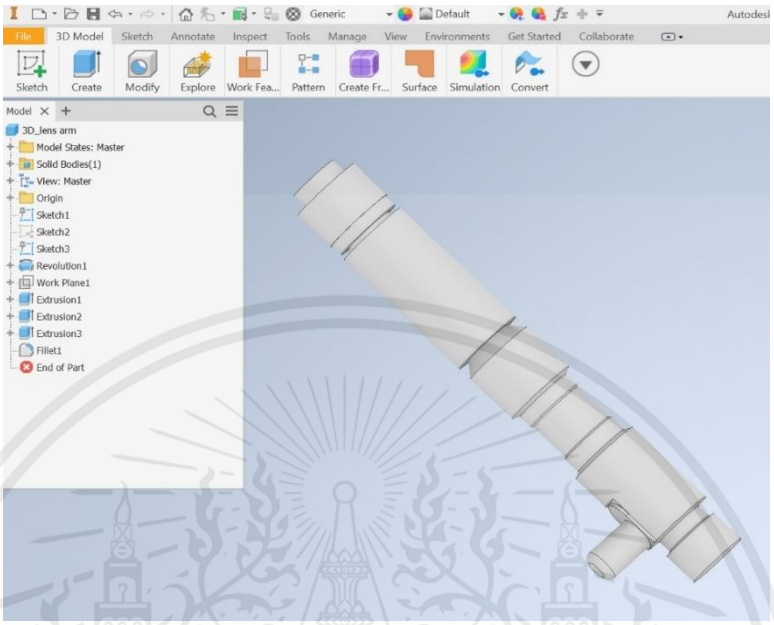


Figure 49 - shows a 3D sketch of the Body Tube.

3.4.1.7 The 3D Sketch of the Nosepiece

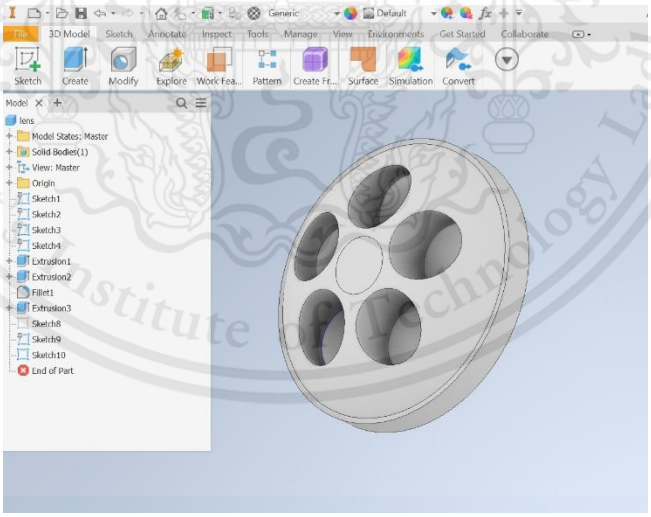


Figure 50 - shows a 3D sketch of the Nosepiece.

There are all of the sketches that use to design the 3D-printing assembly module.

3.4.2 3D-design assembly module

For this topic, we will 3D-design that we use to make a 3D print to combine all the components together.

3.4.2.1 Light source based

This is the part that we use to connect the light source to the breadboard.

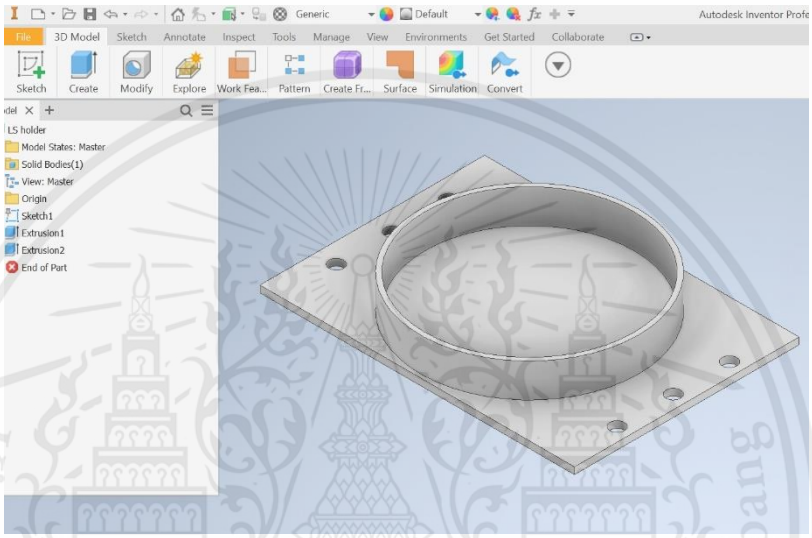


Figure 51 - shows a 3D sketch of the Light source based.

3.4.2.2 LS-Diaphragm Connecter

This is a part that is used to combine the field diaphragm and the light source together. Then it will put it on the light source based.

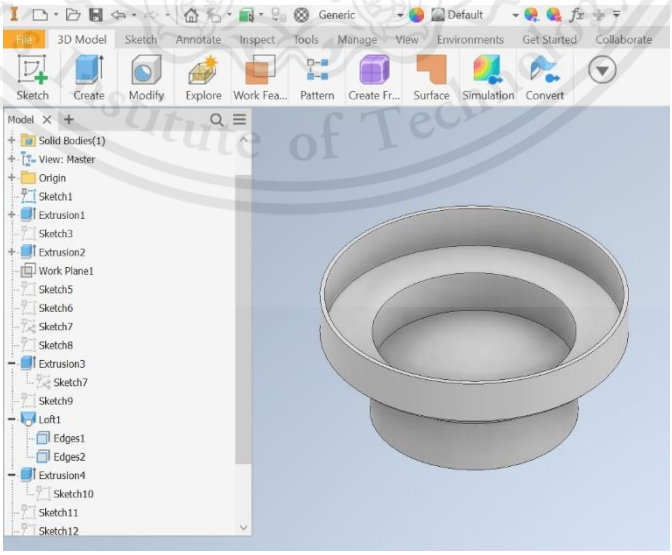


Figure 52 - shows a 3D sketch of the diaphragm connecter.

3.4.2.3 3D-stage based

This is the part that we use to connect the ASI XY-stage to the breadboard.

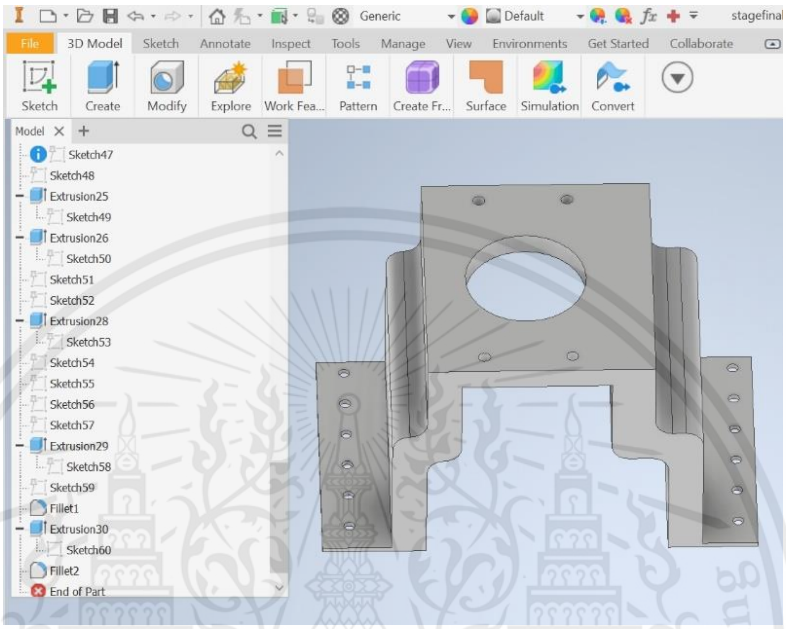


Figure 53 - shows a 3D sketch of stage based.

3.4.2.4 Z-holder

This is a part that is used to hold the condenser holder. This part will mount with the z-stage.

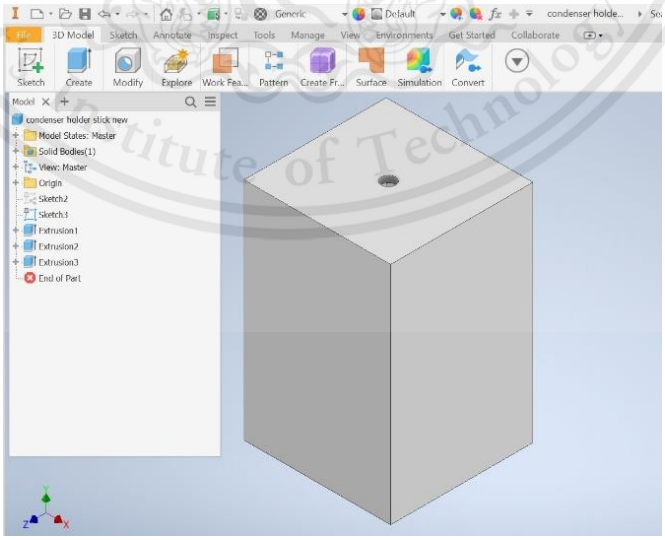


Figure 54 - shows a 3D sketch of z-holder.

3.4.2.5 Z-stage based

This is the part that we use to connect the z-stage to the breadboard.

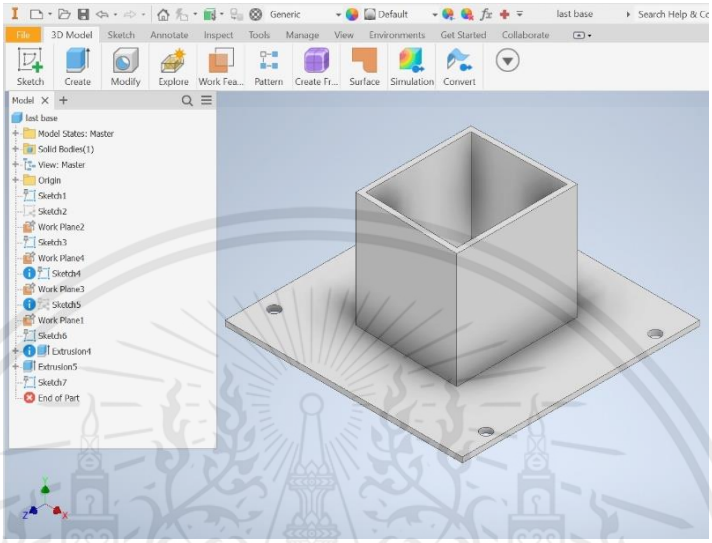


Figure 55 - shows a 3D sketch of z-holder.

3.4.2.6 Z-stage linear connecter

This is the part that we use to connect the moving part of the linear stage and z-stage.

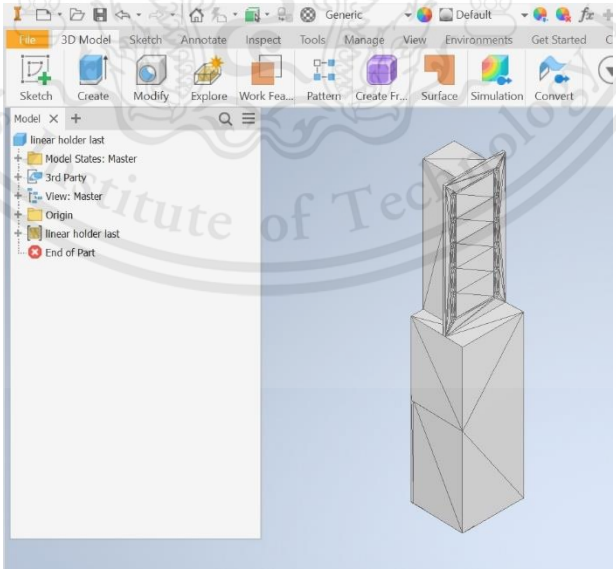


Figure 56 - shows a 3D sketch of z stage linear connecter.

3.4.2.7 Linear-camera holder connecter

This is a part that is used to connect the linear stage and the camera holder.

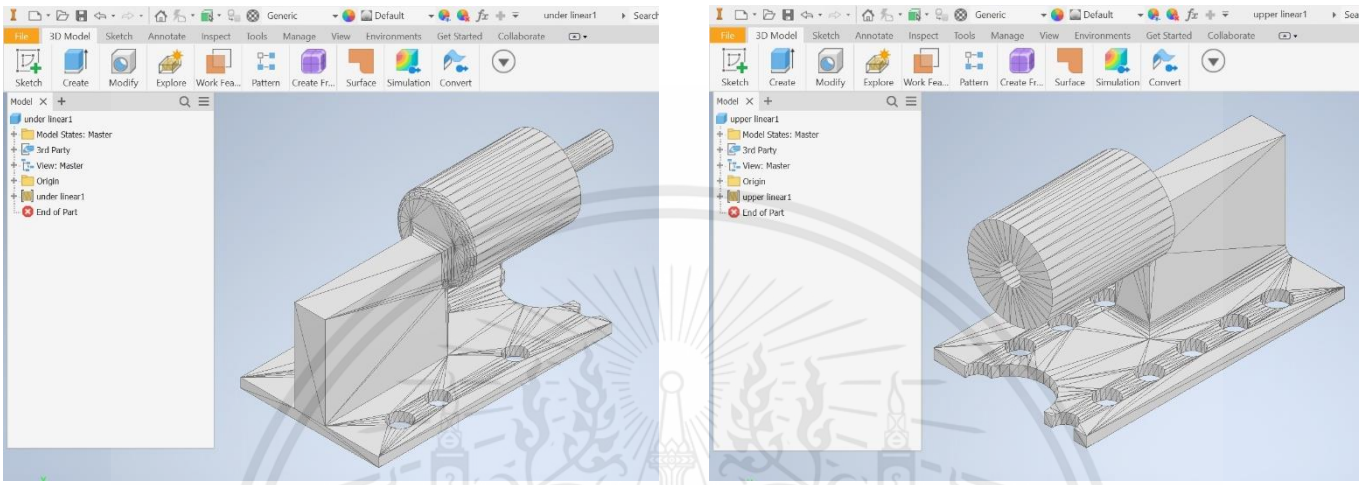


Figure 57 - shows a 3D sketch of Linear-camera holder connecter.

3.4.2.Z- Slide tray

This is the 3D design of the 3D print that we use as a slide tray to place the specimen on it.

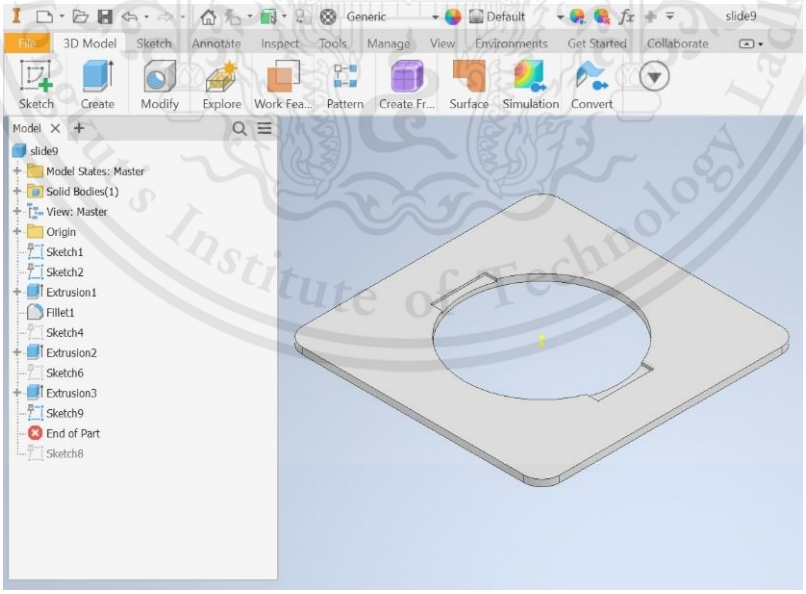


Figure 58 - shows a 3D sketch of a slide tray.

3.5 Component assembly

This part shows how to assemble all the components together.

3.5.1 Mount the z-stage and light source based on the breadboard

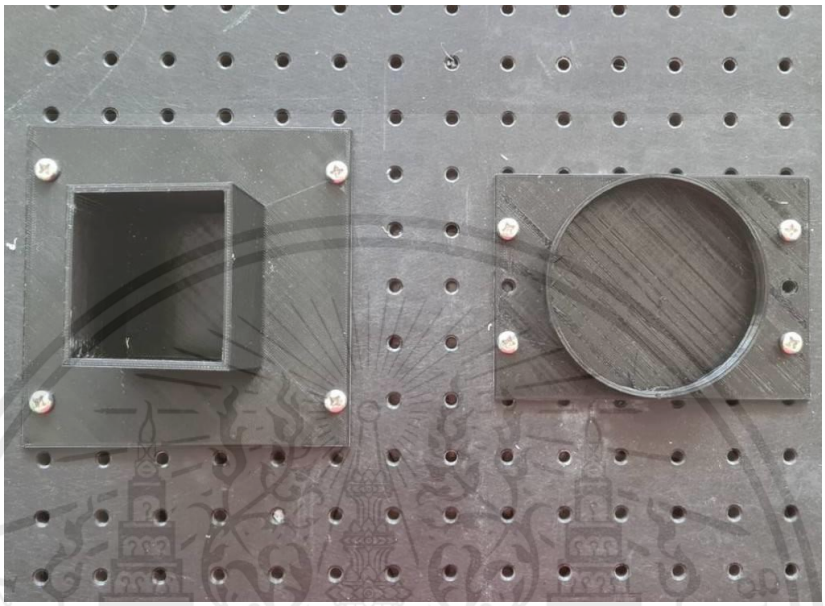


Figure 59 - shows a way to mount based to the breadboard.

3.5.2 Connect the light source with the diaphragm by using the LS-Diaphragm connecter

Figure 60 - shows the assembling of the light source and field diaphragm.

3.5.3 Connect the Z-stage and linear stage together by using the Z-stage linear connecter.

Figure 61 - shows the assembling of the z-stage and linear stage using the Z-stage linear connecter.

3.5.4 Connect the Z-stage and light source to the Z-stage and light source based

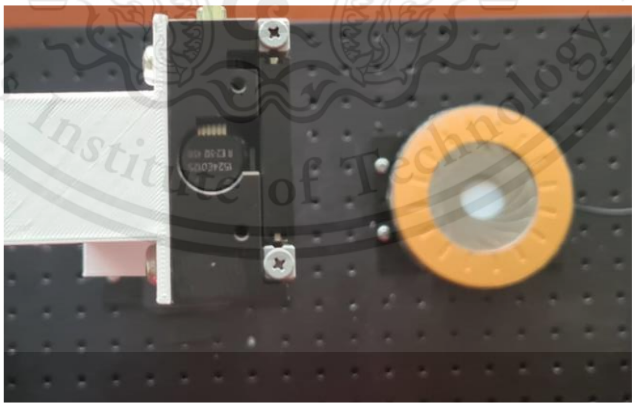


Figure 62 - shows the assembling of the z-stage and light source with base.

3.5.5 Connect the XY-stage to the XY-stage base then connect to the breadboard also place the slide tray on the XY-stage.

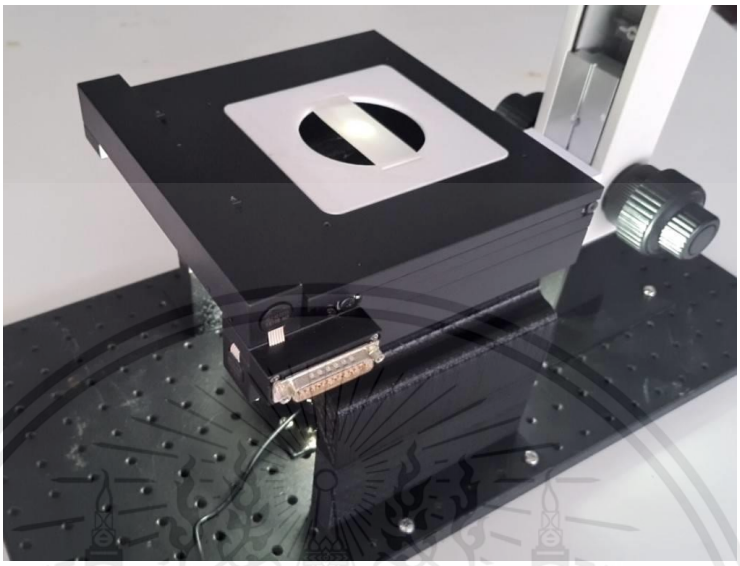


Figure 63 - shows the assembling of the XY-stage that connects to the breadboard.

3.5.6 Connect the condenser holder to the z-stage holder then put the condenser to the condenser holder.

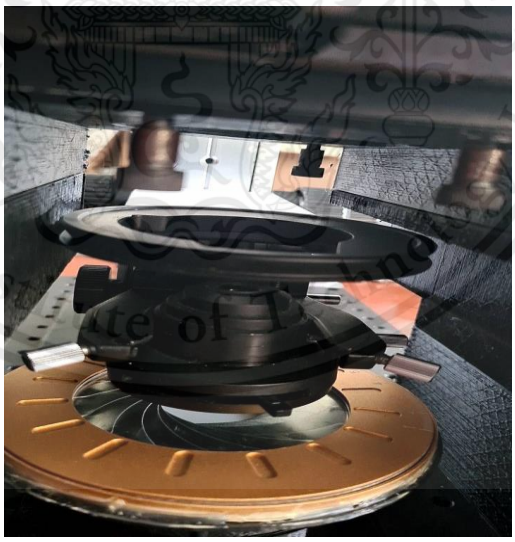


Figure 64 - shows the assembling of the condenser holder and z-stage.

3.5.7 Connect the Body tube, digital camera, and objective lens together then connect to the Linear camera connecter.

Figure 65 - shows the assembling of the camera holder with the linear camera connecter.

3.5.8 Connect the Linear camera connecter to the linear stage then try to capture the image.

Figure 66 - shows the assembling of all components.

CHAPTER 4
EXPERIMENTAL RESULT

4.1 Introduction

This chapter presents the results from the modular custom–made whole slide imaging that we will try to capture images in different magnifications 5x, 10x, and 20x Also we will show the method to capture images in a different magnification and for the stitching image also.

4.2 **The method of scanning**

The method for scanning is different depending on which magnification because in different magnifications it needs to adjust the distance of moving XY-stage and it needs to adjust the focus.

At this moment our LabView is not complete so we need to use ImageView to capture the image also for moving the stage we use TLX4000 move it. The distance that we use to move XY-stage is 0.05 cm for 1 unit on the joystick in TLX4000 so it will be 40,20,10 for the 5x,10x, and 20x magnification respectively

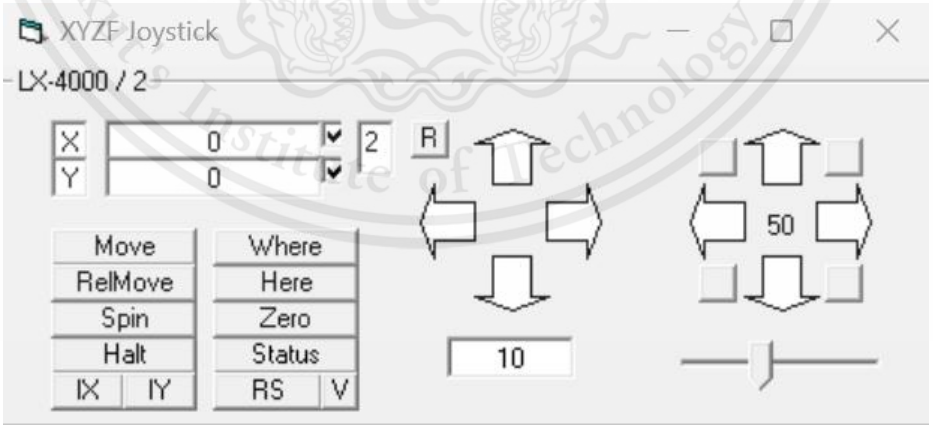


Figure 67 –shows the joystick in TLX4000.

4.3 Serpentine Pattern

The pattern that we use in the scanning is the serpentine pattern it scanning in

zigzag or back-and-forth motion across the target, starting from one edge and moving horizontally or vertically to the opposite edge, and then moving back in the opposite direction, slightly offset from the first pass. This process is repeated until the entire target has been covered.

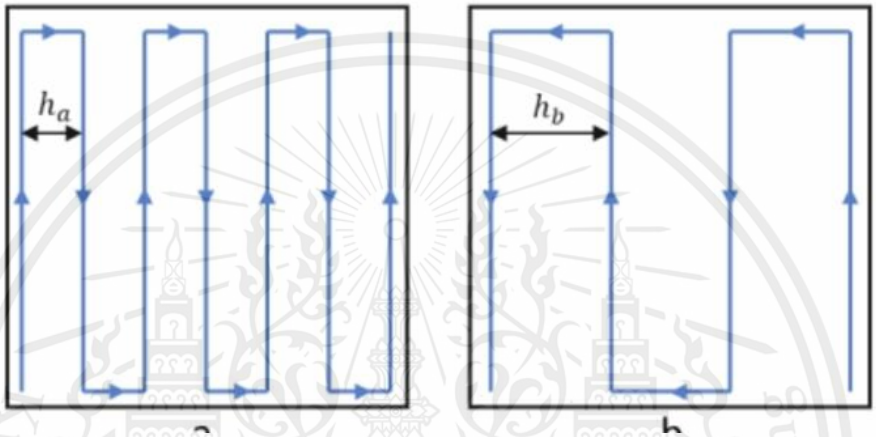


Figure 68 -This figure shows that (a) and (b) are vertical serpentine patterns with hatch distances ha and hb.[28]

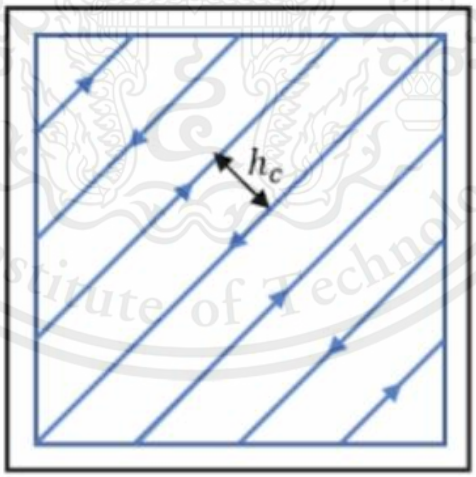


Figure 69 -shows the orthogonal serpentine pattern with a pre-scanned border[28].

4.4 Stitching image

For the stitching image, we decide to make an image of 3x3 that has the 50%
overlap in every magnification both 5x, 10x, and 20x and we decide to pick 3 specimens for the results of the modular custom-made whole slide imaging that is Honeybee wing, Butterfly wing, and locust leg.

2.4.1 Honeybee wing

2.4.1.1 5x Magnification

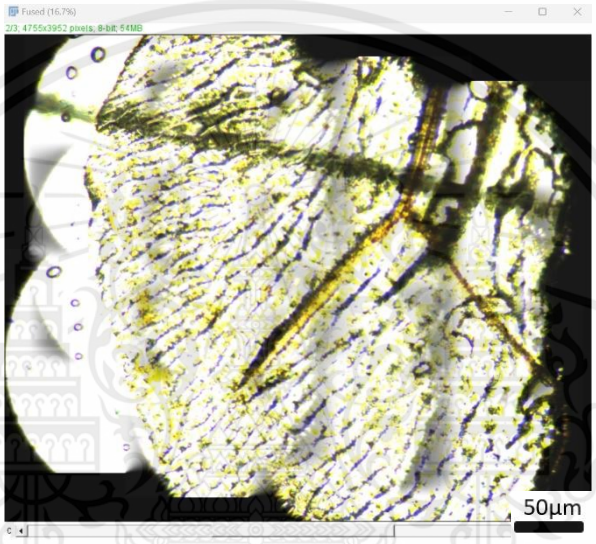


Figure 70 -shows a picture of a honeybee wing with 5x magnification.

2.4.1.2 10x Magnification

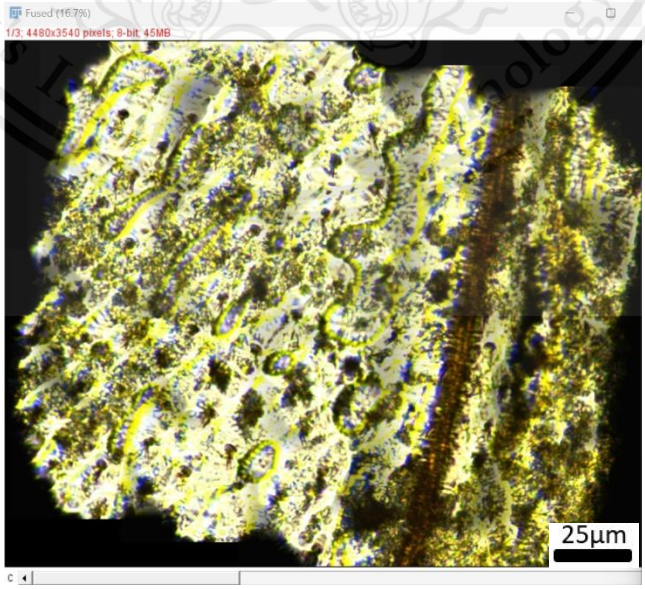


Figure 71 -shows a picture of a honeybee wing with 10x magnification.

2.4.1.3 20x Magnification

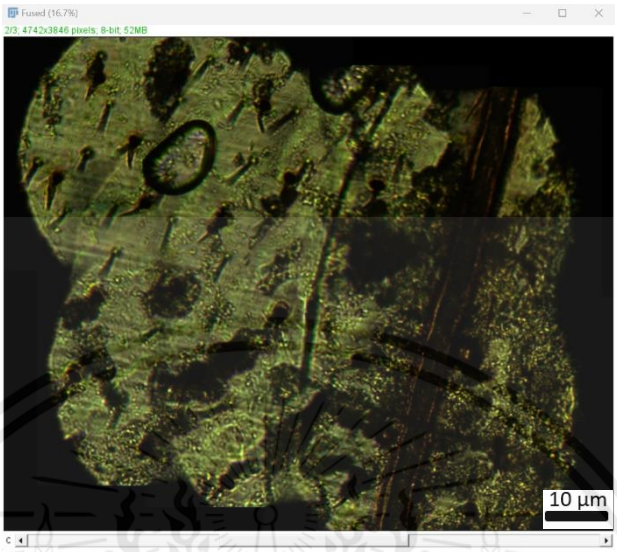


Figure 72 -shows a picture of a honeybee wing with 20x magnification.

2.4.2 Butterfly wing

2.4.2.1 5x Magnification

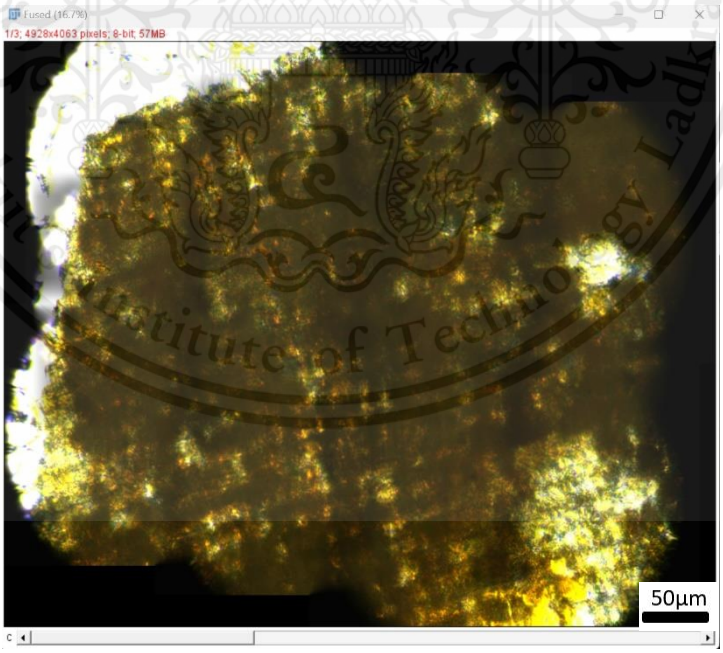


Figure 73 -shows a picture of a butterfly wing with 5x magnification.

2.3.2.2 10x Magnification

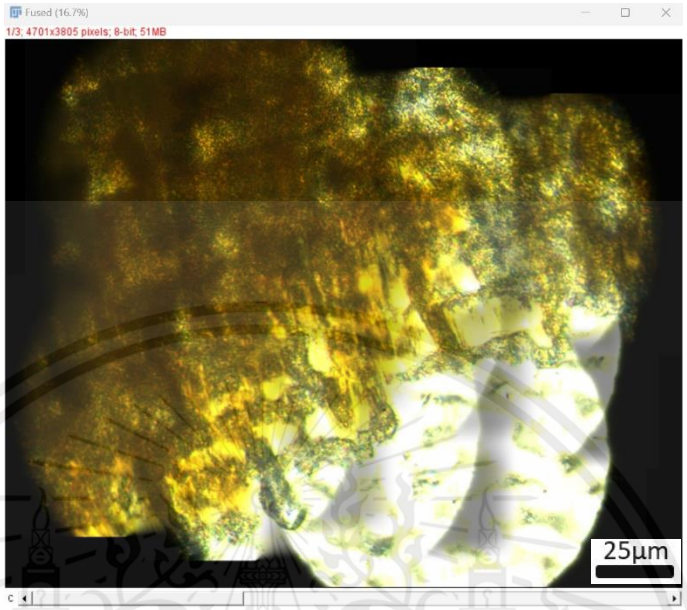


Figure 74 -shows a picture of a butterfly wing with 10x magnification.

2.3.2.3 20x Magnification

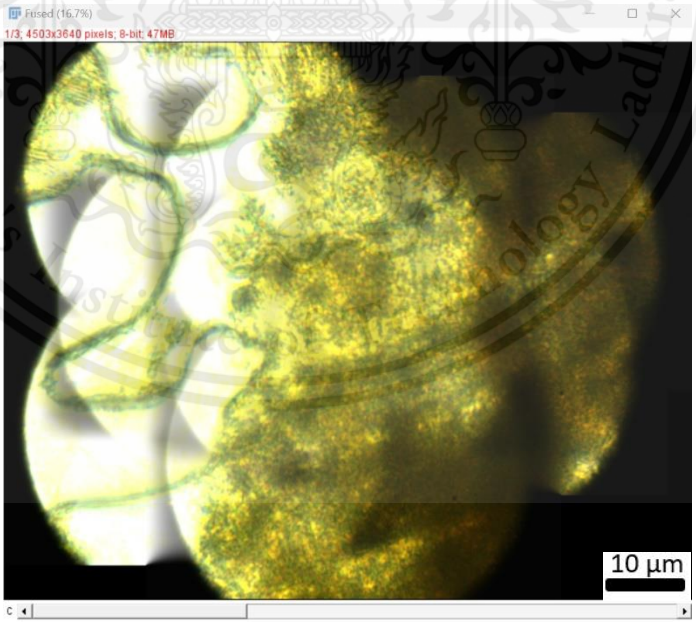


Figure 75 -shows a picture of a butterfly wing with 20x magnification.

2.4.3 Locust’s leg

2.4.2.1 5x Magnification

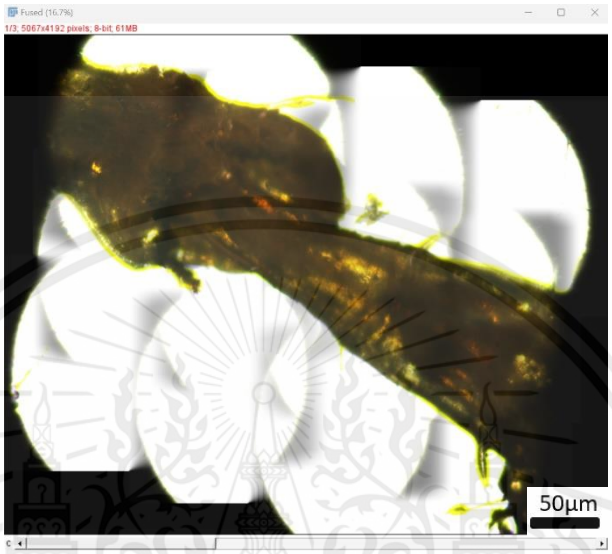


Figure 76 -shows a picture of a with 5x magnification.

2.4.2.2 10x Magnification

Figure 77 -shows a picture of a butterfly wing with 10x magnification.

2.4.2.3 20x Magnification

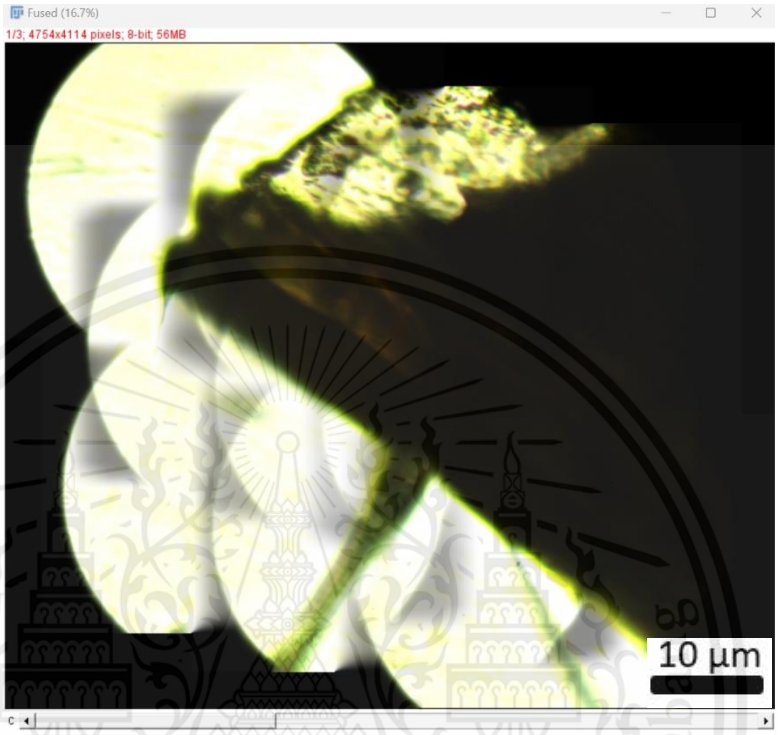


Figure 78 -shows a picture of a butterfly wing with 20x magnification.

CHAPTER 5
CONCLUSION

5.1 Conclusion

In conclusion, this research paper presents a novel custom-made whole slide imaging system that utilizes 3D-printed parts for alignment. The system combines TLX4000 and ImageView software to control the motorized stage and digital camera, respectively, to capture high-quality images of three specimens: honeybee wing, butterfly wing, and locust leg at different magnifications (5x, 10x, and 20x). The acquired images are then stitched using the ImageJ application in a 3x3 grid with 50% overlap in a serpentine pattern.

The developed imaging system demonstrates its effectiveness and potential for high-resolution imaging of various specimens. Additionally, the automation of stage movement and image acquisition using LabView software in the future can further increase the efficiency of the system and reduce manual intervention.

This research paper contributes to the field of whole slide imaging by introducing a custom-made system that can be easily assembled and used in various settings. The system's cost-effective approach and flexibility make it a promising tool for various imaging applications in biology, pathology, and research fields.

5.2 Discussion

For the discussion, the problems that I found are related to the 3D-printing process and the sensitivity of the whole slide imaging system. It was difficult and time-consuming to create a custom-made whole slide imaging system using 3D-printed components. The design and printing of the parts required a considerable amount of time and effort, which may not be practical in certain circumstances. The system's sensitivity to external vibrations, such as touching the table, also caused image shaking and alignment issues.

Despite these challenges, the system showed promising results in capturing high-quality images of various specimens, demonstrating its potential for use in research and diagnostic settings. The use of specific software tools, such as TLX4000 and ImageView, allowed for precise control of the motorized stage and digital camera, respectively. Moreover, the use of the ImageJ application for stitching images proved to be effective in creating a complete image of the specimens.

Future development of the system could focus on improving its alignment and stability. This could be achieved by redesigning the 3D-printed parts to be more stable or implementing a vibration isolation system. Additionally, the automation of the stage movement and image acquisition using LabView software, as planned, could also improve the system's efficiency.

In conclusion, the development of a custom-made whole slide imaging system using 3D-printed parts presents a promising approach to high-resolution imaging of various specimens. Although the system faces challenges related to design, printing, and stability, its potential benefits make it a promising tool for various imaging applications in biology, pathology, and research fields. Further development of the system, including improvements in alignment and automation, can contribute to its wider application and adoption.

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