Custom-Made Cost-Effective Whole Slide Scanner with DSLR Camera for Digital Pathology

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ABSTRACT

Digital pathology had revolutionized the medical field of research, education, and diagnostic resulting in setting of the baseline for the advanced analysis and faster diagnostic. however, the very high cost of the whole slide scanner in the market is the barrier for many of the institution and laboratory to get to this technology. From this problem, this paper is to present the innovative custom-make cost-effective whole slide scanner utilizing DSLR camera. The aim is to push the field of digital pathology into more used in most of the laboratory by providing affordable alternative without compromising the quality of image. Through the design methodology the system integrates the high resolution DSLR camera, the high precision microscope, high preposition moving stage, and a simple imaging algorithms.

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LIST OF SYMBOLS/ABBREVIATIONS

Symbols/Abbreviations	Terms
BME	Biomedical Engineering
SIIE	School of International Interdisciplinary Engineering Programs
IQ	Institute of Qualitative Health Science and Engineering
DSLR	Digital Single-Lens Reflex
ISO	International Organization for Standardization
USAF	United States Air Force
MIL-STD-150A	Military Standard: Photographic Lenses
PPI	Pixels Per Inch
bpp	Bits Per Pixel
WB	White Balance
Mt	Magnification of The Microscope
Mob	Magnification of The Objective Lens
Moc	Magnification f The Oculus Lens
OOP	Object Oriented Programming
AI	Artificial Intelligence
UART	Universal Asynchronous Receiver-Transmitter
USB	Universal Serial Bus
CNNs	Convolutional Neural Networks
VGG	Visual Geometry Group
R-CNN	Region-Based Convolutional Neural Network
OCR	Optical Character Recognition
CCD	Charge Coupled Device

CMOS	Complementary Metal Oxide Semiconductor
CAD	Computer-Aided Design
EOS	Electro-Optical System
ROI	Region Of Interest
RGB	Red, Green and Blue
CNC	Computerized Numerical Control
SRGAN	Super-Resolution Generative Adversarial Networks

CHAPTER 1
INTRODUCTION

Pathology is of vital importance in the investigation and analysis of diseases. The old method to examine tissues, cells, and bodily fluids was using a physical microscope to diagnose the subject slides. It will be an extensive region to diagnose when using extreme magnification and increase the time spending.[1]

Nowadays the issue was resolved by using a whole slide scanner, which can capture the whole slide into digital images. For more practical diagnosis without a physical microscope and reduce time spending. But the cost of the whole slide scanner is still high.

So, we aim to develop the whole slide scanner with cost efficiency but still maintain the high performance.

1.1 Background and significance of the study

There has been an increase in the demand for whole slide scanners with the development of digital pathology. These whole slide scanners play an important role in converting normal slides into digital images for advanced analyses and diagnostics. With the development of artificial intelligence and deep learning algorithms, whole slide scanners are one of the objectives that utilize these algorithms to help with imaging and diagnosis. Most of the whole slide scanners available on the market are costly and out of reach for small laboratories and those located in rural areas. By utilizing cost-effective materials and a DSLR camera, we are able to produce a high-resolution image of the entire slide at a lower cost than most scanners available on the market. With the modular design, provides scalability and customization for further adjustment and upgrade, while maintaining a low cost without compromising performance. Our project aims to improve research, diagnoses, and medical education by overcoming the gap between advanced digital pathology and financial restraints, creating a more accessible and cutting-edge pathology landscape for all.[2]

1.2 Objectives

1. To develop high performance whole slide scanners with a cost efficient system.

2. To perform whole slide scanning in digital pathology.

1.3 Scope of the study

Our study scope cover scanner, slide preparation, scanning process, storage, along with Imaging. For storage we study storing data in local storage and cloud. For Imaging we study software, zooming, and navigation.

1.4 Report outline

The rest of this report is organized as follows:

Chapter 2 Review of theory related

Chapter 3 Methodology

Chapter 4 Experimental result and discussion.

Chapter 5 conclusion.

CHAPTER 2
REVIEW OF THEORY RELATED

2.1 Microscopy

Microscope is the technique of using a microscope to view the details of objects which are too small to observe with the naked eye. In general microscopy was used to advance understanding in scientific knowledge including biological, chemical, and physical phenomena. The microscope can classify to various types but in this project, we will focus on light microscopy.[3]

2.1.1 Principle of microscope

A microscope enlarges the object by using principle of optical lens, the objective and oculus lens. The light passing through the sample object on the slide to an objective lens, the lens will produce between the two lens, and then the oculus lens magnify the real image to magnified virtual image. The magnification of the microscope depends on the magnification of the objective and oculus lens, which can find out with formula $Mt = Mob \times Moc.$

The general light microscope components are composed of eyepiece, objective lens, stage, adjusting knob, light source, and body. The eyepiece contains an oculus lens. the objective lens may have various magnifications to adjust the magnification power according to the magnification formula. From figure 2.1 it has a low power objective lens to view a lower magnified image and a high-power objective lens to view a higher magnified image. The stage is a place to place the sample slide which can adjust the horizontal position to change the viewed field. the adjusting knob used to adjust the vertical position off stage to focal point for the focusing propose, the light source produce the light to the system, it can adjust illumination.[4]

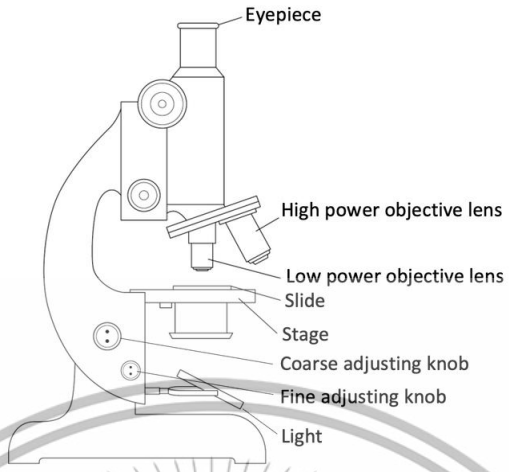

Figure 1 Diagram of light microscope.[5]

2.1.2 Microscope adjustment

To receive the optimal image in microscopy. We need to adjust illumination and focus. Illumination is important to obtain a clear and detailed image. Illumination can be adjusted by controlling light intensity to prevent washed-out images resulting from overexposure of the sample and dim image resulting of underexposure, Control the uniformity and brightness of the illumination across the field of view by adjusting field Diaphragm. Condenser Adjustment to control the contrast and resolution of the image. A smaller aperture provides better resolution but may result in lower light intensity, Used Köhler Illumination technique to aligning the light source, condenser, and field diaphragm to achieve a uniform and focused illumination, and create shadows and highlights to enhancing the visibility of surface details and transparent samples by using Oblique Illumination technique.[6]

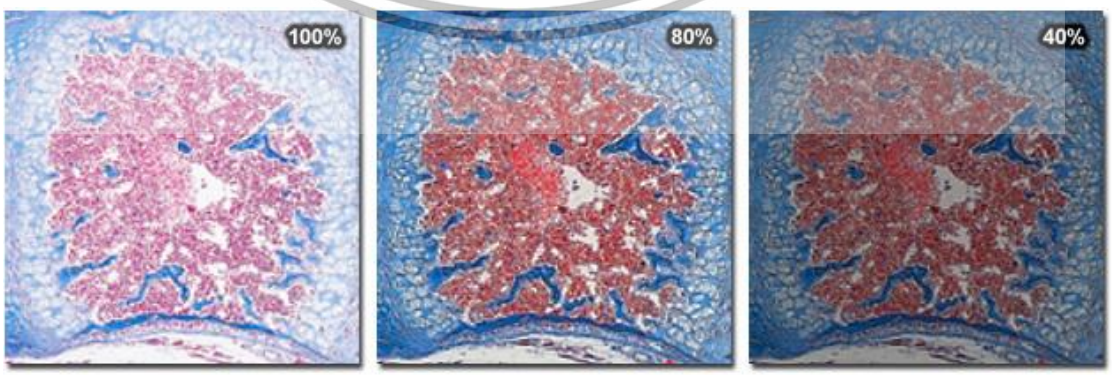

Figure 2 Effect of condenser diaphragm opening size on image contrast and resolution.[7]

Focus adjustment is the process of achieving sharp and clear images of microscopic specimens. Focus in a light microscope can be adjusted by using the Coarse and Fine Focus Knob. The Coarse Focus Knob will make large adjustments to the focus quickly by moves the stage up or down to bring the specimen closer to or farther away from the lens. For the Fine Focus Knob, it precisely adjusts the focus by delicate changes to the distance between the lens and the specimen to achieve optimal sharpness. Adjusting the focus brings the specimen into the focal plane, which is the specific distance at which the microscope's optics converge light rays to form a clear image.[8]

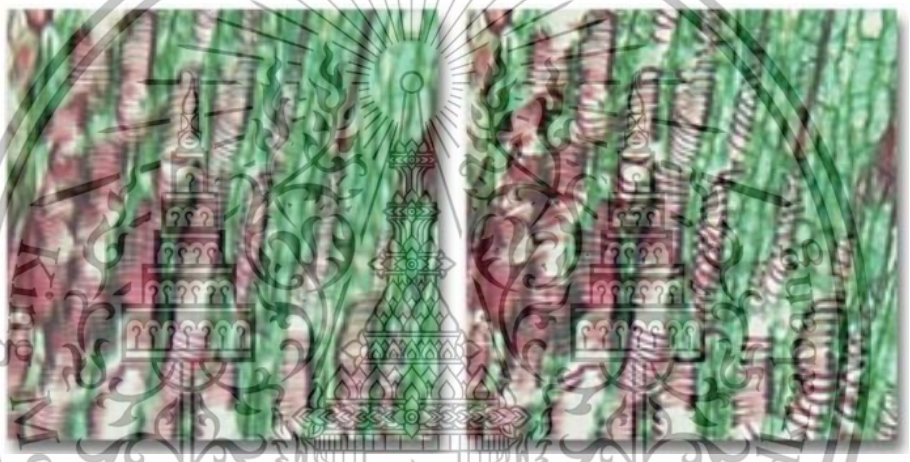

Figure 3 Unsharp image due to focus adjustment error and sharp focus image.[9]

2.2 Slide preparation

Observation microscopic organisms and samples using the compound light microscope commonly fit the sample on a microscope slide. A microscope slide is a thin, rectangular glass plate that holds various microscopic specimens. Microscope slide preparation has several methods depending on the type of viewing specimen.[10]

2.2.1 types of slide preparation

Dry mount: A specimen is placed on a slide with or without a coverslip cover on it. The preparation step is only to place a specimen on a slide and cover the specimen by coverslip if needed. Used to observe a specimen in natural state or a solid specimen that do not require liquid mounting. [11]

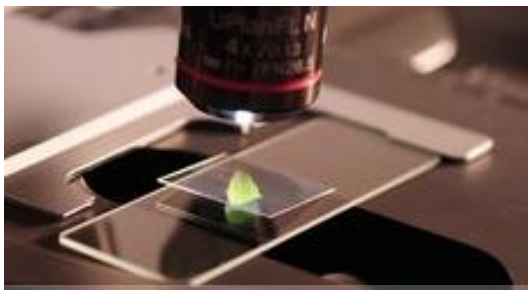

Figure 4 Dry mount.[12]

Wet mount: A specimen is placed on a drop of water on the slide, with a coverslip cover on it. The preparation step starts with placing a specimen on a slide. Next, add a drop of liquid such as water or oil to magnify the specimen with a dropper. Next, set one edge of the coverslip on a slide close to the specimen. And then, slowly lower the coverslip until flat to the slide to prevent air bubbles that can make it difficult to clearly view the specimen. Since liquid is required. Used to observe live microorganisms, aquatic specimens, or any specimens that might change shape or structure when dried.[13]

Smears mount: It is a dried sample that spreads across a slide. The preparation step starts with collecting the small amount of specimen by using a swab or another suitable instrument. Next, place the specimen on the slide and spread it across the slide. Dry it by leaving the slide at room temperature for a period. Apply a stain to the smeared specimen if needed to highlight the specific structure of the specimen. Next, set one edge of the coverslip on a slide close to the specimen. And then, slowly lower the coverslip until flat to the slide to prevent air bubbles. Used to cells, bacteria, and other microscopic structures.

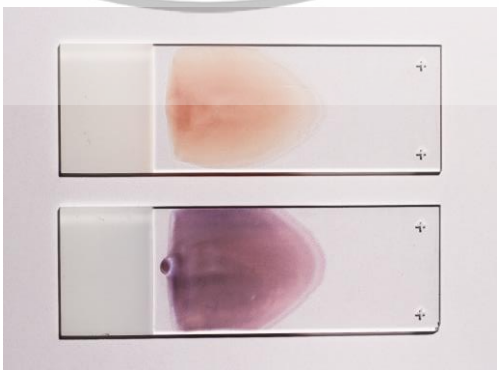

Figure 5 Blood smear [14]

2.2.2 Staining [15]

Staining is a technique using stain to improve a specimen's contrast, making features more distinguishable under a microscope. There are many different stains, which each designed for a different purpose.

Iodine: Used to stain carbohydrates on animal and plant specimens. When detected starch it will stain dark blue.

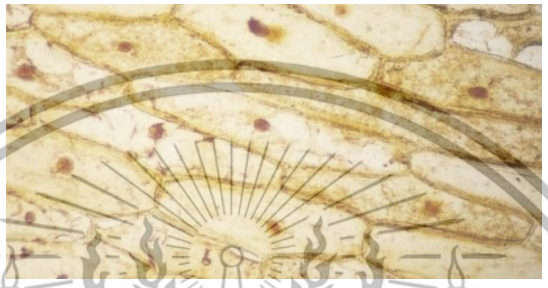

Figure 6 Onion skin with iodine staining.[16]

Methylene Blue: Used to stain nuclei and other acidic cell parts on animal specimens and bacteria. It stains the specimen as blue

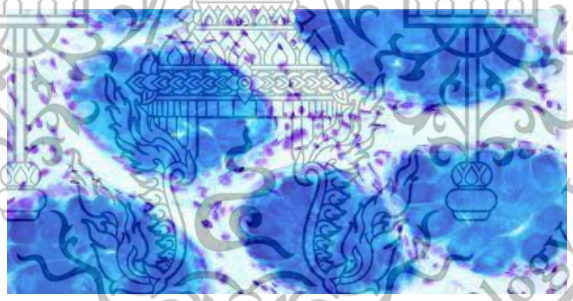

Figure 7 Methylene Blue stain sample.[17]

Crystal Violet: Used to classify bacteria in Gram stains. It stains the specimen as purple.

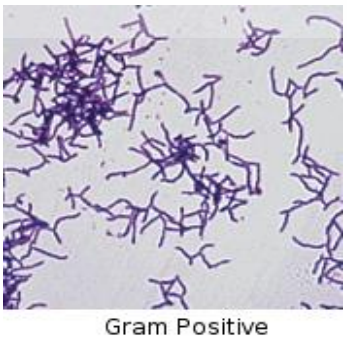

Figure 8 Gram positive staining by crystal violet.[18]

Eosin Y: Used to stain cell membranes, cytoplasm and extracellular structures on plants and animal specimens. It stains the specimen as red or pink.

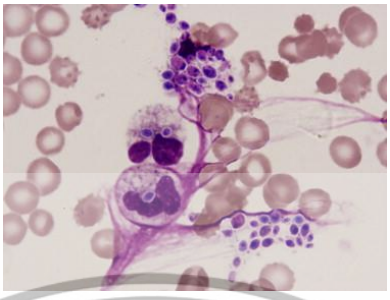

Figure 9 Eosin Y stain sample.[19]

Toluidine Blue: Used to stain acidic cell structures in plant cells to demonstrate mitosis. It stains the specimen as dark blue.

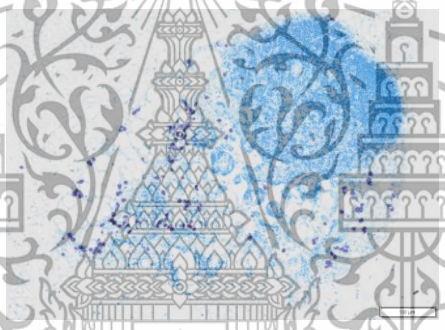

Figure 10 Toluidine Blue stain sample.[20]

2.3 DSLR camera [21]

A DSLR camera is a digital camera. The word DSLR stands for "Digital Single Lens Reflex". It captures the image by using a mirror mechanism, the light passes through the lens and then reflected by the mirror through the viewfinder which is an eyepiece that is used for looking at the image and the image sensor which captures the image by moving the mirror out of the way.

2.3.1 DSLR camera components and working principle.[21]

The components of a DSLR camera consist of 7 main components: lens, reflex mirror, shutter, image sensor, focusing screen, pentaprism, and viewfinder or eyepiece.

The operation of DSLR cameras work by the light passing through the lens into a reflex mirror with angle 45 degree that the light will reflect vertically to the focusing

screen, condenser, and pentaprism. the light will be focused and converted to horizontal and then passed through the viewfinder. The next step is capturing the picture by swinging upward of the reflex mirror to block the vertical pathway and letting the light directly through the shutter.

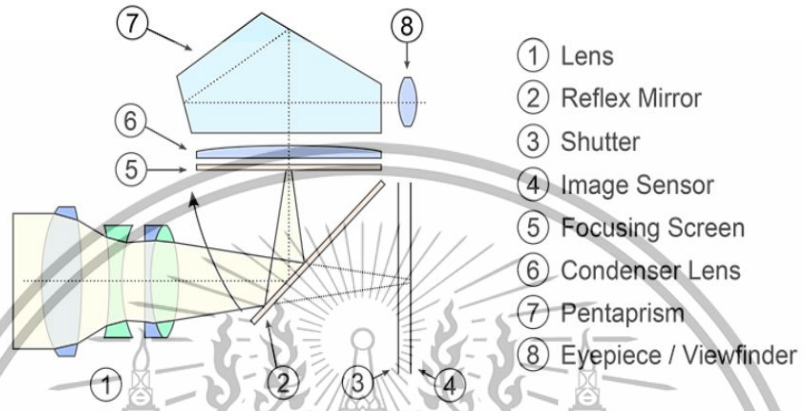

Figure 11 The components of a DSLR camera.[21]

2.3.2 DSLR image capture considering factors.

To capture the image by using a DSLR camera. several factors needed to consider. the factor includes

resolution, it is the level of detail contained in an image, which refers to the number of pixels within an image. The resolution of an image depends on the resolution of the sensor. higher resolution sensors can capture more detail and smoother pictures. Typically, the unit of resolution in DSLR cameras is megapixel (MP). For a digital image, the resolution is measured in pixels per inch (PPI) with Its pixel.[22]

Figure 12 comparing of high-resolution image (The left picture 4715x2779px) and low-resolution image (The right image 200x118px) [23]

Sensor size, the size of the sensor has a significant impact on image quality. Larger size sensors provide bigger areas to receive the light and bigger pixels that produce better image quality. It also provides a lower crop factor, larger depth of view.

ISO sensitivity represents sensitivity to light as a numerical value. Higher number of ISO provide higher sensitivity and a greater ability to capture light. Higher ISO settings used in low-light conditions can introduce noise or graininess into the image. so the ISO sensitivity needed to adjust co-response with the other factors such as aperture, and shutter speed to produce high image quality. [24]

Figure 13 Gain increasing resulting in ISO sensitivity increasing.[25]

Dynamic range, it is the ratio of the darkest shadows to the brightest highlights in the image that a camera can capture. the good dynamic range can preserve details in shadow and highlight regions of the picture.

Lens quality, many characteristics of lenses, have impacts on sharpness, contrast, and overall image quality. Higher quality lenses can reduce distortion and aberration. also, to lens coating, it can reduce lens flare and improve color rendering.

Aperture or f-stop, it controls the size of the lens opening. A wide aperture allows more light to pass and creates a shallow depth of field. A narrow aperture allows less light to pass and increases depth of field.[26]

Figure 14 Depth of field changing resulting in aperture adjustment. [27]

Shuttle Speed, it is the speed at which the shutter of the camera closes., which determines how long the sensor is exposed to light. The fast shutter speed provides shorter exposure that refers to a lower amount of light passing through the camera. The slow shutter speed provides longer exposure that refers to a higher amount of light passing through the camera.

Figure 15 Fast shutter speed (The left picture), Slow shutter speed (The right picture) [28]

White Balance (WB), It is the process to make the color become more realistic by removing unrealistic color casts. it adjusts for the color temperature of the light source.[29]

Figure 16 Color cast picture (on the left), Daylight white balance (on the right) [29]

Focus, it is the sharpness area of the image. also refer to the highlight area on the object by lens working. It works along with ISO sensitivity, aperture, and shutter speed to obtain high quality image [30]

Figure 17 Focus stacking [31]

Color depth, it is the number of colors that can be represented in an image. the unit of color depth is bits per pixel (bpp). Higher color depth provides more accurate color representation and more smoother color transitions.[32]

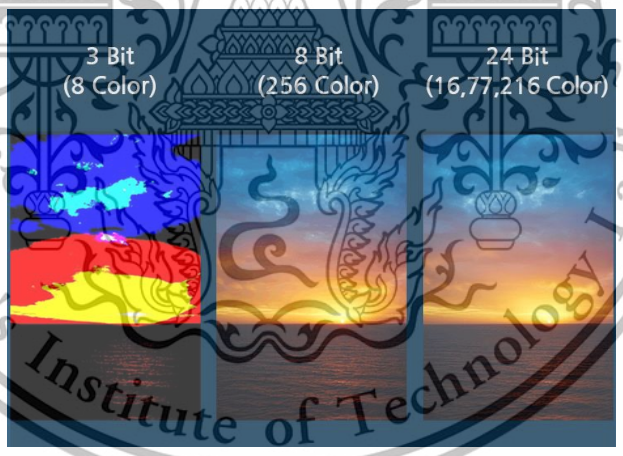

Figure 18 Different color depth image [33]

Image Stabilization, it is the mechanisms of camera or lens that reduce the effects of camera shake. shaking may produce blur or unfocused images.

2.3.4 Image pixel size calculation

The pixels size of an image is depending on two factors that is the resolution of an image and the physical size of the display. The pixel size can calculate by divide the width or height of the medium by the dimension. The unit of pixel size is pixels

per inch (PPI). Higher PPI values provide more detailed and smoother images, while lower PPI values may lead to visible pixilation and reduced image quality.[34]

2.4 1951 USAF (this is calibrator for resolution and pixel size) [35]

A 1951 USAF resolution test chart is a microscopic optical resolution test device originally defined by the U.S. Air Force MIL-STD-150A standard of 1951. The design provides numerous small target shapes exhibiting a stepped assortment of precise spatial frequency specimens. It is widely used in optical engineering laboratory work to analyze and validate imaging systems such as microscopes, cameras and image scanners

These resolution targets consist of a grid of horizontal and vertical lines that serve to determine the resolution capability of an imaging system. Each group within the chart contains six elements, comprising pairs of horizontal and vertical lines. The entire resolution chart is composed of ten of these groups. The illustration below specifically showcases Elements 2 and 3 from Group -2 on a resolution target.

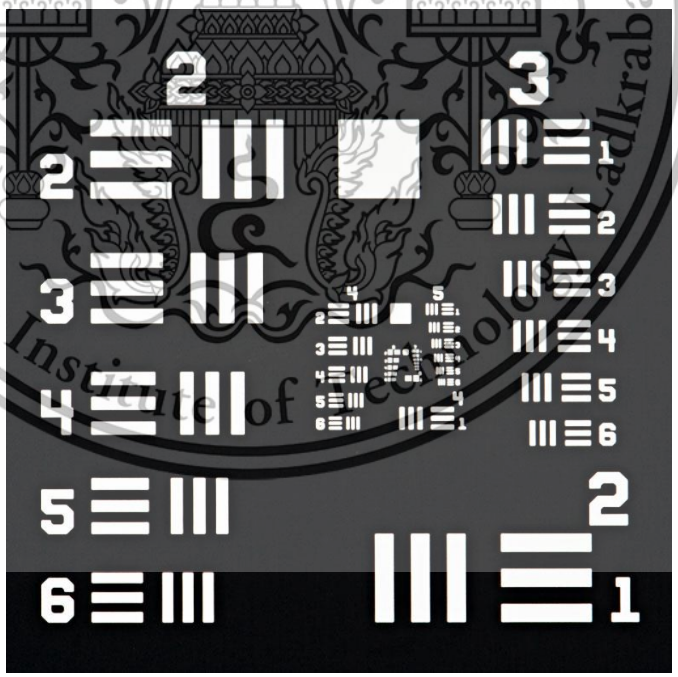

Figure 19 Microscope Image of R1DS1N Negative Test Target. [36]

The utilization of sets of three lines in these targets offers a notable advantage in recognizing instances of false resolution. False resolution, in this context, arises when

a set of lines becomes sufficiently blurred to the extent that the overlap gives the illusion of forming distinct lines that are inverted. This phenomenon can lead to an incorrect assessment of the optical system's resolution, as it might seem that the lines can be differentiated. Furthermore, false resolution creates the optical illusion of there being one less line in the set than there is.

The reason behind this lies in our ability to more easily distinguish between three lines and two lines compared to differentiating between five lines and four lines. Therefore, identifying cases of mistaken clarity is simpler when the target consists of only three lines.[37][38]

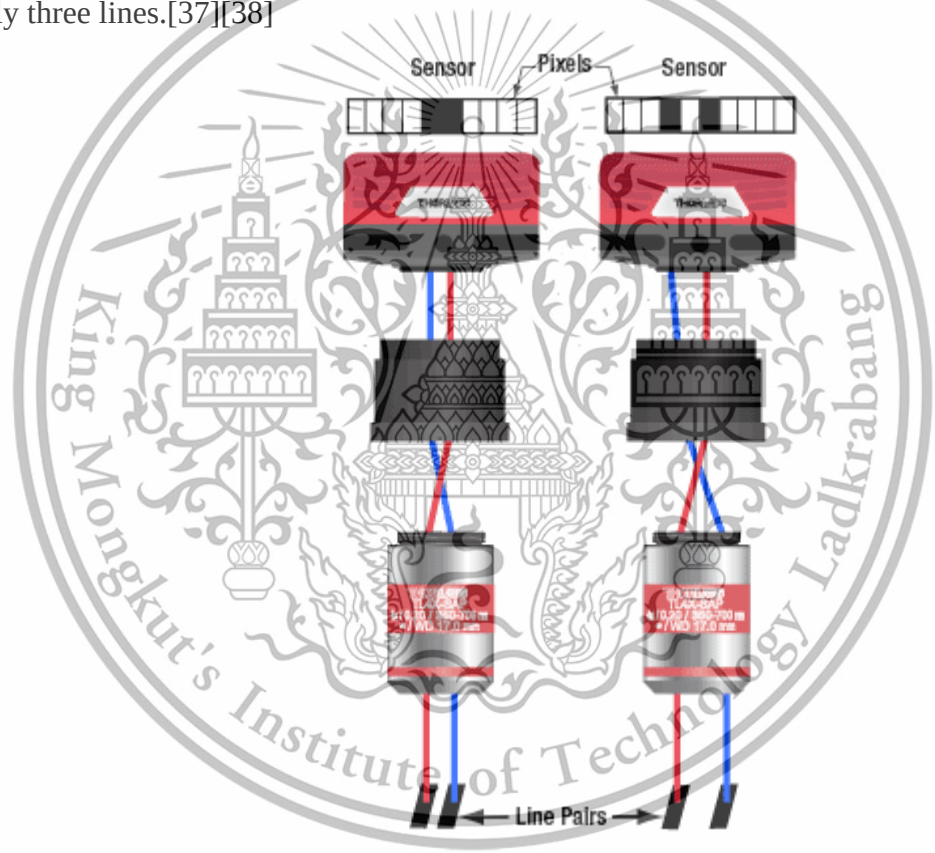

Figure 20 Line pairs help measure how well a camera can distinguish two objects from one another. [36]

The spaces between each line within a single unit are equivalent to the width of the line itself. When the target is captured in an image, we can gauge the resolution capacity of the imaging system by evaluating the sharpness of both the horizontal and vertical lines. The highest count of indistinguishable horizontal and vertical lines sets the limit for the resolving power of the imaging system.

The table provided below presents the count of line pairs per millimeter for a given element within a group based on the provided equation. In our resolution targets, the maximum attainable resolution is 228.0-line pairs per millimeter, approximately translating to 4.4 µm per line pair.

These resolution targets come in various sizes and designs: the 3" x 3" targets encompass ten groups ranging from -2 to +7; the 3" x 1" wheel pattern versions comprise nine targets, each spanning groups +2 to +7; the 3" x 1" birefringent target encompasses six groups, spanning from 0 to +5; the 18 mm x 18 mm (0.71" x 0.71") combined targets include six groups ranging from +2 to +7; and the Ø1" targets cover six groups, spanning from +2 to +7. [39]

$$Resolution\left(\frac{line\ pair}{mm}\right) = 2^{Group + \left(\frac{Element - 1}{6}\right)}$$

Figure 21 Resolution equation from 1951 USAF target.

Element	**Group Number**									
	-2	-1	0	1	2	3	4	5	6	7
1	0.250	0.500	1.00	2.00	4.00	8.00	16.00	32.00	64.00	128.00
2	0.280	0.561	1.12	2.24	4.49	8.98	17.95	36.0	71.8	144.0
3	0.315	0.630	1.26	2.52	5.04	10.10	20.16	40.3	80.6	161.0
4	0.353	0.707	1.41	2.83	5.66	11.30	22.62	45.3	90.5	181.0
5	0.397	0.793	1.59	3.17	6.35	12.70	25.39	50.8	102.0	203.0
6	0.445	0.891	1.78	3.56	7.13	14.30	28.50	57.0	114.0	228.0

Table 2.1 Resolution table observed by line pairs. [36]

2.5 ASI stage

The ASI stage is the movement slide stage. It has stepper motors or other motors to adjust the position of the microscope slide. to make a three-dimensional movement

of the slide, the motors break down to 3 axis including X axis, Y axis, and Z axis to control each axis movement. Typically, ASI 3D stages can be built by combination of 2 axis stage (XY stage) and linear stage (z stage) or complete 3 axis product (XYZ stage). [40]

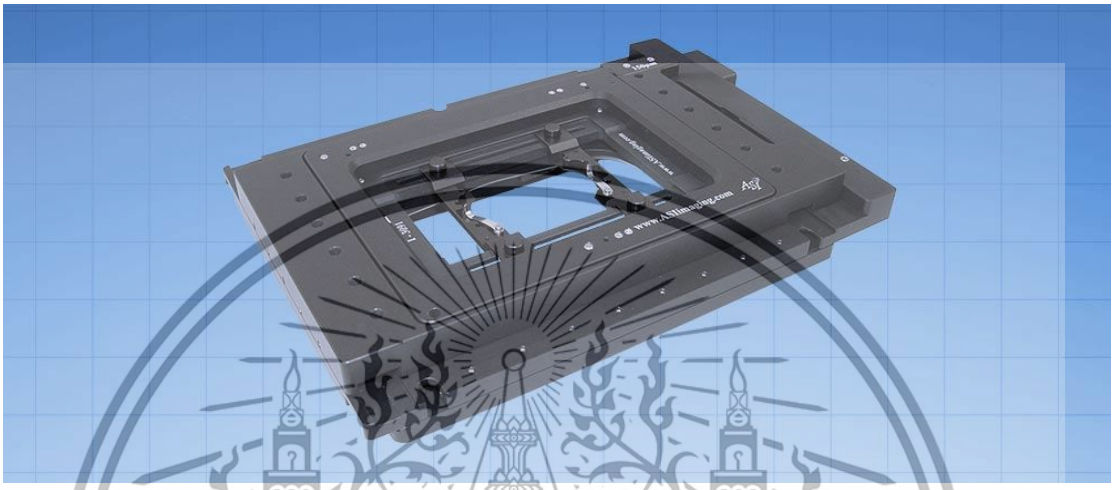

Figure 22 PZ-2000FT SERIES XYZ AUTOMATED STAGE WITH PIEZO Z-AXIS TOP PLATE [41]

Figure 23 LS-SERIES LINEAR STAGES [41]

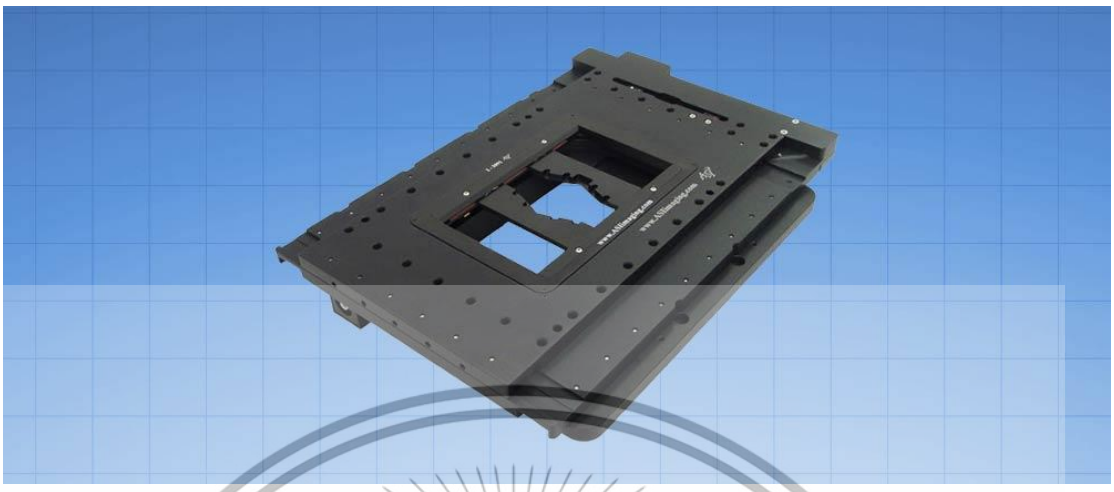

Figure 24 MS-2000 FLAT-TOP *XY* AUTOMATED STAGE [41]

2.5.1 Movement precision [42]

The ASI stage adjusts the slide position by moving the stage in each axis so it needs to calculate the distance for accurate slide position. The movement distance can be calculated by linear motion principle from rotation of screw on ASI stage.

The variable involved in this calculation is Thread pitch, it is the distance between adjacent threads on the screw. Which is equal to the distance of one complete rotation screw along the axis, it typically measures in units of millimeters (mm) or inches (in). from the definition of thread pitch, which is equal to the distance of one complete rotation screw. So the total distance of ASI movement along the axis (D) can be calculate by thread pitch (P) multiply number of rotation

$$Total\ Distance(D) = Thread\ pitch\ X\ Number\ of\ rotation$$

Another method to calculate the distance is encoders, which are more precise than the rotation screw method, due to it providing real time feedback on position. This method operates by the encoder generating count for full revolution, which is called encoder resolution. and then recording the encoder count before and after movement. from the recorded parameter we can calculate the count different by,

$$Count\ different = final\ encoder\ count - Initial\ encoder\ count$$

The final step is to convert the count difference to the physical distance by multiplying the count difference and the distance of each count, which is determined by the mechanic of the system.

$$Movement\ distance = count\ difference\ X\ distance\ per\ count$$

2.6 Python [43]

Python is a high-level programming language renowned for its simplicity and approachability. Developed by Guido van Rossum and first introduced in 1991, Python is designed with a strong emphasis on code readability. This makes it ideal for both beginners and seasoned programmers.

Notable characteristics of Python include:

Simple and Readable Syntax: Python's syntax is intuitive and closely resembles spoken English, making it easier for developers to write and maintain code.

Interpreted Nature: As an interpreted language, Python does not require pre-compilation, allowing programmers to run scripts directly through an interpreter.

Dynamic Typing: The language supports dynamic typing, relieving developers from the need to declare variable types explicitly. Types are determined at runtime.

Extensive Standard Library: Python comes equipped with a vast standard library, offering a range of modules and functions for various tasks like file operations, web development, and networking. This library significantly reduces the need to write codes for common functions from scratch.

Cross-Platform Compatibility: Python runs on multiple platforms, including Windows, macOS, and various Linux distributions, making it versatile for developing applications compatible with different operating systems.

Community and Extensibility: Python's active community contributes to its extensive range of external libraries, enhancing its capabilities in areas like data analysis, machine learning, web development, and scientific computing.

Support for Object-Oriented Programming (OOP): Python facilitates OOP, allowing developers to organize code using classes and objects, enhancing modularity and reusability.

Advanced Data Types: The language offers sophisticated data types like lists, dictionaries, and sets, simplifying complex data structure management.

Indentation-Based Structuring: Unlike many languages that use braces for structure, Python uses indentation, promoting consistent and readable code formatting.

Python is versatile, with applications in web development, scientific computing, data analysis, AI, machine learning, automation, scripting, game development, and

more. Its adaptability, ease of use, and strong community support make it a preferred choice in various fields and industries, aligning well with diverse research needs.

2.6.1 Pyserial [44]

PySerial is a pivotal Python library that enables communication with serial devices, including microcontrollers, sensors, and various hardware elements. It operates through the serial port, a channel for serial communication where data is transmitted bit by bit. This often occurs via a UART (Universal Asynchronous Receiver-Transmitter) port or a USB-to-serial converter.

PySerial equips Python programs with functionalities to interact with these devices. It provides various functions and classes to handle tasks like establishing connections, configuring port settings, reading incoming data, and sending data over serial ports. Its practical uses are evident in several areas:

Microcontroller Interaction: A common use of PySerial is to connect with microcontrollers like Arduino and Raspberry Pi. This connection is crucial for sending commands to the devices and receiving responses such as sensor data.

Sensor Data Collection: Many sensors and measuring instruments use serial communication to send data. PySerial enables the collection of this data, allowing for its integration into Python programs for analysis and processing.

Data Logging and Monitoring: PySerial is instrumental in developing applications that log data from various devices over time, which is essential in fields like environmental monitoring and industrial automation.

Serial Port Communication Emulation: An additional feature of PySerial is its ability to emulate serial communication. This is particularly useful for testing and debugging when direct hardware connections are not available.

Overall, PySerial stands out as a crucial library for enhancing Python's capability to interact with hardware, pushing the boundaries of performance and fostering innovative applications.

2.6.2 OpenCV [45]

OpenCV, or Open Source Computer Vision Library, is a trailblazing toolkit in computer vision and image processing realms, tracing its origins to 1999 under Intel's guidance. Its evolution into a worldwide, collaborative project is marked by contributions from a diverse set of developers and researchers. A standout feature of OpenCV is its remarkable cross-platform compatibility, functioning seamlessly across different operating systems and hardware platforms, including mobile devices. This versatility cements its role in areas ranging from machine learning and robotics to augmented reality.

OpenCV is essentially the digital optic nerve for computers, enabling them not just to see but to interpret visual data. It equips machines with the ability to recognize objects, track movements, analyze patterns, and even perform complex tasks like facial recognition. The subsequent sections will explore OpenCV's extensive toolbox, highlighting its prowess in image manipulation, object detection, machine learning integration, and its broad applications across various industries. Key features of OpenCV include:

1. Object Detection and Recognition: OpenCV employs advanced machine learning techniques for identifying and tracking objects in visual media. Among these, the Haar Cascade Classifier and the Histogram of Oriented Gradients (HOG) stand out. They are commonly used to detect entities like faces in smartphones and moving objects like cars and pedestrians in surveillance. These tools utilize machine learning principles to enhance accuracy and speed in object recognition, crucial for modern facial recognition and security monitoring systems.

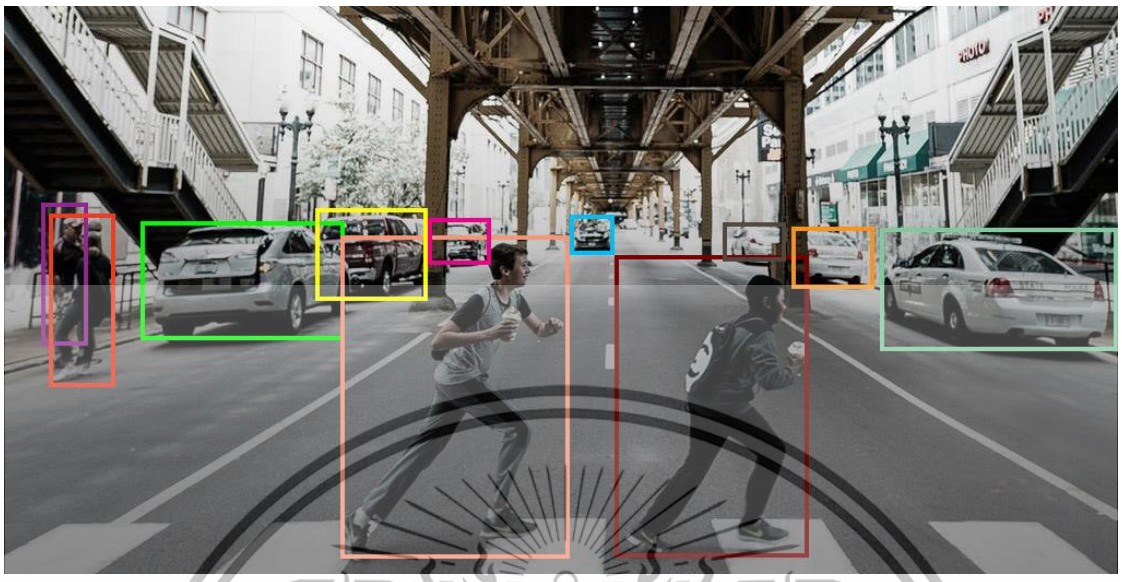

Figure 25 Getting Started with Object Tracking Using OpenCV [46]

2. Image Classification: OpenCV seamlessly interfaces with leading deep learning platforms such as TensorFlow and PyTorch. This integration enables the use of advanced convolutional neural networks (CNNs) for image classification. Users have the option to utilize well-established pre-trained models like ResNet, VGG, and Inception, or to train bespoke models on specific datasets. Such versatility is crucial for various applications, including image retrieval based on content and the analysis of medical imagery.

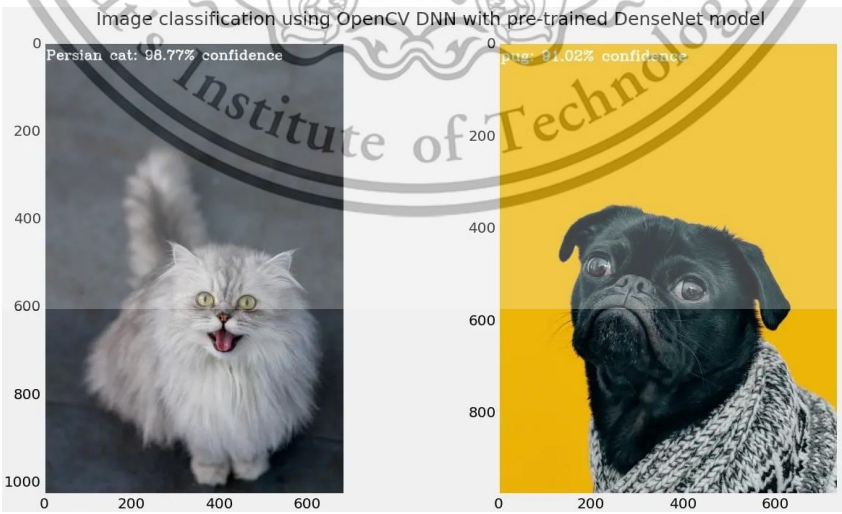

Figure 26 Feature extraction and image classification using Deep Neural Networks and OpenCV [47]

3. Scene Understanding: OpenCV's capabilities in machine learning encompass the understanding of complex scenes, identifying and classifying objects within them. This functionality is especially beneficial in the fields of robotics and autonomous vehicles. It aids these systems in navigating and making informed decisions based on their visual surroundings.

4. Image Segmentation: OpenCV offers sophisticated tools for image segmentation, a key process in dividing images into significant sections, driven by machine learning. It supports both semantic and instance segmentation, employing deep learning models such as U-Net and Mask R-CNN. These advanced segmentation techniques are particularly useful in the field of medical imaging, where they play a crucial role in identifying tumors and delineating organs.

Figure 27 Semantic segmentation with OpenCV and deep learning [48]

5. OCR (Optical Character Recognition): OpenCV has the capability to detect and pull out text from pictures and scanned paperwork using OCR, or Optical Character Recognition, which relies on machine learning. This ability is key in transforming printed stuff into a digital form, making it easy to search through. It's

really handy for a bunch of things, like turning written words into typed text automatically and translating different languages in text.

6. Real-time Processing: OpenCV elevates the performance of machine learning algorithms, making them ideal for dynamic, instantaneous tasks. This enhancement is critical for the effective deployment of applications that depend on visual data processing, particularly in scenarios demanding swift action. Its applicability shines in domains like steering autonomous vehicles and crafting engaging augmented reality settings, where prompt visual data handling is essential for effective operation and user engagement.

7. Continuous Advancements: The dynamic and engaged developer community behind OpenCV plays a pivotal role in its ongoing prominence in the ever-changing landscape of machine learning and computer vision. This energetic group regularly contributes innovative models, methods, and enhancements, maintaining OpenCV's position at the cutting edge of technology. Their contributions ensure that applications developed with OpenCV remain highly advanced and efficient.

In the world of computer vision and image processing, Camera Calibration is a fundamental process. It's key to obtaining precise measurements and securing the dependability of computer vision applications. This process involves determining the intrinsic and extrinsic parameters of a camera, which are critical for converting 2D image data into 3D spatial coordinates. By calibrating the camera, distortions caused by the lens and the camera's positioning are corrected, significantly improving the precision of image analysis.

2.7 ImageJ [49]

ImageJ, an open-source platform, is tailored for processing scientific images across multiple dimensions. It boasts remarkable adaptability, evident in its extensive collection of plugins and scripts that cater to a wide array of tasks. The software is supported by a robust and active user community, enhancing its application in scientific imaging.

2.7.1 Stitching Method: Grid/Collection

Grid/Collection Stitching is a valuable process for handling extensive datasets comprising numerous tiled images. The procedure involves pinpointing specific attributes in the overlap zones of adjacent images and subsequently aligning them based on these identified traits. This technique results in a unified, high-resolution panoramic image that effectively conserves the integrity of the original tiles. Essential for researchers working with broad observation fields, this method facilitates detailed examinations of sizeable subjects or vast landscapes while maintaining the clarity and detail of the images.

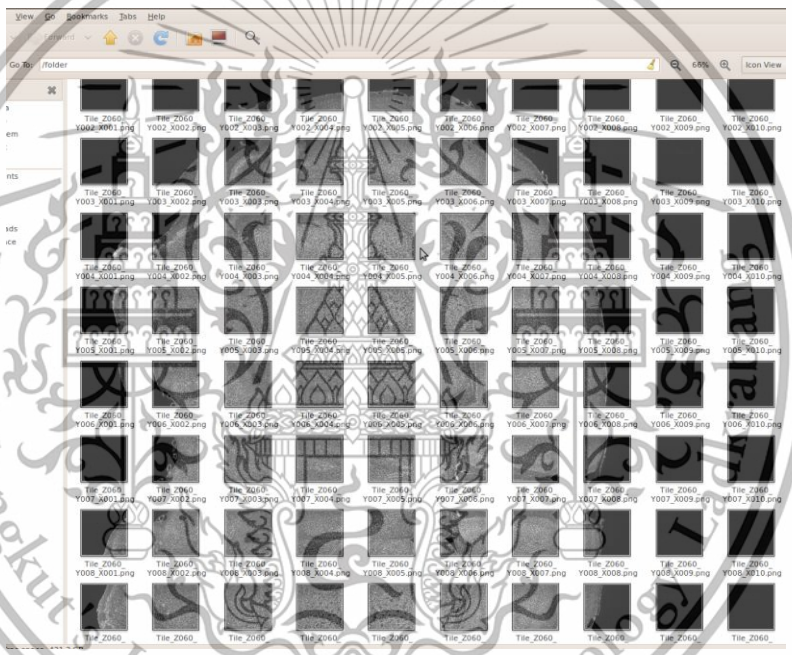

Figure 28 Stitch and Align a sequence of grid images Tutorial [50]

2.7.2 White Balance

ImageJ's white balance feature is key in addressing color discrepancies resulting from varying lighting during photo capture. It fine-tunes the color temperature, ensuring whites in the image are truly represented, thereby keeping other colors in the image authentic. This aspect is particularly vital in scientific imaging, where the exactness of color portrayal is crucial for detailed analysis and correct diagnosis.

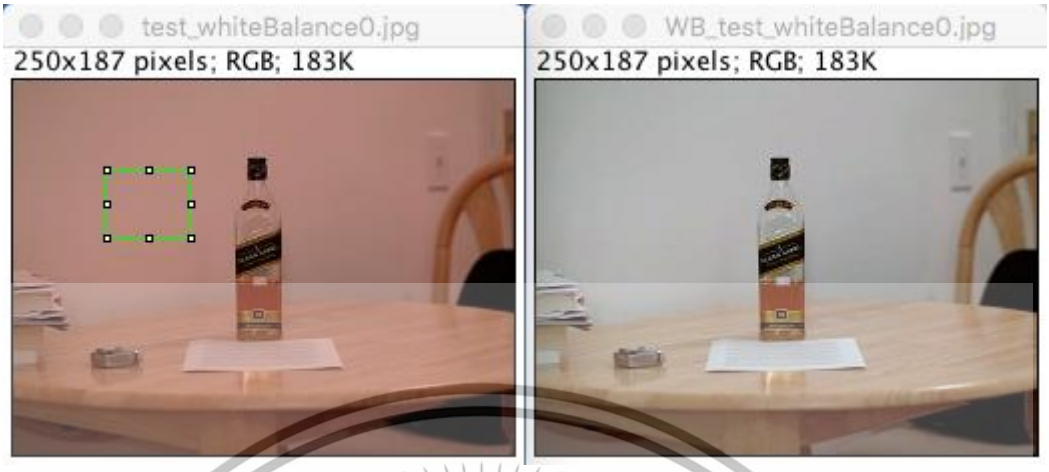

Figure 29 Normalization of True White to Compare Pigment Across Images [51]

2.7.3 Brightness/Contrast

Brightness Adjustment: The ability to adjust an image's brightness in ImageJ is crucial for revealing hidden details in both the shadow and highlight regions of an image. This feature allows researchers to enhance the visibility of specific features in an image, which can be critical for detailed analysis and interpretation. Contrast Adjustment: The contrast adjustment feature in ImageJ enhances the difference in luminance between the various features in an image. By increasing or decreasing contrast, researchers can emphasize or de-emphasize specific elements in the image, making it a versatile tool for tailoring images to the needs of the research.

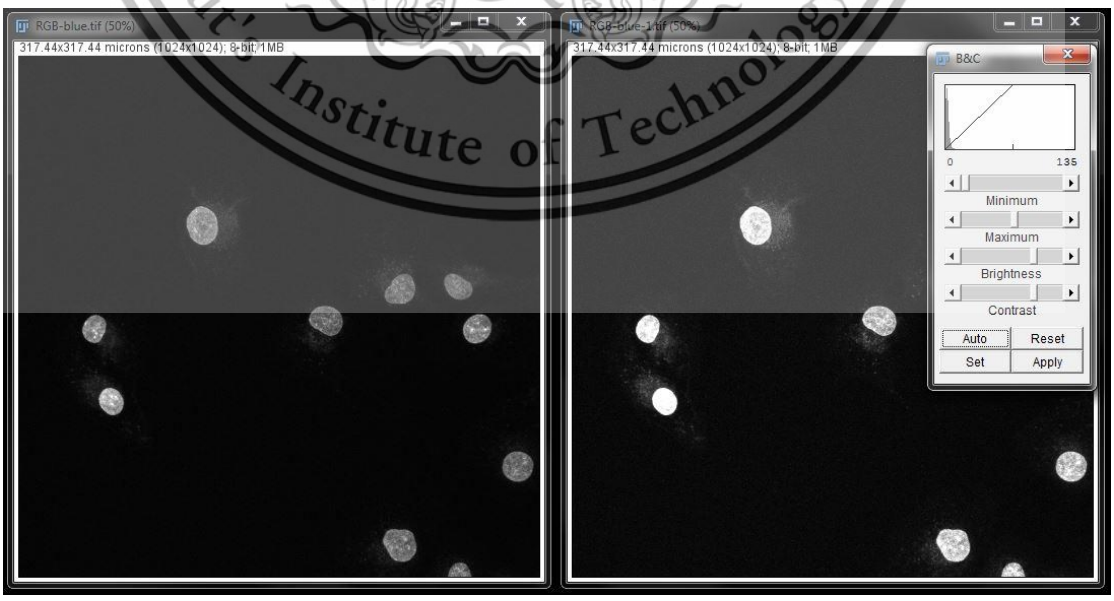

Figure 30 IMAGE ADJUSTMENTS [52]

2.7.4 Application in Research

In this study, ImageJ's capabilities are utilized for processing and analyzing images obtained during the experimental process. The Grid/Collection stitching technique is applied to assemble extensive views of the specimens, facilitating in-depth examination across broad areas. White balance correction is employed to guarantee the accuracy and reliability of color information in the images, a critical factor for the integrity of the study's outcomes. Moreover, modifications in brightness and contrast are executed to uncover concealed details and augment the visibility of distinct features within the specimens, thus enriching the comprehensiveness of the analysis.

2.8 Commercial product [53]

Commercial whole slide scanners from various companies share core functionalities but also exhibit distinct differences. These scanners are designed to digitize entire microscope slides, delivering high-resolution digital images suitable for analysis and storage.

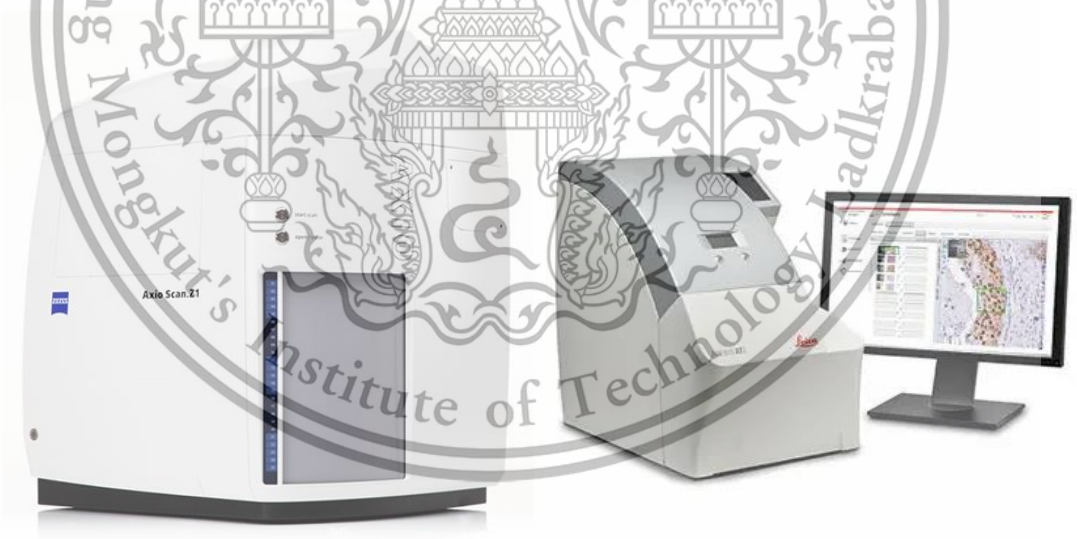

Figure 31 ZEISS Axio Scan.Z1 Slide Scanner (On the left). Leica Biosystems Aperio AT2(On the right).[54]

2.8.1 Component of commercial product [58]

From the similarities and differences between products from each brand. The one similar thing is the main component. Each product will contain the similar main components to do core functionality, which is to digitize entire microscope slides and different in some feature parts. The main component of commercial whole slide scanner including

Slide Holder or Stage, It is part to place the microscope for scanning. The slide holder or stage securely holds the slide in position during the scanning process.

Figure 32 Microscopy Slide and Test Target Holders [55]

Objective Lens, it is a high-quality microscope lens that magnifies the specimen on the slide. It is used to capture detailed images of the entire slide.

Figure 33 Objective lens [56]

Camera, it is a digital camera that is used to capture high-resolution images of the slide. The camera's sensor quality and resolution directly impact the image quality.

Illumination System, it provides uniform and consistent lighting to illuminate the specimen on the slide. It is crucial for capturing clear and well-exposed images.

Slide Movement Mechanism, the mechanism that moves the scanner slide horizontally and vertically to capture images of the entire slide. It is typically using precision motorized stages to produce precise control of slide movement.

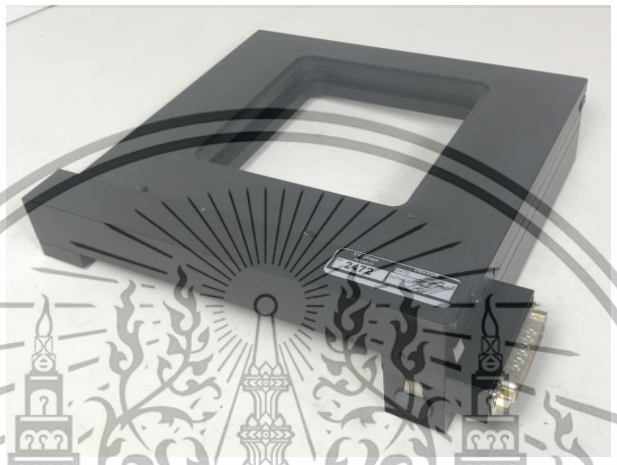

Figure 34 ASI MS 2000 [57]

Focusing Mechanism, It often uses autofocus technology to focus on different areas of the slide. A focusing mechanism to ensure that the entire slide is in sharp focus.

Optical Path, It consists of mirrors, lenses, and prisms that direct and manipulate the light from the slide to the camera sensor to ensure that the image captured is faithful to the specimen on the slide.

Image Sensor, it captures the light from the slide and converts it into a digital image. The sensor's resolution is a critical factor in determining the image's detail and quality. often a high-quality CCD or CMOS sensor.

Microcontroller, it manages all scanning functions, including slide movement, focusing, and image capture. It may also control the illumination system and other components of the scanner.

Cooling System, it used to prevent the image sensor from overheating during prolonged scanning sessions to ensure image quality and consistency in some whole slide scanners.

Software, It manages the scanning process, controls the hardware components, and processes the captured images. It may also include tools for image stitching, annotation, and image analysis.

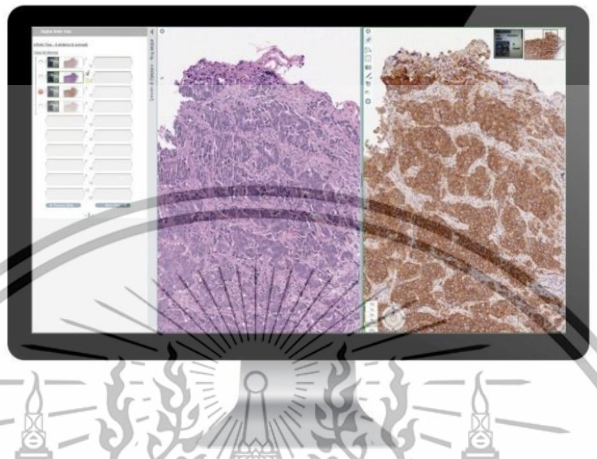

Figure 35 Leica Biosystems microscope slide scanner interface

Data Storage, it is a component to store the captured digital images. It is typically a high-capacity hard drive or networked storage system.

User Interface, it allows users to control and monitor the scanning process, review images, and adjust settings. often in the form of dedicated software or a touch screen on the scanner.

CHAPTER 3
METHODOLOGY

3.1 Introduction

In Chapter 2 we identified the required and related theory for this project include microscopy, slide preparation, DSLR camera, 1951 USAF, ASI stage, Python, data storing, Image J, and commercial product.

3.2 Design Methodology

The design and development of the whole slide scanner system and the Whole slide imaging software.

3.2.1 Whole slide scanner system

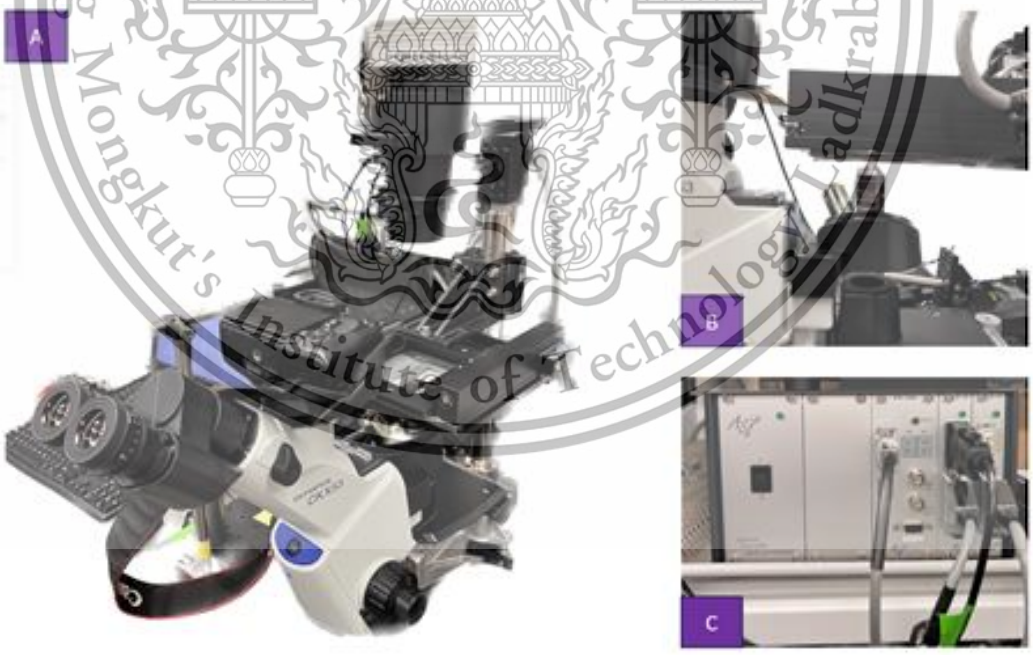

Figure 36 (A) Complete design of the system. (B) side view of the system consists of an ASI Stage and Optical lens facing upward to the slide. (C) Precision ASI control driver.

Microscope

1. The microscope

used in this project is Olympus CKX53, it's an inverted lens microscope, which is known as the popular laboratory microscope due to its reachable cost and the reliable result of magnification. The microscope comes with a linear light source. The stationary slide holder of the microscope then gets disassembled and replaced with the moving state. The optical lens used was 10x magnification and 40x magnification to get the difference in depth of field and more resolution.

2. Moving stage

In this project, a secondhand ASI stage is used due to its accuracy and the minimum moving distance of 10 nanometers. The ASI stage consists of 3 stages, two moving stages (X and Y axis) and one stationary stage(Holding base). Due to the inverted lens microscope, we have to invert the stage upside down with the upper-level stage as stationary and the lower-level stage as a free-moving stage.

3. Imaging system

DSLR cameras provide a high-quality image that is renowned for its high resolution, dynamic range, and color accuracy. The large sensor size and advanced optic allow us to capture the intricate detail on the glass slide. Furthermore, a DSLR camera is easily accessible and is within a reachable cost for the small laboratory. In this project, Canon i3 Reble is used due to the affordable cost and its big sensor.

4. 3D printing part

Some of the parts needed to be customized and print out by a 3D printer. As the ASI stage does not have a slide holder, 3D printing the holder adapter which can attach to the ASI state is more convenient. The slide holder will have two parts which are the slide placer holder, which will attach to the moving stage of the ASI stage, and the slide holder, the part that will hold and lock the slide and be placed on the slide placer holder. The camera mount is also 3D printed due to the specification of the focus length of the optical lens, the height of the camera to the microscope camera mount has to be specified.

The part was built in a CAD program by, an Autodesk inventor professional, and printed in form lab resin printer to maximize the resolution of the printed.

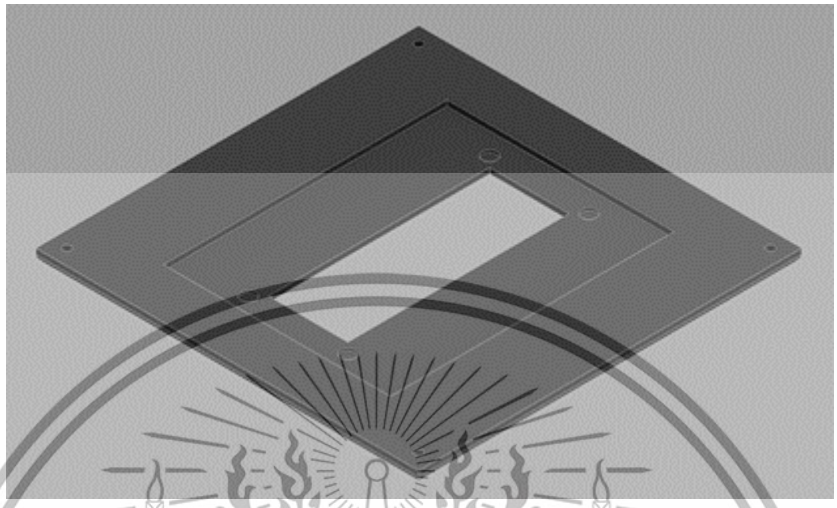

Figure 37 3D design of slide holder placer.

As the stage is inverted, the custom 130 x 130mm square placer holder has to be attached to the free-moving stage which is under the stage. In Figure 37, the rectangle gap in the middle of the holder is to insert the slide placer. The 1 x 5 mm diameter well is made to place the magnetic beads to lock the slide placer in place without manual adjustment.

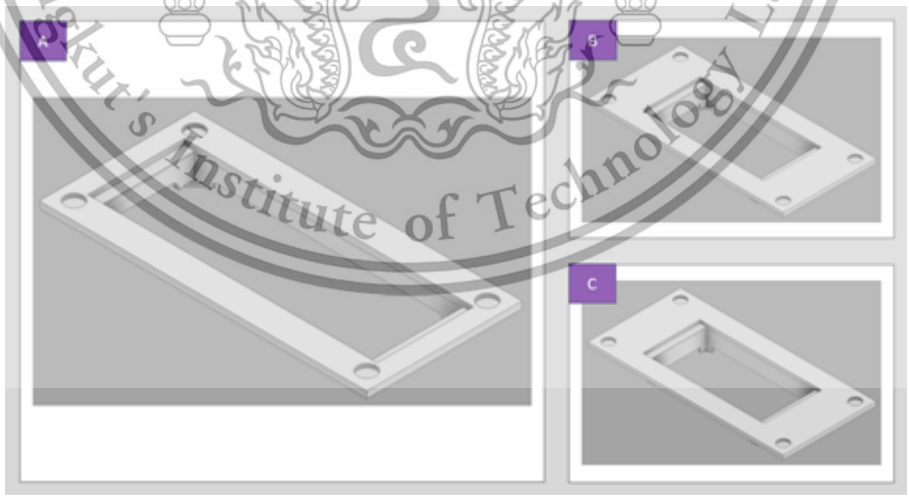

Figure 38 (A) 75 x 25mm glass slide holder, (B) 50 x 25mm Quartz slide holder, (C) Paraffin tissue block holder

The glass slide will not be placed directly to the slide holder and then will be placed on the slide holder placer. To fulfill the varieties of slide shapes and paraffin blocks, three different slide placers were designed as in Figure 38 (A)(B)(C). The holder must place on the placer holder the slide holder was extruded down below the minimum level of the stage to make the slide closest to the lens with no collision with other parts.

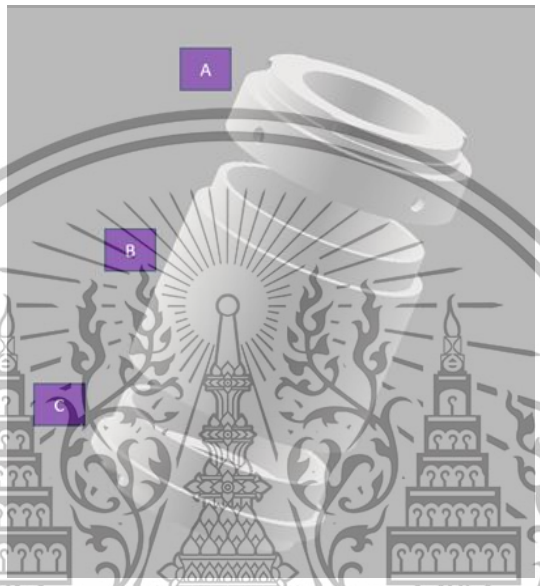

Figure 39 Camera holder (A) Canon EOS C mount (B) 42.45 mm extension (C)base mount to the microscope

The camera holder has 39 parts consisting of a Base mount to the microscope, a Canon EOS C mount, and 42.45 mm extension these will assemble together and form one piece of the camera mount. The purpose of the 42.45mm extension is to set the camera at the optimum focus point of both optical lenses. The design allows the user to tune the orientation of the camera.

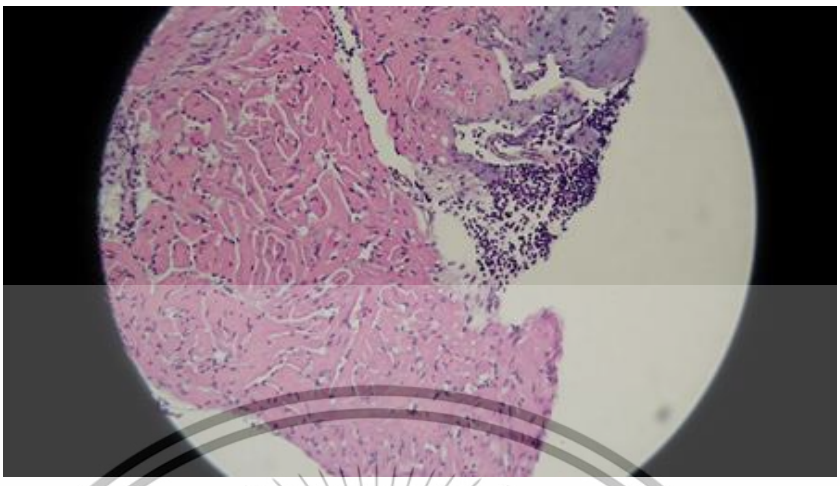

Figure 40 Raw single shot of Zebrafish tissue taken with a 40x optical lens.

Figure 40 shows the full rectangle canvas dimension of the camera is 1280 x 720 pixels which is more than the optical circular area. The black field can be observed on the side of the image, this will be the problem in the process of image stitching.

Figure 41 Raw single shot of Zebrafish tissue taken with a 40x optical lens.

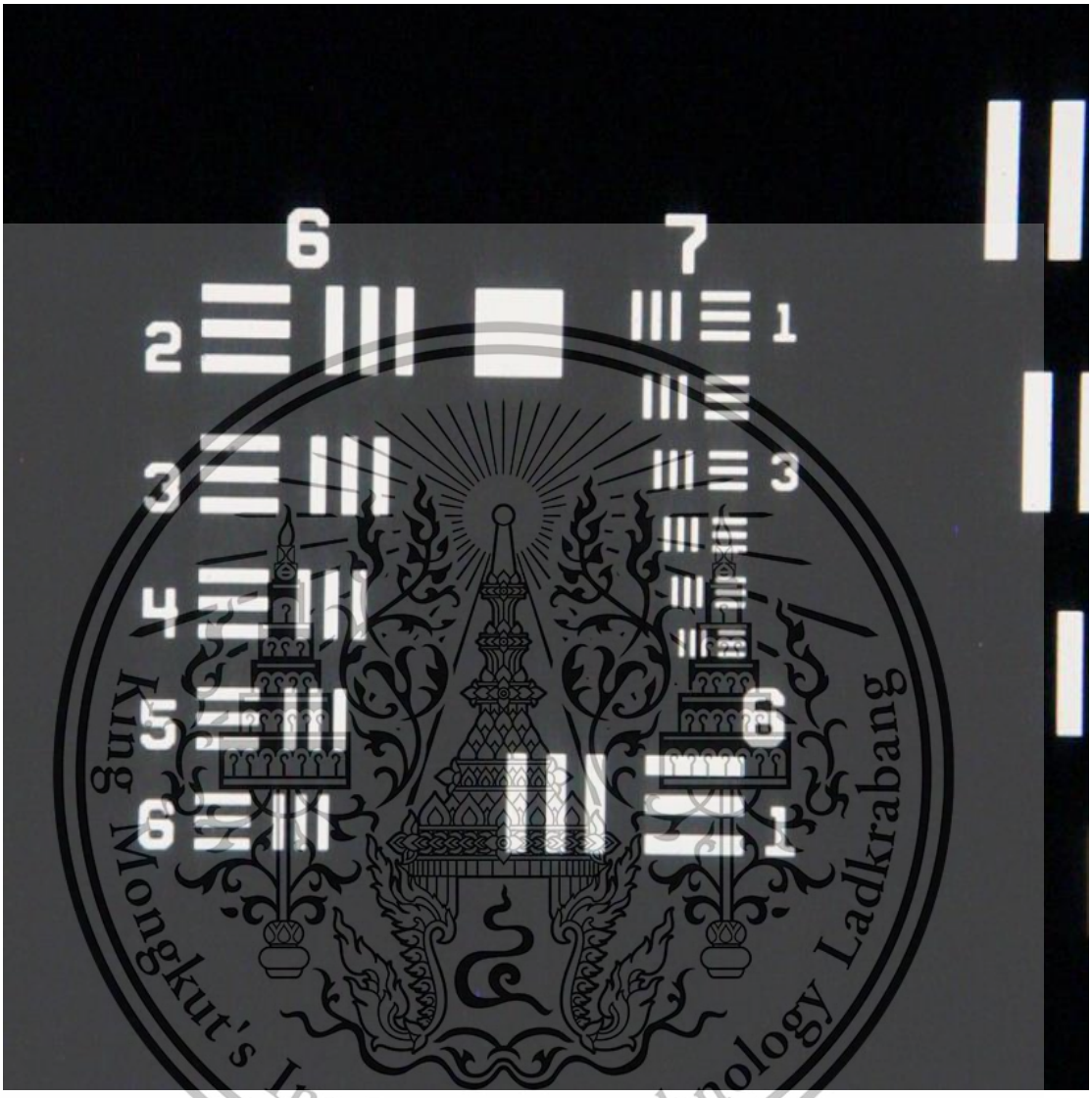

Figure 42 Resolution Calibration by 1951 USAF Target with a 40x optical lens.

Calibrating microscopic optical resolution by using 1951 USAF resolution test device, reading group number and element number is equal to 6. from the result, the resolution can be calculated by using resolution target formula

$$Resolution = 2(Group\ number + \frac{Element\ number-1}{6})$$

hence resolution is equal to 114.0 ip/mm

After using the program to crop the image into a square 700 x 700 pixels which is 352 x 352 um. This square canvas helps image stitching to be more accurate. To calculate the field of the canvas to micron is using the movement of the ASI stage which

is 0.1 um, by capturing the canvas image and then moving the canvas in one direction till the capturing image is out of the field of view. By counting the times of movement of the ASI stage we will know the dimension of the canvas.

3.2.2 Whole slide imaging software

1. Communication software

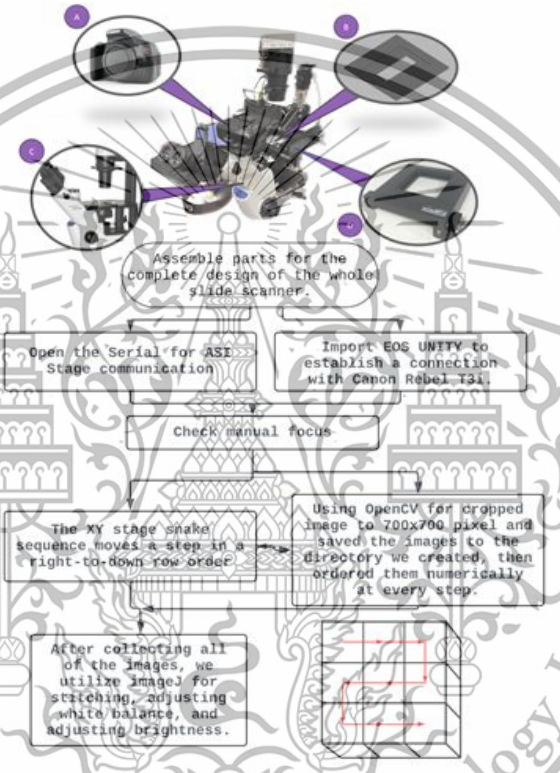

Figure 43 Communication flow of our whole slide imaging software.

After assembling the Whole Slide Scanner and connecting with RS-232 to USB cable for control ASI stage and USB Cable type A to mini 5-Pin type B for imaging, install the EOS UNITY to establish a connection with the Canon Rebel T3i. Python is the primary language used for integration, using PySerial to send the command to the ASI stage and OpenCV for imaging. Utilize the fine focus knob to sharpen the focus quality of the image after it has been brought into focus with the coarse focus knob. then moving the XY stage snake sequence in a right-to-down row order while capturing a single image in each step size movement. Calculate step size by our canvas size is

352x352 um so step size for 50% overlapping is 146 um/step and saving images to the directory we created, sorted numerically

2. Image Processing Using ImageJ

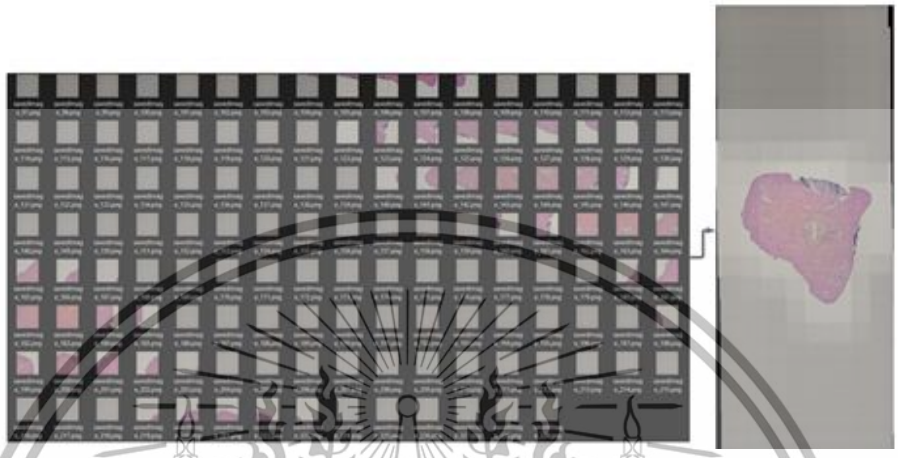

Figure 44 Prepare images for stitching into one image of the whole area

Utilize ImageJ to stitch images using function grid/collection stitching sort by snake sequence movement in a right-to-down row order and 50% tile overlap, 0.30 regression threshold, 2.50 max/avg displacement threshold and 3.50 absolute displacement threshold. Next, We crop the image only of the cell's existing area and then select the ROI regions to adjust the white balance by analyzing the histogram of RGB color in the space area.

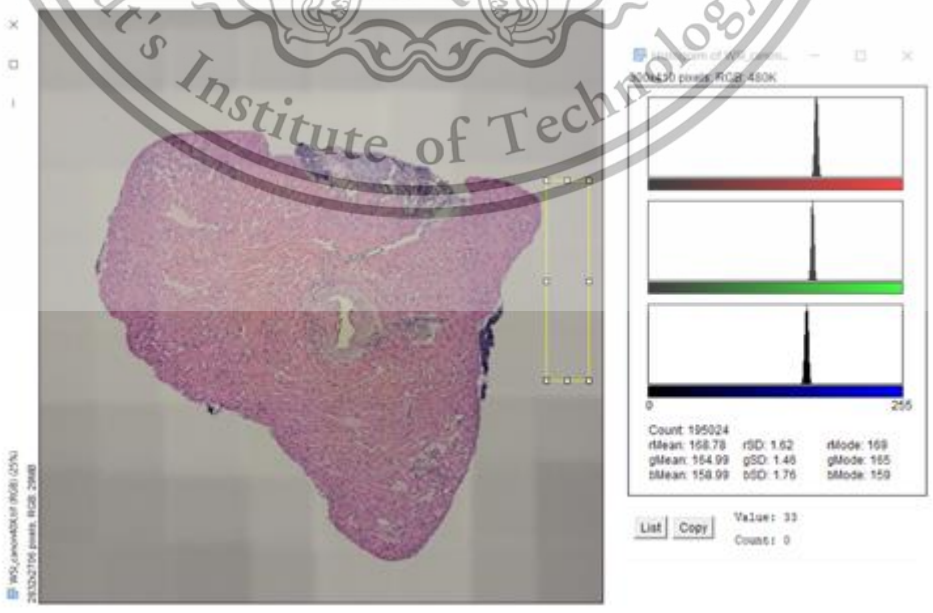

Figure 45 Histogram of Selected ROI section of Stitched RGB image.

The white regions in stitched images should be neutral grey, therefore, the values of RGB channels in these regions shown by the histogram should all have similar grey values. To do so, deselect the ROI regions and adjust the color balance of the RGB channel. When the values approach similarity, a state of white balance is attained, which is essentially the process executed by camera software during a white balance correction.

3.3 Interesting Problems

The main purpose of the whole slide scanner project is to develop high performance whole slide scanners with a cost-efficient system. To solve the problem that the current whole slide scanner has a high price.

3.4 Proposed Solution

Commercial whole slide scanners consist of components. Developing a whole slide scanner, we can substitute components with cheaper components and develop software to reduce the cost of the whole slide scanner. The substituted components include a DSLR camera instead of camera, ASI state instead of slide holder.

3.5 Summary

This chapter described the requirement and design of the whole slide scanner that consist of two major parts including the whole slide scanner system and whole slide scanner software. Whole slide scanner project will solve the high price of the whole slide scanner problem by using cheaper components and developed software to develop high performance whole slide scanners with a cost efficient system.

Development of the whole slide scanner is covered in further detail in Chapter 4 which describes the implementation of the whole slide scanner.

CHAPTER 4
EXPERIMENTAL RESULT AND DISCUSSION

4.1 Introduction

In order to create a whole slide scanner, we break down the working process into two parts including hardware and software parts. For the hardware part we assemble a DSLR camera, ASI stage, and microscope together by utilizing connector parts from the 3D printing method. Next, For the software part we communicate between the DSLR camera and ASI stage interfacing by python to form a whole scanned slide image sequence collecting system. Then we stitch the image sequences to an image of the whole scanned slide image by utilizing ImageJ. the result of two parts of the working process shown below.

4.2 Result and Discussion

4.2.1 3D PRINTING PARTS AND STRUCTURE

Though the 3D design from the CAD program is perfectly accurate in dimension and parameter, the 3D printer still has mechanical flaws which lead to the value of the tolerance that occurs during printing resulting in errors in the dimension of the printed part. In pathology imaging dealing with the microscopy parameter, even a small error can be significant, for example, the flatness of the slide placer and holder can lead to a tilted slide causing an out-of-focus section in the image. Using an iron 3D printer or CNC machine to produce the custom part for higher accuracy in the dimension of the part is recommended.

4.2.2 WHOLE SLIDE IMAGING SOFTWARE

Utilizing an integrated system of a DSLR camera with a 40X magnification objective lens can produce high-resolution images of Zebrafish tissue samples. The cellular structures, which are intricate and often challenging to discern, were distinctly visible. This indicates the enhanced imaging capabilities of the system after stitching the image

using function grid/collection stitching in ImageJ sort by snake sequence movement in a right-to-down row order same as ASI stage movement and 50% tile overlap, 0.30 regression threshold, 2.50 max/avg displacement threshold and 3.50 absolute displacement threshold.

The post-imaging processing of the raw images was conducted using ImageJ software. This method significantly enhanced the brightness and white balance of the images, emphasizing the importance of post-processing for achieving optimal image quality. The RGB channel values of each channel are quite similar meaning that the white balance has already been adjusted

However, there are some dark gray regions of indistinct tissue. This may be caused by the DSLR camera's sensor, which automatically adjusts exposure. Even in the flat tissue sample, there are some out-of-focus regions caused by vibration while we are scanning.

Figure 46 Image of Zebra fish tissue sample in 40X magnification of our whole slide scanner system

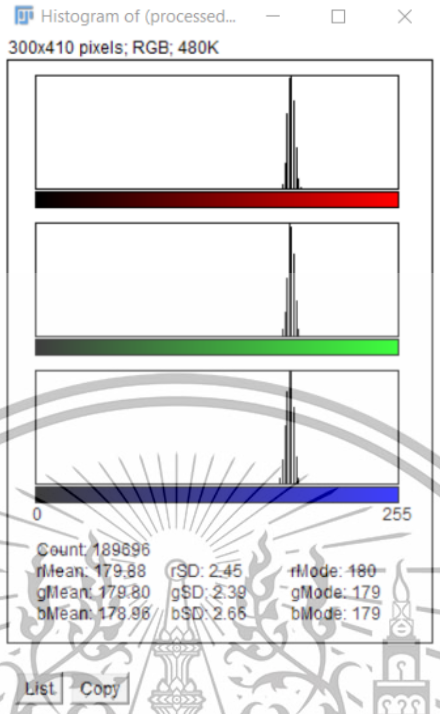

Figure 47 Histogram of white space region of Zebra fish's tissue Image in 40X magnification.

4.3 Summary

The custom-made whole slide scanners' products yielded promising results, showing potential advancements in the digital pathology field. The integration of a DSLR camera with a 40X objective lens, including the modification of 3D printing and image post-processing, offers a promising avenue for future research and development.

CHAPTER 5
CONCLUSION

5.1 Introduction

Integrating a whole slide scanner helps pathologists to save time for scanning, storage samples and to save the cost of the whole slide scanners market. We created our own whole slide scanner by utilizing a DSLR camera setup and ASI stages control system. From the working process and result, We have summarized the project as follows.

5.2 Summary

Examination specimens in pathology using microscopes consume time for scanning the sample. Storing samples in the form of slide waste storage space and difficult to later use. Although presently the whole slide scanner is developed to solve this problem. However, commercial whole slide scanners in the market come with a high price. The Authors aim to develop high performance whole slide scanners with a cost efficient system and perform whole slide scanning in pathology to solve those problems.

Developing high performance whole slide scanners with a cost efficient system and performing whole slide scanning in pathology require knowledge in various fields. The required knowledge for developing the setup and system of the whole slide scanner is the principle of microscope, DSLR camera , 1951 USAF, ASI stage, Python, ImageJ, and commercial whole slide scanner. Performing whole slide scanning requires knowledge of microscope and slide preparation.

Design of the whole slide scanner consists of a whole slide scanner system and whole slide imaging software. The whole slide scanner system is an assembly of microscope, moving stage imaging system and connected together by utilizing connector parts from the 3D printing method. and improve accuracy by utilizing 1951 USAF. The whole slide imaging software communicates with the DSLR camera and ASI stage to collect the whole scanned slide image sequence collecting system. then

stitch the image sequences to an image of the whole scanned slide image by utilizing ImageJ.

The result of performing the experiment according to the methodology is that the out-of-focus section occurs in the image, since mechanical flaws in the 3D printer lead to the value of the tolerance that occurs during printing, Although the 3D design from the CAD program is perfectly accurate in dimension and parameter. Utilizing an integrated system of a DSLR camera with a 40X magnification objective lens can produce high-resolution images of Zebrafish tissue samples. The post-imaging processing of the raw images by utilizing ImageJ significantly enhanced the brightness and white balance of the images, the RGB channel values of each channel are quite similar. However, there are some dark gray regions of indistinct tissue. This may be caused by the DSLR camera's sensor, which automatically adjusts exposure.

5.3 Conclusions

The aim of this project was to develop high performance whole slide scanners with a cost efficient system and perform whole slide scanning in pathology to help pathologists to save time for scanning, storage samples and to save the cost of the whole slide scanners market. We then designed and implemented a system that could perform whole slide scanning composed of a whole slide scanner system and whole slide imaging software which methodology states in chapter 3.

The result of performing experiments is that we can integrate the whole slide scanner system by utilizing a DSLR camera, microscope, ASI stage, and 3D printing connector parts. Utilizing The System with 40X magnification objective lens to Imagining the scanned whole slide image. It can produce high-resolution images of Zebrafish tissue samples. The RGB channel values of each channel are quite similar. However, there are some dark gray regions of indistinct tissue and out-of-focus sections slightly occur in the image.

5.4 Suggestion

To improve the image quality of whole slide scanned image. we can increase a resolution of each image before stitching by utilize a SRGAN (Super Resolution Generative Adversarial Networks) model to train a image into higher resolution. Alternatively, you can also increase the resolution of camera for better result. To reduce out-of-focus regions, vibration isolator is the key for decrease of vibrate in our system that can make a image out-of-focus.

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