Technical University of Crete
School of Electrical & Computer Engineering
Master Thesis

Development of a Robotic, Spectral Microscope for the Automated Microanalysis of Massive Sample Populations

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Abstract

With the rapid advancement of microscopy, new digital imaging techniques continuously emerge, enriching the visualization of diagnostic features. The existing state-of-the-art machinery, which prioritizes diagnostic performance, is noticeably deficient in terms of automation. As a result, the examination of large volumes of samples at microscopic magnification levels becomes impractical and time-consuming in real-world environments. Consequently, evaluations are limited on randomly selected representative areas, leading to subjectivity and significant discrepancies among observers. The convergence of mechatronics technologies, fast-multichannel imaging sensors, and powerful computational systems offers innovative opportunities for automated multimodal microscopy, thereby achieving enhanced diagnostic performance. This thesis focuses on developing an integrated multimodal high-throughput screening microscope that not only enhances spatial resolution but also combines various analysis and imaging techniques into a single instrument. By harnessing mechatronics technologies and advanced imaging capabilities, the proposed microscope revolutionizes microscopy and analysis, significantly reducing examination time while increasing diagnostic accuracy.

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Chapter 1

Introduction

Brief Description In the realm of high throughput screening microscopy for forensic sciences, our innate curiosity to comprehend the microcosmos and macrocosmos has driven us to employ specialized machinery for advanced observation. Throughout history, this drive has expanded in all directions to meet our ever-growing quest for knowledge and understanding, resulting in the development of specialized systems. In the context of forensics sciences and trace analysis, high throughput screening microscopy plays a crucial role in employing various observation techniques to capture valuable imaging data. The progress of examination and diagnosis in this field relies on continuous technological advancements that enhance our capabilities in forensic analysis and investigation.

High throughput screening microscopy is essential in the analysis of forensic evidence, offering various observation techniques for capturing valuable imaging data. This advanced microscopy enables detailed examination of traces such as hair, blood, sand, and glass fibers, aiding in accurate diagnosis and forensic analysis. The integration of polarized light microscopy, fluorescence microscopy, and scanning electron microscopy reveals unique features and properties of the trace materials. These advancements play a crucial role in unraveling complex criminal cases and ensuring justice is served.

Diagnostic procedures in forensics involve the manual examination of numerous samples, requiring meticulous preparation and expert observation. The integration of computer-based image analysis algorithms holds great promise in enhancing digital pathology for forensic analysis, guiding treatment decisions, and predicting outcomes. Additionally, the integration of complementary imaging techniques enables a comprehensive understanding of complex structural and biochemical transformations. This multidimensional approach empowers researchers in materials science, bioengineering, and forensics to uncover critical insights.

Computer-based image analysis has made significant strides in commercial diagnostic sys-

tems. However, continuous progress in image analysis algorithms is crucial to fully unlock the potential of digital pathology in medical research and patient care. Looking ahead, the field of forensics image analysis is anticipated to surpass its current role of streamlining diagnostic workflows and reducing interobserver variability. It is poised to play a more active role in aiding diagnosis, guiding treatment decisions, and even predicting patient outcomes and therapeutic responses. The development of advanced image analysis algorithms holds great promise for advancing the field of forensics sciences and its application in delivering accurate and personalized forensic analysis.

Advanced microscopy encompasses a diverse range of techniques that offer exceptional resolution at the submicron to nanometer scale. This capability enables the detailed examination of biomolecular structures, interactions, and catalytic mechanisms at the fundamental level of proteins, lipids, amino acids, glycans, and other biomolecules. The advantages offered by advanced microscopy techniques make them particularly valuable for forensics imaging. While these techniques find extensive use in biological research and medical applications, their full potential can be realized by combining different modalities. However, the integration of diverse imaging techniques presents challenges, primarily due to the varied hardware requirements. To meet the demand for rapid advancement in microscopy, there is a need to develop technologies that focus on integrating multiple imaging modalities and exploring their applications.

Automation has emerged as a transformative solution in microscopy, streamlining labor-intensive tasks and enabling efficient data acquisition and analysis. By incorporating full motorization, digital imaging, and customized lighting, automated microscopy systems offer exceptional accuracy, repeatability, and advanced features such as data management. These systems revolutionize microscopy, enhancing productivity and scientific advancement.

In summary, the objective of these automated microscopy systems is to provide specialists with a comprehensive tool that integrates various techniques, offering ease of use, speed, accuracy, repeatability, and advanced functionality. By leveraging state-of-the-art technologies, these systems optimize workflow and empower researchers and professionals in their analysis, interpretation, and decision-making processes. Ultimately, these advancements aim to transform microscopy, driving scientific progress and enabling new levels of productivity and efficiency.

Purpose of the Design Our research introduces a cutting-edge high-end digital microscope designed to facilitate rapid and reliable acquisition of multi-modal data. The primary objective of this study is to achieve comprehensive control and automation of the mechanical and optical components that are frequently manipulated by specialists. Through this approach, our aim is to develop an advanced microscope capable of efficiently scanning and analyzing samples using a wide range of imaging techniques, while minimizing the need for manual intervention. By implementing this system, specialists will be empowered to streamline their workflow, improve accuracy and precision, and optimize the overall analysis process.

The integration of automation in key components of a microscope is of paramount importance, offering numerous advantages that enhance the imaging experience compared to traditional manual operation. By automating these components, operators can benefit from a more comfortable and relaxed imaging process. Moreover, the skill requirements for operators are significantly reduced, as the system can be easily configured and controlled through intuitive software, providing a simplified interface for streamlined usage. This automation not only improves the repeatability of image capturing and measurements but also ensures consistent results across operators with varying levels of expertise. Furthermore, by minimizing sample contact, automation enhances the reliability of analysis while reducing associated risks. The overall integration of automation in microscopy significantly enhances efficiency, accuracy, and safety in sample analysis, making it an indispensable tool in modern scientific research and analysis.

Furthermore, automation in microscopy allows for the simultaneous showcase of multiple techniques, eliminating the laborious and time-consuming task of switching between techniques. This system enhances both comfort and speed while ensuring accurate data analysis and image processing, revolutionizing the observation and analysis of samples. The implementation of an automatic system has the potential to improve diagnostics by reducing analysis time. By automating various processes, the system streamlines workflow and enables faster and more efficient sample examination, leading to increased productivity and more effective decision-making in diagnostic and analytical processes.

Structure of the Thesis In the subsequent chapters of this thesis, a comprehensive account of the research and work undertaken is presented. The initial two chapters delve into the technology of microscopy and the associated research, while the ensuing chapters focus on the detailed exposition of our microscope's final outcome and its implementation.

Chapter 1 provides an overview of the current state of microscopy and elucidates the rationale behind the selection of this thesis topic. It offers a concise depiction of the purpose and functionality of our microscope.

Chapter 2 entails a meticulous examination of the research conducted on microscopy and its various techniques. It elucidates the essential terminologies employed in the field of microscopy. The chapter commences with a succinct historical perspective on microscopy and subsequently presents a comprehensive survey of the different microscopy techniques explored. Furthermore, it encompasses an examination of the state-of-the-art advancements in microscopy and its usage in forensics sciences.

Chapter 3 critically evaluates the proposed system design of our microscope. The primary objective is the development of a high end digital microscope. The analysis encompasses a comprehensive review of the hardware and firmware segmentation pertinent to the microscope's development.

Chapter 4, which represents the most extensive section, intricately describes the technical implementation of the microscope's system. It elucidates the high-level requirements and the proposed designs associated with each methodology. The chapter commences by focusing on the design of a modular Stepper Motor Driver, a vital and indispensable component of automated microscopes. Subsequently, it details the fundamental aspects and indispensable procedures pertaining to automated illumination modules and a sophisticated power manager. The chapter concludes with a comprehensive presentation of the microscope developed throughout the course of this research.

Chapter 5 summarizes the results of the research. The chapter ends with suggestions and notes for future work.

These chapters collectively illuminate the intricate aspects of our microscope's design, development, and technical implementation, offering a comprehensive understanding of its features, capabilities, and potential applications.

Acknowledgement *Throughout the course of this research endeavor, I encountered numerous challenges and obstacles that required the invaluable assistance of my colleagues and mentors. I am deeply grateful for the unwavering support and guidance provided by my supervisor, Costas Balas. His profound insights and supervision proved invaluable in navigating the complexities of this thesis, particularly during critical decision-making processes. Furthermore, I extend my heartfelt appreciation to my co-supervisors, Matthias Bucher and Vasilis Samoladas, for their invaluable contributions, thought-provoking discussions, and meticulous review of my research report, which resulted in valuable feedback and improvements. Their assistance has been instrumental in shaping the outcome of this research.*

Significant contributions to my research were made by the engineers of Spectricon and the researchers of the Electronics Laboratory at the Technical University of Crete. I would like to express my sincere gratitude to Thanasis Tsapras, Christos Rossos, and Nikos Kaminakis for their dedicated time and effort in designing and constructing the optic and hardware components of my research on a full scale. Their expertise and commitment have been invaluable in bringing the project to fruition. Furthermore, I extend my heartfelt thanks to Christos Tsiaousis, Babis Boras and all the researchers with whom I closely collaborated throughout this endeavor. Their contributions to the software design and their extensive knowledge were essential in achieving the desired outcomes. I am grateful for their invaluable insights and efforts in evaluating each stage of my research results.

In addition, I would like to express my heartfelt gratitude to my dear friends Theofilos Christodoulou, Yiannis Kritikos and Fotis Giariskanis for their valuable time and contributions throughout the construction of this report. Their ideas, discussions, and support have been instrumental in shaping the final outcome of this research. Last but not least, I want to extend my sincere appreciation to my family for their unwavering patience and unwavering support during this intensive working period. Their understanding and encouragement have been a constant source of motivation and strength throughout my journey.

The successful completion of this research project would not have been possible without the invaluable contributions of the individuals mentioned above. I am deeply grateful for their presence in my life and the profound impact they have had on this endeavor. Their support, guidance, and collaboration have been pivotal in shaping the outcome of this study, and I am truly fortunate to have had the opportunity to work with such exceptional individuals.

Chapter 2

Background

2.1 Microscopy Techniques

Microscopy, as an integral domain of observational technology, employs light and optics to augment the observer's aptitude in comprehending the structural intricacies of samples. Light, serving as the principal medium for energy transmission, facilitates both macroscopic phenomena (within the universe) and microscopic phenomena (within microscopy). Light, as a multifaceted phenomenon, is conventionally expounded through a rudimentary model grounded in the concepts of rays and wavefronts, encompassing a broader category of wave-like phenomena known as electromagnetic radiation. Notably, the visible light range (400 to 700 nanometers) perceptible to the human eye constitutes a minute fraction of the encompassing electromagnetic radiation spectrum. The remaining segments of the electromagnetic spectrum elude human visual perception.

During its nascent stages, microscopy heavily depended on oil lamps and natural sunlight as external light sources to illuminate the early iterations of microscopes, which, despite their rudimentary nature, demonstrated remarkable precision. Regrettably, these approaches failed to consistently deliver reliable illumination, often resulting in excessive field illumination that surpassed the numerical aperture of the objective. As a consequence, issues such as glare and flooding were frequently encountered.

The progression of light sources and optical components employed in specimen observation has substantially improved the quality of imaging and, consequently, the accuracy of assessment. In the realm of modern microscopy, a diverse range of valuable modalities has emerged, catering to the specific requirements of life science research. These modalities have been meticulously designed to facilitate contrast enhancement, enabling superior observation and facilitating the acquisition of high-quality photomicrographs of specimens.

Within the realm of biomedical engineering research, novel microscopy technologies continually emerge alongside the advancement of observation techniques. Figure [2.1] showcases a diagram illustrating the recent developments in microscopy techniques, emphasizing their respective capabilities in terms of Field of View and Spatial Resolution.

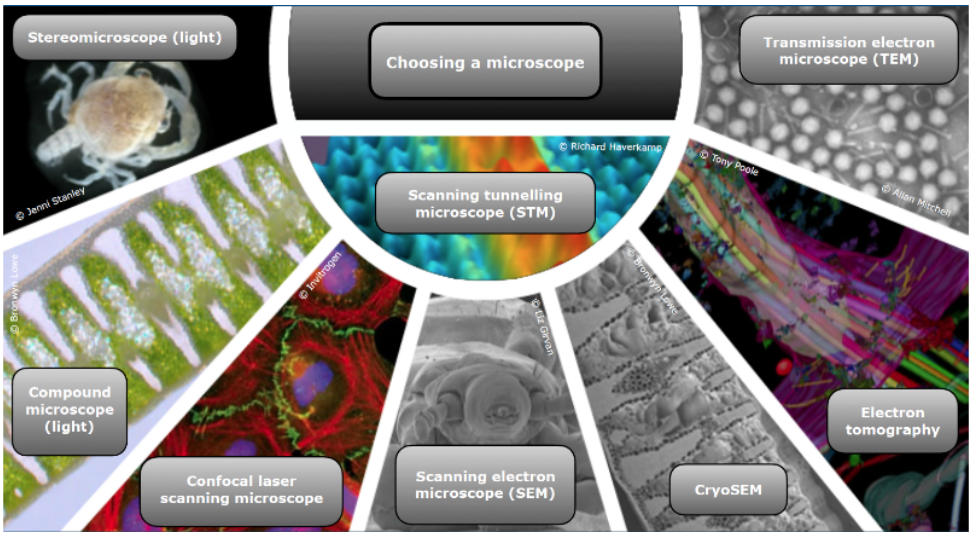


Figure 2.1: Microscopy Techniques
Source: Science Learn - Light Microscopes

Figure [2.1] presents a classification of microscopy techniques based on their illumination and observation methodologies. This research endeavors to explore two extensively employed techniques and examine the cutting-edge optimization methods employed in each. Positioned in the top right quadrant of the diagram is Widefield Microscopy (WF), a fundamental modality widely employed in contemporary microscopy. Advancing towards higher resolution imaging, we encounter Confocal Microscopy, an intricate optical technique that enhances optical resolution and contrast in micrographs by employing a spatial pinhole to eliminate out-of-focus light during image formation [2].
Widefield microscopy encompasses techniques wherein the complete specimen of interest is illuminated by the light source, and the resulting image is observed either directly by an observer or recorded by a camera. This section focuses on the microscope configurations employed for widefield (WF) imaging, including an examination of the light paths involved and the challenges posed by out-of-focus light. Additionally, advanced WF techniques such as WF Super-Resolution will be explored to provide a comprehensive understanding of the topic.

2.1.1 Brightfield Microscopy

The fundamental variant of widefield microscopy is known as 'brightfield microscopy,' wherein the complete specimen is illuminated by white light, either from above (in an inverted configuration) or below (in a standard upright microscope), as illustrated in Figure [2.2]. The chosen microscope configuration significantly impacts the characterization and investigation of the specimen. Upright microscopes are typically employed for applications involving fixed samples mounted on glass slides. Conversely, inverted microscopes have been specifically designed for live-cell imaging, as these cells are commonly cultivated in liquid solutions. The configuration featuring the objective below and the condenser above the specimen ensures the optimal proximity between the objective and the specimen, thereby facilitating precise observations.

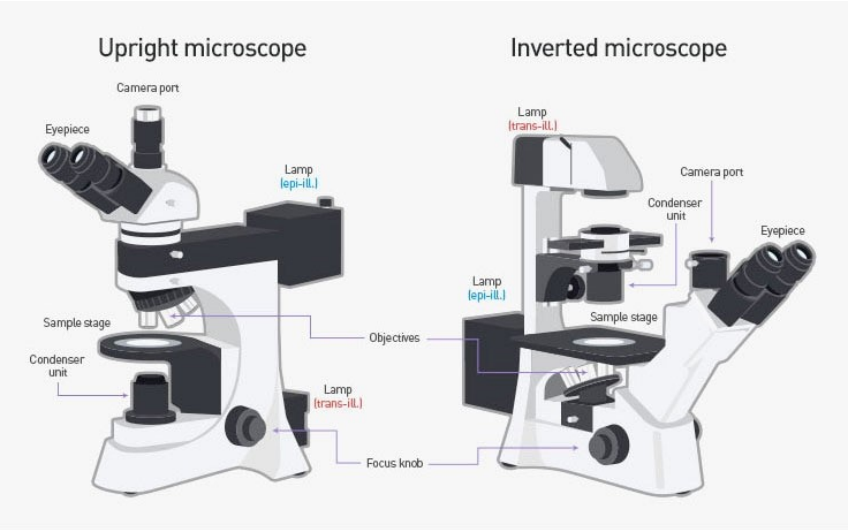


Figure 2.2: Widefield Microscopy Setups
Source: photometrics.com

The construction of a microscope utilizing the brightfield microscopy technique is reliant upon several essential components. These components are as follows:

- **Light Source:** Positioned beneath the sample, the light source provides trans-illumination, which then propagates through the condenser and objective lenses. Broadband sources such as quartz halogen bulbs or LEDs are typically employed for this purpose.
- **Condenser Lens:** The condenser lens collects the trans-illuminated light and focuses it onto the sample, ensuring optimal illumination.
- **Objective Lens:** Functioning as a crucial component, the objective lens collects the light that passes through the sample, magnifying the details of the specimen.
- **Eyepiece/Camera:** The resulting image can be viewed directly through the eyepiece or recorded using a camera for further analysis and documentation.

The brightfield microscopy technique exhibits certain limitations that must be acknowledged. These limitations encompass minimal contrast or the presence of blurriness, particularly in cellular or biological samples, stemming from out-of-focus material. Additionally, the optical resolution achievable through brightfield microscopy is limited due to inherent constraints of light. Lastly, naturally colorless and transparent samples pose a challenge as they are not adequately visualized and often necessitate staining prior to observation.

The resolution of a Brightfield microscope can be enhanced through several straightforward techniques. Firstly, adjusting the amount of light from the source can be achieved by manipulating the iris diaphragm, either reducing or increasing the intensity as required. Additionally, incorporating a colored filter, often in the shade of blue, or a polarizing filter onto the light source aids in highlighting features that may not be discernible under white light illumination. Furthermore, employing an oil-immersion objective lens in conjunction with a specialized immersion oil applied to a glass cover over the specimen contributes to improved resolution.

Nevertheless, the inherent simplicity of the brightfield technique offers significant advantages when initially imaging an unfamiliar sample. In addition, optical microscopy encompasses various other valuable applications, including darkfield illumination, phase contrast, fluorescence, and differential interference contrast [3]. These alternative techniques further expand the capabilities of microscopy, allowing for enhanced visualization and analysis in diverse research domains.

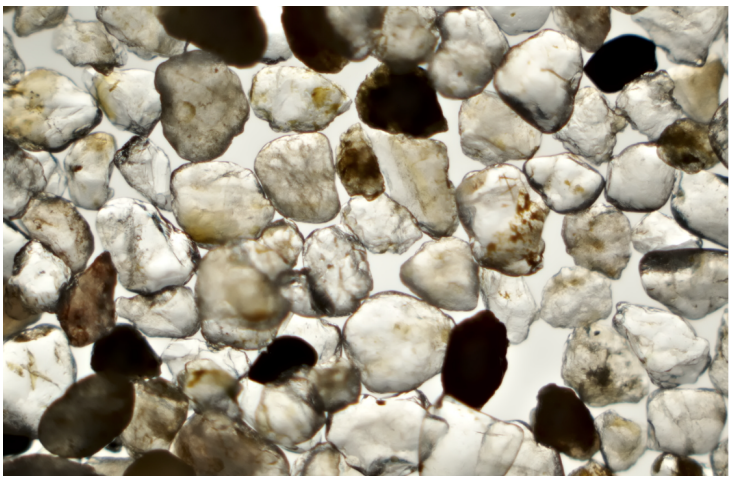


Figure 2.3: Sand from a beach captured in brightfield illumination
Source: microscopyofnature.com

A characteristic feature of a brightfield illumination image is the presence of a dark sample against a white background, wherein the degree of darkness corresponds to the extent of light absorption by different regions within the sample, as depicted in Figure [2.3].

2.1.2 Dark-field Microscopy

Within the realm of optical microscopy, dark-field microscopy is a technique employed to augment contrast in unstained samples. It encompasses various microscopy methods that selectively exclude the unscattered light beam from the resulting image. Consequently, the surrounding field encompassing the specimen, known as the background, typically appears darkened.

Dark-field microscopy proves to be a straightforward yet highly effective technique, particularly well-suited for applications involving live and unstained biological samples. The impressive quality of the resulting images is notable, especially considering the simplicity of the setup, as illustrated in Figure [2.4].

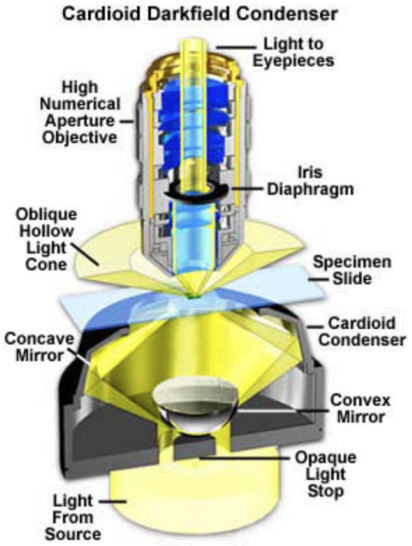


Figure 2.4: Darkfield Microscope Illumination Setup
Source: olympus-lifescience.com

The construction of a microscope utilizing the dark-field modality relies on several key components. These components are as follows:

- **Light Source:** The light source enters the microscope and encounters the dark field patch stop, a disc that effectively blocks light from reaching the condenser while allowing a circular ring of illumination to pass through. Broadband sources such as quartz halogen bulbs or LEDs are commonly employed for this purpose.
- **Condenser Lens:** The condenser lens collects the outer ring of illumination and concentrates it onto the sample, ensuring precise illumination.
- **Objective Lens:** Light interacts with the sample, either by transmission or scattering. The scattered light enters the objective lens, while the transmitted light is not collected by the lens. This differentiation is facilitated by the presence of a direct illumination block.

- **Eyepiece/Camera:** The resulting image can be viewed through the eyepiece or recorded using a camera for further analysis and documentation.

Dark-field microscopy exhibits certain limitations that need to be considered. The low light levels present in the final image necessitate strong illumination of the sample, which can potentially cause damage. Careful interpretation of dark-field images is essential, as common dark features observed in bright-field microscopy images may become invisible, and vice versa. Generally, the dark-field image lacks the low spatial frequencies associated with the bright-field image, resulting in a high-passed rendition of the underlying structure [4]. Conversely, a typical dark-field illumination image showcases a white or brightly illuminated specimen against a dark background, filling the entirety of the image. This is in stark contrast to a bright-field illumination image and proves beneficial for unstained specimens or situations requiring enhanced contrast. Dark-field illumination offers the advantage of preserving the vitality of unstained specimens, allowing them to remain alive and active, unlike their bright-field counterparts that require treatment. Furthermore, this technique facilitates qualitative results through live cellular analysis.

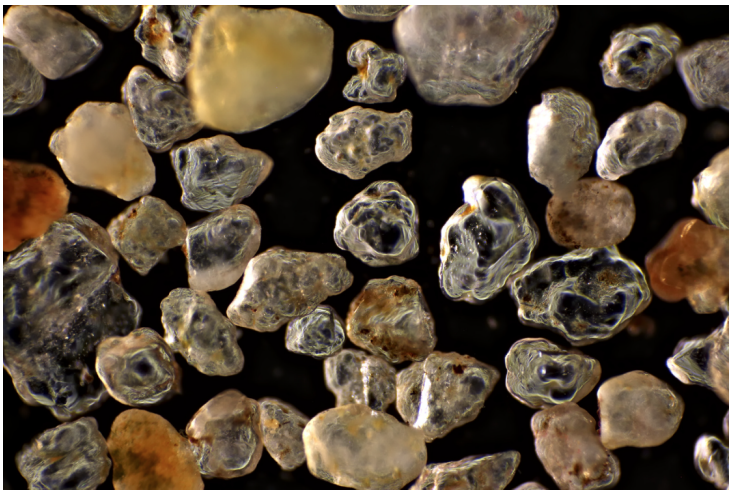


Figure 2.5: Sand from a beach captured with darkfield illumination
Source: microscopyofnature.com

Although the dark-field image may initially resemble a negative rendition of the bright-field image, it reveals distinct visual effects. In bright-field microscopy, features become apparent when a shadow is cast on the surface by incident light or when a portion of the surface exhibits reduced reflectivity, possibly due to the presence of pits or scratches. However, raised features that lack surface irregularities capable of casting shadows remain imperceptible in bright-field images. In contrast, dark-field images capture the light that reflects off the sides of such raised features, making them discernible in the resulting image, as exemplified in Figure [2.5].

2.1.3 Epi-fluorescence Microscopy

Epi-fluorescence microscopy refers to a fluorescence imaging technique wherein a single lens (objective) serves the dual purpose of illuminating the sample and collecting the emitted light. The light path begins with the illumination light emanating from the light source, passing through a filter set, and then through a dichromatic mirror. The shorter wavelength excitation light is reflected by the mirror onto the sample through the objective lens. Subsequently, the longer wavelength emitted light from the sample retraces its path through the objective lens, passes through the dichromatic mirror, and is ultimately visualized through the eyepieces and/or captured by a camera for imaging purposes [5].

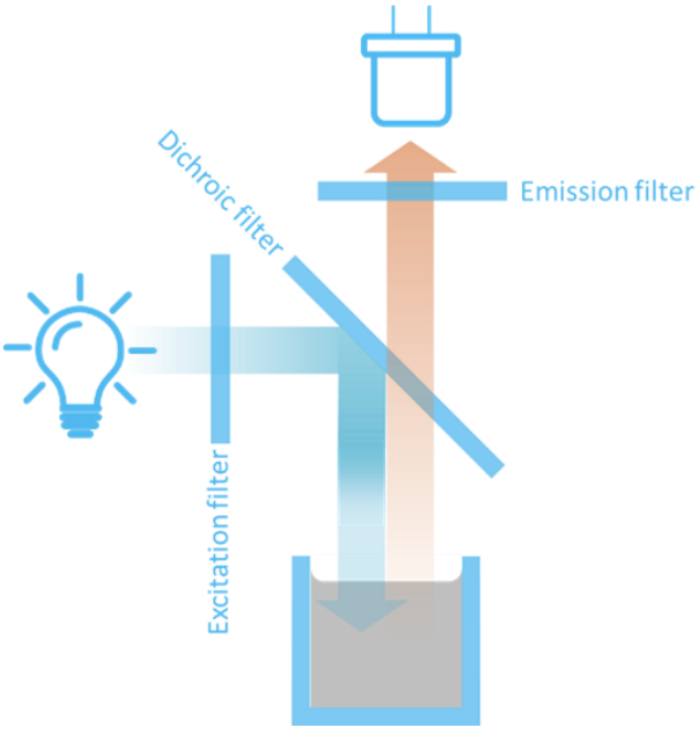


Figure 2.6: Epi-fluorescence Microscopy Setup
Source: deltaopticalthinfilm.com

Construction of a microscope using the Epi-fluorescence illumination technique, is based on these components.

- **Light Source:** Usually a xenon arc or mercury vapor lamp or more recently powerful LEDs.
- **Excitation Filter:** Narrows the wavelengths of the incoming light to only those used to excite the sample.
- **Dichroic beamsplitter or mirror:** Reflects the excitation light to the sample and simultaneously transmit only the emitted light from the sample back to the

detector.

- **Emission Filter:** Transmits only the wavelengths of the emitted light from the sample and blocks all the light passed through the excitation filter.
- **Eyepiece/Camera:** Views or records the image.

A filter set will be designed to capture the maximum excitation and emission wavelengths of a given fluorophore, but will not capture all of the fluorescence. Most modern filter sets are designed so that the excitation filter has a defined band of wavelengths that it allows through. This style of filter is referred to as a band-pass filter. Band-pass filters are normally identified by the middle-value wavelength and the width of a band. In a simple epi-fluorescence microscope setup, once the excitation light leaves the excitation filter it is reflected onto the target. The fluorophores in the target become excited and then emit light that is shifted towards the red end of the spectrum (compared to the excitation light). Not all of this emitted light will be captured by the detector—only what is allowed through the dichroic beam-splitter and also passes through the emission filter. Emission filters are usually band-pass or long-pass filters, depending on the specific fluorophore and imaging experiment. A long-pass filter may be desirable if you need all the light beyond a certain wavelength to pass through to the detector, as shown in Figure [2.7].

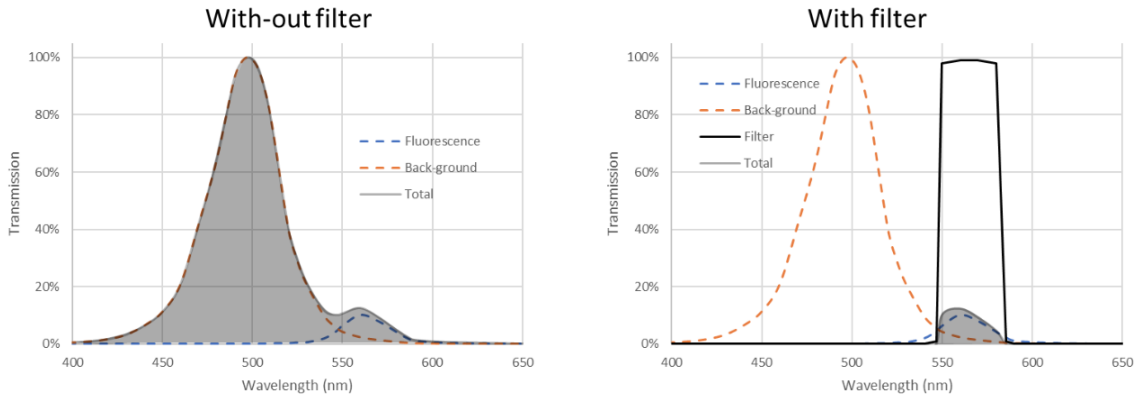
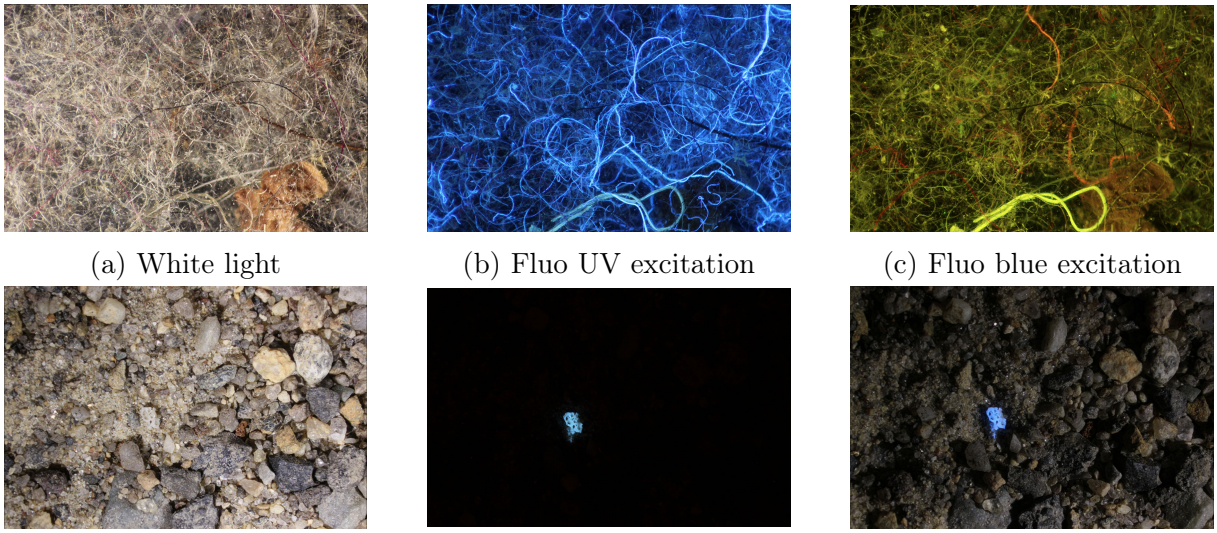


Figure 2.7: Epi-fluorescence Microscopy Spectrum
Source: deltaopticalthinfilm.com

Epi-fluorescence illumination compound microscope also have limitations based on the spatial resolution. It cannot resolve or distinguish between two objects that are less than 200 nm apart. Additionally, because the whole sample is illuminated at the same time, these setups are detecting all of the in-focus and out-of-focus light in your sample. These limitations mean that, depending on the lenses in your objectives, the system will be able to determine that two different-colored probes are present in the same cell, but not always

be able to resolve their spatial relationship to each other without a lot of controls, individual pixel analysis, and math. By understanding and working within the limitations of these systems, designers can be confident about the data and images they collect, as well as being able to fully understand the data and formulate conclusions. The use of lasers in Epi-fluorescence microscopy, narrows the excitation range to 2–3 nm which is around 10 times narrower than the range of wavelengths you get when using excitation filters, as a result we observe increase in resolution, as shown in Figure [2.8].



(a) White light (b) Fluo UV excitation (c) Fluo blue excitation

(d) White light (e) Fluo UV excitation (f) Fluo and White Light

Figure 2.8: (a,b,c) Dust (d,e,f) Small bone fragment in dirt
Source: nightsea.com

A resent study [6] explores the use of fluorescence-based methods for rapid, on-site detection and identification of body fluids in forensic investigations. By analyzing the fluorescent properties of semen, serum, urine and -saliva over time, the study identifies specific fluorescent signatures for each body fluid. The results demonstrate the potential of this non-invasive approach to improve the detection of body fluids in forensic practice, providing a robust and reliable method for body fluid identification, as shown in Figure [2.9].

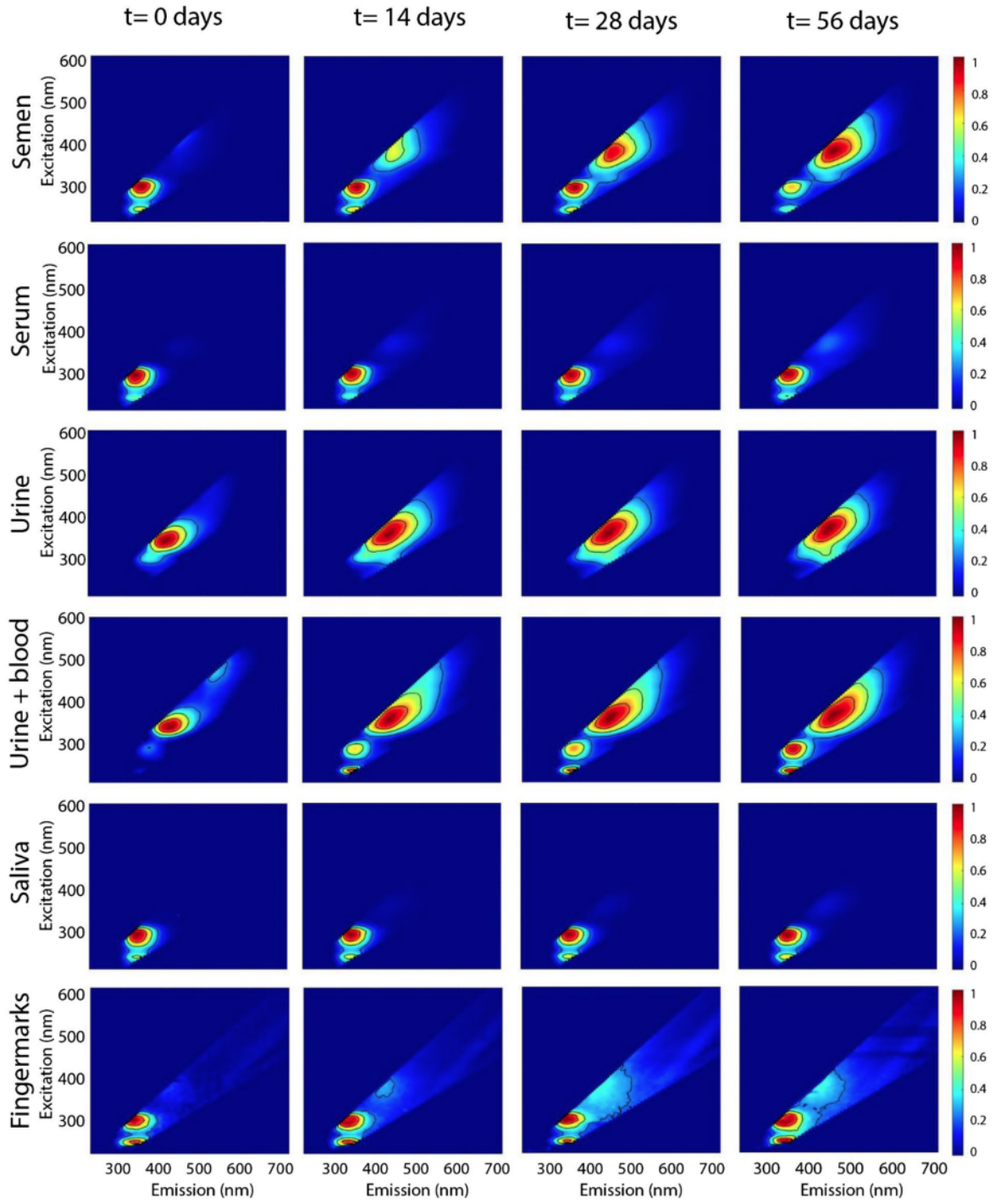


Figure 2.9: Normalized excitation and emission map of five different body fluids
Source: nature.com

2.1.4 Polarization Microscopy

Polarization microscopy is an imaging technique that utilizes the principles of polarized light to visualize and analyze birefringent materials in various scientific disciplines, including materials science, geology, biology, and forensics. This section provides an extended discussion on polarization microscopy, focusing on its technicalities, robustness, and applications in research and industry.

When polarized light interacts with an anisotropic crystal, several optical phenomena can occur. An anisotropic crystal has different refractive indices in different directions, and the interaction of light with such a crystal depends on the polarization direction of the incident light relative to the crystal's optical axis. In this setup, a polarizing light microscope (PLM) is used to study the crystal's properties [7].

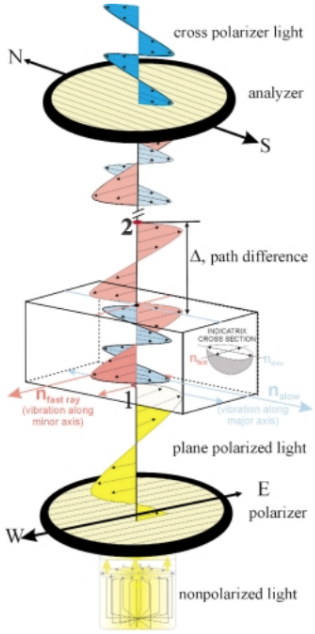


Figure 2.10: Interaction of polarized light with an anisotropic crystal
Source: microscopyu.com

The PLM's rotating stage allows the researcher to align the crystal's vibration direction parallel to the east-west incident light, enabling the measurement of its refractive index. The analyzer, which can be inserted or removed, is essential for different observation techniques. When inserted, it enables the observation of the crystal in cross-polarized light (XPL), which highlights structural and birefringent features. When removed, the observation is conducted in plane-polarized light (PPL), providing information about the crystal's color, transparency, and internal structures. This setup and its versatility in polarization techniques are valuable tools for studying the optical properties of anisotropic crystals. A Polarized Light Setup shown in Figure[2.10].

Construction of a microscope using the polarization microscopy technique is based on similar fundamental components to achieve the desired polarization effects and analysis of birefringent materials. This section will discuss the key components involved in polarization microscopy and their roles in enabling precise and accurate polarization imaging.

- **Light Source:** A crucial element in polarization microscopy is the selection of an appropriate light source. Commonly used light sources include xenon arc lamps, mercury vapor lamps, or more recently, powerful LEDs. These light sources emit broad-spectrum light that can be polarized using polarizers and further manipulated to achieve specific polarization states.

- **Polarizers:** Polarizers play a vital role in polarization microscopy as they selectively filter the incident light to obtain polarized light. A polarizer placed before the light source or in the illumination path allows for the control of the polarization state of the incoming light. This polarized light then interacts with the sample, revealing its birefringent properties.

- **Objective:** The objective lens in polarization microscopy focuses the polarized light onto the sample and collects the transmitted or reflected light. High-quality objectives with a high numerical aperture are essential to ensure efficient light collection and maintain the polarization state throughout the imaging process.

- **Compensators:** Compensators, such as retardation plates or wave plates, introduce controlled phase retardation to enhance the contrast and reveal the birefringent properties of the sample. These optical elements compensate for the varying retardation values introduced by the sample and enable quantitative analysis of the sample's polarization properties.

- **Analyzer:** The analyzer is positioned in the optical path after the objective and plays a critical role in polarization microscopy. It selectively transmits or blocks specific polarization states of the emitted light from the sample, allowing for further control and manipulation of the polarization information.

- **Detector:** The detector in polarization microscopy captures and records the polarized light signals for subsequent analysis and documentation. It can consist of eyepieces for direct visualization or a camera for digital image acquisition. Modern digital cameras provide high-resolution imaging capabilities, enabling detailed analysis and documentation of polarization images.

The polarization microscope combines carefully integrated components to effectively control and analyze the polarization properties of birefringent materials. The light source

provides polarized or unpolarized light, and the polarizers, compensators, and analyzer manipulate the light's polarization states during sample interaction. The objective lens focuses the light onto the sample, and a detector captures the resulting polarized light signals for further analysis. This technique offers various advantages in studying birefringent materials, allowing visualization of structural features, characterization of crystallographic properties, and analysis of optical anisotropy. With applications in materials science, geology, biology, and forensic sciences, polarization microscopy leverages polarized light principles and advanced imaging techniques to deepen our understanding of material polarization properties, facilitating accurate characterization and analysis in scientific research and industrial applications.

The interference color chart, as shown in Figure [2.11], provides a graphical representation of the retardation colors exhibited by birefringent crystals of various thicknesses. This chart serves as a valuable tool in measuring the retardation value, which, when combined with the known or measured thickness, allows for the determination of birefringence.

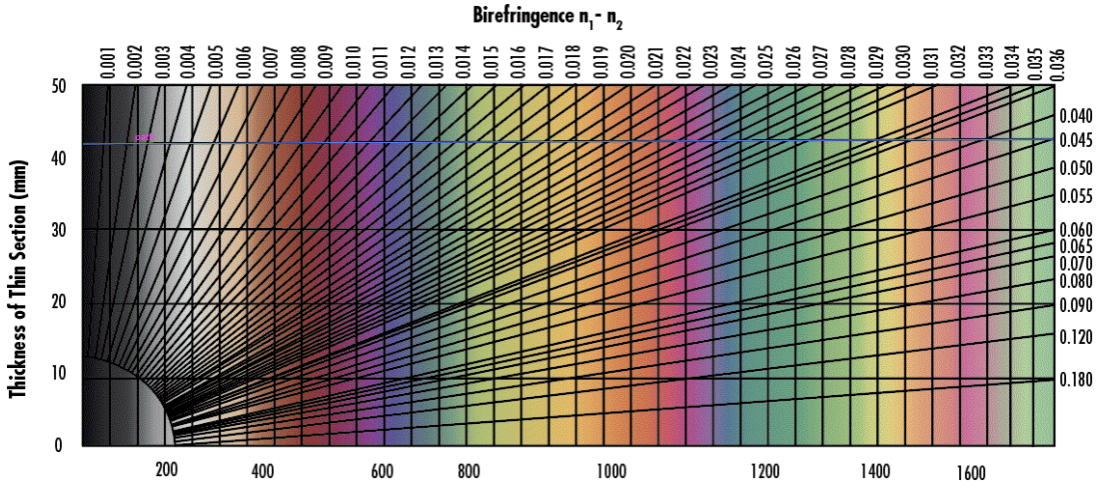


Figure 2.11: Interference color chart showing the range of retardation colors from 0 to 1800 nm.

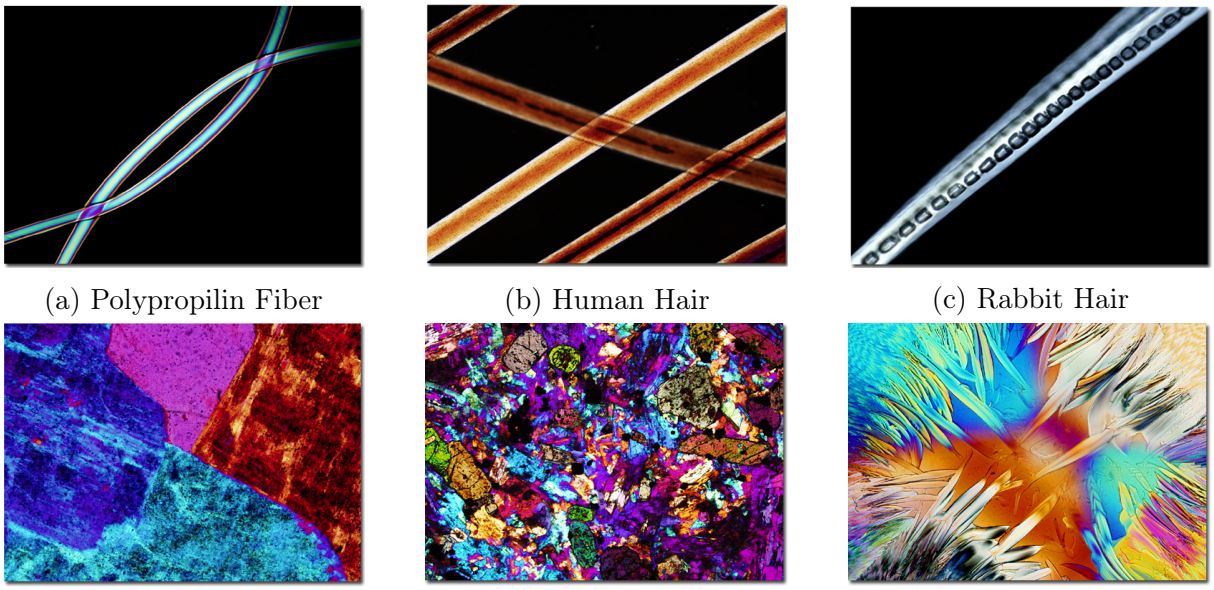
Source: edmundoptics.com

Polarization microscopy (PLM) finds applications in various fields, and Table [2.12] provides a list of material classes and industries that benefit from its use. Hazardous and regulated materials, such as asbestos, silica, white powders, spores, and pollen, can be effectively analyzed using PLM. Minerals, including metal ores, synthetic polymers like PTFE, acrylic, nylon, and electronic and photonic materials like dielectrics and semiconductors, can also be characterized using this technique. PLM proves useful in organic chemistry for the analysis of pharmaceuticals, explosives, herbicides, dyes, adhesives, and organometallic compounds. In the field of forensics, PLM enables the examination of hair, drugs, poisons, munitions, paint, glass, soil, ink, currency, and residues can also be

studied using PLM [8]. These materials are shown in Table[2.12] and Figure [2.13].

Material Class	**Examples**
Hazardous and regulated materials	asbestos, silica, white powders, ash, spores, pollen, mold
Minerals	metal ores (oxides, sulfides, silicates, zeolites, clays)
Synthetic polymers	PTFE, acrylic, nylon, polypropylene, polystyrene, etc.
Electronic and photonic materials	dielectrics, semiconductors, ceramics, composites, metals
Organic chemistry	pharmaceuticals, explosives, herbicides, dyes, adhesives, organometallics
Forensics	hair, drugs, poisons, munitions, paint, glass, soil, ink, currency, residues
Building materials	concrete, wood, asphalt, metal, paint, coatings
Other materials	paper, textiles, food and feed, pigments, bone, fossil, gemstones, pottery

Figure 2.12: Classes of materials benefiting from PLM



(a) Polypropilin Fiber (b) Human Hair (c) Rabbit Hair

(d) Arfvedsonite Granite (e) Alkalic Syenite (f) Vitamin C

Figure 2.13: PLM Microscopy Samples
Source: micro.magnet.fsu.edu

In combination with optical, crystallographic, and physical observations, the information obtained through PLM is usually sufficient to identify an unknown sample or significantly narrow down the possibilities. However, for further confirmation and detailed analysis, additional techniques such as chemical analysis (e.g., EDS or microcrystal tests) or structural analysis (μFTIR, X-ray diffractometry [XRD], selected area electron diffraction [SAED], or EBSD) may be employed. By incorporating these complementary techniques, it is rare for a material to escape identification, as PLM provides a strong foundation for comprehensive material analysis.

2.1.5 Hyperspectral Imaging

Hyperspectral Imaging (HSI) is an advanced technique that captures and processes information across the electromagnetic spectrum, extending beyond human vision. By detecting ultraviolet to infrared wavelengths, similar to the "mantis shrimp's" visual capabilities, HSI enables the identification of subtle differences in objects, such as various types of coral, prey, or predators.

To evaluate hyperspectral sensors, two critical factors are considered: spectral resolution and spatial resolution. Spectral resolution, determined by the width of each captured spectral band, enables the identification of objects even within a few pixels. Spatial resolution, on the other hand, relates to pixel size. Optimizing the balance between spectral and spatial resolutions is crucial, as excessively large pixels can lead to multiple objects being captured in a single pixel, while excessively small pixels compromise signal-to-noise ratio and measurement reliability [9]. Some typical applications of hyperspectral imaging techniques are shown in Figure [2.14].

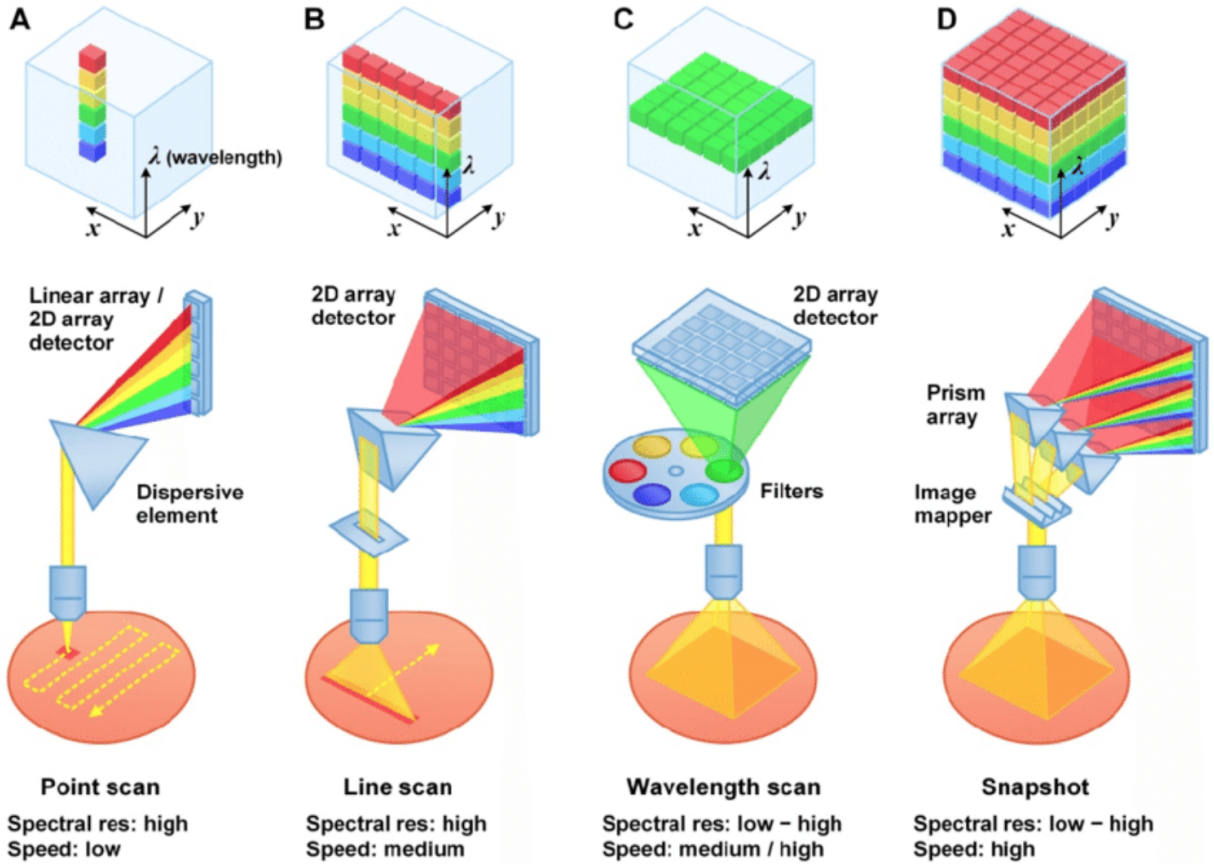


Figure 2.14: Typical (hyper)spectral imaging approaches. (A) Point scan. (B) Line scan (i.e. "pushbroom"). (C) Wavelength scan. (D) Snapshot. REF
Source: [9]

In the field of forensics sciences, HSI has significant applications, providing valuable insights and capabilities for forensic analysis and investigation [10]. By capturing information across a wide range of wavelengths, HSI enables the identification and characterization of materials based on their unique spectral signatures. This technology holds the potential to revolutionize forensic investigations, offering enhanced capabilities for material identification, evidence analysis, and crime scene reconstruction. For example, hyperspectral instruments generate high-contrast images of latent fingerprints while also yielding chemical "signature" information about material left behind that could be associated with the fingerprint, as shown in Figure [2.15].

Figure 2.15: Hyperspectral imaging in forensics.
Source: laserfocusworld.com

Ongoing research and engineering efforts are dedicated to improving both spectral and spatial resolutions in hyperspectral imaging systems. These advancements aim to enhance the accuracy and reliability of collected data, driving innovation across scientific research, resource exploration, environmental monitoring, and other domains requiring precise material identification and characterization.

In the field of forensics, HSI has been successfully applied to various areas. For example, in questioned document analysis, hyperspectral imaging can uncover hidden or altered text by detecting variations in ink composition or paper properties. By analyzing spectral signatures within different document regions, valuable evidence can be revealed, aiding in document authentication and forgery detection.

HSI also shows promise in the analysis of forensic trace evidence, such as fibers, paints,

or gunshot residue. These materials exhibit distinct spectral properties that can be captured and analyzed using hyperspectral imaging techniques. By comparing the spectral signatures of questioned and known samples, connections between crime scenes, suspects, and victims can be established, providing crucial evidence for criminal investigations. A typical setup of a hyperspectral cube is shown in Figure [2.16].

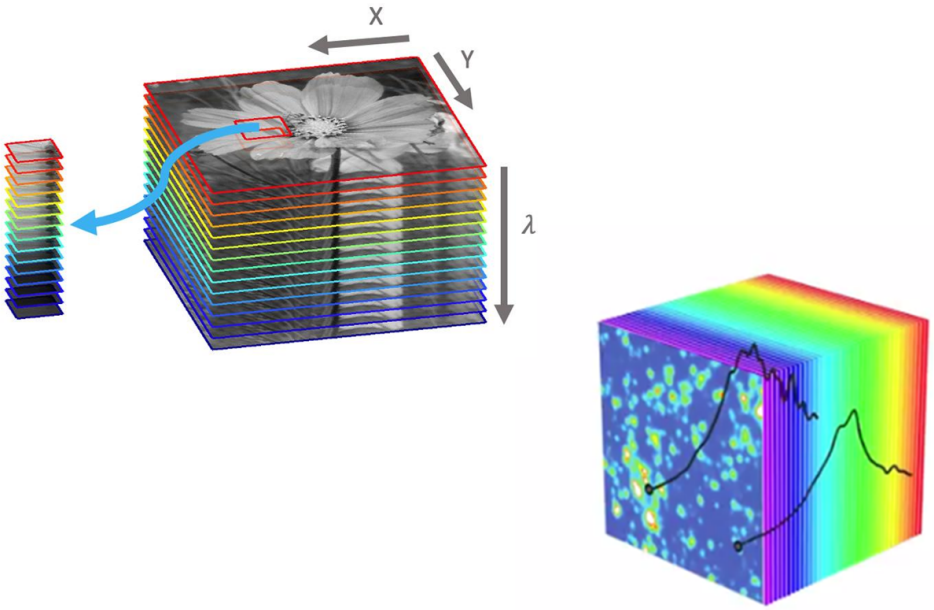


Figure 2.16: Hyperspectral Cube

Furthermore, hyperspectral imaging plays a significant role in the detection and characterization of bloodstains and biological fluids at crime scenes. Analyzing the spectral reflectance properties of these substances enables forensic investigators to differentiate between different biological materials, determine their age, and potentially identify the species of origin. Such information aids in crime scene reconstruction and provides valuable insights into the events that occurred.

2.2 State of the Art Technology

2.2.1 Multimodal Imaging

In recent years, a multitude of optical imaging modalities utilizing diverse contrast mechanisms have surfaced in the scientific landscape. This growing trend can be attributed to the inherent limitations of individual techniques in delivering comprehensive information with regards to both temporal and spatial resolution requirements. To attain a thorough comprehension of the intricate structural and biochemical alterations transpiring in cellulosic materials during physical, chemical, or biochemical processing, the integration of complementary techniques becomes imperative.

In the domain of forensic sciences, a plethora of imaging modalities has gained prominence, finding application in crime scene investigation and evidence analysis. However, to fully harness the power of these techniques, it is crucial to integrate them synergistically. Nevertheless, the integration of diverse imaging modalities presents inherent challenges, primarily stemming from disparate hardware requirements specific to each technique. To overcome these obstacles, it becomes imperative to advance microscopy technologies tailored for seamless integration and explore their potential applications in the field of forensic sciences [12].

Efficiently bridging the gaps between different imaging modalities necessitates the development of innovative technologies capable of facilitating their cohesive convergence. This endeavor involves addressing the intricacies associated with varied hardware demands, while ensuring compatibility and interoperability. The seamless integration of these techniques would unlock numerous advantages, including enhanced spatial resolution, improved sensitivity and specificity, and comprehensive insights into the intricate details of forensic evidence.

Furthermore, the evolution of microscopy technologies must extend beyond hardware compatibility to encompass software and data analysis. The formulation of robust algorithms capable of seamlessly integrating data obtained from diverse imaging modalities is of paramount importance. These algorithms should facilitate efficient data fusion, accurate co-registration, and comprehensive multi-modal image analysis, empowering forensic scientists to extract synergistic information and unravel crucial details that can aid in the investigation and analysis of forensic evidence.

By advancing the integration of multiple imaging modalities, a broader spectrum of applications can be explored within the field of forensic sciences. For instance, combining techniques such as reflectance spectroscopy, X-ray imaging, and infrared thermography

could provide valuable insights into the composition, age, and origin of questioned documents or counterfeit currency. Similarly, the integration of modalities such as scanning electron microscopy (SEM), atomic force microscopy (AFM), and Raman spectroscopy could unravel vital information about the morphology, elemental composition, and chemical nature of trace evidence, enabling precise forensic analysis.

In conclusion, while individual imaging modalities have showcased their significance in forensic sciences, their true potential can be fully realized through the seamless integration of multiple techniques. Overcoming the challenges associated with hardware compatibility, data fusion, and algorithmic development is crucial for comprehensive and transformative imaging capabilities in forensic investigations. By embracing a multidimensional approach, forensic scientists can unlock novel insights, strengthen their analytical capabilities, and ultimately contribute to the advancement of the field.

A notable example of modularity in microscope systems is shown in Figure [2.17].

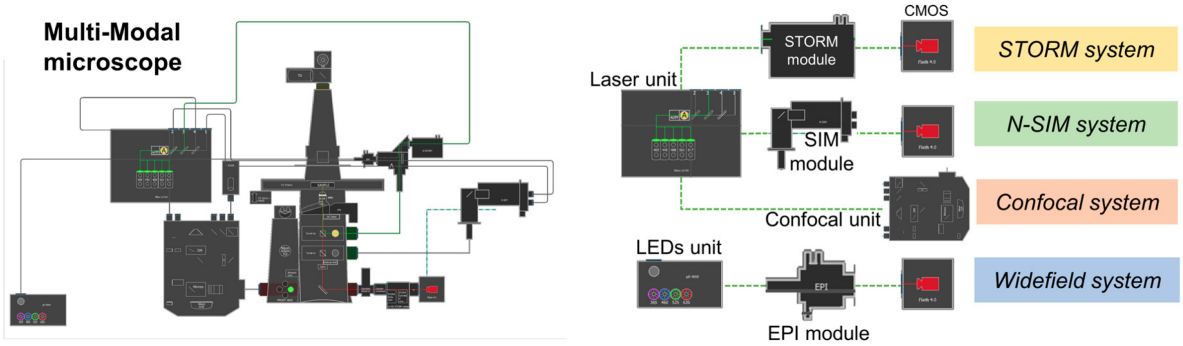


Figure 2.17: Correlative Multi-Modal Microscopy
Source: [13]

The microscope setup in the above image, combines multiple imaging modalities, including fluorescence microscopy, confocal microscopy, and optical coherence tomography (OCT), in a single integrated platform. The multimodal microscope is designed to overcome the limitations of individual imaging techniques by leveraging their complementary strengths. By seamlessly integrating these modalities, researchers can obtain enhanced spatial and temporal resolution, improved sensitivity and specificity, and a more comprehensive understanding of the biological samples or materials under investigation..

Multimodal analysis is powered by the concept of integrating multiple techniques in a single microscope. The unique configuration an integrated microscope enables simultaneous acquisition of both anatomical (structural) and functional imaging information, with particular emphasis for applications in the fields of tissue engineering and cell biology, as shown in Figure [2.18].

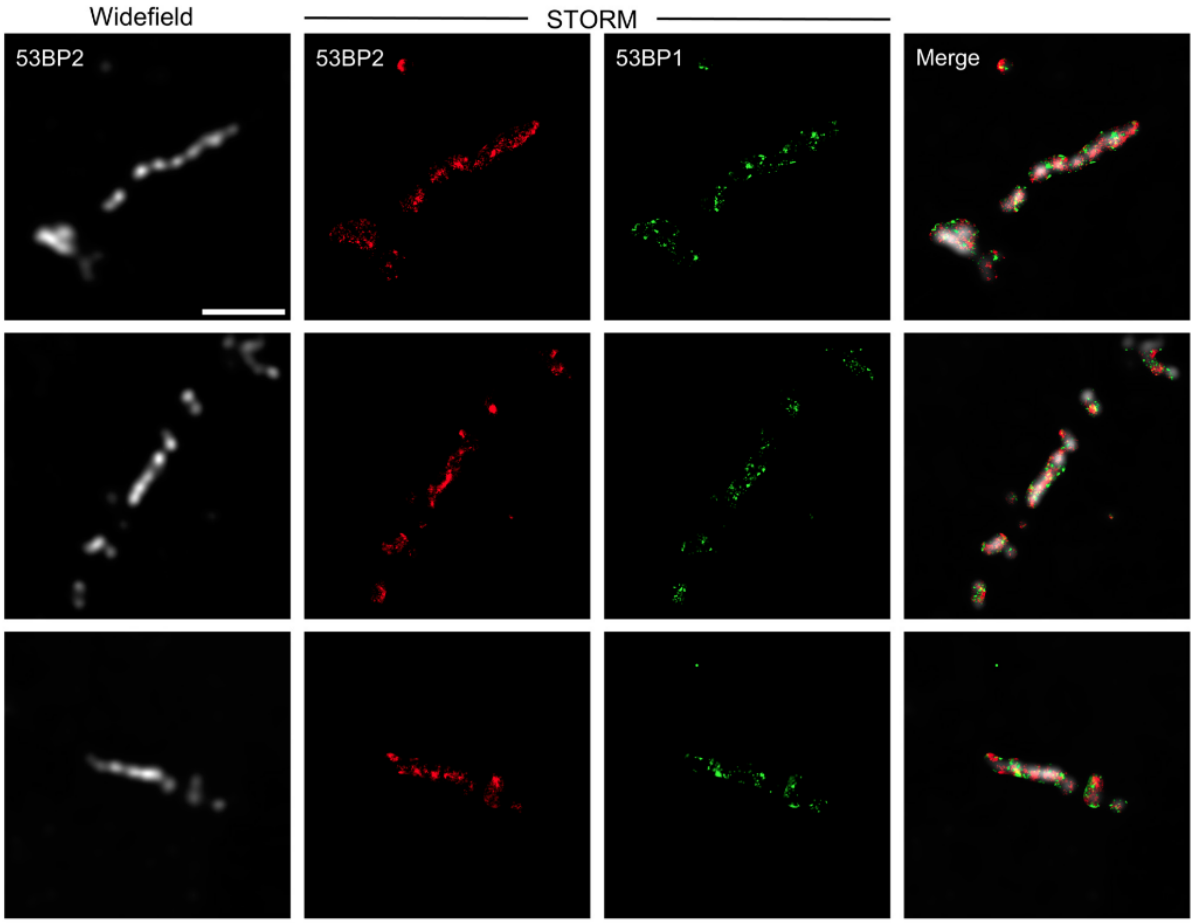


Figure 2.18: Multimodal Imaging using Widefield and dSTORM techniques
Source: www.mdpi.com/2073-4409/12/3/354

In the field of forensic sciences, multimodal microscopy emerges as a formidable imaging technique, offering distinct advantages over traditional methods. Its primary strength lies in its exceptional suitability for high-content real-time imaging, providing forensic investigators with unparalleled capabilities. This unique ability allows for the acquisition of high-resolution visualizations, which hold tremendous value in the examination of forensic evidence and the reconstruction of complex crime scenes.

Multimodal microscopy excels in producing accurate and multi-dimensional data structures of specimens, leveraging the use of straightforward techniques. Through the integration of multiple imaging modalities, such as reflectance spectroscopy, fluorescence microscopy, and confocal microscopy, this approach enables comprehensive visualization and analysis of forensic samples. The resulting information, when subjected to meticulous processing, rivals the reliability of datasets obtained from highly specialized microscopes, while simultaneously mitigating the limitations imposed by instrument construction and analysis specialization [11].

In the context of forensic investigations, multimodal microscopy empowers forensic scientists to extract a wealth of crucial details from the evidence at hand. By seamlessly combining anatomical (structural) and functional imaging modalities, it enables the visualization of microstructures, cellular morphology, chemical composition, and surface properties of forensic specimens. This comprehensive and multidimensional perspective not only enhances the accuracy and reliability of forensic analyses but also enables the identification of subtle features that may have otherwise gone unnoticed using conventional techniques.

Moreover, the simplicity and accessibility of multimodal microscopy techniques hold significant implications for the field of forensic sciences. The straightforward nature of data acquisition and the reduction in complexity associated with specialized microscopes facilitate wider adoption and utilization of these techniques among forensic laboratories. This increased accessibility ensures that the benefits of multimodal microscopy extend beyond research institutions, enabling forensic scientists with varying levels of expertise to leverage its power in routine forensic examinations.

In summary, the advantages of multimodal microscopy in forensic sciences, including its suitability for high-content real-time imaging, high-resolution visualization, and simplicity of data acquisition, make it a valuable tool for forensic investigations. By generating accurate and multi-dimensional data structures through straightforward techniques, multimodal microscopy offers a reliable alternative to specialized microscopes while eliminating constraints related to instrument construction and analysis specialization. Its implementation in forensic sciences empowers investigators to uncover crucial evidence, providing a comprehensive understanding of the complex nature of forensic samples and facilitating accurate and objective forensic analysis.

2.2.2 Automated Microscopy

State-of-the-art microscopy techniques, as previously discussed, have the capability to achieve ultra-high resolution observation in a single imaging region [14]. However, when it comes to examining large areas of a specimen with high magnification and resolution, manual examination by an analyst becomes a time-consuming and labor-intensive process. Moreover, human factors introduce the potential for bias in the results obtained.

To address these challenges, modern-day microscopes have incorporated a range of automated components that significantly enhance the efficiency and reliability of imaging processes. These components include shutters, filter wheels, stages, light sources, and focus control, all of which can be electronically controlled, replacing their manual counterparts.

Achieving an optimally functioning fully automated optical imaging system, however, is a complex task that demands expertise in optics, electronics, and a substantial investment of time. Proper setup and configuration of the system require a thorough understanding of each individual component and their interactions.

Some of the key microscope components that can be automated are as follows:

- **Focus Control:** Fine focus transmission gear sets of microscope stages can be connected to focus motors, enabling automated focus control through the image acquisition software. These motors can also be used for manual focusing in advanced microscopes, decoupling the user control from the stage.
- **Stage Control:** Motors can be employed to control the stage movement along the x- and y-axes. This capability allows for precise positioning of the system in different imaging regions, particularly beneficial for automated scanning.
- **Illumination Control:** Widefield microscopy utilizes various light sources, such as tungsten-halogen sources for transmitted light, arc gas lamps for fluorescence excitation, and lasers for confocal applications. Metal-halide lamps and LEDs have also gained popularity as replacements for mercury arc-discharge lamps. The user can manually select the appropriate light source or utilize software controls for automated switching between light sources in predefined sequences.
- **Shutters:** Electromechanical shutters play a critical role in blocking light sources from illuminating the specimen during camera exposures. This functionality is particularly important in imaging fluorescent or live specimens to minimize photobleaching and phototoxicity. High-performance shutters, controlled by micropro-

cessors and interfaced with a computer, ensure coordinated operation with other microscope components.

- **Wavelength Selection:** Various devices, such as filters, beam splitting units, monochromators, or acousto-optic tunable filters (AOTFs), enable wavelength selection. Manual switching is slow and requires constant user intervention, while automated switching allows rapid selection between desired wavelengths, even in the absence of the user. Sequential or pre-programmed sequences can be employed for wavelength selection.
- **Camera/Detector System:** The operation of cameras or detectors can be controlled through software, enabling image collection even in the absence of the user.

An automated microscope system can be programmed to record images in time-lapse or x-y-z scanning modes without user intervention. The user can select or automate the desired spectral profiles of fluorophores, exposure time, gain, and threshold settings. In confocal or deconvolution microscopy, Z-stack settings can be stored in memory and applied to specific fields. Different settings can be assigned to each field, allowing for customized imaging parameters. Storage of coordinates enables the motorized stage to return to specific fields at appropriate time intervals, while autofocusing compensates for focus drift, especially critical when imaging live specimens and conducting area scanning. A simple automated microscope setup [15] is shown in Figure [2.19]

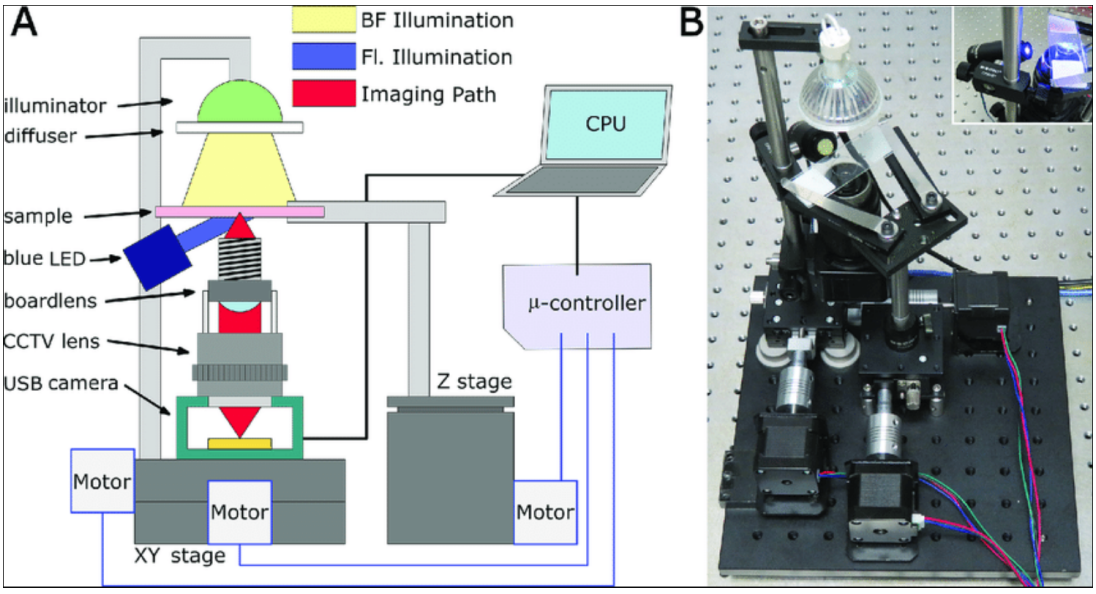


Figure 2.19: Structure of the low-cost automated microscope. (A) Schematic diagram showing electrical connections and optical path. (B) Photograph of the as-built system
Source: researchgate.net

When large fields of view and high resolution are required simultaneously, the microscope can be tiled. The software guides the system to acquire neighboring fields, which are

automatically stitched together to form a single large field of view. Proper archiving of data with metadata and labeling ensures the replication of exact imaging conditions when desired.

In conclusion, automation plays a pivotal role in enhancing the efficiency, accuracy, and reliability of microscopy techniques. Through the integration of automated components, such as focus control, stage control, illumination sources, shutters, wavelength selection mechanisms, and camera/detector systems, analysts can overcome the challenges associated with manual examination of large specimen areas. These advancements in automated microscopy have profound implications for various domains, including forensic sciences, by accelerating analysis workflows, minimizing human biases, and enabling comprehensive investigations of high-magnification and high-resolution imaging.

Field Sequential Imaging Field sequential (FS) imaging is a technique used in image acquisition systems to capture image channels in a temporal sequence, which are then combined to generate the final image, as shown in Figure[2.20]. This approach finds application in various domains, with multispectral imaging being a prominent example. However, in scenarios involving dynamic scenes, the sequential nature of FS imaging introduces motion artifacts, resulting in spatial misalignment of the image channels. Compensating for these motion artifacts is a challenging task due to the violation of the intensity consistency constraint, which is a key assumption in common motion estimation methods [1].

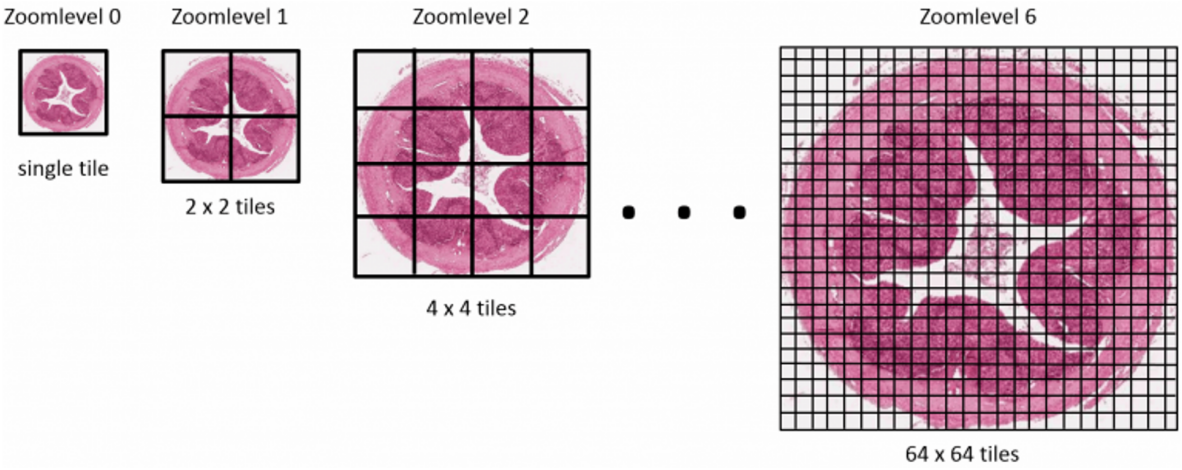


Figure 2.20: Sequential Scanning with different zooming levels
Source: realdata.pathomation.com

In order to address these challenges, specialized FS imaging systems have been developed that prioritize accuracy in handling intensity inconsistent data and, to a lesser

extent, computational speed. These systems employ advanced algorithms and techniques to mitigate motion artifacts and ensure accurate reconstruction of the final image. As FS imaging is often performed in real-time, the efficiency of these algorithms becomes crucial to maintain the desired temporal resolution.

To handle the intensity inconsistencies inherent in FS imaging, novel motion estimation approaches have been proposed. These methods exploit the temporal information available in the sequential image channels and incorporate robust registration algorithms that can effectively align the images despite their varying intensities. Additionally, strategies such as image-based and feature-based motion estimation are explored to accurately estimate the motion between consecutive frames.

State-of-the-art advanced automated microscopy encompasses two fundamental designs that represent cutting-edge technological advancements in the field. These designs are characterized by their sophisticated engineering and capabilities for high-resolution imaging and analysis.

- In the realm of scientific research and education, a modular design tailored for high-resolution multidimensional analysis of localized areas using diverse imaging modalities has gained considerable attention. This design is primarily employed for in-depth investigations and knowledge dissemination. A notable exemplar of this modular automated microscope is the DSX1000, developed by Olympus (Figure [2.21]). The DSX1000 embodies an all-in-one system characterized by an extensive magnification range, facilitating the acquisition of high-resolution images at significant magnifications. It incorporates long working distance objectives and supports multiple observation techniques, contributing to the comprehensive examination and analysis of specimens..

  Furthermore, advanced image fusion techniques are employed to combine the registered image channels and generate a high-quality composite image. These techniques take into account the specific characteristics of FS imaging, such as the temporal sequence of acquisition and the presence of motion artifacts, to optimize the fusion process. The goal is to produce a final image that not only minimizes the spatial misalignment caused by motion but also preserves the spectral information from each channel.

  In summary, FS imaging systems address the challenges posed by motion artifacts in dynamic scenes by emphasizing accuracy in handling intensity inconsistent data. By employing specialized motion estimation algorithms and image fusion techniques, these systems aim to generate high-quality images that faithfully represent the cap-

Figure 2.21: Olympus DSX1000 Digital Microscope
Source: olympus-ims.com

tured scene. The development of efficient and robust FS imaging systems contributes to the advancement of various fields, including remote sensing, medical imaging, and surveillance, enabling detailed analysis and interpretation of complex visual data.

2.3 Technology and Forensics Sciences

Forensic investigations often involve the examination of a large number of traces recovered from crime scenes. These traces can include various types of physical evidence, such as fibers, hairs, glass fragments, paint chips, tool marks, and biological materials. Analyzing and digitizing these traces using microscopy techniques has become essential in modern forensic sciences due to the need for efficient and comprehensive evidence evaluation. This section provides an extended discussion on the significance, technicalities, and robustness of analyzing and digitizing massive numbers of traces in forensic investigations.

- **Significance of Analysis and Digitization:** The analysis and digitization of massive numbers of traces in forensics serve several critical purposes [28]. Firstly, it allows for the identification and comparison of trace evidence, aiding in the establishment of links between suspects, victims, and crime scenes. Secondly, it enables the reconstruction of events, helping investigators understand the sequence of actions and interactions that occurred during the commission of a crime. Additionally, the analysis and digitization of traces provide an opportunity for long-term storage, sharing, and reevaluation of evidence, ensuring its accessibility for future reference or reexamination.
- **Microscopy Techniques for Trace Analysis:** Microscopy techniques play a pivotal role in the analysis and digitization of trace evidence in forensics. Optical microscopy, including stereomicroscopy and compound microscopy, is commonly used for initial screening, visualization, and documentation of traces. These techniques provide information about the morphology, color, texture, and other physical characteristics of the evidence. Advanced microscopy techniques, such as scanning electron microscopy (SEM), transmission electron microscopy (TEM), and confocal laser scanning microscopy (CLSM), offer higher resolution and imaging capabilities for detailed examination of trace materials [29]. By combining these techniques with appropriate sample preparation methods, investigators can extract valuable information from even the smallest and most complex traces.
- **Automation and High-Throughput Analysis:** Due to the large volume of traces encountered in forensic investigations, manual examination and analysis can be time-consuming and prone to human error. The need for robust, efficient, and accurate analysis has led to the development of automated microscopy systems and high-throughput analysis approaches. Automated microscopy systems, equipped with motorized stages, autofocus capabilities, and

image acquisition software, enable rapid and systematic examination of multiple traces. These systems can scan large areas, capture high-resolution images, and generate digital datasets that can be easily stored, retrieved, and shared. High-throughput analysis approaches, such as slide scanners and robotic systems, further enhance the efficiency and scalability of trace analysis, allowing for the processing of a large number of samples in a shorter time frame.

- **Image Analysis and Pattern Recognition:** Digitization of trace evidence through microscopy generates vast amounts of image data that require efficient analysis and interpretation. Image analysis techniques, including pattern recognition, feature extraction, and machine learning algorithms, play a crucial role in automating the identification, classification, and comparison of traces. These techniques enable the extraction of meaningful information from images, such as the presence of specific patterns, colors, textures, or geometric features [30]. By developing robust algorithms and software tools, investigators can streamline the analysis process, improve the accuracy of trace identification, and reduce the potential for human bias.
- **Challenges and Considerations:** The analysis and digitization of massive numbers of traces in forensics also present challenges and considerations. Standardization of protocols, calibration of instruments, and quality control measures are paramount to ensure the reliability, reproducibility, and admissibility of the results in legal proceedings. Additionally, the integration of different microscopy techniques, image analysis algorithms, and databases requires careful consideration to facilitate data sharing, collaboration, and interoperability among forensic laboratories. Data security, privacy, and ethical considerations should also be addressed to protect sensitive information and maintain public trust.

In conclusion, the analysis and digitization of massive numbers of traces in forensic investigations are crucial for effective evidence evaluation, event reconstruction, and long-term storage. Microscopy techniques, coupled with automation, high-throughput analysis, image analysis, and pattern recognition, offer robust solutions to address the challenges associated with trace analysis. By combining technical advancements with rigorous quality assurance measures, forensic scientists can enhance the efficiency, accuracy, and reliability of trace examination, ultimately contributing to the resolution of criminal cases and the administration of justice.

2.3.1 Identification Techniques

Traces found at crime scenes play a critical role in forensic investigations, as they can provide valuable information about the perpetrators, victims, and events surrounding a crime. The identification and analysis of traces require a multidisciplinary approach, incorporating various methods and techniques. This section provides an extended discussion on different methods used in forensic sciences for the identification of traces, highlighting their technicalities and robustness.

- **Microscopy Techniques:** Microscopy techniques are widely employed in forensic sciences for the examination and identification of trace evidence. Optical microscopy, including stereomicroscopy and compound microscopy, allows for the visual inspection of traces, providing information about their morphology, color, texture, and spatial distribution. These techniques are particularly useful for the initial screening and documentation of trace evidence. Advanced microscopy techniques, such as scanning electron microscopy (SEM), transmission electron microscopy (TEM), and atomic force microscopy (AFM), offer higher resolution and imaging capabilities, enabling detailed analysis of trace materials at the nanoscale [29]. These techniques can reveal structural features, elemental composition, surface characteristics, and topographical information, aiding in the identification and comparison of trace evidence.
- **Spectroscopic Methods:** Spectroscopic methods are extensively utilized in forensic sciences for the identification of trace evidence based on their chemical composition and spectral properties. Techniques such as Fourier transform infrared spectroscopy (FTIR), Raman spectroscopy, X-ray fluorescence (XRF), and energy-dispersive X-ray spectroscopy (EDX) enable non-destructive analysis of traces, providing information about the molecular structure, functional groups, elemental composition, and elemental distribution. These methods are valuable for the identification of organic and inorganic materials, including drugs, explosives, pigments, fibers, and glass fragments. Spectroscopic methods offer high specificity and sensitivity, allowing for the rapid and accurate characterization of trace evidence.
- **Chromatographic Techniques:** Chromatographic techniques, such as gas chromatography (GC) and liquid chromatography (LC), are widely employed in forensic sciences for the analysis of trace substances, particularly in cases involving drugs, toxic compounds, and accelerants [31]. These techniques separate complex mixtures into their individual components, allowing for the identification and quantification of specific compounds. Coupling chromatographic

techniques with mass spectrometry (MS) enhances the analytical capabilities, enabling the determination of molecular structures and the generation of unique chemical fingerprints. Chromatographic techniques are highly robust and can provide conclusive evidence in identifying trace substances even at very low concentrations.

- **DNA Analysis:** DNA analysis has revolutionized forensic sciences and has become an indispensable method for the identification of individuals involved in criminal activities. Forensic DNA analysis involves the extraction, amplification, and analysis of specific DNA regions, such as short tandem repeats (STRs), to create DNA profiles. These profiles are then compared to known reference samples, enabling the identification of individuals or establishing associations between individuals and trace evidence [32]. DNA analysis provides a high level of accuracy and can be instrumental in linking suspects to crime scenes, victims, or other individuals involved in a criminal investigation. Advancements in DNA analysis techniques, such as polymerase chain reaction (PCR), capillary electrophoresis, and next-generation sequencing, have further improved the sensitivity, speed, and robustness of forensic DNA analysis.
- **Digital Imaging and Pattern Recognition:** With the increasing digitization of forensic sciences, digital imaging techniques and pattern recognition algorithms have gained prominence in trace identification. Digital imaging methods, such as hyperspectral imaging, multispectral imaging, and digital microscopy, allow for the acquisition of high-resolution images and spectral data of traces. These images can be processed and analyzed using advanced pattern recognition algorithms, including machine learning and computer vision techniques. By extracting features, identifying patterns, and correlating trace evidence with reference databases, these methods can assist in the automated identification and classification of traces, reducing human subjectivity and enhancing the efficiency of forensic investigations.

In summary, forensic sciences employ a range of methods for the identification of traces, each with its specific technicalities and robustness. Microscopy techniques enable visual examination and high-resolution imaging, while spectroscopic methods provide chemical information about trace materials. Chromatographic techniques offer the separation and identification of compounds, DNA analysis allows for individual identification, and digital imaging coupled with pattern recognition algorithms facilitates automated trace identification. The integration of these methods, along with the continuous advancements in instrumentation and data analysis techniques, contributes to the reliable and comprehensive analysis of trace evidence, aiding in the resolution of criminal cases and the delivery of justice.

2.3.2 Analysis of Massive Amount of Traces

Forensic analysis involves the examination and comparison of various types of trace evidence, including fingerprints, DNA samples, fibers, hairs, gunshot residue, and tool marks, among others. Traditionally, forensic analysis has been a time-consuming process, requiring meticulous examination and analysis by forensic experts. The manual examination of each individual trace can be a labor-intensive task, resulting in significant time delays and backlogs in forensic laboratories.

However, recent advancements in high-throughput screening techniques have revolutionized the field of forensic analysis by enabling the simultaneous analysis of multiple traces in a rapid and automated manner. These techniques employ advanced instrumentation, such as robotic systems and automated analysis software, to process and analyze large volumes of evidence efficiently.

One prominent example of a high-throughput screening technique in forensic analysis is the automated fingerprint identification system (AFIS). AFIS utilizes algorithms and databases to compare and match fingerprints against a vast collection of known prints, significantly reducing the time required for identification. This technology has been widely adopted by forensic agencies worldwide and has proven to be instrumental in expediting the analysis of fingerprint evidence.

Similarly, high-throughput DNA sequencing and analysis techniques have revolutionized forensic DNA analysis. Next-generation sequencing (NGS) platforms, coupled with automated data analysis pipelines, enable the rapid and simultaneous analysis of multiple DNA samples. This high-throughput approach has significantly reduced the time required for DNA profiling and identification, thereby expediting the forensic analysis process.

In addition to fingerprints and DNA, high-throughput screening techniques have also been developed for the analysis of other types of trace evidence. For example, advanced imaging systems and pattern recognition algorithms have been employed for the automated analysis of shoeprints and tire tracks. These systems can quickly compare and match patterns against extensive databases, saving substantial time compared to manual examination.

The implementation of high-throughput screening techniques in forensic analysis has yielded significant improvements in terms of efficiency and turnaround time. By automating certain aspects of the analysis process and enabling the simultaneous processing of multiple traces, these techniques have dramatically reduced the time required for forensic analysis. This, in turn, facilitates timely investigations, enhances criminal justice procedures, and helps prevent backlogs in forensic laboratories.

However, it is important to note that high-throughput screening techniques are not without their limitations. The implementation of such techniques often requires substantial initial investments in infrastructure, equipment, and training. Additionally, the development and maintenance of comprehensive databases for comparison purposes are critical for the success of these techniques. Furthermore, the integration of automated systems into existing forensic workflows may require careful validation and quality assurance procedures to ensure accurate and reliable results.

In conclusion, the time considerations involved in forensic analysis of massive amounts of traces have been a longstanding challenge in the field. The advent of high-throughput screening techniques has brought about significant improvements in expediting the analysis process. The utilization of automated systems, advanced instrumentation, and data analysis algorithms has greatly reduced the time required for the examination and comparison of trace evidence. These advancements have not only enhanced the efficiency of forensic analysis but also contributed to timely investigations and the prevention of backlogs in forensic laboratories. However, careful consideration of the limitations and requirements associated with the implementation of high-throughput screening techniques is essential to ensure their successful integration into forensic workflows.

2.3.3 Microscopy Techniques for Trace Analysis

Microscopy plays a crucial role in forensic sciences, providing investigators with valuable insights into physical and biological evidence encountered at crime scenes. The application of microscopy in forensic analysis allows for the identification, characterization, and comparison of microscopic evidence, aiding in the reconstruction of events and the determination of facts in criminal investigations. This section provides an extended discussion on the use of microscopy techniques in forensic sciences, emphasizing their technicalities, robustness, and significance.

- **Optical Microscopy:** Optical microscopy techniques, such as stereomicroscopy and compound microscopy, are commonly employed in forensic investigations. These techniques enable investigators to examine and document macroscopic and microscopic evidence, including fibers, hairs, fingerprints, tool marks, and gunshot residues. The versatility of optical microscopy allows for the visualization and analysis of both organic and inorganic materials, aiding in the identification and comparison of trace evidence. Additionally, polarized light microscopy (PLM) and fluorescence microscopy are used to enhance the visualization of specific materials or stains, facilitating the detection of hidden or altered evidence.
- **Scanning Electron Microscopy (SEM):** SEM is a powerful tool in forensic sciences, providing high-resolution imaging and elemental analysis capabilities. SEM allows for the examination of various types of evidence, such as gunshot residues, paint chips, tool marks, and soil particles. The detailed surface morphology and elemental composition obtained through SEM analysis can assist in the identification, source attribution, and association of evidence with potential suspects or crime scenes [33]. Additionally, energy-dispersive X-ray spectroscopy (EDS) coupled with SEM enables the identification and mapping of elements within the examined samples, further enhancing forensic analysis.
- **Confocal Laser Scanning Microscopy (CLSM):** CLSM is a non-destructive and high-resolution microscopy technique used extensively in forensic sciences. It allows for the three-dimensional imaging of fluorescently labeled samples, facilitating the visualization and analysis of cellular materials, bloodstains, and latent fingerprints [34]. The ability to reconstruct detailed images at different depths within a sample enhances the examination and interpretation of complex forensic evidence. CLSM, combined with advanced image analysis algorithms, enables the identification and discrimination of specific cellular components or stains, aiding in the characterization of evidence.
- **Microspectrophotometry:** Microspectrophotometry involves the analysis of the absorption, transmission, or reflection spectra of microscopic samples. This technique is particularly useful in forensic sciences for the identification and discrimination of pigments, dyes, and fibers encountered in questioned documents, counterfeit currency, or textile-related cases [35]. By obtaining spectral data at specific wavelengths, microspectrophotometry enables the characterization and comparison of materials, supporting forensic examinations and establishing links between evidence and suspects or crime scenes.

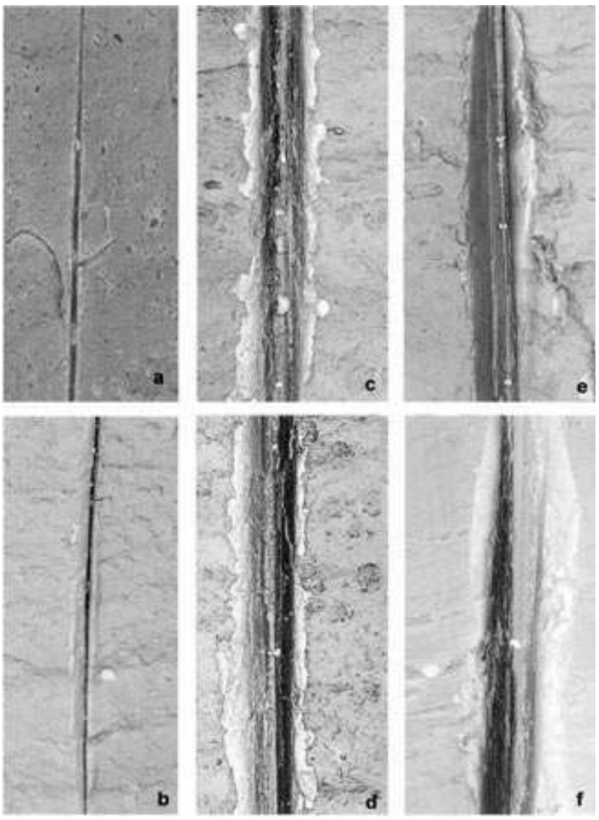


Figure 2.22: SEM images of cut marks made by each knife type in the control and test sample. (a) control sample scalpel blade; (b) test sample scalpel blade; (c) control sample paring knife; (d) test sample paring knife; (e) control sample utility knife; (f) test sample utility knife.

Source: [33]

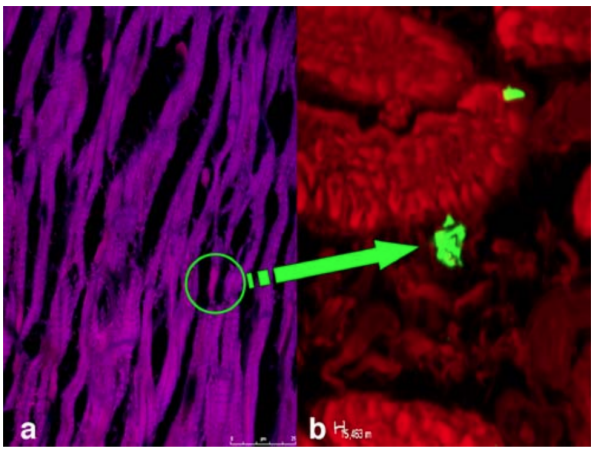


Figure 2.23: CLSM micrographs of contraction band necrosis in the heart (a) with a myocyte (b) showing calcium deposits composed of needlelike crystals (arrow).

Source: [34]

The use of microscopy techniques in forensic sciences requires meticulous sample preparation, appropriate instrument calibration, and adherence to quality assurance and quality control protocols. Robust and standardized procedures are essential to

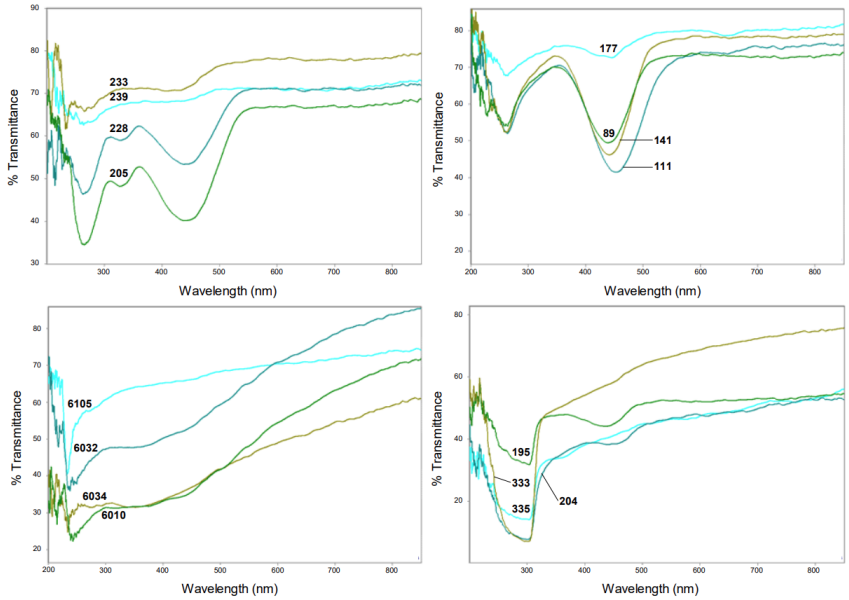


Figure 2.24: UV-VIS spectrum of yellow fibers mounted in glycerin using quartz slides and cover slips: (A) cotton; (B) acrylic, (C) nylon; (D) polyester.
Source: [35]

ensure the reliability, reproducibility, and admissibility of microscopic evidence in legal proceedings. Additionally, the integration of microscopy with complementary techniques, such as spectroscopy, imaging software, and statistical analysis, further enhances the analytical capabilities and interpretation of forensic evidence.

In conclusion, microscopy techniques are invaluable tools in forensic sciences, enabling the analysis, characterization, and comparison of microscopic evidence encountered in criminal investigations. Optical microscopy, SEM, TEM, CLSM, and microspectrophotometry provide investigators with diverse capabilities to visualize, identify, and interpret trace materials, contributing to the reconstruction of events and the determination of facts in forensic examinations. By employing robust methodologies and adhering to strict quality assurance measures, microscopy in forensic sciences continues to advance the field, aiding in the pursuit of justice and the elucidation of complex forensic puzzles.

2.3.4 The essence High Throughput Screening (HTS)

High throughput imaging is a term used to describe automated light microscopy and image analysis of large numbers of samples. It is a useful technique for phenotypic screening assays, evaluating the response of cells, cell-based models, or organisms to various types of perturbagens. When selecting a microscope-based system for high throughput imaging, it is important to not only find a system with the necessary features/capabilities, but also to critically evaluate its reliability and capacity – two factors that have a real-world impact on throughput and efficiency [16].

The essence of High Throughput Screening (HTS) microscopy lies in its ability to rapidly and efficiently analyze large sample libraries, facilitating the discovery and characterization of bioactive compounds, genes, and cellular processes. HTS microscopy has revolutionized the field of drug discovery, functional genomics, and systems biology by enabling the screening of thousands to millions of compounds or genetic perturbations in a relatively short time.

At its core, HTS microscopy relies on the integration of automated microscopy systems with advanced robotics, image analysis algorithms, and data management tools. This integration allows for the parallel processing of multiple samples, enabling the acquisition of large-scale data sets with high throughput and minimal human intervention. One of the key components of HTS microscopy is the use of multi-well plates or microarrays [22], which serve as platforms for sample handling and analysis. These plates can accommodate a large number of samples in a well-organized format, enabling simultaneous imaging and analysis of multiple samples in a high-throughput manner. Additionally, the use of specialized robotic systems enables efficient sample handling, plate loading, and precise positioning for imaging.

Figure [2.25] showcases the different approaches on this technology. Represented are: A. Fully automated high throughput high content imaging systems (ImageXpress, InCell, BD Pathway, ScanR, Opera and the Arrayscan). B. Live cell kinetic imaging systems (IncuCyteFLR and Cell-IQ) and C. Fluorescent tissue slide imaging platform (Scanscope FL)

In HTS microscopy, the choice of imaging modalities depends on the specific application and the information desired from the samples. Fluorescence microscopy is widely used due to its versatility and ability to visualize various cellular processes and molecular interactions. Other imaging modalities, such as brightfield microscopy, phase contrast microscopy, and confocal microscopy, may also be em-

Figure 2.25: High Throughput Screening Technology
Source: europeanpharmaceuticalreview.com

ployed based on the specific requirements of the experiment.

The success of HTS microscopy relies heavily on robust and reliable image analysis algorithms. These algorithms are designed to automate image segmentation, feature extraction, and data analysis, allowing for the rapid extraction of meaningful information from large image datasets. Machine learning techniques, such as deep learning, have been increasingly integrated into HTS microscopy workflows to enhance the accuracy and efficiency of image analysis.

Furthermore, effective data management and analysis are critical in HTS microscopy. Large-scale image datasets generated from HTS experiments require efficient storage, organization, and retrieval strategies. Data integration, visualization, and mining techniques are employed to extract valuable insights and patterns from the vast amount of data generated during the screening process.

HTS microscopy finds applications in various research areas, including drug discovery [18], functional genomics [19], phenotypic screening [20], and personalized medicine [21]. It enables the identification of potential drug candidates, characterization of gene functions, understanding of cellular pathways, and investigation of disease mechanisms. HTS microscopy has played a pivotal role in accelerating scientific discoveries and has the potential to transform the landscape of biomedical

research and drug development.

In conclusion, HTS microscopy represents a powerful and indispensable tool in modern forensics research [17]. Its essence lies in its ability to efficiently analyze large sample libraries, automate image acquisition and analysis, and generate high-throughput data. With its ability to rapidly screen and analyze diverse samples, HTS microscopy holds great promise for advancing our understanding of biological processes, identifying novel therapeutic targets, and accelerating the discovery of new drugs.

2.3.5 Diagnostics and Microscopy

Microscopy plays a crucial role in the field of diagnostics, especially in the context of cancer detection. Traditional diagnostic methods have limitations in accurately identifying cancer at an early stage due to its diverse nature and lack of specific biomarkers. However, advancements in biophotonics have paved the way for the development of novel microscopy techniques that can detect cancer based on the fluorescence, scattering, and absorption characteristics of cells and tissues.

Fluorescence spectroscopy is a technique that has shown promise in cancer detection. It capitalizes on the differences in fluorescence signals between normal and cancerous tissues, which arise from changes in their morphological and biochemical characteristics. By analyzing the fluorescence spectra, it becomes possible to identify diagnostic biomarkers and predict tumor development in various organs such as the lung, brain, skin, gastrointestinal tract, liver, and cervix [23].

Fluorescence microscopy, particularly confocal microscopy and two-photon fluorescence microscopy, has been extensively used to visualize and analyze cancer tissues at a cellular level. These techniques provide high-resolution images and enable the identification of specific features associated with malignancy. For example, confocal endoscopy systems have been employed to detect neoplastic changes during colonoscopy with high sensitivity, specificity, and accuracy. Reflectance confocal microscopy (RCM) has facilitated the detection of neoplastic oral mucosa by determining tissue features and differentiating between melanoma and non-melanoma lesions. These microscopy techniques have the potential to guide treatment decisions and enable real-time monitoring of tumor margins.

Second harmonic generation (SHG) microscopy is another valuable tool in cancer diagnostics [24], particularly for studying morphological changes in non-centrosymmetric structures such as collagen. By quantifying the orientation of collagen fibers, SHG microscopy can provide insights into tissue malignancy. Multiphoton microscopy techniques have also been employed to visualize and analyze cervical tissue, revealing structural information and metabolic changes associated with malignancy.

Raman spectroscopy is a non-destructive technique that offers detailed molecular information about tissues. It has demonstrated the ability to differentiate between benign and malignant tissues in various types of cancer [25]. For instance, confocal Raman spectroscopy has been utilized to distinguish basal cell carcinoma (BCC) from normal skin tissues by detecting distinct spectral differences. Raman microscopy has also shown potential in lung cancer detection by differentiating between healthy and malignant lung tissues based on characteristic spectral features.

Coherent anti-Stokes Raman scattering (CARS) microscopy and stimulated Raman scattering (SRS) microscopy provide high sensitivity and contrast for cellular content studies. CARS microscopy enables the visualization of lipid-rich structures, which can be relevant for understanding cancer progression [26]. SRS microscopy, on the other hand, allows the characterization of lipid droplets in hormone-responsive breast and prostate cancer cells, providing insights into the effects of hormone treatments.

Photoacoustic spectroscopy (PAS) is a technique that combines optical absorption and acoustic wave generation to analyze the molecular composition of tissues [27]. PAS has been employed to detect and analyze volatile organic compounds (VOCs) relevant to lung cancer. By tuning the laser source, PAS sensors can detect specific biomarkers associated with lung cancer, opening up possibilities for early diagnosis.

In conclusion, microscopy techniques such as fluorescence spectroscopy, fluorescence microscopy, SHG microscopy, Raman spectroscopy, and photoacoustic spectroscopy have demonstrated their diagnostic capabilities in cancer detection. These techniques offer high sensitivity, molecular specificity, and the ability to visualize cellular and tissue-level changes associated with malignancy. By leveraging the optical properties of tissues, these microscopy techniques provide valuable insights into cancer biology and have the potential to improve early detection and treatment strategies.

2.4 Limitations of Forensics Traces Analysis

Forensic science is currently facing critical challenges that demand innovative solutions in evidence collection and analysis. One specific challenge arises from the use of adhesive tapes to lift traces and materials for DNA analysis from bodies or crime scenes. While tape collection effectively captures invisible traces, the optical properties of the tapes introduce interference, complicating trace analysis.

In addition to the optical limitations, the extensive use of tape collection presents challenges in the subsequent analysis process, as shown in Figure [2.26]. This manual examination and interpretation of massive amounts of traces is subjective, labor-intensive, and time-consuming. Forensic experts face the daunting task of analyzing and interpreting a significant volume of trace evidence, which can lead to potential delays in investigations and the introduction of human error and inconsistencies in the results.

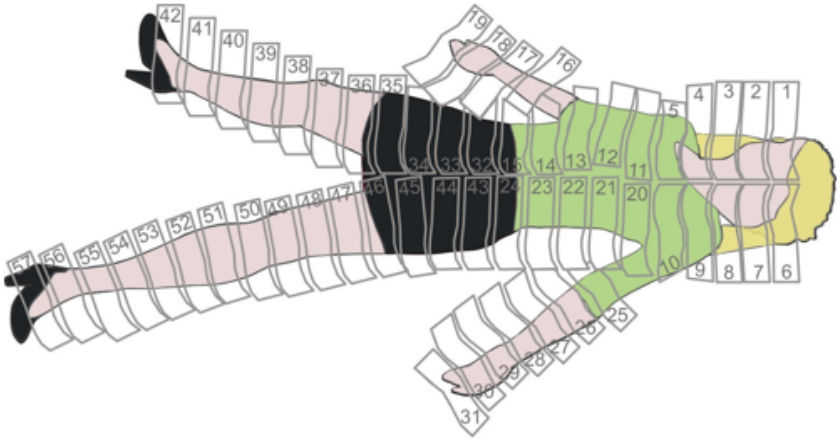


Figure 2.26: Full Body Taping Procudure

To address the challenges faced in forensic science, particularly in the analysis of trace evidence, this research proposes a comprehensive solution. The integration of various analytic and imaging techniques into a single instrument is proposed, offering a multimodal approach to forensic analysis. By combining these techniques within a unified system, a more complete understanding of the materials under investigation can be achieved. By automating routine tasks and leveraging machine learning, the instrument reduces human involvement and improves reaction times. This automation enhances the efficiency, accuracy, and reliability of forensic investigations.

Implementing these solutions in the field of forensic science has the potential to revolutionize forensic investigations and contribute to the advancement of the criminal justice system. The optimized and streamlined collection and analysis processes lead to higher efficiency and accuracy in forensic work, resulting in more reliable and robust results that contribute to the fair administration of justice.

The implications of this research extend beyond forensic science. The integration of various analytic and imaging techniques, along with automation and machine learning, offers opportunities for interdisciplinary applications. Fields such as material science, bioengineering, and environmental monitoring can benefit from the proposed instrument's multimodal approach and high-level scanning technique. The knowledge gained from this research can be applied to different domains, promoting technological innovation and scientific progress.

In conclusion, this research proposes a comprehensive solution to address the challenges in forensic science. The integration of analytic and imaging techniques, along with automation and machine learning, enhances the efficiency, accuracy, and reliability of forensic investigations. The proposed instrument's multimodal aspect and advanced automation hold great potential for improving investigative capabilities and advancing scientific knowledge across disciplines.

Chapter 3

System Design

3.1 Introduction

In the previous Chapter, we discussed the importance of Automated Microscopy and the advancements it has brought to forensics sciences and diagnosis. With the aim of further enhancing the observation experience and enabling multimodal analysis, this chapter focuses on the Proposed System Design of our High Throughput Screening Microscope. The design of this system is intended to address the evolving needs of researchers and forensics Scientists by providing a flexible and adaptable platform for automated microscopy.

The proposed system design represents a significant upgrade from our previous work, as it aims to be more flexible, efficient, and capable of accommodating more sophisticated designs in high throughput screening applications. It encompasses various components and considerations that contribute to the seamless integration of automation into the microscopy workflow. By leveraging the capabilities of modern technology, our proposed design aims to optimize efficiency, accuracy, and reproducibility in the imaging process. Through modular architecture, the system allows for customization and expansion to accommodate diverse imaging modalities, experimental setups, and sample types.

Key aspects of the proposed system design include the integration of advanced imaging techniques, such as fluorescence, polarization, and hyperspectral microscopy, to enable high-resolution imaging and capture specific trace features. Additionally, the design enables the creation of intelligent automation algorithms and image analysis algorithms to facilitate automated sample scanning, focusing, and data acquisition, reducing human intervention and minimizing user bias.

In this chapter, we will delve into the details of the proposed system design, exploring the architectural framework, hardware components and system networking. By elucidating the technical specifications and functionalities of our High Throughput Screening Microscope, we aim to provide a comprehensive understanding of the system's capabilities and its potential impact on forensics microscopy and diagnosis.

Through this System Design chapter, we lay the foundation for the subsequent implementation and evaluation stages of our research. By presenting a detailed and well-structured design, we aim to facilitate the development of an advanced automated microscopy system that can significantly contribute to the field of forensics diagnostics.

3.2 Mark One Design

The previous research introduced a system design that served as a user-friendly motorized extension for digital microscopes. The primary objective was to enable comprehensive control and automation of the mechanical and optical components of the microscope, which are the main areas of interaction for specialists. As a result, the developed microscope extension facilitated scanning and analysis of samples using various techniques, requiring minimal human intervention. The incorporation of motorization in key microscope components proved to be crucial, providing users with a comfortable and relaxed imaging experience, contrasting with the traditional usage of microscopes. The benefits of this design are illustrated in Figure.[3.4].

System's Configuration & Setup The Mark One Automated Microscope design was conceived with the fundamental objective of creating a modular extension for regular microscopes, coupled with user-friendly software, to unlock the full potential of such a design. During the implementation of the Motor Driver and Focusing sections of this research, significant progress was made in establishing the initial setup and integrating it into the main software of the system. This accomplishment was made possible with the invaluable support and guidance provided by the esteemed researchers from the Electronics Laboratory at the Technical University of Crete.

Figure 3.1: (a) Standard microscopy, (b) Advanced Automated Multimodal Microscopy

As an outcome of our previous work, we successfully integrated all the custom made peripherals and established basic functionality for motion enhancements in the automated microscope. This involved installing the hardware, developing effective communication protocols, and constructing robust navigation processes. Additionally, we advanced the microscope's acquisition capabilities by implementing Auto Focus algorithms and evaluating the Absolute Metric for Focus Quality. Furthermore, we introduced an Area Scanning procedure for acquiring consecutive adjacent images, with the potential for future image stitching. These achievements form a solid foundation for our current research. The microscope is shown in Figure [3.2].

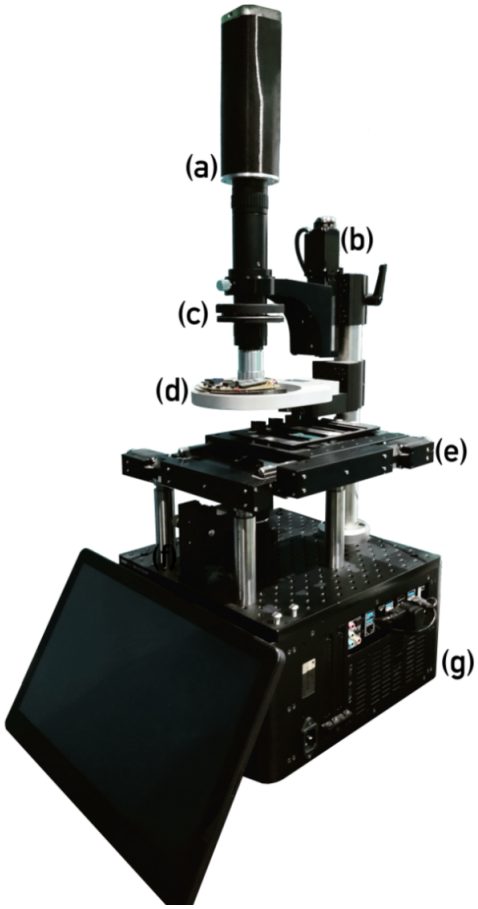


Figure 3.2: Lumnia Updated Hardware

Figure [3.2], presents the final setup of our Mark One microscope. This microscope utilizes (a) Multimodal Camera, (b) Z motor for Focus Control, (c) Variable Magnification and Encoder, (d) Epi Illuminations Controllable Light Source, (e) X Y Stage for Spatial Control, (g) enclosed high performance computer, Three Axes Motor Driver and Transmission Controllable Light Sources. This Mark One microscope design was capable of acquiring basic imaging modalities, as illustrated in the Figure [3.3].

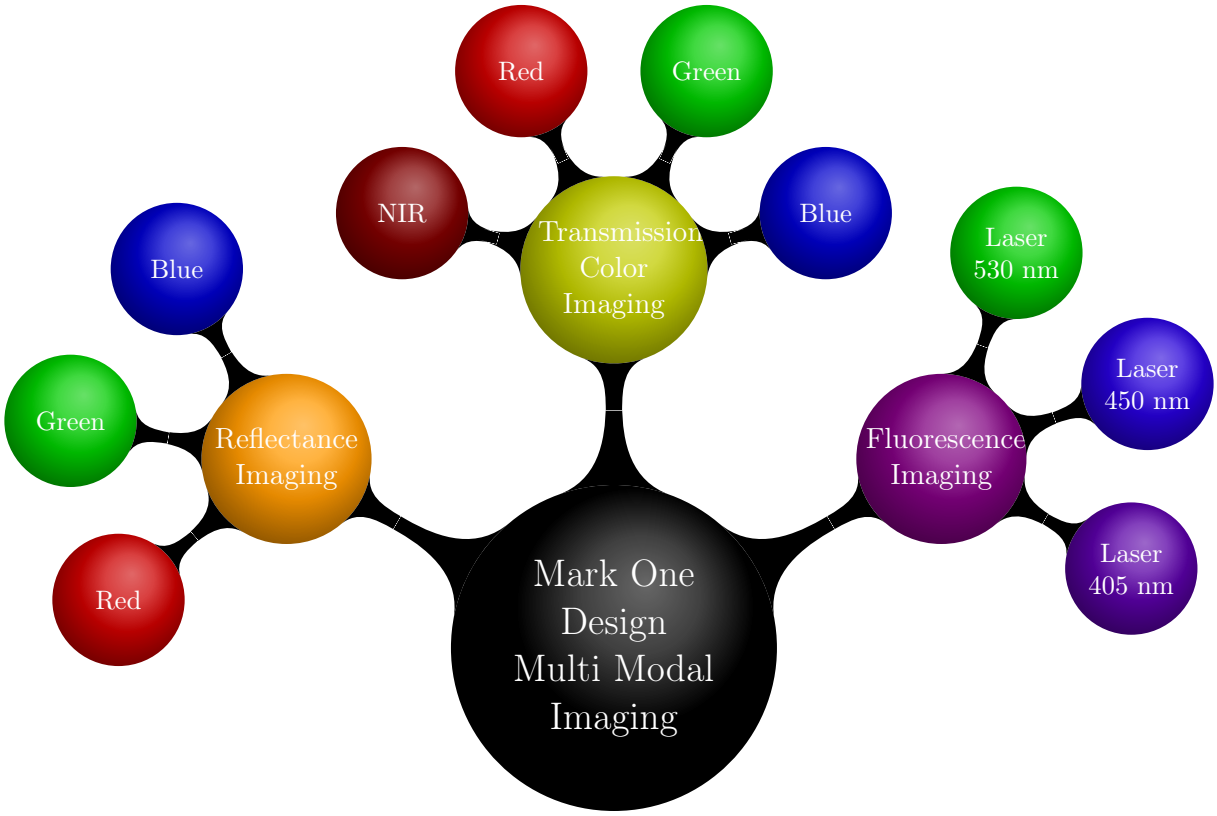


Figure 3.3: Mark One Design Imaging Modalities

The design was inherently constrained by the use of a low-power USB-based peripheral setup and the requirement to function as an easily installable motorized extension for regular microscopes. These limitations, particularly the reliance on USB connectivity, imposed constraints on the scalability and performance of the implemented peripherals. The communication between peripherals within the previous design was hindered by the inherent limitations of USB controllers in the connected computer systems, resulting in slow and unreliable data transfer. This was especially evident when the USB controllers lacked sufficient throughput and power capabilities.

3.3 Mark Two Design

In the realm of trace identification and analysis, the need for efficient and reliable methods to handle massive populations of traces has become increasingly critical. Traditional manual microscopy approaches are often time-consuming and prone to human error, limiting their suitability for high throughput screening applications. To address these challenges, this section focuses on the design of a microscope system specifically tailored for the identification of a massive population of traces, enabling automated scanning and analysis with minimal human interaction.

The key objective of this high throughput screening design is to enhance efficiency and accuracy while minimizing human intervention. By automating the most commonly used parts of a microscope, such as stage movement, focusing, and objective selection, we enable a comfortable and relaxing imaging experience for specialists. The automated extension seamlessly integrates with existing digital microscopes, expanding their capabilities to handle large volumes of samples and perform sophisticated analysis tasks. The design of the system involves careful consideration of the hardware and software components necessary for robust and reliable operation. This ensures consistent scanning of samples and allows for rapid image acquisition over a large field of view.

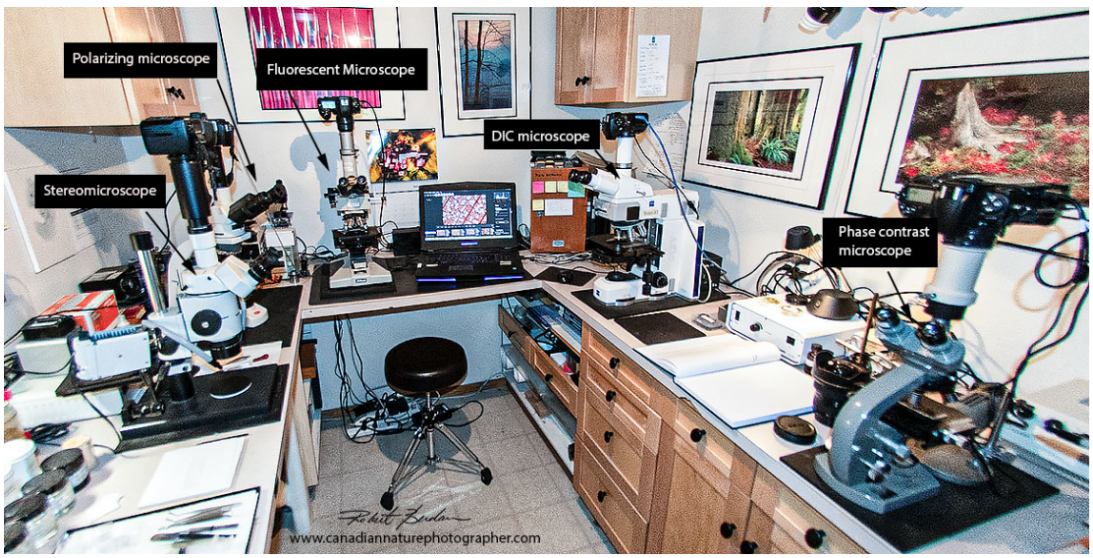


Figure 3.4: Forensics laboratory utilizing different microscopy techniques for traces identification

Source: scienceandart.org

Figure [3.4], demonstrates the transformative potential of the proposed system compared to traditional microscope usage. The motorization of essential microscope components results in a streamlined workflow, enabling specialists to focus on data

analysis and interpretation rather than manual tasks. The system's intuitive user interface and automation capabilities enhance the overall imaging experience, facilitating efficient high throughput screening of trace populations. The outcome of the high throughput screening analysis is a very useful tool for in-depth examination and interpretation. The system is designed to generate comprehensive reports, visualizations, and statistical analyses to aid in the identification and characterization of traces. By providing specialists with a wealth of data and insights, the system supports their research efforts and accelerates the pace of discovery.

3.3.1 System Requirements

The research focuses on the following functionality and system requirements:

- Imaging Requirements.
  1. Transmission Color and Hyperspectral Imaging.
  2. Transmission Polarization Imaging.
  3. Epi Color and UV Imaging.
  4. Epi Fluorescence Imaging.
- Hardware Requirements.
  1. X-Y Movement Resolution < 10 µm.
  2. X-Y Movement Range $> 2500\ cm^2$ ($\sim$ 4 A4 sheets).
  3. Z Movement Resolution < 1 µm.
  4. Z Movement Range $> 5\ cm$ ($\sim$ working distance).
  5. Selective Transmission Illumination of circular polarized light.

Ultraviolet (nm)	**320**	**340**	**365**	**385**	**405**
Visible (nm)	**450**	**490**	**515**	**590**	**630**
Near Infrared (nm)		**780**	**850**	**980**	

  6. Selective Epi Illumination.

Wavelength (nm)	**365**	**405**	**450**	**Visible**

  7. Independent Power Management and Hardware Diagnosis.
- Firmware Requirements.
  1. Real-time decision making system minimizing jerk on each axis of movement.
  2. Fast and reliable illumination condition alternation mechanism.
  3. Parallel hardware architecture with a simple driver.

The Mark Two Microscope is carefully designed to provide rapid and precise acquisition of an extensive array of imaging modalities. This advanced design integrates multiple imaging techniques, enabling the capture of diverse and detailed data, as illustrated in the Figure [3.5].

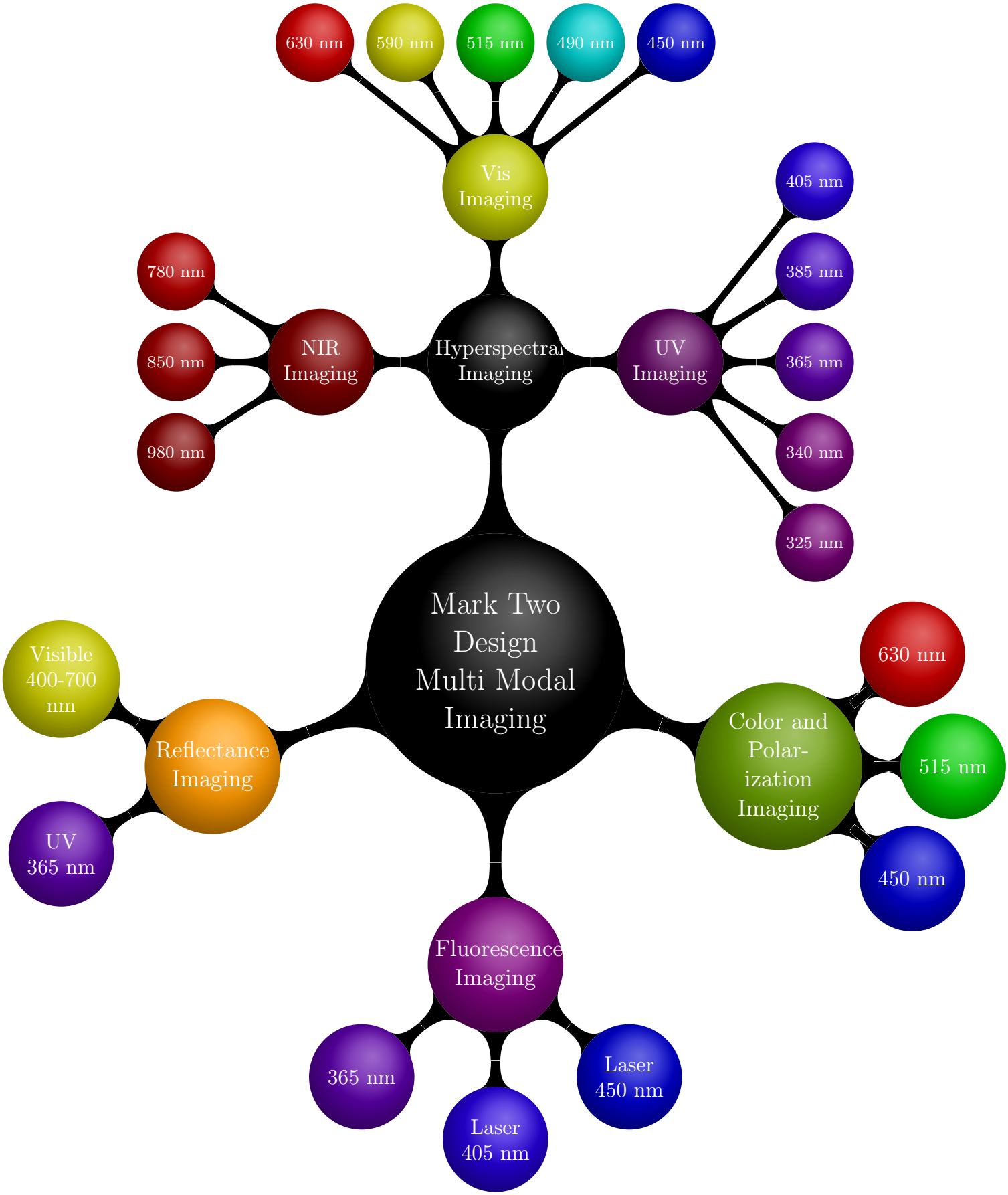


Figure 3.5: Mark Two Design Imaging Modalities

The development of this microscope relies on the integration of high-performance light sources and dedicated imaging sensors capable of robustly acquiring the required imaging techniques. The incorporation of a motorized extension enables rapid acquisition of multiple imaging modalities and facilitates scanning capabilities. Accurate registration and focusing of each acquired image for a given position is crucial in acquiring multiple imaging modalities, and this challenge can be addressed through fast and reliable motion readjustment for focus and 2D positioning. The hardware components utilized in this thesis are shown in Figure [3.6]

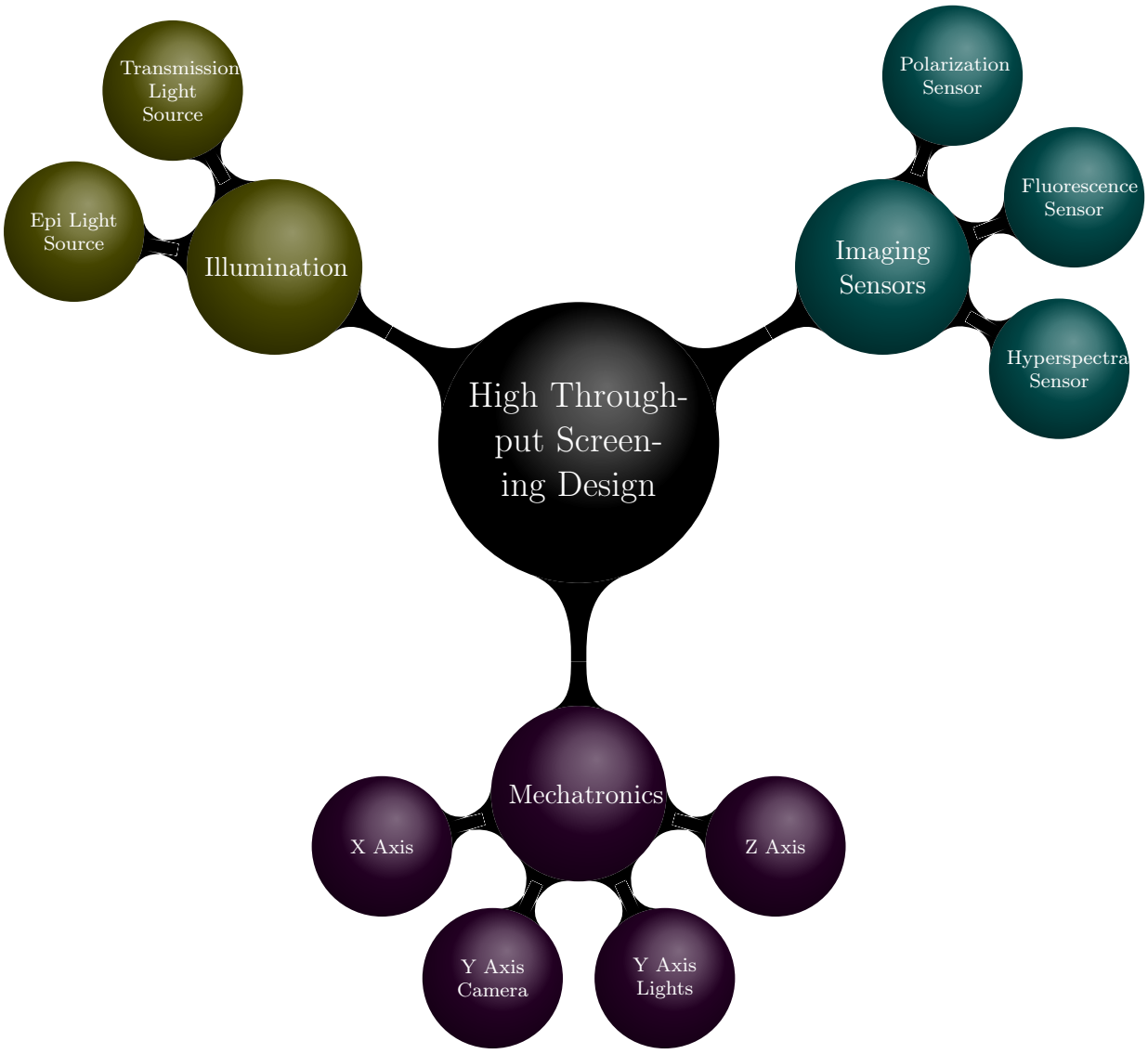


Figure 3.6: Mark Two System Hardware Design

3.4 Architecture

Through the design of this high throughput screening microscope system, we aim to revolutionize the field of trace identification and analysis. By combining automation, advanced imaging techniques, and intelligent data analysis, our system empowers specialists to tackle the challenges posed by massive populations of traces. In the following sections, we will delve into the technical details of the system design, exploring the hardware and software aspects that enable its functionality and usability.

3.4.1 Hardware Architecture

In the context of high throughput screening microscopy for forensic sciences, the system design plays a crucial role in achieving accurate and efficient analysis of massive populations of traces. This chapter presents the proposed system design, focusing on the hardware components that enable movement, magnification, light control, and sample digitization. The following components are key to the system design

- **High Resolution Motorized XY Linear Stage**

  A motorized XY linear stage provides precise and repeatable movement of the microscope's sample. It allows the user to set and save important location points for future inspections. This stage enables large out-of-field-of-view image capture through stitching, facilitating measurement of features that exceed a single field of view. By automating geometric measurements at multiple points, the system improves sample acquisition speed and enhances analysis repeatability.

- **High Resolution Motorized Z Linear Stage**

  A motorized Z linear stage controls the focus of the microscope. It enables fast, accurate, and repeatable camera height adjustments, eliminating the need for manual inspection. Auto-focusing functionality ensures sharp images without human intervention. The Z linear stage also enables extended focal imaging (EFI) by capturing multiple Z focus images, which can be used to generate all-in-focus images, 3D reconstructions, and Z height measurements. Additionally, the Z linear stage contributes to the creation of a focus map during XY plane

stitching, compensating for sample drift and acquisition height data for each image.

– **Custom Light Sources**

Custom-designed light sources are integral to controlling the microscopy techniques employed during sample acquisition. The utilization of various light sources expands the capabilities of the microscope, transforming it into a multimodal scanning microscope with automated light conditioning. This automation enables the system to execute procedures requiring different lighting conditions and to auto-calibrate the correlation between processes and peripherals.

– **Digital Camera**

A digital camera facilitates the capture, processing, and visualization of samples in high-resolution digital format. Unlike traditional binocular techniques, which relied on human observation and memory, the digital camera significantly improves imaging throughput and technical capabilities. With the digital camera, the system can leverage both hardware and software for accurate and repeatable image processing, storage, and analysis.

In summary, the full motorization and digitization of the microscope, combined with custom illumination capabilities, result in a powerful and autonomous system. This system can be programmed to execute specialized acquisitions with exceptional accuracy and repeatability. The proposed hardware design offers a fast, ergonomic, and precise measuring instrument, capable of handling the demands of high throughput screening microscopy in forensic sciences.

3.4.2 Firmware Architecture

In the proposed system design, a network of parallel processors is prioritized over a centralized processor architecture. This decision is motivated by two key factors: modularity and performance.

– **Modularity**Parallel processors offer a modular and scalable system design. Tasks are distributed among multiple processors, ensuring independent oper-

ation and easier maintenance. This architecture enhances fault tolerance and efficient resource utilization.

- **Performance** Parallel processing in a network of processors delivers superior performance compared to a centralized processor. High throughput screening microscopy necessitates real-time processing of large data volumes. Parallel processing enables the distribution of computational load across multiple processors, resulting in significantly reduced processing time. Concurrent execution of tasks in parallel facilitates faster image acquisition, analysis, and decision-making.
- **Real-Time Data Processing and Communication** To efficiently handle high-throughput screening microscopy and real-time processing tasks, a microprocessor with parallel communication data handling capabilities is crucial. It should support high-speed interfaces and industry-standard protocols for seamless data transfer. The microprocessor should also possess ample computational power, memory capacity, and optimized instruction set architectures to handle image analysis, feature extraction, and decision-making. By selecting the right microprocessor, the system can effectively acquire large trace datasets and perform real-time processing tasks.

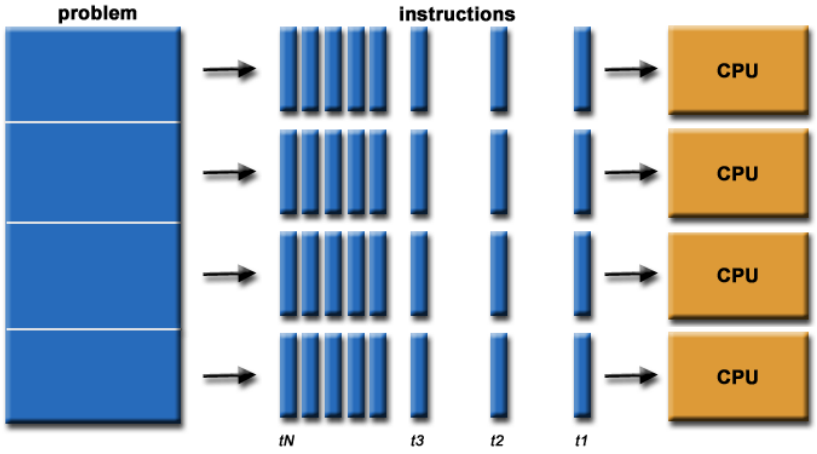


Figure 3.7: Problem Solving over Distributed System
Source: wstein.org

Utilizing a network of parallel processors in the system design offers notable advantages in terms of modularity and performance. The modular architecture enables scalability, flexibility, and fault tolerance, while parallel processing substantially improves computational speed and efficiency. By leveraging these benefits, a high throughput screening microscopy system can be created to meet the demanding requirements of forensic sciences.

3.5 System Structure

The Mark Two system implementation involves the design of a comprehensive automated microscope, incorporating essential motion and illumination related components. The system's block diagram, as depicted in Figure [3.8], highlights the components developed as part of this thesis in green, showcasing their integral role in the overall design.

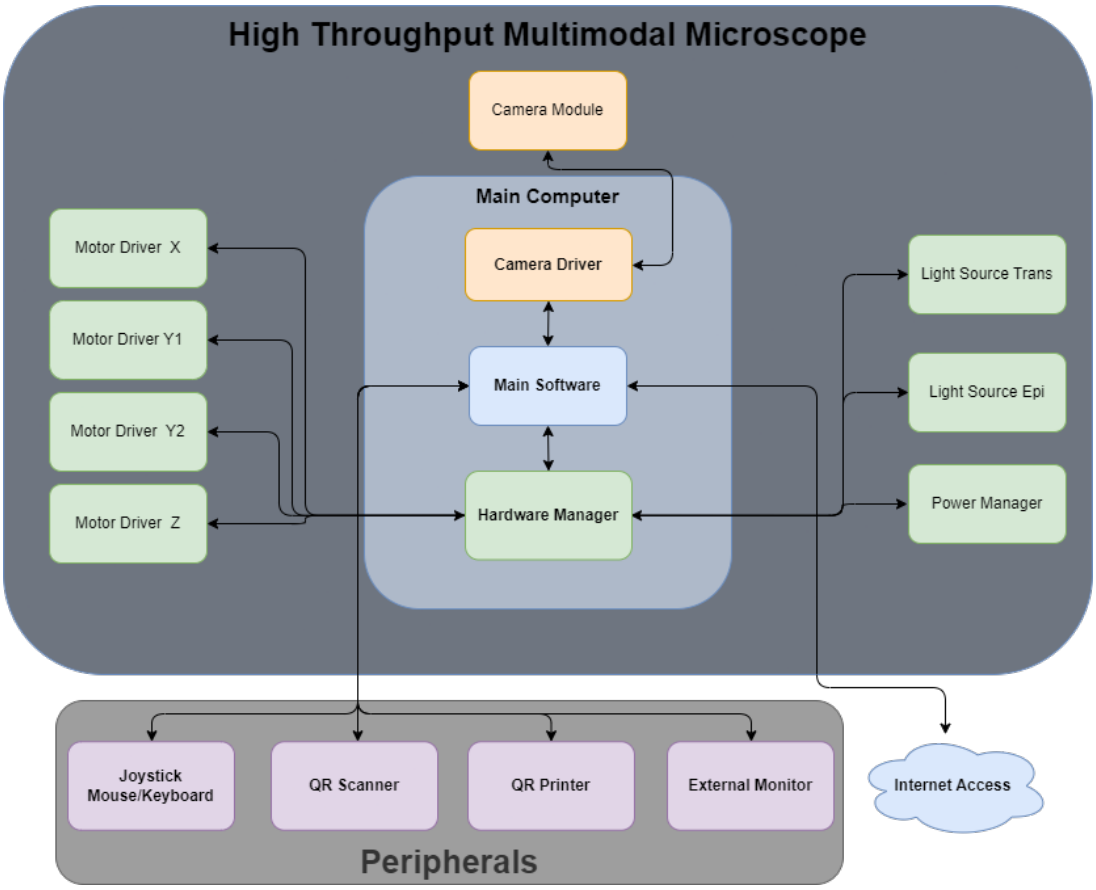


Figure 3.8: System Block Diagram

The X Axis Motor enables movement of the entire camera and illumination system along the X direction. Two independent Y Axis Motors facilitate parallel movement of the Camera Module and Transmission Illumination Module, ensuring that the sample area remains undisturbed. The Z Axis Motor controls the focusing of the camera module. Additionally, the design includes two illumination sources for Epi and Transmission illumination, along with a power manager for efficient measurement and power management. Subsequently, the upcoming sections of this chapter will provide an in-depth analysis of the intricate implementation process involving the individual drivers, their interconnections, and the overall system communication.

Chapter 4

Implementation

4.1 Introduction

This chapter provides a comprehensive description of the implementation procedure and the final structure of the High Throughput Screening microscope that has been designed for this thesis. The construction of such a device entails several crucial aspects, which will be discussed in detail within the following sections.

The centerpiece of this microscope design is the Multimodal Imaging Block, which incorporates an advanced optical setup enabling the real-time acquisition of various imaging modalities. The system's speed and accuracy heavily rely on this imaging block's capability to acquire different modalities simultaneously, with the overall performance being limited by the motion and illumination peripherals' speed and accuracy. Initially, the creation of the imaging block and its core optical characteristics will be discussed in-depth. This section will outline the key components, their configuration, and the principles underlying their integration. The emphasis will be on achieving high-quality imaging and seamless integration of multiple modalities.

Subsequently, the creation of the distributed real-time hardware will be thoroughly documented. This hardware implementation is designed to facilitate the seamless coordination and synchronization of the microscope's various components. The centralized instruction system, which plays a pivotal role in controlling and coordinating these components, will also be described in detail.

By providing a meticulous description of the implementation procedure and the structure of the High Throughput Screening microscope, this chapter aims to offer valuable insights into the design and construction of this advanced imaging system.

4.2 Peripherals Selection

The implementation phase of this thesis involves selecting and integrating key components such as motorized stages, light sources, and MCUs (Microcontroller Units). These components are carefully chosen to meet the specific requirements outlined in previous chapters. The integration of motorized stages enables precise and automated movement for accurate positioning and focusing. High-power LED light sources are incorporated to provide efficient and versatile illumination. MCUs are integrated to effectively control and coordinate the system, enabling real-time data processing and decision-making. The following sections provide detailed insights into the implementation procedures and seamless component integration to achieve the research objectives.

4.2.1 Integration of Motorized Stages

Initially, the selection of linear motorized stages is carried out by considering the specific system requirements outlined in the preceding chapter.

- **High Resolution Motorized X-Y Linear Stage**

  To meet the system requirements, we have chosen the Standa 8MT195 - Long-travel Motorized Linear Stage as the suitable option for the X-Y High Resolution Motorized Linear Stage, as depicted in Figure [4.1]. This motorized stage exhibits a Full Step Resolution of 12.5 μm and can achieve a micro-stepping capability of 1/256. In our configuration, a micro-stepping of 1/16 or 0.785 μm per step is employed. Additionally, this stage offers a maximum speed of 10 mm/sec and possesses a horizontal load capacity of up to 50 kg and a vertical load capacity of up to 40 kg, as specified in the datasheets provided by Standa.

- **High Resolution Motorized Z Linear Stage** To meet the system requirements, we have opted for the Standa 8MT30-50 - Narrow Motorized Translation Stage as the ideal choice for the Z High Resolution Motorized Linear Stage, as depicted in Figure [4.2]. This motorized stage offers a Full Step Resolution of 1.25 μm and supports micro-stepping up to 1/256. In our configuration, a micro-stepping of 1/16 or 0.078 μm per step is employed. Additionally, this stage enables speeds up to 5 mm/sec and possesses a horizontal load capacity of up to 5 kg and a vertical load capacity of up to 3 kg, as indicated in the provided datasheets from Standa.

Figure 4.1: High Resolution Motorized X-Y Linear Stage - 8MTF - Motorized XY Scanning Stage Standa

Source: standa.lt

Figure 4.2: High Resolution Motorized Z - 8MT30-50 - Narrow Motorized Translation Stages

Source: standa.lt

4.2.2 Integration of Light Sources

Next, the implementation process moves towards integrating the necessary light sources for transmission and epi illumination. For transmission illumination, we have chosen a high-intensity LED light source that provides consistent and uniform illumination across the entire sample area for transmission color and hyperspectral imaging. As for epi illumination, high-power laser diode modules with a specific wavelength as well as high-intensity LED light source are selected to ensure precise and focused illumination for optimal fluorescence and reflectance imaging results.

In addition to the light sources, the implementation also involves incorporating a power manager module. The power manager acts as a central control unit for managing the power supply to different components of the system. It ensures proper voltage regulation, current monitoring, and power distribution to maintain stable and reliable operation. The power manager module includes safety features such as over-current protection and thermal management to prevent any damage or mal-

function due to excessive power consumption or overheating.

The integration of these light sources and the power manager module is crucial for achieving efficient and controlled illumination, as well as maintaining the overall stability and safety of the system. The subsequent sections will delve into the detailed implementation and integration procedures, covering aspects such as electrical connections, control interfaces, and synchronization with other system components.

– **High Power LED Sources**

  In order to fulfill the requirements of our system, we have successfully integrated high-power LEDs from Roithner Lasertechnik into our setup, as shown in Figure [4.3]. For broad wavelength range applications, we have selected the surface mount InGaN-based LED models, which offer both high power output and a broad emission spectrum spanning from 400 nm to 1000 nm. These LEDs are housed in SMD packages using PA9T material, ensuring durability and reliability. The soldering pads are silver-plated and lead-free, ensuring a secure connection. To optimize optical performance, the LEDs feature a silicone resin molded flat window and a copper heat sink for efficient heat dissipation. Additionally, for narrowband applications, we have incorporated AlGaInP-based LED models. The integration of these high-power LEDs from Roithner Lasertechnik empowers our system with versatile and efficient illumination capabilities, enabling us to achieve optimal results for our specific application.

Figure 4.3: High Power LED Sources Roithner Lasertechnik
Source: roithner-laser.com

– **High Power Laser Diode Modules** To meet the specific requirements of our application, we have integrated the high power series of Dot Diode Laser Modules from Roithner Lasertechnik, as shown in Figure [4.4]. These laser diode modules have been meticulously designed to prioritize superior beam quality and ensure reliable operation. The modules feature a robust body made of black anodized aluminum, which encloses the laser diode, lens, and driving electronics. The Dot Laser series incorporates a focusable glass lens

optic with a secure locking mechanism, allowing for precise control over the beam focus. The 5 VDC driver circuit integrated into the modules provides support for APC (automatic power control) and TTL modulation up to 20 kHz. This enables us to effectively control the power output and modulation of the laser diode modules, enhancing their versatility and adaptability for our application. The integration of these high-power laser diode modules from Roithner Lasertechnik ensures optimal performance and reliability in achieving our research objectives.

Figure 4.4: High Power Laser Diode Modules Roithner Lasertechnik
Source: roithner-laser.com

4.2.3 Integration of Microcontroller Units (MCUs)

In this section, we will discuss the integration of microcontroller units (MCUs) into the application of the thesis. MCUs play a vital role in controlling and coordinating various components, ensuring precise and synchronized operation of the system. The selection of MCUs is based on their processing power, input/output (I/O) capabilities, and compatibility with the system requirements.

For the implementation, we have chosen MCUs that meet the demanding needs of the application. These MCUs provide sufficient computational power and are equipped with the necessary peripherals to interface with the different system components. Their real-time capabilities enable efficient control and coordination of the light sources, motorized stages, and other peripherals. The MCUs act as the central control units, facilitating communication between different components and enabling synchronized operation. The integration of the MCUs involves programming them to implement the required functionalities. The tasks performed by the MCUs include:

- Control and coordination of the light sources, adjusting their intensity and timing for precise illumination.
- Managing the power modules, ensuring proper voltage regulation, current monitoring, and power distribution.
- Implementing safety features such as over-current protection and thermal management.
- Handling data processing tasks, enabling real-time motion control and decision-making.
- Establishing electrical connections, control interfaces, and synchronization mechanisms for seamless integration into the system architecture.

The main processor used in our application is the ESP32 - Xtensa® dual-core 32-bit LX6 microprocessor. The dual-core processing capabilities of this MCU, combined with the FreeRTOS™ Real-time operating system, make it a powerful choice for our application. The use of dual cores allows for parallel execution of the device's independent functionalities, enabling efficient multitasking and real-time operation. The ESP32 MCU provides a robust platform for our object-oriented design and multitasking requirements. Each core is assigned specific tasks, ensuring optimized utilization of computational resources. This design approach enables seamless communication and execution of commands in real-time, enhancing overall system performance. With the ESP32 MCU, we have achieved high efficiency in both computational and power terms. The capabilities of the MCU, coupled with the object-oriented design and multitasking support, contribute to the development of a reliable and efficient system.

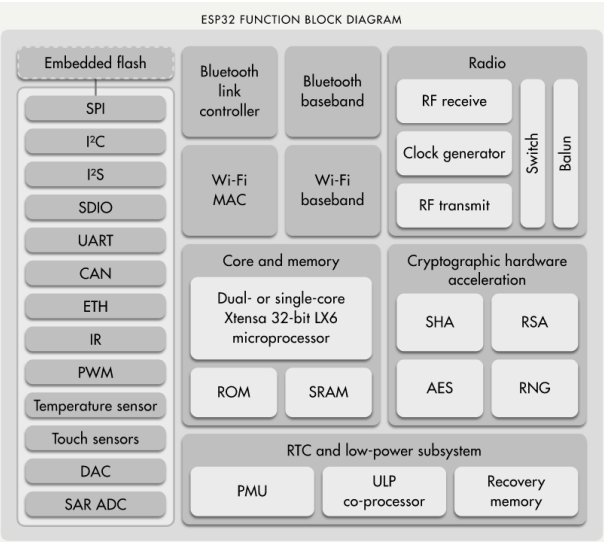


Figure 4.5: ESP32 Function Block Diagram
Source: esp32.net

For our application, we utilized the powerful and fast cores of the ESP32 System on Chip (SoC). This SoC operates at a clock speed of 240 MHz, providing high-speed processing capabilities. We optimized our code to leverage specific functional blocks of the ESP32, as depicted in its functional block diagram (Figure [4.5]). The ESP32 SoC offers a range of functionalities, including WiFi, Bluetooth, radio communications, cryptography, and low-power applications. In our implementation, we focused on utilizing its capabilities for real-time processing. Two specific functionalities that we implemented independently are hardware communication using Network based on the Ethernet and fast GPIO manipulation using hardware drivers.

By utilizing the Ethernet Network communication, we established reliable and efficient data exchange between the MCU and external devices. This enabled seamless integration with various peripherals and facilitated communication protocols. The fast GPIO manipulation through hardware drivers allowed for efficient control and manipulation of GPIO pins, enabling high-speed and precise interactions with external components.

With the ESP32 SoC, we were able to achieve our main objective of implementing independent hardware communication and hardware drivers. The utilization of this SoC's capabilities enhances the overall performance and functionality of our system.

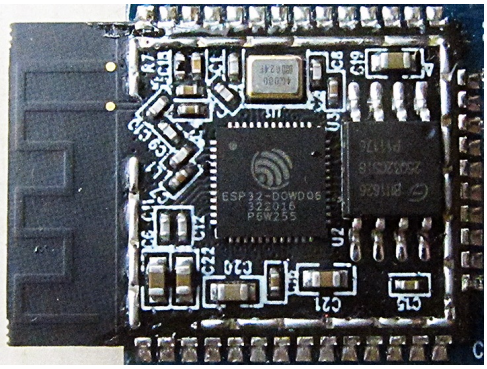


Figure 4.6: ESP-WROOM-32
Source: wikipedia.org

Utilization of the Olimex ESP32-PoE-ISO Board To enhance the connectivity and networking capabilities of our distributed MCU system, we incorporated the Olimex ESP32-PoE-ISO board. This board played a crucial role in establishing Ethernet network connectivity, enabling seamless communication and data transfer

between the different components of our system.

One of the primary reasons for selecting the Olimex ESP32-PoE-ISO board was its built-in Power over Ethernet (PoE) functionality. This feature allowed us to conveniently power the board and provide network connectivity through a single Ethernet cable. By utilizing PoE, we eliminated the need for separate power supplies and significantly simplified the wiring and installation process. Another key advantage of the Olimex ESP32-PoE-ISO board was its galvanic isolation. This isolation provided electrical separation between the Ethernet interface and the rest of the board's circuitry. This feature protected the MCU and other sensitive components from potential voltage surges or electrical noise that may occur on the Ethernet network. The galvanic isolation ensured reliable and stable operation, minimizing the risk of damage to the system.

Additionally, the Olimex ESP32-PoE-ISO board offered a compact and user-friendly design, making it easy to integrate into our system. It provided convenient connectivity options, including Ethernet ports and GPIO pins, allowing for seamless integration with other peripherals and components.

Figure 4.7: Olimex Esp32 POE ISO
Source: olimex.com

By utilizing the Olimex ESP32-PoE-ISO board, we were able to establish robust and reliable Ethernet network connectivity within our distributed MCU system. This connectivity facilitated efficient data transfer, synchronization, and communication between the different nodes of the system, enabling seamless coordination and interaction. The integration of the Olimex ESP32-PoE-ISO board enhanced the overall performance, reliability, and scalability of our system, making it well-suited for the demands of our application.

4.3 Motor Drivers

The motor driver module in our system utilizes the ESP32 microcontroller in conjunction with the TMC5160 motor controller. This integration is achieved by incorporating additional circuitry to handle power sequencing and system interrupts, specifically utilizing the motor's linear stage limit switches. The motor driver module is designed to efficiently and safely operate a diverse range of stepper motors.

4.3.1 Motor Controller

The TMC5160 motor controller is optimized for driving stepper motors, featuring advanced motor control algorithms, high-resolution microstepping, and built-in protection mechanisms. By integrating the ESP32 microcontroller with the TMC5160 motor controller, we create a comprehensive motor driver solution that combines computational power with specialized motor control features.

The Watterott's TMC5160 SilentStepStick, equipped with external transistors, is a robust stepper motor controller specifically designed for applications requiring high dynamic performance and torque. It has the capability to handle up to 3A (RMS) of continuous coil current. Leveraging Trinamic's advanced SpreadCycle™ and StealthChop™ chopper technologies, this motor driver offers silent operation, optimal efficiency, and maximum motor torque.

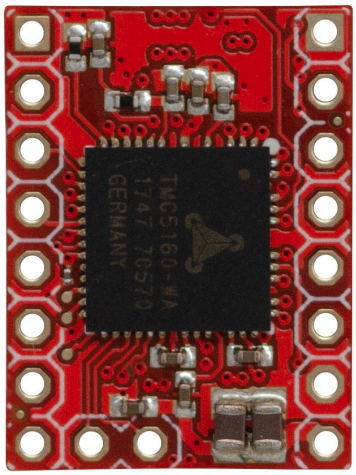


Figure 4.8: TMC5160 Driver
Source: watterott.com

The TMC5160 SilentStepStick provides exceptional motor control by implementing Trinamic's innovative SpreadCycle™ technology. This technology optimizes the cur-

rent control to reduce motor noise and vibrations, resulting in smooth and silent operation. Additionally, the driver incorporates StealthChop™ technology, which enables the motor to operate silently at low speeds by employing a combination of voltage control and sine wave modulation. With its high current handling capacity and advanced chopper technologies, the TMC5160 SilentStepStick ensures efficient and noiseless operation of stepper motors. This driver is suitable for applications that require precise motor control, such as 3D printers, CNC machines, robotics, and automation systems. Its superior performance and reliability make it an excellent choice for demanding industrial and professional applications.

With the inclusion of additional circuitry for power sequencing and interrupt handling, the motor driver module ensures efficient and safe operation of a wide variety of stepper motors. This module plays a vital role in achieving precise motor control and reliable performance across various applications within our system.

4.3.2 Power Sequence

The power sequencing of the TMC5160 motor controller [4.8] is carefully managed using a combination of components. This includes a power relay, a power switch IC (MIC2005), and two adjustable switching regulators (PTN7800WAH). Each of these components plays a specific role in ensuring proper power distribution and control within the TMC5160 system.

The power relay serves the essential function of supplying power to the power MOSFETs, which are responsible for driving the motor coils. This relay ensures the timely and controlled activation of the power circuitry, allowing for efficient and reliable motor operation.

To handle the logic circuitry of the TMC5160, the power switch IC comes into play. This component manages the power supply to the logic circuitry, enabling its proper functioning. By regulating the power flow, the power switch IC ensures the stability and integrity of the control signals and data processing within the TMC5160.

Additionally, the system incorporates an adjustable switching regulators. This regulator provide a stable 5V power supply specifically for the logic circuitry of the TMC5160. Moreover, they facilitate the activation of the internal fan, which aids in maintaining optimal operating temperatures and ensuring the longevity of the

device.

Through the coordinated operation of the power relay, power switch IC, and adjustable switching regulators, the TMC5160 motor controller achieves efficient power management and reliable performance. This integrated power sequencing solution guarantees the proper functioning of the motor control circuitry and supports the cooling requirements of the device.

The block diagram of Figure [4.9], silhouette the system's setup and integration.

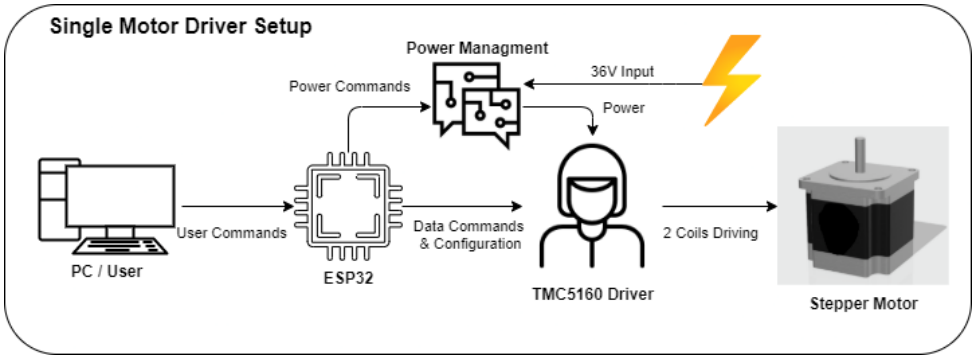


Figure 4.9: Motor Driver Peripherals

4.3.3 Circuitry

Creating our own Motor Driver PCB was created by utilizing the Altium Designer Software. Altium Designer is a PCB and electronic design automation software package for printed circuit boards.

Single Axis Design A single channel Motor Driver was built to prove the principal of operation of our design. Finally, after debugging the Driver we finalize the **Single Channel Motor Driver Schematic** design like shown in the figures [4.10, 4.11, 4.12] below.

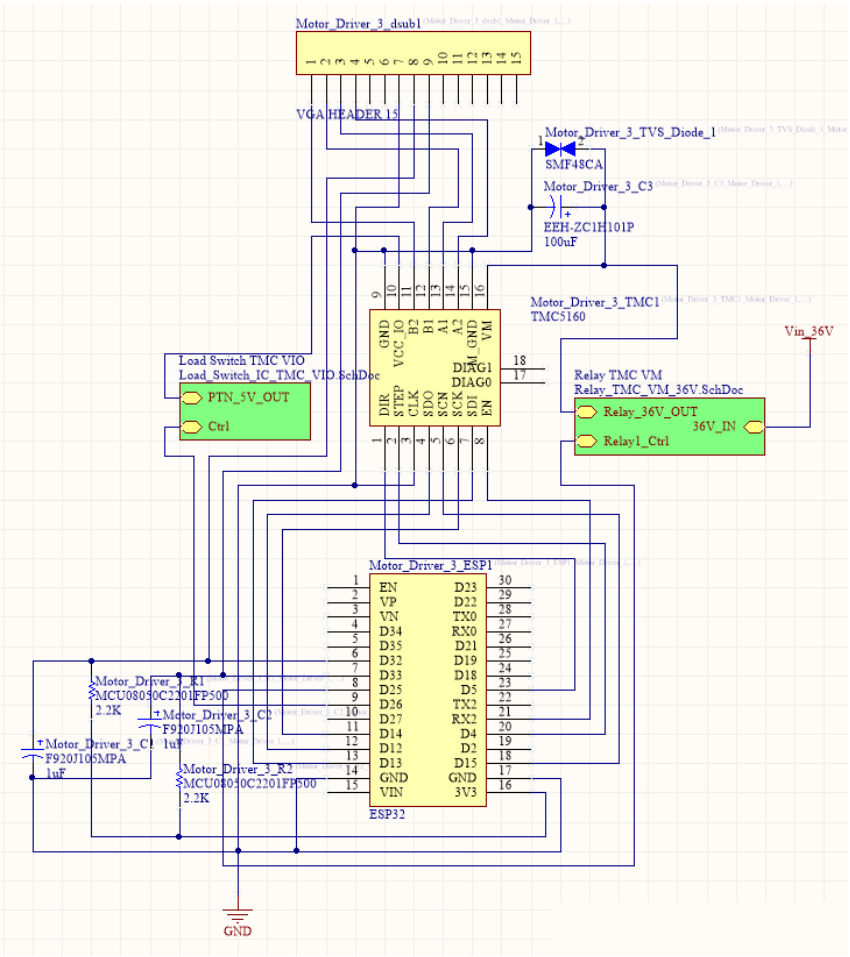


Figure 4.10: Motor Driver Schematic
Source: Altium Designer Motor Driver Project

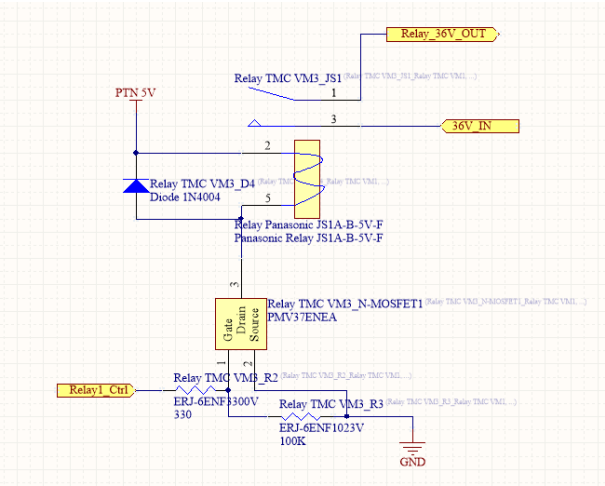


Figure 4.11: Relay Schematic
Source: Altium Designer Motor Driver Project

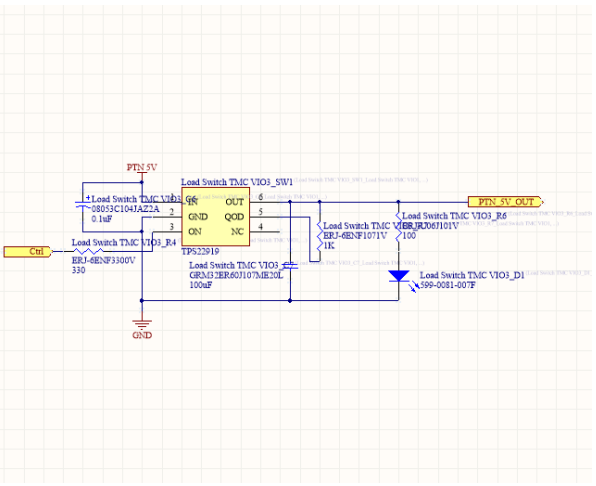


Figure 4.12: Load Switch Schematic
Source: Altium Designer Motor Driver Project

Printed Circuit Board Figures [4.15, 4.16] showcases the Printed Circuit Board (PCB) layout of the network motor driver developed for this project. The PCB design demonstrates a compact and optimized arrangement of components, carefully organized to ensure efficient signal routing and minimal interference. The figure provides a visual representation of the PCB's intricate circuitry, highlighting the precise placement and interconnection of electronic components.

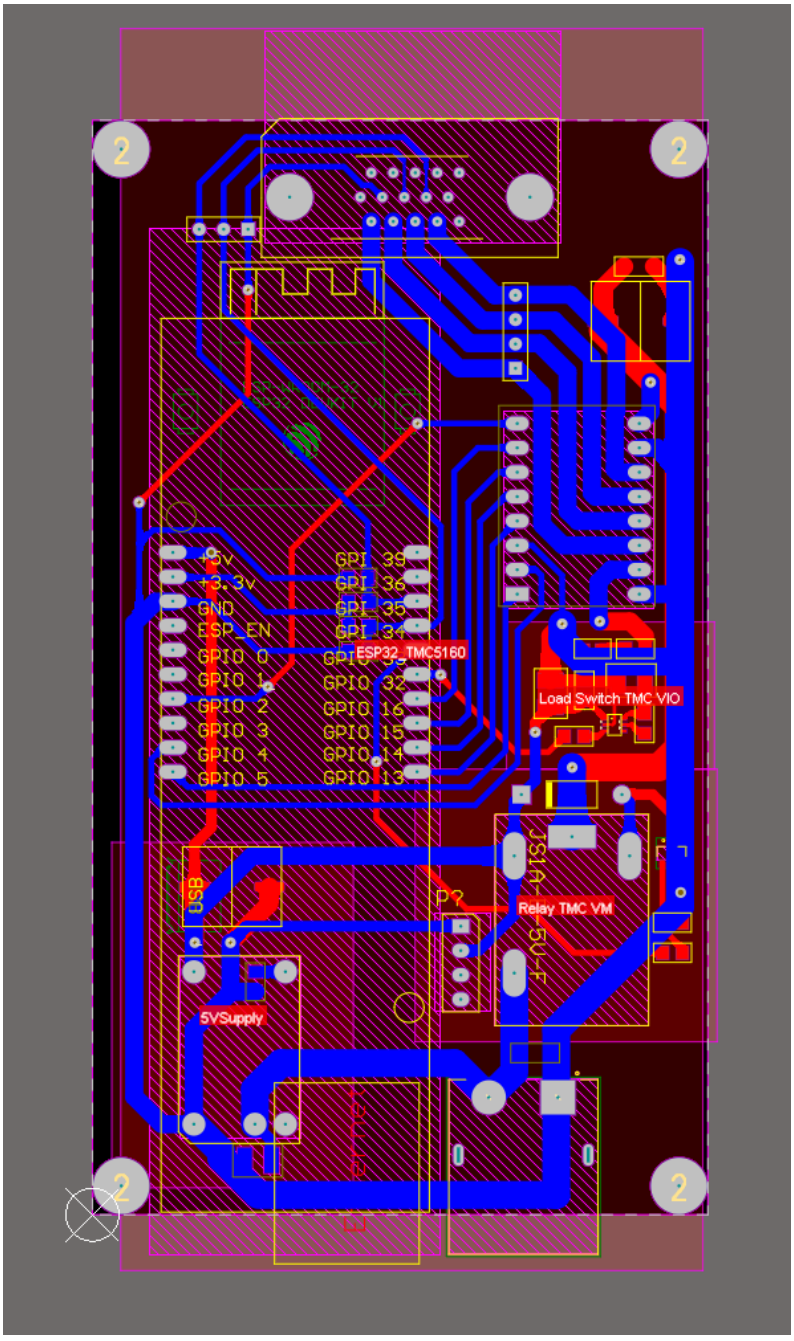


Figure 4.13: Motor Driver PCB
Source: Altium Designer Motor Driver Project

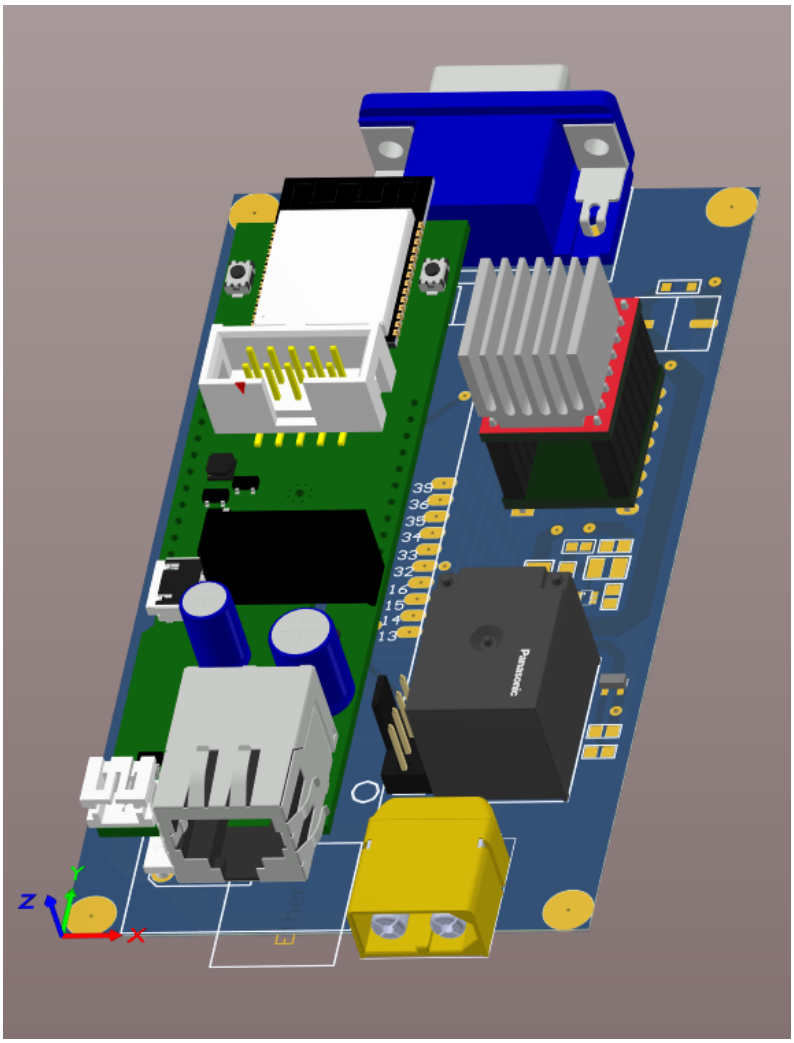


Figure 4.14: Motor Driver PCB 3D
Source: Altium Designer Motor Driver Project

Final Design The final system is enclosed with a robust aluminium case and it is installed inside the microscopes body. A silent but powerful fan is also mounted on the case, to keep the temperature of each Motor Driver within an acceptable range. The motors connectivity to the drivers is accomplished by utilizing a VGA (DB-15) connector. An Ethernet cable manages the communication of the driver and its power is provided through a XT-60 connector.

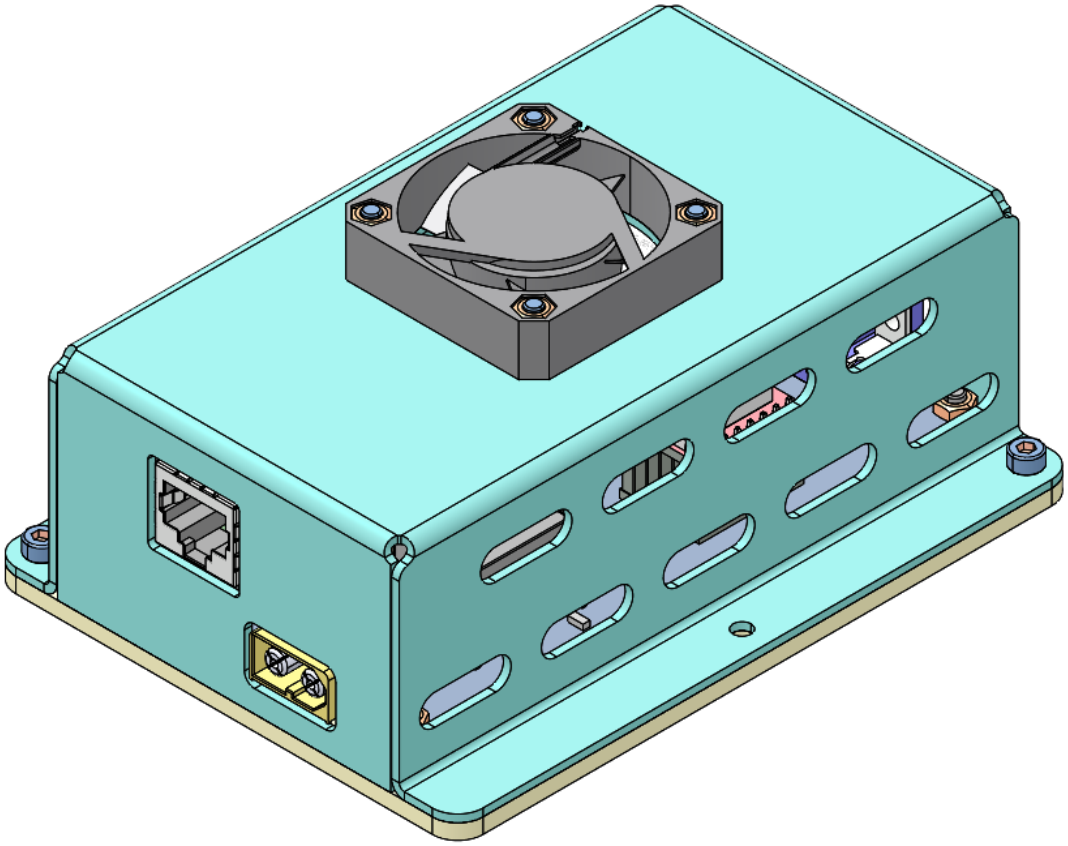


Figure 4.15: Motor Driver Final Design 3D
Source: Altium Designer Motor Driver Project

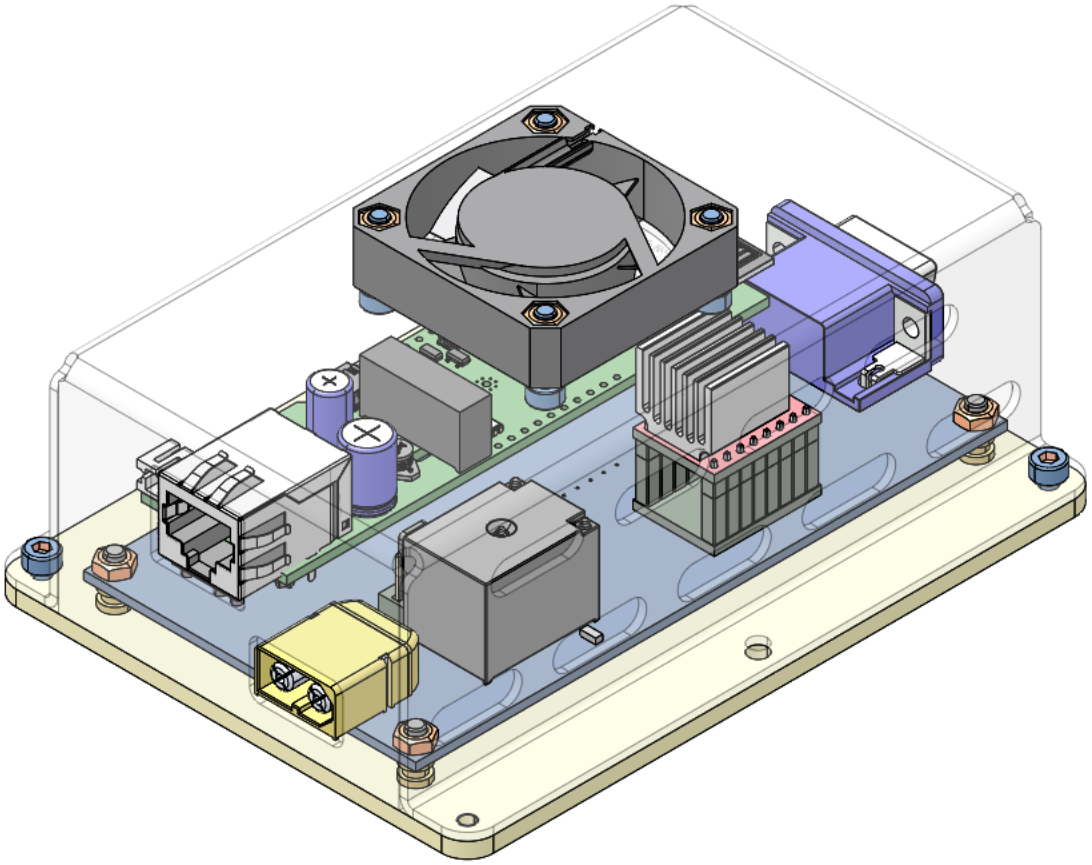


Figure 4.16: Motor Driver Final Design 3D
Source: Altium Designer Motor Driver Project

4.3.4 Functional Segmentation

The **Motor Driver** module is responsible for configuring the TMC5160 driver and controlling the motor based on input commands and motor characteristics. Figure [4.17] illustrates the Functional Block Diagram of the Motor Driver's Design Architecture.

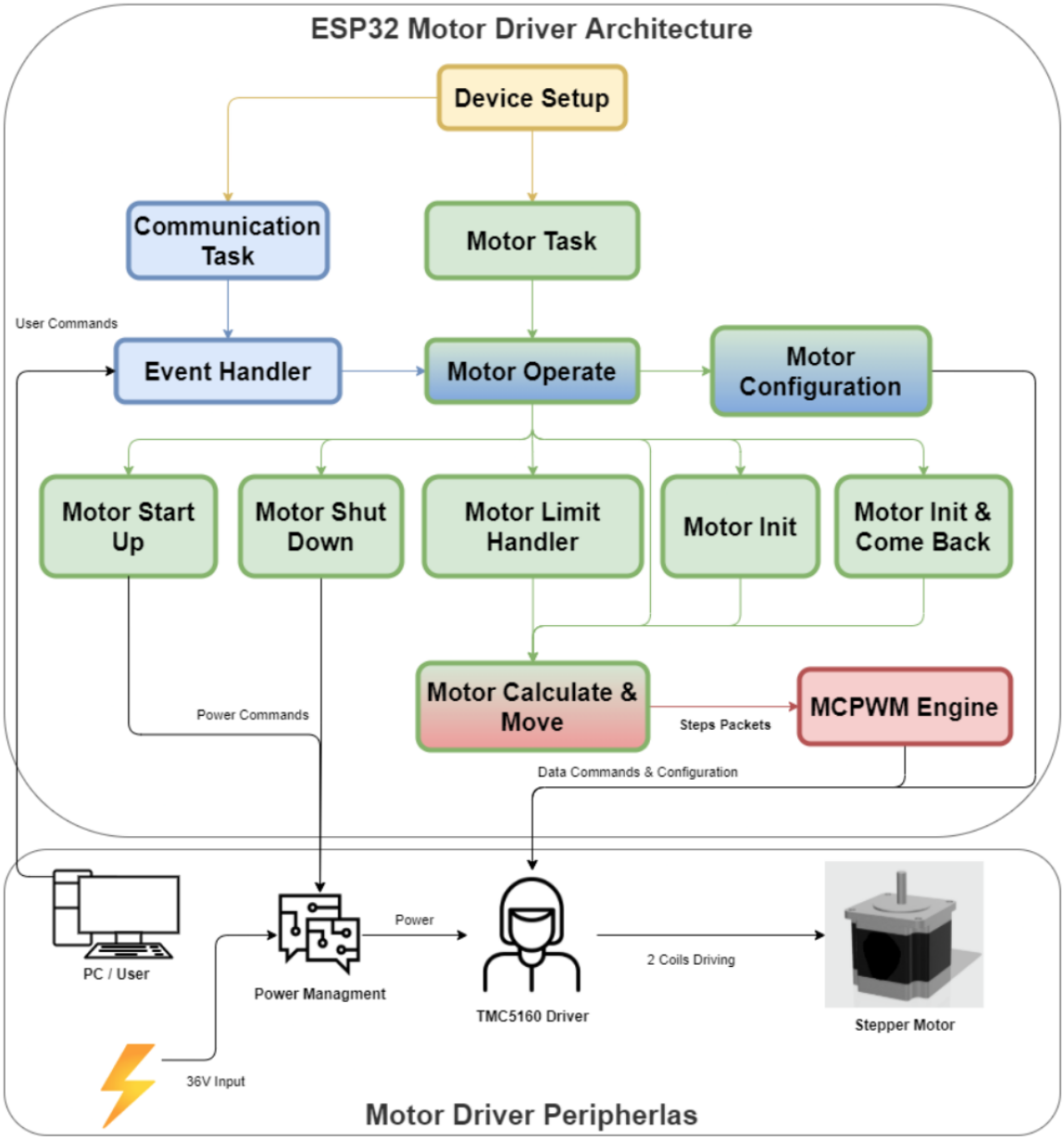


Figure 4.17: Motor Driver Architecture

Figure [4.17] depicts the interface between the ESP32 Motor Driver and its peripheral components. The subsequent sections will provide a detailed analysis of each block presented in the ESP32 Motor Driver Architecture.

The driver encompasses essential components such as **Utility Variables**, **Initial Calculations**, **Motor Operation**, and **Motor Engine**. These elements perform critical functions and facilitate the efficient operation of the driver.

- **Utility Variables**
  The variables in this set enable real-time configuration of the system's behavior, making the driver adaptable for driving various types of stepper motors. By precisely configuring these variables, the driver can efficiently control and operate a wide range of motor systems.

  * The variable `umPerStep` represents the minimum displacement capability of the connected motor, measured in micrometers (um). A typical value for this variable is **0.785 um**. It determines the smallest distance that the motor can move per step, providing fine-grained control over its positioning accuracy.

  * The variable `MOTOR_STEPS` specifies the number of steps required for the connected motor to complete a full revolution of 360 degrees. It is measured in steps, with a typical value of **200 steps** or **1.8 degrees per step**. This parameter determines the angular resolution of the motor, allowing for precise positioning and control.

  * The variable `MICROSTEPPING` determines the number of microsteps into which each individual step of the motor will be divided. It is measured in microsteps, with a range from **1 to 256**. A typical value for `MICROSTEPPING` is **16 microsteps** per step. This parameter allows for finer control and smoother motion of the motor, enhancing the precision and accuracy of positioning.

  * `DRIVER_CURRENT_mA:` Defines the maximum electrical current the driver will push to the connected motor in order to operate it. `DRIVER_CURRENT_mA` is measured in **mA** and a typical value is **670 mA**.

  * The variable `MAX_ACCEL` determines the maximum acceleration constant used by the driver to calculate the acceleration ramp based on the values from the Acceleration Lookup Table. It is measured in revolutions per second per step (RPS/step), with a typical value of **0.0008 RPS/step**. By adjusting `MAX_ACCEL`, the driver can control the rate at which the motor

accelerates, allowing for precise and smooth motion profiles. This parameter plays a crucial role in optimizing the motor's performance, ensuring efficient and reliable operation in various applications.

* The variable `MAX_DECEL` specifies the maximum deceleration constant utilized by the driver to calculate the deceleration ramp based on values from the Deceleration Lookup Table. Measured in revolutions per second per step (RPS/step), a typical value for `MAX_DECEL` is **0.0008 RPS/step**. By adjusting this parameter, the driver controls the rate at which the motor decelerates, enabling smooth and precise motion profiles. The selection of an appropriate `MAX_DECEL` value plays a crucial role in optimizing motor performance, ensuring efficient and reliable operation across various applications.

* The variable `MAX_SPEED_RPS` determines the maximum operating speed of the driver in revolutions per second (RPS) for the connected motor. This parameter sets an upper limit on the rotational velocity achievable by the motor. A typical value for `MAX_SPEED_RPS` is **20 RPS**. By adjusting `MAX_SPEED_RPS`, the driver ensures that the motor operates within the desired speed range, providing optimal performance and meeting the requirements of the application. This parameter plays a crucial role in controlling the motor's rotational speed, enabling precise and efficient motion control for various tasks.

* The variable `MIN_SPEED_RPS` specifies the minimum operating speed of the driver in revolutions per second (RPS) for the connected motor. This parameter sets a lower limit on the rotational velocity achievable by the motor. A typical value for `MIN_SPEED_RPS` is **0.1 RPS**. By configuring `MIN_SPEED_RPS`, the driver ensures that the motor maintains a minimum speed threshold, which is important for smooth and consistent motion control. This parameter allows for precise control over the motor's speed, enabling accurate positioning and motion tasks that require low-speed operation.

* The variable `INIT_SPEED_RPS` specifies the initial speed at which the driver begins its operation, ensuring that any movement starts as quickly as possible. This parameter is measured in revolutions per second (RPS) and has a typical value of **2 RPS**. By setting `INIT_SPEED_RPS` to an appropriate value, the driver initiates motion promptly, minimizing any delay in mo-

tor response. This parameter is crucial for achieving efficient and timely motor control, particularly in applications that require rapid movement or response times. By fine-tuning `INIT_SPEED_RPS`, the driver optimizes the motor's starting performance, enhancing overall system effectiveness.

* The `STOP_SPEED` variable determines the speed at which the driver ceases its operation, enabling rapid and efficient cessation of movement. Measured in revolutions per second (RPS), this parameter plays a crucial role in achieving prompt stopping of the connected motor. With a typical value of **1 RPS**, `STOP_SPEED` ensures that the motor halts swiftly, minimizing any residual motion. By carefully configuring `STOP_SPEED`, the driver optimizes the system's ability to achieve precise and timely motion control. This parameter is particularly important in applications where quick and accurate stopping is critical, such as in robotics or manufacturing processes.

* The `ERR` variable specifies the maximum permissible error in speed calculations that the driver considers when making real-time decisions. This parameter plays a critical role in ensuring smooth and accurate movement of the system. Measured in revolutions per second (RPS), `ERR` defines the acceptable deviation from the desired speed. With a typical value of **1 RPS**, this parameter enables the driver to maintain precise speed control, minimizing any deviations from the intended motion trajectory. By carefully configuring `ERR`, the driver optimizes the system's ability to achieve smooth and consistent movement, crucial for applications requiring high accuracy, such as robotics, CNC machines, or motion control systems.

* The `limitTrigger` variable determines the triggering state that activates the axis limit handling system based on predetermined events. This parameter, expressed as a **Boolean expression**, plays a crucial role in defining the system's response to potential limit events. The acceptable values for `limitTrigger` are **1** for low-to-high transition triggering and **0** for high-to-low transition triggering. By configuring this variable, the system can accurately detect and respond to limit conditions, ensuring safe and controlled operation. This feature is particularly important in applications where precise positioning and boundary protection are critical, such as robotics, CNC machines, or automated systems.

* The `MIN_AXIS_LENGTH` and `MAX_AXIS_LENGTH` variables define the mini-

mum and maximum allowable positions for the motor within the system. These parameters impose virtual boundaries on the motor's axis length, ensuring controlled and safe operation. Measured in **um**, these values determine the acceptable range of motor positions. A typical value for `MAX_AXIS_LENGTH` is **100000 um**, representing the maximum allowed position, while `MIN_AXIS_LENGTH` has a typical value of **0 um**, indicating the minimum allowable position. By setting these parameters, the driver establishes limits on the motor's movement, preventing it from exceeding specified positions. This feature is essential for applications requiring precise control over the motor's range of motion, such as robotic systems, automated machinery, or positioning systems.

– **Lookup Tables**
In this design, two types of tables are employed: the **Speed Tables** and the **Steps Count Tables**. These tables are calculated based on the characteristics of the connected motor and take into account parameters such as the predefined **Utility Variables**. The Speed Tables provide a mapping between desired motor speeds and corresponding step rates, allowing precise control over the motor's rotational velocity. On the other hand, the Steps Count Tables establish a relationship between the desired motor position and the number of steps required to reach that position. By utilizing these tables, the driver can accurately determine the appropriate step rate and step count for achieving the desired motor speed and position. These tables play a crucial role in the driver's operation, enabling efficient and reliable motor control based on the specific characteristics of the connected motor.

* The **speedTable** is a crucial data structure that stores the various speed levels at which the system can operate. It takes into account the predefined minimum and maximum speeds (`MIN_SPEED_RPS` and `MAX_SPEED_RPS`) as well as the sampling rate of the corresponding continuous-time signal (`SamplingRate`). The values within the speedTable are measured in rotations per second (RPS) and provide a comprehensive range of speed options for controlling the motor. By accessing the speedTable, the driver can accurately set the desired speed level for the motor, enabling precise and dynamic control over its rotational velocity. This table plays a crucial role in achieving optimal performance and responsiveness in the motor driver system.

$$speedTable(i) = \left| \frac{(i+1) \cdot (maxSp - minSp)}{SamplingRate} \right| + minSp \tag{4.1}$$

Where `minSp` is `MIN_SPEED_RPS`, `maxSp` is `MAX_SPEED_RPS` and `i` ranges from 0 to SamplingRate.

* The **slope** table is a vital component that stores values representing the acceleration factor over time. It provides a description of the acceleration derivative by determining the relative acceleration factor with respect to the user-defined `MAX_ACCEL`. This table plays a crucial role in controlling the motor's acceleration profile, allowing for precise and dynamic adjustments in the rate of change of acceleration. By accessing the slope table, the driver can effectively modulate the motor's acceleration characteristics, enabling smooth and controlled motion in accordance with the specified maximum acceleration limit. The values stored in the slope table facilitate accurate and efficient acceleration control, contributing to the overall performance and responsiveness of the motor driver system.

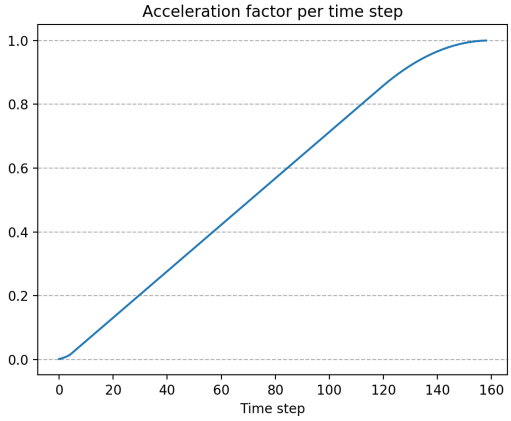


Figure 4.18: Acceleration Curve

* The **accelStepsTable** is a crucial data structure that stores values representing the distance, measured in steps, from the current speed index to the maximum speed defined by `MAX_SPEED_RPS`. This table plays a vital role in the motor driver system by providing the necessary information for determining the number of steps required to accelerate from the current speed to the maximum speed. By accessing the accelStepsTable, the driver can efficiently calculate and execute the appropriate number of steps needed to achieve the desired acceleration profile. The values stored in this table enable precise control over the motor's acceleration, ensuring smooth and reliable motion throughout the operation. The accelStepsTable is an integral component of the motor driver algorithm, contributing to its overall performance and responsiveness.

$$accelStepsTable(i) = \sum_{i=0}^{SamplingRate} \left| \frac{speedTable(i+1) - speedTable(i)}{slope(i) \cdot \texttt{MAX\_ACCEL}} \right| \tag{4.2}$$

Where i ranges from 0 to SamplingRate.

* The **decelStepsTable** is a significant data structure utilized in the motor driver system to store values representing the distance, measured in steps, from the current speed index to the minimum speed defined by `MIN_SPEED_RPS`. This table plays a crucial role in achieving precise deceleration control of the motor. By accessing the decelStepsTable, the driver can determine the number of steps required to decelerate from the current speed to the minimum speed. This information enables the driver to execute the appropriate number of steps to ensure a smooth and controlled deceleration profile. The decelStepsTable is an essential component of the motor driver algorithm, contributing to its overall performance and accuracy.

$$decelStepsTable(i) = \sum_{i=0}^{SamplingRate} \left| \frac{speedTable(i+1) - speedTable(i)}{slope(i) \cdot \texttt{MAX\_DECEL}} \right| \tag{4.3}$$

Where i ranges from 0 to SamplingRate.

– **Calculations & Motor Operation**

The Calculations section of the driver unit plays a crucial role in real-time decision making. By leveraging the Lookup Tables and Utility Variables previously described, the Speed Control Calculations system is capable of processing user commands and translating them into precise motor motion in real time. Each decision made by this unit is converted into packets of steps with a constant speed. These packets are then sent to the Speed Control Queue for execution by the MCPWM engine.

The Calculations section acts as the brain of the motor driver system, ensuring that every user command is accurately translated into the desired motor movement. By utilizing the information stored in the Lookup Tables and the relevant Utility Variables, the system can make informed decisions on motor speed and acceleration profiles. These decisions are then transformed into a series of step packets, allowing for smooth and precise motor control.

The integration of the Calculations section with the Speed Control Queue and the MCPWM engine ensures the efficient execution of motor commands. This streamlined process enables the driver unit to deliver the desired motor motion with minimal delays and precise synchronization. The Calculations section is a critical component of the overall motor driver architecture, contributing to its reliability and performance.

– **Motor Engine**
The MCPWM engine plays a vital role in the motor driver system, facilitating the transmission of step packets as a PWM signal from the Speed Control Queue to the TMC5160 Step Input Pin. This process is carried out asynchronously by the peripheral hardware of the ESP32 SoC. Although the MCPWM has limited speed, it offers a wide range of pulse-width adjustment capabilities.

Due to the inherent inertia and high inductance of motors, they can effectively filter out fast PWM signals, allowing for motor speed control within a range that aligns with their natural filtering properties. The PWM signal generated by the MCPWM engine operates within this range, ensuring optimal motor performance.

In addition to PWM signal generation, the MCPWM engine captures inputs from the motor, enabling the generation of Interrupt Service Routines (ISRs). These ISRs allow for the inspection of various aspects of the motor's operation, including overload detection, load absence, and comparison of actual motor current with expected current. This real-time monitoring provides valuable insights into the motor's performance and facilitates effective troubleshooting and maintenance.

4.4 Illumination Drivers

Light Emitting Diodes (LEDs) have become increasingly popular in various lighting applications due to their energy efficiency, long lifespan, and compact size. In this section of the thesis, we will delve into the implementation of a high stability LED driver to enable rapid control over light emitting diodes (LEDs). The primary objective of this design is to support a fast scanning imaging system capable of dynamically adjusting the intensity of different wavelength light sources in real-time. These drivers will receive feedback from a computerized system and a camera to ensure the desired intensities for microscopic analysis applications are achieved. The implementation of this LED driver is crucial in providing precise and efficient control over the light sources, enabling accurate and reliable imaging for various analytical purposes.

4.4.1 Characteristics of an LED

LEDs are semiconductor devices that emit light when powered by a DC voltage. However, they have specific electrical characteristics that must be carefully considered to ensure their proper operation and longevity.

The forward current, which flows from the anode (+) to the cathode (-), is responsible for producing light in the LED. Applying too much voltage or allowing excessive current to flow can permanently damage the LED emitter. Therefore, it is crucial to control the forward current and voltage within safe operating limits.

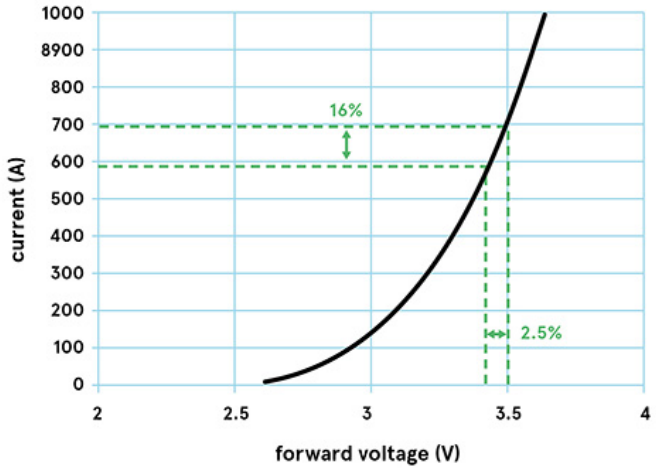


Figure 4.19: Forward voltage against the forward current
Source: avnet.com

The relationship between the forward voltage and the forward current is nonlinear. Small changes in the forward voltage can result in significant changes in the forward current, as shown in Figure [4.19]. This nonlinearity can lead to thermal runaway, where increased current generates more heat, further increasing the current flow and temperature. To prevent this, thermal management becomes essential to maintain the LED's operational reliability. Operating an LED at lower temperatures improves its reliability and extends its lifespan. Therefore, achieving an optimal balance between light output and forward drive current is crucial. Proper thermal management, such as using heatsinks, helps dissipate heat and maintain the LED's temperature within acceptable limits.

In addition to thermal management, controlling the current flow through the LED is a critical factor. By carefully managing the current, we can ensure the LED operates within its specified limits, maximizing its performance and lifespan. For reliable and long-lasting LED operation, it is important to carefully control the forward current and voltage, manage the temperature through effective thermal management, and maintain the current flow within safe operating limits. These considerations are vital for achieving optimal performance and ensuring the longevity of LEDs in our application.

4.4.2 Light Emitting Diodes (LED) Control

To power and control LEDs effectively, LED drivers are utilized. There are two main types of LED drivers: constant voltage (CV) and constant current (CC) drivers. In this section we will compare the characteristics, advantages, and limitations of these two driver types as shown in Figure [4.20].

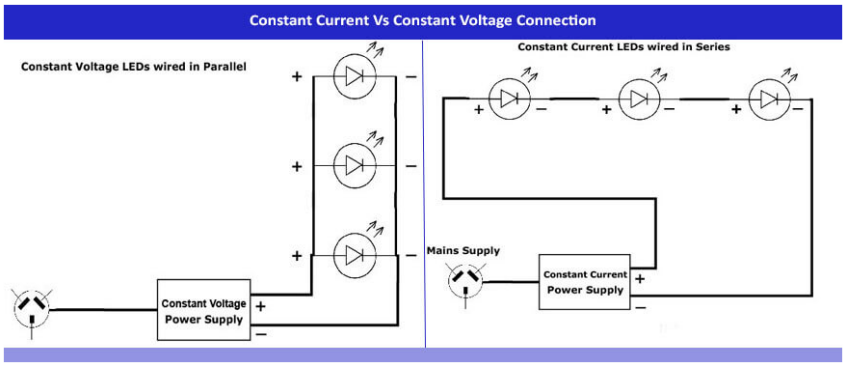


Figure 4.20: Connection of LEDs to CC and CV circuits
Source: specializedlightingconcepts.co.nz

Constant Voltage LED Drivers: Constant voltage LED drivers provide a fixed voltage output to the LED load. The LED driver maintains a steady voltage across the LEDs, and the current flow through the LEDs is determined by its load characteristics. These drivers are commonly used for LED strips, signage, and decorative lighting applications. They are relatively simple, cost-effective, and widely available. However, they may not be suitable for high-power LEDs or applications requiring precise current control.

Constant Current LED Drivers: Constant current LED drivers provide a fixed current output to the LED load. These drivers ensure a consistent current flow through the LEDs, irrespective of load variations or changes in the forward voltage drop. Constant current drivers are commonly used in high-power LED applications, such as architectural lighting, street lighting, and industrial lighting. They offer precise current regulation, better thermal management, and increased LED lifespan. However, they can be more complex, less common, and comparatively more expensive than constant voltage drivers.

Voltage vs Current Control: Constant voltage drivers regulate the voltage across the LEDs, while constant current drivers regulate the current flowing through the LEDs.

- **LED Compatibility**: Constant voltage drivers are suitable for low-power LEDs and applications where precise current control is not critical. Constant current drivers are ideal for high-power LEDs, ensuring stable and accurate current regulation.
- **Flexibility**: Constant voltage drivers can power multiple LEDs connected in parallel, whereas constant current drivers are typically used for individual LEDs or series-connected LEDs.
- **Efficiency**: Constant current drivers generally offer higher efficiency as they can adapt to variations in the forward voltage drop of LEDs, minimizing energy waste and potential damage due to bad regulated power supplies.
- **Thermal Management**: Constant current drivers provide better thermal management by ensuring consistent current flow, reducing the risk of overheating and extending LED lifespan.

- **Cost and Availability**: Constant voltage drivers are more cost-effective and widely available, while constant current drivers may be slightly more expensive and less common.

For our application requiring fast and reliable precision illumination systems, the use of constant current LED drivers is highly recommended. Constant current drivers offer several advantages that align with our specific needs.

Firstly, constant current drivers ensure precise current control, allowing us to achieve accurate and consistent illumination levels, leading to accurate and reproducible results. This is crucial for applications where precise control of light intensity is required, such as in microscopy or imaging systems.

Secondly, constant current drivers provide better thermal management. In high-speed applications, the LEDs may generate significant heat during operation. Constant current drivers regulate the current flowing through the LEDs, preventing excessive heat buildup and reducing the risk of thermal damage. This helps maintain the long-term reliability and lifespan of the LEDs, ensuring consistent performance over time.

Furthermore, constant current drivers offer higher efficiency by adapting to variations in the forward voltage drop of the LEDs. This ensures optimal power delivery and minimizes energy waste. In applications that require fast response times and rapid changes in illumination levels, efficient power utilization is crucial for achieving the desired performance while minimizing power consumption.

While constant current drivers may be slightly more complex and relatively less common compared to constant voltage drivers, their benefits in terms of precise current control, thermal management, and efficiency make them the preferred choice for our application. By using constant current drivers, we can achieve the fast and reliable precision illumination system required for our application, ensuring accurate and high-quality imaging.

Controlling an LED driver

Most LED drivers provide some degree of programmability and control. Some basic features include being able to control or sequence the output to LED lights and strings using a microcontroller-based system. More sophisticated features include

the ability to program or configure the LED driver's current and voltage outputs, and set the over current, over voltage protection limits.

The requirement to control large-scale LED lighting installations has LED to the development of lighting control standards. The most popular of these communications protocol standards used in the industry are the digital addressable lighting interface (DALI) and the digital multiplex (DMX).

The causes of flicker in LED lighting installations The ability to control the light output, such as dimming, is also a popular control requirement although it needs careful attention due to the potential side effect of flicker.

Most LED drivers use a pulse width modulated (PWM) technique to control the LED drive voltage and current. PWM operates by rapidly switching the output voltage at a high frequency. The width of the pulse determines how long the LED is on within a given duration, thereby controlling the duty cycle. This switching reduces the light output, resulting in a dimming effect, as shown in Figure [4.21].

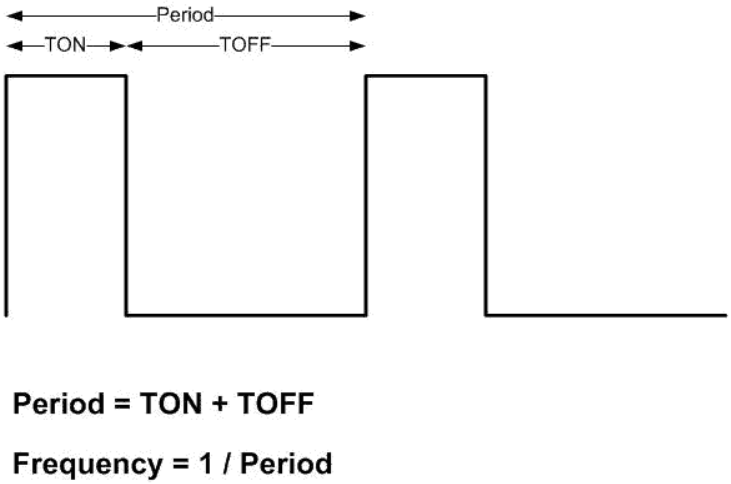


Figure 4.21: PWM from Constant Voltage Led Driver
Source: ledyilighting.com

Flicker is an annoyance and can induce headaches, migraines and cause discomfort for many people. Flicker can also occur due to poorly designed LED drivers. LED drivers utilise switched-mode power supply concepts to convert a mains AC voltage to the required DC LED drive voltage. The power conversion topology can leave small regular low-frequency voltage variations on the DC output. The voltage variations, termed ripple, can be up to 200 mV, and are typically filtered and smoothed

out in well-designed LED drivers. However, such a ripple voltage can represent a significant enough variation to induce flicker in the LED light output.

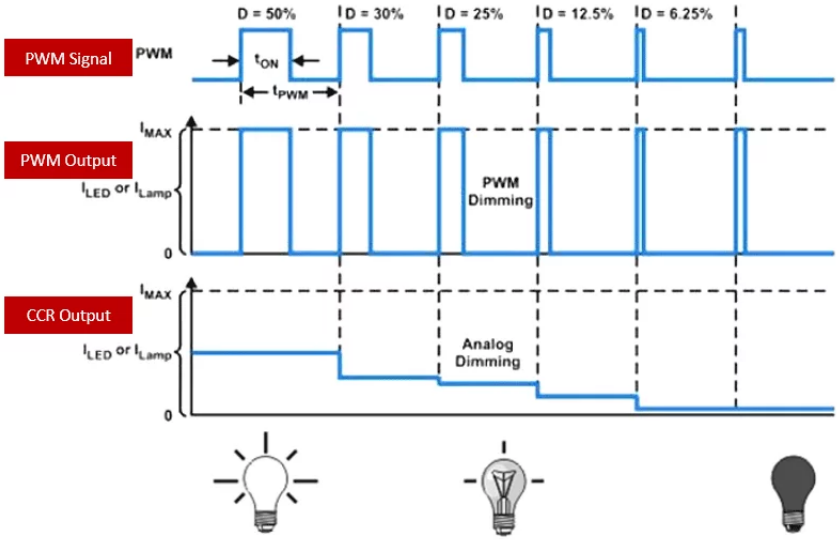


Figure 4.22: PWM conversion to Constant Current flow through Led
Source: upowertek.com

To mitigate flickering when driving LEDs with PWM, the utilization of Constant Current Drivers is essential. These drivers effectively regulate the current passing through the LEDs, ensuring a steady and reliable output, as shown in Figure [4.22]. By maintaining a consistent current level, fluctuations in brightness induced by PWM modulation are minimized, resulting in smooth and flicker-free illumination. Constant Current Drivers enable precise control over the LED current, preventing undesirable visual effects such as noticeable flickering and guaranteeing a superior lighting experience.

4.4.3 Transmission Illumination Circuitry

The circuit design principles for the illumination drivers of the transmission and epi illumination modules in a high throughput screening traces analysis microscope are presented in this thesis.

- Efficient power delivery to the illumination modules
- Precise control of current and voltage for optimal illumination intensity
- Consideration of thermal management to ensure stable performance

To ensure high thermal stability, the driver circuit for the illumination module in the transmission source of the microscope was designed as a single-layer PCB printed on aluminum. This design approach allows for efficient heat dissipation and helps maintain consistent performance. The illumination module comprises thirteen individual LED sources, as detailed in the Table [4.1].

Wavelength (nm)	**320**	**340**	**365**	**385**	**405**	**450**	**490**	**515**	**590**	**630**	**780**	**850**	**980**
Number of Sources	3	3	1	1	1	1	1	1	1	1	1	1	1
Optical Power (mW)	140	210	700	850	710	700	240	700	120	700	800	630	400

Table 4.1: Transmission Source Individual Led Sources

Transmission Illumination Driver Design The circuit design of the transmission illumination driver is based on the AL5816 constant current linear LED controllers, which are utilized for independent control of each wavelength.

The AL5816 is a 5-terminal adjustable constant current linear LED controller with a wide input voltage range of 4.5V to 60V. It offers excellent temperature stability and current capability, making it suitable for medium to high current LEDs. With a low 200mV current sense FB voltage, the AL5816 efficiently regulates LED current while minimizing power dissipation. The controller drives a MOSFETs and supports longer LED chains with low dropout voltage and multiple LED channels. Additionally, the AL5816 features a dedicated PWM pin for precise PWM dimming at frequencies greater than 200Hz. The implementation of this design is depicted in Figure [4.23].

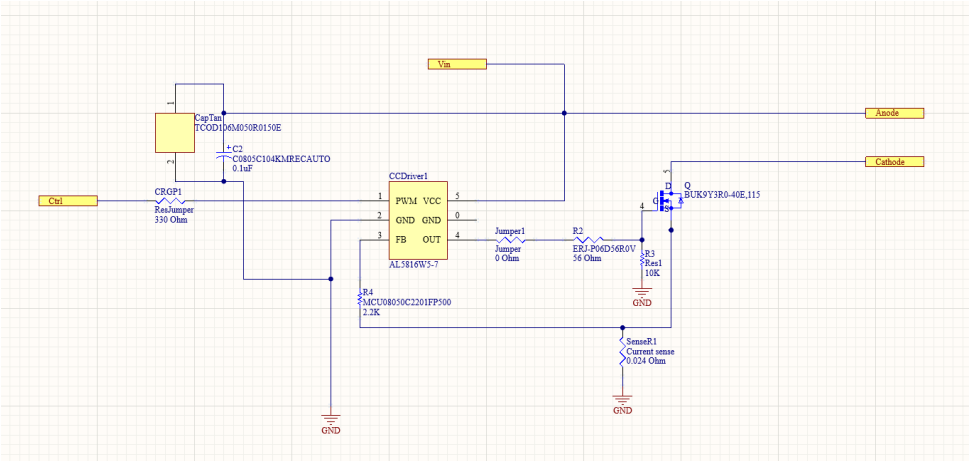


Figure 4.23: Constant Current Driver Schematic
Source: Altium Designer Transmission Illumination Driver Project

To efficiently control a large number of constant current drivers and LED sources using a single microcontroller, we employed the PCA9685 chip. The PCA9685 is specifically designed as a 16-channel LED controller for LED applications. It utilizes the I2C-bus interface for control and incorporates individual PWM controllers for each LED output. This allows for programmable PWM frequencies and precise adjustment of the duty cycle, enabling accurate brightness control. The PCA9685 supports both open-drain and totem pole LED output configurations, capable of providing a 25 mA current sink and a 10 mA source at 5 V. With a voltage range of 2.3 V to 5.5 V and 5.5 V tolerant inputs and outputs, the chip can directly drive LEDs or be used with external drivers for larger current or higher voltage LEDs, while minimizing the need for additional components. Its versatile features make it an ideal choice for our application, enabling efficient and synchronized control of multiple constant current drivers and LED sources.

The schematic diagram of our 13-channel Constant Current driver is illustrated in Figure [4.24]. This driver design incorporates various key components to achieve precise and synchronized control over multiple LED channels.

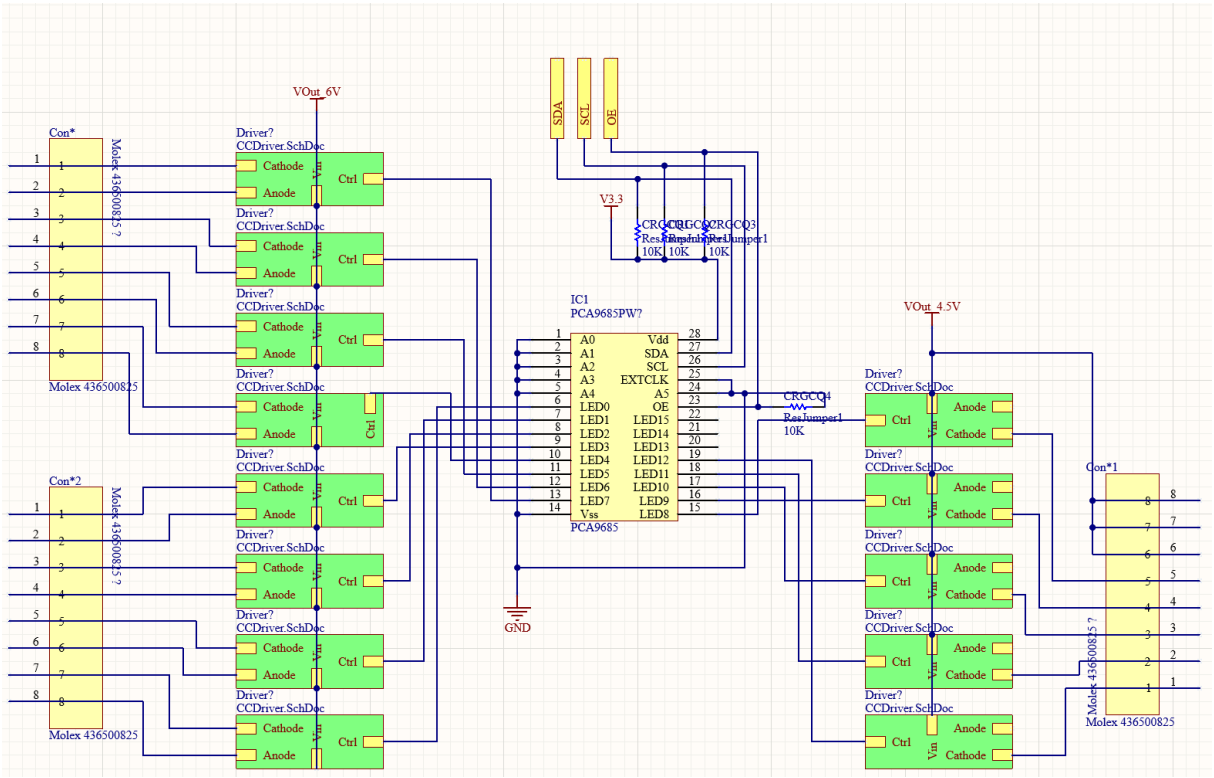


Figure 4.24: Constant Current Driver Multiplexer Schematic
Source: Altium Designer Transmission Illumination Driver Project

To optimize power efficiency and ensure optimal performance of the LED sources, we employed two PTN7820WAH buck converters. These converters were carefully

configured to generate two distinct voltage rails, allowing us to efficiently power the different LED sources. By utilizing separate voltage rails, we were able to minimize power wastage on the MOSFETs of the constant current driver circuit. This approach contributed to the overall energy efficiency of the system while providing reliable and stable power to each LED source. The schematic diagram of the final design of the transmission Illumination Driver is shown in Figure [4.25].

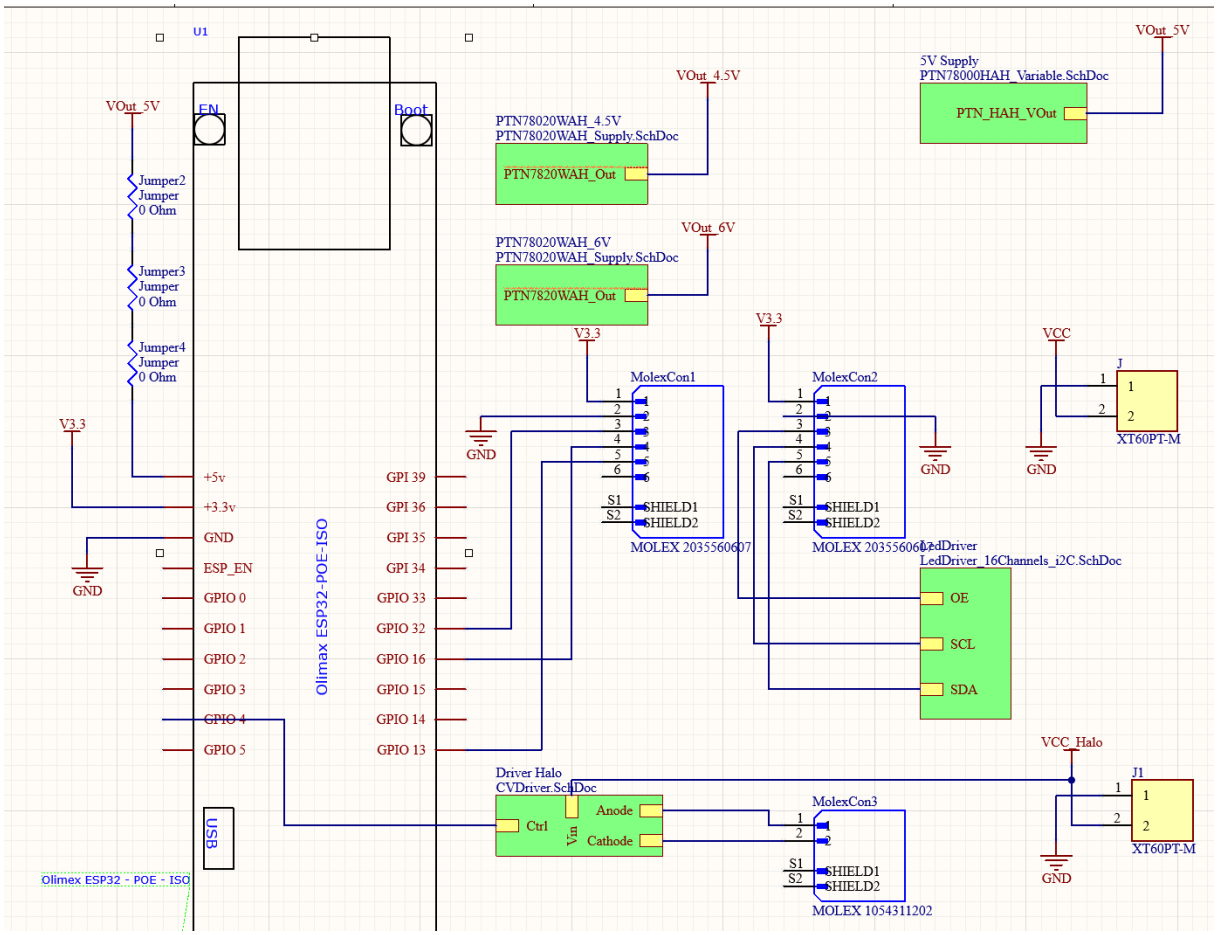


Figure 4.25: Transmission Illumination Driver Schematic
Source: Altium Designer Transmission Illumination Driver Project

Printed Circuit Board Figures [4.26, 4.27] showcases the Printed Circuit Board (PCB) layout of the Transmission Illumination driver developed for this project. The PCB design demonstrates a compact and optimized arrangement of components, carefully organized to ensure efficient signal routing and minimal interference. The figure provides a visual representation of the PCB's intricate circuitry, highlighting the precise placement and interconnection of electronic components.

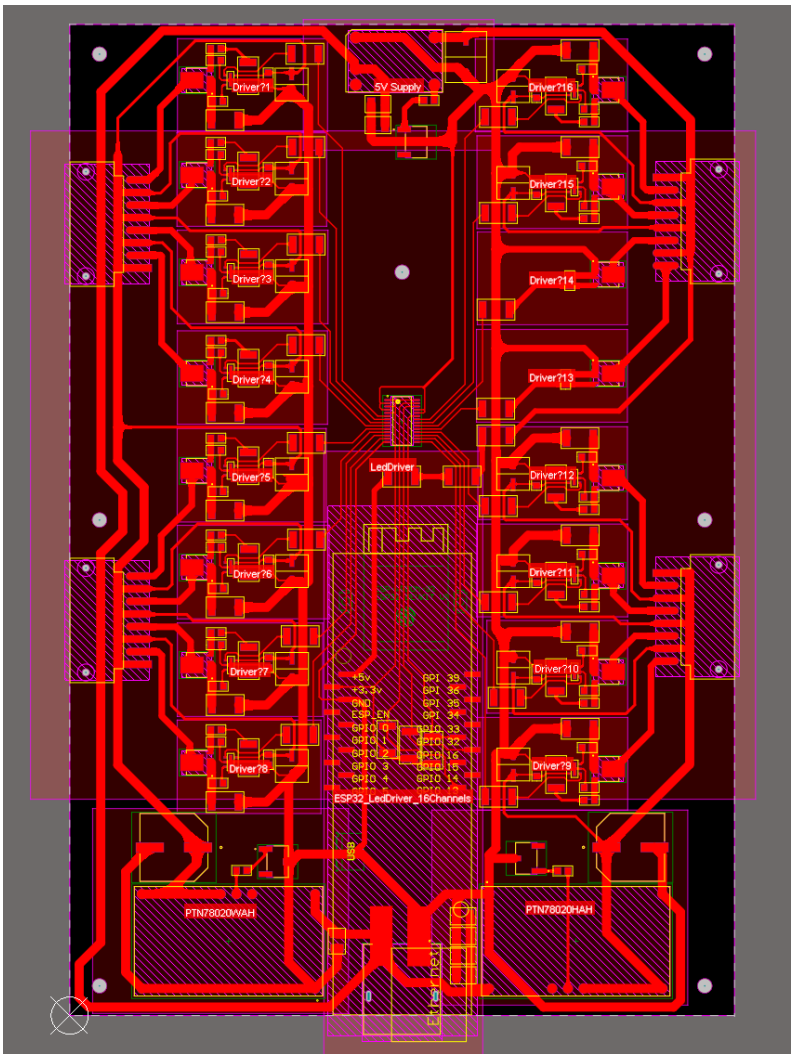


Figure 4.26: Transmission Illumination Driver PCB
Source: Altium Designer Transmission Illumination Driver Project

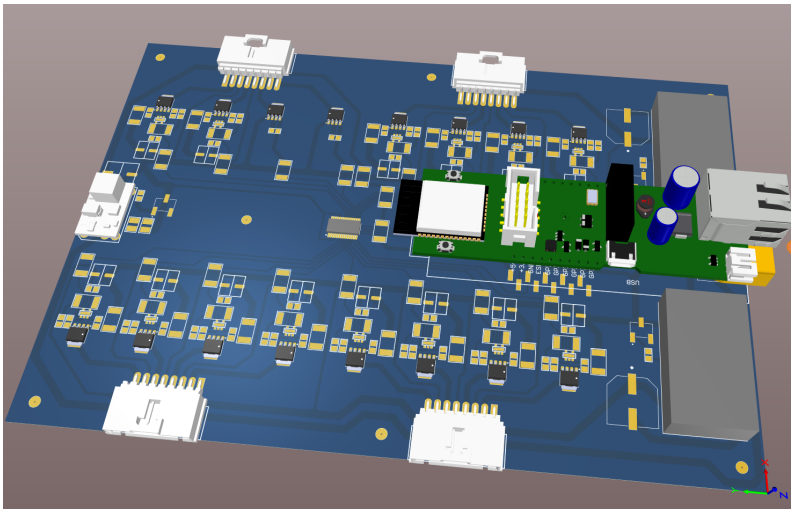


Figure 4.27: Transmission Illumination Driver PCB 3D
Source: Altium Designer Transmission Illumination Driver Project

4.4.4 Optical Setup

To obtain accurate retardance measurements with the use of this illumination source, it is crucial to define the desired ellipsometry or polarization states that our system will detect. To achieve precise measurements of the sample's optical transfer function and retardance, our light source should be capable of providing circularly polarized light across all wavelengths is required. This enables the observation of how circular polarization evolves into elliptical polarization for each specific wavelength, as illustrated in Figure [4.28]. Additionally, to ensure comprehensive information about the polarization state is obtained, the polarisation state detector (PSD) must incorporate four polarization filters (0°, 45°, 90°, 135°).

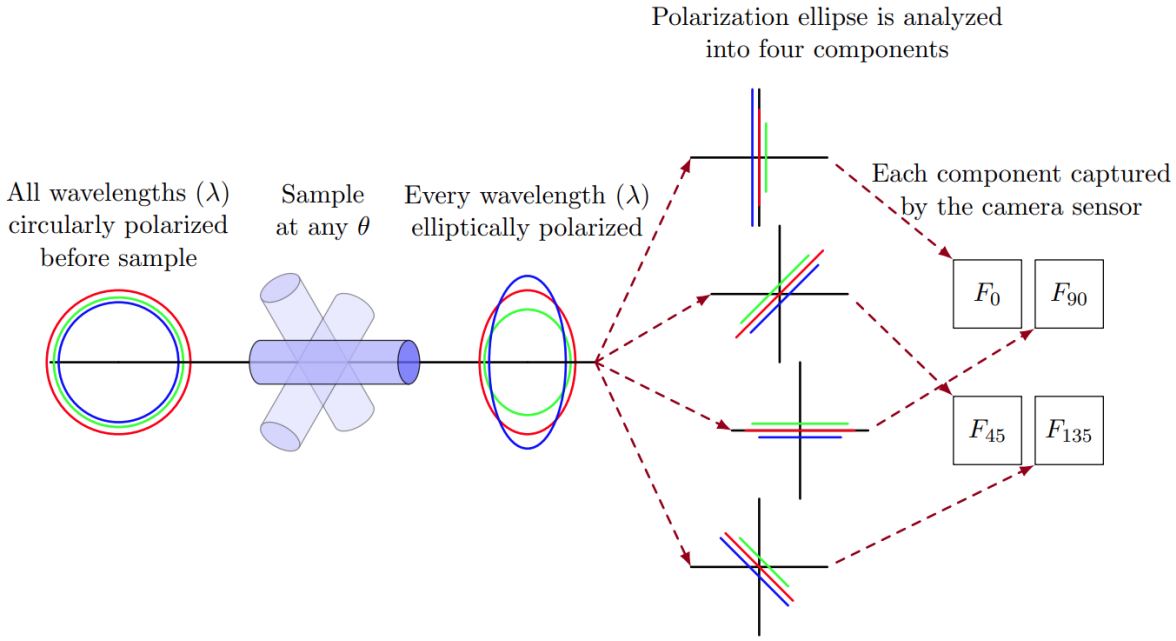


Figure 4.28: The polarization state evolution in the proposed design

The light source utilized in the system is an LED multiplexer, allowing for the selection, multiplexing, homogenization, and emission of specific polarization states across the ultraviolet (UV), visible (VIS) and near-infrared (NIR) spectra.

To achieve uniform illumination, active LEDs are multiplexed with a beam homogenizer, which not only smoothens the light but also serves as an optical waveguide. Light pipe homogenizing rods utilize total internal reflection for homogenizing non-uniform light sources, irrespective of their spectral characteristics. The Illumination Driver enables the selection of active LEDs from the broad spectrum LED array, resulting in a modular light source capable of emitting narrow or broad band light.

To achieve circular polarization, the incoming light should be depolarized. A Quartz-Wedge Achromatic Depolarizer is employed for this purpose, which converts any input polarization component into an unpolarized state. This transformation is illustrated in Figure [4.28].

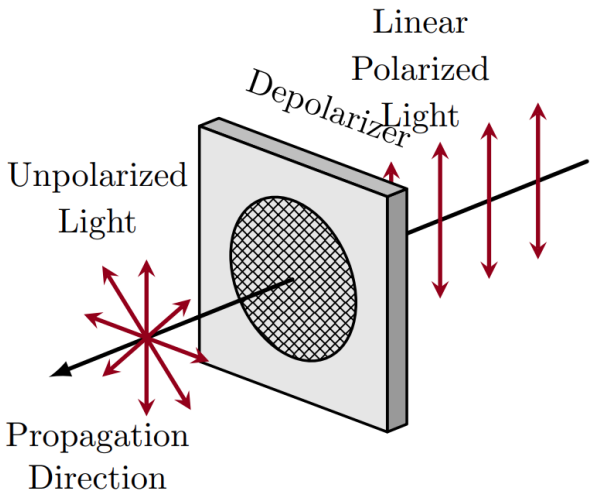


Figure 4.29: The depolarization stage of our design

For the system's operation, a known input and output measurement are required to determine the transfer function. In optical systems, circularly polarized light is desired before entering the sample, allowing the detected polarization state of the light after exiting the sample to be correlated with the sample's optical properties.

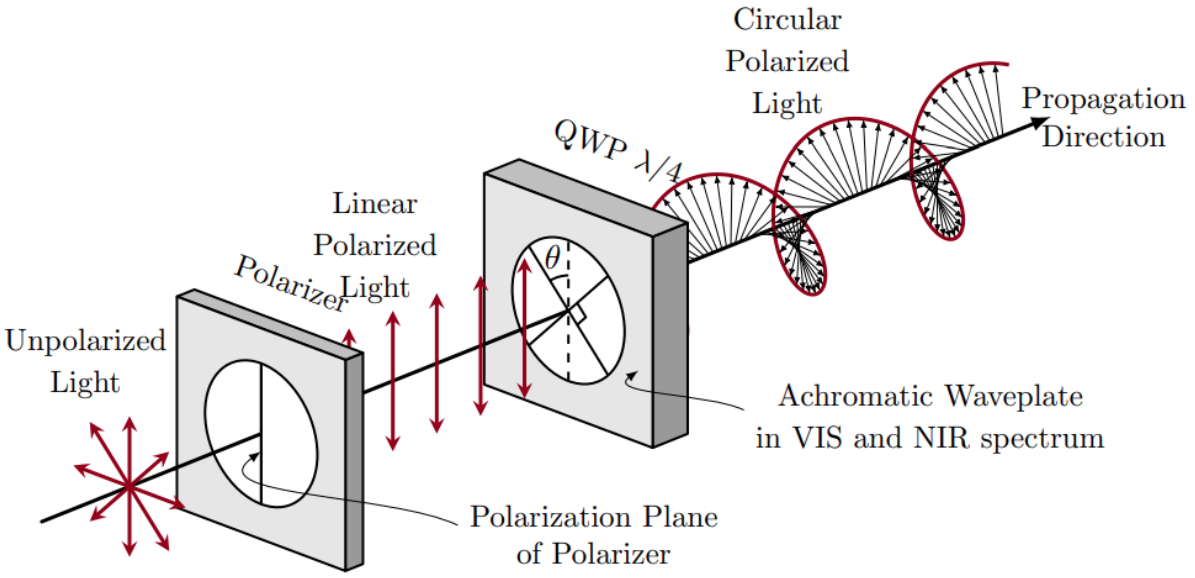


Figure 4.30: Polarization State Generator (PSG)

The PSG includes a linear polarizer to convert any polarization state to a 0°linearly

polarized light beam, followed by an achromatic quarter waveplate to transform the beam into circularly polarized light. Figure [4.30] illustrates the conversion of linearly polarized light to circularly polarized light using the quarter-wave retarder. The exit light is circularly polarized after passing through the quarter-wave retarder, where the light polarized parallel to the slow axis is retarded by $\lambda/4$ relative to the light polarized along the fast axis.

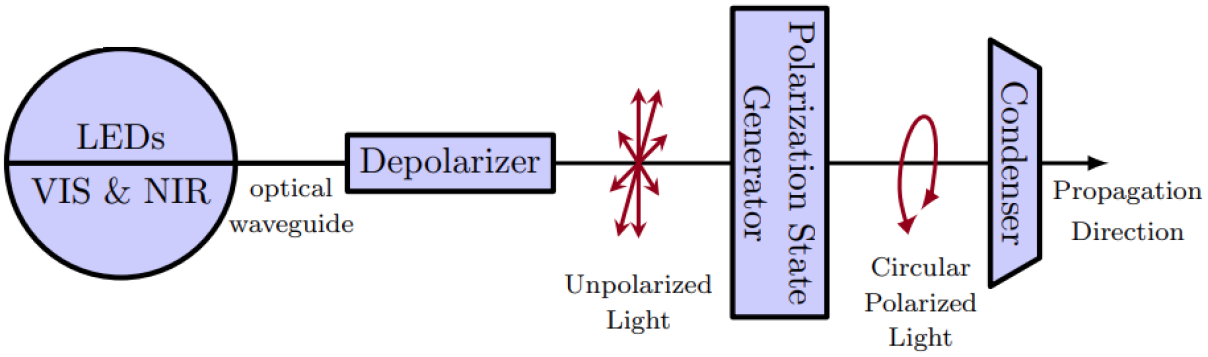


Figure 4.31: The complete polarization schema of the transmission illumination source

The complete schematic of the light source is presented in Figure [4.31]. Individual control of the LEDs is facilitated by the Illumination Driver via Ethernet, while the emission frequency is multiplexed using a waveguide. The depolarizer ensures an unbiased input to the polarization state generator (PSG), which outputs circularly polarized light from the unpolarized input. Additionally, an optional condenser is incorporated for high magnification microscopy, enhancing system performance.

Final Design The final system is housed within a durable aluminum enclosure, securely installed within the microscope body, as shown in Figures [4.32, 4.33]. Communication between the driver is facilitated by an Ethernet cable, ensuring reliable data transmission. Furthermore, the power supply for the system is delivered via an XT-60 connector, enabling efficient power distribution. The choice of an aluminum case, Ethernet communication, and XT-60 connector not only ensures the robustness and integrity of the system but also enables seamless integration and operation within the microscope setup.

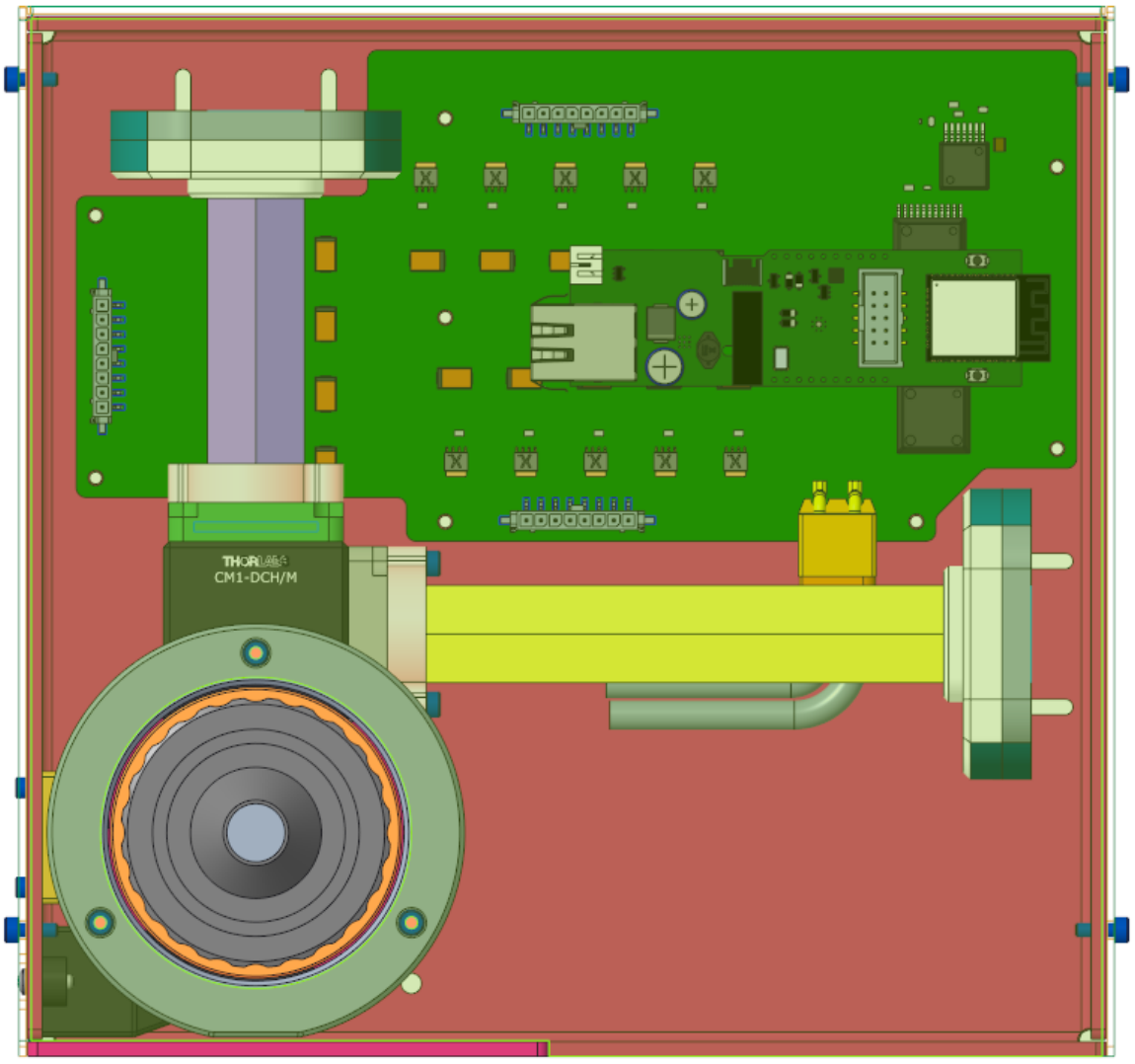


Figure 4.32: Transmission Illumination Source Final Design 3D
Source: Altium Designer Transmission Illumination Source Project

The final design incorporates the Transmission Illumination Driver PCB, which facilitates the control of two distinct illumination sources. The first source is a UV-dedicated light, meticulously filtered to prevent any unwanted leak or fluorescence

interference from the LEDs. The second source generates visible and infrared illumination, which is also subjected to careful filtering and polarization to achieve uniform circular polarized light. These two sources are combined using a dichroic mirror cube, and the resulting beam is projected through a condenser onto the sample plane of the microscope.

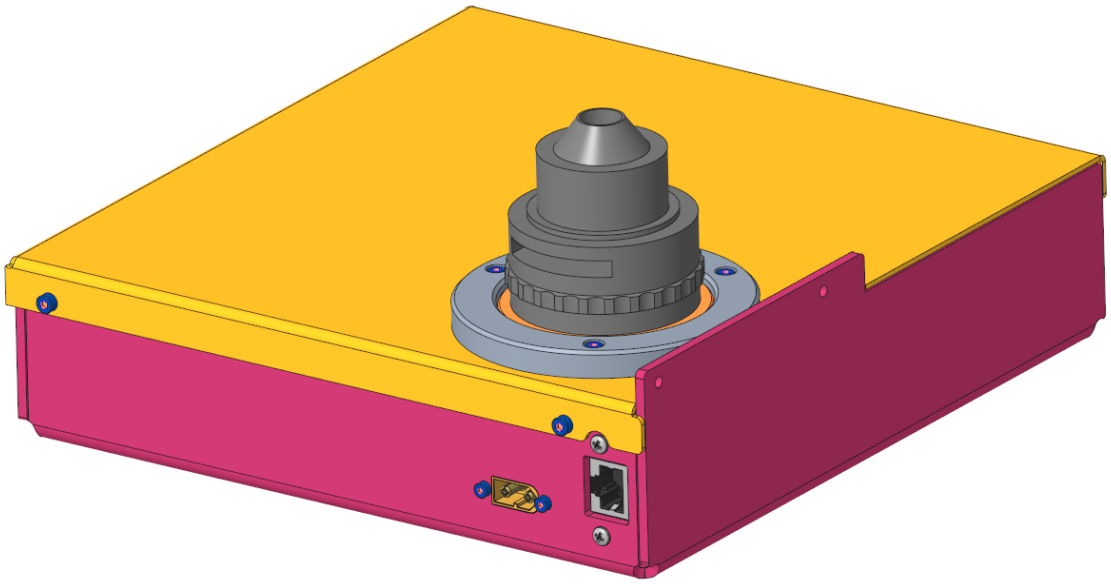


Figure 4.33: Transmission Illumination Source Final Design 3D
Source: Altium Designer Transmission Illumination Source Project

4.4.5 Epi Illumination Circuitry

Utilizing similar principles as the transmission illumination source, we have meticulously designed a power-efficient epi illumination driver that can be powered via Power over Ethernet (PoE). The driver incorporates two Constant Current Drivers, responsible for driving a VisIR LED and a 365 high-power LED for fluorescence applications. In addition, two Constant Voltage PWM drivers are employed to drive two laser modules utilized in fluorescence applications. The specific sources utilized in the driver are outlined in Table [4.2].

Wavelength (nm)	**365**	**405**	**450**	**VisIR**
Optical Power (mW)	1150	100	40	300

Table 4.2: Transmission Source Individual Led Sources

The M365D2 LED from Thorlabs, mounted on a Metal-Core Printed Circuit Board (MCPCB), is specifically designed to deliver high-power output in a compact form factor, with a nominal wavelength of 365 nm. This LED offers a minimum emitted power of 1150 mW and a typical emitted power of 1400 mW. To ensure proper thermal management, it is essential to mount the MCPCB onto a suitable heat sink using two screws and apply a thermal compound to establish effective thermal contact between the MCPCB and the heat sink. The Led is presented in Figure [4.34].

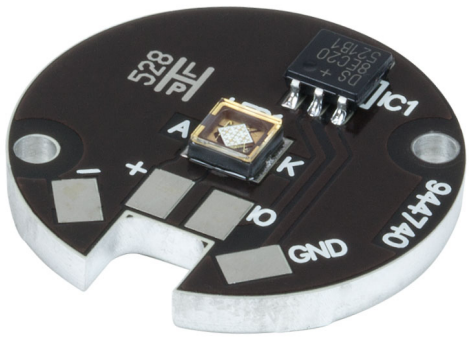


Figure 4.34: 365 nm Led for Epi Illumination module
Source: thorlabs.com

Printed Circuit Board Figures [4.35] showcases the Printed Circuit Board (PCB) layout of the Epi Illumination Driver developed for this project. The PCB design demonstrates a compact and optimized arrangement of components, carefully organized to ensure efficient signal routing and minimal interference. The figure provides a visual representation of the PCB's intricate circuitry, highlighting the precise

placement and interconnection of electronic components.

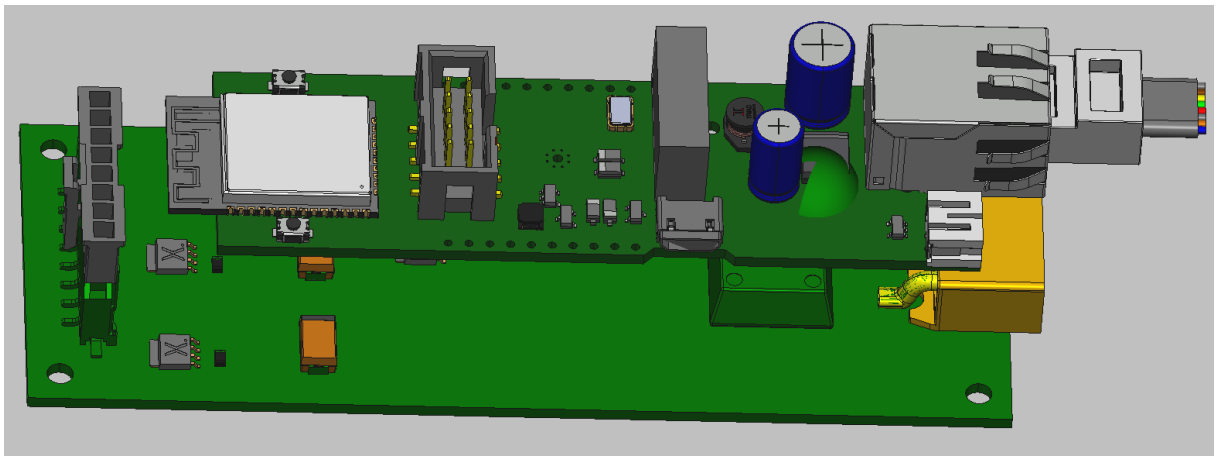


Figure 4.35: Epi Illumination Driver PCB
Source: Altium Designer Transmission Epi Driver Project

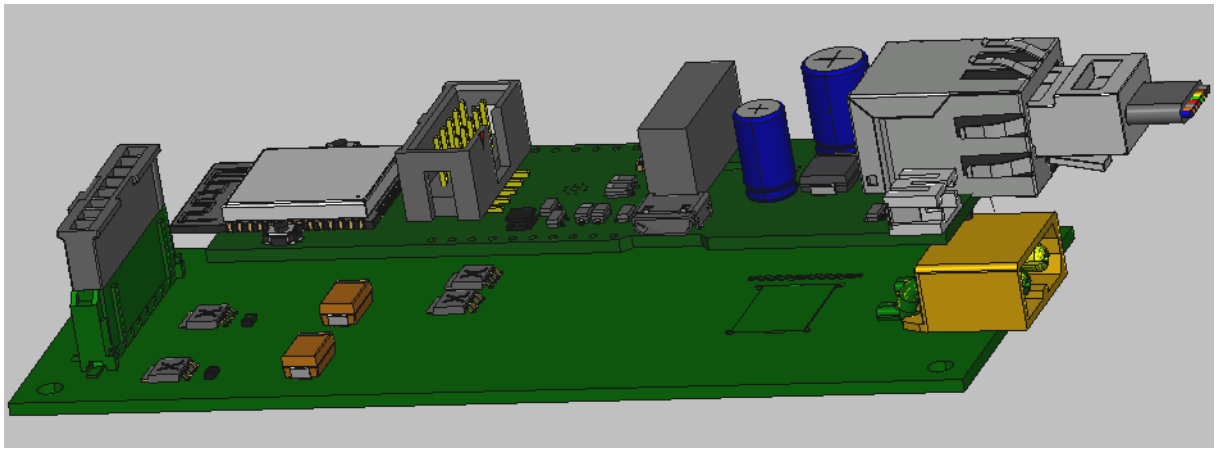


Figure 4.36: Epi Illumination Driver PCB 3D
Source: Altium Designer Transmission Epi Driver Project

Final Design The final system is housed within a durable aluminum enclosure, securely installed within the microscope body. Communication between the driver is facilitated by an Ethernet cable, ensuring reliable data transmission. Furthermore, the power supply for the system is delivered via an XT-60 connector, enabling efficient power distribution. The choice of an aluminum case, Ethernet communication, and XT-60 connector not only ensures the robustness and integrity of the system but also enables seamless integration and operation within the microscope setup.

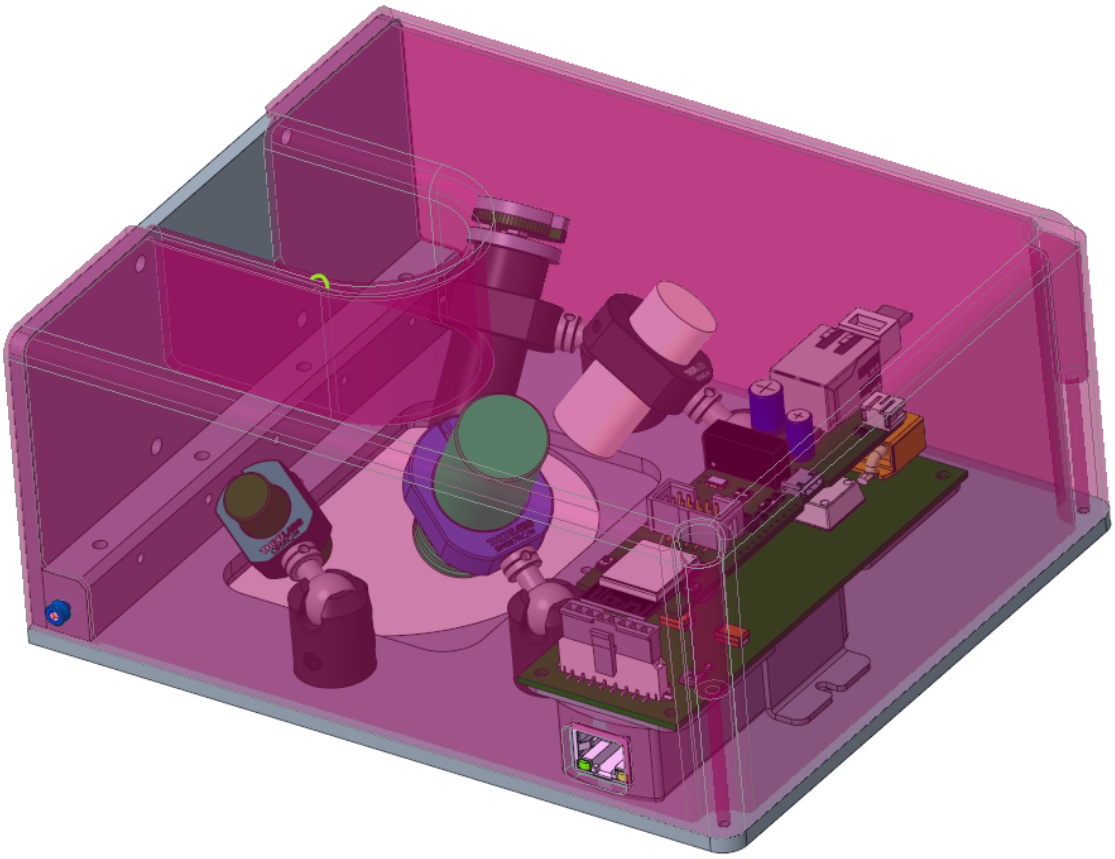


Figure 4.37: Epi Illumination Driver Final Design 3D
Source: Altium Designer Epi Illumination Driver Project

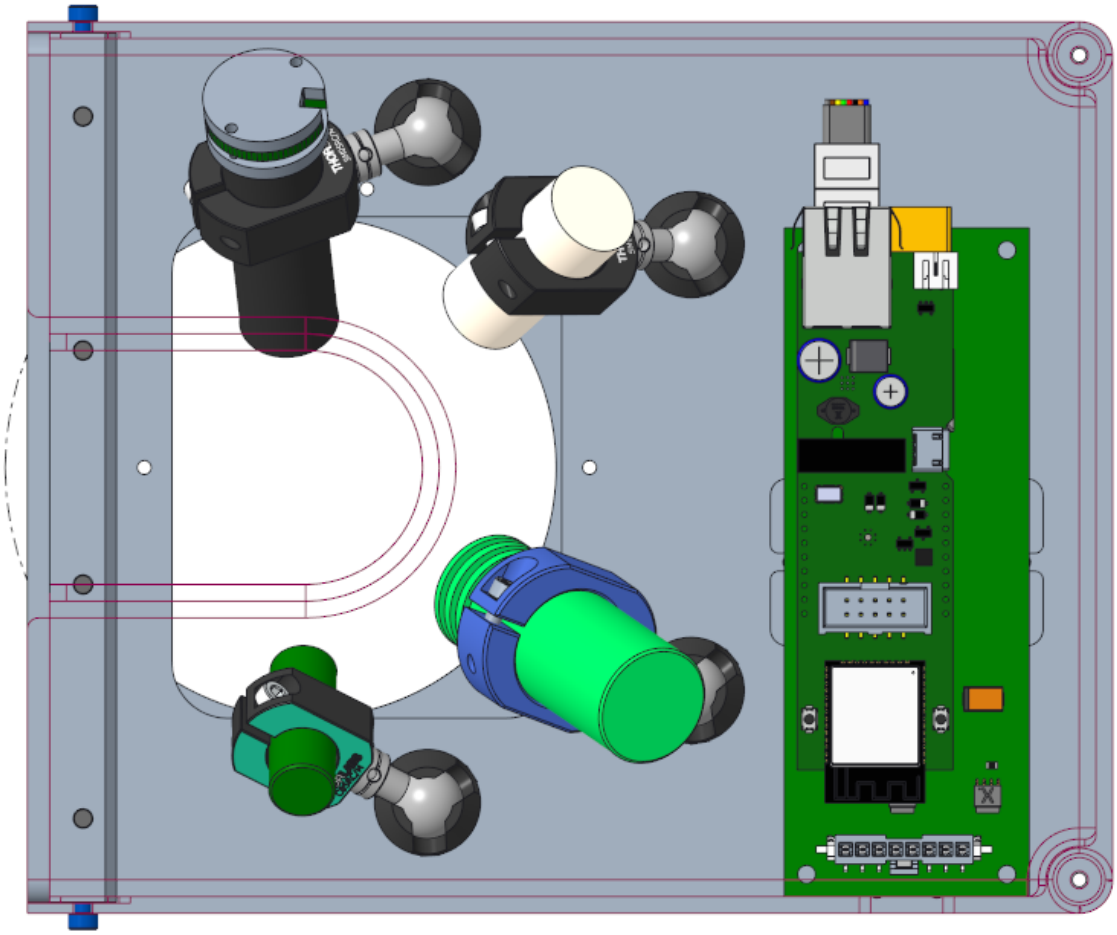


Figure 4.38: Epi Illumination Driver Final Design 3D
Source: Altium Designer Epi Illumination Driver Project

4.5 Power Manager

Automated power management and diagnosis are crucial aspects in the design of automated hardware systems. These features ensure the system's ability to sustain its operational activities while protecting individual components from potential damage. The power manager design, depicted in the block diagram shown in Figure, plays a fundamental role in achieving these objectives. To enable independent measurements and real-time communication, we incorporated the dual-core functionality of our **ERP32 PoE ISO**. This feature enhances the system's performance and responsiveness.

4.5.1 Power Measurements

To enable accurate measurements and real-time feedback on the system's operational status, we incorporated the **INA226** power measurement chips and an environmental measurement chip. The **INA226** is a high-accuracy power monitor chip which features an I2C interface and is capable of sensing bus voltages ranging from 0 V to 36 V. The chip enables high-side or low-side sensing, providing measurements of current, voltage, and power. With configurable averaging options and programmable calibration values, the **INA226** offers precise and direct readouts of current in amperes and power in watts. It operates within a power supply range of 2.7 V to 5.5 V and has a low supply current of approximately 330 µA. The INA226 is available in a 10-pin DGS (VSSOP) package and supports up to 16 programmable addresses on the I2C-compatible interface. It is suitable for various applications and operates reliably in temperatures ranging from -40°C to 125°C. The block diagram of this chip is presented in Figure [4.39].

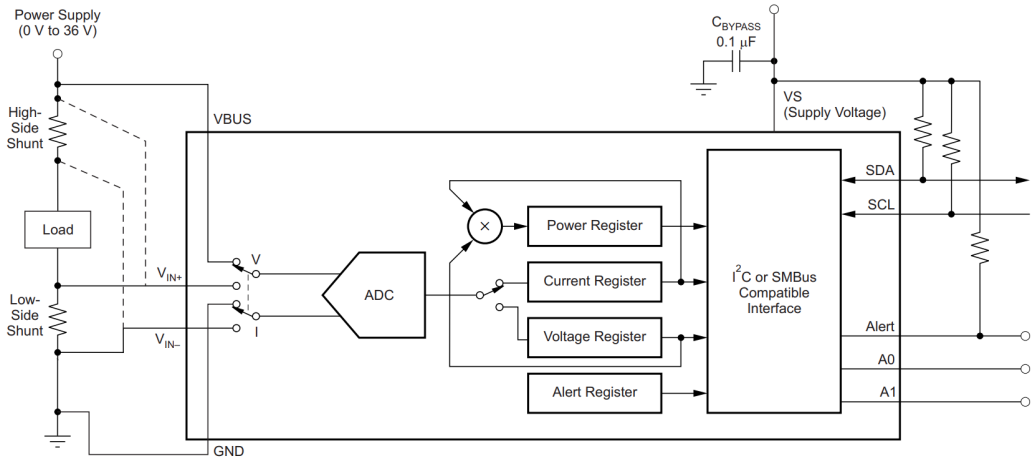


Figure 4.39: INA226 Block Diagram
Source: ti.com

The **INA226** chips provide precise monitoring of power-related parameters, such as current, voltage, and power consumption. These chips facilitate reliable and accurate power measurements within the system. The integration of these measurement chips enhances the overall functionality and performance of the system, allowing for effective monitoring and analysis of the system's power usage.

4.5.2 Environment Measurements

Furthermore, our design incorporates a sophisticated environmental measurement sensor, **BME680**, capable of measuring various parameters, including temperature, humidity, air resistance, and pressure. This enables comprehensive environmental monitoring and enhances the system's overall functionality. The sensor is presented in Figure [4.40].

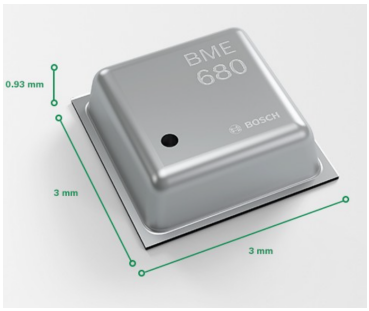


Figure 4.40: BME680
Source: bosch-sensortec.com

The **BME680** is a versatile gas sensor that combines high-linearity and high-accuracy measurements of gases, pressure, humidity, and temperature. Designed for mobile applications and wearables, it offers compact size and low power consumption. With optimized consumption, long-term stability, and strong electromagnetic compatibility (EMC) robustness, the BME680 is well-suited for various use cases. The sensor is capable of detecting a wide range of gases, including volatile organic compounds (VOCs), enabling us to assess air pollution levels and ensure a safe and healthy environment. By leveraging the capabilities of the BME680, we can track air quality trends, identify potential sources of gas emissions, and take appropriate measures to maintain optimal air conditions within the microscope environment as shown in Figure [4.41].

In our design, we have incorporated the functionality to accurately measure temperature and humidity variations, air resistance, and pressure within the microscope. These measurements provide valuable insights into the operational conditions of the system. By monitoring temperature and humidity, we can identify any abnormal

IAQ Index	Air Quality	Impact (long-term exposure)	Suggested action
0 – 50	Excellent	Pure air; best for well-being	No measures needed
51 – 100	Good	No irritation or impact on well-being	No measures needed
101 – 150	Lightly polluted	Reduction of well-being possible	Ventilation suggested
151 – 200	Moderately polluted	More significant irritation possible	Increase ventilation with clean air
201 – 250	Heavily polluted	Exposition might lead to effects like headache depending on type of VOCs	optimize ventilation
251 – 350	Severely polluted	More severe health issue possible if harmful VOC present	Contamination should be identified if level is reached even w/o presence of people; maximize ventilation & reduce attendance
> 351	Extremely polluted	Headaches, additional neurotoxic effects possible	Contamination needs to be identified; avoid presence in room and maximize ventilation

Figure 4.41: BME680 Index For Air Quality Classification
Source: bosch-sensortec.com

heating patterns that may pose a risk to the components or overall system performance. Additionally, by measuring air resistance and pressure, we can detect changes in the concentration of gases emitted by electrical components, such as carbon and other volatile substances. This real-time monitoring allows us to promptly address any potential issues and prevent further damage. In extreme cases, such as the occurrence of a fire, the sensor will immediately alert us, enabling us to implement necessary measures such as power distribution management to mitigate the risk and ensure the safety of the microscope and its surroundings.

4.5.3 Power and Air Flow Management

Power management is a critical aspect of our microscope design, ensuring efficient control of voltage and current flow.

By incorporating Panasonic's **ALQ3F05** power relays, we regulate and distribute power to various components, optimizing energy usage and preventing overloading. This safeguarding approach protects sensitive components from potential damage, enhances overall performance, and improves reliability.

Figure 4.42: Relays Selection
Source: npanasonic.com

The use of power relays enables dynamic power distribution, prioritizing allocation to modules based on operational requirements, thereby ensuring smooth operation

while minimizing electrical failures.

In order to control the whole array of relays from a single microprocessor we once again utilized the PCA9685. The PCA9685 chip is a 16-channel LED controller designed for efficient control of multiple constant current drivers and LED sources using a single microcontroller. It supports programmable PWM frequencies and precise brightness adjustment, enabling accurate control of LED brightness. With versatile output configurations, it can drive LEDs directly or be used with external drivers. Its wide voltage range and 5.5 V tolerant inputs and outputs make it suitable for various applications.

In addition to power management, controlling air flow within the microscope is essential. Noctua's **NF-A14** fans, shown in Figure [4.43], are strategically positioned around the microscope's case facilitate efficient heat dissipation and temperature regulation. This feature plays a vital role in maintaining optimal performance, ensuring that components operate within safe temperature ranges.

Figure 4.43: Fans Selection
Source: noctua.at

By effectively managing air flow, we prevent overheating, extend the lifespan of the microscope, and enhance its overall functionality.

4.5.4 Circuitry

For the purpose of the design of a compact and efficient Power Manager PCB, we utilized the Altium Designer. Once again we worked our design in blocks of components dedicated to different tasks. The Blocks design are named below:

1. **INA226**: This block consists of resistors and capacitors in order to set up the INA226. It safeguards the system from voltage spikes, monitors current consumption, and ensures safe operation within temperature limits.
2. **Power Relay**: This block is the driver circuit for our relays and it consists of a MOSFET, resistors and a diode. Power relays are integrated to control and distribute power to various sections and subsystems of the microscope. It enables efficient power management and allocation.

By structuring the Power Manager PCB design into these blocks, we achieve a systematic and organized approach, enhancing efficiency, reliability, and scalability. This modular design enables easy troubleshooting, maintenance, and future expansions or upgrades. Figures [4.44, 4.45] showcases the Printed Circuit Board (PCB) layout of the Power Manager developed for this project. The PCB design demonstrates a compact and optimized arrangement of components, carefully organized to ensure efficient signal routing and minimal interference.

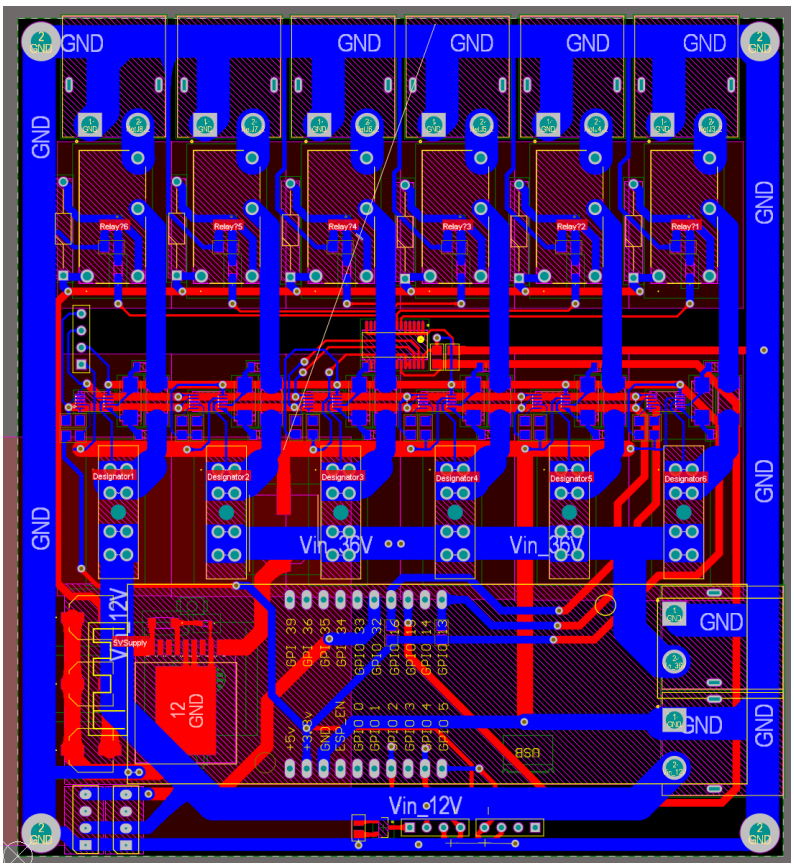


Figure 4.44: Power Manager PCB
Source: Altium Designer Power Manager Project

By integrating these key components into our power management system, we establish a robust and reliable infrastructure that supports the autonomous operation of

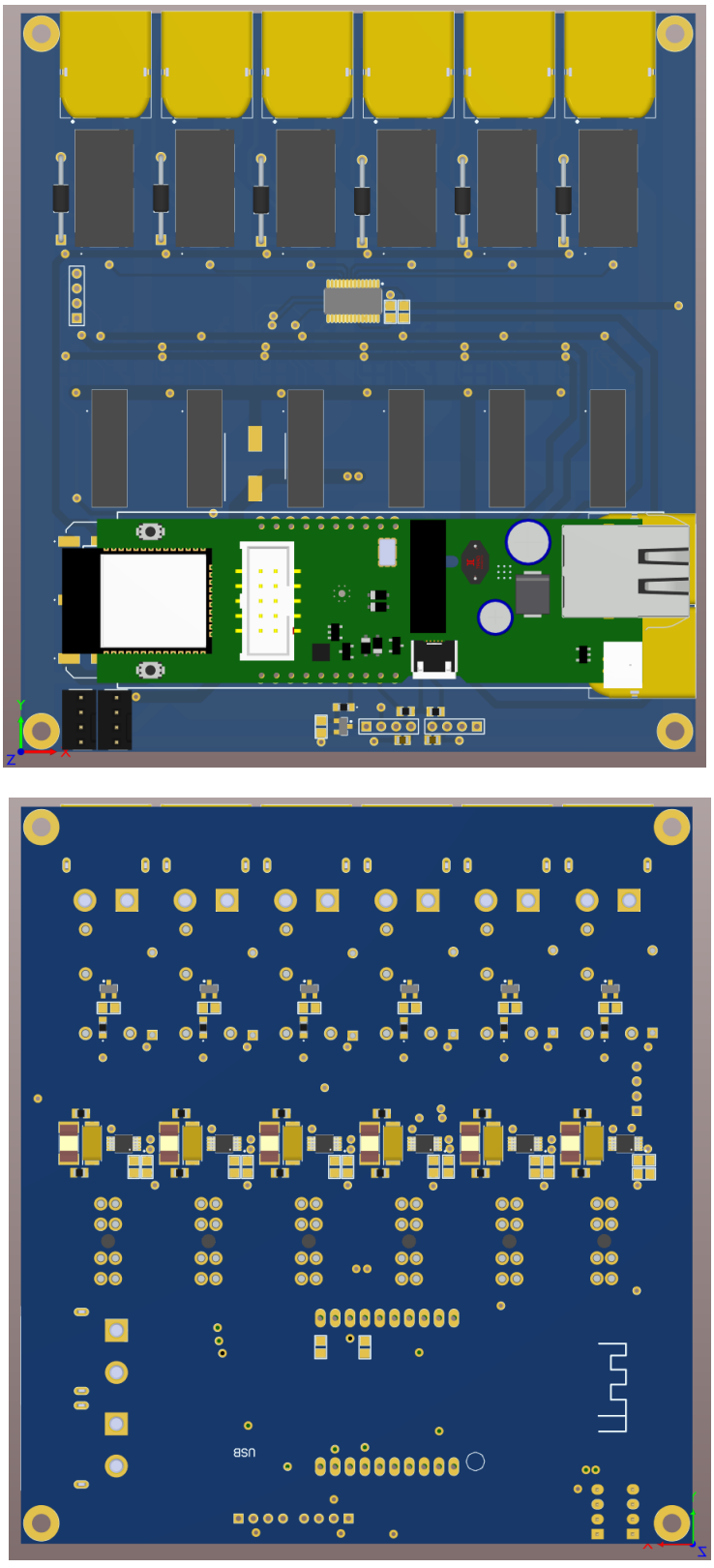


Figure 4.45: Power Manager PCB 3D
Source: Altium Designer Power Manager Project

our system.

4.6 Networking and Communication

A fundamental aspect of this thesis was to develop a comprehensive system comprising various peripherals capable of efficient real-time communication and task handling. The design of such a system necessitated adherence to several key requirements, including ease of hardware implementation and integration, adaptability to new architectures, straightforward software design and setup, and scalability.

4.6.1 Networking

In the proposed system design, networking of the different peripherals of the microscope, plays a crucial role in enabling seamless communication and coordination between various components of the high throughput screening microscopy system. The networking infrastructure facilitates data transfer, control signals, and synchronization among different modules, ensuring efficient operation and data management.

- **Network Architecture** The system should utilize a distributed network architecture to connect and integrate the different components. This architecture enables parallel processing and distributed computing, leveraging the strengths of multiple processors to enhance overall system performance. The network architecture consists of interconnected nodes, each equipped with processing capabilities, memory, and communication interfaces.
- **Data Transfer** Efficient and high-speed data transfer is vital in a high throughput screening microscopy system. Large amounts of image data need to be acquired, transmitted, and processed in real-time. To facilitate this, high-speed data transfer protocols, such as Ethernet or USB, are employed to ensure fast and reliable transmission between the different modules. The use of high-bandwidth connections minimizes data transfer latency and enables real-time processing and analysis.
- **Control and Synchronization** Accurate control and synchronization of the system components are critical for seamless operation. The network infrastructure allows for precise coordination and synchronization of motorized stages, cameras, and other peripherals. Control signals are transmitted over the network to ensure precise positioning, focusing, and capturing of images. Synchronization mechanisms, such as timestamping and triggering, are employed to ensure temporal alignment between different modules and enable coordinated actions.

– **Security and Scalability** In a research environment, data security and scalability are important considerations. The network infrastructure incorporates security measures, such as encryption protocols and access controls, to protect sensitive data and ensure the integrity and confidentiality of the system. Additionally, the system is designed to be scalable, allowing for the integration of additional modules or expansion of the network as the research demands grow.

In summary, the networking component of the system design provides the necessary infrastructure for seamless communication, data transfer, control, and synchronization within the high throughput screening microscopy system. The distributed network architecture, high-speed data transfer, precise control and synchronization mechanisms, efficient data management, and security measures ensure optimal performance and scalability of the system.

4.6.2 Network Protocols

To fulfill these requirements, a critical decision had to be made regarding the communication technology to be integrated into the system. Several technologies were considered, each offering distinct advantages and characteristics. The technologies under consideration included:

– **USB**: USB is an industry-standard interface that revolutionized device connectivity and data transfer. With its various generations and connector types, USB provides fast and efficient connections for computers, peripherals, and mobile devices.

Pros:

1. Versatile and widely supported communication interface.
2. Allows for high-speed data transfer.
3. Enables device interconnectivity.

Cons:

1. Limited in terms of distance coverage.
2. Requires physical connection with cables.

– **I2C**: I2C developed by Philips Semiconductor in 1982, has become a popular bus interface connection protocol for short-distance communication. It is also commonly referred to as the Two-Wire Interface (TWI).

Pros:

1. Simple and easy to implement.
2. Suitable for connecting multiple devices over short distances.
3. Low power consumption.

Cons:

1. Limited in terms of data transfer speed.
2. Not suitable for long-distance communication.

– **Ethernet**: Ethernet utilizes various physical media, including coaxial cables, twisted pairs, and fiber optics, and employs frames for data transmission. It provides services up to the data link layer of the OSI model and supports error checking and retransmission of lost frames.

Pros:

1. Fast and reliable network communication protocol.
2. Extensive infrastructure support.
3. Suitable for high-speed data transfer over long distances.

Cons:

1. Requires network infrastructure and configuration.
2. Higher power consumption compared to some other protocols.

– **WiFi**: Wi-Fi, based on the IEEE 802.11 family of standards, enables devices to connect to local area networks and access the internet through radio waves.

Pros:

1. Enables wireless communication, providing flexibility and convenience.
2. Suitable for various environments.
3. Allows for remote connectivity.

Cons:

1. Limited range compared to wired protocols.
2. Susceptible to interference and signal degradation.

The selection of the most suitable communication technology depends on the specific application requirements, system architecture, data transfer speed, range, power consumption, and compatibility with existing infrastructure. After considering all the different choices, we have ultimately decided to adopt **Ethernet** as the communication protocol for our system. Ethernet offers a range of benefits that align well with our requirements, such as fast and reliable network communication, extensive infrastructure support, and compatibility with existing systems. It provides the necessary capabilities for high-speed data transfer and facilitates seamless integration

into various network environments.

The System Network Configuration of our microscope is an integral part of the overall design, providing efficient connectivity and power management. We have incorporated a high-quality network switch that offers Power Over Ethernet (PoE) functionality, allowing for the simultaneous transmission of data and power through a single Ethernet cable. This eliminates the need for separate power cables, simplifying the setup and reducing clutter. The switch features 8 ports, providing ample connectivity options for various components and devices within the microscope system. Each port serves a specific purpose, ensuring seamless communication and data transfer between the different modules and peripherals. This network configuration plays a crucial role in enabling smooth operation and effective collaboration between the components, contributing to the overall functionality and performance of the microscope. The ports are utilized as shown in Figure [4.46].

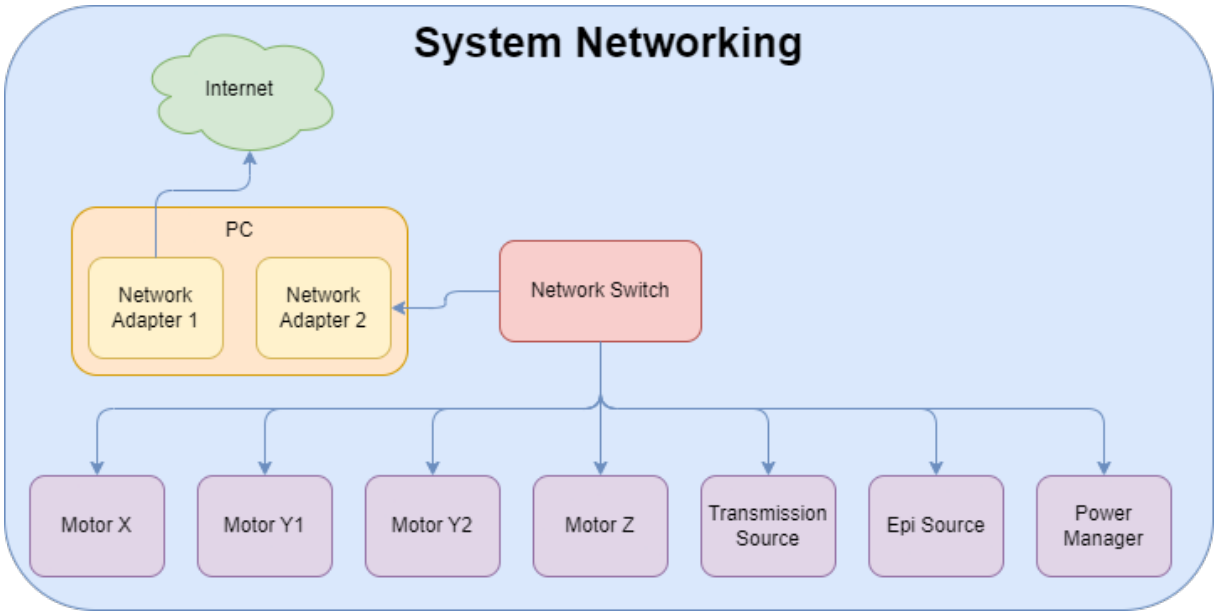


Figure 4.46: System Network Configuration

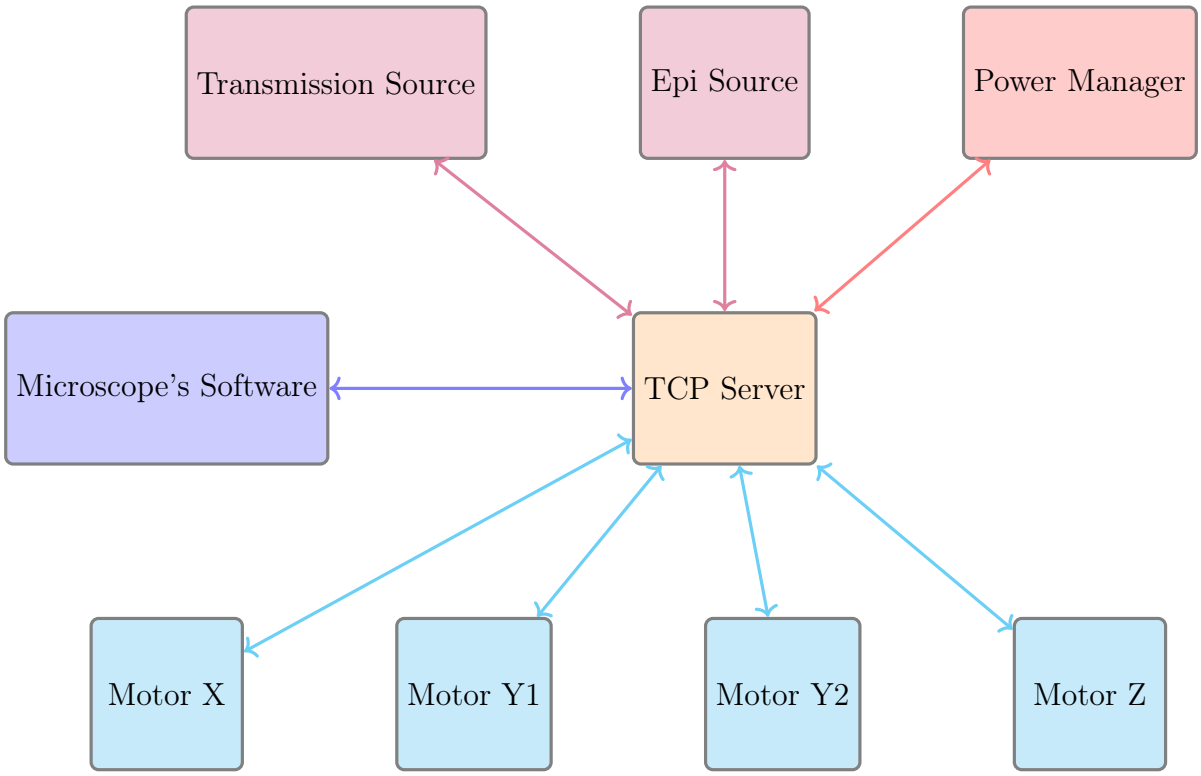
4.6.3 Hardware Network Manager

In order to fulfill the requirements of a versatile, fast, and reliable high-throughput screening microscope, this thesis focuses on the development of a software solution to control the various custom-made drivers necessary for its operation.

The software implementation of a hardware network manager module, created using Qt Creator, is based on several aspects:

- Management of firewall rules.
- Configuration of Ethernet adapter options.
- Create a TCP Server.
- Establish connection with the custom made peripherals.
- Establish connection with the microscopes software.
- Manage and distribute messages from and to different peripherals.

This network manager allows seamless communication between the software and each individual custom made peripheral driver.



The design of the network manager involves a server architecture, where peripheral devices and the main software are connected via TCP sockets. This server manages the status and message exchange between the software and the connected devices.

The implementation of this design follows established principles and protocols to ensure efficient and reliable communication within the system.

By integrating this software solution into the high-throughput screening microscope, it becomes possible to effectively control and coordinate the different drivers, enhancing the overall functionality and performance of the microscope.

4.6.4 Peripherals Interface

In this thesis, we present an innovative interface designed to efficiently control the peripherals developed as part of our research. This interface leverages the software implementation of the Hardware Manager, which was described earlier, and incorporates a basic yet effective Graphical User Interface (GUI) designed using the Qt Creator Designer tool. The Main interface is carefully constructed to effectively manage the different peripherals supported by our software. Figure [4.47] showcases this GUI.

Figure 4.47: Main Peripherals GUI

In the implemented interface, a green circle serves as a visual indicator, representing the device status with green indicating a connected state and red denoting disconnection. Additionally, the interface incorporates strategically placed buttons that enable users to manage the Over the Air Firmware update capabilities of our peripherals and to toggle the Power over Ethernet Capabilities of our Network Switch. Moreover, the interface features individual buttons for displaying or hiding each peripheral's Graphical User Interface, providing users with a convenient and efficient means of interacting with the system.

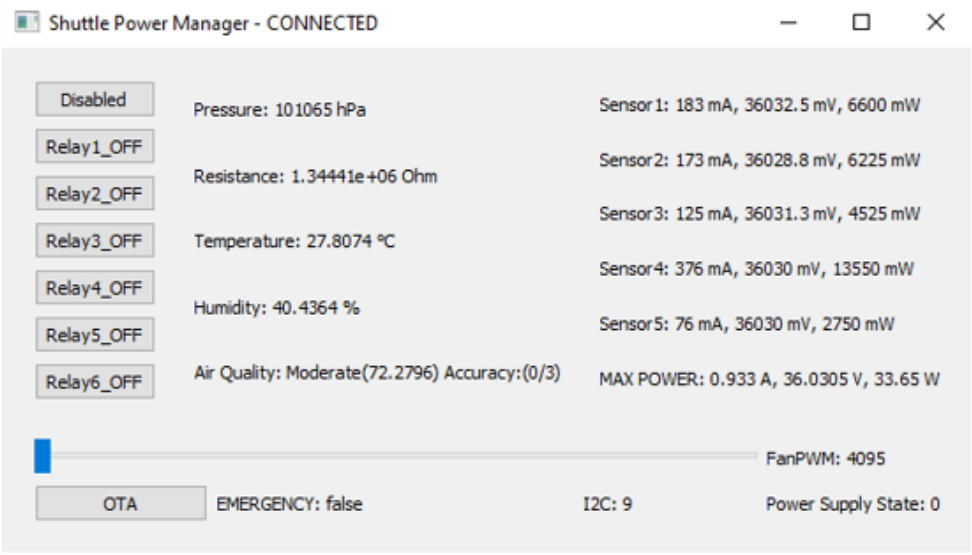


Figure 4.48: Power Manager GUI

The Power Manager unit's user interface is showcased in Figure [4.48]. This Graphical User Interface (GUI) comprises a dedicated section for manual configuration of the power relays used in the Power Manager PCB. Adjacent to these buttons, informative labels provide real-time updates on the air quality within the microscope's casing. Additionally, the interface displays label indications presenting the voltage, current, and power consumption data for each peripheral in our design. Lastly, a slider control is implemented to efficiently manage the PWM duty cycle of the air circulation system, enhancing the overall control and customization options of the Power Manager unit.

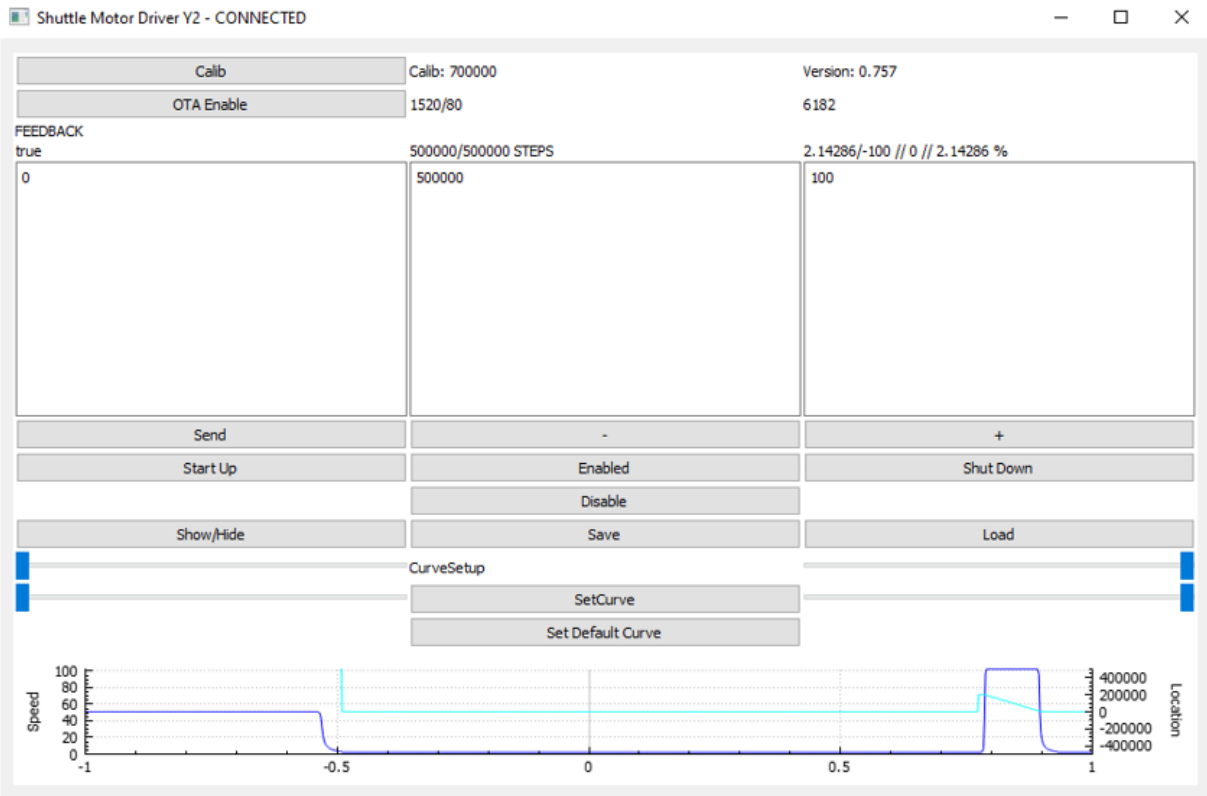


Figure 4.49: Motors GUI

The Motor Drivers GUI, as depicted in Figure [4.49], serves as a powerful tool for real-time interaction with the Motor Drivers. Its fundamental purpose is to grant users the capability to issue custom commands to the Motor Drivers while providing immediate visual feedback on the motor's operation. Each button within this user-friendly interface is assigned a dedicated command, enabling seamless execution irrespective of the current state of the motor driver. Furthermore, the lower section of the GUI features a visualization tool, allowing users to observe in real-time the current status of Speed and Location Variables within the Motor Drivers Engine Core. This sophisticated GUI empowers users with precise control over motor operations and facilitates comprehensive monitoring of vital variables, significantly enhancing the efficiency and effectiveness of the motor control process.

The Graphical User Interface (GUI) of our Transmission Illumination Driver, illustrated in Figure [4.50], offers a comprehensive range of functionalities for configuring diverse illumination conditions. Through intuitive slide bars, users can conveniently adjust the intensity levels for each wavelength. Remarkably, each wavelength provides a staggering 4096 different intensity levels, affording precise control over the illumination settings. Moreover, the GUI allows simultaneous real-time configuration of all wavelengths to the desired intensity, ensuring seamless and instantaneous adjustments. This advanced GUI empowers users with the ability to fine-tune il-

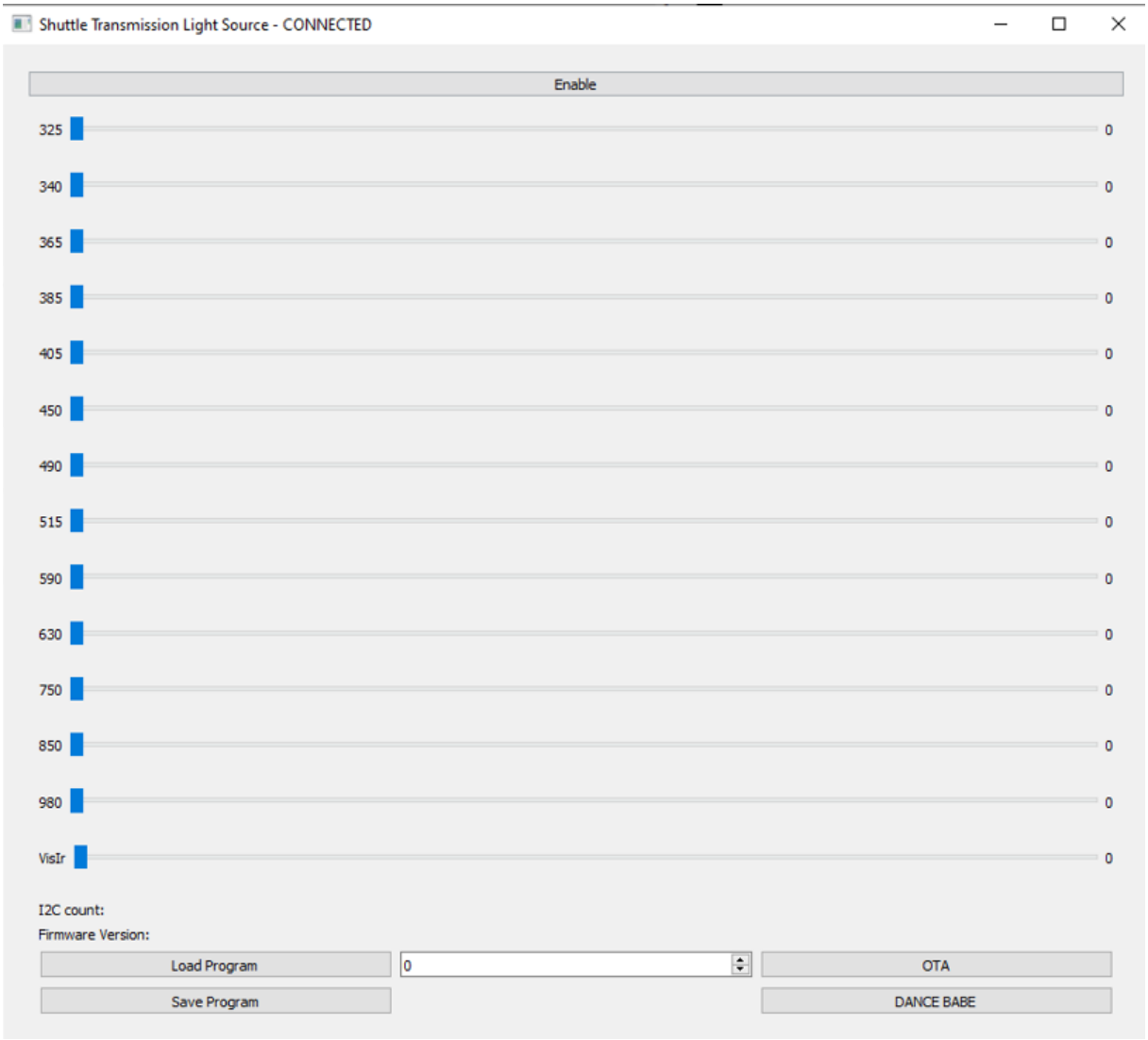


Figure 4.50: Transmission Illumination GUI

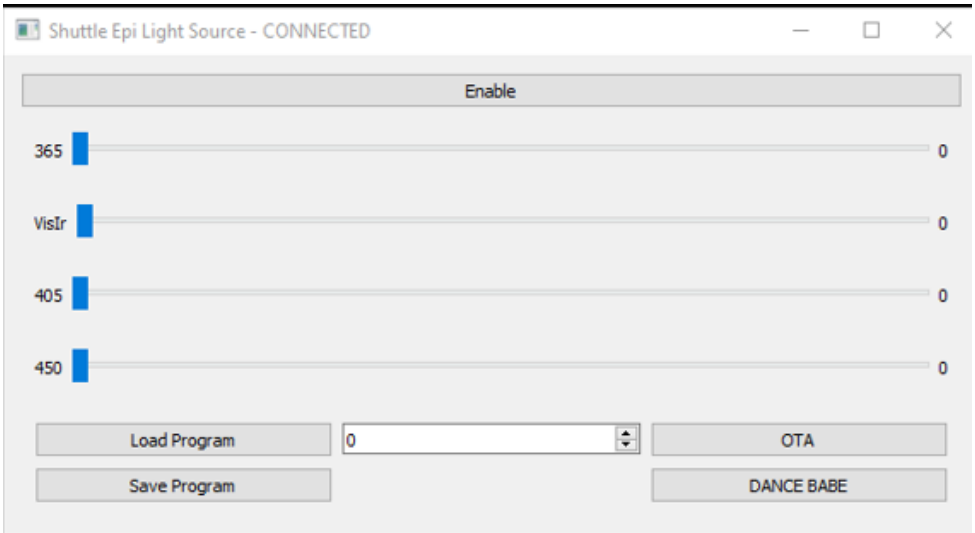


Figure 4.51: Epi Illumination GUI

lumination conditions, facilitating enhanced experimental control and accuracy in various applications. The same principals of operation are followed for the creation of the Epi Illumination Graphical User Interface as shown in Figure [4.51].

Chapter 5

Results

The design of the hardware components, as detailed in the previous chapter, form the fundamental contribution of this thesis. Collaborating with the engineers at the Electronics Laboratory of the Technical University of Crete, we successfully constructed a comprehensive framework for a High Throughput Microscope. The results of this endeavor are extensively presented in the following chapter.

5.1 Design Evolution

In the context of enhancing the Mark One design to the Mark Two version, a comprehensive set of tasks was undertaken to address various aspects and evaluate the resulting improvements. These endeavors aimed to overcome the limitations and challenges encountered in the previous design and achieve significant enhancements in the performance and functionality of the microscope. The outcome of this evolution is depicted in Table [5.1].

Aspects	**Mark One**	**Mark Two**
Scanning Area	$100cm^2$	$2500cm^2$
Imaging Modalities	3	20
Movement Speed	$5mm/s$	$40mm/s$
Data Throughput	$20ms/instruction$	$2ms/instruction$
Acquisition Speed per mm^2	$6sec/mm^2$	$2sec/mm^2$
Speed per Modality	$1sec/mode$	$100ms/mode$
Scalability	max 10 $Devices$	$unlimited$ $Devices$
Power Management	$Unmanaged$	$Managed$

Table 5.1: Mark One vs Mark Two Improvements

5.2 HTS Microscope Configuration

With invaluable assistance from the engineers at the Electronics Laboratory and Spectricon, we successfully integrated the custom-designed peripherals from this thesis into a prototype version of a high-throughput screening microscope. The integration process involved meticulous attention to detail and careful coordination to ensure seamless functionality. The comprehensive design procedure, including the steps taken during integration, is illustrated in the accompanying figures.

The mechanical design of the system was meticulously executed by the skilled engineers from the Electronics Laboratory. Their expertise, combined with advanced CAD software and modern manufacturing techniques, ensured the creation of a robust and efficient design. This carefully engineered design serves as the foundation for the system, providing stability and functionality for various microscopy applications.

The motor drivers, network switch, and power manager, are located within a stable and enclosed compartment of the microscope. This placement ensures optimal performance and protection for these critical elements. Figure [5.1] illustrates the positioning of these components nearby the main power supplies, highlighting the careful consideration given to their arrangement within the overall system design.

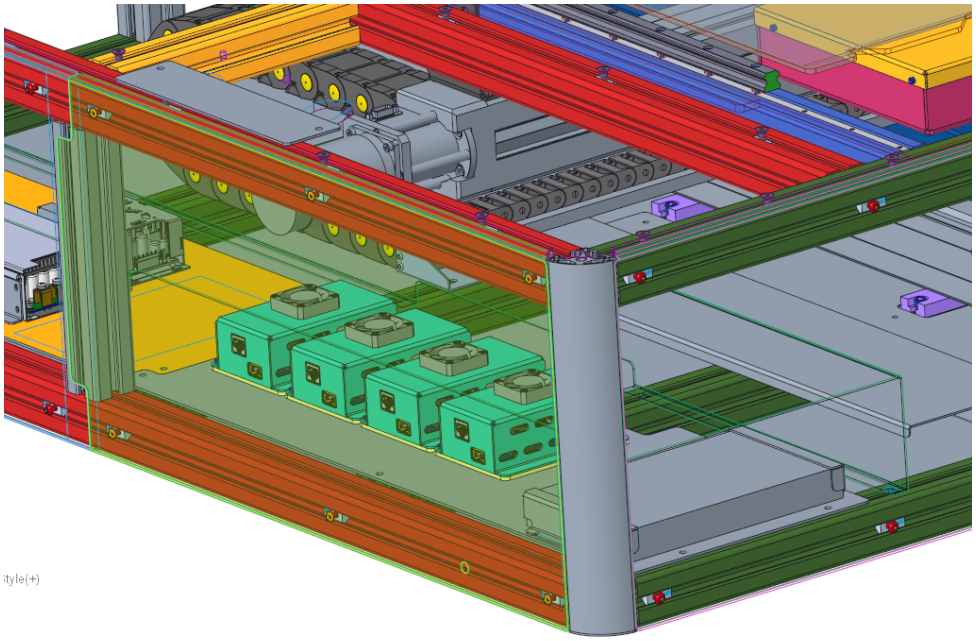


Figure 5.1: HTS Microscope Motor Drivers Arrangement

The illumination sources, as well as the system's camera, are ingeniously integrated and supported by the Y-axis motors of the microscope. This strategic placement ensures optimal positioning and stability, allowing for precise control and alignment during imaging processes. The Y-axis motors enable smooth and synchronized movement of the illumination sources and camera, facilitating seamless scanning and capturing of high-quality images.

The engineers from the Electronics Laboratory meticulously designed and implemented this configuration, considering factors such as weight distribution, mechanical stability, and ease of operation. The result is a well-engineered system that effectively combines the motion control capabilities of the Y-axis motors with the functionality of the illumination sources and camera, ensuring exceptional imaging performance in various microscopy applications. Figure [5.2] illustrates the positioning of these components.

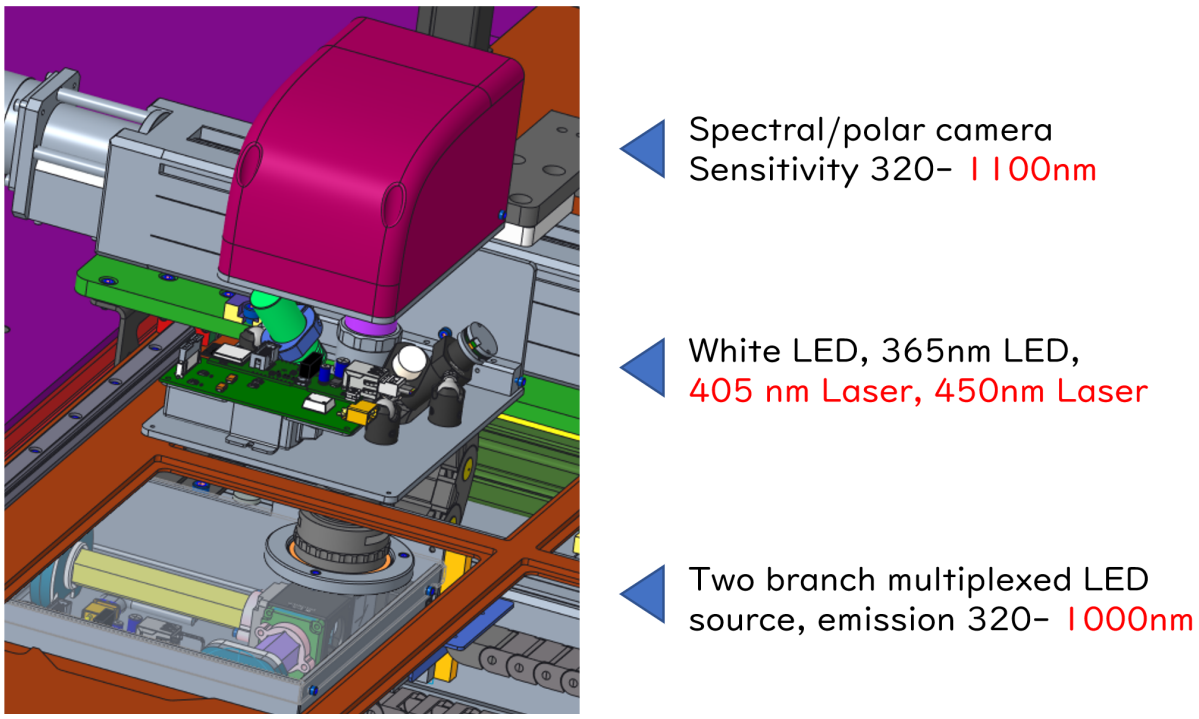


Figure 5.2: HTS Microscope Camera and Illumination System Arrangement

The High Throughput microscope presented in this study represents a significant advancement in the field of imaging systems. Its compact and efficient design allows for the acquisition of Transmission Color/Hyperspectral, Reflectance, Fluorescence, and Polarization Imaging Modalities across a vast 4 A4 area. Under collaboration with Spectricon's engineers the design proposed in the thesis was tested and implemented on a real scale prototype. The final setup of Spectricon's SMMART HTS

Microscope is shown in Figure [5.3, 5.6].

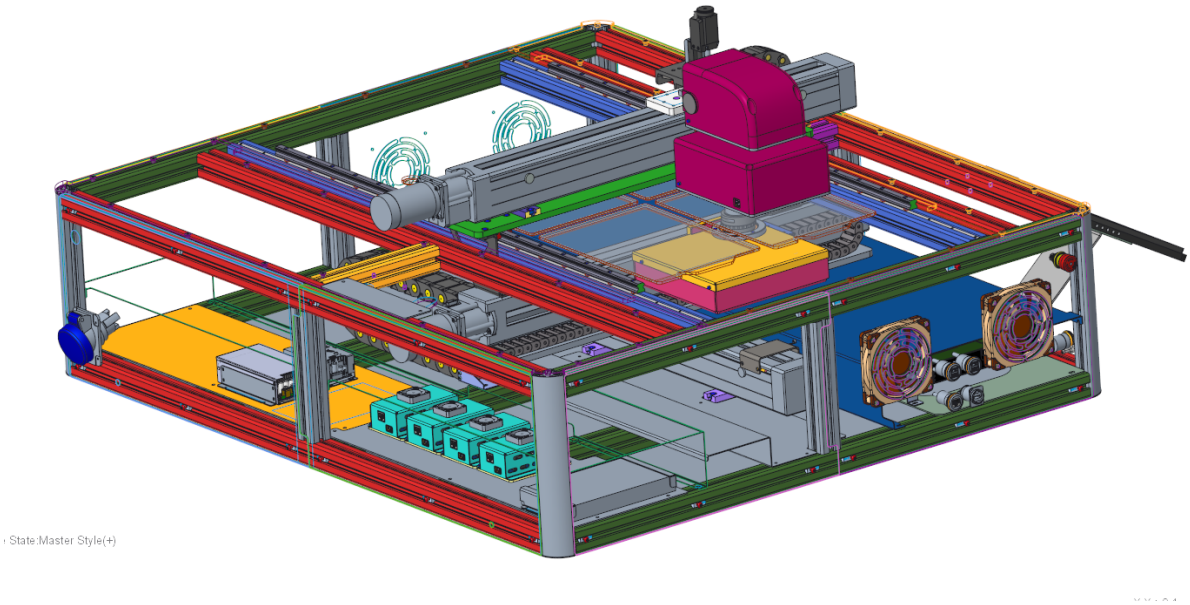


Figure 5.3: HTS Microscope 3D

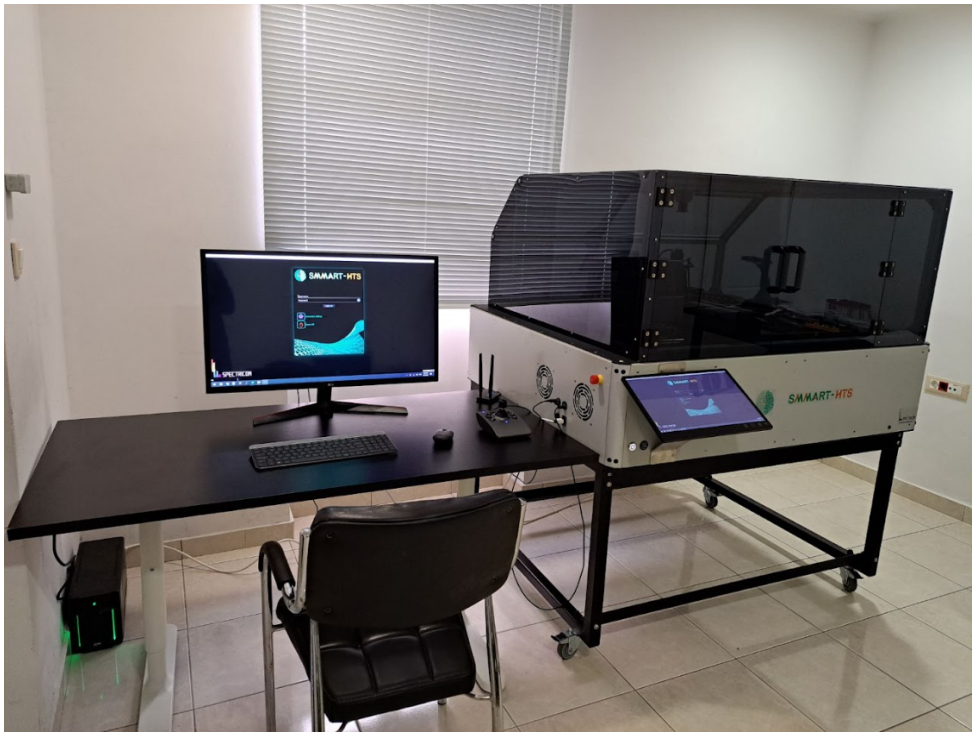


Figure 5.4: SMMART HTS Microscope

5.3 Acquisition Technique

This exceptional hardware and optical configuration, coupled with the sophisticated software developed by the engineers of the Electronics Laboratory, enable seamless control and rapid data acquisition. The integration of the software and the custom-designed Hardware Manager proposed in this thesis has resulted in a groundbreaking achievement: the microscope is capable of acquiring all the different imaging modalities in an astonishingly short timeframe of just 6 seconds. This unprecedented speed not only ensures efficient data collection but also facilitates field sequential scanning of the entire microscope platform in less than a day of work. This remarkable capability opens up new possibilities for high-throughput imaging, enabling researchers to capture comprehensive and detailed data in a fraction of the time previously required.

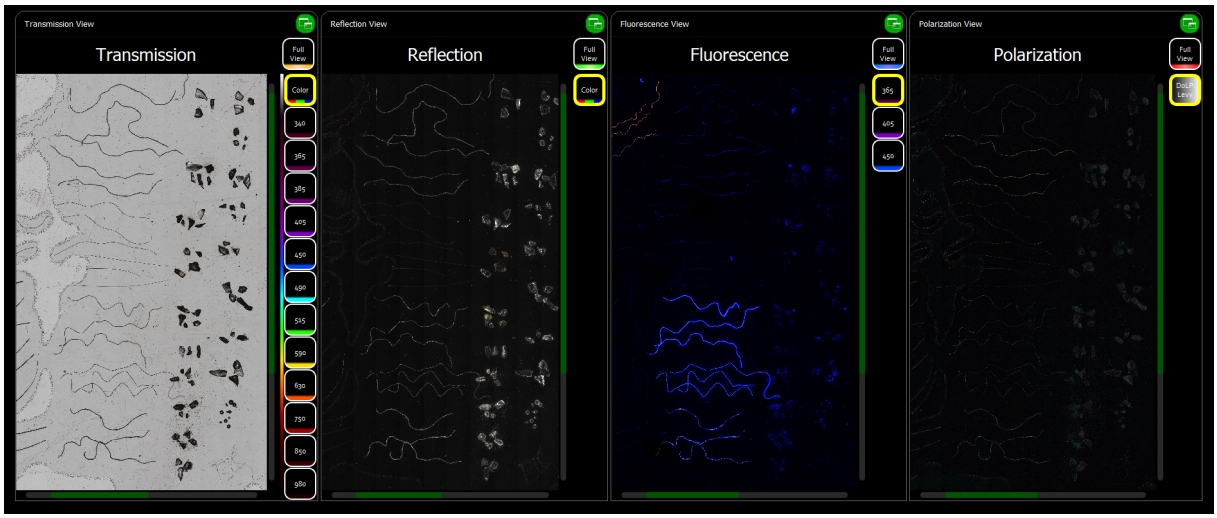


Figure 5.5: Proposed Acquisition Technique Results

The High Throughput microscope's exceptional performance and rapid acquisition capabilities have the potential to revolutionize various fields of research and analysis. It offers researchers and scientists the opportunity to explore and analyze samples with unprecedented speed and efficiency. The ability to acquire multiple imaging modalities, including Transmission Color/Hyperspectral, Reflectance, Fluorescence, and Polarization, within such a short timeframe provides a comprehensive and multifaceted understanding of the samples under investigation. This breakthrough technology not only enhances productivity but also enables new avenues of research and discovery, pushing the boundaries of imaging capabilities. With its compact design, powerful software integration, and exceptional data acquisition speed, the High Throughput microscope opens up new possibilities for scientific exploration and holds tremendous potential for advancing various fields of study, from

biology and medicine to material science and beyond.

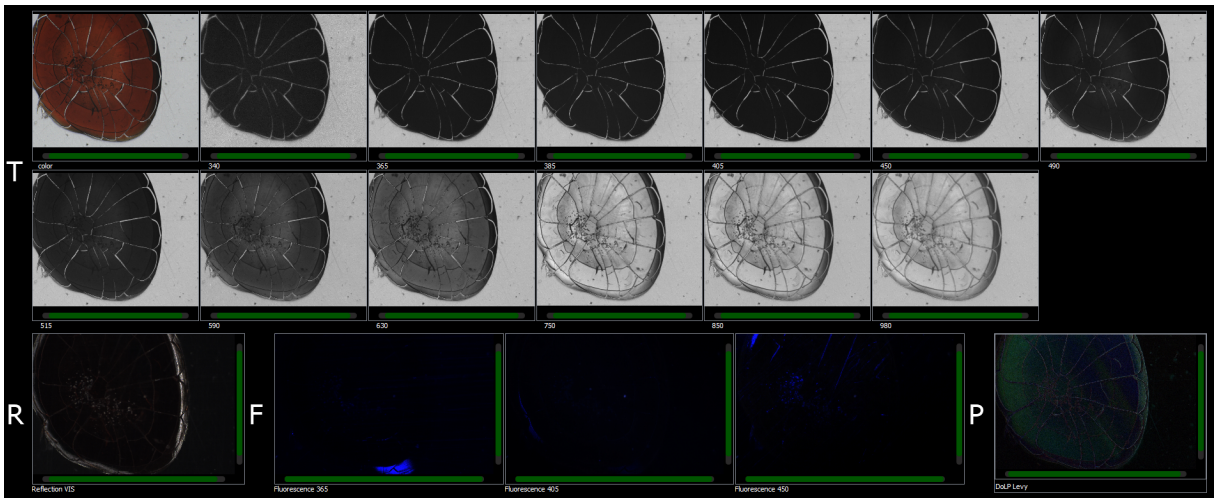


Figure 5.6: Frame Acquisition Results

Furthermore, one of the most remarkable features of the High Throughput microscope is its ability to acquire spectroscopy per pixel, as shown in Figure [5.7]. This advanced functionality allows for the detailed analysis of the spectral characteristics of each individual pixel in the captured images. By capturing and analyzing the full spectral information, researchers gain valuable insights into the composition, properties, and behavior of the samples at a microscopic level. This spectroscopic capability opens up new avenues for studying complex phenomena, such as chemical reactions, material composition, and biological processes. The integration of spectroscopy per pixel in the High Throughput microscope significantly expands its analytical capabilities, making it an invaluable tool for researchers seeking comprehensive and in-depth understanding of their samples.

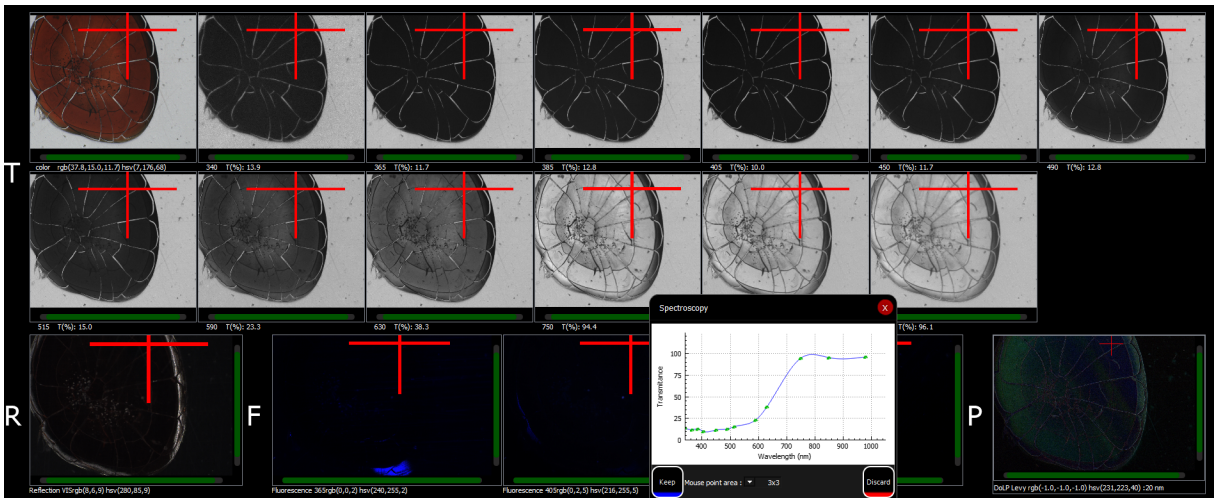


Figure 5.7: HTS Microscope Spectrum per pixel

Figure 5.8: Results of a Complex Sample Transmission, Fluorescence, Polarization

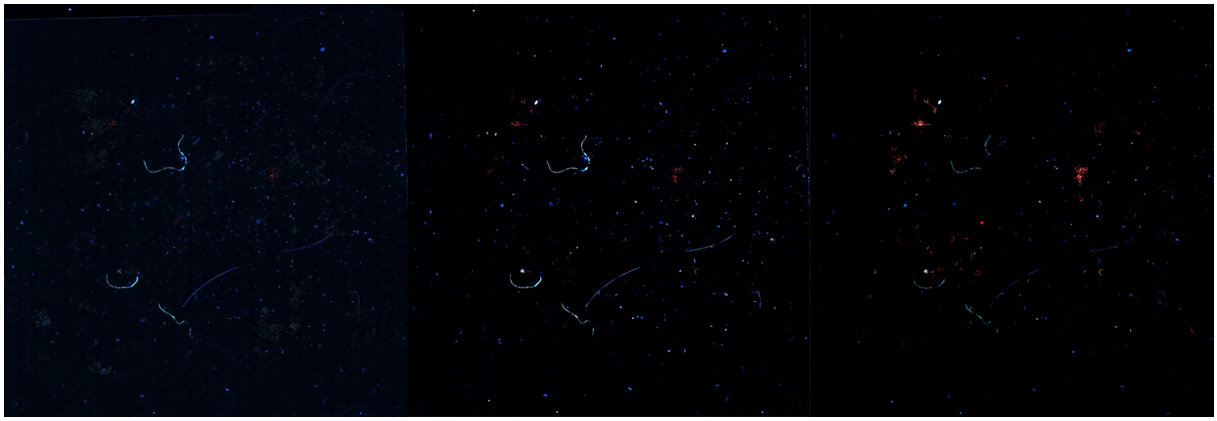


Figure 5.9: Results of a Complex Sample Fluorescence 365nm, 405nm, 450nm

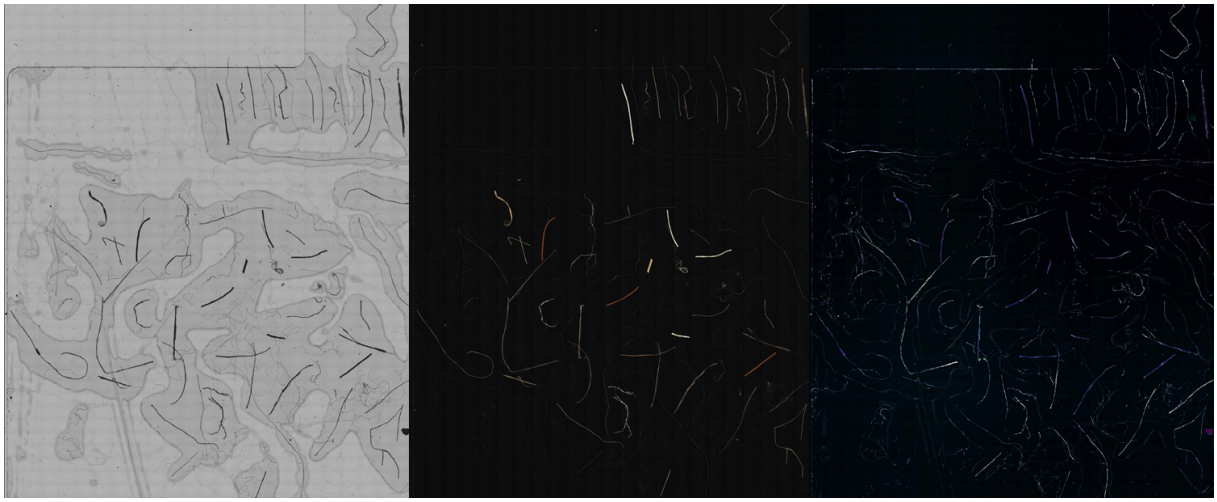


Figure 5.10: Results of a Natural Fibers Sample Transmission, Fluorescence, Polarization

Figure 5.11: Results of a Natural Fibers Sample Fluorescence 365nm, 405nm, 450nm

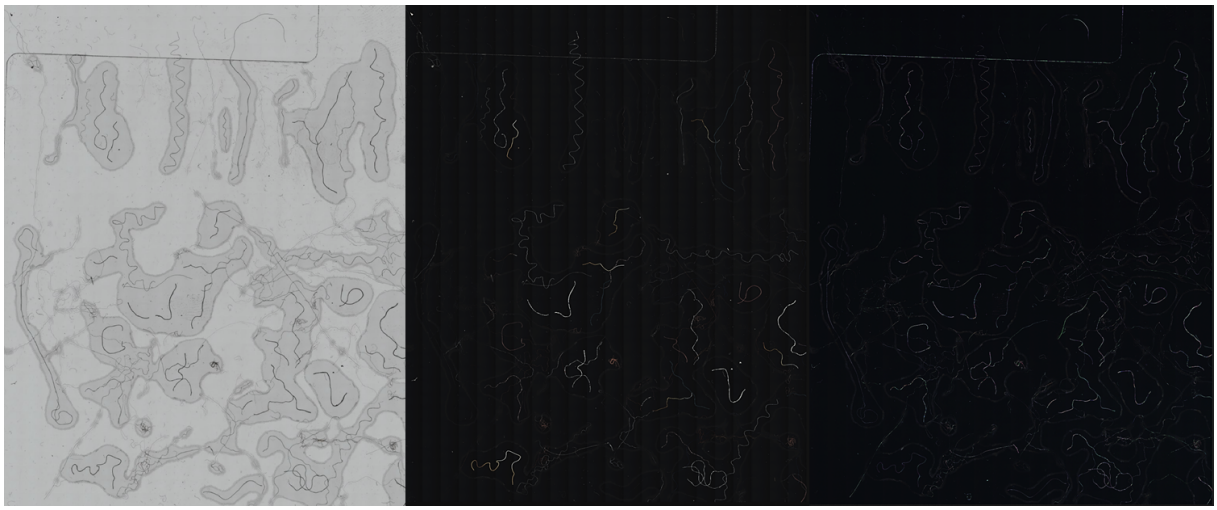


Figure 5.12: Results of a Synthetic Fibers Sample Transmission, Fluorescence, Polarization

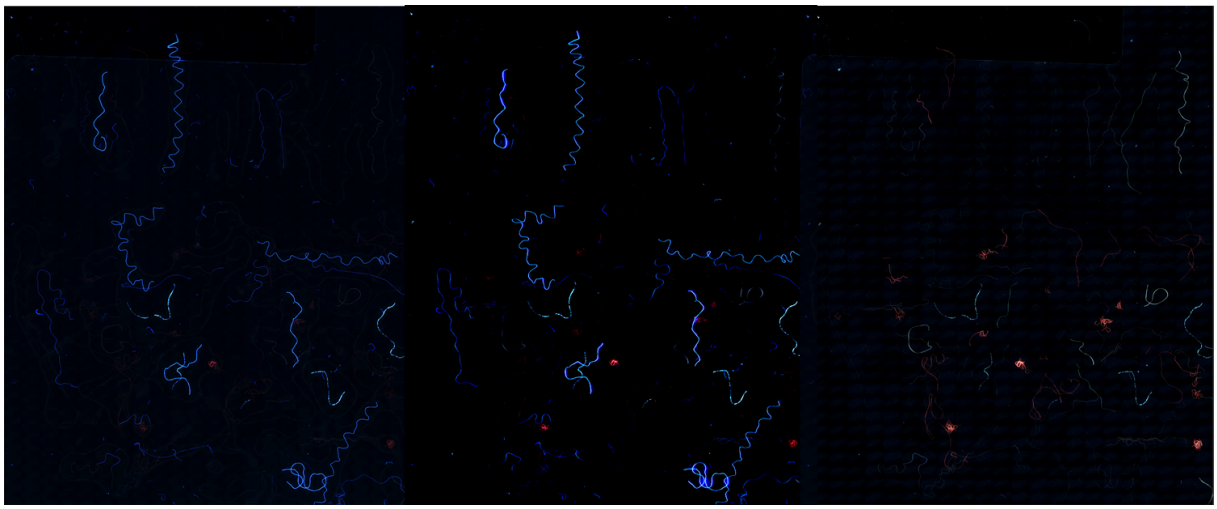


Figure 5.13: Results of a Synthetic Fibers Sample Fluorescence 365nm, 405nm, 450nm

5.4 Conclusion & Future Work

This research introduced a groundbreaking innovation in forensic science, centered around the concept of multi-modal microscopy and high-speed scanning. By integrating various analytic and imaging techniques into a single instrument, and combining it with high-speed scanning capabilities, the proposed approach revolutionizes forensic analysis. This multifaceted device enables simultaneous acquisition of multiple imaging modalities, such as transmission color, hyperspectral, reflectance, fluorescence, and polarization, providing a comprehensive understanding of the samples under investigation. The Multi Modal Feature Space data collection produced from the microscope array is shown in Figure [5.14].

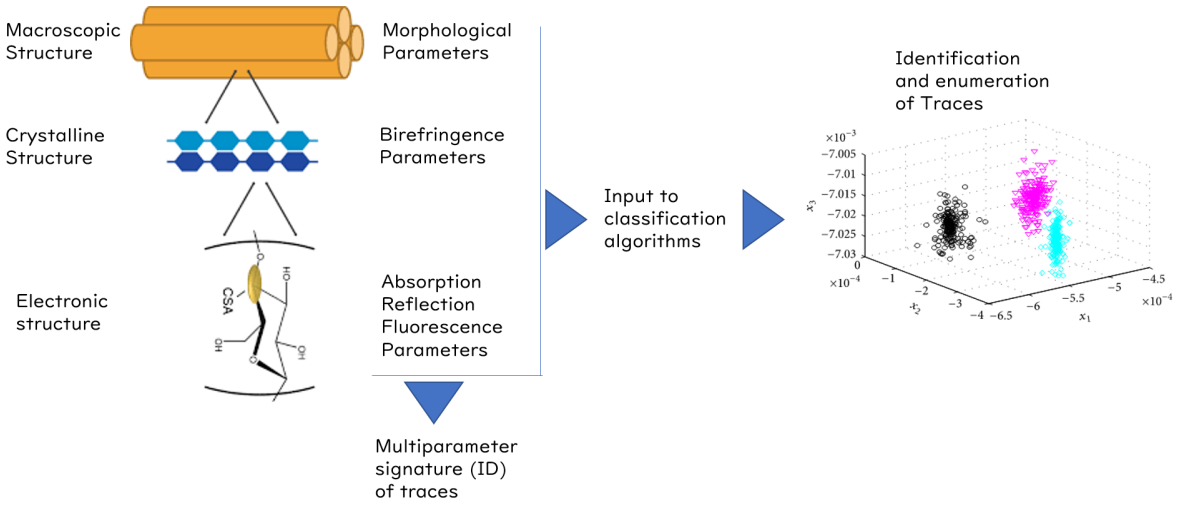


Figure 5.14: Multi Modal Feature Space

Additionally, the high-speed scanning feature allows for rapid data acquisition, significantly reducing analysis time and improving overall efficiency. This cutting-edge technology has the potential to transform forensic investigations, enhancing accuracy, productivity, and the ability to extract valuable information from trace evidence. By leveraging the power of multi-modal microscopy and high-speed scanning, this instrument opens up new possibilities for advancing forensic science and addressing the complex challenges faced in the field.

Moving forward, several areas of future work can be explored to further enhance the capabilities of the instrument and advance forensic analysis:

- Addition of a motorized zooming system: This enhancement would enable selective resolution scanning, allowing investigators to focus on specific areas of interest with higher magnification.

- 3D reconstruction of traces: Implementing techniques for 3D reconstruction would provide a more detailed and comprehensive representation of the scanned traces, aiding in the analysis and interpretation process.
- Advancements in imaging techniques, automation algorithms, and data analysis methods: Continuous improvement in these areas can lead to enhanced image quality, more efficient data processing, and improved accuracy in identifying and characterizing trace evidence.
- Integration of artificial intelligence and machine learning: Incorporating AI and ML algorithms can automate the identification and classification of trace evidence, reducing the reliance on manual analysis and enabling faster and more objective results.
- Exploration of interdisciplinary applications: Investigating the potential use of the technology in other fields, such as material science, bioengineering, and environmental monitoring, opens up new opportunities for innovation and expands the impact of the research beyond forensic science.

In conclusion, this research has presented a novel approach in analysis through the integration of multi-modal microscopy and high-speed scanning. The developed instrument offers a comprehensive and efficient solution for analyzing trace evidence, allowing for simultaneous acquisition of multiple imaging modalities and rapid data acquisition. The results demonstrate the potential of this technology to revolutionize forensic investigations, improving accuracy, productivity, and the ability to extract valuable information from samples.

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