A Low-Cost Multispectral Imaging System for Microscale Applications

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***Abstract*— Multispectral imaging (MSI) is an imaging technology that captures spectral data using multiple individual wavelengths, providing information that would not typically be visible under normal broadband illumination. This has several applications, from astronomy to forensics, but is not widely available due to expensive instrumentation. To address this, a low-cost multispectral imaging device for application in micro-scale imaging was developed. This system uses an array of light emitting diodes (LEDs) on a flexible printed circuit board (PCB) to create a diverse set of wavelengths to illuminate the sample. These PCBs are mounted in an acrylic housing designed to allow illumination in transmission and reflection format. The system is controlled through an ESP32-S3 microcontroller and some peripheral electronics. A monochrome machine vision camera, controlled by Pylon viewer software is used for image acquisition. Multivariate data analysis of the images was done using various freely available software packages. The system was tested on a series of dried milk fluid drops to evaluate the viability of the system in providing chemical information on the droplet pattern.**

***Index Terms*—ESP32, LED, Multispectral imaging, Spectroscopy**

I. INTRODUCTION

Remote sensing technology plays a crucial role in several sectors, such as agriculture, environmental monitoring, disaster management, and urban planning [1]. One such optical technology is known as multispectral imaging (MSI). MSI is where multiple acquisitions of the same image are made under different discrete light wavelengths to extract spectral information, such as material composition, not visible under broadband optical illumination. This can be achieved by various means such as multi-position filter wheels, gratings, and prisms [2]. MSI has several applications as mentioned in [1], and the simplest type of reflective remote sensing is MSI based. The problem with potentially deploying this technology on a wide scale is both the prohibitive cost and delicacy of traditional MSI systems as they involve expensive filters or optics to capture the image. A single set of 25mm band pass filters alone cost close to $2,500 NZD, with the matching filter wheel being an additional $2,188 NZD [3 4].

The realization of a low-cost MSI system capable of imaging at the microscale would open the technology to less financially developed regions or institutes. It would allow for applications such as non-destructive identification of cell cycle phase in cancer cells [5] and other similar applications.

Several existing attempts to create a low-cost MSI system have been attempted by various parties using different methods. The two most common methods are that of using specific band pass filters on one or multiple cameras, or utilizing a specific illumination source to limit what light hits the subject to begin with.

Of the two techniques, filters are best for an environment where the illumination source is unable to be controlled, such as agricultural fields or open areas, and so must filter out the unwanted wavelengths from the optical sensor. As the intended scale of the imaging subject for this paper is able to be fit onto something as small as a microscope slide, it is possible to control the lighting in the environment to only be of one wavelength. That is why the system in this paper utilizes LEDs instead of filters. By utilizing this approach, it is also possible to further allow for imaging under two different lighting conditions: transmission mode, where the light shines through the bottom of the sample through a transparent substrate, and reflection mode, where the light reflects off the surface of the sample. This allows for greater penetration of the subject should it prove too thick to penetrate fully in reflection mode imaging.

To evaluate the system, the application of the technique towards the analysis of dried droplet patterns was utilized. It has been shown by many workers [6] that the pattern left behind when a drop of a complex fluid evaporates can contain information on the chemical composition of the fluid. The potential to add multispectral imaging to the analysis of droplet patterns may hold application for enhanced sensing of the chemical composition of fluids.

We applied the technique towards the analysis of droplet patterns from commercial milk samples. These samples can contain different levels of protein and fat content and our evaluation was a first attempt to see if the developed MSI system could distinguish between samples of different composition.

This project was supervised by Gideon Gouws.

II. Related Work

As previously mentioned, there have been several successful attempts at creating a low-cost MSI system through various means. As part of development, background research was conducted on several existing low-cost MSI systems to determine the best development path going forward. Even if a MSI system wasn't suitable, it could still reveal useful tools that may be adapted for use in another.

During this process, it became readily evident that these systems typically used two approaches to the problem. Filter-based technology, where light of mixed wavelengths is selectively filtered out using some form of filter, or light emission-based technology where the spectrum of light hitting the subject and reflecting into the sensing device is controlled instead.

A. Filter Based Systems

Filter based attempts at creating a low-cost MSI system can usually be broken down into a few key components. One or more imaging devices or sensors are fitted with a system of bandpass filters each allowing a specific wavelength of light to

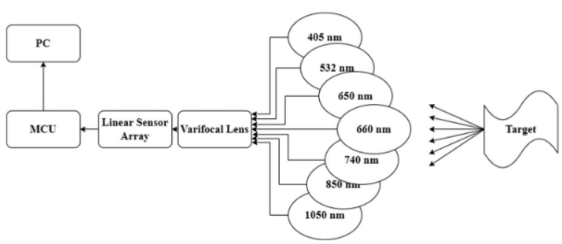


Fig. 1. Block diagram of MSI system shown in [7]

pass through. Images are taken of the subject under each filter and then they are composited into one. The studies [7 8] present two possible ways in which this system may form with [7] utilizing multiple filters on a single capture device, and [8] having multiple capture devices with multiple filters.

The system in [7] was designed with the intention creating an "inexpensive, wideband multispectral imaging device with replaceable and selectable motorized filters and illumination units" and takes the approach of using multiple spectral band filters and attempts to create a working Low-Cost MSI system. It uses a sl1401cl Ams-osramag linear sensor array with a varifocal lens as a camera, paired with a motorized filter wheel to hold and change filters. Figure 1 shows a diagram of the system.

This allows the system a great degree of flexibility over what filters are installed compared to the other study [8] where the system developed only has 18 fixed filters. With this system, the user is able to potentially select up to 40 spectral bands by adding additional filter disks of their own.

This rotating filter disc system is also seen in another similar study [9] where a MSI system was developed for the purpose of detecting leaking CO2 gas from vegetation. It utilized a Thorlabs FW102B six-position rotating filter wheel installed with a red and near infrared (NIR) filter to isolate the required bands while also leaving room for more if needed. Figure 2 shows the completed system in its case.

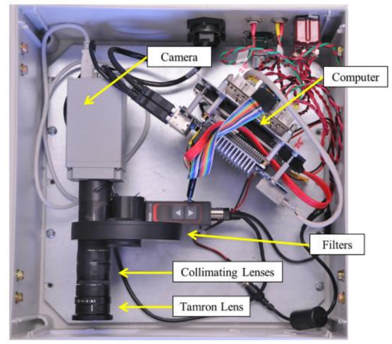


Fig. 2. Complete system as designed in [9]

The advantages of using a filter wheel or similarly interchangeable filter swapping system as seen in [7 9] is that it removes the need for having multiple cameras as seen in a commercial unit [10]. It also allows more flexibility to the user with regards to the specific bands the camera can filter out by making the filters interchangeable by user. However, having only a single camera means that there is a significant time delay between each picture taken, during which the active filter must be swapped out for the next one. This means it is easier for stray movement in either the system or subject to introduce motion artifacts in subsequent images which will affect the final composition.

The solution to this problem is simultaneously capture the subject with all the filters applied at once. The system developed in [8] utilizes a 10.7-megapixel charge couple device (CCD) monochrome camera coupled with a tapped aluminum plate which holds 18 lens assemblies. This is shown in figure 3. Each lens assembly is a simple off the shelf model, each able to be equipped with a narrow band-pass optical filter. The camera when fitted to the plate can capture a subject through all 18 filters simultaneously as seen in figure 4. While this does resolve the motion artifact problem, it does also have a much wider FOV which is unsuited for the micro scale photography which is what the MSI system currently being developed is focused on.

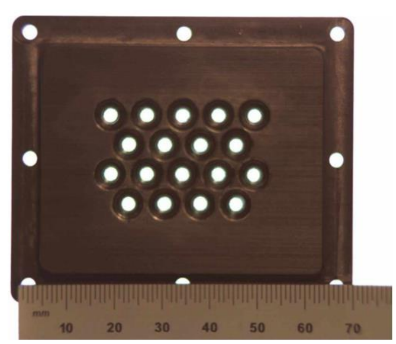


Fig. 3. Photograph of a lens plate containing 18 lens assemblies in a hexagonally close-packed array [8]

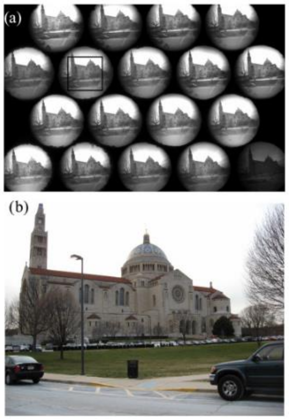


Fig. 4. (a) Complete image of the National Shrine of the Immaculate Conception acquired with the multispectral camera. (b) The Shrine photographed with a conventional digital camera. [8]

Comparing the results of both systems imaging a color chart shows that both methods achieved similar results despite the differences in the designed systems. The spectral response of the system [7] on a color chart for six wavelength bands is shown in figure 5, and the equivalent for the system in [8] is shown in figure 6.

Fig. 5. Spectral data of a color chart for system [7]: (a) image, (b) 405 nm, (c) 532 nm, (d) 650 nm, (e)740 nm, (f) 850 nm, and (g) 1050 nm.

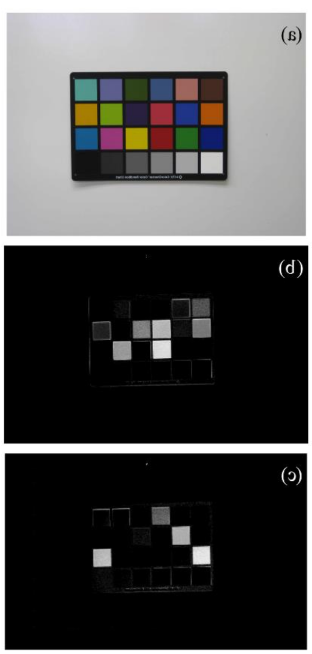


Fig. 6. Spectral response of system [8]. (a) Macbeth color chart photographed with a conventional camera. (b) Composite image showing red enhance-ment. (c) Composite image showing blue enhancement.

While the spectral response of both systems does demonstrate their effectiveness as MSI systems, there are also a few relevant points brought up. Figure 7 shows that the spectral response of the camera used in [7] increased proportionally to the power of the light source illuminating the subject meaning that the quality of the imaging is dependent on the external lighting of the subject.

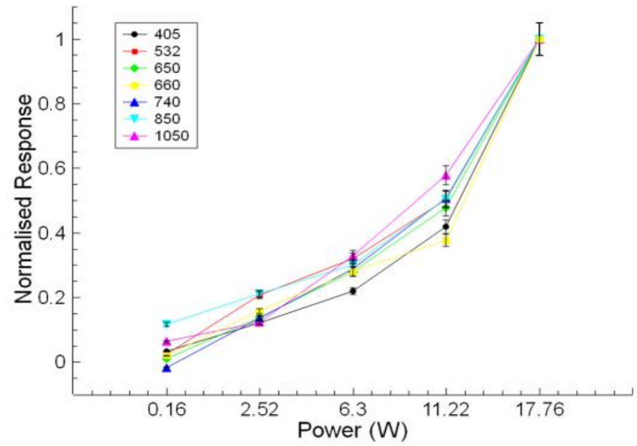


Fig. 7. Spectral response of the multispectral camera for specific bands [7].

For the system in [8], there was a significant amount of noise present in the camera data compared to data gathered from a spectrometer as seen in figure 8.

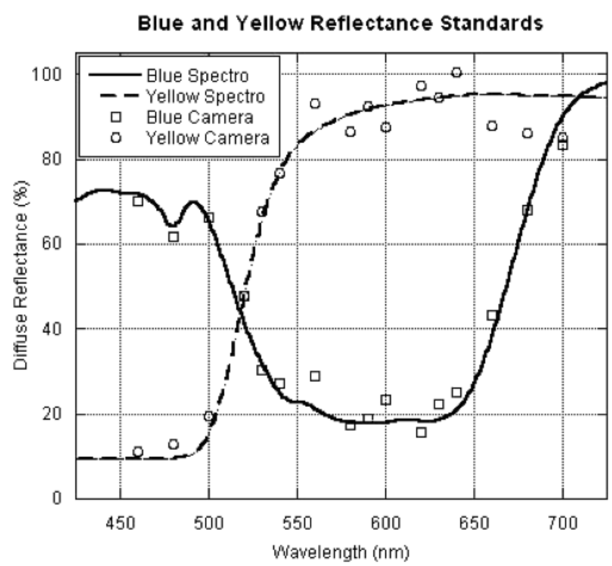


Fig. 8. Diffuse reflectance as a function of wavelength for blue and yellow Spectralon color standards measured using a spectro-photometer and multispectral camera. [8]

The paper states the reason for this is in part due to reduced camera exposure time to avoid saturation in any sub-image, which lead to relatively low pixel values as a result. Notably, the image at 880nm was stated to be so dim as to be unusable except at very long exposures or increased illumination strength which correlates with the data in figure 7.

To summarize, optical filters in MSI are useful when the light hitting the subject is unable to be controlled. Examples include satellite imaging of terrain and monitoring of agricultural fields. However, for subjects that are small enough that the illumination source can be controlled, this advantage is rendered quite redundant. Utilizing filter is still possible, however, depending on the circumstance, an alternative solution may prove more cost efficient and effective overall.

B. LED Emission based Systems

LED emission-based systems invert the paradigm. Instead of limiting the light that enters the camera with a filter, they control the light that hits the subject of the camera. The systems developed in [11 12] all utilize LEDs to illuminate the subject under a specific wavelength of light before imaging it.

The system developed in [11] was created as a general-purpose MSI tool with the idea of being inexpensive, robust, and intuitive to use. It does this by removing the need for an expensive filter wheel system, and instead uses a multiplexed system of LEDs paired with a diffuser and camera to create a rudimentary level MSI scanner. The illuminator assembly is comprised of a donut shaped PCB in which 18 channels of LEDs are installed at a set wavelength per channel. This allows for up to 18 wavelengths of light. A matching diffuser is placed over the PCB to smooth and even out the irradiance pattern on the subject. An optional polarizing layer could also be added to reduce the intensity of surface reflections at the cost of both the filters and needing to cross the lens and illuminator filters. The system is controlled via a NI digital I/O control card connected to a computer. Figure 9 shows the assembled device. The study evaluates the system as being simple enough to be a versatile teaching tool, while also possessing sufficient analytical power to delve into more complex sample analysis.

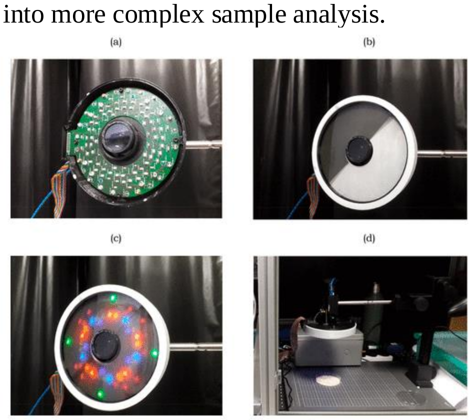


Fig. 9. (a) MSI with front cover and optical components removed. The LED PCB is visible. The camera lens is located at the centre of the instrument. (b) The MSI with diffuser and polarizer added, with the latter shown partially inserted. (c) The MSI fully assembled with LED array fully illuminated. During actual use, the LEDs are illuminated sequentially. (d) The MSI assembly viewed edge-on. The camera is attached to an optical post, and the lighthead is mounted via a 3D printed strut on the camera body. [11]

This system of placing a camera in the center of the illumination source is also seen in [12], only with 8 channels of 4 LEDs as seen in figure 10. A diffuser material is likewise slotted over the illuminator unit to diffuse the light evenly over the subject. A Raspberry Pi 3 is used to automate the lighting and image capture process. This has the advantage of minimizing human interaction with the system during image capture which in turn lowers the chance of motion artifacts appearing.

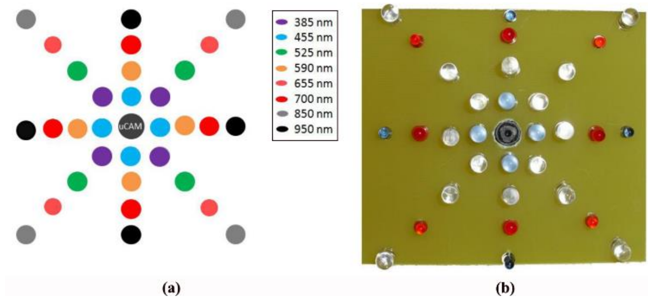


Fig. 10. LED panel and micro-camera disposition scheme (a) and photograph (b) [12]

It is worth noting that both systems [11 12] were tested on relatively large objects such as fruit. The MSI system currently being developed is intended for micro scale imaging of microscope slides, leaving it uncertain as to the ability of the illuminators of both systems to evenly light a sample of the intended size.

The results of both systems are comparable, both having been tested on fruit to determine factors such as bruising or ripeness. Figure 11 shows the application of the system made in [11] on a batch of tomatoes and subsequent analysis of the resulting data cubes taken over a week period.

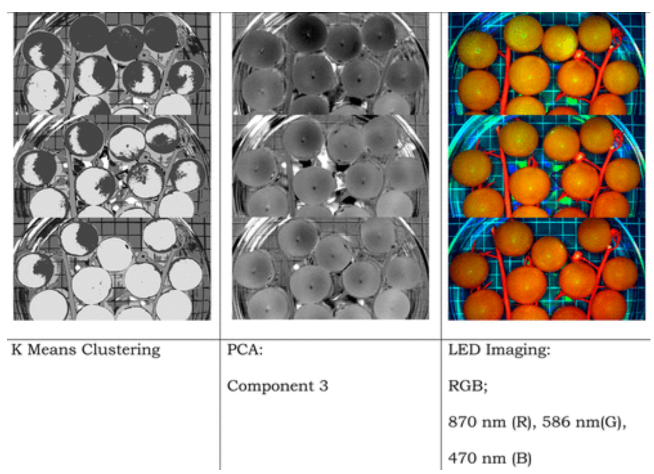


Fig. 11. Vine tomato ripening, with results from clustering and PCA of multispectral images taken of tomato samples. The samples, from top to bottom in each image, are from day 1, day 4, and day 7 of the ripening study. [11]

The study uses free software Spectronon to perform K Means Clustering analysis and Principal component analysis (PCA) on the data cubes to identify the ripeness of the tomatoes. As can be seen in the figure, a clear correlation between the colored areas in both results and the ripeness of the tomatoes was discovered thus demonstrating the viability of the system.

A similar evaluation for the ripeness of peaches was carried out using the system from [12] as seen in figure 12.

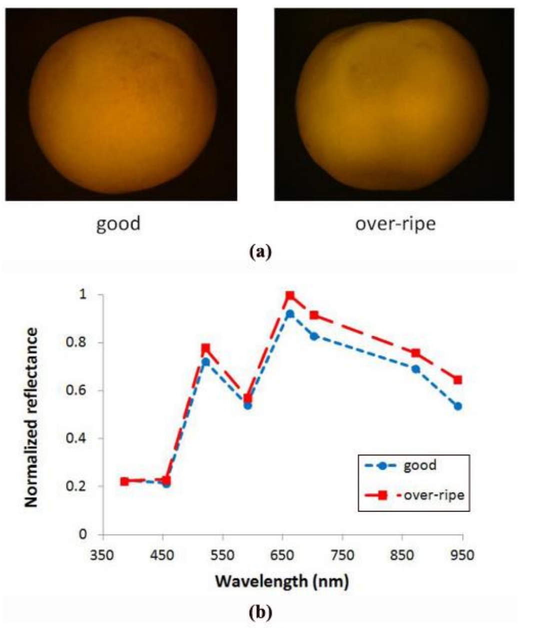


Fig. 12. Images at 590 nm (a) and reflectance spectra (b) of good and over-ripe peaches [12]

Unlike [11], the effectiveness is measured by taking the normalized reflectance of the peach under each wavelength. As can be seen from the graph, there is a deviation between the good and over-ripe curves at the higher wavelengths showing that the system can be useful for determining the ripeness of fruits.

Compared to the filter-based systems in [7 8 9], more control can be exerted over the intensity of the illumination by nature of the LED based design. They are unsuited for large subjects however, the subject imaged in figure 4 would be impossible to achieve using the LED based designs due to the size.

To summarize, using LEDs to perform MSI is most suitable for subjects that can be scanned in a controlled environment. While there is still a delay in changing the active LED, it is much quicker than a filter wheel and eliminates mechanical movement. Care does need to be taken with how the light is distributed. Unlike with filters where there is a single composite light source, the LED based MSI system has multiple tiny light sources of various wavelengths that must be distributed evenly across the subject.

III. Design

Having considered several different existing low-cost MSI systems, the most efficient and best suited methodology is to take the approach of controlling the illumination source instead of filtering it. The intended subject of the system designed in this paper is microscope samples, nothing that should exceed a microscope slide in area. This means the imaging area is small enough that it could be easily shielded against external light allowing full control over the light that does hit the sample, and the camera by proxy.

While using a filter wheel or similar such as in studies [7 8] is also possible, the circumstances are such that doing so would not only cost more, but also not present any objective advantage over simply controlling the illumination source directly. The subject will require a directed illumination source capable of even illumination either way, and the subject is not so large or in a position where controlling the light that falls on it is impossible.

In other words, it is easier to control what light hits the camera to begin with rather than filter out one wavelength at a time in this circumstance.

A. Camera and Illumination Source

The camera and lens provided for the project is a Basler acA250014um with a Navitar 1-60135 zoom lens which will serve as the micro imaging assembly. The output of the camera is displayed on a standard desktop computer using Pylon Viewer. Figure 13 shows a diagram of the current micro imaging assembly with the stage.

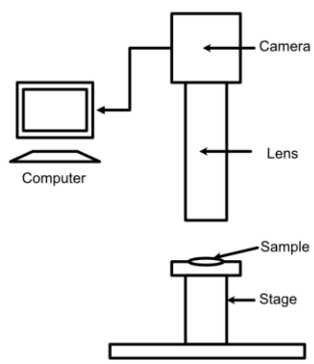


Fig. 13. Existing configuration of the camera assembly.

There are several constraints surrounding the illumination source that had to be observed.

As the camera is positioned directly over the stage where the subject will be placed, it is important that the illumination source be designed in such a way that no shadows or highlights get cast as this would potentially invalidate the results. The illumination system must also be capable of illuminating the sample in transmission and reflection modes meaning that the light must also have a means of shining through the subject into the camera. Finally, the illumination source must be able to switch between multiple different wavelengths.

From this, the lighting system will need to be split into two parts; one that handles transmission lighting, and one that handles reflection lighting. Given that illuminating from one side of the subject would certainly cast shadows and highlight the side it was shining from, a radial lighting assembly that balances the light is required. This is also due to how the only available space in the camera assembly is the area around and under the optical path from subject to camera. A design like that of the one seen in figure 10 is a possibility, however the intended imaging subjects of that system are large enough to warrant the more spread-out LED arrangement which may not work with smaller subjects.

Keeping in mind that the overall objective of this paper is to demonstrate a potential low-cost MSI system, it makes sense to use LEDs as the illumination sources, as they are both cost efficient and able to be purchased in different wavelengths.

B. Power and Control

The biggest consideration for the control system is the intensity of each illumination wavelength. Different LEDs have different intensity characteristics, meaning that some form of power control will be needed to modulate intensity. The electronics used in studies [11 12] are similar in that they both are controlled digitally. [11] uses a series of transistors and resistors interfaced into a standard computer running the control software as shown in figure 14. [12] utilizes a multiplexer circuit controlled by a Raspberry Pi as shown in figure 15.

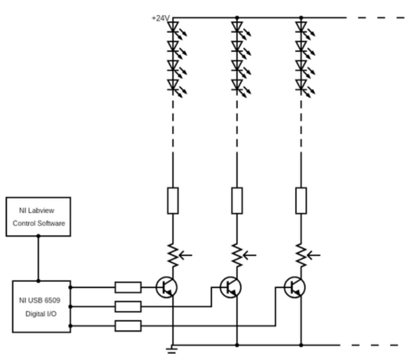


Fig. 14. Schematic of control system for MSI designed in [11]

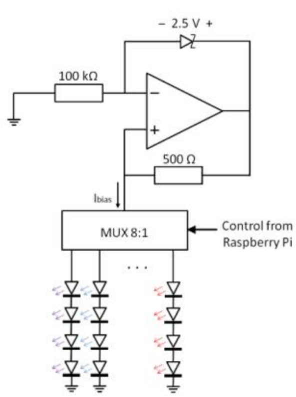


Fig. 15. Schematic of control system for MSI designed in [12]

While both of these would work, the Basler camera is controlled by an external computer, which means a Raspberry Pi would be overkill for just controlling LEDs. A smaller micro controller such as an ESP32-S3 would be a suitable substitute given the surplus of pulse width modulation (PWM) capable pins. A series of transistors like in figure 14 would then suffice to control each series of LEDs.

Given that the ultimate function of the control system is to be capable of controlling LED intensity and cycling through each wavelength series, a complex UI is not needed, and the system could be controlled by something as simple as a button.

IV. Implementation

A. Illumination Source

To solve the problem of even illumination and limited space, it was determined that a radial diffusion device would be the most optimal solution. This took the form of a pair of acrylic rings based on the previous team's work, combined with a flexible PCB designed to hold the LEDs.

In order to provide an even diffuse light, the light from the LEDs, which are direct by design, had to be diffused in some way. This was accomplished by means of a transparent acrylic ring sandwiched between two reflective acrylic disks as shown in figure 16.

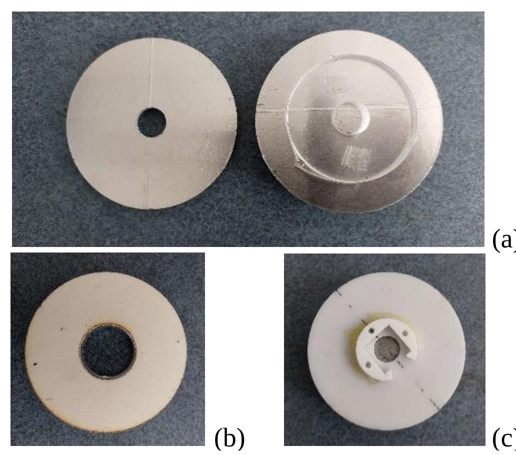
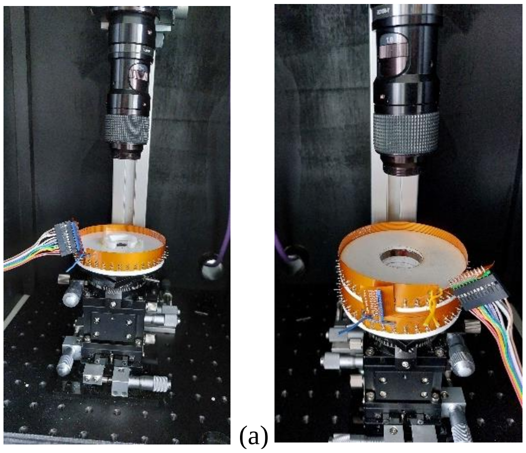


Fig. 16. Internal view of reflection ring (a) and top view of reflection ring (b) and transmission ring (c)

Light injected through the circumference of the disks is diffused by both the reflective interior as well as the internal transparent acrylic disk, the results of which then hit the sample through the respective holes.

A set of flexible PCBs that the LEDs are mounted on were constructed to wrap around each ring and can be secured by a small cable tie. This is so that in the event of breakage, or the LED range needing to change, it is simple to just swap out the PCB by undoing the tie. Each PCB ribbon has ten sets of four LEDs, corresponding to each selected wavelength. The LEDs are connected to the control unit via a ribbon cable that attaches to the header pins on the PCB. Figure 17 shows both rings with the PCBs attached in transmission and reflection configuration.



(a) (b)

Fig. 17. Transmission ring with PCB attached (a). Reflection ring with PCB attached (b) fitted to top of reflection ring.

The transmission ring fits a 17mm square slide in the square slot over the hole where the subject would sit. When reflection imaging is required, the reflection ring simply sits on top of the transmission ring and the connector is changed to the reflection ring PCB. This means the sample doesn't need to be disturbed to change modes.

B. Controller

The control unit of the system was designed around an ESP32-S3 microcontroller. By using a series of PNP transistor array chips and a 3V linear regulator, the onboard LED control functionality could be used to control each channel of LEDs on the PCB despite the ESP32-S3 itself lacking the needed current throughput. Figure 18 shows the block diagram of the system.

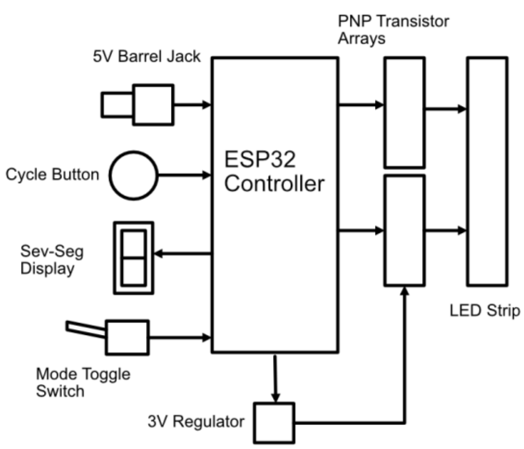


Fig. 18. Block diagram of MSI system control unit.

By pressing a button, the user may cycle through each of the ten LED channels 0-9 which is reflected on the seven-segment display. Each channel corresponds to a specific wavelength of LEDs. As there is a disparity between intensity calibration of reflection mode imaging vs transmission mode imaging, the firmware has been written such that at the flick of a switch the system will switch between the two sets of calibrated duty cycles. Figure 19 shows the completed system in its casing.

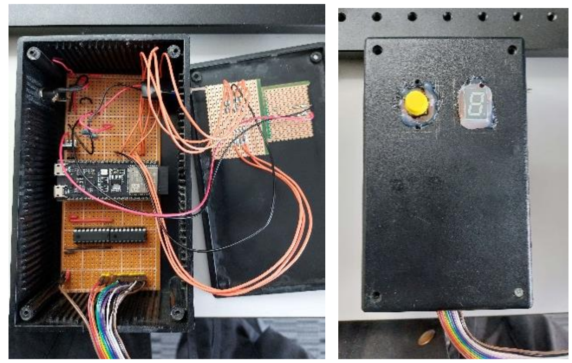


Fig. 19. Completed control system with casing.

The ESP32-S3 is powered from a barrel jack which will take a standard 5V 1A universal serial bus (USB) charger supply. Currently it is powered by using a USB to barrel jack cable connected to a 5V USB charger.

C. Sustainability

By minimizing the production cost of the system, this means that not only would it be able to be deployed on a much larger scale than a commercial MSI system, but it also means that if anything breaks it is very easy to repair instead of needing a wholesale replacement.

Furthermore, each aspect of the design has been designed in such a way that there is a degree of modularity, enough that modifying things such as the LED wavelength range is as simple as removing the PCB and replacing it with another one with different LEDs installed.

V. Evaluation

A. Intensity Calibration

As part of the evaluation process, the intensity of each wavelength's LED channel was calibrated based on intensity analysis of a blank slide imaged under each wavelength in both modes. Table 1 and figure 20 show the LEDs used have varying current to intensity characteristics which needed to be normalized to ensure even results between wavelengths when imaging.

LED Model	Colour	Datasheet peak wavelength	Voltage (V)	Actual peak Wavelength	Current (mA)
SIR-56ST3FF	Infrared	950nm	3.3	950nm	15.83
TSHG5410	Infrared	850nm	3.3	854.7nm	3.81
MTE2077N1-R	Infrared	765nm	3.3	761.9nm	0.78
L-53HD	Red	700nm	3.3	678.6nm	24.8
L-53SRC-J4	Red	660nm	3.3	655.0nm	0.14
HLMP-EH3A-WX0DD	Orange	621nm	3.3	621.2nm	0.35
HLMP-C423	Orange	610nm	3.3	600.9nm	6.3
L-53SYC	Yellow	590nm	3.3	586.6nm	0.53
C503B-GAN-CB0F0792	Green	527nm	3.3	526.6nm	0.06
HLMP-CB3A-UV0DD	Blue	470nm	3.3	462.1nm	0.03

Table 1. Characteristics of used LEDs.

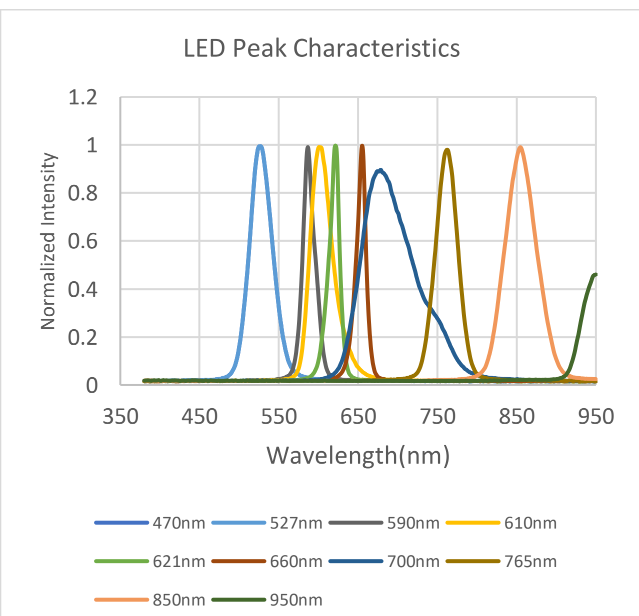


Fig. 20. Intensity characteristics of used LEDs.

Due to how the quantum efficiency of the Basler camera experienced a falloff towards the infra-red (IR) range of the spectrum as seen in figure 21, some exposure time adjustments had to be made for the higher wavelength LEDs, notably the 950nm. Several other LEDs, at 610nm and 700nm, also required increased exposure times to maintain consistent baseline intensity due to being too dim even at full duty cycle. The data in table 1 correlates this.

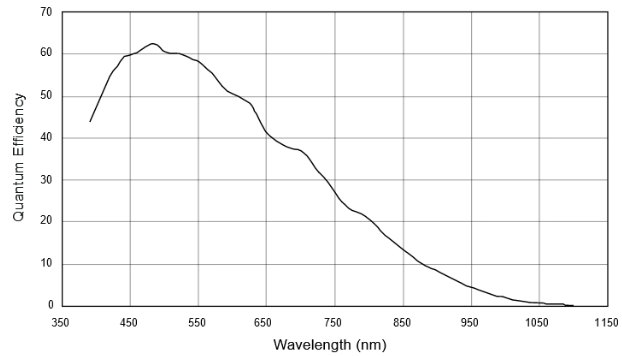


Fig. 21. Quantum efficiency of Basler camera [13]

By using the blank slide as a control sample, the duty cycles of each respective LED channel can be calibrated accordingly to maintain a relatively consistent level of illumination across different wavelengths. The results of this are shown in figure 22, where the mean intensity of the pixels in the blank slide under each wavelength is plotted.

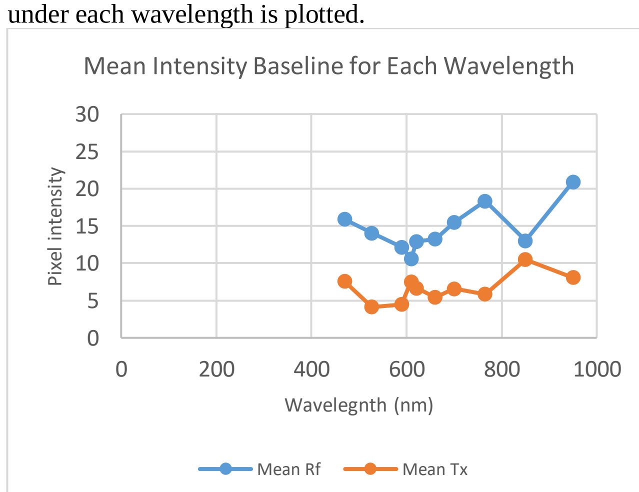


Fig. 22. Intensity of blank slide imaged under calibrated duty cycles.

Establishing this baseline will disprove that any variation in sample intensity from being attributed to disparity in LED intensity.

B. Imaging on Dried Milk Droplet

To evaluate the developed MSI system, a series of prepared milk droplets of differing fat content were imaged in both reflection and transmission mode under each wavelength. The individual imaging results of the high fat milk sample are shown in figure 23.

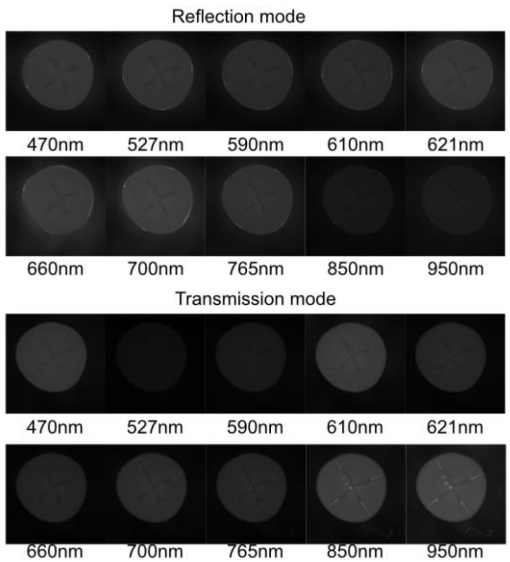


Fig. 23. MSI imaging of high fat image milk fluid drop

Furthermore, taking the intensity of the result images and plotting them shows a distinct correlation with the baseline intensity as can be seen in figure 24.

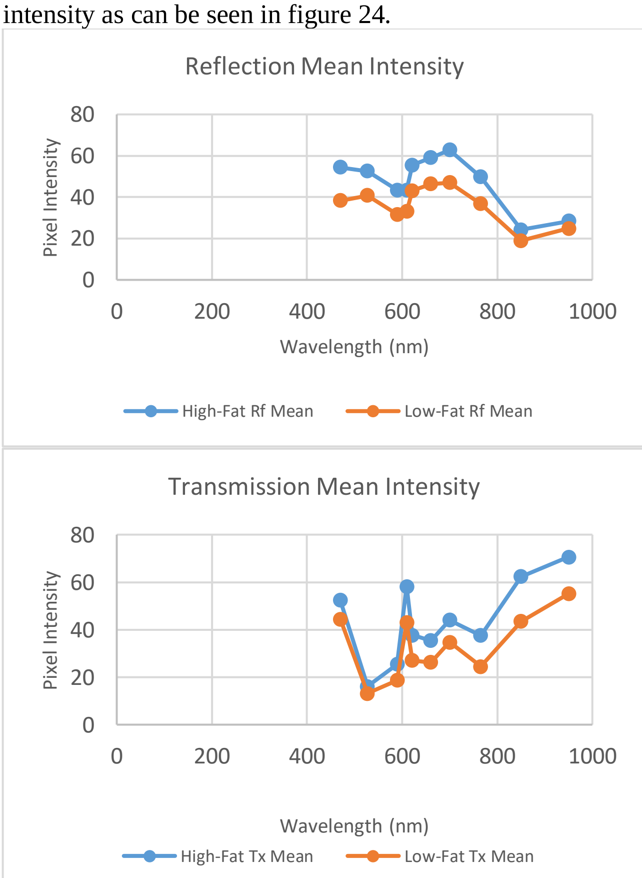


Fig. 24. Pixel intensities of milk samples in transmission and reflection modes

This shows that the intensity variation in the sample images is more affected by the baseline calibration than the sample itself. It also shows that the high-fat sample consistently produces a higher light intensity image than the low-fat sample, which could be a potential correlation.

These images were then processed into data cubes using open-source software Hypercube, a software package made for spectral image analysis. They were then further analyzed in Spectronon, an open-source imaging tool with a wide array of spectral analysis tools, by performing a k-means cluster analysis and principal component analysis (PCA) on both hypercubes. The results of both are shown in figures 25,26.

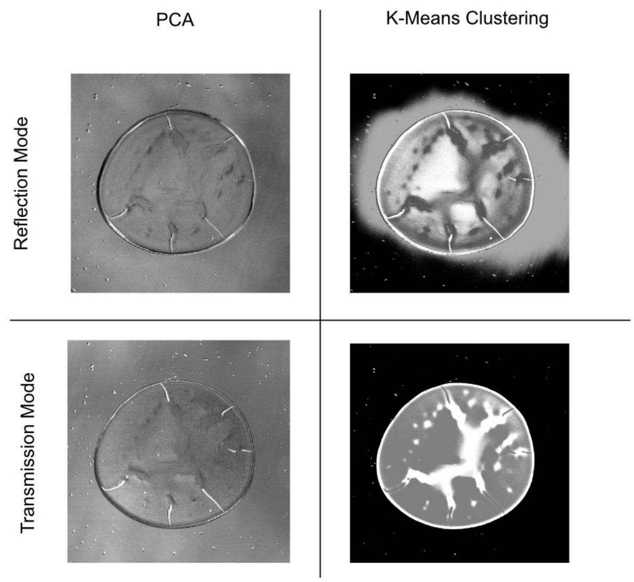


Fig. 25. PSA and K-means analysis of low-fat milk sample

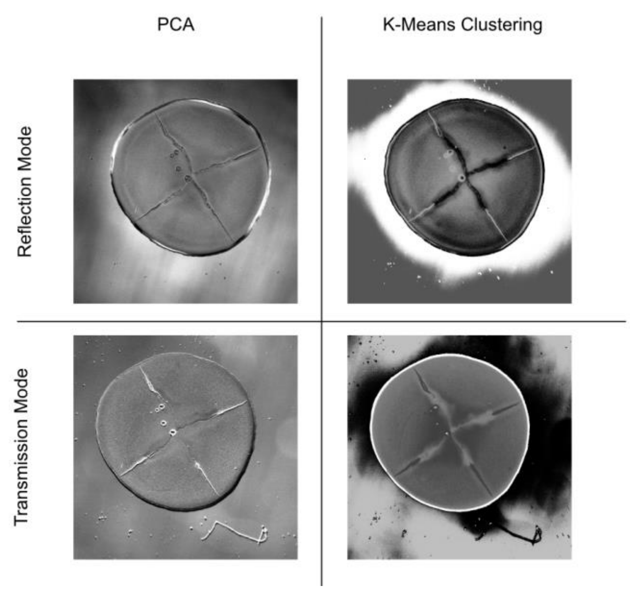


Fig. 26. PSA and K-means analysis of high fat milk sample

From these figures, the application of MSI to the samples does little to indicate the chemical composition of the milk even after PSA and k-means analysis. The drop pattern is quite clearly defined in both cases, however there is a lack of any common data points between the two that could be used as a basis to determine which has the higher or lower fat content or any other frame of reference. There is however a potential correlation based on the average pixel intensity of the sample images as seen in figure 24. The high-fat sample consistently exceeds the low-fat sample in intensity.

VI. Conclusion

Despite inconclusive results with regards to detecting the composition of the milk samples via droplet pattern, the system is demonstrated to be capable of functionally performing MSI imaging. The images in figure 23, particularly those in transmission mode show that additional spectral information appeared towards the IR range that was previously obscured by shorter wavelengths. The overall cost of the entire MSI system is also less than $200 NZD, with the bulk of the cost being the ESP32-S3 module, which puts it definitively in the low-cost range. The system is also modular enough to be easily adapted to other uses if needed.

Further optimizations of the system could also be made in future, such as more robust implementation of the reflection ring which currently sits on the transmission ring to be used. A more detailed analysis into the milk samples the MSI system was applied to would also assist in further determining if the failure to properly display the composition of the two samples was a fault of the system or variance in sample preparation. Further analysis with more powerful software such as Soft Independent Modelling of Class Analogy (SIMCA) [14] could also be used to try and separate the evaporites into categories to compare to other samples. There is also a potential avenue of research in using the average intensity of the images as a metric to determine the type of milk as figure 24 seems to indicate.

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