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作品編號 100018

參展科別 工程學

作品名稱 **Creating a Portable Optical Sectioning
Microscope for Smartphones with Three
Dimensional Viewing Function**

得獎獎項 大會獎：四等獎

就讀學校 臺北市立第一女子高級中學

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關鍵字 **Optical Sectioning、3D、Smartphone
Microscope**

作者簡介



我是梁靖妮，目前就讀北一女中三年級。一直以來都非常喜歡科學，更期待如果有一天，能夠將我心裡稀奇古怪的想像以科學原理實踐出來，那一定是非常快樂的事。

很高興高中的專研課讓我有實現這個願望的機會，讓我能夠去實驗室，真正的動手實驗。縱然中間有挫折，也有過跌倒，但看著自己腦袋裡想像的藍本，真正化為立體的成品在我眼前，那一刻我真的真的覺得好感動。謝謝這一路走來陪伴我的人、幫助我的人，未來我也將秉持著積極向上的精神，努力在科學這個領域中貢獻一己之力。

摘要

本作品首先證明了 HiLo 應用在散射光影像的可行性。目前看到有關 HiLo 的論文都只有提及收集螢光訊號，沒有看到有用在散射光訊號的收集上。本實驗透過架設自製原型機，證明 HiLo 的原理也能夠應用在散射光上，大幅提升 HiLo 光切片的深度與廣度。再者，本作品將自行撰寫之 App 結合雲端運算。再經過驗證後確認處理出來的影像是正確的影像。不僅加速影像處理流程，而且使用者只需攜帶手機便可立即看到處理出來的成果。接著，利用 3D 列表機自製具有光切片功能的手機顯微鏡實體。將原型機微小化並與日常生活常見的手機結合，讓使用者方便使用的同時也實現經濟便利性。最後，實現影像立體化，利用光切片的影像能使得原本平面的 2D 影像，可以呈現 3D 的立體化影像。

Abstract

This project proves the viability of using the HiLo technique on speckle illuminated images. Most of the current papers on HiLo Microscopy works with collecting fluorescence signals, but not so much with diffused illumination. Through experimentation, this project shows that HiLo microscopy can be used with speckle illumination to enhance the application of optical sectioning. Furthermore, through creating a smartphone application, combined with cloud computing, not only has the image processing time been drastically reduced, one only requires a smartphone to see the results. The resulting image has also been proven in this project to be correct. To realize this goal, a prototype was designed and created using 3D printing technology. The objective, to make a compact prototype which can be paired with any smartphone at a reasonable cost, was realized by rigorous designing and creation via 3D printing technology. Lastly, by combining the 2D optical sections, we can view and manipulate the specimen in three dimensions.

Chapter 1 Introduction

1.1 Motive

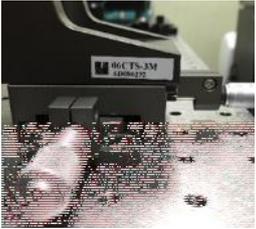
In the FB group “Science Maker”, Professor Chiang designed a smartphone microscope. Since smartphones are now a part of our everyday lives, this portable microscope makes it very convenient to observe and record microscopic things. However, interference from off-focus signals not only reduces the contrast of the image, it can also obstruct the focal plane signal to an extent that we are unable to observe our objective. Confocal microscopes, like the ones used in labs, can deal with this problem, but they are pricey and rather large in size. Thus, I found myself wondering whether it would be possible to use optical sectioning to filter out the off-focus signals, and leave a clear image of the focal plane?

1.2 Research Objectives

1. Understand the principles behind optical sectioning.
2. Experiment to find a viable configuration for optical sectioning.
3. Design a suitable smartphone application for image processing.
4. Design and create a prototype via 3D printing technology.

Chapter 2 Research Equipment and Materials

Experiment equipment

		
Light Source(Phone LED)	Collector Lens	Objective Lens(x10)
		
CCD	CCD Bracket	Beam Splitter
		
Diffuser	Specimen(tissue paper)	Stage
		
CCD Imaging Lens	3D Printer	

Chapter 3 Research Procedures and Methods

3.1 Reading up on optical sectioning microscopy papers

Optical sectioning is the process by which collecting a clear focal image of different depths, one may obtain the microscopic structure of the specimen. Researching papers on this topic reveals many different ways to do this: Confocal Microscopy[1], Light Sheet Fluorescence Microscopy[2], HiLo Microscopy[3]. A confocal microscope uses point illumination and a pinhole to eliminate off-focus signals, and is usually recorded digitally by a computer, thus making it incompatible with smartphones. Light Sheet Fluorescence microscopes are compatible with smartphones, but since it only works well with fluorescent specimens, its application is somewhat limited. HiLo microscopes are compatible with smartphones, the specimens are not limited to fluorescent substances, and the configuration is relatively simple compared to the other two.

In order to find the best method, we listed three core attributes we needed to consider: (1) Imaging method needs to be compatible with smartphones, (2) Imaging signal shouldn't be limited to specific conditions for broader applications, (3) Configuration should be as simple as possible, to reduce the cost and difficulty of fabrication. By organizing the three attributes into the table below, we decided to go with HiLo microscopy for optical sectioning.

	Confocal Microscope	Light Sheet Fluorescence Microscope	HiLo Microscope
Phone Compatibility	Incompatible	Compatible	Compatible
Illumination	Non specific	Fluorescent Specimens	Non Specific
Configuration	Complex	Complex	Simple

3.2 Principles of HiLo Microscopy

HiLo microscopy utilizes the concept of spatial frequencies. Optical sectioning is obtained through the Fourier transform and inverse Fourier transform of the signals. An image can be broadly divided into two parts, the large dimensional blurry edged part, and the small dimensional clear edged part (Fig. 1). The large dimensional blurry edged part has a low spatial

frequency, and the small dimensional clear edged part has a high spatial frequency.



Fig. 1 Spatial Frequency Concept: Wide Field Image (left), High Spatial Frequency (middle), Low Spatial Frequency (right)

Under the microscope, all of the high spatial frequencies originate from the in-focus plane, so in order to acquire this information, we merely need to apply a high-pass filter to a conventional wide field image. The low spatial frequencies occur in both the in-focus and off-focus planes. HiLo microscopes can create a low pass sectioned image, and combine the two to make an image of the focal plane with all the spatial frequencies present, thus realizing an optical section. To achieve this, we require two images, one with uniform illumination (I_{uniform}), and the other with speckled illumination (I_{speckle}). As shown in Fig. 2, by placing a diffuser on the illumination light path, where it creates a conjugate plane with the objective lens back focal plane, the diffuser pattern will be projected onto the focal plane thus creating diffused illumination. By inserting or removing the diffuser, we can obtain I_{speckle} and I_{uniform} respectively, as shown in Fig. 3

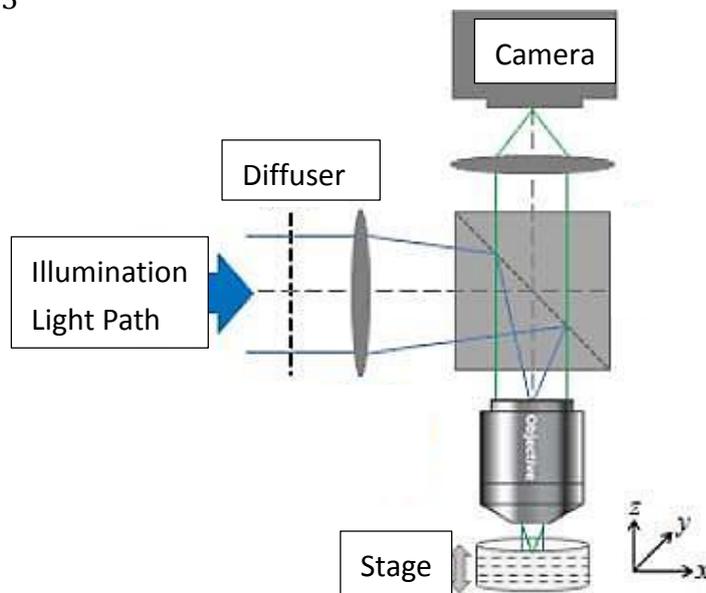


Fig. 1 HiLo Microscope Configuration

Source: <https://www.osapublishing.org/boe/fulltext.cfm?uri=boe-3-1-206&id=226098>

Because the diffuser is on a conjugate plane with the specimen focal plane, the speckle pattern will be in-focus on the focal plane and blurred everywhere else. This may be seen as a weighted equation for contrast in the axial direction, where the weight is at maximum on the focal plane, and decreases as you move away. Using this method, we may filter out all but the focal plane signal. Since the speckle pattern differs from the specimen structure, we need to apply a low-pass filter to remove the spatial frequencies that are higher than the speckle pattern to get our low frequency image. The high frequency signals only occur on the specimen focal plane, so we only need to apply a high-pass filter to I_{uniform} to obtain the high frequency image. A seamless fusion of the both the high and low frequency image contents then leads to a full resolution in-focus image of the specimen focal plane.

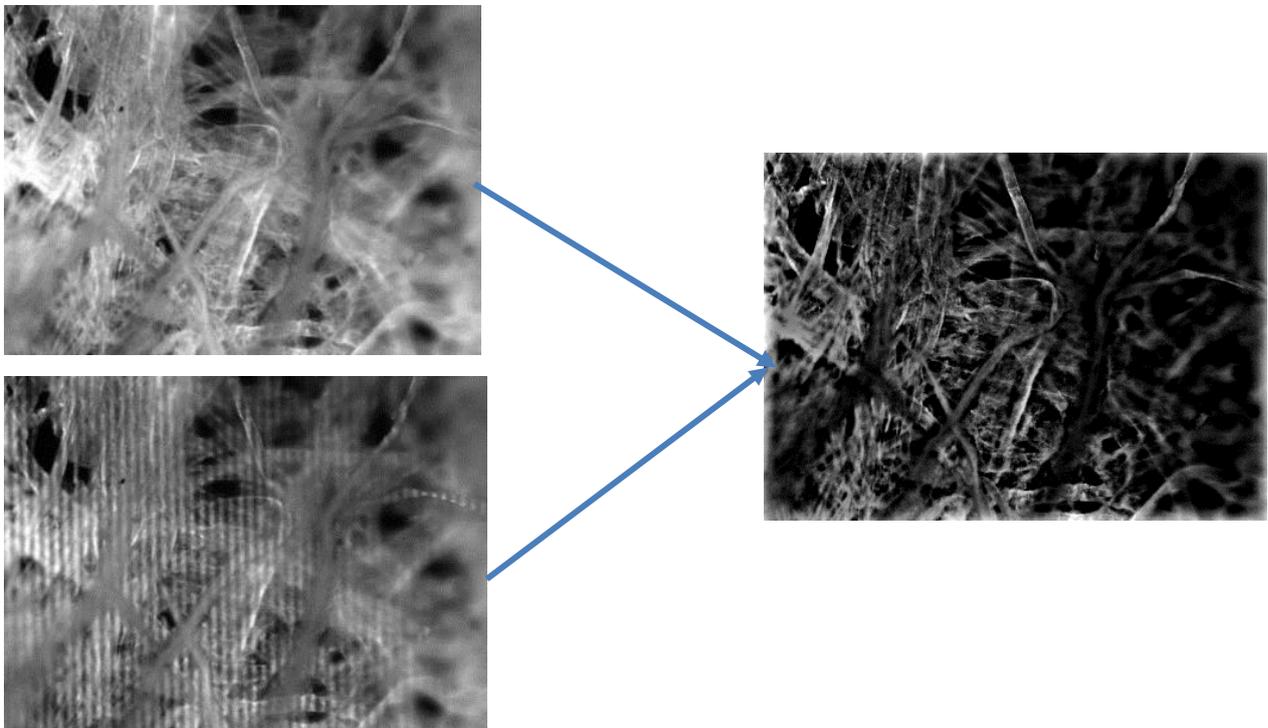


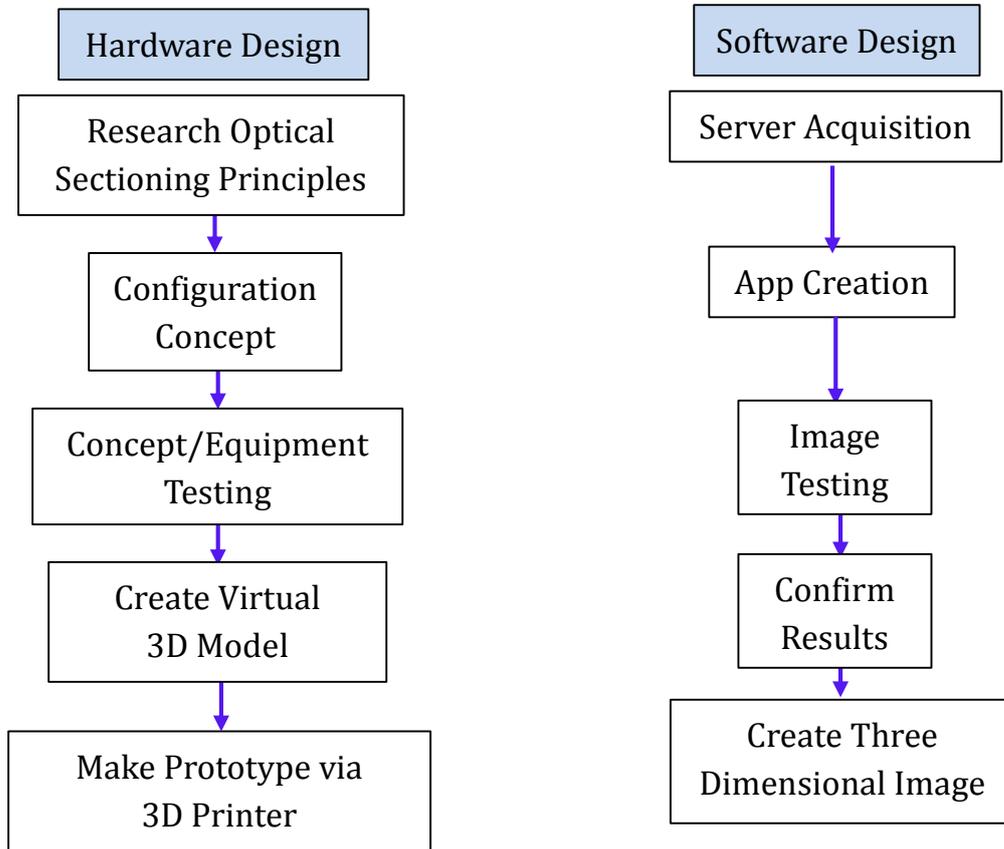
Fig. 2 I_{uniform} (upper left), I_{speckle} (lower left), combined optical section (right)

Source: <http://biomicroscopy.bu.edu/research/hilo-microscopy>

Requirements for stability and resolution are very high in research labs, so the current HiLo microscopes are bulky, and the resulting images need to be processed by high-speed computers. This led me to believe that as long as the requirements aren't that stringent, it would be possible to create a portable microscope that can be cheap, compact and convenient for on the fly observations.

3.3 Research and Design Flowchart

Since this project applies the HiLo technique to a smartphone microscope, we will need to split the research and design process into both hardware and software.



3.4 Hardware Design Process

1. HiLo Microscope Configuration Concept

As shown in Fig. 4, the illumination ray paths through the collector lenses, reflects off the beam splitter to focus on the front focal point of the objective lens. This creates a parallel light path after passing through the objective lens to illuminate the specimen. This is with reference to the Kohler method, which can give very uniform illumination. The imaging ray reflected from the specimen focal plane then paths through the objective lens, beam splitter and imaging lens to be focused on the CCD camera, thus forming the image. The diffuser needs to be placed at the front focal point of the L_2 collector lens for the pattern to be projected onto the specimen focal plane. The diffuser position, specimen focal plane and the CCD camera are all within the image-forming conjugate set, so the diffuser pattern will simultaneously appear on these three positions.

*Conjugate planes : In a microscope, light paths are scattered and focused many times. Each focus plane, for the same light source, is said to be conjugate with the others in that set, because they are simultaneously in focus.

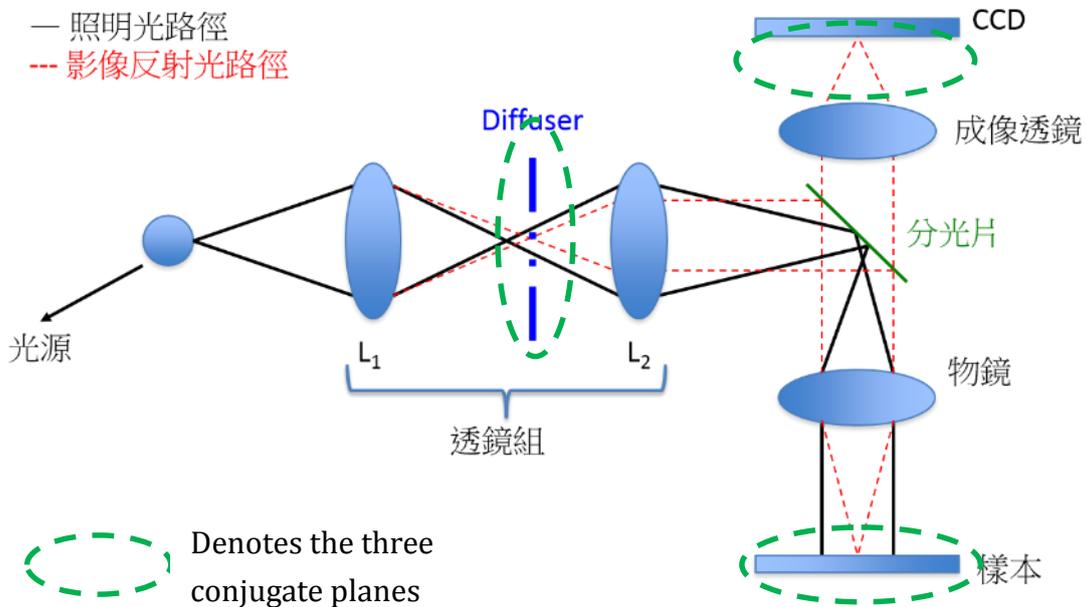


Fig 4. HiLo Microscope Configuration

2. Concept / Equipment Testing

- (1) Place all illumination pathway components, except the diffuser, onto the optical table and fix them in place.
- (2) Place a mirror at the objective lens focal plane so the illumination light may be reflected through the beam splitter.
- (3) Use the reflected beam from step 2. to align the imaging lens and CCD camera.
- (4) Fix the specimen onto a microscopic slide, and clamp it onto the stage.
- (5) After connecting the CCD and computer, adjust the distance between the specimen and objective lens until a clear image shows up on the monitor.
- (6) Place the diffuser in the clamp (Fig. 5) and position it on the front focal point of the second collector lens (Fig. 4), then adjust it axially until a clear image of the diffuser can be seen. This position is the conjugate plane.
- (7) Remove the diffuser from the clamp. Illumination is now uniform.
- (8) Save the image as I_{uniform} .
- (9) Insert the diffuser and save the image as I_{speckle} .
- (10) Turn the knob on the stage to move one step (10 μm) and repeat steps 3 through 9, resulting in a series of Z axis images (I_{uniform} & I_{speckle}).

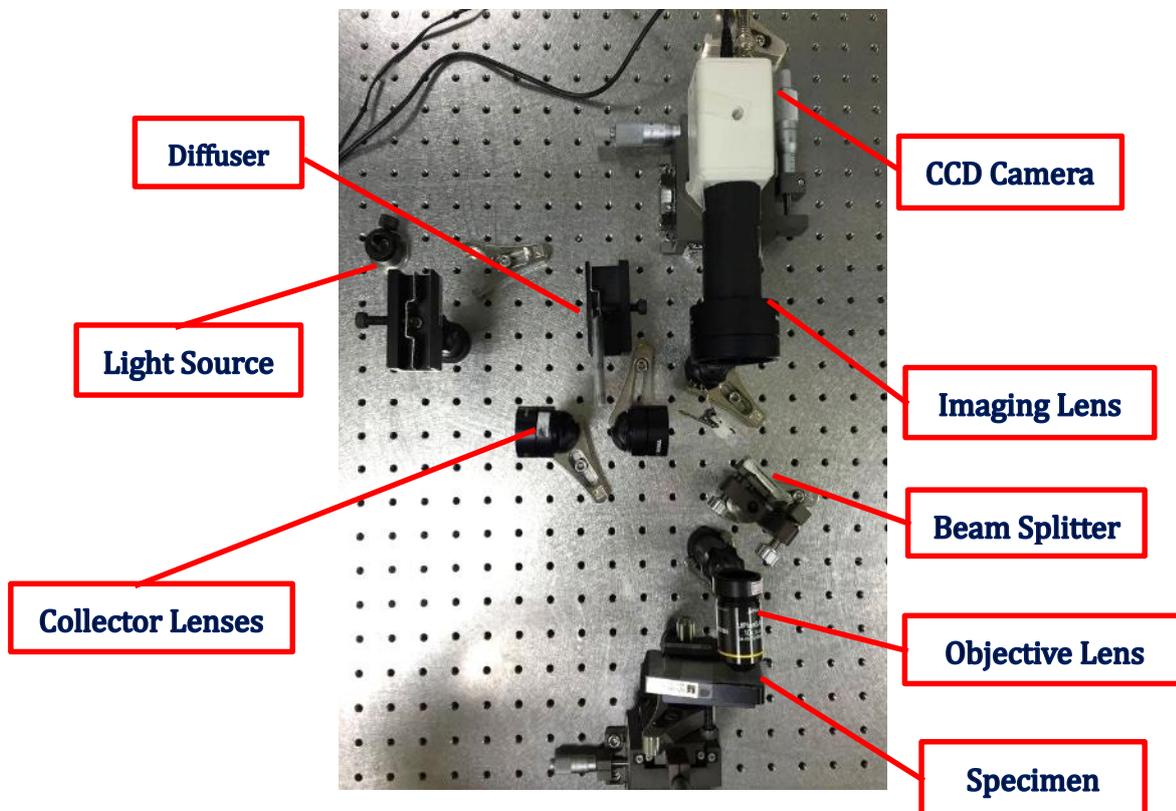


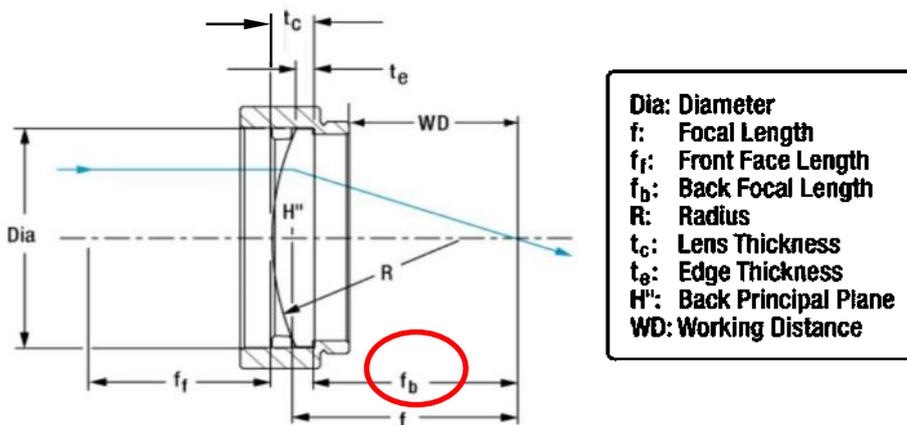
Fig 5. Concept/Equipment Testing

3. Designing the Portable Smartphone Microscope

(1) Designing: The goal is to miniaturize the optical pathways of the experiment, and create a microscope that can be used with smartphones. After purchasing readily available optical components, we used Google's SketchUp to design a casing and stage.

(2) Specifications of the Optical Components

a. Focal length of the collector lenses are 15.0mm. For our design we used the manufacturer's data of 11.6mm for the back focal length.



Item #	Optic Diameter	Focal Length ^a	Diopter ^b	Radius of Curvature	Center Thickness	Edge Thickness	Back Focal Length ^{a,c}	Working Distance ^a
LA1540-A-ML ^d	Ø1/2"	15.0 mm	+66.6	7.7 mm	5.1 mm	1.8 mm	11.6 mm	8.8 mm

Fig 6. Collector Lens

b. The focal length of the objective lens is 1.68mm. However is we were to use it normally, with the specimen on the left hand side (larger lens diameter), the illuminating rays coming from the right hand side will have to go through the small diameter opening in the back, resulting in very weak illumination of the specimen. So in this project, we reversed the direction of this lens.

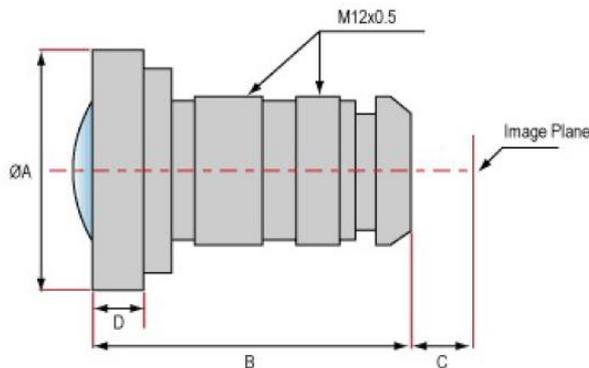


Fig 7. Objective Lens

Stock #		Focal Length (mm)	Aperture (f/#)	A (mm)	B (mm)	C (mm)	D (mm)
No-IR Cut Filter	IR Cut Filter @650nm						
#59-776	#66-88o	1.68	2.5	15.0	13.2	3.3	2.8

(3) Other Design Points

- a. Light Source: Detachable in order to change batteries.
- b. Diffuser: Since both images from uniform and speckled illumination are needed, the design requires the diffuser to be able to engage and disengage between the collector lenses.
- c. Stage Movement: The stage needs to be adjusted axially in very small increments. We implemented an M12x0.5 nut for this purpose. The screw lead is 0.5mm, so for every 5 degrees it is turned, the displacement will be 7um, which is quite ideal for optical sectioning. The calculation is simple:

$$0.5\text{mm} \times \frac{5^\circ}{360^\circ} \approx 0.0069\text{mm} \approx 7\mu\text{m}$$

4. Calculation of the Minimum Length Required

(1) Trying first with a linear optical pathway, we utilize Cauchy inequality and Gaussian lens formula, to estimate the minimum length required for our microscope.

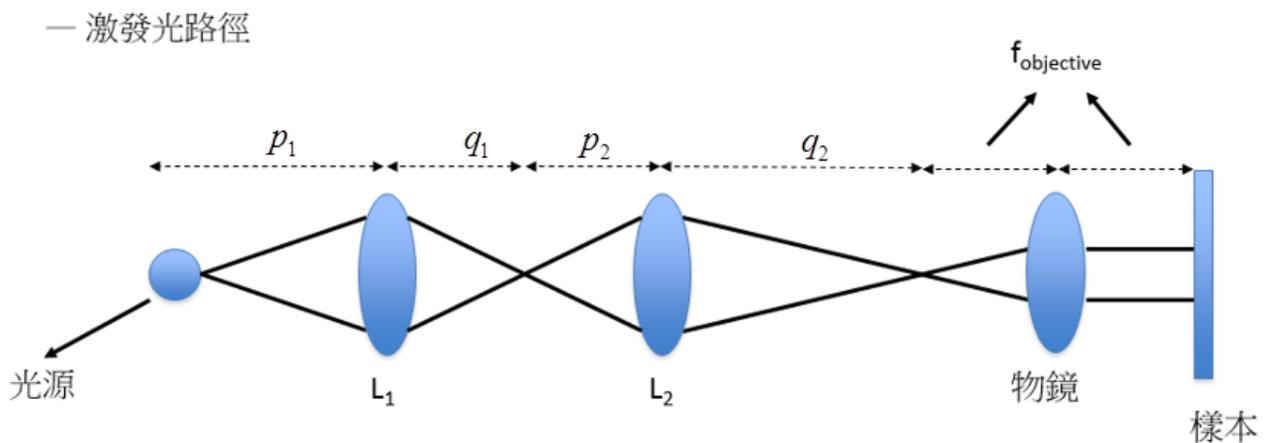


Fig. 8 Simple optical

Assuming the focal length of lens L1 is f1, and f2 for lens L2, then by the thin lens equation

$$\frac{1}{p_1} + \frac{1}{q_1} = \frac{1}{f_1} \dots\dots\dots(1) \quad \text{and} \quad \frac{1}{p_2} + \frac{1}{q_2} = \frac{1}{f_2} \dots\dots\dots(2)$$

From Cauchy inequality (a² + b²)(c² + d²) ≥ (ab + cd)²(3)

we know $(p_1 + q_1) \left(\frac{1}{p_1} + \frac{1}{q_1} \right) \geq \left(\sqrt{p_1 \times \frac{1}{p_1}} + \sqrt{q_1 \times \frac{1}{q_1}} \right)^2 = 4 \dots\dots\dots(4)$

Placing (1) into (4) we derive $p_1 + q_1 \geq 4f_1$, and by similarly placing (2) into (4) we arrive at

$$p_2 + q_2 \geq 4f_2$$

$$\text{so } p_1 + q_1 + p_2 + q_2 \geq 4f_1 + 4f_2$$

- (2) After calculating the minimum length of our optical components, adjusting for tolerances on the optical table, and actually taking pictures with a smartphone to prove that optical sectioning works, we recorded down the optimal measurements for our final design. The focal lengths of our lenses are $f_1 = f_2 = 15 \text{ mm}$, so we estimate the minimal distance between the light source and beam splitter to be $15\text{mm} \times 8 = 120\text{mm} = 12\text{cm}$.

3.5 Software Design

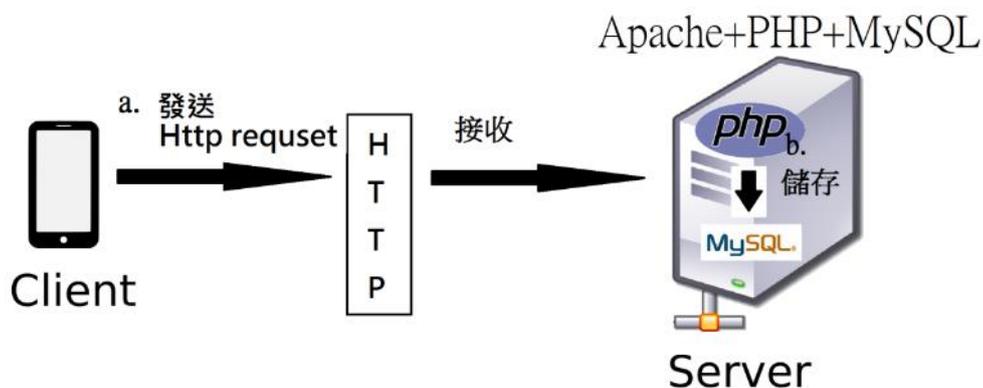
1. Data and Language

- (1) Why are we using a server for image processing?

Although the memory and processing power of smartphones have grown exponentially these past few years, it is still not enough. An actual experiment using a smartphone to process a high-pass filter resulted in a full 6 minutes of processing time. In light of these results, we opted to use a server to speed up this process.

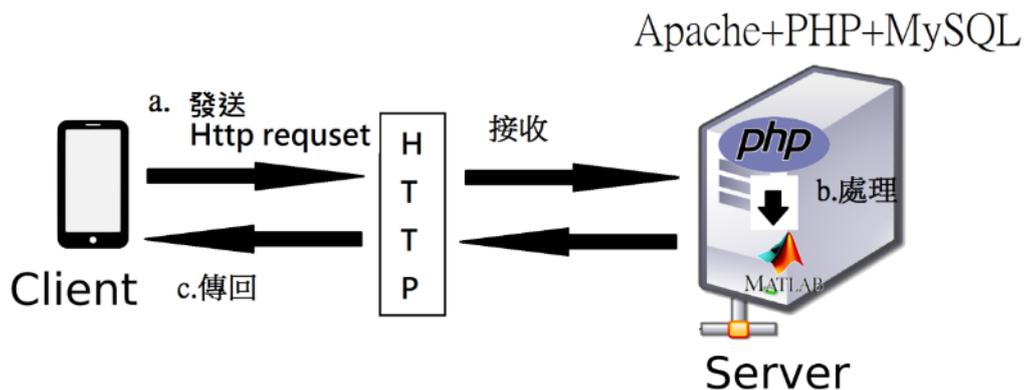
2. Data handling is mainly composed of two processes

- (1) The smartphone transmits the image via internet to the server.
- The phone uses http post protocol to send the image to a php website.
 - The php website sends the image to the server.



(2) The smartphone requests the server to execute HiLo image processing

- a. Smartphone transmits an execute request via http post protocol to the php website.
- b. The php website calls matlab to process the images and saves to the database.
- c. The php website uploads the processed image from the database for display on the phone.



3. HiLo image processing

(1) Writing the App: We used java to write an appropriate app for android systems. The main reference was a paper [4] from Ricky Wai Suen, which had the HiLo processing formulas. Using uniform and speckle illumination to acquire a Hi and a Lo image, and eventually combining the two into one HiLo image.

(2) Three main processing algorithms:

a. Create a Hi image (using a high-pass filter on Uniform illuminated image)

Gray scale the Uniform image to get GrayUniform.

(a) Fourier transform GrayUniform to get FFTUniform.

(b) Apply Fourier low-pass filter on FFTUniform to get LowUniform.

(c) Subtract LowUniform from GrayUniform to get Hi image.

b. Creating a Lo image

(a) Gray scale both Uniform and Speckle to get GrayUniform and GraySpeckle.

(b) Fourier transform GrayUniform and GraySpeckle to get FFTUniform and FFTSpeckle.

(c) Apply Fourier low-pass filter on FFTUniform and FFTSpeckle to get LowUniform and LowSpeckle.

(d) Divide GrayUniform by LowUniform to get impUniform.

- (e) Divide GraySpeckle by LowSpeckle to get impSpeckle.
- (f) Subtract impSpeckle from impUniform to get imp.
- (g) Apply Fourier transform on imp to get FFTimp.
- (h) Apply Fourier band pass-filter on FFTimp to get imLo1.
- (i) Apply inverse Fourier transform on imLo1.
- (j) Apply Fourier band pass-filter (different parameter) on FFTimp to get imLo2.
- (k) Apply inverse Fourier transform on imLo2.
- (l) Subtract imLo2 from imLo1 to get imLo3.
- (m) Multiply imLo3 by GrayUniform to get imLo4.
- (n) Apply low-pass filter on imLo4 to get imLo5.
- (o) Multiply imLo5 by Eta to get imLo.

b. Create the HiLo image

- (a) Combining the Hi and Lo images to get the HiLo image.

4. Software Structure

. (1)Android App

Basic UI: 4 buttons and a display window.

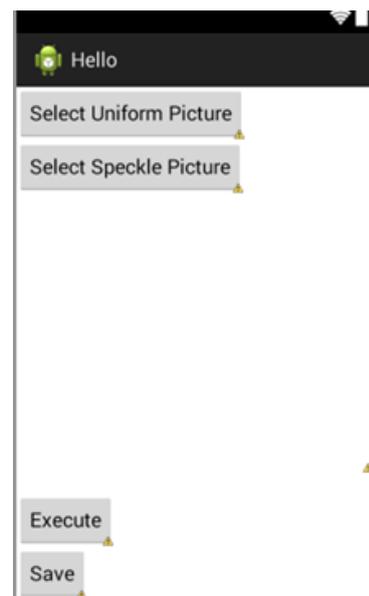


Fig 10. App UI

a. Select Uniform Picture: Select the Uniform image from the phone album and upload to the php website.

b. Select Speckle Picture: Select the Speckle image from the phone album and upload to the php website.

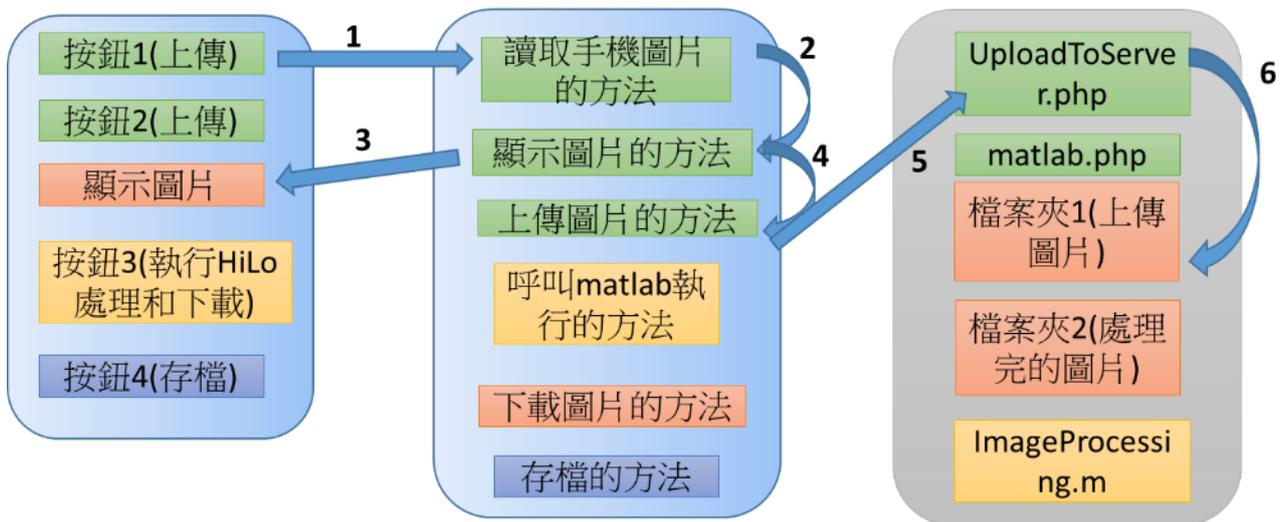


Fig 11. Flowchart for image uploading

Press button 1 (Select Uniform Picture) -> call "read phone image routine" -> select uniform image

-> call "display image routine" -> display image in window -> call "upload image routine"

-> upload image to server and call UploadToServer.php -> place uniform image in Folder1

-> display "File Upload Completed" in window

(Button 2 (Select Speckle Picture) same as above)

c. **Execute: Request** php website to call matlab for image processing, then display the resulting image in the window.

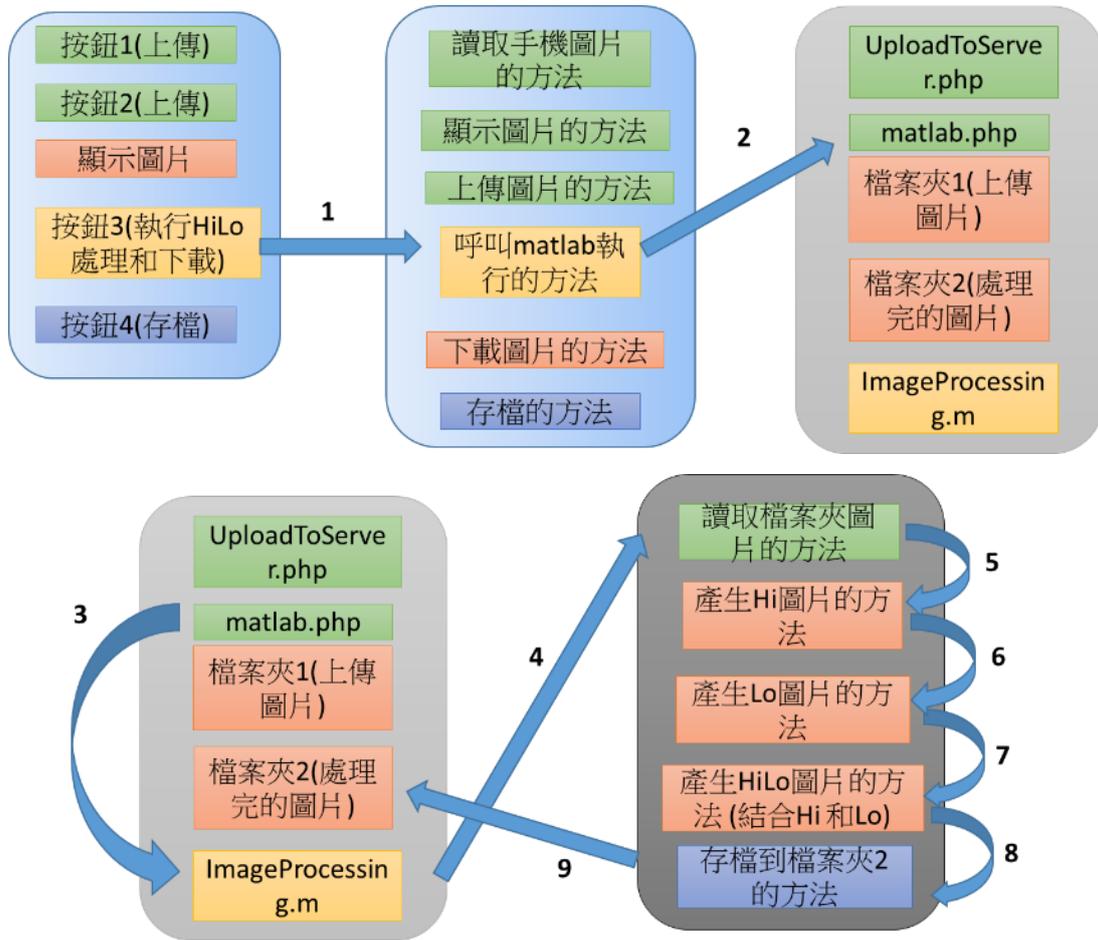


Fig 12. Flowchart for image processing and downloading

Press button 3 (**Execute**) -> call "call matlab execute routine" -> call matlab.php -> auto call matlab to start execution -> call "read image folder routine" -> read uniform and speckle images from Folder1 -> call "create Hi image routine" -> call "create Lo image routine" -> call "create HiLo image routine (combine Hi and Lo)" -> call "save file to Folder2 routine" -> return to button 3 -> call "download image routine" -> read HiLo image from Folder2 -> display "Execute Completed" in window

d. **Save:** Save the image into local memory

(2) PHP website:

PHP website has two main functions, one is responsible for receiving the images android is transmitting and saving them to the database, the other calls matlab to process the images and sends the images back to the phone.

Chapter 4 Results

4.1 Successfully Combined a HiLo Optical Section

1. Processing the images on the phone into a HiLo image through our App.

We took two pictures of the specimen (tissue), one with uniform illumination ($I_{uniform}$), and one with speckle illumination ($I_{speckle}$). Then we used the App to process the HiLo image. The steps are as follows:

Step 1: Upload $I_{uniform}$ image



Press "SELECT UNIFORM PICTURE"

→call "read phone image routine"

→select $I_{uniform}$ image

→call "display image routine"

→display image in window

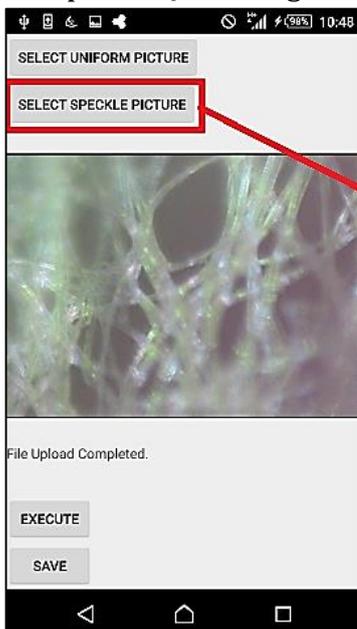
→call "upload image routine"

→upload image to server and call UploadToServer.php

→save uniform image to Folder1

→display "File Upload Completed" on phone

Step 2: Upload $I_{speckle}$ image



Press "SELECT SPECKLE PICTURE"

→call "read phone image routine"

→select $I_{speckle}$ image

→call "display image routine"

→display image in window

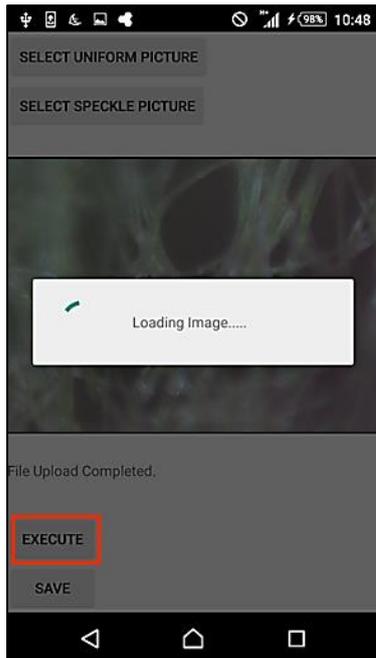
→call "upload image routine"

→upload image to server and call UploadToServer.php

→save speckle image to Folder1

→display "File Upload Completed" on phone

Step 3: Process the HiLo image and download to the phone



Press "EXECUTE"

→call "call matlab routine"

→call matlb.php

→auto call matlab to start execution

→call "read image folder routine"

→read uniform and speckle images from Folder1

→call "create Hi image routine"

→call "create Lo image routine"

→call "create HiLo image routine (combine Hi and Lo)"

→call "save file to Folder2 routine"

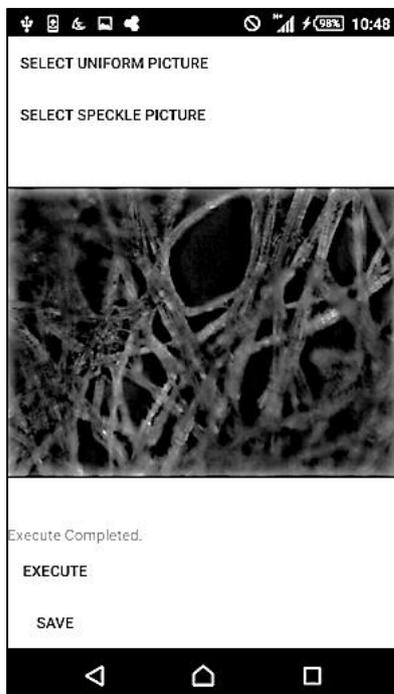
→return to "EXECUTE"

→call "download image routine"

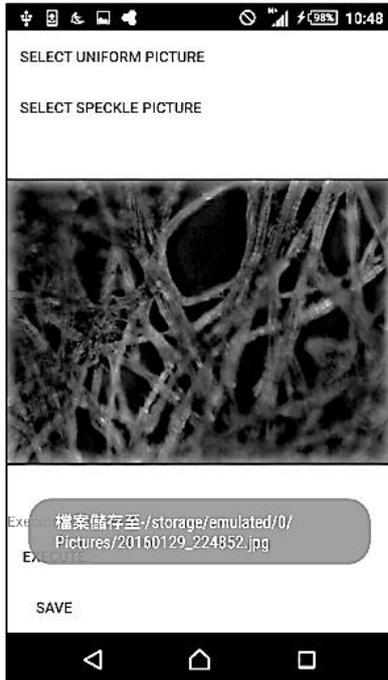
→read HiLo image from Folder2

→display "Execute Completed" on phone

Results:



Step 4: Save image file



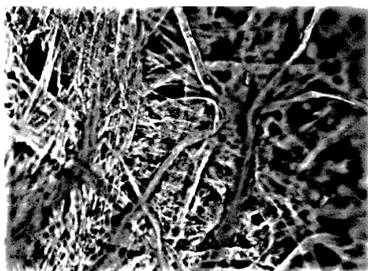
Press "SAVE"

→call "save file routine"

→save image to phone memory

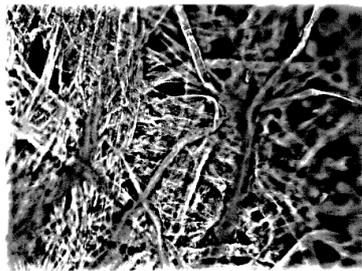
2. Confirming that the Image is Correct

Since the HiLo microscope algorithm has already been published and confirmed to be able to produce optical sections, and the inventor Professor Jerome Mertz has written an image processing freeware, imageJ, for all to use, we can do an image comparison to see whether the results we came up with is correct or not.



imageJ處理的HiLo影像

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自製手機app處理的HiLo影像

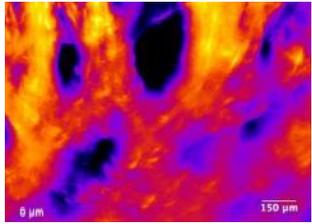
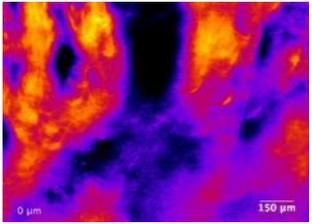
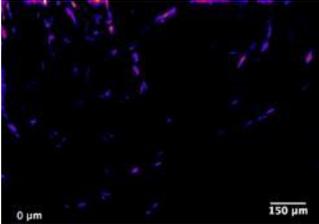
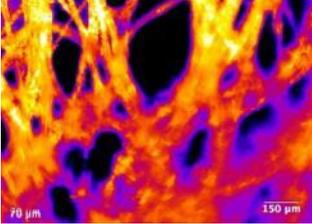
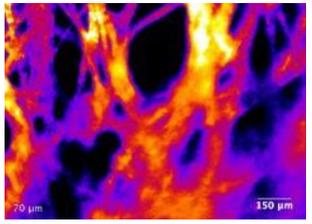
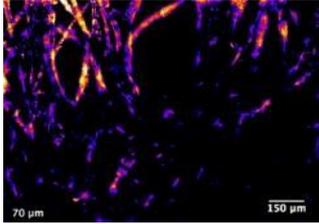
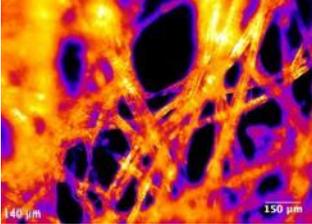
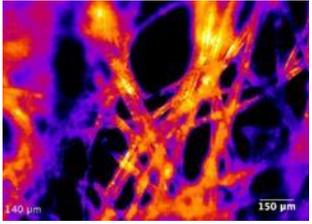
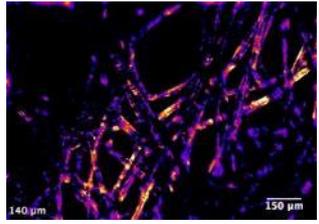
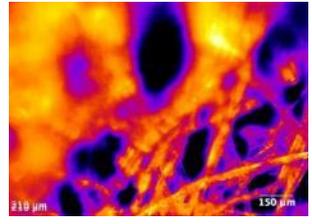
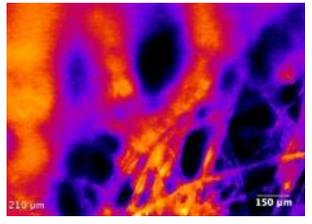
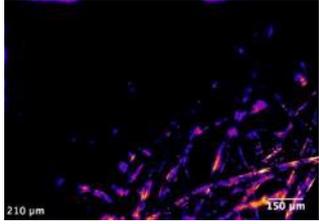
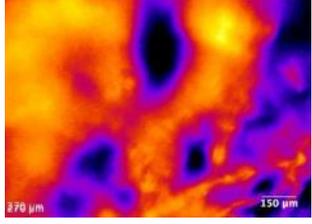
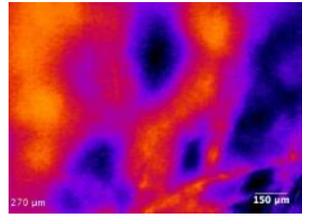
=



Through a simple linear subtraction of the two HiLo images processed by imageJ and our App, we come up with a fully black image, which means that the two images are exactly the same, and thus confirming that the image produced by our App is correct.

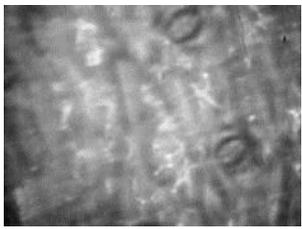
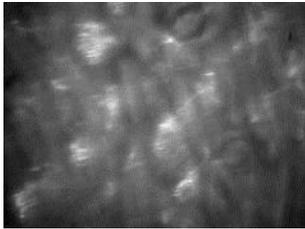
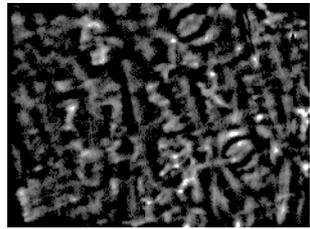
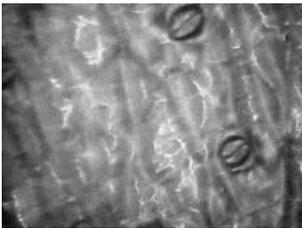
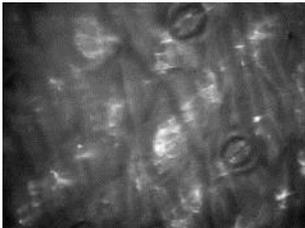
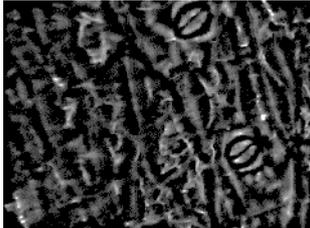
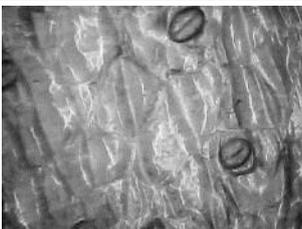
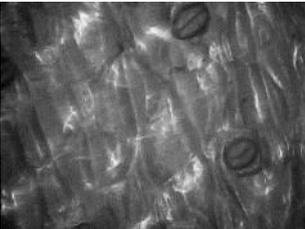
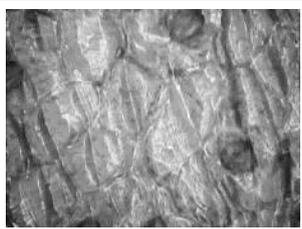
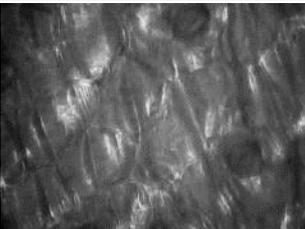
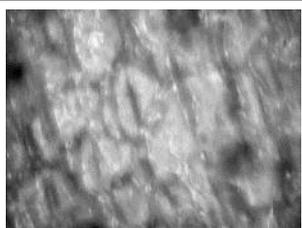
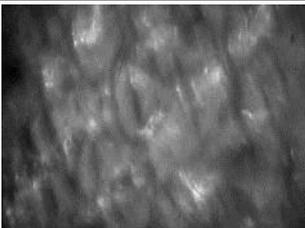
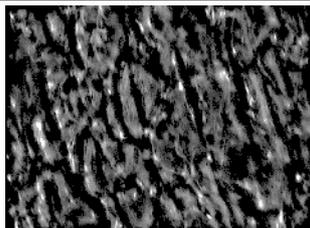
4.2 Image Processing at different depths within the specimen

1. Specimen 1 (tissue paper) at different depths

image depth	Uniform illumination I_{uniform}	Speckle illumination I_{speckle}	Processed HiLo image
0 μm			
70 μm			
140 μm			
210 μm			
280 μm			

We can observe from the I_{uniform} series that at depth $0\mu\text{m}$ and $280\mu\text{m}$, most of the objects are off-focus and blurred, and after HiLo processing, these off-focus items have almost completely been filtered out. At depth $70\mu\text{m}$, the upper left corner is in-focus and the lower right corner is off-focus. The results are opposite while at depth $210\mu\text{m}$. At depth $140\mu\text{m}$, the focal plane is roughly in the middle.

2. Specimen 2 (Plantain dermal tissue) at different depths

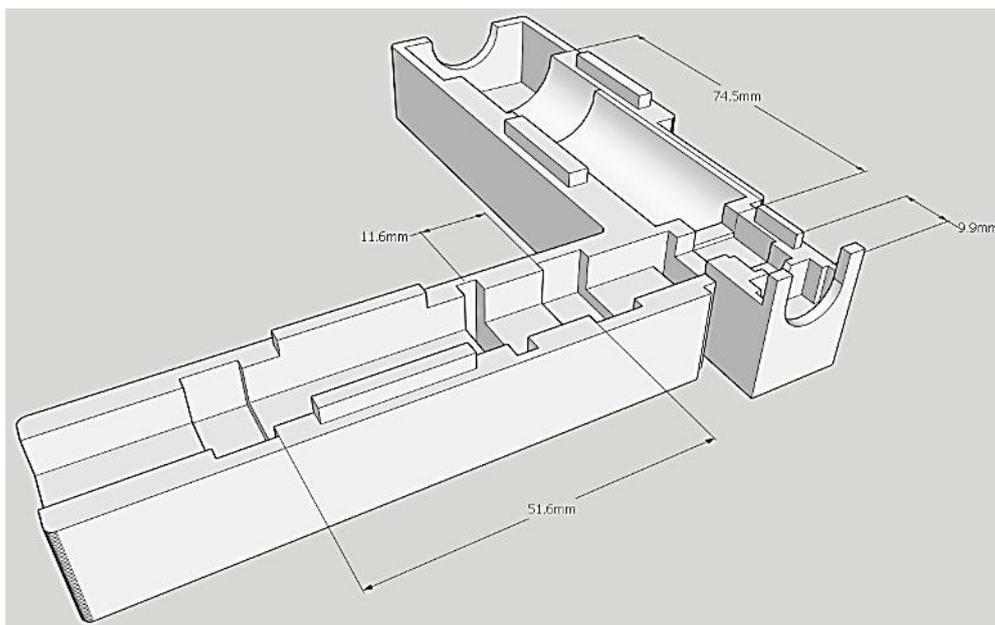
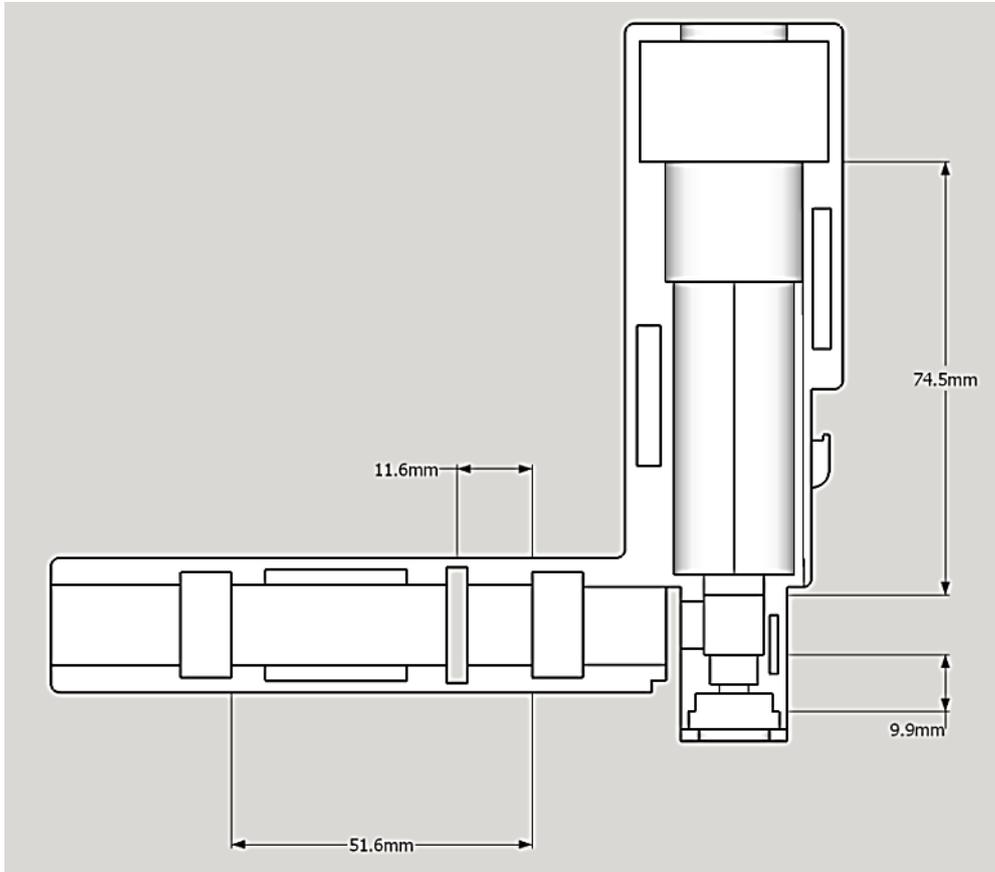
image depth	Uniform illumination I_{uniform}	Speckle illumination I_{speckle}	Processed HiLo image
0 μm			
20 μm			
40 μm			
60 μm			
80 μm			

This is the lower epidermis of plantain, and we can see very clearly as the guard cells come into and out of focus.

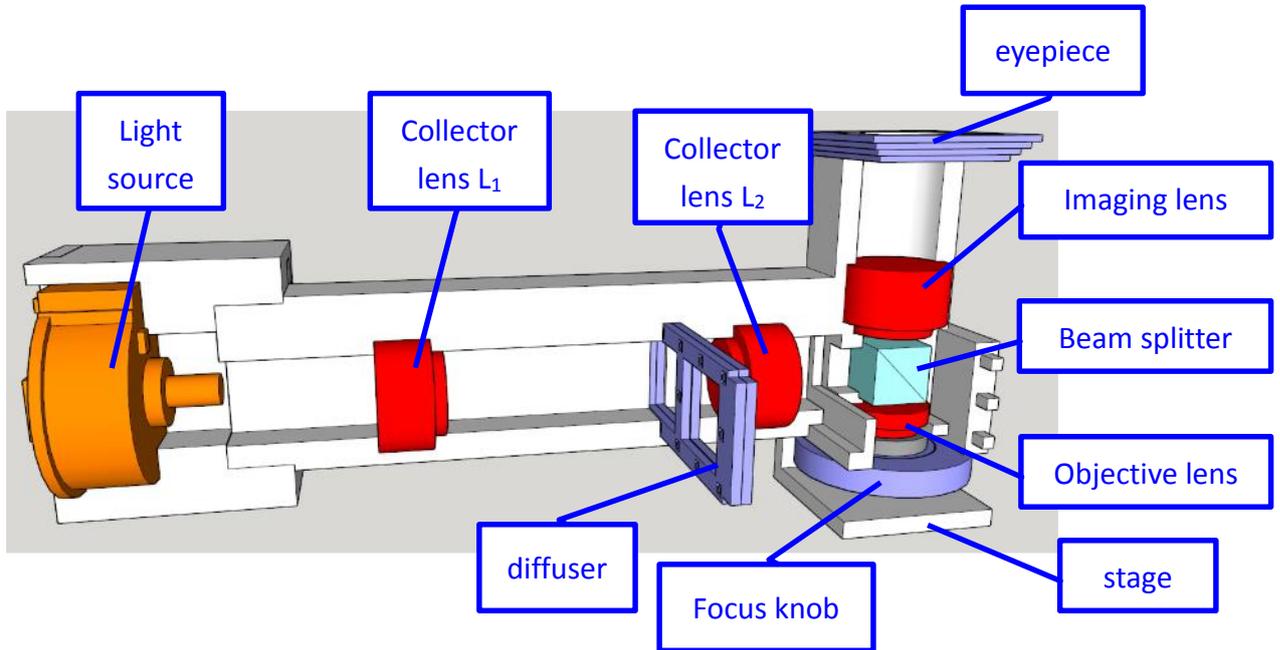
4.3 Creating the Smartphone Microscope Prototype

1. Design Drawings

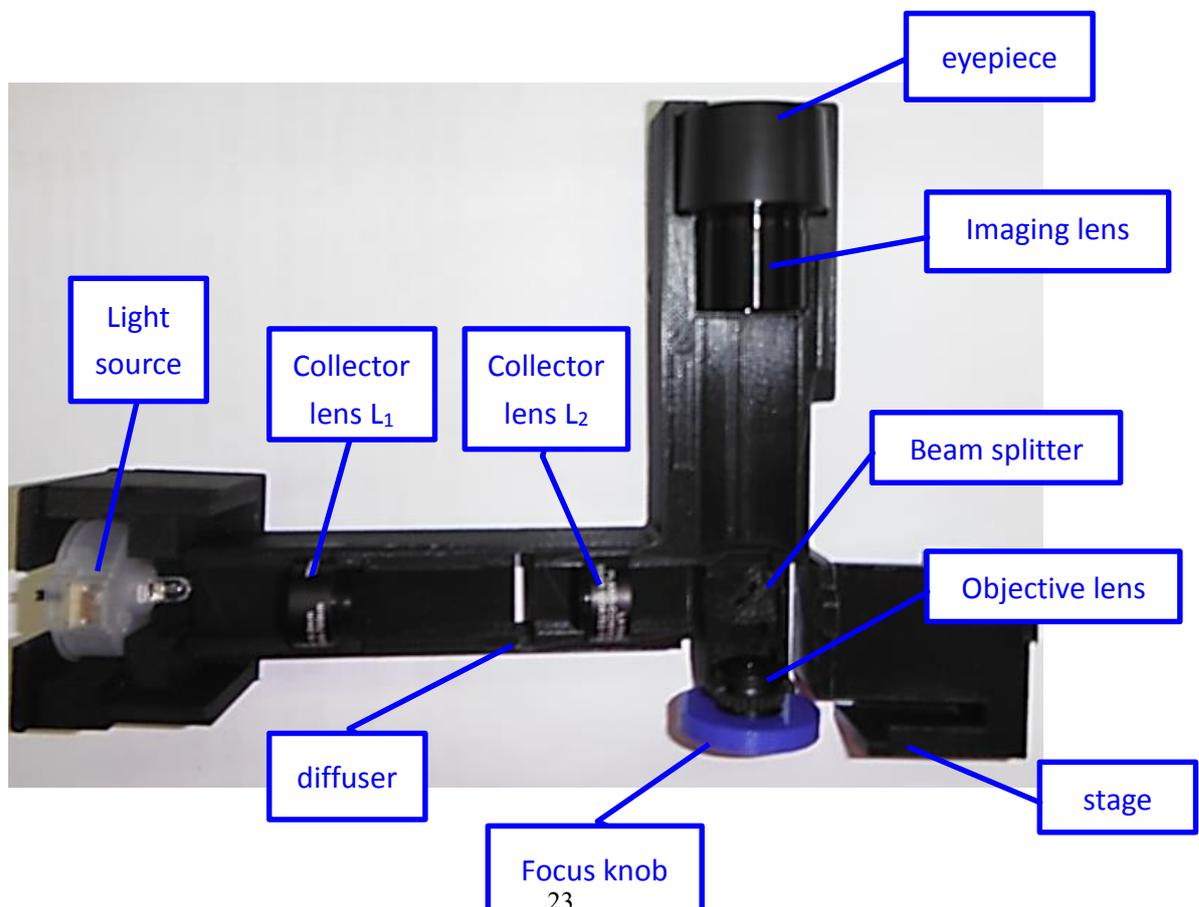
(1) The following drawings depicts the relative positions of the optical components.



(2) Looking at the design in three dimensions, we can trace the light path starting from the left hand side, passing through the two collector lenses, reflecting off the beam splitter and through the objective lens onto the specimen. The reflected light off the specimen then passes up through the imaging lens and into the eyepiece.



2. Printing out the Prototype Using a 3D Printer



Chapter 5 Discussion

5.1 The Effects of Spherical Aberration on Optical Sections

Spherical aberration is a known flaw in the image forming system that needs to be addressed. Please refer to the illustration below. The left side depicts a theoretical perfect lens, where all the beams converge to one focal point. The right side shows, rather exaggeratedly, a lens with spherical aberration, where the beams do not meet at one point, but rather scatters along the optical axis. Spherical aberration will elongate the focus in the axial direction, and increase the focal size in the lateral direction, both of which decreases resolution.

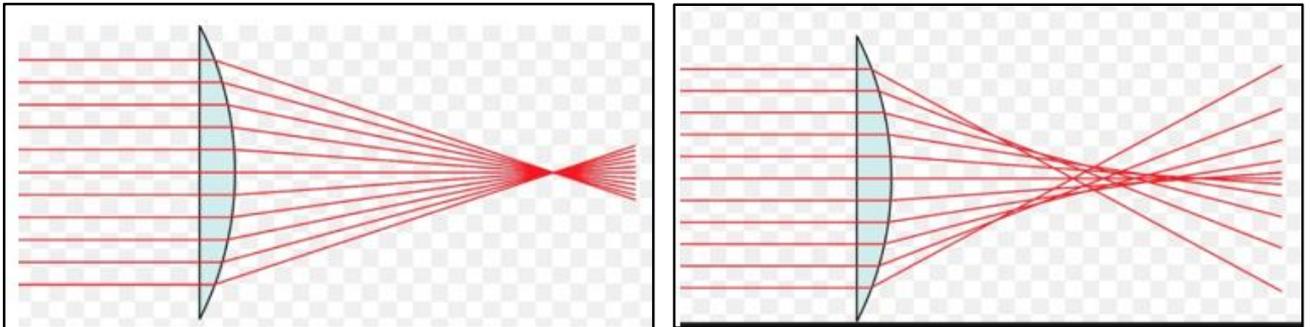


Fig. 13 Illustration of perfect vs. spherical aberration, source:

<https://zh.wikipedia.org/wiki/%E7%90%83%E9%9D%A2%E5%83%8F%E5%B7%AE#/media/Fil>

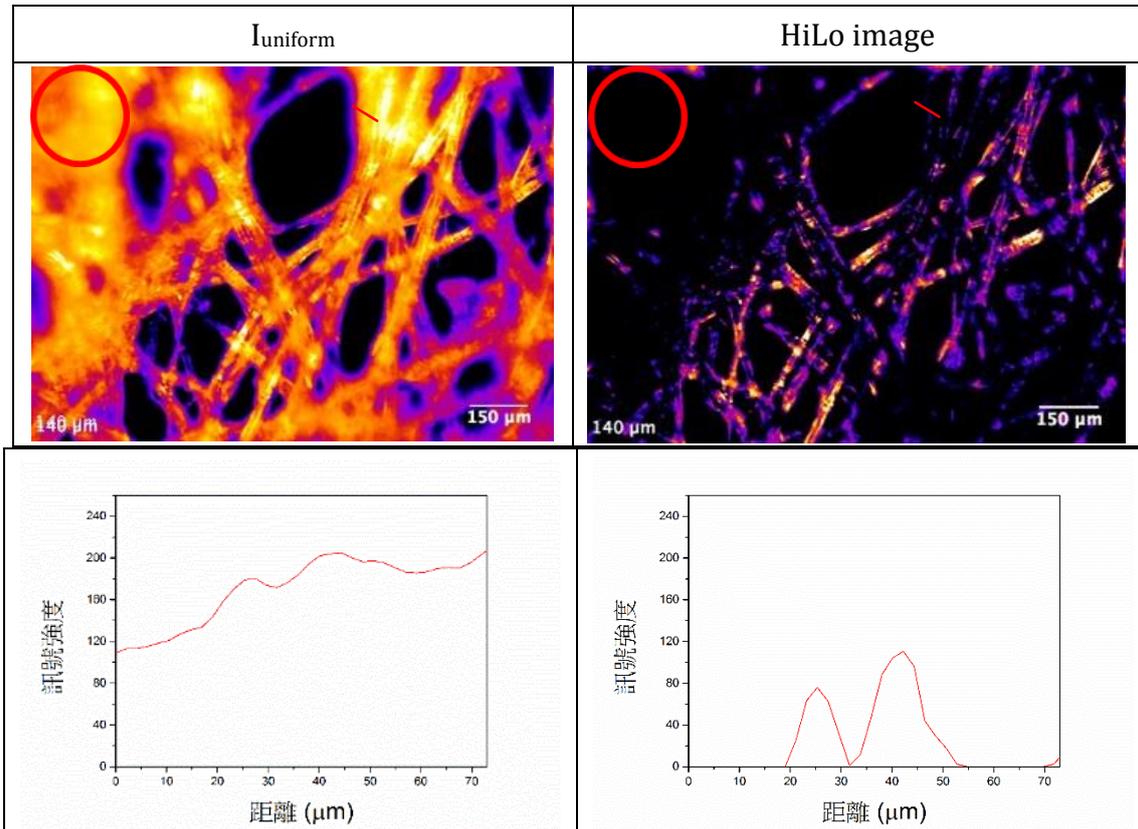
In our application, we can separately discuss the effects of spherical aberration along the illumination light path and the image-forming light path.

1. Since the illumination is not focused onto the specimen, but rather scattered into parallel beams, the effects from spherical aberration mainly appears when the diffuser is engaged, which might affect the edges of the projected structured illumination. Please refer to [5] on quantitative analysis of structured illumination.
2. The image-forming light path is more susceptible to the effects of spherical aberration. This is because the formed image is wide field, and according to Huygen's principle, every point on the sample can be seen as a new light source which paths through the objective lens, imaging lens, phone camera lens to finally arrive at the photo receptors of the camera. Although the objective lens and camera lens are both corrected for spherical aberration, the imaging lens is not. Our current imaging lens has a short focal length (5cm), so the thickness difference between the center and edges of the lens is significant, and prone to the effects of spherical aberration. Although we have tried to minimize its

effects, in order to enhance image quality, a special spherical aberration corrected imaging lens still needs to be introduced.

5.2 Wide field Imaging vs. HiLo Imaging

By revisiting one of the experiments we did, we saw that most of the off-focus patterns were filtered out after HiLo processing. Let's take a depth 140um example to do a comparison between wide field and HiLo imaging.



- Comparison :

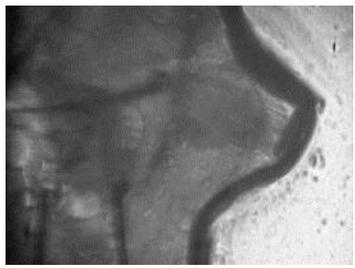
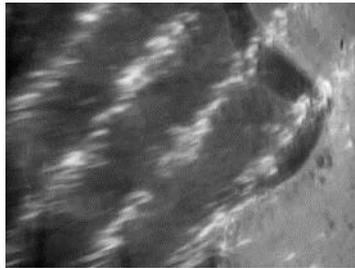
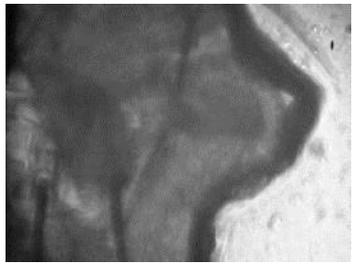
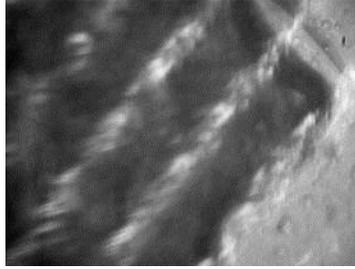
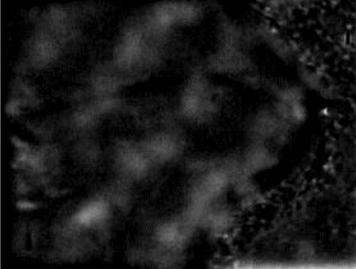
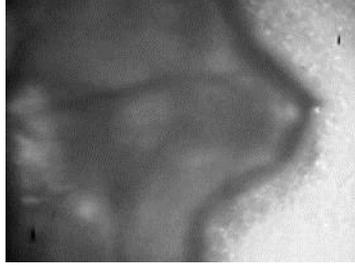
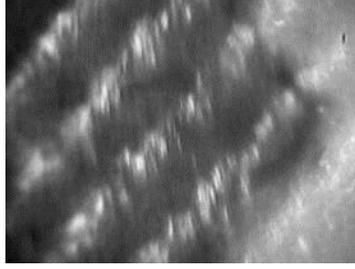
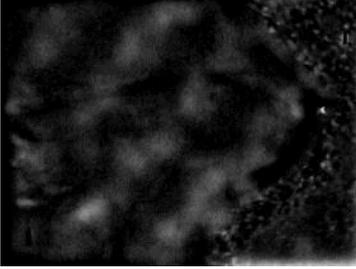
Let's take a look at the part denoted by the red circle. In I_{uniform} we can clearly see that there is significant interference from the off-focus signal, whereas there is considerable improvement after HiLo processing.

Now take a look at the red line in both images. By charting the signal intensity across the length of the red line, we arrive at the two charts below the images. Since there is a lot of interference from the off-focus signals in I_{uniform}, you can hardly make out the two peaks at the 25um and 40um marks. However in the HiLo image, you can clearly see the two peaks which are in the focal plane. This also confirms that after removing the off-focus noise, the HiLo image does in fact have a better contrast.

5.3 Effect of the Diffuser being Offset from the Conjugate Plane

In order to perform an optical section, we require the use of a diffuser, and the diffuser needs to be placed at the image-forming conjugate plane. This part discusses what will happen if the diffuser is offset from the conjugate plane.

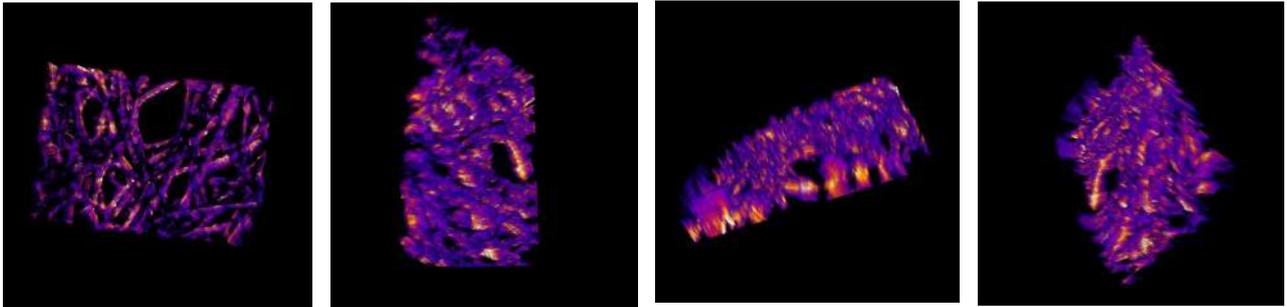
- We take the specimen (vinegar fly head plate) images below as an example

	I_{uniform}	I_{speckle}	HiLo image
0 μm			
20 μm			
40 μm			

From the above images, we can see that I_{uniform} is almost completely defocused, and yet after HiLo processing, we can see the diffuser pattern on the image. We may thus deduce that the diffuser is offset from the conjugate plane, and that is why we can see it on the HiLo image. This can severely affect the analysis of the real image. It was also mentioned in [6] that structured illumination might manifest in the final HiLo image, but can easily be filtered out using an appropriate wave filter. If the sampling depth is too deep in thicker specimens, the structured illumination might be refracted or scattered by the upper specimen structure, resulting in a blurred image and subsequently affecting the contrast of I_{speckle} , which in turn affects the resolution of the HiLo optical section. The vinegar fly head plate above was immersed in saline which contained impurities, so compared to the tissue paper sample, the deformation of the structured illumination was quite severe, affecting the optical sectioning capability, and resulted in incorrect calculations of the HiLo image.

5.4 The Specimen in Three Dimensions

Taking the tissue paper sample and creating a HiLo image every 10um from the surface for 30 steps, we can use the 3D viewer in imageJ software to combine them into a stacked three dimensional image. Through this digital stitching, we can manipulate and view the specimen in 3D, further advancing our knowledge of the specimen's structure as a whole. The following images were taken from different angles as an example.



5.5 Future Aspects

1. Under the current framework, the smartphone requires an internet link to connect with the server, but there are many cases where this is not possible. While using the smartphone's processor is one option for offline situations, another possible solution is to link to a nearby computer via Bluetooth, and have the computer process the HiLo image, then send the image back to the phone.
2. Another enhancement would be to upgrade the App with digital stitching capabilities. It would allow the user to have access to the 3D model, and enable the user to manipulate the sample in virtual space.
3. Far into the future when robots will be doing the exploration of uncharted territories, a portable HiLo microscope might be just the thing to enable a quick analysis of the surrounding environment.

Chapter 6 Conclusion

1. Confirmation that HiLo microscopy can be used with speckle illumination

Most of the current papers on HiLo Microscopy works with collecting fluorescence signals, but not so much with diffused illumination. Through experimentation, this project shows that HiLo microscopy can be used with speckle illumination to enhance the application of optical sectioning.

2. Implementing cloud computing to increase image processing speed

- (1) Developed the software to process the images and link the smartphone to the server.
- (2) Successfully used our software to process the images, and confirmed that the results were the same as imageJ. After HiLo processing, off-focus signals were filtered out, increasing the contrast of the images.

3. Created a portable HiLo microscope prototype using 3D printing technology

- (1) After purchasing miniature lenses, calculating the theoretical relative distances, and adjusting for tolerances on the optical table, we photographed the specimens and successfully processed them into HiLo images.
- (2) Using a 3D printer, we created a 1:1 ratio smartphone microscope, and are currently tweaking the dimensions for final testing.

4. Three dimensional optical sectioning expected soon

We have already successfully used imageJ to combine 30 HiLo images, at 10um steps, into a three dimensional model on the computer, so the next logical step is to implement this technology onto a smartphone.

Chapter 7 References

- [1] J. B. Pawley, "Handbook of biological confocal microscopy", Springer, 3rd edition, 2006.
- [2] M. B. Ahrens, M. B. Orger, D. N. Robson, J. M. Li and P. J. Keller, "Whole-brain functional imaging at cellular resolution using light-sheet microscopy", Nature Methods, 2013
- [3] D. Lim, T. N. Ford, K. K. Chu and J. Mertz, "Optically sectioned in vivo imaging with speckle illumination HiLo microscopy", Journal of Biomedical Optics, 2011
- [4] Optically Sectioned Imaging of Microvasculature of In-Vivo and Ex-Vivo Thick Tissue Models with Speckle-illumination HiLo Microscopy and HiLo Image Processing Implementation in MATLAB Architecture · 2013
- [5] Analyzing speckle contrast for HiLo microscopy optimization, 2011

【評語】 100018

1. 本作品利用 HiLo 影像技術運用在散射光影像的處理，並利用光切片影像呈現 3D 立體化影像，值得鼓勵。
2. 建議針對原理或影像處理或應用範圍，強調創新點並評估與傳統做法的進步程度，例如：探討比較使用與不使用 HiLo 技術在散射與螢光影像之差異，以更彰顯研究的貢獻。