Research Article | Vol. 11, No. 5 / 1 May 2020 / Biomedical Optics Express 2328

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Cell phone digital microscopy using an oil droplet

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Abstract: We introduce an accessible cell phone imaging method using small droplets of microscope immersion oil and consumer-grade oils. Oil droplets were more resistant to evaporation than water droplets, and they resolved cellular structures that were visible using a 20x/0.75 objective. We optically characterized the droplets using a cell phone screen and resolution target. We further obtained cellular resolution images of an onion epidermis and a zea stem cross-section sample. Our droplet-based method enables stable optical imaging for diagnostic and educational purposes without custom setups, specialized components, or manufacturing processes.

1. Introduction

Cutting-edge optical microscopy is currently in high demand in the fields of medicine and biology research. Nevertheless, in low-resource settings where accessibility is limited, the ability to quickly assess the morphology and size of the biological specimen beyond what the human eye can see is of practical interest. In response to the demand for access to low-cost microscopy for educational and diagnostic purposes, several researchers have developed new microscopy devices by attaching lenses and other types of devices to smart phones to perform brightfield, darkfield, fluorescence, and polarized imaging [1–7]. An added benefit of smart phones is that they can be used to transmit high-quality images through Multimedia Messaging Services (MMS). As of 2007-2008, the percentage of people who had MMS in countries across Africa ranged from 1.5% to 92.2% [8]. This increasingly widespread cellular connectivity could be harnessed to facilitate more rapid scientific communication between individuals and to increase accessibility to microscopy for diagnostic and educational purposes on demand.

Previous studies have demonstrated the ability to capture clinically useful microscopic images using a ball lens in front of the camera lens of a cell phone [9,10]. One study developed on-demand lenses by heat-curing polydimethylsiloxane (PDMS) plano-convex lenses to conduct cell phone imaging without using attachments such as ball lenses or accessory devices [11]. While uncured PDMS becomes too thin to function as an effective lens, heat-cured PDMS maintains its droplet shape well, which has been shown to enable a magnification of up to 120 times and a resolution of up to 1 micron [11]. Other studies have explored the option of creating tunable liquid lenses whose focal lengths can be changed through variations in pressure distribution in a liquid-containing chamber using a temperature-sensitive or pH-sensitive hydrogel ring [12]. While these inexpensive lenses enable a vast range of imaging applications with ease of operation, the distribution channels of custom-made lenses to low-resource areas have become a major bottleneck. Moreover, increasing numbers of custom applications call for the on-demand design of accessible, cost-effective lenses. Here, we seek to evaluate the use of simple and accessible materials for optical imaging.

#389345 https://doi.org/10.1364/BOE.389345
Journal © 2020 Received 28 Jan 2020; revised 19 Mar 2020; accepted 26 Mar 2020; published 2 Apr 2020

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Water droplets are easy to make and do not require specialized fabrication processes, so they can serve as useful tools for microscopy [13]. Nevertheless, water droplets have two significant limitations for imaging applications. First, water droplets evaporate rapidly under ambient conditions, which changes their focal length over time. To achieve optical amplification, water droplets often have small volumes, *i.e.*, less than 10 µL. Temperature, air flow, and humidity affect the evaporation rate, which quickly diminishes the optical magnification. Some studies have used methods to reduce the rate of water droplet evaporation, which enables longer imaging sessions while maintaining a consistent focal length. However, even with these methods, water droplet imaging methods are still very time-limited due to water evaporation. One study used a plastic container with wet paper next to the water droplet to maintain a consistent water vapor pressure, which maintained a constant focal length in the water droplet for two hours [13]. Another study used spherical water droplets at the tip of a syringe needle as lenses and coated them with silicone oil to reduce evaporation so that the water droplets could be used for an hour [14]. While each of these studies succeeded in developing a more flexible approach to water droplet microscopy, it may be difficult to conduct certain microscopy experiments using only a one-to-two-hour working time. Therefore, one of our focuses was to develop a method that would enable liquid droplets to be used over a much longer time. In addition to evaporating quickly, water-based lenses display optical aberration due to the index mismatch between water and glass. Since most biological specimens are mounted on a cover glass, which has a refractive index of 1.515 compared to 1.33 of water, optical refraction at the interface could deteriorate the image quality. In this report, we investigate the use of oil droplets that can be used in smartphone microscopy to obtain images of biological samples.

We started by demonstrating the use of index-matched immersion oil droplets for stable optical imaging, and then extended the method by using household cooking oils. We obtained the refractive index values for common household liquids from the International Gem Society [15]. For instance, safflower, peanut, and sesame oil have refractive indices around 1.47-1.48, closely resembling the refractive index of immersion oil at 1.515 [16]. Palm oil has a slightly lower refractive index of 1.46-1.47 [17]. In this study, we decided to compare droplets made of corn oil, canola oil, and olive oil.

2. Methods

2.1. Droplet magnification analysis

To characterize the optical amplification of droplets, we first used cell phone screens for optical illumination and measured amplified pixels through the droplet on a cell phone screen (Fig. 1). To prepare a series of droplet "lenses", we used a micropipette to place droplets with volumes ranging from 1-5 µL in a row on a borosilicate cover glass (Fisher, 12545M, lot 19810). For precise pipetting of the oil droplet, we prewarmed the immersion oil (Nikon Immersion Oil Type F, index = 1.518) in a 37°C water bath for ten minutes to reduce its viscosity. We lifted the cover glass above the cell phone screen through a stack of three glass slides measuring approximately 3 mm in total height. We imaged the droplet by focusing an iPhone 6S camera phone at various distances (Camera App, Photo, Autofocus on, Flash off). For characterizing the droplet, we were able to capture clear pictures by placing the cell phone camera approximately 8 cm above the droplet. We used a solid white cell phone image on an iPhone Xs for capturing the screen pixels.

We then imported the captured images into ImageJ and measured the size of amplified pixels through each droplet lens. The size of the pixel was defined as the distance between adjacent red, green, or blue pixels near the center of the droplet. The dimension of the image was calibrated by the width of the cell phone screen, which was obtained from the manufacturer's specifications. The physical size of each pixel was also derived from the pixels per inch (PPI) data from the manufacturer. We then used our magnified pixel values to calculate the magnification factor that each droplet produced and plotted the magnification factor as a function of the droplet volume.

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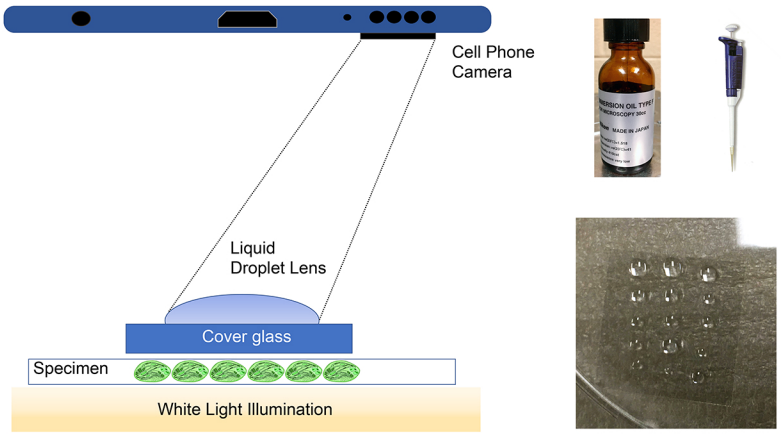

Fig. 1. Schematic illustration of a cell phone capturing an image of a droplet used to magnify a biological sample. The matrix of oil droplets in the sample image sat on a borosilicate cover glass.

For iPhone Xs, we determined the pixel dimension to be 55.46 µm and the screen width to be 6.22 cm.

2.2. USAF resolution target analysis

In order to study the resolution of an immersion oil droplet, we used a white light source and an iPhone 6s Plus and Huawei Honor 7X cell phone to capture images of an oil droplet magnifying a Positive 1951 USAF test target (Thorlabs R1DS1P).

2.3. Imaging biological samples using immersion oil

We used a white light source to illuminate two biological slides containing an onion epidermis and a zea stem cross section (AmScope). We used the Huawei Honor 7X cell phone (Camera App, Photo, Autofocus on, Flash off) camera focused through the droplets. Similarly, the way we imaged the cell phone screen, we moved the cell phone camera up and down until the cell phone was able to focus on the sample slide. Because the biological sample had lower contrast than the cell phone screen, we had to place the cell phone camera slightly higher, approximately 14 cm from the sample, in order to maintain a stable focus. The cell phone images were compared with images taken using a Plan Apo λ 20x NA 0.75 objective on a Nikon Eclipse Ti-E2 microscope. White light illumination was used and a Photometric Prime 95B back-illuminated sCMOS camera captured the image at 50 ms integration time.

2.4. Preparation of cooking oil droplets

To increase the simplicity of the experiment, we used cooking oil as a source of magnification. We obtained consumer-grade corn oil, canola oil, and corn oil.

2.5. Comparison of smartphone images obtained using immersion and cooking oils

Once we compared the images we obtained using immersion oil to those captured with the Nikon microscope, we prepared a series of 1-5 µL droplets of immersion, canola, olive, and corn oil on glass coverslips. We then captured images of the zea stem cross section and onion epidermis using each set of oil droplets.

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3. Results

3.1. Oil droplets are more resistant to evaporation

Using a cell phone camera to acquire magnified images through a plano-convex lens formed by a water droplet practically constitutes a two-lens system. When using water as a droplet lens, evaporation causes constant change to the radius of curvature and effective focal length of the droplet. We compared the evaporation rates of droplets made of water, immersion oil, and corn oil on a glass coverslip. After 20 minutes at room temperature in an indoor laboratory setting, water droplets smaller than 5 µL completely evaporated, whereas both immersion and corn oil droplets maintained their shape and volume (Fig. 2(A)). Furthermore, oil droplets, *i.e.*, those made of immersion oil, sustained their size and shape for extended periods (Fig. 2(B)). For a typical water droplet between 2 and 10 µL, the diameter of the droplet ranged between approximately 0.17 and 0.29 cm, respectively (Fig. 2(C)). Oil droplets showed similar dimensions. It should be noted that the droplet size and shape can vary due to the pipetting error and surface property of the cover glass. For instance, we observed that water and oil droplets spread out more on the glass slide made of water white glass (Fisher, 12-544-4) than on the microscope cover glass made of borosilicate glass (Fisher, 12545M, lot 19810).

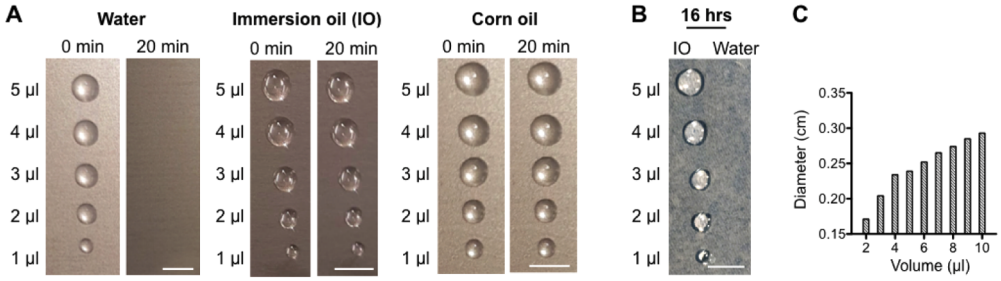

Fig. 2. The droplet morphology. A) Comparison of droplets made of water, immersion oil, and corn oil at room temperature for 20 minutes. Scale bar = 5 mm. B) Comparison between immersion oil and water at room temperature for 16 hours. C) A typical relationship between the volume and diameter of the water droplets.

3.2. Characterizing optical resolution using screen pixels and a resolution target

We utilized known sizes of cell phone screen pixels to quantify the optical magnification. This method of using cell phone pixels to observe the change in magnification upon altering the size of an oil droplet may be useful in a low-resource setting since it can be carried out upon determining the size of a pixel using phone specifications obtained from the cell phone manufacturer. Figure 3(A) illustrates the amplified images of pixels on an iPhone Xs screen through the 5 µL and 2 µL droplets. Of note is the advanced arrangement of red, green, and blue pixels on the iPhone Xs screen. We determined the optimal volume of the droplets to be within this range as droplets smaller than 2 µL created significant aberration, *i.e.* barrel distortion, with a limited field of view while droplets larger than 5 µL exhibited decreasing optical magnification. Specifically, optical amplification of water and oil droplets on the cover glass was found to be similar in the range between 3 and 5x when the volume of the droplet was greater than 3 µL (Fig. 3(B)). The 2 µL oil droplet achieved a ~ 60% higher magnification than the equivalent water droplet. The higher magnification is likely attributed to the higher surface tension as well as the larger refractive index of oil. According to the lens maker formula:

$$\frac{1}{f} = (n-1)\left(\frac{1}{R_1} - \frac{1}{R_2}\right), \tag{1}$$

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where f is the focal length, n is the refractive index, and R_1 and R_2 are the radius of curvature on both sides of the lens, our plano-convex droplet lenses would have the effective focal length f given by:

$$f = \frac{R_1}{n-1}, \tag{2}$$

since $R_2 \approx \infty$ on the flat cover glass. If we simplify the approximation by neglecting the effect from the supporting cover glass, the water ($n = 1.33$) droplet has an approximately 56% longer focal length f than the immersion oil ($n = 1.515$) droplet with the same lens curvature. In addition, the droplet lens (lens 1) and the effective cellphone camera lens (lens 2) form a two-lens system. The overall transverse magnification M can be obtained by:

$$M = \left(\frac{s_{i1}}{s_{o1}}\right)\left(\frac{s_{i2}}{s_{o2}}\right), \tag{3}$$

$$\frac{1}{f_1} = \frac{1}{s_{o1}} + \frac{1}{s_{i1}}, \tag{4}$$

$$\frac{1}{f_2} = \frac{1}{s_{o2}} + \frac{1}{s_{i2}}, \tag{5}$$

where s_{o1}, s_{i1} f_1. and s_{o2}, s_{i2} f_2 are object and image distances and focal lengths for the droplet lens and camera lens, respectively. Combining (3)-(5), we obtain:

$$M = \left(\frac{f_1}{s_{o1} - f_1}\right)\left(\frac{s_{i2} - f_2}{f_2}\right). \tag{6}$$

For the cellphone camera, we assume the second term $\frac{s_{i2}-f_2}{f_2}$ is fixed. Therefore, the overall magnification is governed by:

$$M \propto \left|\frac{f_1}{s_{o1} - f_1}\right| = \left|\frac{1}{\frac{s_{o1}}{f_1} - 1}\right|. \tag{7}$$

In our setup, $s_{o1} \approx 3$ mm. When the droplet volumes are 2 and 3 µL, their focal length f_1 is likely slightly greater than s_{o1}. In this case, M becomes inversely related to f_1 (Fig. 3(C)). Since the oil droplet has a shorter f_1, it would achieve a higher overall M. For the larger droplets at 4 and 5 µL, the focal length is likely much greater than s_{o1}. Figure 3(C) indicates that, in this range, M becomes relatively insensitive to f_1. As a result, both water and oil droplets would achieve the similar M. This derivation is consistent with the measured magnification from different droplets in Fig. 3(B). Of note is that when $f_1 \approx s_{o1}$, the singular value in Fig. 3(C) renders proper image formation difficult. This is likely the case with the 1 µL immersion oil (IO) droplet (Fig. 3(A)).

To demonstrate the enhancement of optical resolution, we compared two images of the USAF resolution target with and without the oil droplet. Figure 3(D) demonstrates a typical image of the resolution target. The right panel demonstrates an image of the same resolution target with an oil droplet outlined by the dotted circle above group 5 elements. While the standard cell phone image failed to resolve any elements within this group, the image magnified by the oil droplet resolved several elements. The line profile plots of the vertical and horizontal elements (Fig. 3(E)) demonstrate the ability of the oil droplet to resolve vertical element 4 and horizontal element 3 in group 5. The resolution is determined by:

$$Resolution\left(\frac{line\ pair}{mm}\right) = 2^{Group+\left(\frac{Element-1}{6}\right)}. \tag{8}$$

As such, group 5 element 3 corresponds to 40.3 line pairs per mm, or 24.8 µm per line pair. We note that this resolution is likely to be sensitive to the distance of the object from the droplet as

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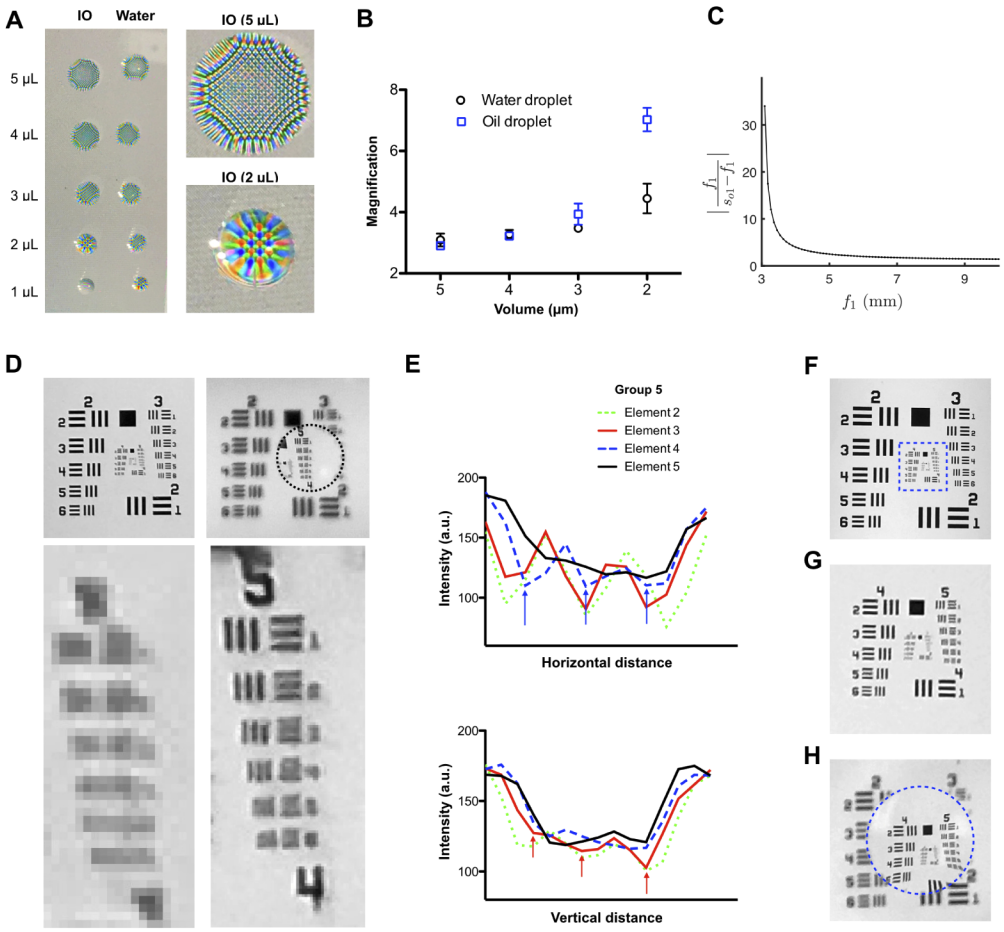

Fig. 3. Optical characterization of water and immersion oil (IO) droplets. A) Immersion oil and water droplet images on a cell phone screen. B) Characterization of the optical magnification of droplets using a cell phone screen for low-resource settings. C) Characterization of the relationship between the focal length of the droplet (f_1, x axis) and the achievable magnification (y axis) assuming the cell phone screen pixels are 3 mm away from the droplet lens, $s_{o1} = 3\ mm$. D) Characterization of the optical resolution using a USAF resolution target. E) Line profiles from the USAF image taken with a droplet in D). F) USAF image taken using a commercial clip-on macro lens. G) High-magnification view of F) showing Group 4 and 5 elements resolved from the image. E) A 2 µL IO droplet image resolving Group 4 and 5 elements.

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well as the distance between the cell phone camera and the droplet. In addition, the blurriness of the resolution target image is likely attributed to the cell phone camera pixel density as well as the built-in image compression algorithm.

Next, we compared the achievable optical resolution between a ~ 2 µL immersion oil droplet and a commercially available clip-on traveler lens kit for cellphone microscopy (AOMAIS, ~$25 USD). The lens kit contains a 18x macro lens for high-resolution imaging. As shown in Fig. 3(F), G, the commercial macro lens was capable of resolving group 4 and some group 5 elements. The optical resolution was found to be similar to that of an oil droplet (Fig. 3(H)). The advantage of the clip-on lens is that the imaging field is relatively flat, while the droplet image showed some field curvature. In particular, the pincushion distortion was apparent when the droplet volume was small and near the edge of the droplet. The optical distortion can be corrected through post-processing. Importantly, the major advantage of our droplet-based technology is that the technology does not rely on specialized distribution channels.

3.3. Resolving cellular structures using an immersion oil droplet

After we examined the resolution of the oil droplets, we used the oil droplets to obtain images of biological samples (AmScope), including a zea stem and an onion epidermis. The cell phone images were taken by illuminating the sample slide with a white light source and using an oil droplet (outlined with a blue circle in Fig. 4(A), B) to magnify the images. The middle images are the high-magnification view of the images on the left, and the images on the right were captured using the Nikon microscope. As shown in Fig. 4(A), B, the images obtained using the iPhone and oil droplets showed the same structures that were obtained using a 20x/0.75 objective on a Nikon Eclipse Ti-E2 inverted microscope. While the Nikon image had a visibly higher resolution than our oil droplet images, the oil droplets enabled us to view biological structures that would have been impossible for us to view using the naked eye. For instance, when the onion epidermis was magnified with the oil droplet, the shapes of individual cells were visible (Fig. 4(A)). In the zea stem cross-section, the xylem and phloem of the plant vascular structure were also visible, although they were less resolved in the oil droplet image than in the Nikon image (Fig. 4(B)). To quantitatively compare the achievable resolution between images obtained by cellphone droplet and commercial microscopy, we examined the regions where cell walls are closely adjacent to each other. The spatial resolution manifests as the capability to resolve distinct but closely spaced boundaries. As shown in Fig. 4(C), while the commercial microscopy image showed higher contrast and more structural details due to the superior optics and sCMOS detection camera, both methods can effectively resolve ~ 20 µm spacing in the cell images. This resolution is consistent with the 24.8 µm per line pair measured from the USAF target.

3.4. Comparing immersion oil and cooking oil droplets for optical imaging

In spite of its ideal properties, the process of obtaining immersion oil may present significant barriers in low-resource settings due to its cost and low accessibility. For this reason, we explored the use of consumer-grade oils, which have historically been used to conduct immersion oil microscopy before synthetic immersion oils were commercially available. Before synthetic oils became the standard for immersion oil microscopy, natural oils, such as cedar tree oil and castor oil, were typically used [18]. More recently, castor oil has been used to obtain immersion objective images of lymphocytes in metaphase to produce images comparable to those taken using synthetic immersion oil [19].

We obtained canola oil, olive oil blend, corn oil, and Nikon immersion oil to evaluate their performance for smartphone microscopy. Two sets of coverslips were prepared for each oil; one coverslip contained unknown volumes of oil and the other coverslip contained known volumes of oil. Approximately 1 mL of each oil was placed into four conical cylindrical centrifuge tubes to be used for the preparation of oil droplets. A pipette tip was then used to transfer small amounts

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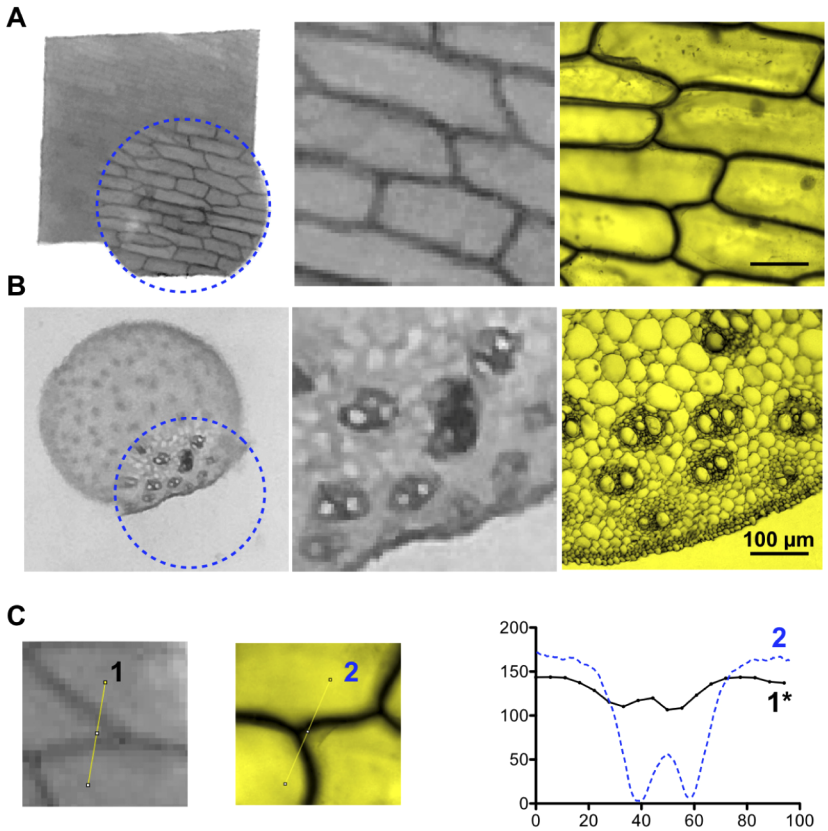

Fig. 4. A comparison between magnified oil droplet images and images obtained using a Nikon Eclipse Ti-E2 inverted microscope with brightfield illumination and a 20x/0.75 objective. A) Droplet (grey) and microscopy (yellow) images of an onion epidermis sample. B) Droplet (grey) and microscopy (yellow) images of a zea stem cross-section. C) Line profile plots of droplet (grey) and microscopy (yellow) images of the onion epidermis sample demonstrating the achievable spatial resolution at the boundary of three adjacent cells. Dimensions in 1* are approximate. Scale bar = 100 µm.

of oil from the centrifuge to the coverslips and form three rows of oil droplets with unspecified volumes. After the droplets of unknown volumes were prepared, the centrifuge tubes containing each oil were heated to 37°C for ten minutes using a hot water bath to reduce the viscosity of the oils. We then prepared one coverslip for each heated oil onto which we placed droplets ranging from 2-4 µL using a micropipette.

Once our oil droplets were prepared, we used them to obtain a series of images of the zea stem and onion epidermis samples. The distance between the camera and the sample was kept at approximately 14 cm. Roughly, similar color and resolution were achieved using cooking oil droplets compared to immersion oil droplets. Generally, images that were taken using 4 µL droplets were less magnified and less resolved than images taken using 3 µL and 2 µL droplets, as expected (Fig. 5). However, several images deviated from this trend; for instance, the 3 µL and 4 µL immersion oil droplets appeared to provide approximately equal resolution.

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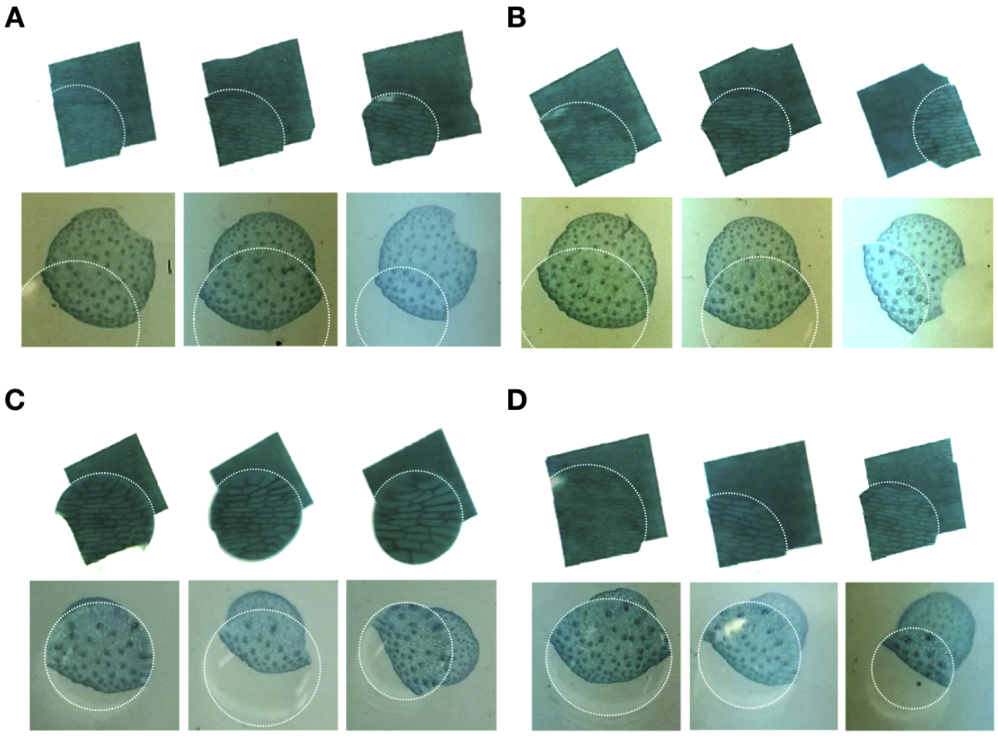

Fig. 5. Imaging using cooking oil droplets of 4, 3, and 2 μL in volume, from left to right. A) Immersion oil, B) corn oil, C) canola oil, D) olive oil.

4. Discussion

In the future, liquid droplets could be used to design flexible, low-cost microscopy systems for educational or diagnostic purposes. Immersion oil, in particular, could be used as a somewhat more permanent lens to view microscopic structures, since it does not evaporate nearly as quickly and has the same refractive index as glass. As can be seen in Fig. 2, while the row of water droplets evaporated almost entirely in about twenty minutes, the oil droplets did not decrease in size over the measured time. Therefore, if an oil droplet lens of a specific size was needed for multiple imaging sessions, the same oil droplet lens could be reused, which would save time and retain the previously used magnification factor. While glass lenses can be expensive and require extensive manufacturing processes, oil droplet lenses only require the use of a coverslip and plastic tool to transfer oil. While we used a micropipette to create our oil droplets, a micropipette is only needed when exact volumes of oil are desired, which is not required to obtain qualitative images of biological samples. Furthermore, since several oil droplets can be made quickly, it is possible to easily prepare a series of droplets that increase in magnification with either a wide or narrow magnification range, depending on what types of images are being captured. Therefore, microscopy using oil droplets and cell phones for sample illumination and image capture are promising yet simple tools that could be refined in the future to potentially conduct quantitative and qualitative analyses of biological samples.

In our experiments, we demonstrated that cooking oil may be a useful, cost-effective method of obtaining images of biological samples in a low-resource environment. In order to address the issue of cost-effectiveness in a low-resource setting, we experimented with the use of cooking oils as oil droplet lenses, since cooking oils, such as castor oil, have a history of being used in immersion oil because their refractive indices are close to that of glass and of synthetic immersion

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oil. We found that images obtained using cooking oil droplets were similar to those obtained using immersion oil droplets, and that oil droplets could be prepared with or without the use of a micropipette. While we used a pipette tip to transfer the oils when preparing droplets of non-specified volumes, a less expensive alternative could be used in a low-resource setting, such as a plastic stick. Although we were able to produce useful images through the use of cooking oil droplets, the resolution and focus levels that we achieved were, at times, inconsistent. This was likely due to settings that are pre-programmed into cell phones that make them less ideal imaging tools for scientific applications. This obstacle is a potential avenue for improvement that may be explored in future studies.

While cell phones are designed to be cost-effective and simple to use, cell phone imaging often sacrifices quality and versatility for simplicity. Studies have shown that cell phones have certain built-in limitations that reduce their accuracy and hinder their use as a quantitative tool for diagnostic microscopy [20]. One of the key differences between scientific and cell phone microscopes is that scientific imaging systems allow users to have full control over the camera or microscope settings, whereas cell phone microscopes are programmed with default settings, some of which cannot be changed easily. Many smartphones use autofocus, which can lead to inaccurate size quantification in microscopy because it can impact magnification by changing the apparent size of a structure as much as 6% [20]. Autofocus had a significant impact on the quality of the images that we were able to obtain. Using the zea stem sample and a 2 µL oil droplet, we found that when working at set distances from the sample, autofocused images showed out-of-focus blur whereas the manual focus feature enabled us to view individual vascular bundles.

Another feature that makes it more challenging to conduct cell phone microscopy is the automatic image processing that is programmed into cell phones, such as noise reduction and image compression. While these features are useful in generating desirable images in cell phone photography, they often result in a loss of information and inconsistent imaging, which can lead to inaccurate quantitative analysis in cell phone microscopy [20]. For instance, the image quality in Figs 3–5 is subject to the image processing algorithm within the cell phone. In future research, it would be useful to design an App that would reduce or enable the modification of automatic image processing that alters image features, such as autofocus, color correction, and blurring of uneven textures. It would also help if the App were to minimize automatic image processing or if it were specifically tailored toward biological imaging.

There are several challenges that may emerge when working with cooking oil droplets. For instance, in an outdoor environment, droplets may attract dust particles, which could potentially deteriorate image quality. While our method is only aimed to be utilized in the short-term, ranging from a few minutes to several days, it may be useful to take measures to account for possible contamination from dust or other particulates. This may be achieved by enclosing coverslips with oil droplets in a container, such as a petri dish, to prevent contamination. Another potential issue is that some vegetable oils such as sesame oil and olive oil are naturally colored, which may impact the apparent color of the biological samples in the images obtained using a cell phone. In our study, there was no noticeable difference in color between the images we obtained using olive oil and other types of vegetable oil. While we did not see a major color difference due to natural oil colors, it may be valuable to conduct smartphone imaging experiments comparing different brands of several types of vegetable oils to see if there is a color difference between them.

In the future, it may also be useful to consider cost-efficient methods that would increase the tunability of vegetable oil droplet lenses, which would provide more flexibility in low-resource cell phone microscopy. For instance, surface properties such as polarity, can affect the wetting and shape of the droplet. These surface properties can be exploited in future studies to explore the tunability of cooking oil droplet lenses. Taken together, the ease of operation in droplet-based

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bioimaging will extend the discoveries from medical and biology researchers to the hands of field workers and educators.

5. Conclusions

We present an accessible imaging method using evaporation-resistant oil droplets and a cell phone camera. The attenable optical resolution enables direct observation of cellular structures in plant tissue samples. We further demonstrate the applicability of using household oils for optical imaging. Combined with the versatility of capturing and sending digital images through the mobile network, our study lays the groundwork for an attractive optical technology for improving healthcare in low-resource settings with a minimal footprint.

Funding

Department of Chemistry at the University of Illinois at Chicago.

Disclosures

The authors declare no competing interests.

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