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Label-free cellphone microscopy with submicron resolution through high-index contact ball lens for *in vivo* melanoma diagnostics and other applications

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ABSTRACT

Developing megapixel large-area CCD and CMOS sensor arrays in the 2000-s stimulated ideas about developing microscope systems operating without heavy and bulky microscope stands and objectives by using microoptics approach in combination with imaging by cellphone cameras. Due to limited magnification, however, the best resolution of such systems is currently limited by the finite size of the pixels at ~1.5 μm level. We propose a novel approach to designing such microscope systems based on using contact ball lenses with index of refraction close to 2, which are capable of imaging biomedical and nanoplasmonic objects with extraordinarily high magnification and resolution. By using ball lenses made from glass with index $n = 2.02$ at $\lambda = 600$ nm we build a cellphone camera-based microscope system with up to ×50 magnification and resolution fundamentally limited at ~600 nm level due to diffraction of light. It is demonstrated that the operation of such system is a subject for strong dispersive effects in glass leading to a complicated tradeoff of magnification, resolution, and field-of-view (FOV) in the proximity to critical index of 2. Using this system, we performed imaging of melanoma samples which shows a potential of developing biopsy-free *in vivo* histology of skin using ball lens-assisted smartphone microscopy.

Keywords: superresolution, cellphone imaging, ball lens, imaging of cells, histology, diagnostics of melanoma

1. INTRODUCTION

From the early 2000s, the development of compact CCD sensors stimulated the ideas of developing hand-held and lightweight cellphone-based microscopes to replace the traditional microscope with heavy stands and bulky objectives for some applications such as, for example, microscopy in remote locations or biopsy-free *in vivo* biomedical diagnostics of skin diseases. [1,2] However, the resolution of cellphone camera is far below the classical diffraction limit and it is determined by the finite size of the pixels. Previous works were mainly focused on fluorescent (FL) imaging [3-6] using compact microoptics designs where an external micro-lens was separated from the object which provided a modest magnification. The highest resolutions of cellphone imaging available in such systems are limited at ~1.5μm. [1, 2] At the same time, the biopsy-free *in vivo* diagnostics of skin diseases requires cellular-level resolution similar to tissue histology which is usually provided by standard (not cellphone-based) reflectance confocal microscopy [7].

In this work, we proposed a different approach to design of cellphone microscopes based on real imaging through contact ball lens with index of refraction close to 2 which provides up to ×50 magnification and resolution fundamentally limited due to diffraction of light. We demonstrate the proposed designs for imaging of melanoma samples. We also demonstrate label-free imaging of nanoplasmonic objects with a submicron resolution ~600 nm which is more than two-fold resolution improvement compared to previous studies of FL imaging by cellphones.

This work builds upon advantages of microspherical superlens nanoscopy developed in recent years for enhancing resolution of standard microscopes up to $\lambda/7$ level [8-15]. These advantages are based on using microspheres with

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diameters about several wavelengths which create magnified virtual images of objects with a participation of their optical near-fields. Such microspherical nanoscopy can be used for both label-free [8-15] and FL imaging [16-20] of biomedical and plasmonic nanostructures. The super-resolution ability of microspherical nanoscopy stems from the magnification of virtual images produced by microspheres evanescently coupled to the objects of studies. More detailed mechanisms are a subject of active theoretical investigation [21-23].

In this work, we propose to use significantly larger, millimeter-scale ball lenses with the properly designed index of refraction close to 2 which are capable of creating magnified real images. We show that these images can be observed directly by the cell phone cameras without using any additional microscope objectives, as schematically illustrated in Fig. 1 (b). Due to extremely high magnification of the real images up to ×50 offered by such contact ball lenses, the resolution of cellphone-based microscopy can be increased up to the diffraction limit. Potential applications of our ball-lens assisted smartphone microscopy include biopsy-free *in vivo* histology of skin.

2. EXPERIMENTAL RESULTS

The resolution of a conventional smartphone camera with CCD sensor is limited by the finite pixel size on the detector. An additional image magnification is required to improve the resolution of cellphone camera. We proposed to use a contact ball lens to introduce additional magnification. In the limit of geometrical optics, the lateral image magnification (M) through ball lens is determined by the formula [12,14]:

$$M(n', D, g) = \frac{n'}{2(n'-1)\left(\frac{2g}{D}+1\right)-n'}, \quad (1)$$

where D is the diameter of the ball lens, g is the gap between the ball lens and object, and $n' = n_{sp} / n_0$ is the refractive index contrast between the ball lens and object space. We propose to use ball lens made from a glass LASFN35 with a refractive index of $n = 2.02$ at $\lambda = 600$ nm which can provide a significant M when it is brought in contact with the object.

The schematic of our proposed smartphone microscopy is illustrated in Fig. 1(b). The illumination is provided by a white light source through fiber at an angle of 30° to normal. The ball lens made from LASFN35 glass is placed on top of the object and form a real image above the object. This image is captured by the smartphone camera. We acquired histological melanoma samples from the company Carolina [24]. The tissue sample was covered with a 200 μm coverslip which created unavoidable separation between the object and ball lens. Fig. 1(a) shows the image of melanoma sample taken by a standard upright microscope using 10x objective. The distribution of lymphocyte cells in melanoma can be observed due to darker spots. Fig.1(b) shows the image of melanoma sample taken by the proposed smartphone microscopy. It is seen that the quality of image obtained using smartphone is very close that that obtained by a standard microscope with 10× objective, but the field-of-view (FoV) is smaller. Imaging larger areas of the sample requires translation of the ball lens. Two such images obtained at different positions of the ball lens are represented in Fig. 1(b).

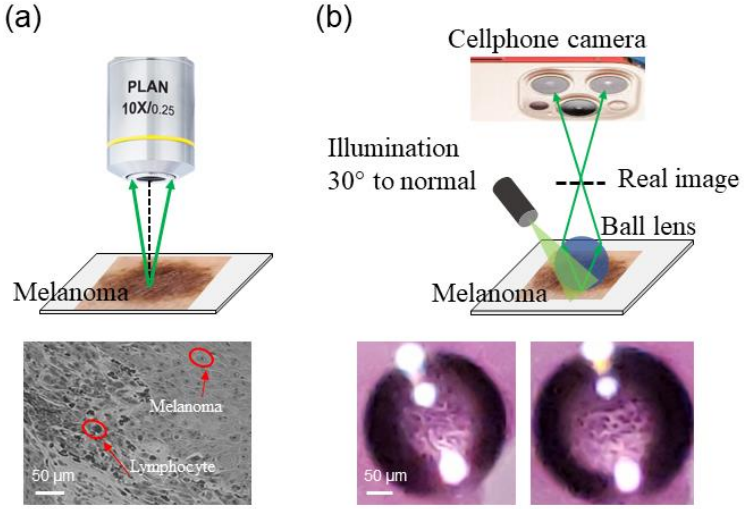

Figure 1. Imaging of melanoma tissue by (a) standard microscope with 10× objective and (b) proposed ball lens-assisted smartphone microscopy.

It should be noted that the magnification of the image in Fig. 1(b) is limited (M~7) due to the 200 μm separation between the histological tissue and ball lens. Eq. (1) shows that it would be significantly increased if the ball lens were in a contact with the tissue sample. Our following analysis allows predicting that the resulting resolution of the smartphone microscopy would be further increased under conditions of close contact with the sample. This indicates a potential application of the proposed smartphone microscopy for diagnoses of diseases such as melanoma by directly placing very compact smartphone attachment containing ball lens on top of the patient's skin and observing the distribution of lymphocyte cells.

The magnification and resolution of the proposed smartphone microscopy was quantified using a series of double-stripe Au arrays deposited on a sapphire substrate as illustrated in Fig. 2(a) (insert (ii)). The dispersion curve of LASFN35 glass in the visible range is also illustrated in Fig. 2(a) (insert (i)). It displays a marked index variation in the visible range that leads to a significant variation of M for different illumination wavelengths. Two Au stripes with width of 1.1 μm were separated with a gap of 0.64 μm. In these studies, the separation between the ball lens of object was absent and the LASFN35 ball lens with diameter of 2.0 mm was placed directly on top of the object. The narrow band pass (~10 nm) filters with the central wavelengths of 430nm, 480nm, 546nm, 589nm, and 632nm were used for illumination. In contrast to Fig. 1(b), the experiments were performed in a transmission geometry with the illumination provided from the opposite side of the sample. To provide a diffusive illumination we used a diffuser inserted sufficiently close to the sample from the illumination side. In our experimental setup, an easily measurable parameter was a distance (d) between the object and the real image obtained through the ball lens. It is simple to show that the magnification (M) of the ball lens is linearly proportional to d, as describe the following equation:

$$M = 2\frac{d}{D} - 1, \tag{2}$$

where D is the diameter of the ball lens. The corresponding dependence is represented by the dashed line in Fig. 2(a). Eq. (2) represents the basic geometrical properties of imaging. However, the absolute values of M determined experimentally by measuring d and using Eq. (2) are found to be approximately three times smaller than the M values which can be estimated for a given index of the lens (insert (i)) based on Eq. (1). The discrepancy with the predications of geometrical optics can be illustrated by the following example: the experimentally determined magnification at λ = 632nm was found to be ~50× whereas the geometrical magnification is expected to be close to 135×. This discrepancy can be explained by the fact that Eq. (1) is obtained in the paraxial approximation of geometrical optics which is not applicable to our experiment where non-paraxial rays, which tend to cross closer to the lens, play a significant role. In general, it should be also mentioned that in the case of microspheres with the diameters on the order of several wavelengths, there is another factor related to mesoscale size of microspheres which does not allow using geometrical optics, but the latter factor did not play a significant role in our case because of the millimeter-scale dimensions of our ball lenses.

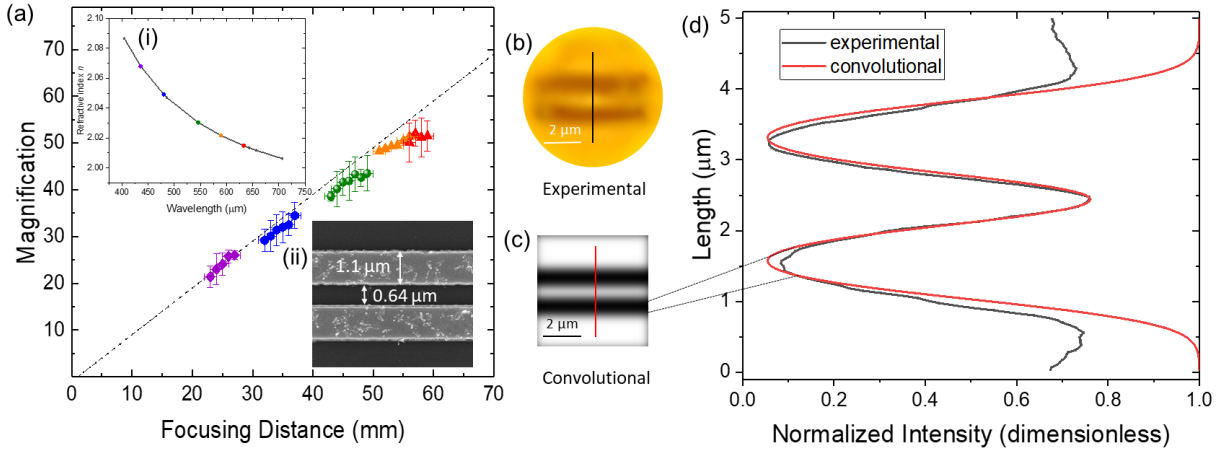

Figure 2 (a) Magnification (M) measured by the ball lens-assisted microscopy using 2.0 mm diameter LASFN35 in a broad range of visible wavelengths. The measurements were performed using a color filter set, as illustrated by points with different colors. Dashed line represents the linear dependence $M(d)$ described by Eq. (2). Insert (i) illustrates a dispersion curve of LASFN35 glass in the visible regime. Insert (ii) illustrates the SEM image of Au double-stripe array used as an object. (b) Image of the double-stripe structure through contact ball lens taken by the cellphone camera at λ = 589 nm. (c) Convolution of the "perfect" drawn double-stripe object with the Gaussian point-spread function with FWHM = 663nm. (d) Comparison of experimental and calculated intensity profiles illustrating good agreement that indicates that according to the Houston resolution criterion the resolution of system is close to 663 nm.

The resolution of our ball lens-assisted smartphone microscopy was quantified based on a Houston resolution criterion according to previously developed procedure [12-14]. Some preliminary results of resolution quantification in this case were presented in Ref. [25]. The "perfect" drawn image of our double stripe object was convoluted with a two-dimensional (2-D) Gaussian point-spread function (PSF). A series of such images was calculated for a various full width at half maximum (FWHM) of the Gaussian PSF. We determined the resolution of our system as FWHM of the Gaussian PSF providing the best fit for the experimental intensity profiles of our images where the stripes manifest themselves as dips, as it is illustrated in Fig. 2(d). More than two-fold better resolution for the ball lens-assisted microscopy in our case compared to previous studies [1, 2] is explained by significantly larger magnifications achieved due to use of high-index ball lenses with index of refraction sufficiently close to 2 under conditions of close contact with the object.

3. CONCLUSIONS

The resolution of cellphone camera with CCD sensor is limited by the finite size of detecting pixels. To transform the cell phone into a powerful microscope, we need to significantly increase the magnification. Previous attempts to use microoptics solutions showed some promising results, but the resolution was still limited at ~1.5 μm level [1, 2]. Dramatically larger magnifications are required, and in this work, we offered a solution to this problem based on using ball lenses with index of refraction sufficiently close to 2 placed in contact with the objects. We demonstrated the giant magnification values ($M \sim 50$) available in such cases. We also showed that is such cases pixilation is not an issue any more, and the resolution becomes to be fundamentally limited by the diffraction of light leading to resolution values ~ λ in our case. The resolution was quantified using rigorous resolution criteria, and it shows that the effective numerical aperture (NA) of our ball lens-assisted smartphone microscope is close to 0.5. The increase of the resolution comes at a prize of FoV. Developing a practical system which can be used for biopsy-free *in vivo* histology of skin or other biomedical applications will require developing a system for translating the ball lenses. It is shown a principal possibility to develop instruments for melanoma diagnostics based on the proposed lens-assisted smartphone microscopy. Currently, the most used ancillary optical imaging tool used by dermatologists are dermatoscopes, but they have a limited resolution. More advanced tools for noninvasive imaging of skin cancers include reflectance confocal microscopes which are bulky, heavy, and expensive instruments. This area represents one example where lightweight and compact ball lens-assisted smartphone microscope proposed in this work can find applications in future.

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