Highly sensitive lens-free fluorescence imaging device enabled by a complementary combination of interference and absorption filters

Kiyotaka Sasagawa,* Ayaka Kimura, Makito Haruta, Toshihiko Noda, Takashi Tokuda, and Jun Ohta

Division of Materials Science, Graduate School of Science and Technology, Nara Institute of Science and Technology, 8916-5 Takayama, Ikoma, Nara, Japan
*sasagawa@ms.naist.jp

Abstract: We report a lens-free fluorescence imaging device using a composite filter composed of an interference filter and an absorption filter, each applied to one side of a fiber optic plate (FOP). The transmission of angled excitation light through the interference filter is absorbed by the absorption filter. The auto-fluorescence of the absorption filter is reduced by the reflection from the interference filter of normally incident excitation light. As a result, high-performance rejection of excitation light is achieved in a lens-free device. The FOP provides a flat, hard imaging device surface that does not degrade the spatial resolution. We demonstrate excitation rejection of approximately $10^8$:1 at a wavelength of 450 nm in a fabricated lens-free device. The resolution of fluorescence imaging is approximately 12 $\mu$m. Time-lapse imaging of cells containing green fluorescent protein was performed in a 5-$\mu$m thin-film chamber. The small dimensions of the device allow observation of cell culturing in a CO$_2$ incubator. We also demonstrate that the proposed lens-free filter is compatible with super-resolution bright-field imaging techniques. These features open a way to develop a high-performance, dual-mode, lens-free imaging device that is expected to be a powerful tool for many applications, such as imaging of labeled cells and point-of-care assay.

© 2018 Optical Society of America under the terms of the OSA Open Access Publishing Agreement

1. Introduction

Lens-free imaging is an imaging technique that does not use any lenses [1]. The lenses in a lens-based wide-area microscope generally lead to a large size and high cost. Thus, a lens-free approach makes it feasible to build a wide-area imaging system with small dimensions. Such a system is suitable for observation of a large number of cells and performance of various biomarker assays. To date, various groups have reported lens-free imaging systems. Especially, many reports address bright-field imaging research [1–9]. By combining variations in illumination conditions and image processing techniques, wide-field (several tens of square millimeters) of view and high spatial resolution (<1 $\mu$m) can be achieved simultaneously. A small imager enables its placement in an incubator, allowing long-term observation of cultured cells [5,10,11]. For fluorescence imaging, few studies have been done. However, fluorescent labeling is a very important technique for observing biological reactions in cells [12] and microarrays [13,14]. To overcome this problem, the performance of fluorescence imaging sensitivity needs to improve. As a way to avoid poor emission filter performance, bioluminescence or chemiluminescence devices have been studied [15–17]. However, the luminescence intensity is typically weak, so the image sensor must have very high sensitivity.

One of the difficulties in lens-free fluorescence imaging is insufficient performance of the emission filter [18–20], which is typically an interference filter or an absorption filter. They work well in a fluorescence microscope system with lenses. However, both of them have intensity and
sensitivity problems when used in lens-free fluorescence imaging systems. This was pointed out in a review article by Greenbaum et al. in 2012 [21].

In this paper, we propose a novel emission filter structure that can overcome the sensitivity problems. The filter is a combination of an interference filter and an absorption filter connected and supported by a fiber optic plate (FOP) and placed directly above an image sensor (Figs. 1(a) and (b)). This works well as a lens-free optical system and shows an excitation rejection ratio of approximately 10^8:1 at 450 nm. The proposed filter shows dramatically improved fluorescence imaging performance. Also, because this filter structure does not use any prism, it has good compatibility with bright-field lens-free imaging techniques. Using a fabricated lens-free imaging device (Fig. 1(c)), we demonstrate time-lapse imaging during cell culturing.

2. Problems of conventional emission filters in lens-free imaging

In fluorescence imaging, an interference (or dielectric multi-layer) filter or an absorption filter is used as an emission filter. The filter rejects excitation light of a specific wavelength and transmits fluorescence. However, these filters have the problems described below.

2.1. Interference filter

Interference filters are widely used in fluorescence microscopes. They are made by alternately stacking dielectrics with different refractive indices in multiple layers. This structure forms a rejection band at a specific wavelength. Precisely fabricated interference filters provide a rejection ratio higher than 10^8:1 at a specific wavelength. Lens-free imaging systems using this type of filter were reported by some groups [18, 22–24].

Important advantages of an interference filter are

- Incident light in the rejection band is reflected. The lack of absorption in this band means
Fig. 2. (a,d) Schematic and photograph of scattered light transmission through an interference filter. Due to a spectrum shift, light incident at an angle is transmitted. Silica beads are used as a diffuser. The laser wavelength in the experiment was 473 nm. The filter was a pass filter 510 nm long. (b,e) Schematic and photograph of an absorption filter. The yellow fluorescence spot in (e) shows how auto-fluorescence from the absorption filter results in effective transmission through it. (c,f) Schematic and photograph of the proposed hybrid filter. The auto-fluorescence of the absorption filter is reduced because the normally incident component is reflected by the interference filter.

- A very steep transmission/rejection band edge can be formed.

The main disadvantage is

- The transmission spectrum is shifted by the incident angle.

Fig. 2(a) shows the schematic of the spectrum shifting problem. An observation target, such as cells, microbeads, or droplets, usually has a refractive index different from that of the surrounding media and scatters incident light a little. As a result, the interference filter transmits the angled component. Note that the scattered light intensity is stronger than the target fluorescence in many cases, such as when imaging green fluorescent protein (GFP) in cells. Fig. 2(d) shows a simple demonstration of 510-nm-long pass interference filter transmission of focused incident laser light at 473 nm on a diffuser made of a layer of 1.5-μm silica microbeads (sicastar 40-00-153; Partikeltechnologie GmbH, Germany) on a cover glass. The round shadow observed on the screen means that highly angled light is transmitted through the filter.

If the absorption and emission peaks are not close, this effect is not significant [25]. However, for example, in the case of imaging cells containing GFP, the peaks are close to each other. Thus, blue excitation light is scattered by cells and transmits through the filter even if the incident light
is normal to the filter.

Such a problem can be solved in lens-based fluorescence microscope systems [26–28]. Scattered light from observation targets is almost collimated by its objective lens and transmitted normally to the interference emission filter. Thus, the interference filter can work with its appropriate condition and reject the excitation light with very high performance. However, a lens makes the imaging device bulky and limits the field of view.

The rejection ratio of an interference filter can be improved by increasing the number of dielectric layers. However, the blue shift with increasing incident angle is basically determined by the effective refractive index of the filter. Thus, the problem discussed above cannot be solved even if the number of layers is high.

2.2. Absorption filter

Absorption filters use dyes or pigments to absorb light at specific wavelengths. Their important features are

- The transmission spectrum is independent of the incident angle of light.
- Almost all the light energy is absorbed and not reflected.

Due to the angle independence, absorption filters are useful for lens-free fluorescence imaging [10,19,20,29–38]. Transmittance is ideally given by

\[ T = \frac{I}{I_0} = \exp(-\alpha L) \]  

where \( T \) is transmittance, \( I \) is transmitted light power, \( I_0 \) is incident light power, \( \alpha \) is an absorption coefficient, and \( L \) is the effective light path length (filter thickness in this case). This equation shows that transmittance decreases exponentially with the thickness of the filter. However, in a real situation, especially in lens-free devices, the result deviates from the equation with regard to thickness. As mentioned above, absorption filters absorb incident light. The absorbed energy is basically emitted by a non-radiative process. However, some of the energy is emitted as fluorescent light. The schematic is shown in Fig. 2(b).

This problem can be easily demonstrated by irradiating intense light onto an absorption filter, as shown in Fig. 2(e). A yellow spot can be observed on the area where the laser beam is focused. This spot is unwanted auto-fluorescence, and it is very significant in lens-free systems. In a lens-based system, the artifact of auto-fluorescence can be reduced by placing the filter out of focus. However, the auto-fluorescence is captured before transmission in a lens-free system because the filter is placed on an image sensor. Auto-fluorescence light is out of the absorption band of the filter. If it is the dominant component of light transmitted through the filter, the rejection performance cannot be improved further by increasing the thickness. Thus, there is a thickness that produces an optimal ratio of fluorescence and excitation light.

3. Hybrid filter: high-performance emission filter for lens-free fluorescence imaging

In this study, we propose a hybrid filter based on the complementary combination of an interference and absorption filter via an FOP. A schematic of the filter structure is shown in Fig. 2(c). The fabrication method is described in Section 4.1. This device overcomes both of the problems discussed above. The photograph (Fig. 2(f)) shows that both excitation light transmission and fluorescence emission are extremely low in this filter. This excitation reduction strategy of this hybrid filter is similar to the prism-based system developed by Coskun et al. [39]. One of the advantages of the proposed structure is that its flat surface allows the use of a simple and small excitation light system. Also, the transmission spectrum can be customized for specific targets by using an interference filter.
An interference filter does not absorb light, but rather reflects it in the rejection band. Thus, it emits almost no auto-fluorescence even when high-power excitation light is incident upon it. Scattered light from observation targets can pass through the filter. This transmitted component is absorbed by an absorption filter. Because its intensity is much lower than that of the directly incident light, the auto-fluorescence intensity of the absorption filter is greatly reduced. As a result, the interference and absorption filters compensate their functions and provide ultra-high selectivity for excitation light and fluorescence.

4. Materials and methods

4.1. Imaging device fabrication

1. A cover glass (24 mm × 24 mm) was coated with the amorphous fluoropolymer CYTOP-M (AGC Chemicals, Japan) by spin coating (Fig. 3(a)). After preparation was complete (steps 1-3), this film could be easily peeled off to form a yellow filter.

2. Valifast yellow 3150 (Orient Chemical, Japan), cyclopentanone (Wako, Japan), and NOA63 (Norland Products, USA) were mixed at a weight ratio of 1:1:1. Surfactant (R-41; DIC, Japan) was added at approximately 1% by weight. The mixture was applied to the CYTOP layer as a yellow filter (Fig. 3(b)). The coated film was cured by UV irradiation and heating to 100°C for 15 min and 150°C for 30 min.

3. An FOP (J5734, Hamamatsu Photonics, Japan) was bonded to the yellow filter with epoxy resin (Z-1; Nissin resin, Japan) (Fig. 3(c)). An interference long-pass filter (cut-on wavelength of 510 nm) was deposited on the FOP beforehand. The FOP surface without the interference filter faced the yellow absorption filter. The epoxy was cured at room temperature for 24 h.

4. The FOP was removed from the cover glass mechanically (Fig. 3(d)). Only the yellow filter was transferred to the FOP. This assembly is the hybrid filter.

5. The hybrid filter was bonded to an image sensor with epoxy resin (Z-1; Nissin Resin, Japan) (Fig. 3(e)). The specifications of the image sensor are shown in Table 1.

| Table 1. Specifications of image sensor |
|----------------------------------------|
| Technology | 0.35-µm 2-poly 4-metal standard CMOS |
| Supply voltage | 3.3 V |
| Chip size | 1.05 mm × 2.7 mm |
| Pixel number | 120 × 268 |
| Pixel size | 7.5 µm × 7.5 µm |
| Pixel type | 3-transistor active pixel sensor |
| Photodiode | N-well/P-substrate |
| Fill factor | 44% |
| Color filter | none |
| Microlens | none |
Fig. 3. Fabrication process for imaging device with hybrid filter. (a) A cover glass was coated with CYTOP. (b) The CYTOP layer was coated with a yellow absorption mixture. (c) An FOP with interference filter was glued onto the absorption filter. (d) The FOP was removed, and the absorption filter was transferred. (e) The FOP with hybrid filter was glued onto the image sensor.

4.2. Fabrication of an ultra-thin film chamber

1. CYTOP-S (CTX-809SP2, AGC Chemicals, Japan) was spin-coated onto a silicon wafer and dried and cured at room temperature.

2. The CYTOP-S film was cut with a knife and peeled off the Si wafer.

3. CYTOP-M (CTL-809M, AGC Chemicals, Japan) was applied as adhesive on the rim of a cloning cylinder (9.5-mm internal diameter × 11-mm length, F37847-0000; AS-ONE, Japan), and the CYTOP-S film was stretched over the top.

4. The CYTOP-M was cured at room temperature for 24 h. The fabricated device is shown in Fig. 4.

Fig. 4. Photograph of a cell culture chamber with a bottom of 5-μm-thick fluorocarbon film (CYTOP-S). The chamber is upside-down in the image. Scale bar is 10 mm.
Fig. 5. Bright-field image examples for each image processing step. The HEK293 cells cultured in an ultra-thin film chamber were observed. (a) Raw image. (b) Image with background subtracted. (c) Image with low-frequency spatial components subtracted. (d) Super-resolved image reconstructed from four images obtained with different illumination angles. Scale bar is 300 µm.

4.3. Cell culturing
HEK293 cells were first cultured in Dulbecco’s Modified Eagle’s Medium – low glucose (DMEM, D5523-10X1L; Sigma-Aldrich, USA) supplemented with fetal bovine serum (CELLect Gold, Canadian Origin M4235; Thermo Fisher Scientific, USA). Gentamicin (G1272-10ML, Sigma- Aldrich, USA) was introduced into the culture chambers and maintained in a 5% CO₂ humidified atmosphere at 37°C. Next, the cells were harvested with trypsin (0.5% Trypsin-EDTA (10×), no phenol red, 15400054; Thermo Fisher Scientific, USA) and resuspended in DMEM. Finally, the cells were seeded onto the ultra-thin bottom chamber.

4.4. Image processing for bright imaging
In bright-field imaging, a high-resolution image can be obtained by using highly directional light, even if the observation target is a little distant from the image sensor surface. Thus, a super-resolution image can be reconstructed by combining images obtained with different illumination angles [5]. We demonstrated that our proposed filter is compatible with such a technique.

The light source was an array of micro-LEDs (ES-CEGHM12A, Epistar, Taiwan) placed approximately 4 cm above the sensor device. Four LEDs emitted light sequentially to change the illumination direction. The frame rate was 2 frames per second, and averages of 2 frames were used. The raw images had low contrast, and the cells were difficult to observe. To increase the resolution, the following image processing procedure was used:

1. A background image was generated by averaging all the obtained frames. Intensity fluctuations between frames were also reduced by normalizing the average value of each frame. The resulting background image was subtracted from the obtained image to reduce the moiré pattern due to the FOP and pixel array of the image sensor (Fig. 5(b)).

2. An image of low-frequency spatial components was generated using an 8 × 8 median filter. The difference between the original and filtered images was obtained (Fig. 5(c)). Thus, the
Fig. 6. (a) Comparison of normalized transmittance of emission filters as a function of absorption filter thickness. The curves show transmission in an absorption filter under the FOP (grey line), an absorption filter on the FOP (orange line), and a hybrid filter (red line). The light source was a 450-nm laser diode (PL450B; Osram, Germany). The spontaneous emission component from the laser diode was reduced by a bandpass filter (MF445-45; Thorlabs, USA). The laser spot patterns observed by an image sensor at points (i) and (ii) are shown in (b) and (c), respectively.

illumination uniformity is reduced and high-frequency spatial components, such as cells, are emphasized.

3. The pixel data from four images were arranged based on the incident angle of the light source and the shadow on the imaging device. Fig. 5(d) is an example of the obtained super-resolution image.

5. Results

5.1. Comparison of effective transmittance

Fig. 6(a) compares the effective transmittance of lens-free imaging devices using various emission filters. A laser diode with wavelength of 450 nm was used as a light source, and it was normally incident on the filter. Orange diamonds show the results for absorption filters comprising yellow dye (Valifast Yellow 3150; Orient Chemical, Japan) and UV curable adhesive (NOA63, Norland Products, USA). This filter has high absorption characteristics for blue light. At thicknesses less than 5 µm, the effective transmittance decreased exponentially with the thickness. However, the decrease was smaller for thicker filters. Gray circles show the result with a 25.4-mm-thick FOP (J5734, Hamamatsu Photonics, Japan) on the absorption filter. The result is worse because the fluorescence of the FOP was added. However, in a practical experiment, the FOP is needed as a cover for the absorption filter to prevent damage from scratches without reducing the spatial resolution.

Figs. 6(b, c) show beam spots observed by the image sensor during the measurement. In this experiment, the light source was a laser. Thus, an interference fringe pattern of the transmitted laser (Fig. 6(b)) is observed for the thin filter at point (i) in Fig. 6(a). With a thicker filter, at point (ii), the fringe disappears. This indicates that the coherence is lost because auto-fluorescence from the filter and FOP is dominant.

The proposed hybrid filter limits the auto-fluorescence, as indicated by the curve with red circles. A long-pass interference filter with a cut-off wavelength of 510 nm is directly deposited.
Fig. 7. Fluorescence images of sample green filter pattern obtained by: (a) fluorescence microscope with GFP filter, (b) lens-free device with an interference filter, (c) lens-free device with an absorption filter under the FOP, and (d) lens-free device with hybrid filter. The scale bar is 100 µm.

on an FOP (Tac Coat, Japan). The proposed filter shows a transmittance of approximately $10^{-8}$. This is almost a 1000-fold improvement against the absorption filter under the FOP.

5.2. Imaging of the green fluorescent film pattern

To verify the performance of the proposed filter, results of an imaging comparison test are shown in Fig. 7. A green resist film coating on a cover glass was used as an imaging sample. Green resist is usually used for color image sensors, and such green filters have been sometimes used as an emission filter [32]. However, the filter we used emits fluorescence that cannot be neglected. In this experiment, we used the green filter as a fluorescence sample. To avoid reduction of spatial resolution, the resist-coated side faced the imaging device. The excitation light source was a high-power blue LED (LZ1-10B202-0000, LED Engin, USA) with an excitation filter (FF01-452/45-25, Semrock, USA).

Fig. 7(a) is an image obtained with a fluorescence microscope with a filter set for GFP. A part of the green resist coating was removed by laser ablation, and the letter “N” was patterned with the resist. Due to fluorescence, the “N” is clearly observed.

Fig. 7(b) is the image by a device with an interference filter. The “N” pattern is darker than the background. This is because the optical density of the filter is about 3, and it could be improved by increasing the number of layers. However, the significant problem is that the edges of the pattern are bright. This originates from scattered components transmitted through the interference filter, and it cannot be improved.

Fig. 7(c) shows the image by a device with an absorption filter. As discussed above, the transmission spectrum of the absorption filter has almost no dependence on incident angle. Thus, the bright-edge artifact seen in Fig. 7(b) is not observed. However, image contrast is low due to a background offset that comes from auto-fluorescence of the absorption filter and the FOP. In some cases, this effect is resolved by enhancing the contrast. However, a high background signal increases photon shot noise, which is proportional to the square root of the signal. When the fluorescence of the observation target is weak, it significantly reduces the signal-to-noise ratio.

Fig. 7(d) shows the image obtained using the hybrid filter. In comparison with the absorption filter, contrast is improved greatly and the “N” can be clearly observed. This result shows that the proposed structure successfully reduces the auto-fluorescence.

5.3. Imaging of fluorescent beads

To investigate the imaging performance for small particles, fluorescent beads were observed. In this experiment, we used a device where one half of the imager had an absorption filter and the other half had a hybrid filter. With this device, filter performance under the same conditions can
be compared directly. Yellow fluorescent beads (FP-4052-2; Spherotech, USA) were dispersed in water and spilled onto the device. The diameters of the beads were 2.5-4.5 µm. These values are smaller than the pixel size of the image sensor used in the device. The light source was the 450-nm laser diode.

Fig. 8 shows the obtained fluorescence image. A movie is also provided in the supplemental information (Visualization 1). As for the green resist pattern imaging, the result shows that the background component is reduced by the proposed hybrid filter. Also, the intensity of the fluorescence signal from the beads is increased because the interference filter reflects the incident excitation light, so the irradiation power to the beads was almost doubled.

We estimated the spatial resolution from the profiles of the beads on the hybrid filter area. The profile data were extracted from the image under dry conditions to evaluate the resolution of beads on the surface of the device. An example of the results is shown in Fig. 9. The average full-width at half-maximum value was 12.0 µm and the standard deviation was 0.59 µm.

5.4. Time-lapse imaging of living cells

One of the advantages of the lens-free imaging system is its inherently small size. To make use of this feature, we performed time-lapse imaging of living cells. For this experiment, we prepared a cell culture chamber with a bottom of 5-µm-thick fluorocarbon film. HEK293 cells partially transfected with GFP were cultured on the bottom film and time-lapse imaging at 10-min intervals was performed for 22 h. During the culturing, the imaging device was kept in a CO₂ incubator.
Fig. 9. (a) Image used for image sensor spatial resolution evaluation where yellow microbeads were dispersed on the image sensor. The image was obtained under dry conditions. Thus, there is no space between the microbeads and filter. (b1)-(b10) Gaussian fitting results of normalized intensity profiles for the fluorescent beads identified by numbers in (a). We chose microbeads that were separated from the others.
Fig. 10. Time-lapse imaging of HEK293 cells partially transfected by GFP. These images were obtained with the proposed lens-free fluorescence imaging device. The top row presents bright-field images, the middle row presents fluorescence images, and the bottom row merges the images from the top two rows. The bright-field image was composed from four different images with different incident angles of illumination. The frame time was 1 sec for each bright-field image and 2 sec for each fluorescence image. The interval time was 10 min. The scale bar is 300 µm. A movie of these images is provided as Visualization 2.

Fig. 11. Comparison of magnified fluorescent cell images obtained with (a) a lens-based fluorescence microscope and (b) the proposed device. The scale bar is 100 µm.
In this experiment, not only fluorescence images but also bright-field images were obtained. The illumination source for bright-field imaging was green micro-LEDs with a center wavelength of 533 nm (ES-CEGH12A; Epistar, Taiwan), at which the filters are transparent. Four LEDs placed at different positions were used as light sources. The distance from the imaging device to the LED was approximately 4 cm. The LEDs were turned on sequentially and the obtained images were combined to create a super-resolution image. The procedure for image processing is described in Section 4.4. The principle of resolution improvement is the same as that in a previously reported method [5]. Thus, our filter is compatible with at least one super-resolution technique for bright-field imaging.

The imaging results are shown in Fig. 10 and Visualization 2. The cells moved and grew during imaging. By comparing the bright-field and fluorescence images, it can be observed the GFP was partially introduced into the cells. Also, the result shows that the intensity of fluorescence from some cells increased gradually. The magnified images obtained with a conventional fluorescence microscope and the proposed device are shown in Fig. 11. The resolution of the fluorescence image is less than 10 µm. However, the shape of the cells can be observed. The bright-field image shows all cells on the imaging area with higher resolution.

6. Discussion

Lens-free imaging techniques realize a wide field of view with a small device. The proposed hybrid filter technique provides a high-performance emission filter without the other disadvantages of a lens-free system. The previously reported lens-free or miniature fluorescence imaging devices used a prism [33, 39–42] or objective lens for high-performance fluorescence imaging. However, a prism or lens makes the system larger. The proposed filter resolves this problem and makes the device simple and thin.

The proposed technique is capable of building a very small device with wide field of view that can be placed in an incubator, as demonstrated in the results section. Incubators are necessary for studying living cells, and almost every biology laboratory has at least one. Our device is small enough to be placed within an incubator. Also, the small size is suitable for point-of-care (bedside testing) devices based on molecular assay using fluorescence imaging [13, 14].

6.1. Limitation of excitation light rejection

From the result shown in Fig. 6, the transmittance of the hybrid filter at the excitation light wavelength achieved almost 10⁻⁸. This value is as high as a high-performance interference filter against a designed incident angle. In this experiment, light density of a few watts per square centimeter was transmitted to the filter. At such a high intensity, photobleaching of fluorophores or phototoxicity to cells can become a problem. Also, fluorescence from other components, such as the chamber or medium, is possible.

In this study, we used a yellow dye with very low fluorescence for the absorption filter. Thus, the fluorescence from the FOP limits the effective transmittance. However, the FOP is necessary to protect the device surface because the absorption filter is very soft and easily scratched. Another problem is difficulty of directly fabricating a high-quality interference filter on the polymer-based absorption filter. The FOP is sufficiently hard and stable because it is based on glass materials. Also, almost all the image sensors have wiring on the same side as the pixel array. The FOP is useful for making the focal plane higher than the wires without degrading the spatial resolution. By using an FOP, it is feasible to mount the hybrid filter on a commercially available image sensor.

6.2. Limitation of spatial resolution

The spatial resolution of fluorescence imaging was approximately 10 µm for fluorescent beads spread on the hybrid filter. To observe fluorescence of nuclei, membranes, or other fine structures
of living cells, higher resolution is required.

Lens-free imaging systems based on holographic microscopy realize sub-micrometer spatial resolution. However, this technique can be applied only to bright-field imaging because it uses the phase information of light scattered by observation targets. Such information is lost when excitation light is converted to fluorescence. Thus, the spatial resolution of fluorescence images taken by the proposed device is determined by the effective distance between the target and image sensor and the effective numerical aperture of the imager. We used a 5-µm-thick fluorocarbon film on the chamber bottom in the cell imaging experiment. However, further improvement is not practical because thin films are easily damaged and very difficult to handle.

Another way to improve spatial resolution is to use a patterned excitation light [10,26,43]. The proposed hybrid filter has high rejection performance for normally incident excitation light. This feature is suitable for patterned light illumination with a micro-lens array. Also, as demonstrated in the cell imaging experiment, super-resolution techniques for bright-field lens-free imaging can be also applied. Although the resolutions of fluorescence images are limited to the cell level, the details of the cell shape can be observed by the high-resolution bright-field mode. However, bright-field images of the present device are slightly distorted by the FOP. The limitation of spatial resolution would be worse than that of the lens-free devices without an FOP.

6.3. Device dimensions and imaging area

The imaging area of the present device is 0.9 mm × 2.0 mm. The dimensions of the imaging head are 30 mm × 30 mm × 55 mm. The peripheral equipment includes a desktop PC and a controller, which is a printed circuit board measuring 300 mm × 220 mm. The dimensions of the power source used for LED emission are 104 mm × 147 mm × 186 mm. Thus, only the head device can be placed in a CO\(_2\) incubator. The computer and controller can be reduced to palm size by using a small computer. Also, the power source can be miniaturized by combining USB power and a digital-to-analog converter integrated circuit.

The proposed filter structure is made by techniques that can easily be scaled to larger areas. In this study, we used a small image sensor designed by our lab [44–46]. By using a sensor with larger area, the imaging area can be increased.

The light source for uniform illumination has very simple optics composed of an LED, a collimating lens, and an excitation filter. Thus, the illumination area is also easy to increase. However, a complicated optics and scanning system is required if a patterned illumination is used to achieve fluorescence imaging with higher spatial resolution. In that case, the device size may be determined by the light source component.

7. Conclusions

We demonstrated a hybrid emission filter with an excitation light rejection ratio of 10\(^8\):1 for a lens-free imaging device. Complementary combination of interference and absorption filters via an FOP compensates for the shortcomings of each filter. Thus, the performance of the emission filter was drastically improved in the lens-free setup. The spatial resolution with uniform excitation light illumination was 12 µm. The cell imaging result showed that individual cells can be identified from the fluorescence images. The proposed emission filter is compatible with super-resolution lens-free imaging techniques [1,2,5]. This makes it feasible to see the details of an observation target in bright-field mode. We suggest that our new emission filter foreshadows the development of a lens-free fluorescence microscope with small dimensions and wide field of view.
Funding

Murata Foundation; Japan Society for the Promotion of Science KAKENHI (18H03519); Japan Science and Technology Agency, Core Research for Evolutional Science and Technology; VLSI Design and Education Center, the University of Tokyo, in collaboration with Cadence Design Systems, Inc.

References

1. A. Ozcan and E. McLeod, “Lensless imaging and sensing,” Annu. Rev. Biomed. Eng. 18, 77–102 (2016).
2. S. Seo, T.-W. Su, D. K. Tseng, A. Erlinger, and A. Ozcan, “Lensfree holographic imaging for on-chip cytometry and diagnostics,” Lab on a Chip 9, 777–787 (2009).
3. O. Mudanyali, D. Tseng, C. Oh, S. O. Isikman, I. Sencan, W. Bishara, C. Oztoprak, S. Seo, B. Khademhosseini, and A. Ozcan, “Compact, light-weight and cost-effective microscope based on lensless incoherent holography for telemedicine applications,” Lab on a Chip 10, 1417–1428 (2010).
4. U. A. Gurkan, S. Moon, H. Heckel, F. Xu, S. Wang, T. J. Lu, and U. Demirci, “Miniaturized lensless imaging systems for cell and microorganism visualization in point-of-care testing,” Biotechnol. J. 6, 138–149 (2011).
5. G. Zheng, S. A. Lee, Y. Atefi, M. B. Elowitz, and C. Yang, “The ePetri dish, an on-chip cell imaging platform based on subpixel perspective sweeping microscopy (spsm),” Proc. Natl. Acad. Sci. 108, 16889–16894 (2011).
6. O. Mudanyali, W. Bishara, and A. Ozcan, “Lensfree super-resolution holographic microscopy using wetting films on a chip,” Opt. Express 19, 17378–17389 (2011).
7. Z. Göröcs and A. Ozcan, “On-chip biomedical imaging,” IEEE Rev. Biomed. Eng. 6, 29–46 (2013).
8. R. Stahl, G. Vannmeerebeek, G. Lafruit, R. Huys, V. Reumers, A. Lambrechts, C.-K. Liao, C.-C. Hsiao, M. Yashiro, M. Takemoto, T. Nagata, S. Gomi, K. Hatabayashi, Y. Ohshima, S. Ozaki, N. Nishishita, and S. Kawamata, “Lens-free digital in-line holographic imaging for wide field-of-view, high-resolution and real-time monitoring of complex microscopic objects,” in Imaging, Manipulation, and Analysis of Biomolecules, Cells, and Tissues XII, vol. 8947 (International Society for Optics and Photonics, 2014), p. 89471F.
9. D. Vercruysse, A. Dusa, R. Stahl, G. Vannmeerebeek, K. de Wijis, C. Liu, D. Prodanov, P. Peumans, and L. Lagae, “Three-part differential of unlabeled leukocytes with a compact lens-free imaging flow cytometer,” Lab on a Chip 15, 1123–1132 (2015).
10. C. Han, S. Pang, D. V. Bower, P. Yiu, and C. Yang, “Wide field-of-view on-chip Talbot fluorescence microscopy for longitudinal cell culture monitoring from within the incubator,” Anal. Chem. 85, 2356–2360 (2013).
11. D. Jin, D. Wong, J. Li, Z. Luo, Y. Guo, B. Liu, Q. Wu, C.-M. Ho, and P. Fei, “Compact wireless microscope for in-situ time course study of large scale cell dynamics within an incubator,” Sci. Rep. 5, 18483 (2015).
12. M. Chalfie, Y. Tu, G. Euskirchen, W. W. Ward, and D. C. Prasher, “Green fluorescent protein as a marker for gene expression,” Science 263, 802–805 (1994).
13. D. M. Rissin, C. W. Kan, T. G. Campbell, S. C. Howes, D. R. Fournier, L. Song, T. Piech, P. P. Patel, L. Chang, A. J. Rivnak et al., “Single-molecule enzyme-linked immunosorbent assay detects serum proteins at subfemtomolar concentrations,” Nat. Biotechnol. 28, 595 (2010).
14. S. H. Kim, S. Iwai, S. Araki, S. Sakakihara, R. Iino, and H. Noji, “Large-scale femtoliter droplet array for digital counting of single biomolecules,” Lab on a Chip 12, 4986–4991 (2012).
15. H. Eltoukhy, K. Salama, and A. E. Gamal, “A 0.18-/spl mu/m CMOS bioluminescence detection lab-on-chip,” IEEE J. Solid-State Circuits 41, 651–662 (2006).
16. A. Roda and M. Guardigli, “Analytical chemiluminescence and bioluminescence: latest achievements and new horizons,” Anal. bioanalytical chemistry 402, 69–76 (2012).
17. A. Roda, M. Mirasoli, E. Michelin, M. Di Fusco, M. Zangheri, L. Cevenini, B. Roda, and P. Simoni, “Progress in chemical luminescence-based biosensors: a critical review,” Biosens. Bioelectron. 76, 164–176 (2016).
18. K. Sasagawa, K. Ando, T. Kobayashi, T. Noda, T. Tokuda, S. H. Kim, R. Iino, H. Noji, and J. Ohta, “Complementary metal–oxide–semiconductor image sensor with microchannel array for fluorescent bead counting,” Jpn. J. Appl. Phys. 51, 02BL01 (2012).
19. H. Takehara, K. Miyazaki, T. Noda, K. Sasagawa, T. Tokuda, S. H. Kim, R. Iino, H. Noji, and J. Ohta, “A CMOS image sensor with stacked photodiodes for lensless observation system of digital enzyme-linked immunosorbent assay,” Jpn. J. Appl. Phys. 53, 04EL02 (2014).
20. H. Takehara, M. Nagasaki, K. Sasagawa, H. Takehara, T. Noda, T. Tokuda, and J. Ohta, “Micro-light-pipe array with an excitation attenuation filter for lensless digital enzyme-linked immunosorbent assay,” Jpn. J. Appl. Phys. 55, 03DF03 (2016).
21. A. Greenbaum, W. Luo, T.-W. Su, Z. Göröcs, L. Xue, S. O. Isikman, A. F. Coskun, O. Mudanyali, and A. Ozcan, “Imaging without lenses: achievements and remaining challenges of wide-field on-chip microscopy,” Nat. Methods 9, 889 (2012).
22. R. R. Singh, D. Ho, A. Niclei, G. Gulak, P. Yau, and R. Genov, “A CMOS/thin-film fluorescence contact imaging microsystem for DNA analysis,” IEEE Transactions on Circuits Syst. I: Regul. Pap. 57, 1029–1038 (2010).
23. D. Ho, M. O. Noor, U. J. Krull, G. Gulak, and R. Genov, “CMos spectrally-multiplexed fret-on-a-chip for DNA analysis,” IEEE Transactions on Biomed. Circuits Syst. 7, 643–654 (2013).
24. A. Hassibi, R. Singh, A. Manickam, R. Sinha, B. Kuimelis, S. Bolouki, P. Naraghi-Arani, K. Johnson, M. McDermott, N. Wood et al., “A fully integrated cmos fluorescence biochip for multiplex polymerase chain-reaction (pcr) processes,” in 2017 IEEE International Solid-State Circuits Conference (ISSCC). (IEEE, 2017), pp. 68–69.

25. K. Imai, M. Nishigaki, Y. Onozuka, Y. Akimoto, M. Nagai, S. Matsumoto, and S. Kousai, “A lens-free single-shot fluorescent imaging system using cmos image sensors with dielectric multi-layer filter,” in 2017 19th International Conference on Solid-State Sensors, Actuators and Microsystems (TRANSDUCERS). (IEEE, 2017), pp. 139–142.

26. S. Pang, C. Han, J. Erath, A. Rodriguez, and C. Yang, “Wide field-of-view talbot grid-based microscopy for multicolor fluorescence imaging,” Opt. Express 21, 14555–14565 (2013).

27. G. Zheng, R. Horstmeyer, and C. Yang, “Wide-field, high-resolution Fourier ptychographic microscopy,” Nat. Photonics 7, 739 (2013).

28. J. Kim, B. M. Henley, C. H. Kim, H. A. Lester, and C. Yang, “Incubator embedded cell culture imaging system (EmSight) based on Fourier ptychographic microscopy,” Biomed. Opt. Express 7, 3097–3110 (2016).

29. M. Beiderman, T. Tam, A. Fish, G. A. Jullien, and O. Yadid-Pecht, “A low-light CMOS contact imager with an emission filter for biosensing applications,” IEEE Transactions on Biomed. Circuits Syst. 2, 193–203 (2008).

30. Y. Dattner and O. Yadid-Pecht, “Low light cmos contact imager with an integrated poly-acrylic emission filter for fluorescence detection,” Sensors 10, 5014–5027 (2010).

31. L. Blockstein, C. C. Luk, A. K. Mudraboyina, N. I. Syed, and O. Yadid-Pecht, “A PVAc-based benzophenone-8 filter as an alternative to commercially available dichroic filters for monitoring calcium activity in live neurons via Fura-2 AM,” IEEE Photonics J. 4, 1004–1012 (2012).

32. S. Pang, C. Han, L. M. Lee, and C. Yang, “Fluorescence microscopy imaging with a fresnel zone plate array based optofluoric microscope,” Lab on a Chip 11, 3698–3702 (2011).

33. K. Sasagawa, S. H. Kim, K. Miyazawa, H. Takehara, T. Noda, T. Tokuda, R. Iino, H. Noji, and J. Ohta, “Dual-mode lensless imaging device for digital enzyme linked immunosorbent assay,” in Frontiers in Biological Detection: From Nanosensors to Systems VI, vol. 8933 (International Society for Optics and Photonics, 2014), p. 89330N.

34. A. K. Mudraboyina, L. Blockstein, C. C. Luk, N. I. Syed, and O. Yadid-Pecht, “A novel lensless miniature contact imaging system for monitoring calcium changes in live neurons,” IEEE Photonics J. 6, 1–15 (2014).

35. L. Blockstein and O. Yadid-Pecht, “Lensless miniature portable fluorometer for measurement of chlorophyll and CDOM in water using fluorescence contact imaging,” IEEE Photonics J. 6, 1–16 (2014).

36. M. Kim, M. Pan, Y. Gai, S. Pang, C. Han, C. Yang, and S. K. Tang, “Optofluoric ultrahigh-throughput detection of fluorescent drops,” Lab on a Chip 15, 1417–1423 (2015).

37. H. Takehara, O. Kazutaka, M. Haruta, T. Noda, K. Sasagawa, T. Tokuda, and J. Ohta, “On-chip cell analysis platform: Implementation of contact fluorescence microscopy in microfluidic chips,” AIP Adv. 7, 095213 (2017).

38. H. Takehara, Y. Nakamoto, N. Ikeda, K. Sasagawa, M. Haruta, T. Noda, T. Tokuda, and J. Ohta, “Compact lensless fluorescence counting system for single molecular assay,” (2018). Submitted.

39. A. F. Coskun, I. Sencan, T.-W. Su, and A. Ozcan, “Wide-field lensless fluorescence microscopy using a tapered fiber-optic faceplate on a chip,” Analyst 136, 3512–3518 (2011).

40. A. F. Coskun, I. Sencan, T.-W. Su, and A. Ozcan, “Lensless wide-field fluorescent imaging on a chip using compressive decoding of sparse objects,” Opt. Express 18, 10510–10523 (2010).

41. A. F. Coskun, I. Sencan, T.-W. Su, and A. Ozcan, “Lens-free fluorescent on-chip imaging of transgenic caenorhabditis elegans over an ultra-wide field-of-view,” PloS one 6, e15955 (2011).

42. I. Sencan, A. F. Coskun, U. Sikora, and A. Ozcan, “Spectral demultiplexing in holographic and fluorescent on-chip microscopy,” Sci. Rep. 4, 3760 (2014).

43. S. Pang, C. Han, M. Kato, P. W. Sternberg, and C. Yang, “Wide and scalable field-of-view Talbot-grid-based fluorescence microscopy,” Opt. Lett. 37, 5018–5020 (2012).

44. J. Ohta, T. Tokuda, K. Sasagawa, and T. Noda, “Implantable CMOS biomedical devices,” Sensors 9, 9073–9093 (2009).

45. M. Haruta, C. Kitsumoto, Y. Sunaga, H. Takehara, T. Noda, K. Sasagawa, T. Tokuda, and J. Ohta, “An implantable CMOS device for blood-flow imaging during experiments on freely moving rats,” Jpn. J. Appl. Phys. 53, 04EL05 (2014).

46. Y. Sunaga, H. Yamamura, M. Haruta, T. Yamaguchi, M. Motoyama, Y. Ohta, H. Takehara, T. Noda, K. Sasagawa, T. Tokuda et al., “Implantable imaging device for brain functional imaging system using flavoprotein fluorescence,” Jpn. J. Appl. Phys. 55, 03DF02 (2016).