Lensfree color imaging on a nanostructured chip using compressive decoding

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We demonstrate subpixel level color imaging capability on a lensfree incoherent on-chip microscopy platform. By using a nanostructured substrate, the incoherent emission from the object plane is modulated to create a unique far-field diffraction pattern corresponding to each point at the object plane. These lensfree diffraction patterns are then sampled in the far-field using a color sensor-array, where the pixels have three different types of color filters at red, green, and blue (RGB) wavelengths. The recorded RGB diffraction patterns (for each point on the structured substrate) form a basis that can be used to rapidly reconstruct any arbitrary multicolor incoherent object distribution at subpixel resolution, using a compressive sampling algorithm. This lensfree computational imaging platform could be quite useful to create a compact fluorescent on-chip microscope that has color imaging capability. © *2010 American Institute of Physics*.
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Microscopy, in general, has found widespread use in biomedicine by providing a toolset that can routinely image various biological processes with desired spatial and temporal resolution, as well as specificity and sensitivity levels. Despite the recent advances in microscopy,[1–4] there is still a need for designing new imaging modalities for various biomedical applications. Among these, one area is lensfree digital microscopy which in general aims to provide compact and lightweight on-chip microscopes that are better suited to image lab-on-a-chip platforms, especially targeted for point-of-care and field settings. For this end, several lensfree on-chip microscopy modalities have been introduced so far, covering both bright-field and fluorescent imaging.[5–13] Along the same lines, we have recently demonstrated lensfree incoherent imaging on a chip using nanostructured substrates.[14] This recent on-chip imaging modality involved spatial modulation of the diffraction pattern for each point at the object plane which created a calibrated and yet spatially varying lensfree point-spread function (PSF) between the object and the sensor planes. Such a spatially varying PSF was the key to achieve subpixel resolution in lensfree incoherent on-chip microscopy, and therefore constituted a promising step forward to create high-resolution lensfree fluorescent microscopes on a chip.[14] On the other hand, these initial results were restricted to quasimonochromatic fields, which create an important limitation to image multicolor objects, as would be frequently encountered in fluorescently labeled specimen. We should also emphasize that unlike conventional lens-based microscopy, color imaging capability at the subpixel level is rather challenging to achieve in a lensfree on-chip imaging geometry since the diffraction patterns of multicolor objects all mix with each other at the far-field, which makes the objects lose their natural colors at the detector plane.

To release this limitation, here we demonstrate subpixel color imaging capability in lensfree incoherent on-chip microscopy. Toward this end, we use a similar nanostructured substrate as before[14] to modulate the lensfree incoherent PSF of our on-chip microscope. If this spatial modulation is color sensitive (as one would observe in e.g., plasmonic substrates) then a multicolor incoherent object on the chip could be imaged by calibrating the PSFs of the nanostructured chip at three major wavelengths, corresponding to red, green, and blue, to decode the objects' diffraction patterns into a lensfree color image. While this would be feasible by designing, e.g., appropriate plasmonic nanostructured substrates,[15–17] such resonant transmission behavior would potentially be affected by the presence of the objects on the chip, which could make the lensfree PSFs of the structured substrate object dependent. Since this is an undesired feature for a microscope in general, instead of taking this plasmonic approach, we have actually used a color sensor-chip for recording the lensfree diffraction patterns of multicolor objects. This optoelectronic sensor-chip has red, green, and blue (RGB) filters installed at the pixels, such that each period contains one red and one blue pixel together with two green pixels forming a repeating RGB pattern across the entire sensor active-area. Using this configuration, the spatially varying PSFs of a nanostructured substrate would exhibit color sensitivity that is now independent of the object or its near-field, such that an *arbitrary* multicolor incoherent emission from the object plane can be decoded into three distinct colors (RGB), yielding a lensfree color image at the subpixel level. This digital imaging approach would be especially important to create compact fluorescent on-chip microscopes that can simultaneously image various colored fluorescent probes.

To experimentally confirm this approach, we used a structured metallic thin-film slab, composed of an array of nanoislands (see Fig. 1). These nanostructures were fabricated using focused ion-beam milling (NOVA 600 at UCLA Nanolab) on borosilicate cover slips (150 μm thick) that were coated with ~200 nm gold layer. The design of the nanostructures was made using finite-difference time-domain (FDTD) simulations (FULLWAVE from RSOFT) in order to minimize the spatial correlation among the PSFs for closely spaced points on the chip.[14] Figure 1 schematically presents

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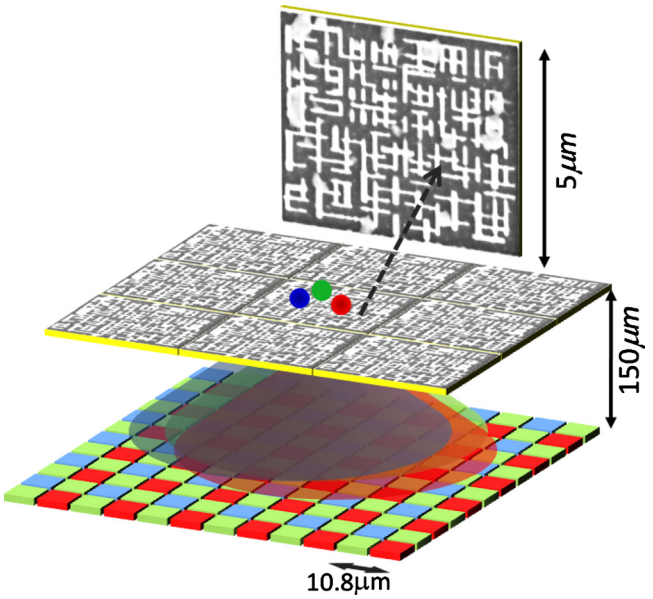

FIG. 1. (Color online) Schematic diagram of the lensfree incoherent color imaging platform is shown. This setup achieves subpixel level color resolution based on spatial modulation that is introduced using nanostructured substrates. Notice that the diffraction pattern of a multicolor subpixel object that is sampled at the detector plane unavoidably loses its original colors, which in general makes lensfree color imaging at the subpixel level rather challenging to achieve.

the experimental setup. The incoherent light emitted from a *multicolor* object is transmitted through the nanostructured substrate and propagates a vertical distance of ~0.15 mm to be sampled by a RGB optoelectronic sensor-array [i.e., a charge-coupled device (CCD)-Kodak, KAF 8300, pixel size: 5.4 μm)]. Due to the color filters installed on the pixels, each sampled lensfree diffraction image is actually composed of three raw images corresponding to RGB channels. These lensfree RGB diffraction images of the objects, along with the calibration data of the nanostructured chip, are used to reconstruct multicolor images of the objects on the chip.

To calibrate the spatially varying and color-sensitive PSFs of the fabricated nanostructured chip in Fig. 1, we measured the lensfree diffraction pattern of a point source that is created by focusing of a light emitting diode while the CCD and the structured surface assembly were being scanned in two dimensions (x and y), as illustrated in Fig. 2. This calibration process was repeated for three different illumination wavelengths (RGB), and quite conveniently needs to be performed *only once* for each nanostructured chip. As for the calibration point source, we used fiber-coupled light emitting diodes (at 470, 530, and 670 nm with a bandwidth of ~20–30 nm each) focused to a spot size of <2 μm (full width at half maximum) on the top surface of the nanostructured chip. In these calibration experiments, the detector-array together with the structured surface was scanned using a piezostage controlled by a LABVIEW code. Using a scanning step size of ~0.5 μm in both x and y directions, we acquired a total of $N=3\times120$ calibration frames over 6 μm$\times$5 μm area of the nanostructured surface. Figure 2 exhibits representative sets of diffraction patterns for each color channel captured at different points on the nanochip. As expected, these lensfree diffraction images at each color channel are significantly different from each other, validating the spatially varying and color-sensitive nature of our lensfree PSFs, which are key features to achieve subpixel level color imaging on a chip.

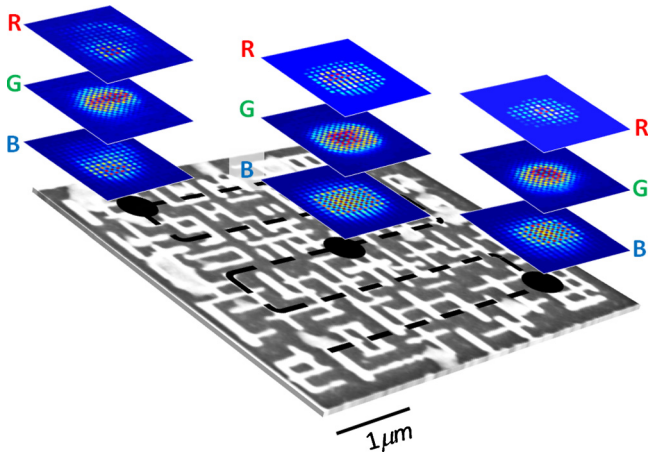

FIG. 2. (Color online) The calibration procedure for a given nanostructured substrate is summarized. A point source at each color (RGB) scans the surface of the structured substrate, while the sensor-array is recording the resulting lensfree diffraction patterns corresponding to each spot on the structured chip. The figure also shows some of these lensfree diffraction patterns corresponding to three representative locations indicated by the black circular spots.

After this calibration step, to demonstrate the resolving power of this lensfree color imaging modality, we illuminated the same nanostructured chip with a multicolor object containing three spots at RGB, which were spatially separated from each other by ~1–2 μm. This implies a subpixel three color object since the pixel period at the CCD chip is 10.8 μm (see Fig. 1). The results of this lensfree color imaging experiment are summarized in Fig. 3. For comparison purposes, a conventional reflection microscope image of the same multicolor object is shown in Fig. 3(d), which was acquired using a 40× objective lens (numerical aperture (NA): ~0.6). For this subpixel multicolor object, the raw lensfree diffraction pattern that is sampled at the CCD chip is shown in Fig. 3(a) which, due to diffraction, is quite broadened with an extent of ~100 μm. Because of the color filters installed at each pixel, this raw image in Fig. 3(a) exhibits a mosaic pattern (also known as the Bayer pattern), which is normally demosaiced to yield a regular color image. The output of this digital demosaicing process is illustrated in Fig. 3(b) that now indicates an almost uniform white diffrac-

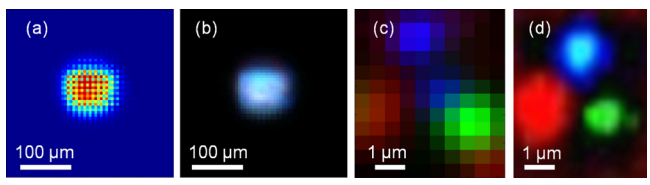

FIG. 3. (Color online) (a) Raw lensfree diffraction image of a subpixel multicolor object is illustrated. This object is composed of RGB spots that are separated from each other by 1–2 μm. The same subpixel object is also imaged using a conventional reflection microscope (40× objective lens, numerical aperture=0.6) as illustrated in (d) for comparison purposes. (b) presents the same lensfree image as in (a) after a demosaicing algorithm is applied to the raw image shown in (a). As expected, the far-field diffraction pattern of closely packed RGB spots creates a white-looking enlarged pattern as shown in (b). (c) successfully demonstrates our compressive decoding results based on processing of the raw lensfree diffraction pattern shown in (a). Notice that the scale-bars in (a) and (b) are 100 fold larger than the ones in (c) and (d).

tion spot, spanning a width of ~100 μm. This is expected since a closely packed set of RGB spots at the subpixel level would look as white in the far-field (assuming similar power levels in each color).

Here, we would like to emphasize that neither Fig. 3(a) nor Fig. 3(b) exhibit any visible sign of the subpixel multicolor objects located at the nanostructured surface. While this is true for the bare eye, it is actually feasible to decode, using a compressive sampling algorithm,[18–20] the raw lensfree diffraction image of Fig. 3(a) into a much higher resolution image to recover the subpixel multicolor object distribution located at the structured chip. The result of this numerical decoding process is illustrated in Fig. 3(c), which now clearly resolves the three distinct subpixel sources at each color, providing a decent match to the reflection image of the same chip acquired with a conventional microscope [Fig. 3(d)]. The computation time of this decoded image was <10 s using a dual-core processor (AMD Opteron 8218) at 2.6 GHz, which can be significantly improved by employing a graphics processing unit.

In conclusion, we have presented a subpixel color imaging scheme using lensfree incoherent on-chip microscopy. We have utilized a nanostructured substrate that spatially modulated the far-field diffraction patterns corresponding to each point at the object plane. These lensfree diffraction patterns were sampled using a color optoelectronic sensor-array, where the pixels had three different types of color filters at RGB. In order to reconstruct an arbitrary multicolor object located on the nanostructured chip, calibration of the spatially varying and color-sensitive PSFs of the nanochip was required. For this calibration process, we used three different point sources at RGB colors that were independently focused and scanned on the structured surface. The resulting diffraction patterns at each color were recorded and used as a basis in compressive decoding of an arbitrary multicolor object located on the chip. Our experimental demonstration of this lensfree color imaging platform achieved a spatial resolution ~2 μm, which is around five times smaller than the resolution that our pixel period at the CCD chip (10.8 μm) would normally permit in direct contact color imaging. We believe that this lensfree computational imaging platform could be quite useful to create a compact fluorescent on-chip microscope which has true-color imaging capability.

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