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ARTICLE *in* ACS NANO · OCTOBER 2013

Impact Factor: 12.88 · DOI: 10.1021/nn405167q · Source: PubMed

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Toward Single-Molecule Microscopy on a Smart Phone

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ABSTRACT Thanks to fluorescence, single nano-objects down to individual fluorophores can now be imaged in optical microscopes. Fluorescence imaging is still restricted to laboratory facilities as it usually involves expensive and bulky instrumentation. A report by Wei *et al.* in this issue of *ACS Nano*, however, shows that a sensitive, cost-effective, and portable device can be developed to image individual nano-objects as small as large viruses. This work opens the fascinating prospects of single-molecule microscopy and spectroscopy on a smart phone. We speculate on the possible applications of such a portable imaging device and on the perspectives it may open in different fields of science and technology.

Optical imaging and spectroscopy of single nano-objects have become important tools for studying many complex systems in biology, chemistry, and materials science as it provides direct access to the nanometer scale.[1,2] Depending on the question under investigation, a wide variety of nanoprobes can be used. Prominent examples are single molecules, organic nanoparticles (clusters of molecules), semiconductor quantum dots, and metal nanoparticles. Over the years, many optical techniques have been developed to detect these nanoparticle probes through their absorption, scattering, and fluorescence. Absorption-based techniques, in particular photothermal imaging, are sensitive enough to detect small metal nanoparticles and even weakly fluorescent molecules,[3] but the required experimental setup is relatively complex and expensive. Scattering-based techniques are simpler and easier to implement. However, scattering is difficult to detect and to discriminate from background scattering for small particles. Even metal particles, which present high index contrast with their dielectric environment, are difficult to detect for diameters below 30 nm.[4] The most widely used technique to detect nanoparticles is therefore based on their fluorescence. As the red-shifted fluorescence can be easily separated from the excitation light, even faint objects down to single fluorophores can be imaged.[5] Thanks to charge-coupled devices (CCD), fluorescence images are acquired rapidly, are virtually background-free, and present high contrast. The key requirements of fluorescence imaging are (1) a sufficient emission rate of the nano-objects to overcome the dark count of the detector, and (2) an efficient rejection of the excitation light, usually with suitable dielectric band-pass filters. A typical fluorescence imaging setup, such as found in many cell biology laboratories, consists of a laser as the excitation source, a high-quality objective lens to excite individual nano-objects and to collect their emission efficiently, a long-pass or notch filter to block the excitation light, and a sensitive detector, either an avalanche photodiode (APD) for high time resolution or a CCD camera for parallel imaging of many objects in a wide field of view.

As described in this issue of *ACS Nano*, Wei *et al.* have built a simple, low-cost, and portable fluorescence imaging system that can be mounted on a smart phone.[6] Their detection system includes a diode laser, a lens to collect the fluorescence signal from the sample, and a long-pass filter to block the excitation laser. The complementary metal-oxide semiconductor (CMOS) sensor of the smart phone's camera works as the detector and the lens images a large sample area (0.6 × 0.6 mm^2) onto the detector. The optical components are mounted on a small, lightweight holder (made with a three-dimensional laser printer) that is

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Published online October 10, 2013
10.1021/nn405167q

attached to the smart phone. One of the important features of the design is the near-grazing incidence of the excitation laser beam, which impinges on the sample at a shallow angle of approximately 15° with respect to the sample plane through the glass sample holder. Because of this oblique excitation geometry and total internal reflection, only scattered excitation light can be collected by the lens, which considerably reduces the background. The authors demonstrate the device capabilities by imaging single nano-objects including dye-doped polystyrene beads that are 100 nm in diameter, and human cytomegaloviruses labeled with dye molecules. The large field of view makes it possible to search for fluorescent objects in a macroscopic region of interest, easily identified by eye. This may make the overall detection process easier and faster. While its low cost and portability are unique advantages of this design, its main limitations are its comparatively low detection efficiency and limited spatial resolution, about 1.5 μm. These qualities are sufficient to detect beads or viruses labeled with thousands, or at least hundreds, of fluorophores, but smaller or dimmer objects, in particular single fluorophores, cannot be imaged with this device. Yet, single-molecule sensitivity would open many applications in biomedical fieldwork among other uses.

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Thanks to its low production cost, user-friendliness, and portability, the smart-phone fluorescence microscope promises a wide range of applications, especially for fieldwork in remote areas where laboratory facilities are inaccessible. The proposed detection of specifically stained viruses and bacteria is particularly interesting, not only for human cytomegalovirus, HIV, rabies, or influenza viruses, which present similar sizes, but also for smaller viruses such as Hepatitis B or Poliovirus, which would be difficult to detect with the present device. Other appealing applications could be found in food or water industries to detect fluorescently labeled bacteria or impurities. Therefore, improving sensitivity and resolution of the device would give access to fluorescence or scattering images of even smaller individual objects, and even, perhaps, of single molecules. Several classes of interesting objects spring to mind, in particular fluorescent or photoluminescent molecules and organic nanoparticles, and a broad variety of scatterers, among which Raman scatterers are probably the most interesting. Weak emitters, such as single molecules, require improved detection efficiencies, whereas Raman scatterers require spectral resolution, which the present device cannot provide. For both kinds of applications, coupling to small metal nanostructures (*i.e.*, plasmonics) would be extremely useful. We briefly discuss these different directions below.

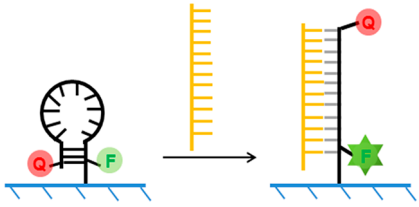

Figure 1. A scheme for the detection of DNA fragments. F and Q represent a fluorophore molecule and a quencher molecule, respectively. The folded construct is thus dark. The unzipping of DNA upon hybridization with a complementary DNA strand results in a change of distance between the fluorophore and the quencher, leading to bright fluorescence.

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Single fluorescent molecules are currently detected in many laboratories around the world at ambient conditions thanks to commercial optical microscopes and sensitive detectors.[5] Detecting single molecules in the field in remote areas, far from any scientific laboratory, would open a wide range of potential applications, many of which we can only dream today. Think, for example, of the detection of allergens with labeled antibodies or of imaging short DNA strands through their complementary sequences,[7] with hybridization leading to a fluorescence resonance energy transfer (FRET) signal (see Figure 1). Their detection in the field and in real time would revolutionize pollution monitoring, forensics, the authentication of products, and a myriad of other uses. 'Democratized' single-molecule detection would be primarily appealing for epidemiologic purposes. Low-priced alternatives to expensive analysis techniques would make it possible to follow the propagation of pathogens

through parallel monitoring by many observers, even before the first symptoms of an infectious disease manifest themselves. Decentralized single-molecule observations would replace the strength of having a few high-end laboratories by the massive acquisition and parallel use of (probably) lower quality, but statistically more reliable observations. Even more importantly, because the requirements of purification, sterilization, and shipping would be relinquished to a large extent, this massive monitoring could occur, if not instantaneously, at least on a much shortened time scale than today. The same technical progress would apply not only to molecules, but to a broad range of other emitters, including gold nanoparticles,[8] semiconductor quantum dots,[9] rare-earth-doped nanoparticles,[10] or nanodiamonds.[11]

Another attractive direction opened by cell-phone-borne devices is spectrally resolved microscopy. Spectral resolution of fluorescence creates many multiplexing possibilities in the detection of particles or molecules, which would enrich the above possibilities. Moreover, spectral resolution also opens the coupling of microscopy with Raman spectrometry, thus allowing quantitative chemical analysis *in situ*. For this application, the numbers of available molecules are very large, but the Raman scattering process is so weak that the scattered intensities are comparable to, and often even weaker than, single-molecule fluorescence. Yet, the prospects are again tantalizing. Think, for example, of a geologist identifying minerals within their rocky matrix or of a repair workshop immediately able to choose the proper glue for an unknown polymer part. To achieve suitable spectral resolution, many more channels than the three colors of a cell phone camera are needed. They could be obtained by attaching a small grating or a dispersing prism to a cell phone microscope,[12] at no great increase in cost, and by imaging a whole line of a sample on the two-dimensional detector array. Such Raman microscopes are currently in use in only a handful of cellular biology laboratories.[13]

The coupling of nanoemitters to plasmonic structures opens promising perspectives. Metal nanoparticles and nanostructures can concentrate electromagnetic fields,[14−16] thereby enhancing the interactions of emitters with propagating light waves by large factors, which can reach several orders of magnitude. Enhancement may work both for excitation by light and for emission of light. A well-known example of such enhancement is surface-enhanced Raman scattering, where the intensity of a Raman line can be amplified by up to billions of times, so that the Raman scattering of a single molecule becomes detectable.[17] The fluorescence of weak emitters can also be considerably enhanced by nanostructured antennas[16] or even by simple gold nanorods.[18] It would be imaginable to employ arrays of many plasmonic nanostructures to couple to weak emitters, either nanoparticles or even individual molecules. Enhanced fluorescence or scattering could then be detectable in a cell phone microscope. Disposable plasmonic arrays could be produced at low cost for simple nanoparticle antennas, and recyclable ones could be made for more sophisticated structures.

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The work of Wei *et al.*[6] is a first step in bringing single-nanoparticle optical imaging techniques, now mostly confined in sophisticated laboratory environments, to real-world applications in remote areas. The ever-growing popularity of smart phones, with already more than a billion in use, coupled with great technological advances in making faster processors, and more sensitive cell phone cameras will certainly extend the reach of such devices in the future. Increasingly faster wireless networks will also aid remote data analysis and sharing of information through the cloud. One would, however, desire to improve the sensitivity of the current device up to the detection of a single molecule or at least detection of a few molecules. We believe this could be tried through some simple modifications without sacrificing the portability of the device. Two possibilities come to mind. First, a high-numerical-aperture (NA) objective lens would considerably increase the brightness and the spatial resolution of the smart-phone microscope. Although the best quality of imaging is obtained with refractive, multilens objectives, simple reflective objectives can reach NAs as high as 0.6.[19] Interestingly, reflective objectives are completely achromatic and can be manufactured out of molded polymers in great numbers at low cost.[20] The increase in sensitivity and resolution would of course come at the cost of a reduced field of view, but this would not necessarily be a severe limitation for biomedical assays, where only minute quantities of analytes are usually available. Second, the regular glass coverslip could be replaced by a glass substrate bearing a dense array of plasmonic nanoantennae, for example gold bowtie nanoantennae.[16] One would thus take advantage of plasmonic enhancement of the excitation and of the fluorescence or scattering. Such substrates are still expensive to produce with the required quality, as the gap between the metal islands has to be tuned precisely and reproducibly to some tens of nanometers. However, the structures could be easily cleaned by ozone or oxygen plasma and could be recycled many times without increasing unduly the cost of the device. Alternatively, simple

gold nanorods, which are easy to produce massively, could be used as plasmonic enhancers in limited spectral ranges. Whichever practical solutions prevail in the long term, a safe bet is that the future of smartphone optical devices is very bright indeed.

Conflict of Interest: The authors declare no competing financial interest.

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