

Francesco Baldini · Arnaldo D'Amico
Corrado Di Natale · Pietro Siciliano
Renato Seeber · Luca De Stefano
Ranieri Bizzarri · Bruno Andò
Editors

Sensors

Proceedings of the First National
Conference on Sensors,
Rome 15-17 February, 2012

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on Sensors, Rome 15–17 February, 2012

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ISSN 1876-1100

ISBN 978-1-4614-3859-5

DOI 10.1007/978-1-4614-3860-1

Springer New York Heidelberg Dordrecht London

ISSN 1876-1119 (electronic)

ISBN 978-1-4614-3860-1 (eBook)

Library of Congress Control Number: 2013940913

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Printed on acid-free paper

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Foreword

The National Conference on Sensors was an important event held in Rome from 15 to 17 February, 2012 at the headquarters of the National Research Council in Rome. The most important scientific associations active in the area of sensors, actuators and microsystems were involved, for the first time altogether, in the organization of this national event: the Italian Society of Optics and Photonics (SIOF), the Electrical and Electronic Measurement Italian Association (GMEE), the Italian Association the Ambient Assisted Living Italian Association (AitAAL), the Italian Chemical Society (SCI), the Italian Physical Society, the Italian Association of Sensors and Microsystems (AISEM), the Italian Association for the Information and Communication Technologies (AICT), the Italian Association of Photobiology and the Italian Society of Pure and Applied Biophysics.

The driving idea in the organization of the event was the creation of a gathering moment at the national level, in order to favor the birth and the consolidation of interdisciplinary interactions among the different groups working in this field, and to strengthen the relationship between the sensor developers, the manufacturers and the final users. We consider both these aspects essential elements to create a real step forward in the research.

The Conference was a very successful interdisciplinary event, with more than 150 attendants coming from different disciplines, ranging from physics, engineering, chemistry, material science, biotechnology and biophysics. The Conference numbered 5 international plenary talks, 13 keynotes, 62 oral presentations and 81 poster presentations with a large participation of academic institutions, institutes of the National Research Council, and other national governmental research organizations. Particularly important was the substantial participation of companies involved in the design and development of sensors.

This book collects a selection of 100 papers presented at the conference and offers an exhaustive view of the state of the art in Italy in this field.

Particular thanks is expressed to the National Research Council, which hosted the Conference and to Assobiotec, the Italian Association for the Development of

Biotechnology, within the Italian Federation of the Chemical Industry (Federchimica), which strongly supported the event. The event was also financially supported by Datamed and AMS Technology.

Special thanks to Dr. Antonella Tajani, Dr. Ambra Giannetti, Dr. Sara Tombelli and Dr. Cosimo Trono for their helpful commitment to the conference organization.

The Editors

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Part I

Plenary

Chapter 1

Beyond Human Senses: Technologies, Strategies, Opportunities, and New Responsibilities

Arnaldo D'Amico and Corrado Di Natale

Abstract Natural senses evolved to be adapted to the life conditions of our ancestors. Eventually, as an example, sight is tuned to the sunlight and olfaction is aimed at quickly discriminating between eatable and non eatable foodstuff. Technological progress changed the human environment, and the growth complexity of the surrounding world requires senses whose characteristics are rather different with respect to those provided by Nature. From this simple observation sensor science stems. Current technologies, in particular those related to the nano-world, are expected to provide a substantial leap towards the fulfillment of such a requirement. In this short paper, some considerations about the relationship between nanotechnologies and sensors are introduced and discussed.

Introduction

Senses are important components for life and its evolution. In human body, millions of them form the olfaction, the sight, the hear, the taste, and tactile just to mention the most relevant but many others do exist. Human brain under the sensorial signals, improved through averages, promotes the perception mechanism and the actuation actions according to the necessities of the moment, such as: reflection, attention, alarm, or pleasure [1].

Living beings use their senses to get information from the environment, in order to understand it and shape it. The sensorial capacity of the living beings appears limited and as a consequence the amount of perceived knowledge is *limited*.

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In particular, human sensors, even if excellent are not sufficient at all to locate instability generators and detect chemical, physical or biological quantities to the extent needed for the full satisfaction of the global requirement of the overall knowledge.

The desire of *novelty* and *beauty* together with a higher security level need, has led the mankind to develop technologies able to increase the capabilities of looking to the world; one of the main consequence is the fact that the ambient small or large, it does appear worth amplified.

The Peculiar Contribution of Nanotechnology

Artificial sensors which enlarge the physical, chemical and biological bandwidth are more than necessary. Furthermore, miniaturized systems are becoming more and more requested due to their tested peculiarities which are: production cost reduction, greater reliability, reduced material consumption, more possibility for large scale integration and better overall performances.

The possibility of imaging individual molecule or even single atoms by the atomic force and the scanning tunnel microscopies has led to see what we are doing in extreme details when a given material is constructed starting from its elementary constituents [2].

The basic approaches called top-down and bottom-up are still valid along the technology development especially in the frame of dimensions shrinking.

The top-down technology attempts to develop the lithographic tools and the related processes (deposition etching, etc.) which are necessary to reach the final dimensions of the desired materials. To this regards, the starting point is determined by the well consolidated micro-technology.

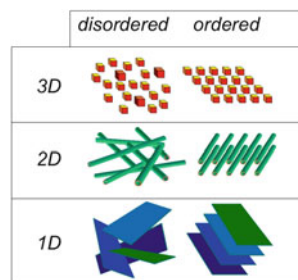
The bottom-up approach tends to grow structures starting from single atoms or molecules. The two procedures together may represent the ideal approach for the construction of the final device or structure.

In fact by the bottom up approach one can first grow a thin and uniform material onto which the application of the high resolution bottom up approach can determine the designed structure. On the other hand starting from the bottom up approach one can define the structure dimensions even applying the surface or bulk micromachining, if necessary, and leaving to the bottom up strategy the duty of performing some additional detail.

In any case the nanotechnologies whatever the implementation strategy could be, should satisfy in case of fabrication of a number of identical structures, the following basic statements: single devices should be located to the same distance, should have the same dimensions, same homogeneity and same optical or electronic properties. Figure 1.1 illustrates this principle comparing ordered and disordered nanostructures of different dimensionalities.

In both strategies the chemical properties and their control play a fundamental role in nanotechnologies.

Fig. 1.1 Orders and disorders in tridimensional, bidimensional, and unidimensional structures



Some interesting aspects are encountered when dimensions of materials are reduced, which have very sound relevance in the sensor technology context.

One of them, the surface to volume (S/V) ratio does really play a crucial role and for this reason it deserves a bit of discussion. As the single dimension L of a cube reduces to L/K (with $K > 1$), the volume L^3 and the surface $6L^2$ whose S/V ratio is given by $6/L$, reduces to $(L/K)^3$ and $(L/K)^2$ whose new S/V ratio is K/L .

So the surface becomes more and more important as K increases. This means that the thermal dissipation processes are more consistent and small devices dissipate heat more rapidly than larger devices of the same shape.

If a device dimensions are reduced of a factor K , the mass reduces of K^3 . So the rigidity coefficient reduces as K ; as a consequence the mechanical forces are reduced more slightly with respect to the inertial forces that object can generate, being these scaled as K^3 . From the above consideration we can say that small MEMS can sustain very high accelerations.

Also in the nano-frame with reference to the fluid-dynamics context, we can say that mixing compounds in solution and in small channels should be easier performed due to the reduced Reynold number. This can reach values less than 2,000, which means less turbulences and, as a consequence, less heat dissipation.

In small devices small defects are also very important so their reduction or total elimination, if possible, does represent the main concern. One of the consequences is that the kinetics of oxidation-reduction processes must be controlled with a great precision. In particular, the speed of reactions can greatly be reduced by appropriate chemical buffers into the solution.

Once a sensitive material has been grown and located onto a suitable substrate the main problem becomes how to contact it and how to utilize it in a real context. This problem is growing and has nowadays reached the point where new sensor paradigms are becoming more than necessary. Global functionalities are considered as one of the main concerns.

Nature tells us how to proceed or at least it gives us some indications. According to these suggestions, it is worth while to think first to the low dimensionality, observe a new property, if any, connect locally a foreseen number of these nano-sensor till the point where a macroscopic level is reached from which the overall output can be finally utilized. Our eye is a superb example of this strategy. In fact only one receptor would have been not useful at all to see a sufficiently large field

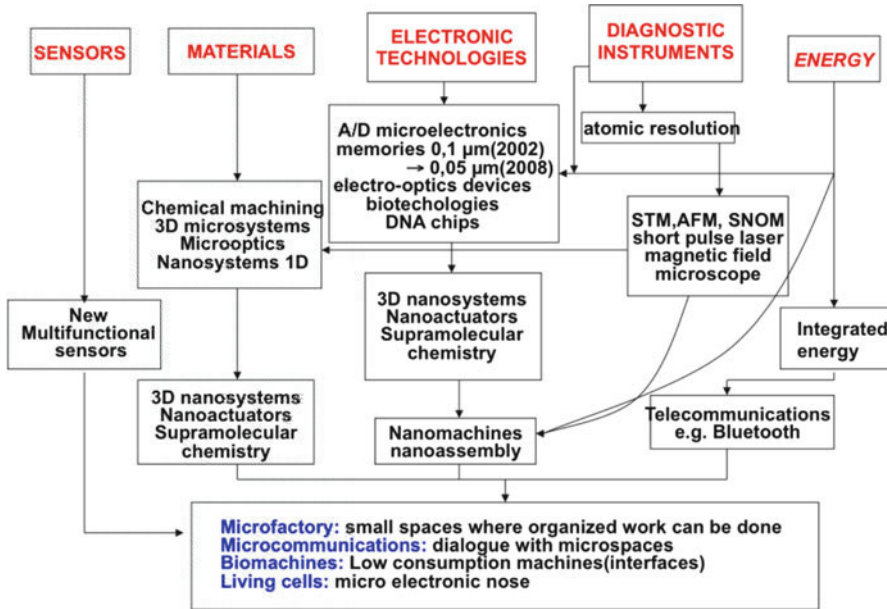


Fig. 1.2 Roadmap for complete nanosensors development

of view, but many of them is performing an almost perfect job till the point where another mechanism related to the image construction takes the floor with the final aim to give us the image perception. This bio-mechanisms are evident for all our fundamental senses which on the other hands do converge to about the same final perceptions strategy.

Global functionality could become a must in a not far away future. Figure 1.2 shows a possible roadmap for sensors development. Such a map indicates that future challenges are related to the possibility of doing useful and finalized work into nano-spaces, perform the information syntheses and express in the real macroscopic world the strictly necessary data. These could be for instance, light pulses, or electric field distributions, or even localized thermal distributions. In other words, all the preprocessing actions included elementary computation aspects should be performed at nano-levels with the final aim of reaching, as much as possible, the final utilization objective with the minimum number of connections and maximum data weight.

Compatibility with living tissues or even with single cells is a paramount characteristics that future nano-sensors should have so the search for biologically compatible nano-materials or nano-structured ones, does represent a real challenge for future new sensor class development. To this regard, nanoparticles for their capability to penetrate into the recesses of human body have a great potential to target drugs but also they constitute a new threat to human health [3, 4].

Another observation comes directly from the nano-sensors used to detect chemical species using, for instance, the conductivity change as transduction principle. The material which constitutes the substrate in very small dimension conditions, is

no longer self protected, rather it is almost directly in contact with its surface and with the sensitive surface. So nano-corrosions may take place inducing changes in the intrinsic transduction property.

Another problem which arises in nano-sensors is the following: even if the surface to volume ratio may have a rather high value, the surface itself may be relatively small, and in case of volatile compound detection expressed at very low pressure, the rate of impacts of the detectable species with the nanosensor surface may be so small that the interactions, and consequently the output response, could require a non tolerable response time [5].

Future strategies in this field which do represent the new frontiers along the next generation of nanotechnologies will consider among others the following paths: the investigation of small size particle and their influence on the so called step change of some intrinsic properties (small size effects). An example is here given by the highly compressed ferrous alloy powder able to greatly absorb high frequency electromagnetic waves [6]. Applications are foreseen in the fields of cell phones, game console, very high resolution digital camera and so on. Also the diamond is an excellent candidate for nanotechnology context; in fact it can be precisely machined into nano-structures. Key characteristics are the very high acoustic velocity, very high thermal conductivity, and extremely high hardness. But in particular one of the most outstanding property as nano-material is the fact that it can be machined with very high precision. Nano-emitters also do represent an extraordinary product for the future development of new class of very high frequency devices in the region of terahertz.

One of the main concern with nanostructures is that their so small dimensions are getting far away from the perception sphere of those persons involved in all the related technological aspects and this could have the effect to increase the gap between the real distances to the extent where the disaffection towards small sized devices could take place. New teaching paradigms and approaches to the nano-world science should be envisaged in order to reduce the effects of the mechanical interfaces and live with it on the basis of both a better acceptance and reduced psico-problems.

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Chapter 2

Lensfree On-Chip Fluorescence Microscopy for High-Throughput Imaging of Bio-Chips

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Abstract On-chip fluorescence microscopy is an emerging platform that enables high-throughput screening of bio-chips over a wide field-of-view without the use of any lenses, thin-film filters or mechanical scanners. In this review, we summarize the recent advances in lensfree fluorescence microscopy and also discuss some of its unique capabilities toward high-throughput screening applications, including rare-cell imaging, on-chip cytometry as well as micro-array research.

Introduction

Optical Microscopy has become an indispensable tool for many scientific disciplines especially in biomedical sciences. Although rapid advancements in modern microscopy techniques allow us to visualize microscale structures and processes in

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unprecedented details, they are still relatively bulky and low-throughput, necessitating a tedious mechanical scanning to image e.g., large-area microfluidic devices and biochips [1]. To provide an alternative imaging toolset for this high-throughput screening challenge of bio-chips, we have recently introduced an on-chip fluorescence imaging platform that can rapidly monitor fluorescently labeled cells or small animal models over an ultra-wide field-of-view (FOV) of e.g., $>9 \text{ cm}^2$ without the use of any lenses, thin-film filters or mechanical scanners [2–6]. This emerging lensfree fluorescence microscopy platform, achieving $<4 \text{ }\mu\text{m}$ spatial resolution, provides at least an order of magnitude larger FOV compared to a conventional $10\times$ objective lens and lends itself to a compact architecture that can easily be integrated with microfluidic chips for massively parallel screening of fluorescently labeled cells or small animals. This high-throughput lensfree fluorescence microscopy platform, combined with the state-of-the-art bio-chips, could pave the way toward rapid on-chip diagnostic systems for biomedical applications, including rare-cell research, high-throughput cytometry as well as micro-array analysis.

Materials and Methods

Our on-chip lensfree fluorescent imaging modality utilizes an excitation interface (e.g., a prism, a hemisphere or a planar waveguide) to pump the objects of interest located within a bio-chip, where the excitation light is mostly rejected through total internal reflection (TIR) occurring at the bottom facet of the sample holder (Fig. 2.1[a1, b1, c1]). In addition to TIR rejection, an inexpensive absorption filter is also used to remove the weakly scattered excitation light that does not obey the TIR process. Upon removal of the excitation, only the fluorescent emission from the objects is collected using e.g., free-space, fiber-optic-faceplate (FOF) or fiber-optic-taper (FOT) based optics, and is then delivered to a large-format sensor-array (e.g., CMOS or CCD) that has an active area of e.g., $>9 \text{ cm}^2$, which is also equivalent to the sample FOV. Finally, by using an image reconstruction method (e.g., deconvolution or compressive decoding), the detected lensfree fluorescent images are rapidly processed to yield higher-resolution microscopic images of the specimen across a wide FOV. Typical reconstructed images of this lensfree fluorescence microscopy platform are demonstrated in Fig. 2.1[a3, b3, c3], where $4\text{--}10 \text{ }\mu\text{m}$ diameter micro-beads are imaged using the lensfree on-chip imager.

Components of the Lensfree Fluorescence Imaging Platform

In this sub-section, we will discuss some of the key components of the lensfree on-chip fluorescence microscopy platform.

Excitation/Illumination Design: Fluorescently labeled specimen located within micro-fluidic devices can be probed with various illumination configurations: the

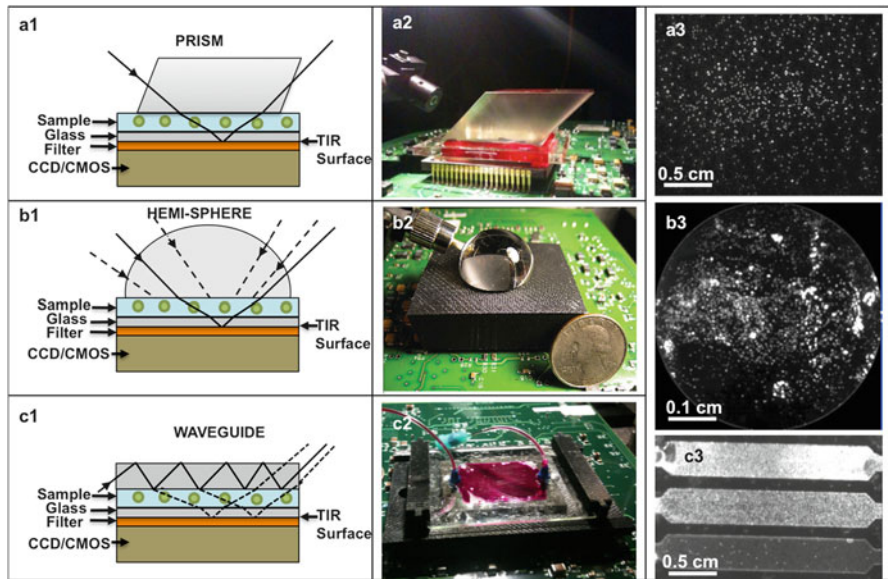


Fig. 2.1 The schematics (a1, b1, c1), corresponding experimental set-ups (a2, b2, c2) and typical wide-field lensfree fluorescence images (a3, b3, c3) of various excitation methods are shown

fluorescence excitation can be achieved through e.g., a prism (e.g., rhomboid, dove prisms – Fig. 2.1[a1, a2]), a hemi-sphere (Fig. 2.1[b1, b2]) or a waveguide (Fig. 2.1[c1, c2]), where incoherent sources such as simple light emitting diodes (LEDs) can be used to provide uniform illumination over a wide FOV.

Light Collection and Sampling: In this on-chip imaging platform, once the specimen is excited through one of the illumination methods presented above, the fluorescence emission is collected and is then delivered to an optoelectronic sensor-array. As for the collection of the fluorescence signal, three different configurations can be utilized, incorporating free-space, an FOF or alternatively an FOT (see Fig. 2.2).

Although free-space collection enables monitoring of bio-chips over a wide FOV, since the fluorescent emission is not directional and rapidly diverges, the detected raw lensfree images become rather broad at the sensor plane. Therefore, to better control the spatial spreading of fluorescent signal in our platform, we can employ a planar optical component, i.e. an FOF, which is located between the object and the sensor planes [3, 4]. A typical FOF (Fig. 2.2[b1, b2]) is composed of a 2D array of fiber-optic cables that carry two-dimensional optical intensity information from one plane to another. Its main function in lensfree imaging is to engineer the point-spread function (PSF) (Fig. 2.2[b3]) of the on-chip imager, improving the signal-to-noise ratio (SNR) and the spatial resolution of the microscopy platform. As an alternative to a regular FOF, an FOT (Fig. 2.2[c1, c2]) can also be used, which has a larger density of fiber-optic cables on its top facet compared to the bottom one [5]. FOT not only provides a better PSF (Fig. 2.2[c3]), but also achieves

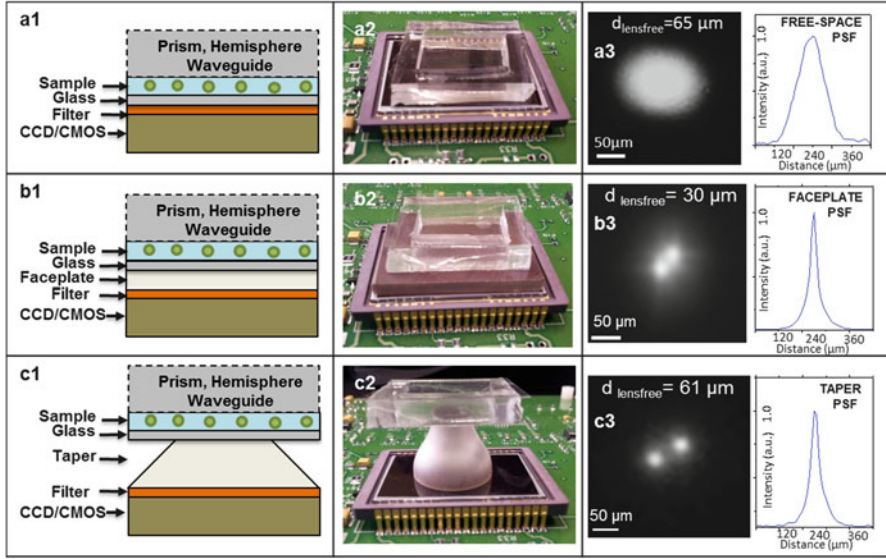


Fig. 2.2 The schematics (a1, b1, c1), corresponding experimental set-ups (a2, b2, c2), lensfree images and PSF analysis (a3, b3, c3) of the various light collection methods are shown

magnification in our platform (e.g., 2–3 \times), which further helps us to increase spatial resolution, despite the reduced FOV due to the taper geometry. Typical lensfree images of micro-particles and the PSFs of various configurations are demonstrated in Fig. 2.2[a3, b3, c3].

As for sampling of the fluorescent signal, once the emitted photons are transmitted through one of the collection methods described above, a sensor-array is used to digitize the fluorescence signal. For lensfree fluorescent imaging, CCD sensors can in general provide better sensitivity and larger FOV, while CMOS sensors can be employed for relatively cheaper and lighter weight designs (e.g., for field use).

Bio-Chip Design: To handle fluorescently labeled specimen, various bio-chip designs can be used, including glass-tape-glass based devices, PDMS (Polydimethylsiloxane)-channel-glass devices, or wide-area glass capillary arrays. One can select any of these device designs and then combine it with e.g., surface-chemistry protocols to achieve highly specific and sensitive on-chip lensfree fluorescence microscopy and/or biosensing that could potentially be useful for e.g., rapid detection of pathogens, sub-population of cells as well as molecular assays.

Reconstruction Methods

Lensfree fluorescence raw images look blurry due to diffraction, and therefore, to partially undo the effect of diffraction and create higher resolution microscopic

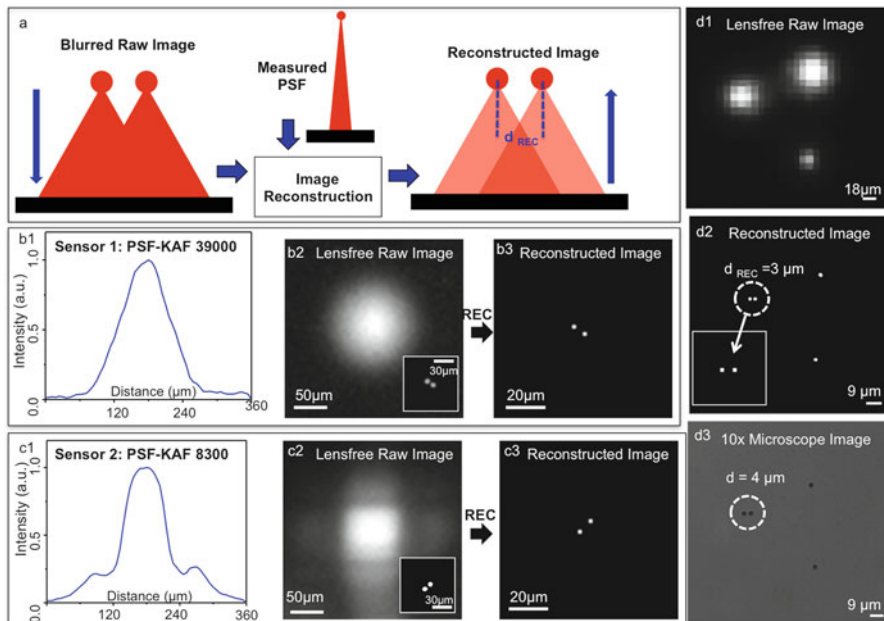


Fig. 2.3 The image reconstruction process (a) and PSFs of the different sensors (b1–3, c1–3) are shown. The resolving power of the imaging platform is quantified (d1–3)

images, these raw images are processed using image reconstruction methods, employing e.g., a *Lucy-Richardson deconvolution method* [2, 7, 8] or a *compressive sampling based decoding algorithm* [3, 9, 10]. Starting with an initial measurement of the incoherent PSF of the on-chip system, lensfree images are reconstructed within a few minutes (e.g., ~ 10 min for 9 cm^2 FOV using a standard PC – Fig. 2.3[a]). To quantify the spatial resolution, closely packed fluorescent bead pairs are reconstructed, verifying $< 4 \mu\text{m}$ spatial resolution based on an FOT collection platform (Fig. 2.3[d1, d2, d3]) [5]. Furthermore, to demonstrate the sensor independent performance of this platform, the PSFs of the two different CCD sensor-arrays are measured, showing a noticeable variance in their 2D patterns; however the reconstruction of closely packed fluorescent beads can still be achieved as illustrated in Fig. 2.3[b1–3, c1–3].

Results

This on-chip fluorescence imaging platform, combining a compact experimental set-up and rapid image reconstruction algorithms, together with its wide field imaging capability could especially be useful for high-throughput screening applications. To demonstrate its proof-of-concept, we performed experiments with

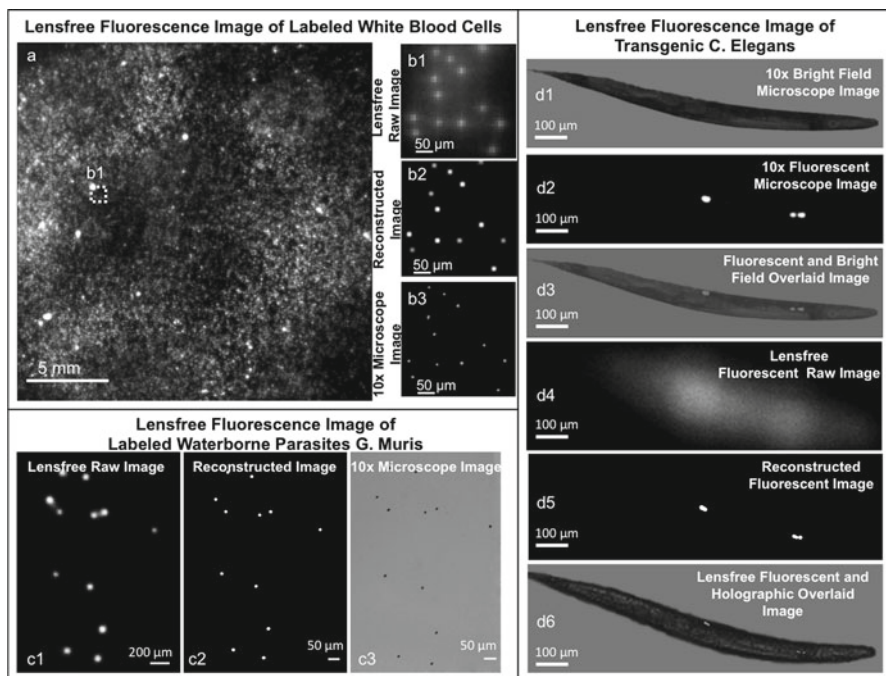


Fig. 2.4 Lensfree fluorescence images of white blood cells (**a**, **b1–2**), *G. muris* parasites (**c1–2**) and *C. elegans* (**d4–6**) are demonstrated, which agree well with 10× microscopic comparisons

bodily fluids (e.g., whole blood samples with labeled white blood cells) [6], water-borne parasites (e.g., *Giardia muris*) [5] as well as genetically modified small model animals (e.g., transgenic *Caenorhabditis elegans*) [4].

The results of these experiments are presented in Fig. 2.4 which also includes comparisons against lens-based conventional microscope images of the same specimen. In Fig. 2.4[a] wide-field fluorescence image ($\sim 9 \text{ cm}^2$ FOV) of labeled white blood cells are shown. Figure 2.4[b2] illustrates the reconstruction results of digitally zoomed images of some white blood cells (Fig. 2.4[b1]), providing a decent agreement to a conventional fluorescent microscope image (Fig. 2.4[b3]). For water quality applications, in Fig. 2.4[c1] lensfree imaging of water-borne parasites is also presented, with the reconstructed results and microscope comparisons as shown in Fig. 2.4[c2, c3], respectively. Finally, we also present lensfree imaging of a *C. elegans* sample in Fig. 2.4[d4–6].

Conclusions

In this chapter, we reviewed an emerging wide-field lensfree fluorescent imaging modality, achieving $<4 \mu\text{m}$ spatial resolution over a large FOV of e.g., $>9 \text{ cm}^2$, that can rapidly monitor the state-of-art microfluidic chips toward high-throughput

screening applications, including on-chip cytometry, rare-cell analysis as well as microarray research. The presented imaging platform can also leverage other techniques to further increase its spatial resolution, incorporating e.g., higher-magnification FOTs to increase the resolving power of the imager, pixel super-resolution approaches [11] by shifting the fluorescent specimen to effectively create smaller size pixels, or through the use of nano-structured surfaces [12] to spatially modify the PSF of the on-chip imager. Such a lensfree fluorescence imaging platform, combined with better optical components and computational approaches could in general be useful for wide-field imaging of bio-chips.

Acknowledgements Ozcan Research Group at UCLA gratefully acknowledges the support of the Presidential Early Career Award for Scientists and Engineers (PECASE), ARO Young Investigator Award, NSF CAREER Award, the ONR Young Investigator Award and the NIH Director's New Innovator Award DP2OD006427 from the Office of The Director, NIH. The authors also acknowledge the support of the NSF BISH program (under Awards # 0754880 and 0930501). S. A. Arpali acknowledges the partial financial support provided by the Scientific Technological Research Council of Turkey (TUBITAK) as a postdoctoral research scholarship.

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