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# Satellite Nanoscope and Cellular BioASICs for Quantitative Biomedicine

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***Abstract*** – This paper presents satellite nanoscopes and biological application specific integrated circuits (BioASICs). Satellite nanoscope is developed for spectroscopic imaging of living cells, molecular optogenetics (i.e. the remote control of gene regulation and protein expression), and therapeutic applications. Fundamental understanding of living cells via satellite nanoscopes will provide insights of molecular dynamics and signaling pathways in living cells. BioASICs are accomplished to permit precise and repeatable characterizations of cellular activity. These BioASICs are facilitating significant advancements in quantitative cell biology and the foundation for precision biology, cell reprogramming, high-speed biologic microprocessors, drug screening, molecular diagnostics, and personalized medicine.

***Keywords*** - Nanoscope, biophotonics, microfluidics, cell culture chip, NASBA, spectroscopic imaging, nanoplasmonics, ONCOS, molecular optogenetics, molecular diagnostics, quantitative biology, personalized medicine.

## I. INTRODUCTION

THIS is critical time to invent new tools to explore our inner life (i.e. living cells) in order to understand the functions of gene and proteins in living cells, and challenge to overcome the barriers of accomplishing quantitative biomedical science and personalized medicine for better healthcare.

Our understanding of biological systems is increasingly dependent on our ability to visualize and precisely measure the dynamics of molecular, biological, biophysical events with high spatial and temporal resolution, within the context of a living cell. In this regard, the development of biologically inspired optical systems for cellular and molecular imaging techniques are of considerable interest in many areas of research, from molecular and cellular biology to medical diagnostics and molecular medicine [1-3]. Label-free nanoplasmonic optical or nanomechanical probes offer multiple advantages over traditional molecular imaging techniques: stability, biocompatibility, selectivity, and spectroscopic imaging capability [4-13] By visualizing nanoplasmonic probes within a living cell, we obtain snapshots of what we refer to as the *cellular galaxy* (Fig. 1). By focusing on a specific probe within this "galaxy" we can probe localized biochemical structural and kinetic data from each of the nanoplasmonic light sources, which function as "satellite nanostars or nanoscopes" that can be explored in the living cellular environment (Fig. 2).

Moreover, we have accomplished a new remote control switch of gene interference in living cells by using oligonucleotides on a nanoplasmonic carrier-based optical switch (ONCOS), short interfering oligonucleotides, and a near-infrared (NIR) laser transmitter [14]. The ONCOS allows us to create new field called molecular optogenetics.

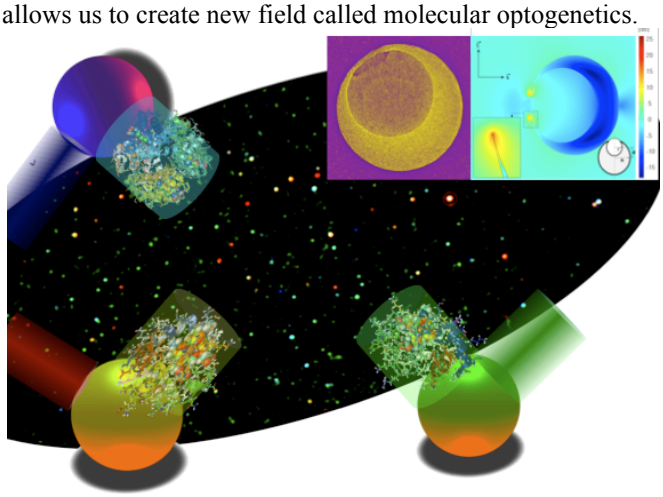

***Figure 1.*** *Satellite nanoscopes for label-free spectroscopic imaging of living cells: interfacing nanoscale biological space to the real world via nanoplasmonic optical antennas.*

In order to accomplish physiologically relevant high-throughout screening and *in vitro* diagnostic systems, we have developed Biological Application Specific Integrated Circuits (BioASICs) by connecting novel microfluidics and nanofluidic circuits [15-32]. The BioASICs can impact on high-speed and high-content precision biology, and quantitative medicine in new ways. We are creating a library of these "building blocks" to develop innovative single cell array, physiologically relevant dynamic cell culture array, and biological microprocessors with integrated optical controls and detections capability. As an example of quantitative real time nucleic acid detection and molecular diagnostics, Integrated Nucleic Acid Sequence-Based Amplification (*i*NASBA) is described [29]. Multiplexed iNASBA with a sample to answer platform can impact on systems biology as well as global healthcare research and technology. In this paper, we report on the development of satellite nanoscopes, BioASICs, and recent progress in molecular diagnostic applications of these areas.

## II. SATELLITE NANOSCOPES FOR LIVING CELLS

### *A. Nanocrescent Optical Antenna*

As the first example of satellite nanoscopes for living cell imaging, Lu *et al.* developed a novel gold nanocrescent optical antenna with a sub-10 nm sharp edge, which can enhance local electromagnetic field at the edge area (Fig. 2). The formation of unconventional nanocrescent structure (20-500 nm) is accomplished by using a sacrificial nanosphere template and conventional thin film deposition method, which allows an effective batch nanofabrication and precise controls of different resonant frequencies of nanocrescents for multiplexed wireless communications between inside and outside of living cells.

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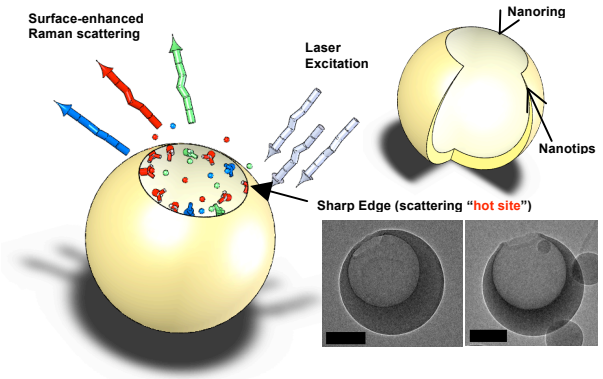


***Figure 2.*** *Nanocrescent optical antenna as a satellite nanoscope for spectroscopic imaging of living cells.*

The Raman scattering spectrum of molecules adsorbed on the single gold nanocrescent probes can be characterized and the Raman enhancement factor of single gold nanocrescent probe is estimated larger than $10^{10}$, which suggests the potential applications of gold nanocrescent probes in ultrasensitive biomolecular detection and cellular imaging using surface enhanced Raman spectroscopy.

## B. PRET

Recently we have discovered a new phenomenon called ***Plasmonic Resonant Energy Transfer*** (PRET) between a biomolecule and a nanoplasmonic particle.

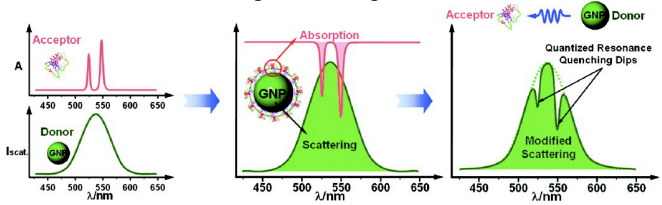


***Figure 3.*** *The biophysical principle of PRET for quantized plasmon quenching dips nanospectroscopy.*

When we match the optical window of plasmonic resonance spectrum of nanoplasmonic probe and the absorption spectrum of molecules, the mirror image of absorbance spectrum of biomolecules results in a dip in the scattering spectra due to PRET (Fig. 3). As a new innovative molecular imaging technique, PRET overcomes many of the limitations of conventional fluorescence microscopy, such as photobleaching and molecular labeling. Label-free PRET nanospectroscopy is capable of acquiring dynamic chemical information within a living cell, with nanoscale spatial (<20 nm) and fast temporal (<1 ms) resolution. By matching the electronic transition energy of a target biomolecule to the resonance frequency of a nanoplasmonic particle, multiplexed PRET probes that measure the kinetics of specific molecules can be accomplished (Fig. 4).

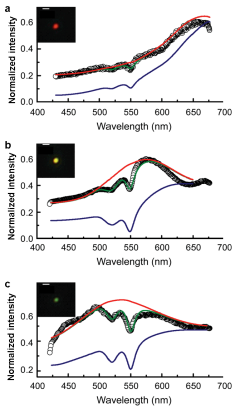


***Figure 4.*** *The PRET spectrum from different nanoplasmonic probes with cytochrome c (reduced).*

## III. BioASICs

As semiconductor-based application specific integrated circuits (ASICs) have been embedded into everyone's life in personal computers, cell phones and numerous consumer-based electronics, we envision customized BioASICs for a particular biomedical use can make impact on quantitative biology, molecular diagnostics, personalized cancer diagnosis and treatment. So far we have developed patch clamp array [15], single cell analysis [16-21], cell-cell communications on a chip [22], dynamic cell culture array [23-25], open access microfluidic patch-clamp array [26], single cell electroporation array with feedback control [27], artificial liver sinusoid for primary hepatocyte culture on microfluidic chip [28], integrated NASBA with sample preparation [29], and integrated optofluidics for Raman activated cell sorting device [30]. Few examples of above BioASICs are highlighted as following.

### A. Integrated Microfluidic Patch-clamp Array Chip (IMPAC)

To improve visualization and the control of cell position we have developed a simple and effective patch-clamp device based on microfluidic junctions between a main chamber and lateral recording capillaries using PDMS [15]. The geometry of the recording capillaries permits high quality stable whole-cell seals despite the hydrophobicity of the PDMS surface.

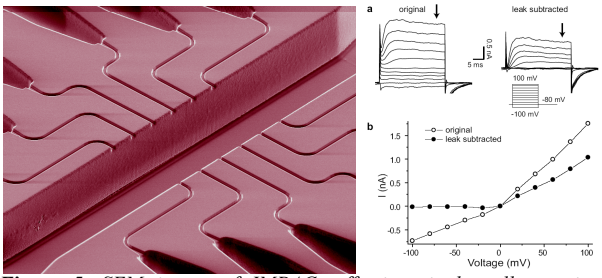


***Figure 5****. SEM image of IMPAC, effective single cell trapping, and electrophysiological characterizations of voltage sensitive potassium channel Kv2.1.*

### B. Single Cell Electroporation Chip

Khine *et al.* demonstrated the effectiveness of using a PDMS microfluidic device with individual lateral cell trapping sites to selectively and locally electroporate single cells in parallel [16]. This easy-to-use chip focuses the electric field and therefore achieves dielectric breakdown of the cell membrane using low applied voltages (< 1.0 *V*). This design also enables the electrodes to be placed a distance from the cell, eliminating the need to incorporate electrodes on chip and the potential of adverse products from electrode reactions.

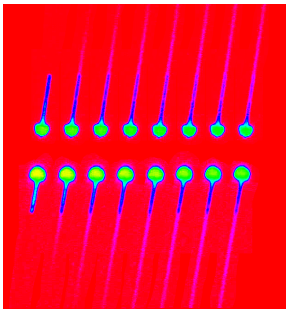

***Figure 6.*** *Single cell electro oration array: resealing is achievable and this electro oration array provides an efficient means of introducing otherwise impermeant material, such as drugs, DNA, and protein, into individual cells.*

### C. Dynamic Single Cell Array

Di Carlo *et al.* reported a high-density single cells array (Fig. 7), which can isolate singe cells in a purely hydrodynamic fashion within a microfluidic devices allowing uniform convective reagent control and reduced image processing [18]. Cell loading is quick (<30 s) and easy to perform. Using this technology, we reported novel data on the single-cell concentration distribution of carboxylesterases within three different human cell lines, as well as on the inhibition of intracellular esterases by the nonspecific inhibitor nordihydroguaiaretic acid (NDGA). These types of analyses are enabled by interrogating spherical suspended cells with known volumes as opposed to adherent cells.

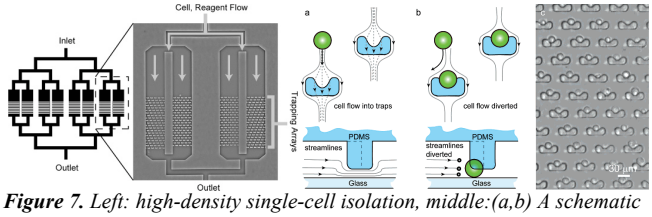


***Figure 7.*** *Left: high-density single-cell isolation, middle:(a,b) A schematic diagram of cell trapping using flow-through arrayed suspended obstacles. (c) A phase contrast image of an array of single trapped cells is shown.*

### D. Cell-Cell Communication Array

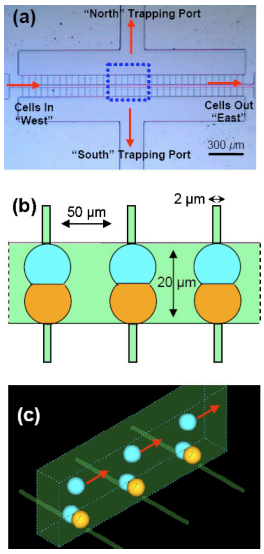


***Figure 8.*** *(a) Layout of micro fluidic cell-trapping device. (b) The trapping design allows two independently controlled sets of channels for localizing different cell populations either to the north or south trapping sites. (c) Three-dimensional schematic view depicting channel geometry, trapped cells, and cell flow.*

Current biological techniques are difficult to scale up for high-throughput screening of cell-cell communication in an array format. In order to provide an effective biophysical tool for the analysis of molecular mechanisms of gap junctions that underlie intercellular communication, Lee *et al.* have developed a microfluidic device for selective trapping of cell-pairs and simultaneous optical & electrical characterizations (Fig. 8) [22].

Two different cell populations can be brought into membrane contact using an array of trapping channels with a 2 $\mu$m by 2 $\mu$m cross section. Device operation was verified by observation of dye transfer between mouse fibroblasts (NIH3T3) placed in membrane contact (Fig. 9).

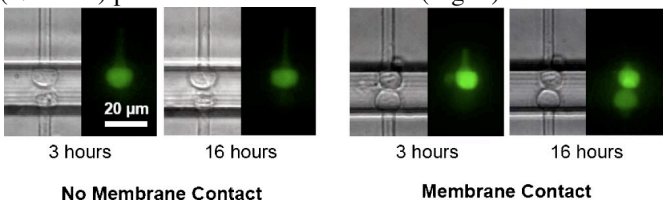


***Figure 9.*** *Diffusion of intracellular dye between NIH3T3 fibroblasts in membrane contact.*

Integration with lab-on-a-chip technologies offers promising applications for cell-based analytical tools such as drug screening, clinical diagnostics, and soft-state biophysical devices for the study of gap junction protein channels in cellular communications. Understanding electrical transport mechanisms via gap junctions in soft membranes will impact quantitative biomedical sciences, systems biology and clinical applications.

### E. Dynamic Microfluidic Cell Culture Array

Lee *et al.* have demonstrated a nanoliter scale microbioreactor array was designed for multiplexed quantitative cell biology [25]. An addressable 8x8 array of three nanoliter chambers was demonstrated for observing the serum response of HeLa human cancer cells in 64 parallel cultures (Fig. 10). The individual culture unit was designed with a ''C'' shaped ring that effectively decoupled the central cell growth regions from the outer fluid transport channels. The chamber layout mimics physiological tissue conditions by implementing an outer channel for convective "blood" flow that feeds cells through diffusion into the low shear "interstitial" space.

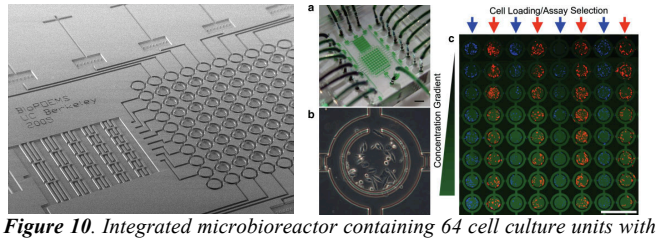


***Figure 10****. Integrated microbioreactor containing 64 cell culture units with concentration gradient generator.*

Scale up and automation of the device to perform 96 and 384 well assays is currently in progress. Application of this platform for multiparametric dynamic cell response quantification can potentially close the gap in cell level integration for systems biology [31].

### F. Artificial Liver Sinusoid

Lee *et al.* have implemented a physiologically relevant microfluidic design for primary cell culture [28]. This microscale culture unit mimics many of the mass transport properties of the functional liver sinusoid such as extensive cell-cell contact, defined tissue and fluid transport regions, and continuous nutrient exchange (Fig. 11). Primary rat and human hepatocytes were maintained in this format for over 7 days without diminished viability while retaining metabolic activity.

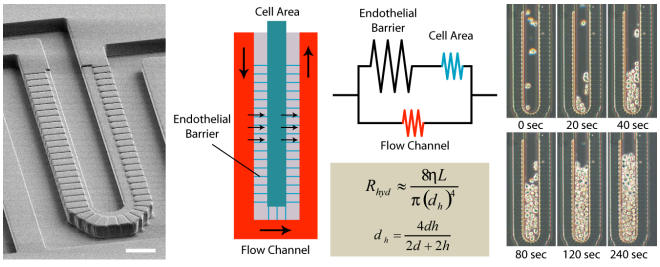


***Figure 11.*** *Microfluidic endothelial barrier properties. (a) SEM micrograph depicting the microfluidic sinusoid unit. (b) Mass transport properties of the microfluidic liver sinusoid. The transport properties of this flow were modeled using an equivalent resistance circuit. (c) Hepatocyte loading into the artificial sinusoid. Rat hepatocytes ($10^6$ cells/ml suspension) were introduced via the cell inlet channel.*

Continued development of this microfluidic cell culture method may prove useful for providing a better experimental platform for studying primary cells, stem cells, cancer stem cells, and tissue behavior.

## IV. Conclusion

We have demonstrated satellite nanoscope and PRET probes that can capture the kinetics of specific molecules. We also accomplished more than twelve different BioASICs to solve different biological experiments. As ASICs are mainly developed as an electrical engineering category, BioASICs could potentially open a new field in bioengineering. This will revolutionize the *in vivo* molecular imaging of living systems for quantitative biology, medical diagnostics, drug discovery, therapeutics, and personalized medicine.

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