SINGLE CELL GENOMICS

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An exciting emerging area revolves around the use of microfluidic tools for single-cell genomic analysis. We have been using microfluidic devices for both gene expression analysis and for genome sequencing from single cells. In the case of gene expression analysis, it has become routine to analyze hundreds of genes per cell on hundreds to thousands of single cells per experiment. This has led to many new insights into the heterogeneity of cell populations in human tissues, especially in the areas of cancer and stem cell biology. These devices make it possible to perform "reverse tissue engineering" by dissecting complex tissues into their component cell populations, and they are also used to analyze rare cells such as circulating tumor cells or minor populations within a tissue.

We have also used single-cell genome sequencing to analyze the genetic properties of microbes that cannot be grown in culture—the largest component of biological diversity on the planet—as well as to study the recombination potential of humans by characterizing the diversity of novel genomes found in the sperm of an individual. We expect that single cell genome sequencing will become a valuable tool in understanding genetic diversity in many different contexts.

SINGLE CELL GENE EXPRESSION ANALYSIS BY RNA SEQUENCING COUPLED WITH SINGLE CELL CDNA LIBRARY ARRAY

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We have developed a gene expression analysis method using a single cell cDNA library on beads for qPCR and RNA sequencing. Although the method gives very accurate gene expression profiles even for genes expressed at low levels, the analyzable number of cells is limited. For analyzing so many cells by sequencing, we need a new tool. We have developed a new tool for producing a single cell cDNA library array coupled with the bead technology. It consists of many reaction chambers containing beads immobilizing capture probes to produce cDNA libraries. The produced single cell cDNA libraries are got together for sequencing. The number of reaction cell can be increased tens of thousands which may be enough for various applications of single cell gene expression analyses. The detail of the device together with the result will be presented in the workshop.

STRATEGY FOR HIGH-THROUGHPUT SINGLE-CELL GENOMIC SEQUENCING

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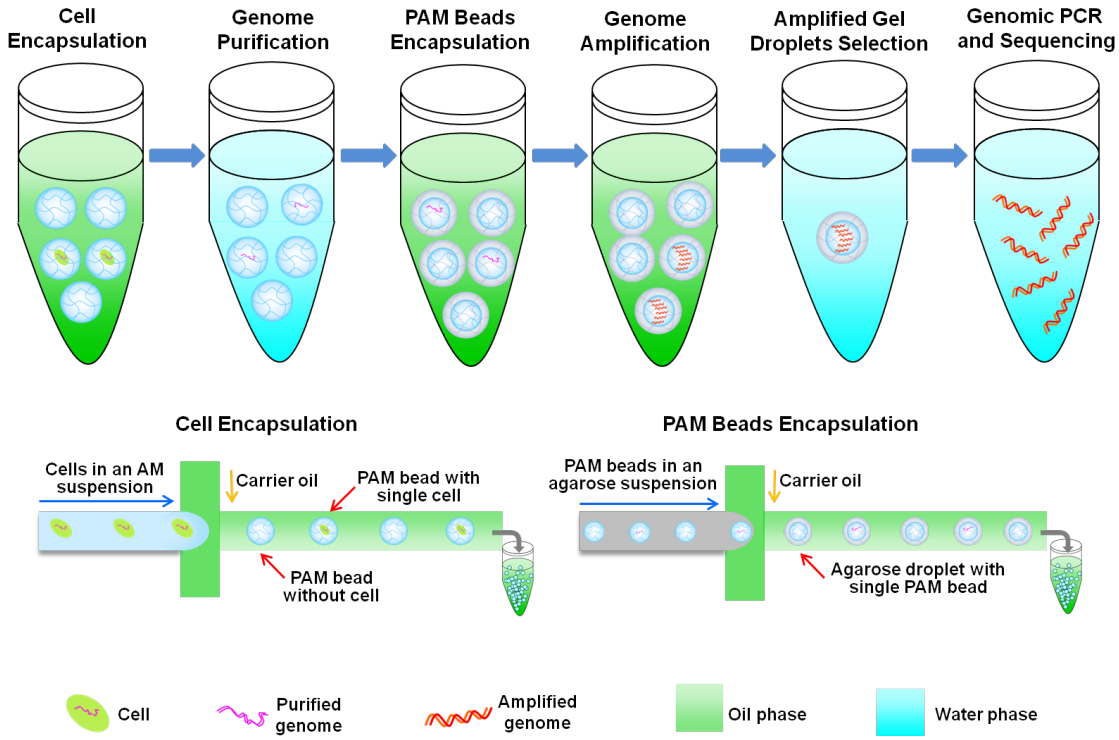
The need is well-recognized for performing whole genome analysis (WGA) for single cells to understand heterogeneity in cell communities. In spite of this fact, this challenge has not been fully met owing to difficulties in achieving full coverage and the burden of removing all DNA contamination from reagents and from the handling process. We outline below a procedure we are exploring to overcome these problems:



Scheme 1. Workflow diagram of strategy for high-throughput single-cell genomic sequencing

The steps are the following: (1) Individual cells are microfluidically encapsulated in poly-acrylamide(PAM) gel beads; (2) After removing the oil, the PAM gel beads are dispersed in a standard cell lysis solution to release the genome of single cells; (3) Single PAM gel beads are microfluidically encapsulated in the agarose gel droplets with genome amplifying reaction solution; (4) After genome amplification, single-cell genomes are amplified in the single agarose gel droplets; (5) The gel droplets with amplified genome are selected; (6) Following the another step of genomic amplification, the genomic amplified products are sent to genomic sequencing. AM= acrylamide; PAM= polyacrylamide.

ULTRA-LOW INPUT WHOLE-GENOME BISULFITE SEQUENCING: A STEP TOWARD SINGLE CELL METHYLOME ANALYSIS

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Background: DNA methylation plays a key role in epigenetic regulation of eukaryotic genomes. Hence the genome-wide distribution of 5-methylcytosine, or the methylome, has been attracting intense attention. In recent years, whole-genome bisulfite sequencing (WGBS) has enabled methylome analysis at single-base resolution. WGBS is rapidly becoming the method of choice; the International Human Epigenome Consortium decided to use it to collect the methylome data. While WGBS has been proven to be powerful, the current method has some practical limitations: it typically requires ~5 μg of DNA (*i.e.*, ~10^6 mammalian cells) as well as global PCR amplification. These features not only preclude its application to samples of limited amounts (*e.g.*, mammalian eggs, early embryonic tissues, and stem cells), but also invite the risk of skewed representation that may lead to inaccurate estimation of the methylation level. It would therefore be ideal to have a PCR-free method that is applicable to a minute amount of DNA.

Results: We assumed that the inefficiency of WGBS is presumably because bisulfite treatment of adaptor-tagged templates, which is inherent to current protocols, leads to substantial DNA fragmentation. To circumvent this loss, we conceived an alternative method termed Post-Bisulfite Adaptor Tagging (PBAT) wherein bisulfite treatment precedes adaptor-tagging. PBAT can be achieved by two successive rounds of random primer extension on bisulfite-treated genomic DNA. The method can start with as little as 125 pg of DNA (*i.e.*, ~20 mammalian cells) to generate a substantial number of unamplified reads. It typically requires only ~30 ng of DNA for amplification-free, 30-fold coverage of mammalian genomes. Indeed, it has been successfully applied to WGBS from 1,000 mouse eggs, notably, without using any global PCR amplification.

Conclusions: The PBAT method has enabled various novel applications that would not otherwise be possible. With a little help from microdevice technology and minimal amplification, it will provide a step toward the single-cell methylome analysis.

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QUANTITATIVE ASPECTS OF 3D SUPER-RESOLUTION IMAGING IN CELLS WITH SINGLE MOLECULES

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Using the fact that light acts at a distance with little perturbation, single-molecule fluorescence imaging has enabled a wide array of biophysical measurements in living and fixed cells. In particular, the single-molecule emitter can also act as a nanoscale light source which yields enhanced spatial resolution beyond the diffraction limit when combined with optical control of the concentration of the single emitters. The methods of PALM/STORM etc. build first upon the ability to image a single molecule[1], combined with a broad array of methods for controlling the emitting concentration such as photoactivation, photoswitching, blinking, enzymatic conversion of a fluorogen, etc.[2]. For example, using the native photoinduced blinking and switching of enhanced yellow fluorescent protein[3] we achieved sub-40 nm precision super-resolution imaging of a variety of protein structures in the bacterium *Caulobacter crescentus*[4][5]. With a fluorescently-labeled toxin, the locations of voltage-gated sodium channels on the surface of neuronal cells can be observed in near-real time[6]. In terms of methods, our scheme for 3D imaging based on a double-helix point spread function (DH-PSF) enables quantitative tracking of single mRNA particles in living yeast cells with 15 ms time resolution and 25-50 nm spatial precision[7], and this approach has been used to define the 3D spatial structure in bacterial[8] and mammalian cells[9]. Using a precise image registration protocol and fixed phase masks, multicolor 3D super-resolution colocalization of several different protein superstructures can now be achieved[10]. With polarization-resolved microscopy, the DH-PSF provides a method to reduce localization errors for orientationally restricted single-molecule labels[11]. The degree of localization error depends critically upon the orientational freedom of the single-molecule labels, a property that should be optimized for highest accuracy[12]. The examples provided here illustrate some of the challenges and opportunities where the power of optics and lasers applied to single-molecule microscopy can lead to new insights into the behavior of cellular systems.

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SINGLE CELL GENETIC AND FORENSIC ANALYSIS USING NANOLITER DROPLET MICROFLUIDICS

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Single cell genetic analysis using nanoliter emulsion droplet technology offers the ultimate in digital sensitivity and an unprecedented view of the characteristics of cell populations that are masked by the ensemble average. In many cancers, small subpopulations of distinct cell types cause or drive the disease but are difficult to study using conventional biomolecular methods. In forensics, the ability to type individual cells and to analyze cellular mixtures cell-by-cell offers unprecedented identification capabilities. To enable this new analysis technology, we have developed microfluidic droplet generators that produce highly uniform 1-5 nL droplets at very high production rates that are subsequently used to perform PCR and RT-PCR analyses [1]. A significant challenge is the release of genomic DNA from single cells in these nanoliter droplets. This challenge has been addressed by encapsulating cells together with primer functionalized beads in nanoliter agarose droplets which can then be used as porous carriers for sequentially performing cell lysis and cleanup followed by microemulsion PCR [2]. The subsequent analysis of the individual beads by flow cytometry, capillary electrophoresis or sequencing techniques then reveals the target presence, size or sequence, as desired. Specifically, in one application we have developed a method for high-throughput single cell DNA purification and detection of the t(14;18) translocation, an approach for investigating the frequency of rare mutation events within individuals prior to the onset of clinical symptoms. In a second application, methods have been developed for performing a 9-plex short tandem repeat amplification in the nanoliter emulsion droplets that provides high fidelity forensic typing of single cells in cellular mixtures [3]. These single cell techniques enable the quantitative study of cellular behavior with unprecedented sensitivity that should lead to a better mechanistic understanding of cellular processes such as cancer onset and progression. The application of single-cell analysis to forensics should similarly revolutionize human identification with digital sensitivity and unprecedented mixture analysis capabilities.

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MAPPING PROTEINS AND PROTEIN INTERACTIONS IN SINGLE CELLS

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Analysis of molecular functions within cells, and of crucial differences between cells, necessitate molecular analyses at single cell levels. Our group is developing highly resolving molecular tools for analyses at levels of DNA, RNA and protein. In particular we establish proximity assays for protein level analyses. The assays permit sensitive measurement of levels of specific proteins and protein interactions or modifications, or imaging the distribution of these targets between and within cells.

Briefly, proximity ligation assays (PLA) employ affinity reagents such as antibodies, directed against proteins or their posttranslational modifications, and equipped with strands of DNA. Upon coincident binding by pairs of affinity reagents to target molecules, the attached DNA strands are brought in proximity and can undergo ligation or polymerization reactions to give rise to amplifiable reporter DNA molecules.

The reactions can be designed to produce circular DNA strands that template local rolling circle amplification reactions (in situ PLA). This permits digital detection of individual proteins or protein complexes, and several targets can be interrogated in parallel, further illuminating molecular events during signaling at the single cell level. We have established assays for interactions and modifications of proteins during signaling cascades, in order to assess activation of cells by TGFb and other stimuli, and the assays serve to monitor responses to targeted therapy.

We also perform highly sensitive parallel detection of around 100 proteins via proximity extension assays to analyze expression levels of proteins in lysates from single isolated cells, and we compare the levels of protein to mRNA measurements from these same cells. This research is part of a larger program to investigate therapeutic opportunities in malignancy, where small groups or even individual cells are treated with drugs and assessed for responses at mRNA and protein levels.

CELL FUNCTION MANIPULATION USING MEMS DEVICES: FROM CELLS TO SHEETS

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Advances in tissue engineering have opened exciting new possibilities to generate tissues for replacement therapy. Recently it has become possible to generate tissues and organs in vitro using scaffolds made from biomaterials, and embedded with cells from the body, as templates to guide the growth of new tissues. However, upon implantation, the small pore size of most scaffold materials hinder immediate interaction between cells grown on the scaffold and host cells, resulting in poor grafting. To overcome this limitation, in this study we are exploring a new platform for continuous cell culture and cell sheet formation using large-mesh scaffolds that would allow for direct cell-cell contact when implanted.

Using microfabrication technology, we fabricated a large-mesh scaffold with micro-strands (5 mm in width) interspaced with 100~200 mm wide open spaces (4-5 times larger than a normal round cell) and used it to culture cells. The meshed scaffold was suspended in a culture medium and then seeded with epithelial cells (mouse embryonic fibroblast cells (MEF cells) and TIG120 cells). While a majority of cells would fall off to the dish bottom during seeding, a few that managed to attach on the micro-strands underwent elongation and proliferation, expanding dynamically to cover the open spaces of the scaffold to generate a nearly monolayer cell sheet after 2 weeks of culture. Stunningly, mouse embryonic stem cells (mESCs) grown on the scaffold also showed a similar trend, forming a sheet-like structure that was distinctively different from the typical embryoid body that these cells usually form when cultured on a culture dish.

Considering that the sparse scaffold provides less attachment area, the observed cell sheet formation is largely due to cell-cell adhesion and interconnectivity. Thus, the generated cell sheets are expected to have improved mechanical strength and thus easier to handle compared with those formed on a culture dish. Future developments include generating cell sheets of different cell types and incorporating them in a microfluidic platform to develop tissues/organ-on-chip models for functional characterization of body tissues and organs.

MICROENGINEERED DEVICES FOR ADVANCING PRECLINICAL AND CLINICAL RESEARCH

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Crucial to understanding the molecular processes controlling cell behavior is the ability to experimentally influence and analyze single cells. The Allbritton Group has pioneered a number of analytical tools that provide the experimentalist with unprecedented opportunities for cell manipulation and analysis down to the single-cell level. One focus of the research program is directed at assay platforms that enable the selection, interrogation, and lysis of single cells followed by chemical separation of each cells' contents. The platforms can now perform fast serial measurements of single cells and hold the promise multiplexed assays of signal transduction pathways in a high-throughput manner. A key aspect to this research is the development of a variety of single-cell biochemical probes that report the enzymatic activity of kinase, lipases, and proteases with the end goal of creating clinical diagnostic and prognostic assays in patients. Another focus area is the creation of a variety of microengineered platforms for cell manipulation and analysis. High density arrays comprised of transparent, microfabricated, releasable elements enable the culture, analysis and subsequent isolation of cells or colonies. The arrays bring the power of mating cell sorting capabilities to high-content-screening platforms. Applications for this technology are envisioned for sorting of stem cells, capture of circulating tumor cells, creation of cell lines and other uses. In addition, μTAS systems are being combined with advanced stem-cell culture systems to develop organ-on-a-chip platforms that will enable exquisite experimental control and detailed study of the intestinal stem cell niche and stem cell differentiation.

SINGLE CELL LIPIDOMICS WITH IMAGING MASS SPECTROMETRY

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SIMS is a powerful method for imaging biological samples with high spatial resolution. We have focused a great deal of effort on single cell experiments aimed at new developments in neuroscience. Here, SIMS will be presented with complementary data from single cell electrochemistry experiments related to neurotransmitter release via exocytosis.

First, neurons isolated from Aplysia californica, an organism with a well-defined neural network, were imaged with secondary ion mass spectrometry, C60-SIMS. A major lipid component of the neural membrane was identified as 1-O-hexadecyl-2-oleoyl-sn-glycero-3-phosphocholine [PC(16 : 0e/18 : 1)] using tandem MS. The assignment was made directly off the sample surface using a C60-QSTAR instrument; a prototype instrument that combines an ion source with a commercial ESI-MALDI mass spectrometer. Normal phase liquid chromatography mass spectrometry (NP-LC-MS) was used to confirm the assignment. The mass spectrometry images reveal heterogeneous distributions of intact lipid species, PC(16 : 0e/18 : 1), vitamin E and cholesterol on the surface of a single neuron. These might be involved in the immune response.

Second, we are using the small spot size of the bismuth ion source with an aim to examine cells where the internal vesicles have been exposed. In mast cells, these vesicles can average 700 nm and in PC12 cells around 150 nm. The goal of this work is to develop the technology to where we can delineate between the protein dense core in the vesicle and the halo of solution around the dense core. We have begun by looking at liposome models and are developing an artificial protein core in these models to mimic the cellular vesicle. This work will have impact on our understanding of transmitter storage in cells and vesicles.

Third, we have used small electrodes to measure neurotransmitter release from single cells, a method that has become commonplace after twenty years. Here, we have analyzed amperometric peaks corresponding to release at PC12 cells and found stable plateau currents during the decay of the peaks (post-spike feet), indicating closing of the vesicle after incomplete release of the vesicular content. From careful analysis of these data, we propose a process for most exocytosis events where the vesicle partially opens to release transmitter and then closes directly again, leaving the possibility for both a stable pre and post spike feet to be observed with amperometry. This strongly suggests that the dynamics of vesicle opening, controlled largely by lipids, might be an important characteristic in neurotransmission.

ASSESSING CLONAL HETEROGENEITY AT THE SINGLE CELL LEVEL IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Clonal evolution is a key feature of cancer progression and relapse. Recent studies across cancers have demonstrated the extensive degree of intratumoral heterogeneity present within individual cancers. We hypothesized that evolutionary dynamics contribute to the variations in disease tempo and response to therapy that are highly characteristic of chronic lymphocytic leukemia. We have recently investigated this phenomenon by developing a pipeline that estimates the fraction of cancer cells harboring each somatic mutation within a tumor through integration of whole-exome sequence (WES) and local copy number data (Landau et al., *Cell* 2013). By applying this analysis approach to 149 chronic lymphocytic leukemia (CLL) cases, we discovered earlier and later cancer drivers, uncovered patterns of clonal evolution in CLL and linked the presence of subclones harboring driver mutations with adverse clinical outcome. Ongoing studies seek to directly characterize individual evolving cells within subpopulations by using a microfluidics-based platform that integrates detection of mutations and quantitation of mRNA expression at the single cell level. As CLL is a disease marked by a high degree of variability across patients and within patient tumors, only by delineating the distinct cell populations at the level of mutations and transcriptional profiles can we address important questions about the biological mechanisms underlying the competition and evolution of genetic subclones.

THE SECRET LIVES OF SINGLE CELLS

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The transcriptional landscape of single cells is quite complex showing many types of variability. Data distinguishing this variability and it's biological function will be presented. Particular emphasis will be placed on assessing cytoplasmic intron retaining transcripts (CIRTs) that have been found in single cell cytoplasmic mRNA populations and methodologies for assessing the functional role of these CIRTs. Understanding of multigenic RNA abundances requires a functional genomics approach that permits the analysis of multiple mRNAs simultaneously with an ability to alter mRNA abundances. With this goal in mind we have developed procedures for RNA transfection that have permitted an analysis of cellular phenotype (TIPeR, transcriptome induced phenotype remodeling) and elaboration of the dynamics of *in vivo* mRNA translation. Further we have worked towards development of the transcriptome *in vivo* analysis (TIVA) methodology for analysis of individual and populations of RNAs within live cells in their natural microenvironment. *In toto* these experimental and theoretical approaches have facilitated unprecedented insight into how cells modulate transcriptome variability to create stable interacting cellular systems.

APPROACHES THAT ENABLE THE CELL BY CELL NEUROCHEMICAL CHARACTERIZATION OF THE BRAIN

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In the postgenomic era, one expects the suite of chemical players in a brain region to be known and their functions uncovered. However, many cell-to-cell signaling molecules remain poorly characterized and for those that are known, their localization and dynamics are oftentimes unknown. Significant challenges in characterization of intercellular signaling molecules arise in part from their large chemical diversity and broad range of concentrations. Neurotransmitters and neuromodulators vary from small gaseous molecules such as nitric oxide to larger peptides that are only bioactive with particular posttranslational modifications. The enormous biochemical complexity of nervous system where even adjacent cells often have very different and dynamic metabolic profiles necessitates development and application of technologies capable characterization of the neurometabolome on the individual cell and even the subcellular level.

Here, we present a suite of bioanalytical approaches that allow the investigation of individual neurons. These approaches include capillary electrophoresis with laser induced fluorescence and mass spectrometric detection, and direct mass spectrometric-based profiling and imaging. Several applications of single cell microanalysis are highlighted: investigating novel indolamine neurochemistry, determining the role of d-Aspartate in the brain, and characterizing the peptides in single cells. Specifically, new serotonin-related compounds and literally hundreds of new neuropeptides have been characterized in well-defined neuronal networks, and in several cases, the functional roles of these molecules described. Discovery of new neurochemical pathways often relies not only on structural information provided by traditional mass spectrometry but also requires knowledge on the spatial and temporal dynamics of these signaling molecules in the brain. Imaging mass spectrometry and dynamic sampling of the extracellular environment are used for elucidating novel cell to cell signaling molecules in a range of neuronal model systems. Current work involves extending the depth of metabolome coverage and adapting these analytical approaches to higher throughput cell assays. Our overarching goal is to uncover the complex chemical mosaic of the brain and pinpoint key cellular players in physiological and pathological processes.

AT THE NANO-BIO INTERFACE: PROBING LIVE CELLS WITH NANO SENSORS

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The rapidly evolving field of nanotechnology creates new frontiers for biological sciences. Recently, we and other groups show that vertical nanopillars protruding from a flat surface support cell survival and can be used as subcellular sensors to probe biological processes in live cells. In particular, we are exploring nanopillars as electric sensor, optical sensors, and structural probes. As an electric sensor, nanopillars electrodes offer several advantages such as high sensitivity, subcellular spatial resolution, and precise control of the sensor geometry. A sensitive measurement of cellular electrical activities requires strong coupling between the cell membrane and the recording electrodes. We found that nanopillars electrodes deform the cell membrane inwards and induce negative curvature when the cell engulfs them, leading to a reduction of the membrane-electrode gap distance and a higher sealing resistance. The 3D topology of the nanopillars electrodes is crucial for its enhanced signal detection. A new approach explores nanoelectrodes of a new 3D geometry, namely nanotubes with hollow centers. The nanotube geometry further enhances membrane-electrode coupling efficiency and also significantly increases the time duration of intracellular access. Interestingly, nanopillars serve as focal adhesion points for cell attachment. The presence of high membrane curvature induced by vertical nanopillars or nanotubes affects the distribution of curvature-sensitive proteins. Those studies show a strong interplay between biological cells and nano-sized sensors, which is an essential consideration for future development of interfacing devices.

A DEFINED "STRUCTURE" FOR THE IMMUNE SYSTEM THAT REFLECTS IMMUNE SURVEILLANCE & MECHANISTIC PROCESSES

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Analysis of molecular functions within cells, and of crucial differences between cells, necessitate molecular analyses at single cell levels. Our group is developing highly resolving molecular tools for analyses at levels of DNA, RNA and protein. In particular we establish proximity assays for protein level analyses. The assays permit sensitive measurement of levels of specific proteins and protein interactions or modifications, or imaging the distribution of these targets between and within cells.

Briefly, proximity ligation assays (PLA) employ affinity reagents such as antibodies, directed against proteins or their posttranslational modifications, and equipped with strands of DNA. Upon coincident binding by pairs of affinity reagents to target molecules, the attached DNA strands are brought in proximity and can undergo ligation or polymerization reactions to give rise to amplifiable reporter DNA molecules.

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SINGLE CELL GENOTYPING AND CLONAL COMPETITION IN MYELOID LEUKEMIA

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Natural selection and evolution drives the process of cancer. The importance of clonal evolution, the selection for and emergence of increasingly malignant clones of cells in cancer metastasis and relapse has been highlighted recently for a variety of cancers. Recent work has employed next generation sequencing (NGS) to quantify changes in mutant allele frequencies over the course of therapy, including comparing diagnosis to relapse [1-4], and between primary tumor sites and metastases [5-7]. These allele frequencies of wild type and mutant genes have been extrapolated to describe clonal distributions within tumors via a set of basic assumptions regarding the heterozygosity of mutations that are sequentially acquired and maintained throughout the evolution of the disease [1]. We have recently performed mutation genotyping on bulk and single cells from cases of acute myeloid leukemia. Our data suggest the presence of far more complex clonal distributions than previously assumed. We identify concurrent mutations and the zygosities of the mutations in each of three genes frequently mutated in AML using multiplex PCR and fragment analysis. Using this sensitive, but relatively simple technique, we can describe the true clonal distribution in the entire leukemic cell population with respect to these three mutations by directly determining which mutations occur simultaneously, rather than inferring it mathematically from the data derived of examining thousands of cells. Our data suggest that for AML, previous assumptions regarding mutations occurring in a heterozygous state, and in a pattern consistent with the sequential acquisition of mutations forming a clonal "tree" are not universally applicable. This work highlights the caveats in using bulk samples and NGS data to mathematically infer clonal distributions and subsequently describe clonal evolution in tumors. However, the data lend strong support to the prediction of existing NGS data of the existence of far more complex clonality in AML than a single leukemic clone. The documentation of a complex clonal structure in cancer has obvious implications in treatment options, particularly the concept of "targeted" therapy.

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NANOVELCRO-EMBEDDED MICROCHIPS FOR RARE CELL BIOLOGY: PRACTICAL APPLICATIONS IN ONCOLOGY AND OB/GYN CLINICS

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Our research team at UCLA has demonstrated a highly efficient cell-affinity assay (known as NanoVelcro MicroChip) capable of detecting and isolating rare cells, e.g., circulating tumor cells (CTCs) and fetal nucleated red blood cells (fNRBCs) in blood samples collected from cancer patients and pregnant women, respectively. In addition to conducting the enumeration of these rare cells, we have been exploring the use of NanoVelcro Chips to isolate single CTCs and fNRBCs without contamination by white blood cells (WBCs) and cell-free DNA in the blood. The individually isolated CTCs and fNRBCs can be subjected to subsequent molecular analyses by FISH, RT-PCR, microarray or next-generation sequencing, enabling a wide range of advanced applications for cancer and prenatal diagnosis.

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SINGLE SINGLE CELL GENOME SEQUENCING: LIFE AT THE SINGLE MOLECULE LEVEL

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DNA are single molecules in individual cells. Point mutation and copy number variation, which are two major dynamical changes of DNA, can now be studied at the single cell level by whole genome amplification and sequencing. Experiments probing the biology of meiosis and cancer will be described. We demonstrate the proof of principle of selecting oocytes in in vitro fertilization in order to avoid miscarriage and genetic diseases. We also show that individual circulating tumor cells can be sequenced, providing tumor genetic signatures for personalized therapy.

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