

#1

SINGLE-CELL TRANSCRIPT ANALYSIS WITHOUT PRE-AMP USING HIGH PRECISION, ULTRA-SENSITIVE DROPLET DIGITAL PCR (ddPCR™)

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Over the last decade, it has become increasingly evident that gene expression profiles can vary from cell to cell, even within an apparently homogenous population. The analysis of this heterogeneity has become a focus of interest in various fields of biology, especially in stem cell research. The main obstacles to analysis of gene expression at the single-cell level are the low amount of starting material and the low abundance of many transcripts of interest. This requires a high level of confidence in results obtained from unique samples, making it difficult to be done accurately by traditional quantification methods such as qPCR. A quick and simple quantification method that does not require pre-amplification or purification of crude cDNA from individual cells, yet gives reliable measurements of transcript copy numbers, is desirable.

Droplet digital PCR (ddPCR™) provides absolute quantification of individual molecules with high precision, and without the requirement for standard curves or pre-amplification steps. Using Bio-Rad's QX100 ddPCR system, we developed a quick and simple method that measures single-cell gene expression in multiplexed assays with high sensitivity and reproducibility, thus enabling us to simultaneously analyze expression of different targets in unpurified cDNA from the same cell. In order to perform this, we carefully evaluated various cell lysis and cDNA synthesis methods and developed a protocol with flow-sorted Jurkat cells that is fully compatible with ddPCR, easy to use and capable of analyzing gene expression in single cells without pre-amplification. We show that high, medium and low abundance transcripts (< 20 copies/cell) can be reproducibly measured with ddPCR and that the system is well-behaved for quantifying transcripts from 1 to 100 cells per lysate. We further used this protocol to measure cell cycle-specific genes and revealed distinctive gene expression patterns in populations of single-cells that may reflect the contribution of cell-cycle phase to transcript copy number. This method allows us to easily and quickly measure the expression of multiple genes of interest in single cells, minimizing the stochastic effect of sampling and empowering us to accurately and sensitively detect and quantify low-expressing genes in single cells.

#2

DETECTION OF MICRORNA HETEROGENEITY IN SINGLE CELLS USING AN AUTOMATED MICROFLUIDIC CHIP SYSTEM

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MicroRNAs (miRNAs) are short (18–24 nucleotides), non-coding RNAs that regulate gene expression by disrupting messenger RNA (mRNA) stability and inhibiting their translation. The expression of miRNA species in cellular populations is thought to drive downstream gene expression and protein functionality. Our goal was to determine the variability in miRNA expression at the single-cell level using a microfluidic system that automates single-cell capture and miRNA preamplification for downstream expression analysis. We have developed a simple, modular workflow for streamlined analysis of cell populations down to the single-cell level. The workflow is centered on two key components: 1) cDNA preparation from isolation of single cells, and 2) highly parallel gene expression analysis. Using this workflow, up to 96 individual cDNA samples are analyzed in parallel with up to 377 different miRNA expression assays. We demonstrate the ability to detect significant variations in the expression of discrete miRNA species in a population of single cells from a single phenotype. Comparison of phenotypically distinct populations [human embryonic fibroblasts, human induced pluripotent stem cells (iPSCs), neural progenitor cells (NPCs) derived from the iPS, and fetal human neurons] demonstrates more dramatic differences in addition to the heterogeneity of expression within each group. In particular, while heterogeneous on a cell-to-cell basis, miRNA species miR-372 and miR-222 were expressed more frequently and at a higher level in iPS than in NPC cells. In contrast, we noted that miRNA species miR-218 and miR-24 showed different patterns of expression in NPC, and were expressed at a lower level and less frequently in iPS cells.

DIFFERENTIATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS TO NEURAL PROGENITOR CELLS AND NEURONS ON MICROFLUIDIC CHIPS

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Since *in vivo* cell fate determination and cell-cell signaling involve multiple genes, extracellular factors, and intracellular signaling pathways, finding the right combinations for *in vitro* cell culture conditions is invaluable for studying fundamental cell biology questions and for developing cell-based therapies. However, using traditional cell culture systems, multi-factorial experiments are often laborious and difficult to reproduce. Microfluidic technologies allow for precise control of microenvironment of cells, facilitate studies of multi-factorial combinations, and enable development of robust, reproducible, and chemically-defined cell culture systems. We have designed and fabricated a prototype microfluidic chip and an automated instrument that can culture cells on chip for extended periods of time and deliver multiple combinations of different factors to cells. Each chip includes 32 cell culture microchambers and 32 reagent inlets. Reagents can be automatically multiplexed to desired concentrations and combinations at various pre-defined time points. Cells can be cultured and stained on chip, or harvested from the chip for continued off-chip culturing, single-cell genomic analysis, and/or functional assays. We have demonstrated growing human induced pluripotent stem cells (hiPSCs) on chip for over a week; the hiPSCs retain pluripotency markers and can form colonies. The hiPSCs can also be differentiated on chip to neural progenitor cells or nociceptor neurons by scheduled combinatorial dosing of small signaling molecules. The identities of cells at different stages were profiled with immunostaining and single-cell gene expression analysis of targeted genes. The results were consistent with published reports and were confirmed in large well-dish format. In summary, the prototype automated microfluidic system reported here provides the potential to study and screen the precise combinatorial effects of multiple factors on stem cell culture maintenance, reprogramming, and differentiation—and therefore could be a valuable tool for the stem cell community.

Key words: New technology, Single-cell genomics, Human iPSC

#4

mRNA SEQ OF SINGLE CELLS REVEALS VAST HETEROGENEITY IN SINGLE-CELL GENE EXPRESSION AND PROVIDES INSIGHT INTO THE MOLECULAR MECHANISMS OF NEURAL DEVELOPMENT

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The ability to measure gene expression in single cells is crucial for understanding the molecular and cellular complexity of living cells, cell populations, tissues, and organisms. Most living tissues, such as the developing brain, are composed of a diverse range of cell types. However, our understanding of gene expression dynamics is typically based on measurements made from the entire population of cells. These bulk measurements may mask single cell gene expression states important for lineage progression, patterning, or tissue organization. To address this technical obstacle and explore heterogeneity within and between diverse cell types, we have performed mRNA transcriptome analysis of > 300 single cells across a wide sampling of > 10 phenotypically distinct populations. Using the C₁TM automated system for the capture, imaging, and routine preparation of full-length mRNA sequencing libraries of single cells, we delineated the transcriptional heterogeneity within and between cell populations at the level of the individual cell. After sequencing single-cell cDNA libraries on Illumina instruments, mRNA Seq mapping was conducted using both RSEM and the Tuxedo Suite (TopHat v2.0/Bowtie 2/Cufflinks 2). Finally, the single-cell gene expression data was analyzed using the Fluidigm SINGuLARTM 2.0 Analysis Toolset to perform outlier identification, principal component analysis, and hierarchical clustering, along with several other analyses. By comparing single-cell cDNA libraries sequenced at various depths [MiSeq (~10⁵ reads per cell) and HiSeq (~10⁷ reads per cell)], we discovered that many key gene expression signatures can still be identified in single cells even at extremely low depth (~10⁵ reads per cell). We next examined whether single cell analysis could identify cells of known types in heterogeneous samples representing several stages of neural development: neural progenitor cells derived from iPS cells using three small molecules (LDN-193189, SB431542, and cyclopamine), and primary human fetal cortex at gestational weeks 16 and 21. Remarkably, principle components analysis with either high or low coverage data separated cell types along an axis of neuroepithelial differentiation. The major genes distinguishing cell types included known markers of radial glia (*PAX6*, *SLC1A3*, *FABP7*) and differentiating cortical neurons (*MAPT*, *SNAP25*, *SATB2*) as well as novel factors. Hierarchical clustering of cells based on these genes allowed us to reclassify cells from heterogeneous tissue samples into known cell types and to elucidate the distinctive changes in gene expression patterns that occur as these cells progress through stages of neural development. Thus single-cell sequencing, even at low coverage, reveals the molecular identity of individual cells from heterogeneous populations and can be used as a tool to identify previously unrecognized properties of important cell populations.

DEVELOPMENT OF A SINGLE-CELL ANALYSIS TECHNIQUE FOR PLANT CELLS
--- A technique for extracting small tissue sections for site-specific gene expression analysis---

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Although an enormous quantity of genome data have been obtained so far, there are still many things that cannot be elucidated from averaged data obtained from multiple cells. Single cell analysis may provide the solution to this. Global gene expression analyses of single-cells will enable us to understand the mechanisms of living systems and the differentiation of site-specific responses in a tissue.

However, preparing single cells or small cell pools from a plant tissue is problematic because the cells are covered with and connected to cell walls. To overcome this difficulty, we developed an efficient method for extracting a small piece of plant tissue with a needle while controlling the decomposition of mRNA within 1 minute [Fig. 1(a)]. The needle is 31 gauge (0.28 mm in diameter and 0.13 mm in inner diameter). The end of the needle is sharpened so as to have four tips [Fig. 1(b)]. The size and the weight of an extracted tissue piece was about 130 μ m and 1.9 μ g, respectively.

The experimental flow of a site-specific gene expression study is shown in Figure 2. Site specific cDNA libraries on magnetic beads were synthesized according to this procedure. These cDNA libraries are reusable for analyzing multiple gene expressions by real time PCR. We plan to obtain a map of gene expression distributions for multiple genes.

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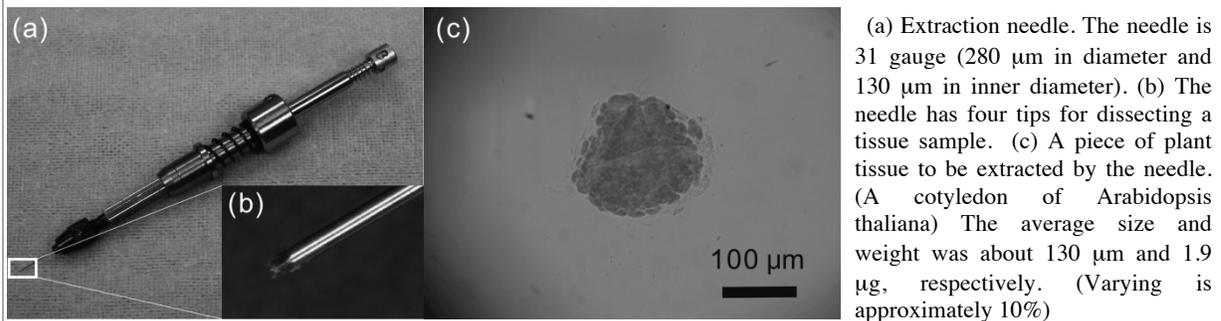


Fig. 1. Extracting plant tissues.

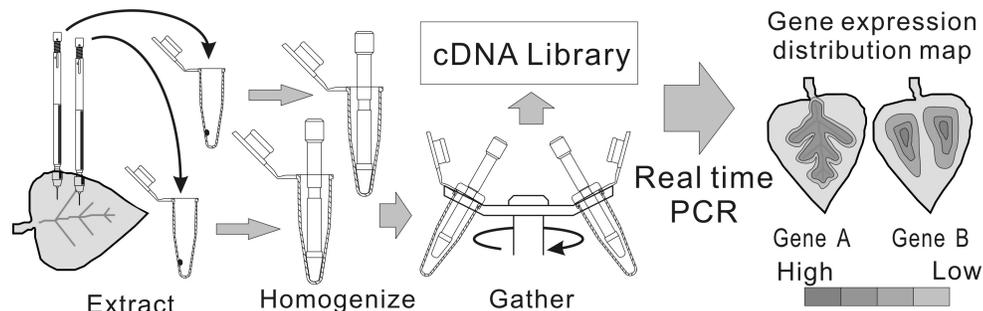


Fig. 2. Experimental flow of the site-specific gene expression analysis.

Small pieces of tissue samples are cut off with a needle and put into a drop of buffer and then are homogenized by a pestle. This pestle is able to operate to centrifugalize with tube by using a cap. Each sample is recovered with a centrifuge. A cDNA library is constructed on magnetic beads in each tube. A distribution map of gene expressions is obtained by carrying out a multiple gene expression analysis.

#6

USING NGS WITH RNA AMPLISEQ™ TO CORRELATE SIRNA TRANSFECTION WITH KNOCKDOWN IN SINGLE CELLS

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When analyzing gene expression profiles from large numbers of cells the average RNA expression profile may not be a true representation of the many different profiles that exist in the cell population (ex. in different states of growth or differentiation). In addition, transcriptional variability of individual cells can complicate any insight into the relationship between specific genes. This is especially true when looking at gene knockdown by siRNA transfection. Variables such as transfection efficiency, target gene expression level, and gene knockdown can vary so much from cell to cell to give an extremely heterogeneous population. The question has long been asked: if there is 50% knockdown, then is it that 50% of the population is 100% knocked down, or that 100% of the population is 50% knocked down.

Analyzing many genes from a single sample can be challenging especially from limited samples. Whole Transcriptome sequencing can be biased due to the need for Whole Transcriptome Amplification resulting in the assignment of incorrect expression levels. In addition, different methods produce different biases due to the mechanism of amplification that can lead to the inability to correlate samples. We have developed a targeted sequencing methodology call Ampliseq™ RNA that allows for the unbiased amplification and sequencing of transcripts using the PGM™ and Proton™ sequencing instruments.

We have utilized the Ampliseq™ RNA technology to monitor target gene knockdown and then correlate these levels to transfection level of a fluorescently labeled siRNA. The ability to monitor many genes within the same cell has given a better idea of cell state and target gene knockdown and finally answers the question of gene knockdown in individual cells.

#7

WILDFIRE: AN IN-SITU ISOTHERMAL PCR METHOD FORMING BILLIONS MONOCLONAL COLONIES FOR NGS

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We have developed a new massive parallel monoclonal technology, called “WildFire”, where sequencing libraries are *in-situ* isothermal amplified directly on the surface of a 5500 flowchip. Sequencing libraries (~ 40 uL per lane) are added directly to the 5500-series Genetic Analyzer flowchip, whose surfaces have been coated with a special library-adaptor capture oligonucleotide. A DNA polymerase reaction mix is added, and in a single isothermal step lasting ~ 30 minutes, single templates are amplified *in-situ* on the flowchip. The net density of sequencing-colonies created in this manner far exceeds anything currently utilized in next-generation sequencing, reaching ~ 1.5 million colonies per mm² per flowchip surface. During *in-situ* amplification, the capture oligonucleotide is “consumed”, and each individual nucleic-acid fragment “spreads” (like a WildFire) inside the flowchip until reaching an adjacent library fragment(s). When the individually-growing fragments “meet”, the amplification step terminates, because all of the surface-bound primer was consumed. These “self-assembled”, spatially resolved, monoclonal colonies, are then sequenced by SOLiD chemistry. The resulting colony-sequencing reads maintain the same high accuracy as our bead-based method. Full genomes (from bacterial to human), exomes (human), and transcriptomes (human) have now been sequenced using WildFire technology. WildFire technology greatly improves NGS workflow, increases throughput, and significantly decreases net cost-per-genome.

#8

SINGLE CELL TARGETED DNA SEQUENCING AND ANALYSIS USING ION TORRENT SEMI-CONDUCTOR NEXT GENERATION SEQUENCING PLATFORM

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Target enrichment for coding regions of genomic DNA is a cost effective way to study genetic variations without the need of sequencing the entire genome. Typical hybridization-based exon enrichment has extensive sample preparation time and is challenged by applications that require analysis of scarce and highly degraded DNA materials from sources such as single cell, fine needle biopsy or archival FFPE sample.

Ion AmpliSeq™ technology combines the specificity, sensitivity, and efficiency of PCR technology to enrich tens to thousands of genomic targets from less than 10ng of FFPE or gDNA in a reaction for next generation sequencing (NGS).

The unique amplicon preparation workflow sharply reduces side-products in multiplex PCR and thereby enables this technology to venture into extremely high levels of multiplex PCR for NGS.

The workflow consists of target amplification using primer pools designed by Ion AmpliSeq™ Designer software. The amplicons (150bp ~ 200bp) are subsequently ligated to sequencing adapters and purified. The resulting DNA libraries can typically be completed in 3.5 hours with as little as 30 minutes of hands-on-time using the Ion AmpliSeq™ Library Kit 2.0.

An early demonstration of amplicon preparation with extremely low level DNA input using the Ion AmpliSeq™ Technology suggests the possibility of highly multiplexed, rapid targeted re-sequencing for single-cell research.

#9

IMMUNE CELL CAPTURE BY NEGATIVE DIELECTROPHORETIC ATTRACTION TO AN ION ENRICHMENT ZONE GENERATED BY A BIPOLAR ELECTRODE

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This poster describes dielectrophoretic capture of single immune cells at a bipolar electrode (BPE). Simple alteration of the applied electric field led to controlled cell swelling and lysis *via* the localized depletion of ionic strength. Importantly, the captured cells were shown to be viable following capture. In the system presented here, the electric field gradient employed for dielectrophoretic attraction is generated via faradaic manipulation of the ionic strength of the solution surrounding the BPE. A BPE is a conductive phase that in the presence of an electric field drives both oxidation and reduction reactions at opposite ends. A key advantage of BPEs is that they do not require wire leads and are therefore amenable to patterning in an array format. BPEs are therefore attractive candidates for cell capture in an array format by dielectrophoresis.

MOLECULAR COMPONENT DISTRIBUTION IMAGING OF SINGLE LIVING CELLS BY RAMAN MICROSPECTROSCOPY COMBINED WITH MULTIVARIATE CURVE RESOLUTION ANALYSIS*Masahiro ANDO*¹ and Hiro-o HAMAGUCHI^{1,2}¹Consolidated Research Institute for Advanced Science and Medical Care, Waseda University²Dept. of Applied Chemistry and Institute of Molecular Science, National Chiao Tung University
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Raman microspectroscopy is now widely used in label-free and molecular-level investigations of single living cells. Although Raman spectra contain rich information on molecular structure, detailed interpretation of space-resolved spectra is often difficult because of their complexity. Raman spectra are usually interpreted as the superposition of several biochemical component spectra, as well as those of background and fluorescence. In order to decompose the complicated spectral data set into tractable component spectra, a number of chemometric methods have been developed. Especially, the multivariate curve resolution – alternating least squares (MCR-ALS) method can be a powerful tool for the molecular component distribution imaging of single living cells. Under appropriate model constraints, such as non-negativity and norm regularization for spectral profiles and their concentrations, MCR-ALS technique provides physically interpretable spectral components, without a priori information on chemical components. For example, as shown in Fig. 1, MCR-ALS analysis of yeast cell and eosinophil provides the spectral components interpreted as proteins, nucleic acids, lipids, heme enzymes, and so on, as well as their distribution images. In this presentation, the principles of the MCR-ALS method and their applications to single living cell analysis are discussed.

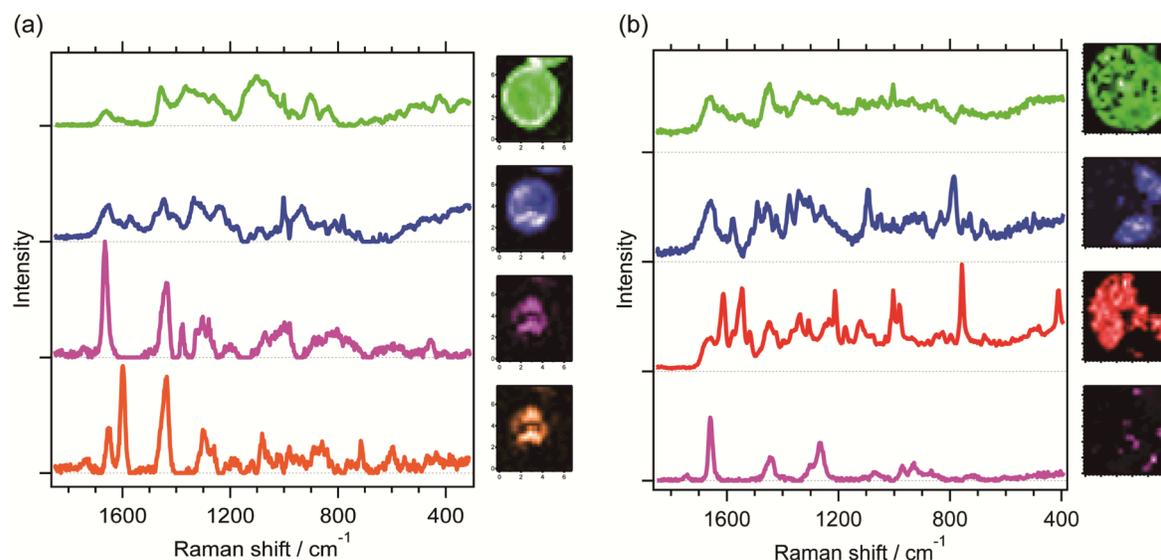


Fig. 1. Raman spectral components and distribution images obtained by MCR-ALS analysis: single living (a) yeast cell and (b) eosinophil.

SINGLE-CELL RESOLUTION ANALYSIS OF GRN DYNAMICS IN CARDIOMYOCYTE DIFFERENTIATION FROM HEALTHY AND PATIENT IPS CELLS

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ABSTRACT

Induced pluripotent stem cells (iPSCs) offer a new promise, not only for regenerative medicine, but to expand our understanding of the normal and disease development in humans. However, a major hurdle is the elucidation of the fundamental molecular processes that are involved in cellular development required to efficiently steer the differentiation of iPSCs to a desired cell type. Work by Eric Davidson and colleagues in sea urchin development suggest that cell fate determination is tightly regulated by expression of sets of transcription factors within gene regulatory networks (GRN). However, in contrast to invertebrates, a stable mature cell types in mammals arise through complex developmental paths that involve discrete intermediate states of the GRN. Current attempts to define gene regulatory networks (GRN) in development and disease based on whole-tissue measurements are caricatures because each individual cell in a cell population occupies a distinct GRN state, leading to cell population heterogeneity. The major bottleneck to identify these discrete GRN configurations is that cell population heterogeneity blurs the unique features of functionally distinct cells and their cellular states. Building on this, we hypothesize that (1) the differentiation process occurs through the dynamic creation of a series of discrete populations of cells that can be defined by the multidimensional analyses of quantified transcriptomes (or “quantized cell populations”) that interact with one another; that (2) the observed heterogeneity within seemingly homogeneous populations can be explained by the presence of these quantized cell populations; and that (3) in genetic diseases, mutations perturb the gene regulatory networks – altering both the quantized cell populations and their subsequent interactions – leading to abnormal differentiation and diseased phenotypes. The differentiation to mature cell types from iPSCs constitutes a model system that is uniquely well-suited for this study. Therefore, we aim to study iPSC-to-cardiomyocyte differentiation using single-cell analysis to understand, at a new resolution, the development of cardiomyocytes and how genetic mutations cause congenital heart defects. We envision that single-cell resolution measurement of differentiating cardiomyocyte populations will reveal new facets of developmental dynamics. We will perform single-cell transcript analysis of thousands of iPSCs undergoing cardiomyocyte differentiation to compare temporal appearance and weight of quantized states in normal vs. patient-derived iPSCs carrying a mutation that causes cardiac defects using Fluidigm’s microfluidics-based BioMark™ RT-PCR platform. In parallel, traditional multi-omics analysis of epigenetic marks and the proteome will provide a genome-wide context to embed our single-cell analysis, and will complement the systems analysis. Our overall goal is to demonstrate the new value of single-cell analysis of cellular development, and thereby better understand cardiac diseases caused by the GATA4-mutation.

SINGLE CELL MASS CYTOMETRY APPLIED TO THE IMMUNOPHENOTYPIC ANALYSIS OF MYELODYSPLASTIC SYNDROME

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Introduction: The clinical course of patients with myelodysplastic syndrome (MDS) is quite heterogeneous with some patients surviving for many years with minimal symptoms and others rapidly progressing to acute myeloid leukemia. While a great deal of progress has been made in the understanding of MDS pathogenesis, current models remain incomplete. **Objective:** The objective of this study was to extend previous flow cytometry studies by applying the novel technology of mass cytometry, which is capable of measuring up to 40 simultaneous parameters on a single cell basis. This pilot study was designed to allow selection of the most predictive surface markers to utilize in future experiments combining surface and intracellular functional markers.

Methods: Analysis was performed on 11 samples from 4 patients with high-risk MDS, 4 with low-risk MDS, and 2 patients with cytopenias not due to MDS. In addition, 3 bone marrow samples from healthy donors were analyzed. A panel of 33 surface markers was measured by mass cytometry on each cell.

Results: Mass cytometry allowed for measurement of 33 cell surface markers across all major immunophenotypic populations in human bone marrow. Despite the small sample size, significant differences were observed in several markers across the total cell, CD34+ cell and mature cell populations. The total bone marrow cell populations of patients with high-risk MDS demonstrated significantly increased staining for CD34 ($p=0.021$) and CD44 ($p=0.015$) compared to normal samples. High-risk patient samples were also significantly different from low-risk MDS patients with regard to expression of CD34 ($p=0.015$) and CD99 ($p=0.042$). Comparison of mature cell populations also revealed significant differences with high-risk MDS samples characterized by higher levels of co-expression of CD44 ($p=0.051$) on CD15+ cells compared to control samples. CD34 expression on mature myeloid cells (CD14+ and CD15+) was also significantly different between high-risk and low-risk MDS samples ($p=0.036$ and $p=0.019$, respectively). CD99 expression on CD15+ cells was also significantly different between high-risk and low-risk patients ($p=0.04$). Analysis of the CD34+ cell population revealed that CD321 was significantly elevated in patients with MDS compared to normal donors ($p=0.026$). Three out of four patients with high-risk disease also demonstrated high expression of HLA-DR on CD34+ cell populations compared to normal donors (average median expression of 155 counts versus 24 counts, respectively), however, this difference was not statistically significant. These differences could also be appreciated in SPADE, which was employed to visualize immunophenotypic differentiation across each patient sample. Lastly, PCA was used to define the surface markers that best captured the variance across the MDS and normal samples.

Conclusions: This is the first application of mass cytometry for the analysis of immunophenotypic variation in myelodysplastic syndrome. Despite the small sample size of this pilot study, mass cytometry analysis was able to detect a variety of previously reported immunophenotypic patterns in MDS and defined CD321 as a novel marker of abnormal CD34 cells in patients with MDS. These data will allow for the creation of staining panels for more detailed mass cytometry studies of MDS.

PROPAGATING AND INFLATING INDIVIDUAL VIRAL LINEAGES USING DROP-BASED MICROFLUIDICS

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The study of how viruses propagate is important for curing disease and preventing viral outbreaks, yet is challenging due to inability to access enough variants in the population, including ones that do not survive genetic drift and selection. In nature, viruses can compete with one another, and the most evolutionary fit virus usually takes over a population. Yet there exist variants in the population that can escape subjected evolutionary pressures and eventually dominate the population. Successful studies of viral epidemics *in vitro* hinges on the ability to access these variants, in order to obtain a comprehensive understanding of fitness in an evolving population and to allow the exploration of multiple virus trajectories, as well as prediction of their direction. Unfortunately, measurements of fitness trajectories can currently be observed in the laboratory only when the mutations have a large effect on fitness in the population. Accessing the richness of the background genomic diversity has only been performed upon a small number of species, since these experiments have been both labor intensive and time-consuming due to their large population sizes. There remains a need for a simple experimental method that allows greater access to the genetic diversity in a large population, in order to allow exploration of fitness space.

Here, we present the use of droplet-based microfluidics as a simple method to segregate and propagate a viral population as individual viral lineages from single cell host cells, effectively eliminating competition between viral strains and minimizing natural selection. From deep sequencing results, we discover that the population obtained from isolating viruses in drops is more diverse than that from bulk culture, thus allowing access to mutations and variants that would not otherwise occur be seen from natural selection events, allowing the potential to control mutation rates and accelerate evolution *in vitro* or *in silico*. This general method of permitting smaller population sizes to propagate, similar in vein to mutation accumulation experiments, can be widely applied to other genetic systems to revolutionize our understanding of evolution and the distribution of fitness.

MAGNETIC MANIPULATION OF AXONAL TRANSPORT IN LIVE NEURONS

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Retrograde neurotrophic signals, from the axon terminal to the cell body, are essential for the survival and function of neurons. Axonal microtubules serve as polarized tracks for molecular motor proteins driving the signaling endosomes from the axon terminal to the cell body. The robustness of this long-distance transport and the direction specificity are attributed to the cooperative mechanics of multiple motors and/or specific regulators in vivo. Noninvasive external force control of axonal endosomes in live neurons is a technical challenge that can elucidate the axonal transport machinery and possible regulation mechanisms in vivo. Here, we present an integrated methodology based on microfluidic neuron culture, high-gradient magnetic trapping and pseudo-TIRF imaging that permits external control of axonal endosome transport in live neurons via magnetic forces. We fabricated a novel microfluidic device for neuron culture by patterned electrodeposition of soft micromagnets on glass coverslips. In the presence of an external magnetizing field, the soft micromagnetic pattern gives rise to local zones of high magnetic gradients. By culturing neurons in this device, with axons aligned along these high gradient zones, we can exert pN forces on axonal endosomes carrying magnetic nanoparticles (<100 nm). The magnetic forces can be designed to either assist/oppose the molecular motor forces driving the axonal endosomes. We have successfully compartmentalized DRG neurons in prototype magnetic devices and captured the axonal transport dynamics under load by pseudo-TIRF imaging. High-resolution tracking and stochastic modeling of axonal endosome transport under load reveal several key insights into the cooperative mechanics of motors and the regulation of axonal transport. We also present the prospects of our methodology in deciphering the role of transport in retrograde neurotrophin survival signaling of neurons.

#15

SINGLE CELL IN SITU METHODS FOR QUANTITATIVE CANCER DIAGNOSTICS AND TUMOR HETEROGENEITY ANALYSIS

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Cell-to-cell phenotypic variability in tumors has been observed since the early days of pathology, yet single cell, quantitative analyses of tumor heterogeneity are just beginning to emerge. Here, I will show how two methods developed in our lab – High-Definition DNA FISH (HD-FISH) and Single-Molecule RNA FISH (smFISH) – can be used as powerful cancer diagnostic tools and allow studying tumor heterogeneity in a robust and quantitative fashion.

First, I will demonstrate how dozens of libraries of HD-FISH probes can be designed in a rapid and flexible manner, and be used to monitor the relationship between gene copy number and transcriptional activity as well as gene or chromosome positioning within the nucleus at the diffraction limit in hundreds of individual cells. Second, I will show how application of smFISH enables reliable simultaneous assessment of *ERBB2* and estrogen receptor-1 abundance and spatial heterogeneity in breast cancer tissue sections. Third, I will present a powerful method for rapid, quantitative detection of expressed gene fusions based on smFISH and unbiased correlation analysis (FuseFISH), discussing an application of the method to measure *BCR-ABL1* expression heterogeneity and dynamics in single leukemia cells exposed to the kinase inhibitor Nilotinib.

#16

EXPLORING EPHB SIGNALLING IN NEURAL STEM CELLS

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Eph receptors are tyrosine kinases that bind and cluster with membrane bound ephrin ligands on apposing cells to signal juxtacrine. Eph-ephrin signalling is traditionally known to play an important role in many aspects of brain development, including cell migration, axon guidance, and stem cell proliferation. Recently, our collaborators in the Schaffer lab have reported a previously unknown role of EphB signalling in regulating neural stem cell differentiation: pre-clustering Fc-ephrinB2 ligand in solution induces neuronal differentiation. An earlier study from our lab has demonstrated that EphA signalling is sensitive to the spatial reorganisation of receptor-ligand clusters, resulting in altered downstream cellular response. This suggests the possibility that EphB signalling is also spatio-mechanosensitive, since EphA and EphB receptors share similarities in their physical structures and ligand interactions. Altogether, these results motivate the hypothesis that neural stem cell cellular behaviour may be regulated by EphB signalling in response to the receptor-ligand spatial reorganisation. To evaluate this possibility we employ a hybrid interface that couples live cells with a synthetic supported bilayer well-suited for studying Eph-ephrin juxtacrine signalling. This setup reconstitutes the physical geometry of Eph receptors and ligands between apposing membranes, and accommodates the method of spatial mutation, whereby physical constraints—engineered using lithographically patterned micro-corrals—are introduced to restrict receptor-ligand mobility and precisely control the spatial patterns of receptor-ligand organisation.

#17

ASSESSING CHROMOSOMAL ABERRATIONS FROM LIMITING INPUT OF CELLS USING AFFYMETRIX COPY NUMBER AND SNP ARRAYS

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Measurements generated from traditional RNA/DNA profiling methods typically represent an amalgam of signals compiled from tens to thousands or even millions of cells within a multi-cellular population. These approaches require a bulk amount of test material and assume that there is minimal heterogeneity among seemingly similar cells in order to draw a global conclusion. In contrast, the ability to measure signal from a single cell allows unprecedented ability to resolve genomic differences in complex mixture of individual cells, monitor circulating/rare cells often in a non-invasive manner, and allow early access to the genome during development in the form of cells taken from pre-implantation embryos. Cumulatively, this expanded spectrum of applications has an important impact on therapeutic management and development of diagnostics tests by offering (1) increased discrimination of heterogeneous tumors (2) early and routine monitoring of disease progression through access to circulating tumor cells (3) non-invasive diagnosis of prenatal health through monitoring circulating fetal cells and (4) selection of healthy embryos for assisted reproductive technologies. Here, we utilize Affymetrix copy number and single nucleotide polymorphism (SNP) arrays to detect chromosomal imbalances originating from serial dilutions of cellular input. Single copy gains and losses are examined from different protocols for amplification of genomic DNA. A comparative map of copy number alterations is presented for evaluation of the data.

**ULTRAHIGH-THROUGHPUT SINGLE-CELL PCR
AND SORTING WITH PACS***Dennis J. Eastburn*¹, Adam Sciambi¹ and Adam R. Abate¹¹ Department of Bioengineering and Therapeutic Sciences, California Institute for Quantitative Biosciences, University of California, San Francisco, CA 94158
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Investigations of large, clinically relevant populations of heterogeneous cells require tools capable of both single-cell analysis and extremely high throughput. We introduce PCR Activated Cell Sorting (PACS) as a new ultrahigh-throughput method for the examination of heterogeneous cell populations. PACS is analogous to Fluorescent Activated Cell Sorting (FACS), except that rather than antibody-based sorting of intact cells, it isolates up to 1 million individual cells into individual aqueous picoliter-volume drops suspended in oil and performs PCR reactions on their lysates. Using multiplexed TaqMan PCR assays, the system is able to interrogate each cell for the expression of specific combinations of transcripts, mutations or non-coding RNAs. Moreover, similar to FACS, this system can sort the cell lysate into different containers, allowing the lysates of cells with a unique transcriptional signature to be recovered for additional analysis using microarrays, Next Gen Sequencing, or other methods. A key technical advance with our PACS system is a novel microfluidic workflow that overcomes lysate-mediated inhibition of RT-PCR in picoliter drops, enabling massively-parallel RT-PCR on millions of single cells isolated in distinct drops. With this method we have already demonstrated 100-fold higher single-cell RT-PCR throughput than can be achieved with existing methods. Additionally, we have shown that our approach is highly specific, enabling the unambiguous detection of specific cell types from a mixed cell population. Ongoing work is focused on increasing the throughput of our method and demonstrating robust sorting and recovery of cell lysate. We envision PACS as a complimentary method to FACS, allowing cells to be differentiated and sorted based on transcriptional and genomic biomarkers impossible to detect with current methods.

#19

SINGLE-CELL MASS CYTOMETRY REVEALS A HIGHLY STRUCTURED CELLULAR IMMUNE RESPONSE IN PATIENTS WITH SURGICAL TRAUMA

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Surgery elicits a complex immune response that, if deregulated, compromises clinical recovery. High-dimensional single-cell mass cytometry enabled a system-wide functional *in vivo* characterization of the cellular immune response in whole blood from six patients undergoing hip arthroplasty. Longitudinal analysis of circulating leukocytes revealed distinct changes in cell population sizes, cell phenotypes, and intracellular signaling responses. Serial changes in activation states of six key signaling regulators of immune function (STAT1, STAT3, STAT5, p38, rpS6, and CREB) occurred in cell subsets that coordinate the primary response to surgical trauma. Activation of cell signaling pathways within the innate and adaptive immune system were detected as early as 1 hour after surgery, a finding challenging the current view that innate activation precedes adaptive changes. This study presents a system-level single-cell analysis platform for patient immune profiling. This approach has the potential to guide individualized immune-modulatory strategies designed to improve health outcomes.

#20

APERTURELESS NEAR-FIELD LASER ABLATION MASS SPECTROSCOPY TOWARDS SINGLE CELL ANALYSIS

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Many biochemical analyses of biological systems are performed on groups of cells based on the assumption that the cells are sufficiently similar that an ensemble average from the cell group will yield a useful result. However, this approach can obscure the underlying heterogeneity of the individual cells and masks important differences in gene expression, protein levels, and small-molecule distributions. Bringing modern methods of analysis to new levels of sensitivity and spatial resolution is necessary if single cell biochemical analysis is to be achieved. Mass spectrometry is a key analysis technique for cell biochemistry, but there are technological barriers in sampling scale that must be overcome for it to be used to its full potential with single cells.

The goal of this research is to construct and test a system for nanometer scale laser ablation sampling of single cells and tissue coupled with electrospray ionization mass spectrometry. We have started this project with nano-sampling of small molecules using a heated tip in the AFM by capturing the thermally desorbed molecules in SPME followed by detection in GC-MS. Moreover, detection of multicomponent mixture has also been performed using polycyclic aromatic hydrocarbons (PAHs). The next step was apertureless near field laser ablation mass spectroscopy of small molecules using the same SPME set up and detection in GC-MS. Presently, we are designing method to detect multiple chemical components.

Our ultimate goal is to capture proteins and peptides in mm size droplet followed by detection in electrospray ionization mass spectroscopy. Hence, our next step will be capturing small molecules in a mm size solvent droplet to validate the proof of principle. Finally, we will perform apertureless near-field laser ablation on large biomolecules. Nanoscale laser ablation sampling developed in this project will have applications not only in mass spectrometry but also in microfluidics. Laser ablation sampling with droplet capture will provide a new method for spatially resolved sampling into a microfluidic device. It will also allow a separation step to be added to mass spectrometry imaging that will enable the imaging of minor biomolecule components of tissue.

#21

STRUCTURE AND DYNAMICS OF THE BACTERIAL CHROMOSOME IN *E. COLI*

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Despite a great number of studies on bacteria, and especially *E. coli*, our knowledge of the scheme by which the bacterial chromosome is physically organized is at best incomplete. We address the question of whether the *E. coli* chromosome is folded into a self-adherent nucleoprotein complex, or alternately whether it is based on an essentially unfolded DNA molecule. Through *in vivo* visualization using an inducible GFP fusion to the nucleoid-associated protein Fis, to non-specifically decorate the entire chromosome, we have been able to observe the global chromosome structure in live cells, where the dynamics of structure and positioning of the chromosome as a whole could be followed in real time. Our observations indicate that for a range of growth conditions, the chromosome is a self-adherent, folded object with persistent small-scale features, including an overall coiled shape. In addition to the *in vivo* studies, we have developed an assay to carry out mechanical experiments on nucleoids removed from cells. The further objective would be to study the effects of the major nucleoid-associated proteins mutations on the chromosome by probing changes in the mechanical properties, and get a better understanding of the roles played by major chromosome-folding proteins in organizing the physical state of the chromosome.

**METHODS FOR COUPLING MICROFLUIDICS WITH
SINGLE-CELL MASS SPECTROMETRY**

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Cell heterogeneity among a eukaryotic cell line or within a region of uniform tissue is the main driving force behind the recent development of analyses for single-cells. At the tissue-level, a set of cells may seem entirely homogenous. However, at cell level, the difference in a protein, organelle or strand of DNA from one cell to the next can affect major pathways involved in stress or stimulus responses and even the overall function of the cell. In this way, the tiny variations in cells that cause this heterogeneity to occur can control the fate of the organism to which the cells belong. This becomes extremely important when discussing rare cells, such as tumor cells. Such cells carry unique markers useful for singling them out, leading to early diagnoses and a more structured ways of determining the proper treatment. Markers can exist at extremely small concentrations per cell, causing them to be missed by ensemble analysis of large groups of cells; isolating and quantizing markers by single-cell methods allows for more sensitive detection.

Currently development is underway on a method which combines the sensitivity of Time of Flight-Mass Spectrometry (TOF-MS) with the sample delivery capabilities of a microfluidic chip. A simplistic T-junction design has demonstrated stable, aqueous plug generation in a fluorinated oil continuous phase. By integrating a fused silica capillary at the outlet of the chip, droplet delivery into the mass spectrometer was also achieved. The small volume and compartmentalization of the plugs allows for sensitive mass analysis without over-diluting the sample. Applying charge at the end of the droplet emitter allows for either electrospray ionization (ESI) or large, charged droplets to be emitted into the mass spectrometer. Thus far results have been promising in compartmentalization and sample delivery, but much work is still needed for single-cell analysis on such a setup can be achieved.

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#23

PREDICTING RATES OF CELL STATE CHANGE DUE TO STOCHASTIC FLUCTUATIONS USING A DATA-DRIVEN LANDSCAPE MODEL

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We develop a potential landscape approach to quantitatively describe experimental data from a fibroblast cell line that exhibits a wide range of green fluorescent protein (GFP) expression levels under the control of the promoter for tenascin-C. Quantitative time lapse live cell microscopy provides data describing short term fluctuations in promoter activity in single cells. And, flow cytometry measurements provide data about the long term kinetics as isolated subpopulations of cells relax from a relatively narrow distribution of GFP expression back to the original broad distribution of responses. The landscape is obtained from the steady state distribution of GFP expression and is connected to a potential-like function using a stochastic differential equation description (Langevin/Fokker-Planck). The range of cell states is constrained by a “force” that is proportional to the gradient of the potential, and biochemical noise causes movement of cells within the landscape. Analyzing the mean square displacement of GFP intensity changes in live cells indicates that these fluctuations are described by a single diffusion constant in log GFP space. This allows application of the Kramers’ model to calculate rates of switching between two attractor states, and enables an accurate simulation of the dynamics of relaxation back to the steady state with no adjustable parameters. With this approach, it is possible to use the steady state distribution of phenotypes and a quantitative description of the short term fluctuations in individual cells to accurately predict the rates at which different phenotypes will arise from an isolated subpopulation of cells.

USING DIGITAL DROPLET PCR FOR QUANTITATIVE ANALYSIS OF LINEAGE-MANIFESTED CNVs UNCOVERED IN HUMAN INDUCED PLURIPOTENT STEM CELLS BY WHOLE-GENOME SEQUENCING

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We have performed whole-genome sequencing based CNV analysis in 7 fibroblast samples and 20 corresponding induced pluripotent stem cell lines obtained from two families [Abyzov et al., Nature. 2012 Dec 20;492(7429):438-42].

We found that on average an iPSC line has two LM-CNVs (lineage-manifested CNVs). We defined the term LM-CNV to describe CNVs detected by genome-wide analyses in an iPSC line but not in the fibroblast culture from which the given iPSC line was derived but without making a statement as to the nature of the CNV-forming event (i.e. de novo formation during reprogramming from fibroblast to iPSC or unmasking of a somatic variant present in mosaic fashion in the fibroblast tissue).

After detecting LM-CNVs by sequencing based analysis in the iPSC lines we investigated the masked, mosaic presence of the same CNVs in the fibroblast tissue of origin, using regular PCR and Sanger sequencing as well as digital droplet PCR (ddPCR).

We determined that more than half of the LM-CNVs detected in iPSC lines were already present at low allele frequency, mosaic somatic CNVs in the fibroblasts and that up to 40% of fibroblast cells carry such medium-sized to large somatic CNVs.

Using ddPCR we were able to determine that the somatic allele frequency of the detectable mosaic variants in our sample ranges from ~1% to ~15%.

Therefore, de novo CNVs in iPSCs may not be an obligate consequence of reprogramming. Our analysis unexpectedly revealed extensive somatic copy number variability in fibroblasts carrying over into iPSC and becoming unmasked in the process.

Our results underline the necessity of carrying out high-resolution genome analysis during iPSC-model based studies and demonstrate that whole-genome sequencing allows for detection and managing of the potential confounds caused by genomic variation such as LM-CNVs.

DEVELOPMENT OF DROPLET-BASED SINGLE-CELL ANALYSIS SYSTEM FOR ENZYME SCREENING FROM METAGENOME LIBRARY

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Microorganisms are known to play an important role in industry and pharmaceuticals. However, the field of microbiology has been limited by the restricted ability to culture microorganisms in the laboratory whereby it is estimated that more than 99% of microorganisms identified to date are considered uncultivable. Accordingly, the metagenomic approach, which has the potential to resolve this problem, has been performed for various applications recently.

The most common technique to screen for potential bacterial enzymes from marine or soil metagenomic libraries is plate screening. However, this approach has significant drawbacks since it is tedious and time consuming for culturing organisms that exhibit slow growth or require environmental cues. To overcome such limitations, microfluidic devices are noted since they have the advantage in rapid mixing, automated processing, and the reduction of the consumption of analytes and the duration of analyses to allow high time-resolved single-cell analysis. In this study, a microfluidic device was proposed for encapsulating single bacterial cells in aqueous droplets via emulsification for high-throughput enzymatic assay.

A cross-junction microfluidic device was fabricated from polydimethylsiloxane (PDMS) using a SU-8 mold. Microdroplets were formed using LB medium containing 1% low melting point agarose in the presence of fluorinated oil containing fluorosurfactant, which utilized to stabilize the microdroplets and prevent coalescence. *E. coli* cells were added to the LB medium containing agarose immediately prior to microdroplet formation. Then, the sample and carrier oil were introduced into the device to generate microdroplets by flow focusing. It was shown that uniform microdroplet formation was attained and the average of diameter was 52.0 μm . Cell encapsulation follows a Poisson distribution that defines the microdroplet occupancy statistics, and resulted here in approximately one-third of the droplets being empty and one-third containing a single-cell. The incubation of microdroplets containing single cells led to the formation of microcolonies within the agarose. In order to evaluate the activity of lipase for metagenome screening, *E. coli* cells harboring a plasmid for the expression of lipase were encapsulated in microdroplets with a fluorogenic substrate (fluorescein dicaprylate). The microdroplets were incubated at 37 °C for 24 hours to allow time for cell growth and substrate hydrolysis by the enzyme. Following incubation, the fluorescent product of the lipase reaction spread throughout the droplets. The droplets containing lipase-expressed cells were significantly more fluorescent than either empty droplets or those containing wild type cells. Therefore, our system has potential as a tool for high throughput screening of metagenome library by the microdroplet-based enzymatic assay at the single cell level.

**SINGLE CIRCULATING TUMOR CELL ISOLATION USING
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Abstract. Based on the NanoVelcro microfluidic system, a universal platform for circulating tumor cell (CTC) capture and molecular analysis is developed for investigating melanoma, prostate cancer and pancreatic cancer. Single cell level molecular analysis was carried out on captured CTCs from cancer patient fluid biopsy, which gives the deep insight of cancer progression. By utilizing nano-imprinting approach, we developed a new next-generation polymer NanoVelcro Chips by stamping the PDMS replicates of embedded nano-features onto a LCD slide with pre-deposited PLGA film. The nano-structure of PLGA nano-pillar array is precisely controlled for improved capture performance and preservation of cell viability. Under the optimized conditions, the captured single CTC are identified and isolated by Laser Capture Microdissection (LCM) for further Whole Genome Amplification (WGA). Based on whole exom or genome sequencing on the single isolated CTC, our extensive study will aim on several aspects of oncological researches. 1) We will use a real-time single-CTC genomic approach that will overcome the challenges associated with tumor heterogeneity and the difficulties of serial tumor biopsies. 2) By comparing the genomic signatures of CTCs with those of tumor biopsies, we will measure the surrogacy of CTCs as a source of surrogate tissue. 3) Pairing the technology development and validation of CTC genomics with our trial of target therapy will allow us to describe the dynamic evolution of CTCs in predicting drug resistance. 4) Careful validation of CTC genomics will be performed using both targeted sequencing and Complete Genomics' non-amplification whole genome sequencing to avoid potential false discoveries. 5) Phylogenetic analyses of serial CTC samples will unveil the dynamic evolution of cancer, and may lead to the discovery of dominant clones pre-therapy and at-progression. By performing a differential comparison of the genomic signature among tumor cells pre-therapy and at-progression, we will identify genetic alterations associated with resistance to therapy.

SINGLE-CELL WESTERN BLOTTING

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We report the **first single-cell western blot via ~2,000 simultaneous separations**, bringing western blotting towards the density of protein microarrays, yet with single cell resolution. To achieve western blotting on this scale for single cells **we introduce a micropatterned photoactive polyacrylamide (PACTgel) microdevice** for single cell seeding into microwells, *in situ* lysis of cells, separation of cell contents in the PACTgel and subsequent antibody probing. In a first of kind study, we utilize the single cell western to elucidate cell-to-cell heterogeneity in signaling and differentiation of thousands of single neural stem cells.

We introduce an “open” format PACTgel stippled with 6,720 microwells, each seeded with single cells. The 30 micron thick PACTgel supports the fully integrated four-stage western blot comprised of: **STAGE 1: Seeding of cells** into the micropatterned device, achieving single cell occupancies of >40%. **STAGE 2:** Lysis and SDS-PAGE of single cells through microwell walls into the PACTgel, with log-linear protein sizing over the 20-150 kDa analyte range in separation distances of ~500 μm . **STAGE 3:** Photo-blotting with ~30% gel capture of endogenous proteins from single cells. The PACTgel capture efficiency range rivals conventional electrotransfer blotting efficiencies on polymer membranes. **STAGE 4:** Probing by diffusion of primary and secondary antibodies into the PACTgel, with rapid kinetics (45 min probing/washout) facilitated by 3D scaffolded analyte capture via our nanoreactive PACTgel. Importantly, the single cell western allows 3-plex protein analyte quantification in 4 hours. This performance fills a critical gap in protein analysis, allowing study of previously intractable biological hypotheses.

Our single cell western blot advances integrated targeted proteomics in four areas. Firstly, in **capability** – single cell western blotting is **not currently possible with existing assays** and has not been reported previously, except for unusually large cells such as *Xenopus laevis* oocytes. Secondly, in unprecedented sensitivity – 10^4 - 10^5 molecules (**tens of zmol, low fg**) suitable for analysis of ~50% of the mammalian proteome. Thirdly, in microarray-like throughput – 2,000-plex separations compared to 54-plex microchannel separations, 12-plex for commercial capillaries and 1-plex for native 2D microblotting. Fourthly, in analyte co-probing – 3 analytes assayed, with the capability to **re-probe blotted single cells** for additional targets >5 times, making sophisticated single cell signaling pathway analyses via western possible.

Biological studies of neural stem cell differentiation using the single cell western reveal **striking cell-to-cell heterogeneity in neural stem cell differentiation marker expression** over a 6-day differentiation time course. Phosphorylation timing of the signaling proteins ERK and MEK are quantified after growth factor stimulation. Our single cell western blotting is poised to advance proteomics, from analysis of rare circulating tumor cells to elucidating heterogeneous response to small molecules.

CE-SELEX OF CHIMERIC DNA-BASED APTAMERS

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XNA (xeno-nucleic acid) aptamers are candidates for therapeutic drugs and diagnostic agents.¹ Therefore, enzymatic syntheses of XNAs² and their application to SELEX (systematic evolution of ligands by exponential enrichment) methods³ have been attempted. 2',4'-BNA/LNA (2',4'-bridged/locked nucleic acid)⁴, a form of XNAs shows high duplex stability and high nuclease resistance. Furthermore, 2',4'-BNA/LNA analogs are expected to be applicable to controlled release drug delivery systems, because those bridged rings can be opened and closed depending on external stimuli.⁵

We have obtained chimeric artificial nucleic acid aptamers specific to human thrombin from a chimeric artificial nucleic acid library using a CE (capillary electrophoresis)-SELEX method.^{6,7} This library contained 2'-O,4'-C-methylene-bridged/linked bicyclic ribonucleotides (B/L nucleotides) in the primer region and C5-modified thymidines bearing N⁶-ethyladenine in the non-primer region. Some obtained aptamers are found to completely lose their binding activities in the absence of B/L nucleotides. This emerging characteristic binding property will enable the creation of a direct selection methodology for DNA-based molecular switches that are triggered by chemical conversion of B/L nucleotides introduced to constant sequence regions in ODN libraries. Nonequilibrium capillary electrophoresis of equilibrium mixtures (NECEEM) was used for separation of active species in selection process and affinity analyses of obtained aptamers. In our presentation, we report results of sequence analyses, target-binding properties and nuclease resistances of obtained aptamers.

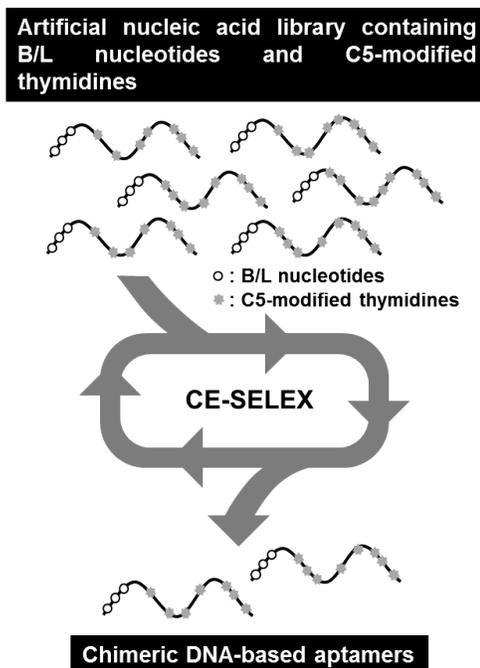


Figure 1. Selection of chimeric DNA-based aptamers using CE-SELEX

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**SINGLE CELL GENOMIC STUDY OF *DEHALOCOCCOIDITES* IN
DEEP SEA SEDIMENTS OF PERU MARGIN 1230**

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Dehalogenating *Chloroflexi*, such as *Dehalococcoidites* (*Dhc*) were originally discovered as the key microorganisms mediating reductive dehalogenation of the prevalent groundwater contaminants tetrachloroethene and trichloroethene. Molecular and genomic studies on their key enzymes for energy conservation, reductive dehalogenases (*rdh*), have provided evidence for ubiquitous horizontal gene transfer. A pioneering study by Futagami et al. in 2009 discovered novel putative *rdh* phylotypes in sediments from the Pacific, revealing an unknown and surprising abundance of *rdh* genes in pristine habitats. The frequent detection of *Dhc*-related 16S rRNA genes from these environments implied the occurrence of dissimilatory dehalorespiration in marine subsurface sediments.

Despite being ubiquitous in those environments, metabolic life style or ecological function of *Dhc* in the absence of anthropogenic contaminants is still completely unknown. We therefore analyzed a non-contaminated deepsea sediment sample of the Peru Margin 1230 site by a single cell genomic (SGC) approach. We present for the first time data on three single *Dhc* cells, helping to elucidate their role in the poorly understood oligotrophic marine sub-surface environment. Deepsea *Dhc* seem to be no halorespirers like their terrestrial relatives.

**DYNAMIC REGULATION OF SINGLE-CELL CODING AND NONCODING
TRANSCRIPTOMES DURING REPROGRAMMING**

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Epigenetic reprogramming of somatic cells into induced pluripotent stem (iPS) cells is clonal in nature, involving widespread changes in the coding and noncoding transcriptomes of individual cells. Numerous transcription factors and chromatin-modifying enzymes, some of which associate with long noncoding RNAs (lncRNAs), facilitate reprogramming of the somatic epigenome to pluripotency. Genome-wide transcriptome studies of reprogramming cell populations have been informative, yet are confounded by the stochastic and asynchronous nature of this clonal process. We performed ultra high-throughput RNA sequencing (RNA-seq) of all polyadenylated RNA species in single cells to investigate the identity, order, and coordination of changes triggered by ectopic Oct4, Sox2, Klf4, and Myc (OSKM). Induced cells faithfully activated known pluripotency transcription factors at early time-points, which coincided with a large global decrease in transcriptome variation between such cells at subsequent stages of reprogramming. We found that germ cell regulators are re-activated after the endogenous pluripotency network is established, as confirmed by single-molecule RNA FISH (smFISH) measurements in hundreds of single cells. Gain-of-function experiments using germ cell regulator genes increased reprogramming efficiency at early stages, highlighting molecular similarities between somatic cell reprogramming and epigenetic reprogramming during germ cell development. Furthermore, we characterized the dynamic progression of lncRNA activation and repression in induced cells, including hundreds of lncRNAs that are associated with chromatin regulatory proteins. These lncRNAs, which have emerging roles in epigenetic regulation, may exert broad effects in reprogramming the epigenome to the pluripotent state. Finally, our findings highlight a host of novel coding and noncoding gene sets, whose activity in individual cells begins to parse divergent and shared responses to reprogramming stimuli.

ACCURATE GENOME ANALYSIS OF SINGLE CELLS

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DNA sequence analysis and genotyping of biological samples using next-generation sequencing (NGS), microarrays, or real-time PCR is often limited by the small amount of sample available. A single cell comprises only one to four copies of genomic DNA, depending on the organism (haploid or diploid organism) and the cell cycle phase. The DNA amount of a single cell ranges from a few femtograms in bacteria to picograms in mammals. However, a thorough analysis of the genome requires a few hundred nanograms up to micrograms of genomic DNA. Consequently, accurate whole genome amplification (WGA) is required for reliable genetic analysis (e.g., NGS) when genomic DNA is limited, as in the case of single cell DNA. Usually single-cell genomic analysis, and in particular single-cell sequencing suffers from incomplete or biased genome amplification with missing or underestimated sequence information. In order to overcome these typical drawbacks, we developed an easy-to-apply single cell WGA method. This method is based on isothermal multiple displacement amplification (MDA) and consists of an innovative lysis and use of an optimized form of the Phi 29 Polymerase. To prove the method's robustness for single cell amplification, we amplified a variety of human and bacterial single cells, and checked the resulting genome coverage with NGS and qPCR methods. Discussed are experiments on cell-to-cell variations, GC content in comparison to genomic DNA, percentage of genome coverage with respective error rates, and genome-wide real-time PCR analysis. Overall the new method results in the effective lysis of cells, complete DNA denaturation, and reliable amplification of the whole genome of a single cell with high accuracy and minimal amplification bias.

NUCLEIC ACID APTAMERS FOR SMALL MOLECULES

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Nucleic acid aptamers that bind to small molecular targets will be applicable to biomarker detection and metabolome analysis. To date, various modifications of DNA/RNA base, sugar, and phosphate have been proposed to improve performance of nucleic acid aptamers¹. However, extremely few examples of base-modified DNA/RNA aptamers that recognize a small molecule have been published². For example, Battersby *et al.*, in 1999, first reported a modified DNA aptamer containing a 5-(3-aminopropyl)-uracil base that forms a 1:2 complex with adenosine-5'-triphosphate (ATP)^{2a}. Subsequently, Vaish *et al.* described an ATP-binding RNA aptamer in which 3-aminopropyl groups were introduced at the C5 position of the uracil base^{2b}. Unfortunately, those results failed to demonstrate the effectiveness of base modification in either target-binding affinity or specificity. In 2007, we have reported a modified DNA aptamer that included 5-(6-aminohexyl)-carbamoylmethyl-uracil bases specifically bound to the (*R*)-isomer of a thalidomide derivative^{2c}. The results demonstrated that modified aptamers could exhibit high enantioselectivity for a highly symmetric, low-molecular-weight target. Furthermore, we have recently demonstrated the superior efficacy of base modification in affinity enhancement^{2h}. By targeting the camptothecin (CPT) derivative, high affinity aptamers with dissociation constants (K_d) in the low nanomolar range were acquired from a base-modified DNA library containing an *N*-(2-(*N*⁶-adeninyl)ethyl) carbamylvinyl group at the C5-position of the uracil base. In contrast, aptamers from a natural DNA library exhibited only ordinary affinities to the target with K_d values in the low micromolar range, comparable to those generally observed in aptamers for small molecules.

Structural and affinity analyses of those CPT-binding aptamers were investigated using circular dichroism (CD) spectroscopy and fluorescence polarization (FP) assay. One of obtained DNA aptamers forms a parallel G-quadruplex structure³, and its binding activity is greatly affected by a few nucleotides at the 3'-tail. Interestingly, the T_m value was elevated from 73°C to 80°C when CPT derivative associated with the G-quadruplex aptamer, indicating a significant contribution to the thermodynamic stability owing to π - π stacking interaction between the G-quartet plane and heterocyclic rings of the target. Meanwhile, the modified DNA aptamer with the highest affinity was predicted to form a stem-loop structure, which was experimentally confirmed by the competitive assay using a short strand with sequence complementary to 3'-terminal bases of the stem region. We believe that the insights derived from our results will provide clues for devising a more universal approach for small ligand recognition of nucleic acid aptamers

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SELECTIVE DETECTION OF DOPAMINE USING NANOPARTICLE-BIOMOLECULE CONJUGATES*Markita P. Landry*^{1‡}, Sebastian Kruss^{1‡}, Michael Strano^{1*}¹Department of Chemical Engineering, Massachusetts Institute of Technology
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The detection of neurotransmitters is of fundamental importance for the development of platforms to study signal transduction in neuronal pathways. Temporal and spatial changes of neurotransmitter concentrations are central to information processing both within and amongst neuronal cells. Therefore, biosensors for neurotransmitters are necessary tools to understand how our brain works, particularly at the most fundamental single-cell scale. Here, we present fluorescent single-walled carbon nanotube (SWCNT) based sensors for neurotransmitters such as dopamine. We functionalized and suspended single-walled carbon nanotubes with a library of different polymers including phospholipids, nucleic acids and amphiphilic polymers and studied how neurotransmitters affect their intrinsic band-gap near-infrared (nIR) fluorescence. We found several polymer/SWCNT conjugates that enable detection of neurotransmitters. Most important, we found that catecholamines such as dopamine increased the fluorescence of nucleic acid wrapped SWCNTs in solution by a factor of up to 80 upon addition of 100 μ M dopamine for (9,4) chirality SWNT at their signature emission at 1132 nm. Furthermore, we identified single-stranded-(GT)₁₅-DNA and single-stranded-(GU)₁₅-RNA wrapped SWCNTs as the most promising optical dopamine sensors. In solution the limit of detection is < 100 pM with a K_d of 83 nM. Mechanistic studies showed that the turn-on response is due to an increase of the fluorescence quantum yield and is not related to covalent modification or reactive oxygen species. When immobilized to a surface, the fluorescence intensity of single (GT)₁₅- SWCNT was enhanced by a factor up to xyz and xyz for GU₁₅-SWCNT, and both fluorescence signals were reversible. Our findings indicate that the DNA/RNA backbones act as redox switches on the SWCNT surface and show a strong turn-on fluorescence response in the presence of dopamine. We attribute this sensing mechanism to a SWCNT corona-phase molecular recognition imparted by the DNA and RNA polymers. These findings pave the way for fluorescent neurotransmitter sensors in the near-infrared, which may find many applications in studying neurotransmitter release or exocytosis of single neuronal cells.

#34

SINGLE-CELL ANALYSIS REVEALS UNIQUE TRANSCRIPTION PROGRAM IN HUMAN METASTATIC BREAST CANCER CELLS

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Although surgical resection is a highly effective treatment for patients with early stage, organ-confined breast cancer, there are currently no effective treatments for patients with metastatic disease. 90% of breast cancer related mortality is due to metastasis, and median survival for metastatic patients is less than three years. This is due to the inherent complexity of metastatic disease, which can consist of tumor cells in many different peripheral tissues, often with unique clonal properties and therapeutic susceptibilities. In this study, we have utilized the new nanofluidics-based technology developed by Stephen Quake at Stanford University and Fluidigm Corporation to explore transcriptional heterogeneity of metastatic breast cancer cells at the single cell level. In collaboration with the Alana Welm lab at the University of Utah, we have generated a bank of human patient-derived xenograft (PDX) models of breast cancer that maintain many of the molecular, genetic, and metastatic properties of the original patient tumor. Using these models, we have developed a Fluorescence Activated Cell Sorting (FACS) based assay to identify and isolate human metastatic cells in the peripheral tissues of xenografted mice. 96x96 dynamic array chips were designed comprised of primer sets recognizing genes involved in mammary stem cell function, differentiation, cell cycle, and signaling pathways commonly implicated in breast cancer. We have examined multiple mice from each of five different PDX models. Interestingly, in all mice analyzed, clustering analysis of single-cell data sets shows that metastatic cells possess significantly different transcriptional signatures from the primary tumor cells from which they are derived. Metastatic cells in different peripheral tissues, such as the lung, lymph node, and peripheral blood, also possess distinct gene expression patterns within the same animal. Through this analysis, we have identified several new therapeutic targets upregulated on specific metastatic cell populations. We are beginning to test whether inhibition of such targets can block tumor cell dissemination to peripheral tissues. The long term goal of this work is to use this system as a model for personalized medicine; to determine if it is possible to define the clonal diversity that constitutes the metastatic landscape in individual mice, and use that information to inform individualized therapy decisions.

MASS SPECTROMETRIC IMAGING FOR ACHIEVING SINGLE CELL ANALYSIS

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We have developed a new and simple method for ambient ionization mass spectrometric analysis named laser desorption ionization droplet delivery mass spectrometry (LDIDD-MS), which requires no or minimum sample preparation. It utilizes a pulsed laser for desorption of analytes bound to substrates. Liquid droplets serve as the ion carrier of the desorbed and ionized species to the inlet of a mass spectrometer. This approach minimizes the loss of ions in ambient air. Repeated short pulses of laser light directed to the surface induce the desorption/ionization of analytes of interest bound to the surface while the directed sprayed liquid droplets on the surface formed by a nebulizing gas flow pick up and carry the desorbed ions to the mass spectrometry. As the region of desorption/ionization of LDIDD-MS is spatially limited to the laser beam spot size, the spatial resolution can be ideally reduced to several micrometers. By placing the sample on an XY translation stage a spatial chemical map is obtained.

We employed LDIDD-MS to image printed standard samples, as well as a mouse brain and a pancreas. We obtained 2.4 μm resolution for microcontact-printed samples and 7 μm for tissue imaging. We are currently investigating the analysis and imaging of heterogeneity of cell apoptosis and the photosynthesis process of Arabidopsis. We also applied the LDIDD-MS technique to analyze the samples in liquid phase which allows the direct analysis of biofluids in real-time. The exocytosis of PC12 cells under biochemical or electric stimulation is under investigation for the real-time imaging/analysis of exocytosis of neuropeptides. The developed mass spectrometric technique would provide new opportunities for imaging and analysis of biological samples at the single cell level.

STUDY OF ALTERNATIVE SPLICING IN SINGLE CELLS

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Alternative splicing plays an important role in many cellular processes and functions. However, the study of alternative splicing on the single cell level remains poorly understood. Here we study the alternative splicing of the *S. cerevisiae* PTC7 and SUS1 genes, two of three known alternatively spliced genes in the organism. Splicing efficiency of PTC7 is affected by different carbon sources on the protein level, while splicing efficiency of SUS1 is affected by heat shock on the mRNA level. By using single molecule FISH, we can determine the splicing efficiency of these two genes under the different conditions. We also treated yeast cells with nocodazole and performed smFISH, to look at splicing at two sites of PTC7 transcription in the same cell, thereby determining whether differences in alternative splicing is due to intrinsic or extrinsic factors. We have also utilized the MS2 and PP7 techniques to tag the intron and exon of the PTC7 and SUS1 genes to look at alternative splicing in living cells. We have also tagged the constitutively spliced RPL28 gene with MS2 and PP7. Using these techniques, we hope to study how environmental stresses affect splicing in single cells, as well as how factors such as transcription rate affect splicing of alternatively and constitutively spliced genes.

#37

SYSTEMS ANALYSIS OF MOUSE HEMATOPOIETIC STEM CELL DIFFERENTIATION *IN VIVO* AT THE SINGLE CELL LEVEL

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Hematopoietic stem cells (HSCs) are responsible for regenerating the blood and immune systems and play a central role in bone marrow transplantation. HSCs sustain the blood and immune systems through a step-wise lineage commitment, during which HSCs become progressively more specified in their potential and eventually give rise to mature blood and immune cells with distinct functions. While this process has been extensively characterized at the population level, little is known about the lineage commitment of individual HSCs. In particular, how these stem cells, few in number and residing in different bones, are coordinated in regenerating a common blood pool remains an unsolved question.

Here, we use a recently developed single cell tracking system to simultaneously track many HSCs *in vivo* through multiple stages of lineage commitment. This technique combines genetic barcoding and high-throughput sequencing. It offers the high sensitivity necessary for the direct examination of the clonality of HSCs and other rare hematopoietic progenitors. It also provides the capacity to simultaneously track many HSCs in a single mouse. Our data provides a comprehensive view of *in vivo* HSC clonal differentiation for the first time.

We show that in a pre-conditioned mouse ubiquitously used to study HSCs, a small subset of HSC clones always dominantly differentiates to supply the majority of blood cells. Dominant differentiation at distinct lineage commitment steps initiates unbalanced blood production, namely lineage bias. Individual HSCs producing distinct quantities and varieties of blood cells are coordinated in maintaining a balanced blood supply. In contrast, if the recipient mouse is not treated with any conditioning, all HSCs uniformly differentiate and do not exhibit dominant differentiation or lineage bias. We have identified the cellular origins of dominant differentiation and lineage bias, and have uncovered the discrete and mutually compensating lineage commitment pathways that lead HSC clones to differential blood production. These discoveries based on single cell analysis in conjunction with a systems biology approach are unexpected and unobtainable from conventional studies. They demonstrate new opportunities for understanding cellular regulation and coordination through tracking single cell activities that are indiscernible at the population level.

#38

TOWARDS SINGLE-CELL SYSTEMS BIOLOGY THROUGH SUPER-RESOLUTION IMAGING AND MOLECULAR BARCODING

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Fluorescence microscopy is a powerful quantitative tool for exploring regulatory networks in single cells. However, the number of molecular species that can be measured simultaneously is limited by the spectral overlap between fluorophores. We have demonstrated a simple but general strategy to dramatically increase the capacity for multiplex detection in single cells by labeling with unique combinations of fluorophores using fluorescence in situ hybridization (FISH) and resolving these barcodes using optical super-resolution microscopy (SRM). We have used this technique to measure mRNA levels of 32 genes simultaneously in single *Saccharomyces cerevisiae* cells. Ongoing work to scale this methodology up for the high-throughput analysis of gene regulatory networks in single cells will be presented.

SINGLE CELL GENE EXPRESSION PROFILING OF FIBRO-ADIPOGENIC PROGENITORS AS A NEW PROGNOSTIC TOOL IN DUCHENNE MUSCULAR DYSTROPHY

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Emerging evidence points to fibro-adipogenic progenitors (FAPs) as key cellular determinants of Duchenne Muscular Dystrophy (DMD) disease progression and response to pharmacological treatment in dystrophic muscles using HDAC inhibitors. FAPs comprise a heterogeneous population of skeletal muscle interstitial cells that retain a partial pluripotency within mesodermal lineages and can alternatively contribute to compensatory regeneration or fibroadipogenic degeneration of injured or diseased muscles. Because of their heterogeneous nature, the relative contribution of different subpopulations of FAPs to either muscle regeneration or degeneration is unknown. The lack of knowledge on the molecular network that controls the functional phenotype of FAPs hampers the attempts to selectively modulate their function for therapeutic purposes.

We are currently implementing a single cell gene expression profiling of FAPs in order to identify gene regulatory networks operating in FAPs. Our preliminary data analysis comparing the single FAPs isolated from wild type mice and dystrophic mdx mice showed that several sub-populations of FAPs can be identified, some of which are disease-specific. Using this strategy, we plan to identify specific gene expression profiles that can lead to the isolation and characterization of specialized FAPs subpopulations in skeletal muscles. Our ultimate goal is to identify molecular signatures and biomarkers of disease progression and response to experimental therapies in clinical trial. To this purpose, we will export the knowledge gained from single cell analysis of FAPs from mdx mice to muscle biopsies of DMD patients that are currently participating in a clinical trial with the HDAC inhibitor Givinostat.

BIFURCATION ANALYSIS OF SINGLE-CELL GENE EXPRESSION DATA REVEALS EPIGENETIC LANDSCAPE

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Waddington's epigenetic landscape has been widely accepted as a fundamental concept to describe the development process in complex systems. However, the molecular underpinnings of the epigenetic landscape remain poorly characterized. Here we present a novel mathematical approach to reconstruct the epigenetic landscape from single-cell gene expression data, combining dynamic clustering and bifurcation analysis. By analyzing two different datasets, obtained from mouse early embryos and bone marrow, respectively, we provide strong evidence that dynamic changes in gene expression during cell differentiation are highly organized and can be well described by the bifurcation theory. Characterization of the epigenetic landscape identified candidate regulators driving the initial events during cell differentiation, without relying on prior knowledge about the underlying gene regulatory network. Furthermore, we predicted the effect of perturbations of transcription factor expression levels on cell differentiation. Our molecular characterization of the epigenetic landscape may serve as a useful guide for future mechanistic studies of developmental regulation.

#41

IN VIVO DETECTION OF AMPHOTERICIN B PRODUCED BY *STREPTOMYCES NODOSUS* USING RAMAN MICROSPECTROSCOPY

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Bacteria are known to be one of the main producers of bioactive secondary metabolites that are highly important to pharmaceuticals. However, since 99% of all currently identified bacteria are uncultivable, the identification of such producers is extremely challenging. Thus, techniques for rapid and efficient screening are in demand. In this research, we approached this challenge by employing Raman microspectroscopy, a noninvasive technology applicable in the identification of molecular structures at the single-cell level, and applied it in the detection of antifungal antibiotic, amphotericin B within *Streptomyces nodosus*.

Fig. 1 and Fig. 2 shows the microscope images of *S. nodosus* and the Raman images of amphotericin B screened within bacterial aggregates and in single cells respectively. It was shown that amphotericin B exists locally within the bacterial aggregate but was not distributed throughout the bacterial population. Instead, the compound was only identified in specific areas within the population indicating the possible relation between amphotericin B production and cell growth stages. In the case of single cells, the detection of amphotericin B showed a much more discrete where it was found to be localized within vesicles distributed throughout the cell.

To summarize, we conclude that the successful detection of amphotericin B suggests that Raman microspectroscopy has the potential to be an efficient screening technique for secondary metabolites directly from bacteria.

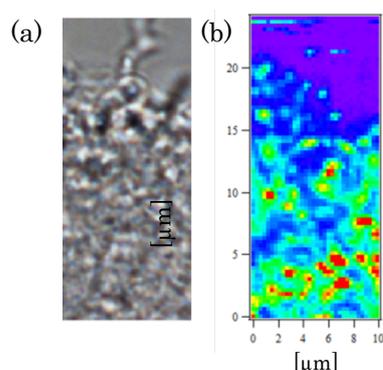


Fig. 1 (a) Microscope image and (b) Raman image of amphotericin B in aggregated *S. nodosus*

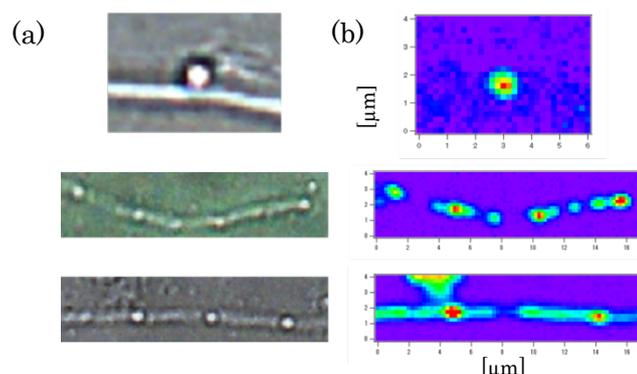


Fig. 2 (a) Microscope images and (b) Raman images of amphotericin B in single cells of *S. nodosus*

MICROCAVITY ARRAY SYSTEM FOR SIZE-BASED ENRICHMENT OF CIRCULATING TUMOR CELLS FROM SMALL CELL LUNG CANCER PATIENTS

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Circulating tumor cells (CTCs) are defined as tumor cells circulating in the peripheral blood of patients with metastatic cancer. The number of CTCs in peripheral blood has prognostic value in patients and can be used to evaluate therapeutic effects. We have developed a microcavity array (MCA) system integrated with a miniaturized device for the enrichment of CTCs without relying on Epithelial Cell Adhesion Molecule (EpCAM) expression. The MCA system allows enrichment of tumor cells from whole blood on the basis of differences in the size and deformability between tumor and blood cells. In previous study, we have developed circular MCA with an 8 μm diameter and have shown that the MCA system is potentially superior to the conventional epithelial antigen-based method for detecting CTCs in lung cancer¹. Recently, we further optimized the structure of MCA to improve the number and purity of small-sized tumor cells as in small-cell lung cancer (SCLC) recovered from whole blood. We developed the novel rectangular MCA to prevent the occupancy of microcavities by single cells and increase porosity of the substrate. Here we report the results of optimization of the rectangular MCA and the results of clinical study in metastatic SCLC patients using MCA system².

A microfabricated nickel filter with a rectangular MCA (10^4 cavities/filter) was integrated with a miniaturized device. We used NCI-H69 SCLC cells for spike-in experiments. The recovery rate of spiked SCLC cells in 1 ml of whole blood with the rectangular MCA ($80 \pm 5\%$) was significantly higher than that with the circular MCA ($67 \pm 7\%$) ($p < 0.01$, t-test). In addition, the number of leukocytes captured on the rectangular MCA (width of 8 μm , length of 30 μm) was 7-fold lower than that on the circular MCA (8 μm in diameter). To evaluate the effect of occupancy of microcavities, we measured the pressure drop across the MCA. The fluctuation of the pressure drop during filtration with the rectangular MCA (<1.5 kPa) was smaller than that with the circular MCA (2.3 kPa); the rectangular MCA enabled a reduction in the flow resistance and pressure drop across the microcavities.

For the clinical evaluation, paired peripheral blood samples were collected from 16 patients with metastatic SCLC for detection of CTCs by MCA. There were no significant difference in the number of recovered CTCs between circular MCA and rectangular MCA ($p = 0.60$, Wilcoxon test). In contrast, the number of leukocytes captured with the rectangular MCA (median, 854 cells/ml) was 2-fold lower than that with the circular MCA (median, 2269 cells/ml). This result suggests that implementing the rectangular MCA diminishes a considerable number of carryover leukocytes.

Our results suggest that the MCA system has potential as a tool for the efficient recovery of CTCs with high purity in patients with small cell-type tumors, while offering additional advantages in cost, portability, and capacity for further detailed analyses of CTCs.

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IDENTIFYING AND CHARACTERIZING LUNG CANCER TUMOR INITIATING CELLS USING SINGLE CELL ANALYSIS

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Lung cancer, with more than one million deaths, is the number one killer among cancers in both men and women worldwide. Despite numerous advances in our knowledge during the recent years, early detection and treatment of lung cancer have not yet been fulfilled and need to be improved. A group of cancer cells called tumor-initiating cells (TICs) are a subpopulation of cells believed to drive the progression of solid tumors. TICs are proposed to persist in tumors as a distinct population and cause relapse and metastasis by giving rise to new tumors. To find out more about tumor initiation in lung cancer, we looked into gene expression of individual cells. This will help us identify a specific gene expression pattern in TICs. As TICs are a rare population, their study at the single cell level may shed light to the tumor initiation process, which has been neglected in bulk cell studies currently applied. For this purpose, we used enriched TIC lines from different lung cancer patient tumors as well as circulating tumor cells (CTCs) obtained from cancer patients' blood. CTCs are rare cells, which have separated from the initial tumor and move around in the blood stream, spreading to other parts and causing metastasis. Using Fluidigm Biomark HD, we analyzed single cells from patient specific TIC enriched cell lines. Furthermore, we looked into CTCs gene expression pattern for 96 different genes selected on the basis of different criteria. This study will lead us to understand the tumor initiation in lung cancer, a phenomenon which is not well understood. On the other hand, this will aid us in identifying biomarkers which would potentially improve prognosis, diagnosis as well as treatment of lung cancer.

NON-DESTRUCTIVE HANDLING OF CHROMATIN FIBERS ISOLATED FROM SINGLE CELLS UTILIZING AN OPTICALLY DRIVEN MICROTOOL

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In eukaryotic cells, DNA molecules form chromatin fibers with histone proteins; gene expression is regulated by the degree of chromatin folding and histone tail modifications such as methylation, acetylation, and phosphorylation. Epigenetic phenomena are determined by the partial unfolding/tight folding and histone modifications along the chromatin fibers, and by the timing of these events. There are few methods for investigation of individual intact chromatin fibers isolated from single cells, because intact chromatin fibers (typically several hundreds of microns long) break easily due to hydrodynamic shear during isolation and handling.

In this study, we report a novel method for non-destructive handling of, and biochemical experiments with, individual intact chromatin fibers, as well as their isolation from single cells, utilizing a specifically designed microfluidic device with an optically driven microtool. Spheroplasts of recombinant yeast cells expressing fluorescent protein-tagged core histones were employed, and isolation of chromatin fibers was conducted by cell bursting via changing from solution condition to hypotonic condition in the microfluidic device. The isolation of chromatin fibers was confirmed by the fluorescence-labeled core histones of chromatin fibers. For the non-destructive handling of the isolated chromatin fibers in the microfluidic device, we developed antibody-conjugated microspheres, which had affinity to the fluorescent protein-tagged core histones, and the microspheres were manipulated using optical tweezers, which functioned as optically driven microtools. With the aid of the microtool, isolated chromatin fibers were manipulated non-destructively, and were tethered at the microstructures fabricated in the microfluidic device with straightened conformation by the flow.

INVESTIGATION OF COLON ONCOGENESIS OF INFLAMMATION-INDUCED MOUSE TUMORS BY SINGLE-CELL EXPRESSION ANALYSES.

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Recent reports indicate that intestinal tumors can originate from stem cells and their progenitor cells located at the bottom of intestinal crypts. However, it is not clear how these intestinal stem-related cells expand and acquire tumorigenic characteristics during early stages of cancer development. In order to understand early oncogenesis of colon cancer, it will be important to clarify the process of dynamic transformation of stem-related cells. We used a mouse model of inflammation-induced colon tumors, and performed single-cell expression analyses of epithelia during the course of colon carcinogenesis, and by investigating cellular heterogeneity, attempted to understand how normal stem cells altered their expression profiles to generate tumors. ApcMin mice were treated with dextran sulfate sodium (DSS) to generate colon tumors, and EpCAM-positive epithelial cells derived from each stage of tumor development were selected by flow cytometry. Subsequently, single-cell expression profiles were determined by performing multigene qPCR (Fluidigm, Biomark HD System). Development of adenoma was associated with a marked increase of LGR5-positive cells and a decrease of cells of differentiated lineages. Detailed analyses of stem cell dynamics during colon oncogenesis are in progress.

TRIALS AND TRIBULATIONS IN SINGLE CELL ANALYSIS OF LEUKEMIA

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The importance of clonal evolution - the selection for and emergence of increasingly malignant clones of cells during therapy, resulting in cancer metastasis and relapse - has been highlighted recently for a variety of cancers. Preliminary work tracking mutant alleles from diagnosis to relapse, or primary site to metastasis has relied primarily on bulk samples and large scale next generation sequencing. While bulk leukemia sample analysis of mutations and allele frequencies can give valuable information about mutation burden in cancer; understanding how these mutations interact intracellularly and how unique clones and their progeny compete requires more detailed analyses, specifically at the single-cell level.

To study genetic clonal heterogeneity present in leukemia, we focused on detection of mutations in specific genes commonly mutated in acute myeloid leukemia (AML) and correlated with prognosis concurrently in single cells. We validated a straightforward single-cell, multiplexed PCR method downstream of flow cytometry based cell selection. As all of the mutations we were investigating were insertions or deletions, we adapted existing bulk methods used clinically to identify FLT3 and NPM1 mutant samples to reliably assess single cells as input (both mutations occur in approximately a 1/3 of cytogenetically normal, adult AML). This method allows us to efficiently examine the zygosity of all clones in a tumor sample with respect to specific genes of interest, including wild type or rare clones in a large number of cells per patient. The simplicity of the analysis allowed for a robust, cost-effective method to be applied to actual AML patient samples directly, focusing on the distribution of known mutations within a single leukemic cell population.

We applied the technique to obtain a snapshot of the clonal distribution in six diagnosis AML samples with respect to FLT3, NPM1, along with WT1 in one matched relapse sample and demonstrated more clonal diversity than previously assumed. While the technique employed was fairly direct and uncomplicated, it is clear that given the extent of potential diversity in leukemia, further considerations regarding the validation, application and statistical analysis of single cell data from clinical samples are required. Ideally, a framework for appropriate validation of single cell genetic data could be generated in order for this technically challenging data to be more applicable to actual clinical samples. To this end, we have begun additional layers of validation from assessing potential bias due to sources of technical variability such as flow sorting and PCR failure rates, allele dropout, and sample size determination given those sources of error. This additional validation work for a clinically focused technique has brought to light a variety of issues that will be crucial to address in future single cell genotyping.

Understanding the change in clonal structure due to chemotherapy selective pressure might allow us to develop clinical diagnostics to quickly quantify clones at diagnosis and during therapy, and adapt therapy as the clonal population adapts to target the surviving clones. Using the insight gained by single cell genotyping in the context of bulk sample sequencing data, we can connect what is realistic to assess clinically (bulk samples), to what is occurring at a population level during remission and relapse. However, this will require the development of robust, high throughput, well-validated targeted single cell assays and the computational framework to interpret the data and extract clinically relevant information.

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DIRECT SINGLE-CELL / SINGLE-MOLECULE MEASUREMENT OF TCR TRIGGERING BY AGONIST pMHC IN LIVING PRIMARY T CELLS

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The central question in immunology is how T cells distinguish self from foreign antigens. At the center of this question is the T cell receptor (TCR), which is constantly challenged with the daunting task of recognizing foreign antigens among countless self-peptides presented by MHC molecules. While the molecular interactions between TCR and its ligands are well characterized in vitro, quantitative measurements of these interactions in living cells are required to accurately resolve the physical mechanisms of TCR signaling. We report direct single molecule measurements of TCR triggering by agonist pMHC in hybrid junctions between live primary T cells and supported lipid membranes. Every pMHC:TCR complex over the entire cell is tracked while simultaneously monitoring the local membrane recruitment of ZAP70, as a readout of TCR triggering. Mean dwell times for pMHC:TCR molecular binding of 5 and 54 s were measured for two different pMHC:TCR systems. Single molecule measurements of the pMHC:TCR:ZAP70 complex indicate that TCR triggering is stoichiometric with agonist pMHC in a 1:1 ratio.

**SIGNALING DYSFUNCTION IN DUCHENNE MUSCULAR DYSTROPHY
REVEALED BY SINGLE-CELL MASS CYTOMETRY**

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Duchenne Muscular Dystrophy (DMD), the most common inherited muscular dystrophy, is caused by mutations in the dystrophin gene, a cytoskeletal protein essential for the stability of membranes of muscle myofibers. The disease is a lethal X-linked recessive disorder that affects 1 in 3,500 boys and leads to death by the third decade of their life. A conundrum has been that mice lacking dystrophin (*mdx*) manifest minimal symptoms, limiting our understanding of the disease and hindering the development of therapies. Recently, we hypothesized that telomere length differences between mice and humans could account for this discrepancy and developed a mouse model with somewhat shortened telomeres (*mdx/mTR^{KO}*). Our laboratory has demonstrated that these mice exhibit both skeletal muscle and cardiac defects seen in DMD patients. Notably, their skeletal muscle stem cells (MuSCs) are functionally defective and fail to maintain the damage-repair cycle initiated by dystrophin deficiency.

In order to understand whether alterations in the relative abundance of MuSCs subsets and/or in the regulation of their signaling networks underlies the functional regenerative defect observed in our mouse model of DMD, we capitalized on a new technology, multiparametric single-cell mass cytometry (also known as CyTOF). Single-cell mass cytometry is a technology at the interface between mass spectrometry and flow cytometry, which allows quantitative, high-throughput analysis of multiple (>50) biological cell surface and intracellular markers at the single cell level, by using antibodies labeled with transition element isotopes instead of fluorophores. This cutting edge technology allows discovery of novel cell subsets within rare stem cell populations, as well as single-cell studies of signaling dynamics. We aimed to define distinct cell subsets within the MuSC population based on surface markers, and identify the signaling pathways that go awry within those subsets during the onset and progression of DMD.

We performed a high throughput flow cytometric screen to identify novel surface markers that label the murine MuSC population and developed a panel of 30 isotope-chelated antibodies against surface proteins and intracellular signaling molecules for the analysis of muscle cells isolated from *mdx/mTR^{KO}* mice and controls (*mdx/mTR^{Het}*, *mTR^{G2}*). Importantly, using new marker sets and the Spanning-tree Progression Analysis of Density normalized Event (SPADE) algorithm for data analysis, we identified previously unrecognized stem cell subsets in mouse. In addition, CyTOF analysis of MuSCs from *mdx/mTR^{KO}* mice revealed dysregulation of key signaling pathways. These studies are providing novel insights into the molecular mechanisms responsible for MuSC dysfunction that will inform the development of more efficacious therapies for DMD.

ENHANCING FLOW CYTOMETRY BY INTELLIGENT DATA PROCESSING USING PLATEANALYZER VISUAL PROGRAMMING TOOLKIT

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Traditionally, flow cytometry (FC) experiments provided low to medium content, as typical assays utilized only a few separate FCS files to represent individual samples. The standard practice was to analyze each file individually making it not only time consuming and but also reducing the opportunity to integrate data. Recently, a significant progress in FC practices brought by new technologies such as hyperspectral detection, atomic mass spectroscopy-based detection (CyTOF) and use of robotics to drive high-throughput systems allowed massive multiplexing capabilities resulting in vastly increased number of variables and data files. In fact, the modern HT FC devices are capable of collecting thousands of samples per day.

The new FC collection modalities designed to take advantage of systems biology approaches created a need for innovative, next-generation data analysis tools allowing manipulation, processing and rapid visualization of multifactorial and multiparametric FC data ensembles. This is because both the throughput and available dimensionality provided by these techniques have increased well beyond the processing, mining or visualization ability offered by current FC analytical software.

PlateAnalyzer is a modern FC-oriented data processing and visualization environment, which offers FC practitioners ability to rapidly process large number of experimental files organized in multidimensional arrays according to various experimental conditions. PlateAnalyzer directly operates on data ensembles containing multiple FCS files. It maintains logical structure and hierarchies of large data collations and provides unique ability of performing cytometry “what-if” analysis.

In contrast to other available solutions PlateAnalyzer is fully programmable. The PlateAnalyzer Canvas – a design component of PlateAnalyzer – is essentially a visual programming toolkit allowing a cytometry analyst to combine various processing steps, control the input for the algorithms, and apply processing operators graphically, interacting with the design canvas, rather than encoding the processing pipeline by using all-purpose data-manipulation and statistical analysis language such as R. In the PlateAnalyzer system small icons or boxes are representing programmatic entities (snippets of functional code), and lines (or pipes) connecting these objects allow for flow of information and indicate relationships between operators. These entire arrangements of data processing code snippets and connecting pipelines create so called FC logic maps, which are autonomous, reusable segments of instantly executable code. The data processing tools developed and distributed under this program are interactive, intuitive, and capable of operating in real-time even on huge data sets.

The poster demonstrates the use of PlateAnalyzer in the context of traditional florescence-based FC, and for processing and exploration of complicated CyTOF experiments. PlateAnalyzer is available free for academic use (http://www.cyto.purdue.edu/Purdue_software) and a license can be requested at wombat@purdue.edu.

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SINGLE CELL CHIP-SEQ USING DROP-BASED MICROFLUIDICS

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Populations of cells have substantial heterogeneity that is important for their function and understanding. This variability is reflected in cell to cell variations of epigenetic features that are quickly averaged when characterized without single-cell resolution. We developed a method for single-cell ChIP-Seq that combines drop-based microfluidics with genomics and DNA barcoding. The method is based on encapsulating individual cells in micron-size droplets, lysing the cells inside the droplets, and labeling the DNA fragments with molecular barcodes unique to each droplet. When sequenced, each barcode is used to group DNA fragments that originate in the same cell, yielding multiple density maps for individual cells.

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SUPER-RESOLUTION FLUORESCENCE IMAGING OF INTRACELLULAR MUTANT HUNTINGTIN PROTEIN REVEALS A POPULATION OF FIBRILLAR AGGREGATES CO-EXISTING WITH COMPACT PERINUCLEAR INCLUSION BODIES

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The identities of toxic aggregate species in Huntington's disease (HD) pathogenesis remain unclear. While polyQ-expanded mutant huntingtin (Htt) is known to accumulate in compact inclusion bodies inside neurons, this is widely thought to be a protective coping response that sequesters misfolded conformations or aggregated states of the mutated protein.

To define the spatial distributions of fluorescently-labeled Htt-exon1 species in the cell model PC12m (terminally differentiated into sympathetic-neuron-like cells with nerve growth factor), we employed highly sensitive single-molecule-based and stimulated emission depletion (STED) super-resolution fluorescence imaging modalities. In addition to inclusion bodies and the diffuse pool of monomers and oligomers, fibrillar aggregates ~100 nm in diameter and up to ~1–2 μ m in length were observed for pathogenic polyQ tracts (expression experiments with 46 and 97 repeats) after targeted photo-bleaching of the inclusion bodies. These short structures bear a striking resemblance to fibers described in vitro. We identified a sharp cutoff behavior of maximum fibril length and documented the ensuing bundling of these fibers into denser arrangements of varying complexity, both in the cytosolic space and inside the neuritic processes.

Definition of the diverse Htt structures in cells will provide an avenue to link the impact of pharmacological agents to aggregate populations and morphologies. The latest observations w.r.t. co-localization of Htt with various quality control proteins such as the chaperone Hsp70 are presented.

STUDY OF CELL REPROGRAMMING BY ELECTROFUSION OF SOMATIC CELLS WITH PEG-FUSED ESCS

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Somatic cells can be reprogrammed toward pluripotent state by fusion with embryonic stem cells (ESCs). To investigate the process of reprogramming, we performed one-to-one fusion of somatic cells (mouse embryonic fibroblasts, MEFs) with ESCs using an electrofusion device and observed the fusants with a time-lapse microscope. However, reprogramming did not occur in this case of one-to-one fusion, although fused MEF expressed a red fluorescent protein which was expressed only in the nuclear of ESCs. Based on this result, we hypothesized that a single MEF fused with one ESC cannot gain sufficient amount of reprogramming factors. Then, we attempted to fuse one MEF with several ESCs. The method of one-to-several fusion is as follows. First, ESCs were fused using PEG (polyethylene glycol) on a culture dish to obtain homokaryon of ESCs. Next, we fused these fusants with MEF using an electrofusion device. We observed that fused cells had three or more nuclei suggesting that fusion between somatic cell and ESC fusant was successful. In the future we will observe fused cells further and investigate the number of ESCs needed for reprogramming.

SINGLE CELL TRANSCRIPTOMICS

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Even ‘identical’ cells can exhibit substantial differences in their gene expression, protein levels and cellular phenotypes, with important functional consequences. Existing studies of cellular heterogeneity, however, have typically measured only a few pre-selected RNAs or proteins simultaneously, because genomic profiling methods could not be applied to single cells until very recently. Using single-cell RNA-Seq, we investigate heterogeneity in the response of mouse bone marrow-derived dendritic cells (BMDCs) to lipopolysaccharide (LPS), a component of gram-negative bacteria. We discover extensive variation between individual cells in both the abundance and splicing of RNA transcripts, which we independently validate by RNA-fluorescence *in situ* hybridization of selected transcripts. In particular, hundreds of key immune genes are bimodally expressed across individual cells, a phenomenon driven by differences in both cell state and cell circuit usage: some of the observed bimodality can be attributed to closely related, yet distinct, developmental states; other portions reflect differences in the usage of key regulatory circuits. We also discover high levels of cell-to-cell variation in splicing patterns, with some cells primarily expressing one isoform, and others expressing another. Our study demonstrates the potential for unbiased single-cell genomics to uncover extensive functional diversity between cells. Towards realizing this promise, we discuss high-throughput experimental strategies for achieving the statistical power necessary to reconstruct intracellular circuits, enumerate and redefine cell states and types, and transform our understanding of cellular decision-making on a genomic scale.

MULTI-FUNCTIONAL PROBES FOR MESSENGER- AND MICRO-RNA ANALYSIS OF TISSUE MODELS AT SINGLE CELL LEVEL

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We have been developing multi-functional probes to collect mRNA from adherent cells at single cell levels, including Pt-ring electrode [1], double-barrel carbon electrode probe (DBCP) [2], and microfluidic probe (MFP) [3]. Scanning probe microscopy (SPM) is advantageous to capture high-resolution living cell images based on not only topography but also electrochemical and optical responses, although current SPM system has not achieved the mapping of a relatively larger (more than tens or hundreds) numbers of species. The multi-functional probes have potential to be applied for high-throughput single-cell analysis by combining a dynamic array device integrating microfluidic circuit [4], mass spectrometry, or electrochemical array device [5].

In the present study, we applied a glass pipette probe and the DBCP to collect micro-RNA and mRNA from human umbilical vein endothelial cells (HUVEC). HUVEC were seeded on Matrigel layer and were cultured for 4–24 h. Tube-like network formed within 4h-culture and the elongation length decreased with time course after 8 h-culture. The pipette probe or DBCP was set on oil-derived three-axis manual micromanipulator. HUVECs at elongation and aggregation points were collected by using either probe. For electrolysis, the medium was exchanged for 0.25 M sucrose for the electrical cell lysis in experiment. The DBCP was positioned 5 μm above the targeted cells. Then, an electric pulse was applied at +150 V between the two electrodes of DBCP for a 10 μs period using ECM 2001 Electro Cell Manipulator (BTX-Harvard Apparatus, USA). After the cell lysis, content was collected inside probe with aspiration using syringe. The collected solution was transferred into a PCR tube. Total RNA purification was performed according to the protocols for RNeasy Micro Kit (Qiagen). The RT reaction was carried out according to the protocols for the QuantiTect reverse transcription kit (Qiagen). Pre-amplification and realtime PCR was performed by using Thermal Cycler Dice® Real Time System (Takara) and /or LightCycler 1.5 System (Roche). For micro-RNA analysis, miRNeasy Micro Kit (Qiagen), TaqMan Micro-RNA Reverse Transcription Kit (Applied Biosystems), and specific primers (TaqMan MicroRNA Assays, Applied Biosystems) were used. RNU48 was used as normalizing control.

For the analysis of large number of cells (ca. 10^4 cells), the expression levels of mRNA (DLL4, KDR, FLT1, FAS, NOTCH1, INGB1) and micro-RNA (miR-92a, miR-221, miR-210, miR-21, and miR-126) were quantified at various cultivation period on Matrigel. DLL4, KDR and miR-92a increased depending on the culture period. FLT1 and FAS showed the highest value at 4 h-culture and decreased. miR-221 was highest at 8-hr culture, NOTCH1, ITGB1, miR-210, miR-21, and miR-126 did not change during the tube formation. The expression levels of mRNA and micro-RNA collected with the probes were in good agreement with those of larger sample volume. Basically, no significant difference was observed between at elongation and aggregation points.

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INSIGHTS INTO THE B CELL DECISION TO DIVIDE OR DIE REVEALED WITH A NOVEL CELL TRACKING TOOL

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The B lymphocyte response to pathogens is a multi-cellular dynamic process essential for an efficient immune response, however variability in individual cell decisions and timing makes deconvolution of the robust population behavior quite challenging. We have developed a novel semi-automated cell tracking tool which we have applied to week-long, time-lapse microscopy datasets of primary CpG stimulated B lymphocytes. Treating cells as time-continuous physical blobs, the tool enabled us to confidently measure cell size, mitosis, and apoptosis for hundreds of cell tracks even in the presence of high cell motility, cell clustering, division, and apoptosis. Analysis of cell tracks revealed that cells which choose to grow and divide are protected from death, suggesting that cell fate is decided relatively early. The analysis further revealed that the timing of the decision process is variable in progenitors, while proliferating daughter cells showed minimal delay before cell-cycle re-entry in subsequent divisions. In addition, the timing of decision making, division, and death was highly correlated between siblings, corroborating and extending previous studies. Analysis of average generational cell sizes for responders and non-responders revealed that progenitor cells that chose to grow and divide grew by more than five-fold and underwent two to three rounds of nearly perfect size doubling and division followed by typically several rounds of poor growth and division. Interestingly, decreasing the concentration of the stimulus did not affect the growth rate or the probability that cells would respond in the first few divisions, however, the fractions of cells entering later divisions were dramatically reduced, arguing for the existence of a minimal response. These results are being incorporated into ongoing multi-scale modeling studies, exploring how molecular variability can result in the observed single-cell and population response

COMBINED PHENOTYPIC, FUNCTIONAL, AND GENOMIC ANALYSIS OF LEUKEMIC SUBCLONES REVEALS A CONSERVED DIFFERENTIATION TRAJECTORY

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Acute myeloid leukemia (AML) can present with multiple concurrent subclones at diagnosis. Subclone-specific mutations may confer resistance to molecular-targeted drugs through loss of antigen expression or rewiring of intracellular signaling pathways, leading to relapse. Deep sequencing approaches improve the detection of rare subclones, but the ability to prospectively identify subclones (i.e. without relapse material) is limited, and the effects of subclone-specific mutations on phenotype are poorly understood. In the course of a broader study of oligoclonal pediatric AML patients, we focused investigation on a diagnosis bone marrow sample from a patient harboring 3 presumed subclones (two with distinct NRAS-G12D, NRAS-G13D mutations) as determined by whole-genome sequencing (WGS). We employed a combination of 31-parameter mass cytometry, deep sequencing, FACS sorting, and computational modeling to produce a detailed profile of the subclonal genotypes and phenotypes in this patient.

The 3 anticipated subclones did not correlate with a clear subset of surface markers in the mass cytometry analysis. Instead, we observed a single continuous 'differentiation trajectory' from progenitor-like to monocyte-like blasts. To dissect this trajectory, 7 distinct myeloid/progenitor subsets were FACS-sorted, plus T/B cells as a non-leukemic control. For each subset we performed capture-based deep sequencing of 307 tumor-specific variants (Tier 1: 13; Tier 2: 33; Tier 3: 261; median depth: 1420x). Shifts in allele frequencies among the FACS-sorted subsets provided critical information to a Bayesian mixture modeling algorithm, allowing identification of 5 subclones, as opposed to the 3 subclones anticipated by WGS. The inferred subclonal genotypes were validated and further refined by targeted single-cell Sanger sequencing of multiple Tier 3 loci.

Although all 5 subclones were present throughout the differentiation trajectory, some were enriched in certain phenotypic states or 'reservoirs'. For example, the NRAS-G12D subclone was enriched in a progenitor-like subset, but its daughter subclone, which harbored an additional mutation in RAC2 (implicated in HSC engraftment), was enriched in the more differentiated subsets, suggesting an opposing effect. Notably, the T/B cell population harbored 2 tumor-specific Tier 1 mutations at >15% allele frequency, suggesting its presence in a preleukemic, multilineage-competent HSC.

Taken together, subclone-specific mutations appear to skew cells toward either progenitor or mature phenotypes, but the developmental trajectory enforced by parental mutations is resistant to change. Furthermore, characterization of subclones is possible at diagnosis, and may improve the selection of targeted therapies.

MULTIPLEXED ANALYSIS OF PROTEIN INTERACTIONS IN SINGLE CELLS***Ola Söderberg***

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Measurements in single cells are required to identify cell-to-cell variation in a heterogeneous cell population such as a tissue section. The activation status of proteins is in most cases regulated by post-translational modifications (PTMs) such as phosphorylation that cause structural changes in the proteins and thereby expose catalytic sites or promote interactions with other proteins. By binding partner proteins, i.e. forming protein complexes, they will gain additional function such as the ability to target substrate molecules. The activity status of a protein or signaling pathway can be visualized with *in situ* Proximity Ligation Assays (*in situ* PLA) using a pair of antibodies targeting the interacting proteins or PTM, using an attached DNA oligonucleotide to the antibodies to template the creation of a circular DNA molecule that is a surrogate marker for the interaction. It can then be amplified by rolling circle amplification (RCA) and be detected, with a single-molecule resolution, in fixed cells or tissues (Söderberg *et al.*, Nature Methods, 2006, **3**(12): 995). To enable parallel detection of multiple alternative protein interactions, e.g. for measurements at multiple nodes in a signaling network, we have recently developed a multiplexed version of *in situ* PLA (Leuchowius *et al.*, Mol Cell Proteomics, 2013, **12**(6): 1563). In this version of the assay we incorporate tag sequences in the PLA probes (antibodies conjugated to oligonucleotides), which is subsequently propagated into the RCA products. By targeting the different tags with different fluorophore labelled detection oligonucleotides the identities of the interacting proteins can thus be determined and enumerated. Multiplexed *in situ* PLA was used to determine levels of EGFR/HER2/HER3 dimers in tissue sections from breast cancers, showing increased levels of HER2 containing dimers in HER2 positive cases.

**LIGHT-CONTROLLED GLUCOSE REPRESSION SIGNALING
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Recent advances in fluorescence microscopy allow us to investigate time-resolved single cell behaviors [1, 2, 3]. Quantitative time-resolved information is particularly useful to understand dynamic features of gene regulation. Transcription factors change phosphorylation status response to external stresses to control gene expression by relocating between cytoplasm and nucleus. By tagging with fluorescence proteins, it is possible to track localization events of transcription factors. Using time-lapse fluorescence microscopy, we previously discovered frequency-modulated signaling system where signaling events occur in burst-like manner [4]. By a calcium stress, a transcription factor, Crz1 is dephosphorylated by a phosphatase Calcineurin, localizing into nucleus like a spike. The frequency and duration of the spike are determined by the strength of external stresses, coordinating downstream gene expression. To further extend the previous study, we investigated other stress-response transcription factors and their mechanism of frequency-modulated pulses. Under the low glucose condition, *S. cerevisiae* rapidly responds to glucose deprivation by a relief of Mig1 repressor, yielding expression bursts of the downstream genes such as *SUC2*. Using fluorescence time-lapse microscopy, we found that Mig1 localization oscillates between cytoplasm and nucleus under the adapted low glucose condition. To exam its mechanism, we employed optogenetic toolkits (PhyB/PIF6 [5] and CRY2/CIB1 [6]) by making c-terminal fusion constructs of responsible upstream regulating proteins such as kinase (Snf1), phosphatase (Glc7) and regulating subunit (Reg1). Upon the irradiation of light, specific pairs of proteins are recruited to interact, affecting Mig1 localization via catalyzing (de)phosphorylation reactions. We confirmed a class of pairwise interactions affecting Mig1 localization known from biochemical studies. Importantly, activation of a specific protein-protein interaction also induces localization change on non-labeled Mig1 protein, indicating the presence of global equilibrium perturbation in the Mig1 signaling pathway. Perspectives and future direction of the current project are also discussed.

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THE PROLIFERATION-QUIESCENCE DECISION IS CONTROLLED BY A BIFURCATION IN CDK2 ACTIVITY AT MITOTIC EXIT

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Tissue homeostasis in metazoans is regulated by transitions of cells between quiescence and proliferation. The hallmark of proliferating populations is progression through the cell cycle, which is driven by Cyclin-dependent kinase (CDK) activity. Here we introduce a live-cell sensor for CDK2 activity and unexpectedly found that proliferating cells bifurcate into two populations as they exit mitosis. Many cells immediately commit to the next cell cycle by building up CDK2 activity from an intermediate level, while other cells lack CDK2 activity and enter a transient state of quiescence. This bifurcation is directly controlled by the CDK inhibitor p21 and is regulated by mitogens during a restriction window at the end of the previous cell cycle. Thus, cells decide at the end of mitosis to either start the next cell cycle by immediately building up CDK2 activity or to enter a transient G0-like state by suppressing CDK2 activity.

MICROMECHANICS OF HUMAN MITOTIC CHROMOSOME*Mingxuan Sun*¹ and John Marko^{1,2}¹Department of Molecular Biosciences, Northwestern University, Evanston IL 60208
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Eukaryote cells completely reorganize their long chromosomal DNAs to folded mitotic chromosomes to facilitate precise DNA segregation during mitosis. Errors in chromosome folding and segregation can result in severe chromosome damage during cell division. The question of how DNAs are folded into the compact X-shaped chromosomes has been an outstanding problem intricately related to control of cell growth and proliferation and gene regulation, and consequently a problem closely related to many genetic and developmental disorders and cancers. However, the internal organization of mitotic chromosomes remains unclear.

We report biophysical experiments on single mitotic chromosomes from human cells, where isolated single human chromosomes were studied by micromanipulation and nanonewton-scale force measurement to understand chromosome connectivity and topology. This approach can be applied to study the micromechanics of chromosomes from cancer versus non-cancer cells.

We further looked into the effect of RNAi knockdowns of a major chromosome-organizing protein—condensin on mitotic chromosome organization. We found the stiffness of human chromosomes goes down by almost 10 fold in condensin depleted cells, compared to wildtype cells. Immunofluorescent staining of condensin shows a punctuated pattern along the chromosome arms, indicating individual condensin clusters. Our studies provide a quantitative analysis of the effect of condensin on mitotic chromosome condensation.

INTERACTION SURVEY BETWEEN RECEPTOR ON CELL SURFACE AND LIGAND BY ATOMIC FORCE MICROSCOPE

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Atomic Force Microscope (AFM) is the unique tool for surveying a single cell. We focused on measuring an affinity force between a sample and a probe in pN order with imaging one. By this function, we developed the new methodology how to analyze the interaction of receptor-ligand by AFM.

In this research, the target receptor is one of G protein-coupled receptors (GPCRs). GPCRs are well known as target molecules for drug development. However, knowledge of interactions between a ligand and GPCRs located on the surface of cell membrane is not enough for designing of drug. As a model host strain in this study, we used *Saccharomyces cerevisiae* BY4741, which expresses only one GPCR, Ste2p. It is resulting protein expressed from the *STE2* gene. The binding of the peptide pheromone (α -factor) initiates the cascade reactions that lead to the mating of haploid yeast cells.

We measured the interaction force between yeast cell variants (BY4741, BY4741(*ste2* Δ), and BY4741/*pGK421-STE2* which over-expresses of Ste2p) and the cantilever modified with α -factor or buffer (a negative control). For comparing, we used α -factor conjugated Cy2 with Flow Cytometry and measured mean of fluorescence intensity (Fig. 1). On the other hand, the histogram of adhesions was made after analyzing the results of AFM (Fig. 2). As a result, the differences in Ste2p expression level could be indicated by AFM. AFM will be the strong tool for researching a cell surface and a cell response in a single cell level.

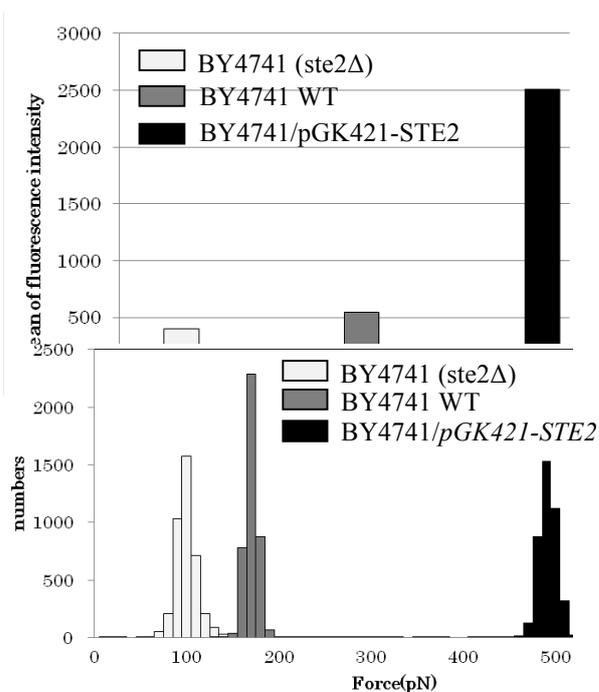


Fig. 2 Histograms of adhesions

**MULTI-ANGLE CONFOCAL ANALYSIS OF SINGLE ADHERENT CELLS
WITH MAGNETICALLY HANDLEABLE MICROPLATES**

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In this study, we propose the magnetically handleable microplate technology to manipulate single adherent cells toward the multi-angle confocal observation. In the field of cell biology, single cell analysis has attracted a great deal of attention in recent years. With expanding single cell research, multidimensional imaging including the cross-sectional image, especially, provides detailed information about spatiotemporal pattern of gene and protein expression inside the cells. The multidimensional images are obtainable by confocal microscopy, and three-dimensional (3D) images for the cellular cross-sectional view are assembled from a focal stack of 2D images. However, conventional acquisition of 3D confocal images requires many scanning x - y planes and is too slow to capture biological events, which results in a photobleaching or causes artifacts. Furthermore, spatial resolution in z axis is generally inferior to that of x - y plane. To overcome this limitation, we prepared the mobile substrate to culture and manipulate single adherent cells under the magnetic fields. Applied magnetic fields can rotate and tilt the cell-laden microplates in the desired orientation, thereby facilitating the observation of the cross-sectional intracellular structure in scanning x - y planes.

Through the microfabrication process, we fabricated the microplates with 50 μ m diameter by utilizing a transparent polymer, Parylene, and permalloy. The Parylene surfaces are modified with fibronectin to enhance cell attachment, growth and stretching. To isolate single cells on these microplate arrays, cells are initially placed in suspension at an optimized density and are allowed to settle, stretch, and grow on individual microplates. An alginate hydrogel sacrificial layer coated underneath the microplate array can be dissolved on a time scale of several seconds without cytotoxicity. By putting hollow magnets under objective lens, we formed magnetic fields around microplates and manipulated single cell-laden microplates in order to immobilize, rotate and tilt them without disturbing the microscopic optical path. During the manipulation, this mobile substrate also preserves their adhesive physiological properties. We applied the microplate system to tilt single cells to the observation in the confocal microscopy.

By utilizing confocal microscopy, we were capable of obtaining x - y scanning images inside the cells in the desired orientation after we tilted the cell-laden microplates. This system enables high-resolution cross-sectional imaging with single-molecule sensitivity at single image scanning. In this system, we successfully observed the intracellular structure composed of immune-stained actin fibers and microtubules inside single adherent cells. As the demonstration of high resolution observation, we applied this microplate handling technology to analyze the parasite invasion into host cells. We tested infection assay by using *Toxoplasma gondii* and their host HFF cells cultured on microplates. In the microplate system, single host cells infected by parasites were tilted in 90 degrees under the magnetic field, which facilitates to obtain the cross-sectional images scanned in x - y layer. Compared with reconstructed z -axis images in conventional two-dimensionally cultured cells in petri dishes, the confocal x - y scanning image of 90 degrees rotated single host cells clearly shows the cell membrane boundary between host cells and parasites, and reveals the relation between host cell morphology/cycle and parasite invasion efficiency. This method can be an attractive platform for the manipulation of host cells, the observation of invading microbes and the analysis of microbe-host cell interaction.

#63

RADIOLUMINESCENCE MICROSCOPY: MEASURING THE HETEROGENEOUS UPTAKE OF RADIOTRACERS IN SINGLE LIVING CELLS

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Radiotracers play an important role in interrogating molecular processes both *in vitro* and *in vivo*. However, current methods, such as PET, autoradiography, and scintillation counting are limited to measuring average radiotracer uptake in large cell populations and, as a result, lack the ability to quantify cell-to-cell variations. To overcome this limitation, we apply a new technique, termed *radioluminescence microscopy*, to visualize radiotracer uptake in single living cells, in a standard fluorescence microscopy environment.

In this technique, live cells are cultured sparsely on a thin scintillator plate and incubated with a radiotracer. Radioluminescence microscopy utilizes the scintillator plate, which is in contact with the cells of interest, to convert ionizing radiation from emitted beta particles into visible-range photons detectable in a sensitive microscope. The optical signal can be measured with an EM-CCD with a high resolution and enables the detection minute concentrations of radiotracers. The transparency of the plate in the visible range allows for conventional fluorescence and brightfield microscopy, which provides a rich environment to characterize the biological status of a population of living cells. Radioluminescence microscopy revealed strong heterogeneity in the uptake of [¹⁸F]fluorodeoxyglucose (FDG) in single cells, which was found consistent with fluorescence imaging of a glucose analog. We also verified that dynamic uptake of FDG in single cells followed the standard two-tissue compartmental model. Last, we transfected cells with a fusion PET/fluorescence reporter gene and found that uptake of FHBG (a PET radiotracer for transgene expression) coincided with expression of the fluorescent protein.

Together, these results indicate that radioluminescence microscopy can visualize radiotracer uptake with single-cell resolution, which may find a use in the precise characterization of radiotracers. We also envision using radioluminescence microscopy to explore the metabolism of cancer cells.

MEASUREMENT OF SINGLE CELL PROTEASE ACTIVITY*Lidan Wu*¹ and Jongyoon Han^{1,2}¹Department of Biological Engineering, Massachusetts Institute of Technology (MIT),
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The heterogeneity of individual cellular behavior in response to physical and chemical stimuli has raised increasing attention and exerted significant impact on current understanding of disease progression. While many previous studies primarily focused on the intracellular signaling events, people have more and more recognized the importance of extracellular signaling events in regulating cellular responses. Particularly, A disintegrin and metalloproteinases (ADAMs), which are membrane-bound proteases, play a significant role in the extracellular signaling network. The active ADAMs on cell surface could affect the cellular behavior through both positive and negative feedback by shedding growth factors, cytokines or receptors from cell surface via proteolysis. While the activities of ADAMs have been associated with breast cancer progression, the increased shedding of EGF ligands by ADAMs has also been linked to different clinical pathologic process. The EGF family members released from cell surface by ADAMs are known to activate specific EGF receptors connected to diverse intracellular signaling pathways, regulating important cellular processes, such as cell proliferation, migration, differentiation and survival. Furthermore, recent study revealed that the autocrine signaling via protease-mediated ligand shedding could stimulate the directed migration of individual cells without affecting their close neighborhood. These evidences suggested that variability in single cell protease activity could lead to diverse intracellular kinase activation profile or cell migration pattern in response to same stimuli. However, most of available single cell techniques for extracellular microenvironment measurements, including the microengraving method, fluorescent nanosensor platform and multi-electrochemical sensory array, focus on the quantification of the amount of diffusive target molecules and thus are not directly applicable when the enzymatic activity of extracellular molecules including those on cell surface are the matter of interest.

Therefore, given the need in context-dependent single cell study and the special information provided by extracellular enzymatic activities, we'd like to build a single cell protease activity measurement platform that is compatible with other types of molecular detection methods using fluorescent probes. In this work, we built the platform on a microwell array format where the spatial address of each well acts as index to correlate the measurements at different time frame back to the same cells. The designed platform has the capability to isolate individual microwells rapidly on demand so that one can control the paracrine signaling between cells in different microwells and perform the cell-surface protease activity measurement using diffusive FRET-based substrates. As a proof-of-concept, we tested the developed platform using HepG2 cells treated with PMA or inhibitors. And to study the temporal behavior of PMA-induced protease response, we monitored the extracellular protease activity of same single cells during two different time periods and showed that upon PMA stimulation, the extracellular protease activity of individual cells could either decrease or increase over time. While previous study on the EGF-stimulated ERK activation, another major component of ADAM-mediated autocrine signaling, discovered a nuclear-cytoplasmic ERK oscillation, our result suggested that the oscillation-like behavior might also exist in the protease activity level within the microenvironment of individual cells. More efforts are required to study the protease response more deeply. And we envision that this platform would be helpful in answering the questions of how individual cells coordinate their molecular events in a context-dependent manner, by allowing the examination of correlations between multiple cellular parameters.

SOLID PHASE NUCLEIC ACID QUANTIFICATION BY PCR AND MDA*Liyi Xu*^{1,2}, Linda Griffith¹ and Paul Blainey^{1,2}¹Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139
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Characterizing the phenotypic and genetic heterogeneity of cells in their natural biological context is of significant interest, but remains technically challenging. Progress has been made in exploring heterogeneous gene expression and genetic diversity in cells¹, but many of today's approaches require upfront isolation of single cells²; and the removal of cells from their native microenvironment, thus losing important spatial information³. In order to push forward applications such as characterizing the effect of different microenvironments on single-cell differentiation, epigenetic modification, and tumor chemo-resistant gene distribution, new technologies are urgently needed.

We developed a solid phase nucleic acid quantification and amplification method to provide a more versatile technology that avoids isolation of single cells. Using PEG hydrogel, we achieved *in vitro* digital counting of genome nucleic acids by PCR and MDA (multiple displacement amplification). In order to optimize the technology for sub-cellular volume, we characterized DNA amplification colony size's dependence on various parameters. We also accomplished single cell whole genome amplification by MDA on *E. coli* and environmental microbe samples. The results demonstrated the feasibility and the great potential of this technology to tackle single cell applications on mammalian cells and tissue samples. In the future, we aim to focus on *in situ* analyses allowing the characterization of a population of cells within tissue sample and the characterization of genetic information in sub-cellular level. The development of this technology will push forward many applications on single cell differentiation, epigenetics, and tumor heterogeneity characterization and exploration.

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SINGLE CELL TRANSPLANTATION UNVEILS SELF-RENEWING MYELOID-COMMITTED PROGENITORS AND NON-STEPWISE DIRECT DIFFERENTIATION PATHWAYS FROM HEMATOPOIETIC STEM CELLS TO MYELOID-COMMITTED PROGENITORS

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While hematopoiesis has provided important conceptual models for basic processes of cell differentiation, early differentiation pathways of hematopoietic stem cells (HSCs) remain obscure. Consensus holds that HSCs (CD34⁺KSL) give rise to multipotent progenitors (MPPs, Flt3⁺CD34⁺KSL) of reduced self-renewal potential and that MPPs eventually produce lineage-committed progenitor cells in a stepwise manner. However, without an assay system that permits clonal assay of potentials for differentiation into all types of mature blood lineage cells, definitive experimental evidence for hierarchical relationships among HSCs, MPPs, and lineage-committed progenitor cells is lacking. To address this issue, we generated a transgenic mouse line in which platelets (Plt) and erythrocytes (E) in addition to neutrophils/monocytes (nm) and T and B lymphocytes express Kusabira-Orange fluorescent protein. We used single cell transplantation with tracing of five mature blood lineages derived from individual cells to define the hematopoietic hierarchy. Unexpectedly, in the phenotypically-defined HSC compartment, we could detect not only HSCs but also progenitors of megakaryocytes, of megakaryocyte-erythroid cells, and of common myeloid cells and these progenitors exhibited long-term repopulation activity (MkRPs, MERPs, and CMRPs respectively). This indicates that loss of self-renewal activity in HSCs is not an essential step for lineage commitment. Furthermore, paired daughter-cell assays combined with single cell transplantation clearly revealed that HSCs undergo both symmetric division (yielding two HSCs) and asymmetric self-renewal division (yielding HSC-MkRP or HSC-CMRP pairs without passing through conventional MPP status). On the other hand, in the phenotypically-defined MPP compartment, Flt3⁺ MPPs tended to produce myeloid-restricted progenitors, whereas Flt3^{high} MPPs tended to produce progenitors with nm and/or B-lineage potentials. In addition, Flt3^{low} MPPs tended to produce progenitors with nm, E, Plt and B-lineage potentials in addition to the same kind of progenitors as in both Flt3⁺ MPP and Flt3^{high} MPP fraction. Importantly, multipotent cells with 5 blood lineages were extremely rare in the MPP fraction, indicating that cells in this fraction are oligo- or mono-potent progenitors at a single cell level. The results provide proof of symmetric and asymmetric self-renewal division in HSCs and demonstrate novel non-stepwise early differentiation pathways from HSCs to myeloid lineages. Single cell analysis *in vivo* is a powerful tool for precise understanding of functions of cells.

CELLULAR POPULATION ANALYSIS OF SINGLE-NUCLEUS GENOMES RESOLVES CLONAL AND SUBCLONAL ALTERATIONS IN GLIOBLASTOMA

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Glioblastoma, like other solid cancers, have been shown by histomorphologic, cytogenetic, and genomic studies to contain heterogeneous tumor cell populations. Such heterogeneity has been hypothesized to account for failure of targeted therapy in glioblastoma. Here, we present a cellular population-based framework to characterize tumor heterogeneity by single-nucleus sequencing and allele-type-based clustering of subclonal populations. Our analysis revealed distinct patterns of somatic copy number alterations within glioblastoma subclones, and clonal *EGFR* amplification followed by independent subclone-specific evolution of distinct *EGFR* variant II rearrangements. This heterogeneous evolution of a single gene has potential functional implications as the EGFR variant II protein exhibits transforming and inhibitor-sensitive properties. This study shows that single-nucleus sequencing can elucidate multiple forms of genomic heterogeneity within primary glioblastomas and can clarify the complexity of oncogenic drivers.

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LIGHT-CONTROLLED MAPK SIGNALING PATHWAY REVEALS A MEMORY EFFECT IN PC12 CELL NEURITE OUTGROWTH

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With a limited set of signaling pathways, cells exploit temporal control in order to ensure proper conversion of specific environmental stimuli into certain cellular output. It is well established that nerve growth factor (NGF) and epidermal growth factor (EGF) mediate distinct cell fates because they induce different time kinetics in mitogen-activated protein kinase (MAPK) signaling pathway. However, a quantitative linkage between the time kinetics of MAPK activation and the cellular response is still lacking, due to difficulties in precisely controlling the time kinetics of intracellular signaling pathway. Here, we constructed a light-gated protein-protein interaction system that uses light to regulate the activation and inactivation of MAPK signaling pathway. We showed that sustained MAPK activation through continuous light stimulation induces significant neurite outgrowth in PC12 cells in the absence of NGF. Interestingly, the same amount of neurite outgrowth can also be achieved by intermittent MAPK activation through pulsed ON/OFF light stimulation. Precise control of the ON and OFF time reveals a 45-minute “memory” period for MAPK-induced neurite outgrowth in PC12 cells. Within the “memory” period, a full speed of neurite growth is maintained despite that MAPK signaling pathway is turned off. This study of using light to precisely control intracellular signaling pathways shows a great promise in dissecting temporal dimension of signal transduction in cells.

SENSITIVE AND HIGH-THROUGHPUT DETECTION, ISOLATION AND ANALYSIS OF RARE CELLS BASED ON ENSEMBLE-DECISION ALIQUOT RANKING (EDAR)

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The analyses of rare cells, such as circulating tumor cells (CTCs), are often challenging, considering the desired sensitivity and throughput. For example, CTCs are extremely rare events in peripheral blood, usually 1-10 cells per 1 mL of blood. Another challenge is the heterogeneity of the CTCs, whose physical and biological attributes can vary significantly. To address those challenges, we developed an “all-in-one” platform for rare-cell analyses, ensemble-decision aliquot ranking (eDAR).¹ eDAR integrates the line-confocal detection, active sorting, on-chip purification, imaging and downstream analysis onto a microfluidic chip. The whole blood sample was labeled with antibodies conjugated with fluorophores, and then introduced into the eDAR chip. The sample was virtually divided into 500,000 aliquots per 1 mL of blood, with a typical aliquot size at 2 nL, and then ranked as “positive” or “negative” based on the immunostaining detected by the line-confocal microscopy. After this step, an active sorting mechanism was applied to isolate those “positive” aliquots, while discarding all the “negative ones”, which represented more than 99.999% of the blood. Those isolated aliquots were transferred to another region on the same microchip, so the CTCs could be further purified, trapped and enumerated.

We have developed two generations of eDAR to improve the performance, imaging quality and capacity. Our detection scheme was able to detect single CTCs surrounded by thousands of blood cells in the microchannel.² In the newest generation of eDAR,³ the recovery efficiency was 95% with a zero false positive rate. The throughput was high enough for clinical applications, up to 4.8 mL of whole blood per an hour. With an over 1 million fold enrichment ratio, cells were trapped on a very small area with a high purity, so we could image and enumerate the CTCs efficiently, typically less than 10 min for a chip. Using eDAR, we could easily perform downstream analyses such as multiple rounds of immunostaining,⁴ or pick up the single CTCs for more bio-functional assays. We applied eDAR on analyzing CTCs from metastatic breast cancer patients, and showed that our method was more sensitive than the FDA-approved method, CellSearch, based on a side-by-side comparison. We also successfully applied this method on analyzing peripheral blood samples from patients with metastatic lung cancer or pancreatic cancer, and found the detection efficiency was about 90%. In summary, we believe this sensitive, fast and robust method is useful in isolation, enumeration and downstream analyses of CTCs from patients' blood. eDAR can also benefit the analyses of other rare cells besides CTCs.

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#70

PHYSICAL GENE CLUSTERING IN BACTERIA BY SINGLE MOLECULE DNA FISH

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We study the *E.coli* chromosome in single cells by fluorescence microscopy. Our goal is to label and image multiple genes simultaneously in order to reconstruct chromosome structure. With an improved single molecule DNA FISH protocol, we can label genes in a sequence specific manner. Super resolution microscopy allows us to resolve multiple genes in the same diffraction limited region. The results indicate that the bacterial chromosome structure is sensitive to most general, and many specific stresses. In addition, we observe that some genes located far apart on the genome map are clustered in physical space. We found that other bacteria species, for example *Pseudomonas aeruginosa* and *Bacillus subtilis* but not *Caulobacter crescentus*, have similar gene clustering.

QUANTITATIVE STUDY OF CELLULAR HETEROGENEITY IN DOXORUBICIN UPTAKE AND ITS PHARMACOLOGICAL EFFECTS ON CANCER CELLS

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Genetically identical cells vary significantly in response to drugs even in a uniform environment, which can result in drug resistance and therapy collapse. In this study, the cellular heterogeneity in doxorubicin uptake and its pharmacological effects on cancer cells were quantitatively investigated by single-cell analysis for the first time.

An in vitro experimental model was established by treating human leukemia K562 and breast cancer MCF-7 cells with different schedules of doxorubicin with or without P-glycoprotein inhibitor verapamil. The cellular heterogeneity in doxorubicin uptake was quantitatively examined by single-cell analysis using capillary electrophoresis coupled with laser-induced fluorescence detection. The corresponding cytotoxic effects were tested by cellular morphology, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium and flow cytometry assays. The expression of cellular membrane surface P-glycoprotein was determined by flow cytometry.

Results exhibited that the cellular heterogeneity in doxorubicin uptake existed within both K562 and MCF-7 cells populations. Most importantly, the cellular heterogeneity in doxorubicin uptake was found negatively correlated with drug cytotoxicity on cancer cells and surface P-glycoprotein expression. Quantitative regression equations ($|r|=0.7680\sim 0.9587$) were obtained, which means reduced heterogeneity in cellular drug uptake could lead to better treatment outcomes. Verapamil as P-glycoprotein inhibitor reduced the cellular variation in doxorubicin uptake, suggesting cellular surface P-glycoprotein may be responsible for the cellular heterogeneity in doxorubicin uptake.

This research demonstrates the importance of quantitative study of cellular heterogeneity in drug uptake by single cells analysis and its potential applications in drug response prediction and therapy modulation.

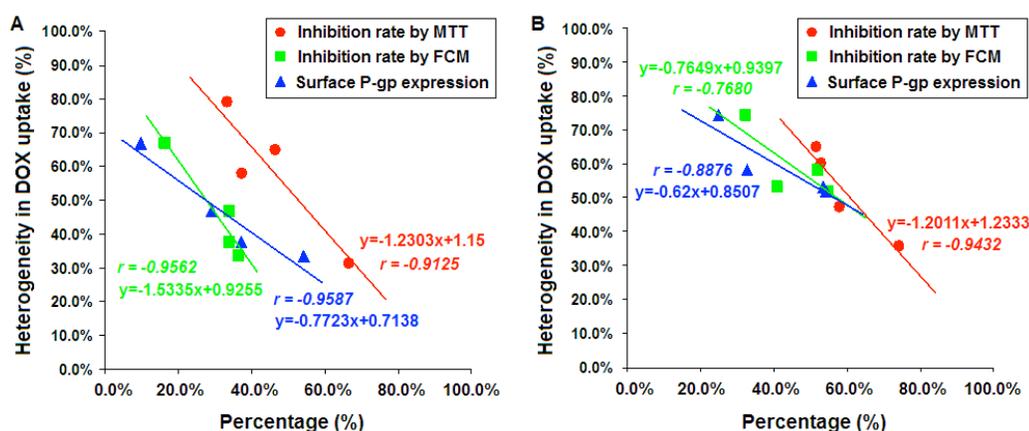


Figure 1. The negative correlations between cellular heterogeneity in DOX uptake and DOX cytotoxicity, and surface P-glycoprotein expression in (A) K562 and (B) MCF-7 cells

A BINDING-INDUCED GRAPHENE PROTEIN SENSOR PROMOTED BY EXONUCLEASE III-AIDED ISOTHERMAL AMPLIFICATION

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The sensitive and accurate detection of proteins on cell surface is critical for understanding their biological functions and discovering disease markers and drug targets. However, it is technically challenging to measure trace amounts of cell surface proteins in complex biological matrix. Herein, we present a sensitive protein sensor enabling homogeneous, isothermal detection of low concentrations of proteins. The sensor shows great potentials for amplified detection of cell surface proteins.

The sensor is built on a binding-induced DNA assembly strategy we proposed, in combination with the recent advances in Exonuclease III (Exo III)-aided isothermal amplification and graphene oxide (GO)-mediated fluorescence quenching. To build the sensor, we designed three DNA oligonucleotides (oligos), a triggering DNA, an assistant DNA, and a signal DNA. The triggering and assistant DNA oligos are conjugated to affinity ligands, forming probes to recognize target proteins. Three DNA oligos contain complementary sequences which are so short that DNA oligos are unable to assemble together in the absence of target proteins. In the presence of target proteins, two probes bind to the same target molecule, inducing hybridization between the triggering and assistant DNA. This binding-induced DNA hybridization consequently leads to ensemble of all three oligos, forming a T-shape DNA structure, which thereby triggers Exo III-aided recycling cleavage of the signal DNA. As a consequence, hundreds to thousands of fluorescently-labeled single nucleotides can be generated in response to a single target binding event. The fluorescently-labeled nucleotides are not adsorbed onto GO, offering fluorescence signal for amplified protein detection.

To demonstrate the proof of concept, we choose streptavidin and prostate specific antigen (PSA) as initial targets. We were able to detect as low as 1 pM streptavidin and 5 pM PSA. The analysis can be conducted under 37 °C in homogeneous solutions without the need for separation, which is promising for sensitive detection of specific proteins on cell surface.

**SCREENING OF PHARMACEUTICAL COMPOUNDS IN MULTIPLE
PATIENT-DERIVED GLIOMA STEM CELL LINES**

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Glioblastoma multiforme (GBM) is a highly heterogeneous type of brain cancer, in which glioblastoma cells have characteristic of glial cells. GBM has been emerged as one of the key study areas in the field of medical systems biology that paves a way to better understand the biology of this tumour. Glioblastoma tumor appear in naturally occurring sub-groups also indicate a potential for patient-selective targeting based on systems biology techniques. Furthermore, the study of glioblastoma is facilitated by a reliable range of model systems, such as mouse models and newly established cancer stem cell lines. The goal of our study is to develop the first 'quantitative handbook of drugs and siRNA's' for targeting glioblastoma derived tumor stem cells, applicable to individual patients. As a first step, we studied the effect and sensitivity of 1544 FDA-approved compounds in nine patient-derived brain tumor stem cell lines from the Human Glioma Cell Culture consortium. Initial analysis reveals multiple hits that effectively kills the 80% of the cancer cells, hits specific to individual patients and molecular subclasses. In conclusion, our pilot results indicate that it will be possible to systematically map and predict drug vulnerabilities in glioma, finding new uses to existing drugs in well-defined patient subgroups.

RNA-SEQ ANALYSIS KITS FOR IMPATIENT EXPERIMENTALISTS

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The rapid decrease in cost for high throughput sequencing has accelerated its adoption by a broad group of basic and clinical researchers. In particular, RNA-seq is becoming a tool of choice for exploring gene expression patterns, discovery of new biomarkers, and recognizing regulatory links between genes. A wide variety of effective open-source tools have been created to meet the growing “Big Data” challenge, yet most of these methods assume at least some bioinformatics expertise and familiarity with UNIX environments. As a result, biologists must often recruit or rely on external expertise from a limited pool of available bioinformaticians, creating a significant hurdle to achieving timely data analysis goals.

To address this problem, we develop a biologist-friendly cloud-based platform with end-to-end RNA-seq analysis kits that researchers can obtain gene expression analysis results from raw sequencing data and explore them in interactive heatmaps. In addition to visualizing analytic results in the private UCSC genome browser [1,2] framework, scientists can explore public datasets in the context of their own data. Users are also able to share data and results across labs and institutions within a secure environment, or distribute their data to broader communities. By supporting any plant, metazoan, or microbial genome of interest, our platform provides maximum flexibility to researchers to make breakthrough discoveries in drug development, targeted therapies, personalized medicine, biofuels, and basic research.

[1] Meyer L.R., Zweig A.S., Hinrichs A.S., et al. (2013) The UCSC Genome Browser database: extensions and updates 2013. *Nucleic Acid Res.* **41**(Database Issue):D64-D69.

[2] Chan P.P., Holmes A.D., Smith A.M., et al. (2012) The UCSC Archaeal Genome Browser: 2012 Update. *Nucleic Acid Res.* **40**(Database Issue):D646-D652.