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2	Blue B. Lake, Rizi Ai, Gwendolyn E. Kaeser, Neeraj S. Salathia, Yun Yung, Rui Liu, Andre Wildberg, Derek Gao, Ho-Lim Fung, Song Chen, Raakhee Vijayaraghavan, Julian Wong, Allison Chen, Xiaoyan Sheng, Fiona Kaper, Richard Shen, Mostafa Ronaghi, Jian-Bing Fan, Wei Wang, Jerold Chun and Kun Zhang; University of California, San Diego, Scripps, Illumina	Using single-nucleus RNA sequencing to reveal neuronal subtypes and diversity in the adult human brain
3	Olga Ornatsky, Qing Chang, Eric Swanson, Bedilu Allo, Alexandre Bouzekri , Alexander Loboda, Vladimir Baranov; Fluidigm Canada, Inc.	Multiparametric analysis of cells in tissue sections using imaging mass cytometry
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5	Camille Lombard-Banek, Sushma Reddy, Sally A. Moody, Peter Nemes; George Washington University	Quantitative, Untargeted Proteomics for Single Embryonic Cells in the 16-cell Xenopus Embryo
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40	Jeremy L. Norris, Bo Yang, Jeff Spraggins, Richard M. Caprioli; Vanderbilt University School of Medicine	Single Cell Analysis using High Spatial Resolution Imaging Mass Spectrometry
41	Alan J. Simmons, Amrita Banerjee, Eliot T. McKinley, Cherie R. Scurrah, Charles A. Herring, Jeffrey L. Franklin, Leslie S. Gewin, Ryota Masuzaki, Seth J. Karp, Michael J. Gerdes, Jonathan M. Irish, Robert J. Coffey, Ken S. Lau;	CyTOF-based single-cell analysis of intact signaling reveals divergent TNF-induced behaviors in the intestinal epithelium
42	Peter Nemes, Rosemary M. Onjiko, Erika Portero, Sally A. Moody; The George Washington University	Second-generation Single-cell Mass Spectrometry Finds Metabolic Cell Heterogeneity along the Left-Right Body Axis in the Developing Frog (Xenopus) Embryo

Poster 1: Innovative reporters to characterize heterogeneous states among cells

Li He and Norbert Perrimon

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Gene activation patterns vary widely in complexity. Some are straightforward and static, such as the pattern of many housekeeping genes that are expressed in all cells at all times through life. Others are extraordinarily intricate with expression fluctuating from minute to minute or from cell to cell. Changes in gene expression reflect changes in the transcriptional landscape of the cell, which in many cases occur in response to extracellular signals. Characterizing the spatiotemporal changes in gene expression is therefore a critical question in cell and developmental biology. Transcriptional reporters that drive the expression of fluorescent proteins (FPs) are commonly visualized to analyze the activation of signal pathways; however, the stability of these reporters makes it impossible to achieve the temporal resolution needed to dissect dynamic nature of gene expression. And even with some particular short-lived reporters, live imaging is usually required to reveal the active gene expression changes, which is hard to be adapted for large scale screen.

To address some of the limitations with current technologies, we first developed a series of new fluorescence reporters (Dynamic Fluorescence Reporters) that improve the temporal visualization of gene activities. In addition, we combined both dynamic and stable reporters to reveal signal changes without doing live cell imaging. Using the new reporters, we were able to visualize more precisely where and when particular signal is activated. And we also found that the due-color dynamic reporter will more sensitively and faithfully reflects the physiological and pathological changes in *Drosophila* intestinal stem cells. To expand the application of our reporter system, we also generated transgenic flies with due-color reporter controlled by UAS regulatory sequence, which allowed us to discover new expression dynamics of the vast Gal4 collections. In addition, we also created enhancer trap lines of the due-color dynamic reporter, mobilized it within fly genome, and collected novel insertions showing interesting dynamic patterns in various fly tissues. These new lines will serve both as reporters to monitor heterogeneous states among cells and as tools to identify the underlying genes responsible for the dynamics. We believe our new reporter system will become a useful tool for researchers to study the signaling heterogeneity in complex organisms.

Poster 2: Using single-nucleus RNA sequencing to reveal neuronal subtypes and diversity in the adult human brain

Blue B. Lake[1], Rizi Ai[2], Gwendolyn E. Kaeser[3,5], Neeraj S. Salathia[4], Yun Yung[3], Rui Liu[1], Andre Wildberg[2], Derek Gao[1], Ho-Lim Fung[1], Song Chen[1], Raakhee Vijayaraghavan[4], Julian Wong[3], Allison Chen[3], Xiaoyan Sheng[3], Fiona Kaper[4], Richard Shen[4], Mostafa Ronaghi[4], Jian-Bing Fan[4]*, Wei Wang[2]*, Jerold Chun[3]* and Kun Zhang[1]*

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The human brain has enormously complex cellular diversity, yet the underlying transcriptional landscape at the level of single cells is not completely understood, in part reflecting difficulties of interrogating individual neurons. We developed a scalable approach to sequence and quantify RNA molecules in isolated neuronal nuclei from post-mortem brain, generating 3,227 sets of quality-filtered, single neuron data from six representative Brodmann Areas (BAs) of the cerebral cortex. We find that the nuclear transcriptome can accurately predict cellular identity and, using an iterative unsupervised clustering approach, have identified 16 subtypes of cortical neurons that were further annotated based on known neuronal markers and cortical cytoarchitecture. Single neuron transcriptomes revealed shared genes sufficient to distinguish both neuronal subtypes and cortical region identity. These data demonstrate a robust and scalable method for identifying and categorizing single nuclear transcriptomes, revealing both new neuronal subtypes as well as heterogeneity indicative of uniqueness at the level of the single neuron.

Poster 4: *In situ* detection of a single bacterium in complex environment by hyperspectral CARS imaging

Weili Hong[1], Chien-Sheng Liao[1], Hansen Zhao[1], Waleed Younis[2], Yinxin Zhang[1], Mohamed N. Seleem[2], and Ji-Xin Cheng[1]

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Rapid detection of individual microbial cells in their natural environment is an important yet challenging task for clinical infectious disease diagnosis and food safety purposes. Conventional methods require long bacterial culture times, lack accuracy due to limited information, and are not sensitive in complex environments. Newly developed molecular techniques like polymerase chain reaction (PCR) reduce the detection time; however, these techniques take multiple steps and thus need a specialist, suffer from contaminations, and depend on the availability of suitable probes.

Vibrational spectroscopic imaging, which measures molecular vibration spectra, has emerged as a high-speed imaging platform for biology and medicine. In this project, we report for the first time a compact fiber-laser based hyperspectral coherent anti-Stokes Raman scattering (CARS) imaging system for rapid, *in situ* detection of a single living bacterium. By phase retrieval and multivariate curve resolution analysis, we demonstrated that a single *Escherichia coli* (*E. coli*) bacterium can be identified in a complex environment without the need of culturing, labeling, or sample contact.

Poster 5: Quantitative, Untargeted Proteomics for Single Embryonic Cells in the 16-cell *Xenopus* Embryo

Camille Lombard-Banek[1], Sushma Reddy[1], Sally A. Moody[2] and Peter Nemes[1]

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Deciphering cell-to-cell differences promises to help understand normal embryonic development. This goal, however, requires new analytical technologies capable of high sensitivity to probe limited amounts of biomolecules in single cells. Particularly needed are instruments enabling the untargeted interrogation of the metabolome and the proteome, the set of molecules that maintain homeostatic balances and carry out important molecular functions. With the NIH Single-cell Analysis (SCA) Common Fund, we recently developed a single-cell metabolic mass spectrometer that uncovered surprising metabolic cell heterogeneity in the early developing embryo (Onjiko-Moody-Nemes, PNAS 2015, 112, 6545).

Here, we report the development of a SCA platform that extends discovery measurements on single embryonic cells to proteins, filling an important technological gap. The platform was developed based on capillary electrospray high resolution mass spectrometry (CE-μESI-HRMS), and enabled the handling of nanoliters of samples with high sensitivity (25 amol lower limit of detection) and high reproducibility (<15% S.E.M.) for standard peptides. We then adapted a bottom-up proteomic workflow to the finite protein content of single embryonic cells (blastomeres) that were identified and dissected from the 16-cell frog (*Xenopus laevis*) embryo. By systematic evaluation of each step underlying sample preparation to data acquisition–processing, we were able to identify 480 different protein groups from 20 ng, or <0.2% of the total protein content of the blastomeres.

After adapting multiplexing quantification to the workflow, we compared protein expression in three different blastomeres from the 16-cell Xenopus embryo; the D11, V11, and V21 blastomeres give rise to different tissue types. These measurements resulted in the identification of 1,709 protein groups (<1% false discovery rate) and the quantitation of ~150 protein groups consistently across each biological replicate. An appreciable number of these proteins were statistically and biologically significantly differentially expressed between the blastomeres, revealing translational differences between cells along the animal–vegetal and the dorsal–ventral axis of the embryo. This finding complements results from deep transcriptomic measurements that found cell heterogeneity only along the former axis.

In summary, we successfully developed a single cell platform for the interrogation of proteins from single blastomeres in 16-cell *Xenopus* embryos. With continuous developments in analytical sensitivity, we envision this technology to be applicable also to smaller blastomeres and other cell types as well as cells from different models.

Poster 6: Single-cell detection of secreted Aβ and sAPPα from human iPSC-derived neurons and astrocytes

Mei-Chen Liao[1], Christina R. Muratore[1], Todd M. Gierahn[2], Sarah E. Sullivan[1], Priya Srikanth[1], Philip L. De Jager[1], J. Christopher Love[2,3], and Tracy L. Young-Pearse[1,*]

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Secreted factors play a central role in normal and pathological processes in every tissue in the body. The brain is composed of a highly complex milieu of different cell types, and few methods exist that can identify which individual cells in a complex mixture are secreting specific analytes. By identifying which cells are responsible, we can better understand neural physiology and pathophysiology, more readily identify the underlying pathways responsible for analyte production, and ultimately use this information to guide the development of novel therapeutic strategies that target the cell types of relevance. We present here a method for detecting analytes secreted from single human iPSC-derived neural cells, and have applied the method to measure Aβ and sAPPα, analytes central to Alzheimer's disease pathogenesis. Through these studies, we have uncovered the dynamic range of secretion profiles of these analytes from single iPSC-derived neuronal and glial cells, and have molecularly characterized subpopulations of these cells through immunostaining and gene expression analyses. In examining Aβ and sAPPα secretion from single cells, we were able to identify previously unappreciated complexities in the biology of APP cleavage that could not otherwise have been found by studying average responses over pools of cells. This technique can be readily adapted to the detection of other analytes secreted by neural cells, which would have the potential to open new perspectives into human CNS development and dysfunction.

Poster 21: Analysis of cellular heterogeneity in activated macrophages reveals hidden modes of state-specific gene regulation

Andrew J. Martins[1*], Manikandan Narayanan[1*], Thorsten Prüstel[2], Bethany Fixsen[1], Yong Lu[1], Rachel A. Gottschalk[1], Cindi Pfannkoch[1], Kyemung Park[1], William Lau[4], Katherine Wendelsdorf[1,5], John S. Tsang[1]

**Equal contribution.*

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Macrophages are critical innate immune cells, known for diverse physiological roles and heterogeneous, dynamic phenotypic adaptations to external signals. The underlying gene regulatory programs that control cellular behavior in these distinct adaptive states are typically examined by utilizing average changes across conditions, ignoring heterogeneity between individual cells within a particular state. We hypothesized that utilizing the natural heterogeneity across single cells to construct state-specific gene regulatory networks would reveal hidden functional connections missed by approaches relying on cross-state comparisons. We have examined state-specific gene-gene correlation networks in human macrophages using 96-plex microfluidic qPCR. We observed striking changes in gene-gene correlation networks in different activation conditions. Some “hub” genes in these networks, often involving factors not previously known to play a role in these conditions, exhibit substantial differences in connectivity across conditions without a concomitant large change in average gene expression. These include activating transcription factor 2 (ATF2), a component of the activating protein (AP)-1 complex, which we find to be a hub specific to interleukin (IL)-10-activated macrophages. Microscopy and flow cytometry experiments revealed a higher level of phosphorylated nuclear ATF2 after IL-10 activation, and ChIP-Seq analysis showed increased ATF2 binding and enhancer activity near ATF2-connected genes after IL-10 treatment, suggesting that an post-translational/epigenetic mechanism mediates the observed differential connectivity for ATF2. Our results show how cellular gene expression variation may differentially propagate through a gene network, in a condition specific manner, through post-translational/epigenetic mechanisms independent of large population level changes in gene expression, and suggest that such differential connectivity may be a key underappreciated aspect of cellular adaptation to different environmental conditions.

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Poster 22: Mechanism Inference from Single Cells (MISC)

Rachel Haggerty, Jeanette Baran-Gale, and Jeremy Purvis

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Individual cells show variability in their signaling dynamics. This variability has been correlated with predictable downstream responses, indicating that cell-to-cell variability is not merely noise but represents functionally meaningful differences. Based on this observation, we reasoned that cell-to-cell variability could result from a single signaling mechanism that converts different upstream signals into a corresponding set of downstream responses. If true, then repeated measurements of upstream and downstream signaling in single cells may provide information about the underlying signaling mechanism for a given pathway, even when no prior knowledge of that mechanism exists. To test this hypothesis, we analyzed paired time series measurements of transcription factor dynamics and target gene expression from single yeast cells under various stress conditions. Using a novel computer algorithm to infer the underlying mechanisms, we found that each type of stress evoked a different decoding mechanism to induce target gene expression. The ability to infer the underlying mechanism became poorer when a cell's upstream signal was randomly paired with another cell's downstream response, demonstrating that averaging measurements across a population obscures information about the signaling mechanism. In contrast, predictions became better as more cells were added to the analysis. These results provide evidence, based on experimental data, that mechanistic information about cellular signaling networks is embedded within the dynamical patterns of single cells.

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Poster 23: Mononuclear Single Cell Mass Spectrum Following Stem Cell Transplantation: Predicting Clinical Outcomes

Kevin Leslie [1], Catherine Roberts [2], Roy Sabo [2], Amir Toor [2] and Jason Reed [1,2]

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Patients with cancers affecting the blood and bone marrow are seldom cured once they develop a recurrence. A procedure called stem cell transplantation (SCT), which exploits the genetic differences between tissue compatible donors and blood cancer patients may however help cure a large number of these patients. The donor's immune system plays a key role in these kinds of transplants, by identifying recipient tissues as non-self and attacking them, and in so doing eliminating the recipient's cancer cells. This process also puts recipients at risk for donor immune cells targeting normal tissues such as the skin, liver, lungs and the gastro-intestinal tract, a condition called graft versus host disease (GVHD). At the current time it is not possible to discern in real-time, which patients will be at risk for GVHD, particularly as post-transplant immunosuppression is weaned. This is partly because the kinetics of immune recovery following SCT are not well understood.

Previously we proposed a model in which a small proportion of donor immune system cells will become activated and grow to form the bulk of the immune system following transplant, with the rest remaining relatively quiescent. Importantly, this model predicts that the proportion of cells growing rapidly can serve as a biomarker to help adjust immunosuppression over time to avoid GVHD or prevent relapse of cancer. We will present preliminary data from an ongoing pilot clinical trial designed to test this mass/growth biomarker hypothesis. To implement this diagnostic strategy, we have adopted a new technology called Lived Cell Interferometry (LCI) that can inexpensively identify individual rapidly growing immune cells present in patent blood samples. We will study the recovery of immune system cells following SCT, and test the predictions of the immune system regeneration developed by our group. By allowing discrimination of activated versus quiescent immune system cells, once perfected, this inexpensive technique to measure immune cell recovery may allow accurate prediction of GVHD risk with different tissue matched donors. Combining the LCI assay with knowledge of the degree of genetic difference between different individuals, will allow optimization of their post-transplant care and improvement in cancer survivability.

Poster 24: Nuclear pre-mRNA Analysis of Single cells in Brain slice

Jacqueline Morris[*], Youngji Na[†], Jaehee Lee[*], Jennifer Singh[*], Stephen Fisher[†], James Eberwine[*], Junhyong Kim[†], Jai-Yoon Sul[*]

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Transcription of a gene produces precursor mRNA containing each exon and intron of that particular gene. For proper gene function, these precursor mRNAs (pre-mRNA) are subjected to processing mechanisms such as splicing that control the exonic and intronic contents of the mature mRNA. These processes begin in the nucleus but can continue once the pre-mRNA is exported to the cytosol where its final form can be translated into functional protein. Many of these steps in mRNA maturation are regulated by external signaling factors, which can initiate global changes in transcriptional programs as well as changes in intronic contents of pre- and mature mRNA, ultimately resulting in changes in protein and thus cellular function. A previously unachievable single cell approach for measurement of gene expression profiles of single nuclei in vivo is made possible by use of our unique mRNA capture compound (TIVA-Nuc). Here, we perform pre-mRNA analysis of single nuclei from different cell types from multiple brain locations in order to build a database of cell-type specific pre-mRNA profile and to correlate pre-mRNA signatures with their resultant cellular phenotype in vivo. Our hypothesis is that because different microenvironments are exposed to different signaling patterns, pre-mRNA contents will be differentially regulated among samples and that these differences can be correlated with or used to find new specific functions within that microenvironment. High resolution microscopy and optical manipulation of the TIVA capture molecule was employed to capture polyadenylated pre-mRNA of nuclei or soma with and without physiological stimulation. Using genomic alignments and comparison of nuclear versus total cell RNAseq, we are creating an atlas of locus-specific pre-mRNA structures for each cell type and stimulation. We show data that indicate cell-specific multi-genic variation in pre-mRNA structures. These results will generate a novel dataset for assessing the role of pre-mRNA, which will greatly facilitate understanding their specific roles in regulating and maintaining brain function.

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Poster 25: Power of atomic force microscopy in study of circulating tumor cells biology

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Atomic force microscopy (AFM) is a type of scanning probe microscopy belonging to stylus profilometry. Recent technological advances allowed applying AFM to image live cells with exceptionally low forces and reasonably short scanning times. We routinely use this technique commercially known as peak force quantitative nanomechanical (PF-QNM) AFM to biophysically phenotype single cultured cells or cells obtained ex vivo. Additionally, we adapted this AFM technology to bridge single cell and single molecule probing by measuring forces controlling ligand-receptor interactions on a surface of single cells using probes decorated with specific antibodies. Moreover, using AFM we also precisely quantify forces responsible for cell-cell adhesion with cantilever-attached cells probing surface-growing cells. Nanomechanical characterization of circulating tumor cells (CTCs) represents one of the most innovative applications of PF AFM, since it gives an access to untapped properties of exceptionally rare and diverse cells blamed for seeding metastases. These properties constitute a biophysical mirror that amplifies physiological changes in individual CTCs. Therefore, AFM may help to establish predictive biomarkers that can improve identification of cancer patients at risk of developing metastasis. CTCs shed from tumor sites are considered an attractive “liquid biopsy” obtained from bloodstream with minimal invasiveness. Their enumeration is the FDA approved method to follow cancer progression. Here we extended these capabilities by showing in retrospective studies that AFM detected mechanical properties of CTCs can successfully distinguish between castration sensitive and resistant prostate cancer patients, including a case where resistance was detected by AFM before biochemical confirmation. Such stratification is of prime importance since successful colonization by CTCs depends on their transition from the epithelial to mesenchymal (EMT) phenotype, and such transition seems to be eagerly detectable by nanomechanical profiling. Indeed, the early identification of pre metastatic disease assures successful targeting of the potential therapy responders. Currently, to recapitulate how CTCs acquire EMT traits needed to execute multiple steps of metastasis, we molecularly and biophysically characterize CTCs collected from human xenografts EMT mouse model.

To summarize, the modern multi-parameter AFM offers a unique platform for precise biophysical characterization of single live cells.

Poster 26: Analysis of DNA repair pathway choice upon zinc finger nuclease induced double-strand breaks

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DNA double-strand breaks (DSBs) are the most dangerous class of DNA damage. If DSBs are left unrepaired they can result in genomic instability or cell death. Therefore, cells have evolved complex DNA damage response pathways to repair DSBs. In mammalian cells, two major and mechanistically distinct DSB repair pathways are non-homologous end-joining (NHEJ) and homologous recombination (HR). NHEJ and HR can be regulated by multiple factors including cell cycle phase. Although, cell cycle regulation of the DSB repair pathway choice on a single-copy locus is thought to be restricted to G1 phase for NHEJ and to late S/G2 phase for HR, it remains unclear for a multi-copy locus. Here, we have investigated DSB pathway choice by developing and utilizing multi-copy and single-copy DSB reporter cell systems. Using these systems we initiated a DSB with a specific zinc finger nuclease and then visualized DSB repair as it relates to cell cycle phase. We show by single cell analysis that both the NHEJ and HR pathways can simultaneously repair DSBs at the reporter locus. The frequency of recruitment of the HR repair protein Rad51 is high at a multi-copy versus a single-copy DSB-substrate. This suggests that, availability of donor templates near a DSB repair site could influence its HR vs NHEJ repair pathway choice. Consistent with this, molecular analysis of repair at the single-copy reporter by SMRT sequencing, revealed an increase in gene editing frequency by HR with increasing donor template concentration. Interestingly, HR can occur in G1 phase of the cell cycle, if donor template is available for repair. Our findings suggest a competition between the HR and NHEJ repair machineries throughout interphase of the cell cycle, which could be a critical factor for the stability of mammalian genomes that are highly enriched with repeat sequences. Insights from these studies will help in understanding genomic instability disorders, cell-to-cell variations in DNA repair and to improve gene therapy approaches.

Poster 27: Single Cell Dissection of Transcriptional Codes for T Cell Identity

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T cell development is a prominent model system to understand developmental choice and progression control from mammalian stem cells. Powerful genome-wide technologies such as RNA-seq and ChIP-seq have mapped out the regulatory networks that are formulating the developmental code to launch the lymphocyte cell identity. Although the gene regulatory factors and their interactions have been extensively studied in the ensemble level, it remains to be elucidated if these relationships are exhibited uniformly across the population, or alternatively, distributed among distinct subsets of cells.

To this end, we have used an emerging single cell transcript profiling technique, termed as Sequential Coding anALYSis of Fluorescent *In Situ* Hybridizations (FISH SCALYS), in T cell development to obtain quantitative correlations of previously established transcription factors within individual cells. Here, we have purified primary immune cells that are extracted from thymus for our experiments using flow cytometry to collect only developing T cells, and then identified the development stage of each immature T cell based on immunostaining of surface markers, followed by the FISH SCALYS measurements on the same single cells which were done by sequential processing of single molecule FISH based transcript detection. Our preliminary results show that specific subsets of early T cells exhibit coregulation patterns that were not previously observable with other techniques. Statistical analyses also reveal combinatorial activity of gene regulators in single cells. The low copy nature of T cell and stem cell genes (some of which have less than 20 molecules per cell) in developing mammalian cells benefits from the high detection sensitivity of our FISH SCALYS approach which would have been challenging for accurate quantification of such transcripts by other amplification based single cell sequencing methods. The cell-to-cell variability in the immune cells will provide insights into how cells obtain distinct cell fates in multicellular organisms.

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Poster 28: SMART-Seq v4 Ultra Low Input RNA Kit for the Fluidigm® C1™ System: improved chemistry for single cell transcriptome studies

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In single cell transcriptome studies, it is critical to obtain high-quality cDNA libraries from individual cells that represent the original *in vivo* mRNAs as closely as possible. To achieve this, we improved the existing commercially available mRNA-Seq chemistry for single cell transcriptome studies (the SMARTer Ultra Low Input RNA Kit for the Fluidigm C1 System).The new chemistry employs locked nucleic acid (LNA) technology integrated in our proven SMART® technology, which greatly enhances the first strand cDNA synthesis reaction. The new chemistry also utilizes efficient SeqAmp™ polymerase, which excels even in GC- and AT-rich regions. All procedures (including lysis, RT, and all PCR steps) are designed to be performed in the Fluidigm C1 System using Open App IFC.

In this poster, we present data derived from K562 (human leukemia) cells. The results show a higher yield of cDNA, a larger number of genes detected, and an improvement in high-GC gene detection compared to the existing chemistry. The new chemistry also produces higher consistency among replicates. This is important because the reduced technical variations will increase the likelihood of discovering true biological variations. Altogether, these results indicate that the new chemistry can robustly produce high-quality and highly reproducible cDNA from single cells for meaningful transcriptome analysis.

Poster 29: Single cell analysis of circulating tumor cells from metastatic breast cancer patients revealed presence of heterogeneous breast cancer stem cells with EMT, MET and or dual EMT-MET phenotypes

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Cancer stem cells (CSCs) are believed to be responsible for tumor initiation, recurrence, metastasis and drug resistance. Circulating tumor cells (CTCs) that are tumor cells shed into blood circulation from primary site may serve as markers of cancer progression and as a "liquid biopsy" to provide information on tumor biology at single cell resolution. Current methods of CTC capture are not fully inclusive to isolate all phenotypic variations of CSCs among the CTCs. Using newly developed microfluidic methods to isolate CTCs from blood samples of metastatic breast cancer patients, we assessed gene expression profiles in these cells at single cell level. The single cell gene expression signatures of isolated CTCs were determined using a highly sensitive microfluidic-based 96-plex RT-qPCR method. Analyzed multiplex RT-qPCR data revealed distinct gene expression patterns among CTCs isolated from different breast cancer patients. Cancer stem cell markers such as CD44 and CD24 as well as ALDH1a1, and ALDH1a3 were differentially expressed in isolated CTCs. In addition, markers of the epithelial to mesenchymal (EMT) and mesenchymal to epithelial (MET) phenotypes such as Vimentin, CDH2, TGFb1, EpCAM, HER2, CDH1 and cytokeratins were also expressed variably in the CTC samples. Furthermore, MMPs, TIMPs, cMET, LGALS3BP, EGFR, and TP53 genes which play important roles in tumor initiation, promotion, metastasis and drug resistance were also differentially expressed in the isolated CTCs. Overall, single cell studies further our understanding into the heterogeneity of CTCs and CSCs as well as providing a potential tool for real-time monitoring of cancer patients on clinical trials.

Poster 30: High-Throughput, Single-Cell Whole Transcriptome Sequencing Analysis of Cancer Cells with the New BD FACSseq™ Cell Sorter and BD™ Precise Assay

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Single-cell mRNA sequencing is a powerful method for defining cell-to-cell variation, either within a cell population or among different cell populations. The information uncovered at the single-cell level can give insights on functional mechanisms such as oncogenesis. Optimal sample preparation is critical to obtain high-quality gene expression data from single cells. Previously, BD's fluorescence activated cell sorting (BD FACS™) technology has been applied to accurately target and isolate single cells or a small number of cells for use in next generation sequencing (NGS) or transcriptome analyses.

In this study, the new, easy-to-use BD FACSseq™ cell sorter was applied to sort single cancer cells. Individual cells were isolated into each well of the 96-well BD™ Precise WTA (whole transcriptome analysis) encoding plates that are pre-filled with reagents. These WTA plates are newly developed assays for single-cell whole transcriptome sequencing studies. Each cell was lysed directly in the plate where the mRNA content is barcoded with a unique molecular index and sample index during the reverse transcription step. We interrogated the whole transcriptome of approximately 100 Jurkat cells (a T-leukemia cell line), 100 T47D cells (a breast cancer cell line), and 100 cells from mixed Jurkat and T47D cell samples. One sequencing library was generated by pooling the products of the BD Precise plates and sequencing on an Illumina® NGS instrument. A streamlined computation pipeline was created, and automated data analysis was performed on the Seven Bridges Genomics Platform. Principle component analysis clustered the sorted Jurkat and T47D cells into distinct populations. Cells from the mixed sample clustered into their respective cell types based on their gene expression profiles.

Our study combined the FACS sorting technology and WTA assays to streamline the single-cell isolation and transcriptome sequencing analysis. These results demonstrate a powerful tool for researchers to better understand otherwise complicated biological samples at the single-cell level.

Poster 31: Identifying the intercellular networks regulating estrogen receptor expression with a high definition single cell printer

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The long-term objective of this proposal is to reinvent live-cell printing by combining advances in droplet microfluidics, robotic automation, and microscopy techniques. The immediate scientific goal of this proposal is to recreate the in situ microenvironment by building combinatorial cellular interaction arrays using primary or limited life-span human mammary epithelial cells. Specifically, we will apply the multicellular interaction arrays to dissect the cellular network that contributes to heterogeneous patterns of estrogen receptor expression. Estrogen receptor (ER) is the key regulator of human mammary gland growth and is necessary for the most prevalent forms of human breast cancer. Remarkably, however, the estrogen receptor is only expressed in one cell type in the human mammary gland – luminal epithelial cells. More remarkably still, the receptor is expressed in a binary fashion, and only in 5-20% of luminal epithelial cells in a normal tissue. Little is known about the mechanisms through which ER expression is regulated within the luminal population, but available evidence suggests that multiple other cell types in the microenvironment are critical. We will use the proposed high definition single cell printer to explore a large combination of primary human cell types, soluble factors, and ECM components to identify the minimal intercellular circuit necessary to sustain ER expression in the mammary gland. We hypothesize that ER expression in the luminal population is regulated by a combination of cell-cell contact with myoepithelial cells and a paracrine circuit involving fibroblasts and at least one other stromal component responsible for stabilizing estrogen receptor expression.

Poster 32: Highly Selective Mitochondria-Specific Fluorescent K^+ Sensor

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Monitoring the concentration changes of intracellular K^+, Na^+, Cl^- and Ca^{2+} ions is of great importance to physiological processes, including heartbeat, muscle contraction, nerve processes, cancer apoptosis, and secretory tissue function. Ion influx and efflux controlled by different ion pumps and channels maintains the functionality of life. Abnormalities in ion channel function can lead to health issues such as epilepsy, ataxia, migraine headaches, heart arrhythmia and cystic fibrosis. Different fluorescent probes of Na^+, and Ca^{2+} ions with variable affinities and sensitivities have been developed and investigated in cellular biology. In comparison, only a few K^+ ion fluorescent probes are commercially available. Our research center has successfully developed a highly selective fluorescent K^+ sensor, **KS6**, to monitor K^+ ion dynamics in mitochondria. **KS6** possesses good response to K^+ in the range of 30-500 mM, large dynamic range (F_{max}/F_0: ~130), high brightness (ϕ_f: 14.4% at 150 mM of K^+) and insensitivity to pH (5.5-9.0) and other metal ions under physiological conditions. Co-localization tests of **KS6** with MitoTracker Green confirmed its predominant localization in the mitochondria of HeLa and U87 MG cells. K^+ efflux/influx in the mitochondria was observed in HeLa and U87MG cells upon stimulation. These studies demonstrate the promise of the **KS6** as an effective tool for measuring potassium channel behaviors in a wide range of single cell biological pathway studies such as inflammasome formation, insulin secretion, and neuron tumor treatment.

Poster 33: Laser Fabricated Cell Patterning Stencil

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A major challenge in single cell experimental methods is the difficulty in obtaining representatively large quantities of isolated single cells. We are exploring innovative methods for patterning mammalian cells into wells of fused silica microarrays for use in high throughput single cell assays. Microwell arrays created using standard photolithography methods were secured beneath a stretched region of X-Ray Fluorescence (XRF) film. A 355 nm laser was tuned to an energy below the ablation threshold of the fused silica and positioned concentrically with the microwells of the array. The low fluency laser light passed through the fused silica and was partially absorbed by the XRF film during 500 ms exposures. The film was redistributed away from blast site (through ablation or thermal expansion processes) creating a pore just slightly larger than the laser beam diameter. Cells are then seeded normally and the porated film restricts cells from the underlying substrate except inside the wells.
Cells seeded onto and through the mask exhibited healthy morphologies while the stencil was in place and after removal of the stencil. This method dramatically reduced the probability of cells being deposited on the lips or outside of the microwells. Preparation of the stencil is low cost, simple, and arrays of 2980 microwells can be prepared in less than an hour. While the current method does not achieve single cell occupancy rates as high as many microfluidic methods, it does successfully isolate low counts of cells within predefined geometries and does not cause more cell stress than traditional seeding methods. Future work will aim towards increasing single cell occupancy rates in comparison to random seeding.

Poster 34: High-throughput robotic analysis of integrated neuronal phenotypes

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In our project, we set out to develop robotic approaches to acquire simultaneously the gene expression patterns, morphologies, and electrophysiological properties of single cells in brain tissue, in an automated fashion. First, we designed and built a robot that performs "hands free" serial patch clamp recordings in vivo that dramatically increases the throughput of in vivo cell-type classification. This robot has recorded from dozens of cells (average duration 25 minutes, maximum duration 110 minutes, yield 10%) in an effort to characterize the orientation tuning and intrinsic properties in mouse V1 pyramidal cells with simultaneous biocytin labeling. Second, we made major progress in transcriptomic and morphological characterization of neurons from mouse brains. We completed a single-cell transcriptomic study of 1,679 mouse visual cortical cells and generated transcriptomic cell type taxonomy for this region. This work was recently published in *Nature Neuroscience*. We reconstructed the 3D full morphology of in vivo labeled neurons, generating pilot datasets of various types of excitatory and inhibitory cortical neurons from in vivo DNA electroporation, viral injection and transgenic labeling. These datasets demonstrate the feasibility of our entire full morphology characterization process, from strong fluorescent labeling, large-scale imaging, to reconstruction and morphology analysis and classification. Finally, we refined our approaches for simultaneous multiple-cell patch clamp neural recording in awake mice, as well as image-guided whole cell patch clamp in intact brain tissues. We aim to completely develop a powerful, easy-to-use toolset that enables the integrative phenotyping of cells of the brain.

Poster 35: WOLF Cell Sorter Isolates Target Cells for Single Cell Analysis

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Fluorescence-activated cell sorters (FACS) are instruments that provide pure cells for molecular analysis workflows in gene expression, sequencing, and protein analysis where homogenous cells are essential. They can also be used for sorting and dispensing individual cells into separate wells for single-cell genomic analysis or for clonal expansion (ex CRISPR). Traditional sorters force cells through high-pressure nozzles to create aerosols with charged droplets that are directed by electric fields into collection tubes. However, the process of forming and deflecting droplets significantly damages cells and creates biohazardous aerosols. Unlike traditional flow cytometers, the WOLF Cell Sorter directs cells through a microfluidic channel where the fluid path is briefly diverted into collection channels by a pulse from a piezoelectric actuator. NanoCellect's WOLF Cell Sorter is a small, affordable, easy to use personal benchtop system that uses sterile disposable microfluidic chips for gentle cell sorting and no aerosols. This results in safer, on-demand sorting to allow users to perform more experiments with improved workflows without worrying about cell damage or sample-to- sample contaminations.

Here, we demonstrate some of the capabilities of the WOLF Cell Sorter. One of the most common uses of sorting is to isolate GFP positive cells from GFP-negative cells. We sorted MCF-7 GFP+ target cells from unlabeled MCF-7 cells while maintaining high viability of the cells after sorting. Cell viability is even more critical when sorting very fragile and precious cells like human embryonic stem cells. Stem Cells sorted by the WOLF resulted in high cell viability (78.8%). In contrast, BD's FacsAriaII droplet-in-air sorter resulted in 31.3% cell viability after sorting. The WOLF is also useful in sorting single cells into 96 well plates. We performed single-cell isolation where we exhibit ~90% of wells with a single cell. Users can also control the number of sorted cells per well to enable various downstream applications.

Poster 36: Whole-body tracking of single cells by positron emission localization

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Methods for spatiotemporal tracking of cells are becoming increasingly important as interest in cell-based therapies continues to grow. Such methods can shed light on biodistribution and viability of cells, which may be important markers of treatment efficacy [1]. In addition, cell tracking is potentially valuable for studying circulating tumor cells, a key to understanding cancer metastasis [2]. Current *in vivo* cell tracking methods are not effective for following the trafficking of single cells throughout the whole body. We have developed a novel algorithm for processing raw PET data (list-mode format) and extract the position of single cells from very sparse measurements. This statistical estimation problem is simplified because we can assume that the radioactivity is only contained within the single cells being tracked. However, even with this new method, there is still a gap between what we can do and what we would like to do. We have successfully demonstrated that we can track single-cell-mimicking droplets containing 100-200 Bq of radioactivity. This level of radioactivity is however still too high compared to what is achievable with current cell radiolabeling protocols. Labeling with ^{18}F-fluorodeoxyglucose (^{18}F-FDG) yields approximately 5-10 Bq per cell. We are therefore aiming to close this tantalizing small gap with a multipronged approach. During this past year, we have investigated the use of a novel PET system made from bismuth germanate (BGO) detectors, which have exceedingly low background count rate, suitable for tracking few cells. We have also refined our algorithm to better handle various sources of noise such as gamma ray scatter and cosmic radiation. This should allow us to track sources using less radioactivity. In parallel, we are also optimizing cell radiolabeling using a different radiotracer known as ^{18}F-HFB. This radiotracer has previously been shown to label cells more efficiently that ^{18}F-FDG, reaching up to 50 Bq per cell [3]. Combining these approaches should allow us to close the labeling radioactivity gap and enable the tracking of single cells *in vivo*, anywhere in the body.

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Poster 37: Measuring genome-wide replication timing in single cells

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DNA replication proceeds in a spatiotemporal order where clusters of origins fire synchronously at different times during S-phase. This divides the chromosomes into distinct domains that replicate early or late during S-phase. Replication timing is regulated during differentiation in units of topologically associating domains (TADs) and changes in replication timing represent stable epigenetic transitions corresponding to changes in sub-nuclear compartment.

To date replication timing has been studied only in cell populations. Since, each cell uses a different cohort of origins to achieve the temporal order of replication, it is imperative to study cell-to-cell variation of the replication timing program. Here we devise a strategy to measure single cell replication timing by whole genome amplification of S phase cells followed by copy number detection. Surprisingly, despite cell-to-cell variation in origin usage, the replicating timing program is remarkably conserved. Scaling up to several cells will allow us to determine regions of the genome with higher and lower variability in replication timing. Using a hybrid cell line (Cas/129), combined with deeper sequencing will enable us to measure intrinsic variability between alleles within single cells.

Poster 38: Imaging A to I editing of individual mRNAs in mammalian cells using iFISH

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Adenosine to inosine (A -to -I) editing is the most prevalent form of RNA editing in mammals, and it is mediated by ADAR enzymes, which deaminate adenosine bases. A to I editing in an mRNA may lead to a change in the amino acid composition of its encoded protein by modulating splicing or by altering codon identity. The biological importance of editing is clearly highlighted by the extreme phenotypes resulting from ADAR perturbation, including neurological dysfunction and cell death. Despite its prevalence and importance, basic information about A to I editing in RNAs, such as localization and timing, remains a mystery.

Current knowledge about A to I editing comes primarily from bulk RNA sequencing or restriction digestion-based techniques. These methods lack the resolution to observe important properties such as single-cell variability in editing rates and subcellular localization. Recently, our laboratory described a fluorescence *in situ* hybridization (FISH) method for visualizing and quantifying single nucleotide variants (SNV) in individual cells using a unique probe hybridization strategy. We applied this method towards visualizing and quantifying known A to I editing sites on mammalian cells by exploiting the chemical structure of inosine as a guanosine analog in a method we refer to as inosineFISH (iFISH). Using this new tool, we successfully distinguished between edited and unedited transcripts, and observed editing rates consistent with sequencing and restriction-digestion based methods.

Poster 39: The CellRaft System for Single Cell Isolation: Applications and Advances

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Analyzing single cells requires unique sample preparation methods relative to those used for preparing bulk cells and tissue. New techniques must ensure that specific cell populations, however rare, are efficiently separated from a given sample. Cell Microsystems has developed the CellRaft System, based on technology originally pioneered in the laboratory of Nancy Allbritton, MD, PhD at the University of North Carolina at Chapel Hill. The CellRaft System employs a microwell array on which cells can be seeded and subsequently isolated at the single cell level without a minimum cell input requirement. An investigator then images the array on a standard inverted microscope, and when a single cell of interest is identified, the CellRaft Release Device actuates a needle through the elastomeric bottom of the array thereby releasing the individual CellRaft. A magnetic wand is then used to both retrieve the released CellRaft, which is loaded with paramagnetic particles during manufacturing, and deposit it in a well plate or other collection vessel. Here, we will provide details regarding our research funded by the NIH's Single Cell Analysis Program, which has significantly advanced our capabilities in using the CellRaft System in a single cell whole transcriptome workflow. We will also share representative data from various investigators using the CellRaft System for applications such as single cell whole genome analysis, clonal propagation of engineered cell lines and stem cell differentiation. During 2016 we will continue these developments toward expanding the applications of the CellRaft System, as well as develop a fully-automated platform for the CellRaft System. We will also present our work in developing CellRaft Arrays in new form factors for rare cell isolation, alternative CellRaft Array materials for high resolution imaging, and variations on our automated system for high-throughput isolation of nearly 10,000 single cells.

Poster 40: Single Cell Analysis using High Spatial Resolution Imaging Mass Spectrometry

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Imaging mass spectrometry is a powerful technology that combines the molecular measurements of mass spectrometry with the spatial information inherent to microscopy. This unique combination of capabilities is ideally suited for the analysis of metabolites and lipids from single cells. Here, we present a methodology for the sample preparation and analysis of single cells using high performance MALDI mass spectrometry. Using this approach, we are able to analyze hundreds or thousands of individual cells in one measurement and generate profiles and molecular images of lipid and metabolite expression from single cells that characterize cellular heterogeneity. This approach also enables the detection of variations in the expression profiles of lipids and metabolites induced by chemical stimulation of the cells. These results demonstrate that MALDI IMS provides an insightful view of lipid and metabolite expression that will be useful in the characterization of a number of biological systems at the single cell level. This presentation will provide perspective on the current state of the technology and provide specific examples of the analysis of single cells in culture and the study of tissue at cellular resolution.

Poster 41: CyTOF-based single-cell analysis of intact signaling reveals divergent TNF-induced behaviors in the intestinal epithelium

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TNF (tumor necrosis factor) is a central cytokine with pleiotropic functions in many contexts, from inflammatory disease to cancer. A central biochemical property of TNF is its ability to engage in the cell death signaling program to induce cell apoptosis. TNF induces apoptotic cell shedding in the differentiated intestinal epithelium, but it is not understood why apoptosis occurs only intermittently throughout the differentiated compartment and never in patches, even though all cells are equally exposed to TNF. To understand heterogeneous cellular behaviors in a complex tissue requires the evaluation of signaling networks at single-cell resolution. However, probing signaling in epithelial tissues using cytometry-based single cell analysis has been confounded by the necessity of single cell dissociation, where disrupting cell-to-cell connections inherently perturbs native cell signaling states. Here, we demonstrate a novel, relatively high throughput strategy (Disaggregation for Intracellular Signaling in Single Epithelial Cells from Tissue – DISSECT) that preserves intact signaling for Cytometry Time-of-Flight (CyTOF) and fluorescent flow cytometry applications. A 21-plex CyTOF analysis encompassing core signaling and cell-identity markers was performed on the small intestinal epithelium after TNF stimulation. Multiplex single cell data consisting of hundreds of thousands of data points were quantitatively analyzed with unsupervised and supervised methods to robustly select signaling features that identify a unique subset of epithelial cells that are sensitized to TNF-induced apoptosis in the seemingly homogeneous enterocyte population. Specifically, p-ERK and apoptosis are divergently regulated in neighboring enterocytes within the epithelium, suggesting a mechanism of contact-dependent survival. We propose a model where dying cells signal to their direct epithelial neighbors to activate a survival program, in order to prevent large contiguous areas of epithelium from dying.

Poster 42: Second-generation Single-cell Mass Spectrometry Finds Metabolic Cell Heterogeneity along the Left-Right Body Axis in the Developing Frog (*Xenopus*) Embryo

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We recently developed a single-cell mass spectrometry platform based on cell dissection, capillary electrophoresis, electrospray ionization, and high-resolution mass spectrometry to enable the analysis of 40 different metabolites between single embryonic cells (blastomeres) in the 16-cell *Xenopus* embryo. Using this platform, we discovered small molecules that are able to alter the tissue fate of blastomeres, which are precursors to nervous and epidermal tissues. Here, we implement technological–methodological advances to uncover metabolic heterogeneity between blastomeres also along the left-right axis of the embryo. First, we developed multi-step small-molecular extraction for the single cells to advance metabolite detection to hundreds of different signals and confidently identify 56 of them. With higher-sensitivity detection, we were able to test whether there are metabolic differences between blastomeres that occupy the left and right hemisphere of the 8-cell embryo, at which developmental stage body asymmetry is already exists but the underlying molecular players are still elusive. By reproducibly quantifying ~80 different small-molecule signals, we identified 10 different metabolites that were statistically ($p < 0.05$) and biologically significantly (fold change < 1.5) differentially accumulated between left vs. right D1 blastomeres (fated to become nervous tissues). Furthermore, we extended these single-cell measurements to later stages of embryonic development (e.g., 32- and 64-cell embryo) by integrating microcapillary sampling into the workflow. As a result, we were able to minimize chemical interferences from the media, allowing us to obtain signals that have comparable–to–higher signal/noise ratios than detected from manually dissected cells despite sampling only a fraction of the cell's cytoplasm using the microcapillary. The ability to measure broad diversity of small molecules in cells in the complex body of the embryo using microcapillary-sampling single-cell mass spectrometry raises an opportunity to help better understand molecular processes underlying cell differentiation and development of the normal vertebrate embryo.

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