High throughput flow cytometry for discovery at UNMCMD and the NIH Molecular Libraries Initiative

Commercial flow cytometers became widely established in research and clinical laboratories in the 1980s. Once the domain of immunologists and hematologists for cell sorting and analysis, the need for sample handling beyond manual delivery of single flow cytometry samples became apparent. Automated sample handling carousels subsequently evolved into multiwall plate-based sampling systems. In parallel, data acquisition and data analysis were advanced with automated and batch processing systems.

By Dr Larry A. Sklar and Dr Bruce S. Edwards

Flow cytometry has long been recognised for its multiparameter capability (now up to 20 colours) as well as its high speed analysis and sorting capabilities (tens of thousands of particles per second). In addition, its multiparameter applications distinguish tens of cell or particle subsets in multiplex format. Thus, while flow cytometry has found application in several stages of drug discovery including compound profiling, pathway analysis, toxicology, and the like, the platform was considered unlikely to achieve throughput compatible with small molecule discovery from large libraries. A team at the University of New Mexico Center for Molecular Discovery (UNMCMD) pioneered the HyperCyt platform for screening compound libraries. The platform delivers samples from a multiwell plate as a single data file by using an autosampler and pump to insert air bubbles between samples from individual wells. The platform takes advantage of the multiplexing capabilities of the flow cytometer as well as its ability to distinguish the fluorescence associated with a cell or particle from the fluorescence of the illuminated volume surrounding the particle. This allows homogeneous detection of macromolecular assemblies or other binding interactions. The platform is particularly well-suited to cell suspension assays including single components as well as complex mixtures of blood cells, bacteria, yeast and other fungi and cell-virus interactions. We anticipate that the platform will provide drug discovery opportunities in immunological and infectious diseases, as well as blood cancers and personalised medicine. Technological advances in sample delivery are expected to enable highly multiplexed targets to be delivered from 1536 well plates in 10 minutes. The UNMCMD will host and support the screening of targets requiring complex sample handling including combinations of cell surface and intracellular staining for collaborators worldwide.

Flow cytometry principles

The development of flow cytometry arose from a need for the rapid characterisation of cell numbers, sizes, cell cycle and expression of cell surface

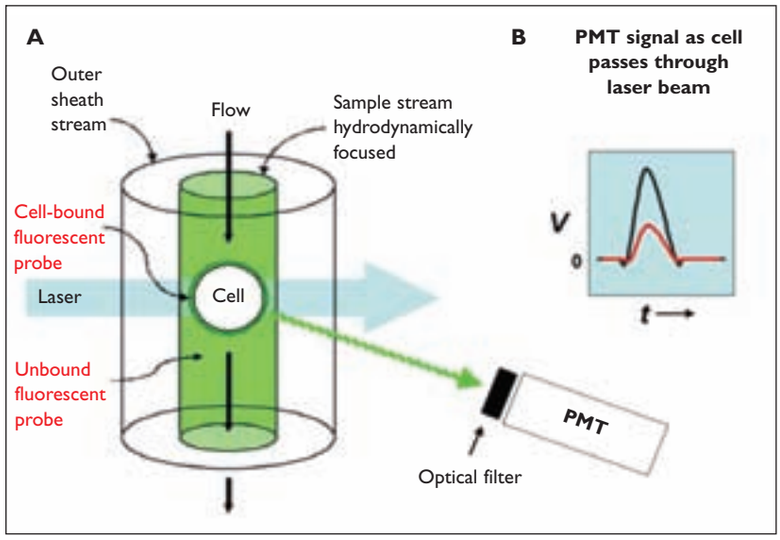

Figure 1
Flow cytometry principles compatible with homogeneous mix and measure assays. **A:** A small volume of sample stream surrounds a cell as it passes through the excitation laser beam so that the fluorescence signal from unbound fluorophore in the stream may be small relative to that of fluorophore bound to the cell. **B:** The voltage pulse signal elicited by the cell represents the difference between cell and sample stream fluorescence. Although pulse height may be reduced in the presence of background fluorescence (black vs red pulse), it is a quantitatively accurate measure of bound fluorophore relative to other cells measured under the same conditions. This allows fluorescent probes with affinities up to 100nM to be detected without requiring a wash step

markers, as well as the sorting and collection of individual cell populations. Flow cytometry is based on aligning particles or cells in a stream flowing across the path of a laser beam. The cells or particles are individually interrogated to detect the optical signals of molecular stains or antibodies that have been associated the cells. Flow cytometry can analyse tens of thousands of cells or particles per second, and during 5-10 microseconds of illumination, up to 20 series of optical characterisations can be performed. Cells can also be sorted on the basis of these optical characteristics.

The versatility of the flow cytometry platform is remarkable and well-known. Capabilities range from >10 colour immunological assays and extend to numerous cell-based assays that can be performed fluorimetrically or microscopically including cell proliferation, differentiation, viability, signaling, adhesion, ligand-binding, transport, etc. There are published protocols, stains and markers for surface, cytoplasmic and nuclear components. Flow cytometry excels at phenotypic analysis, such as antibody staining and light scatter (size and shape) discrimination used for complex cell populations in haematology, immunology, cancer biology, reproductive biology, marine biology, microbiology, pharmacology, toxicology, etc. In principle all of these features are compatible with high throughput flow cytometry[1].

In practice, the flow cytometer requires only several hundred particles for an accurate determination of mean value of a fluorescence signal for a particle population. Fluorescence signals are usually detected when there are a few thousand fluorophores per particle. High affinity fluorescent molecular assemblies (<500nM) can typically be assayed in homogeneous, no-wash format (**Figure 1**). For example, expensive epitope tagged protein reagents are conserved when associated with beads which use <1 pmole per assay. Less precious reagents such as fluorescent small molecules, peptides, and oligonucletodies can be supplied at concentrations above their dissociation constant. In contrast, other homogeneous binding methods typically require both sets of reagent concentrations to be near their dissociation constant. These elements are discussed elsewhere in more detail[2]. For high throughput applications, 1-2µl volumes are sampled from 10µl well volumes with particle densities ~1,000-10,000 cells or beads per µl.

Plate-based and high throughput flow cytometry

Sample delivery for multiwall plates in flow cytometry has become available through most manufacturers of flow cytometers (Accuri, Beckman-Coulter, Becton-Dickinson, Guava, IntelliCyt, Luminex) as well as flow cytometry device companies (eg Cytek). Since most flow cytometers are pressurised, plate-based systems typically depend upon two common principles: 1) samples from individual wells are injected into the flow cytometer one well at a time; and 2) the contents of each well are treated as a single data file. Generally, the contents of individual wells are drawn into a syringe prior to injection; in one case, the wells are sealed and pressurised (Cytek) for sample delivery; and recently in another case the contents of a well are sipped by the flow cytometer (BD HTS system, Accuri), and the plate is repositioned for each well.

High throughput flow cytometry evolved from an interplay between technology and biology at Los Alamos National Laboratory National Flow Cytometry Resource (NFCR) and the University of New Mexico (UNM).The Cytometry group at the University of New Mexico along with colleagues at the NFCR began to consider new delivery schemes for flow cytometers, as well as opportunities for evaluating small molecule interactions[3]. Through collaborations, industrial partnerships and NIH support, subsecond time resolution and high throughput (HT) flow cytometers were introduced.

In collaboration with Axiom Biosciences, the UNM team developed the first generation of high throughput flow cytometry (Plug Flow Cytometry[4]) in 1999. Plug Flow used a reciprocating multiport flow injection valve to deliver 10 endpoint assays per min, four on-line mixing experiments per min and, in secondary screens, a 15-point concentration

gradient of soluble compound in ~2 min[4]. The plug flow technology led to funding at UNM from the National Institutes of Health to develop HyperCyt. HyperCyt uses a peristaltic pump in combination with an autosampler to deliver biological samples to a flow cytometer. As the sampling probe of the autosampler moves from well to well, a peristaltic pump sequentially aspirates suspended cells or particles from each well. Between wells, the pump draws a bubble of air into the sample line and delivers a series of bubble-separated samples[5]. Data from each microplate is represented as a single data file. The time-resolved data, with periodic gaps corresponding to the passage of the air bubbles, are analysed by software. 384 well plates with multiplexed data sets are routinely sampled in 10min with sample volumes of 1-2µls. The particle counting ability of flow cytometry can be used for determinations of compound solubility.

HTS has been typically defined as screening rates of 100,000 samples or more per day. Flow cytometry systems can create a data file from each sample at rates of 5-10 samples per minute. The HyperCyt platform for HTS flow cytometry, currently available at 50 or more sites around the world through IntelliCyt[6], can acquire samples at rates up ~40 wells/min. These sampling rates are currently compatible with throughput to many tens of thousands of samples per day, depending upon the number of hours of screening, the number of cytometers available, the extent of automation, and the degree of multiplexing.

Sites actively reporting usage of flow cytometry in a discovery mode in addition to UNMCMD include: 1) Vivia[7] which performs reprofiling of approved drugs; 2) Rigel which screens SiRNA[8]; and 3) The Center for Chemical Genomics at the University of Michigan Life Science Institute. To our knowledge, the Rigel site is unique in having a fully automated and integrated platform that was developed in collaboration with IntelliCyt and Agilent[7]. IntelliCyt provides a benchtop HyperCyt flow cytometry system. A partnership between IntelliCyt and Beckman Coulter[9], the international distributor of the HyperCyt platform links the Beckman sample handling Biomek platform,

HyperCyt and the Cyan flow cytometer (recently acquired by Beckman Coulter). The platform is also beginning to appear in cellular applications in academic laboratories that are seeking the collaboration of UNMCMD for HTS and small molecule discovery.

UNM Center for Molecular Discovery and the NIH's Molecular Libraries Initiative

In the United States, the Human Genome Project served as a model of the integration of technology and biology for the acceleration of biomedical research. Through the NIH Roadmap, the Molecular Libraries Initiative (MLI[10]) in chemical genomics serves as a follow up to the Human Genome Project where small molecule libraries are used to probe biological structure, function and diversity. As a public initiative, a variety of information about the MLPCN funding mechanisms[11-16], probes[17], and databases[18] is available online.

UNM was chosen as one of 10 pilot sites for the MLI. Currently in the MLI Production Phase, UNMCMD is a specialty probe production centre focusing on HTS flow cytometry[19]. The MLI Production Phase is generating biological probes, potentially as drug discovery leads, for several hundred biological targets. UNMCMD screens are intended to identify active molecules in a library of several hundred thousand molecules, and to follow up leads for chemical optimisation. HyperCyt allows multiparameter analysis, as well as real-time measurements of cell response.

Our experience indicates that many molecular assemblies or suspension cell responses (except for topography) can be displayed in a format compatible with flow cytometry. Moreover, by creating a suspension array of particles, assays and responses can be highly multiplexed or performed on complex cell populations without compromising throughput. For example, in a blood sample with multiple subsets of white cells it is practical to evaluate each of the subsets simultaneously. Alternatively, cell lines can be engineered to examine multiple targets simultaneously. In the US, federal funding can support discovery on cells in suspension including white blood cells, blood cancers, as well as the role of white blood cells in infection, allergy, and inflammation through NIH Institutes such as the NCI (Cancer), NHLBI (Heart, Lung and Blood), and NIAID (Allergy and Infectious Diseases).

As a specialty centre for screening by flow cytometry for the Molecular Libraries Network, UNMCMD is responsible for identifying biologically active small molecule or 'probes'. UNMCMD works with other centres and with target providers around the world in target development and screening. The MLI provides an entree for academia into the drug discovery enterprise and expands the scope of diseases that will be studied and targeted for drug development. As UNMCMD is firmly entrenched in a university, centre members have academic portfolios defined by target areas such as GPCR[20-23] and transporters[24-26], as well as diseases foci such as immunology and oncology.

UMNCMD is also charged with outreach for identifying target providers. As these investigators may also want access to HyperCyt technology in their own laboratories, UNMCMD creates synergy for the MLPCN and IntelliCyt by being available to make presentations about the platform capabilities as well as opportunities for target development and screening. UNMCMD also has the opportunity to develop its own research specialties that augment the utility of the platform. These specialisations have included the development of a portfolio of tools that take advantage of the power of yeast genetics as models systems for drug discovery. We are now assembling two portfolios: 1) drug resistance transporters relevant to resistance in pathogenic organisms as well as cancer in suspension cells; and 2) unique approaches to identify non-canonical ligands for GPCR as well as canonical ligands for orphan GPCR.

In the current economic environment, the US Government has made available stimulus funding on a competitive basis through the ARRA (American Renewal and Recovery Act). UNMCMD was fortunate to compete successfully at NIH to allow UNMCMD to renovate a building to house UNMCMD team members (target development, discovery, cheminformatics and medicinal chemistry) in contiguous space. Through integration of the HTS platform with updated modular equipment, UNMCMD is poised to provide enhanced collaborative discovery capabilities based on HyperCyt worldwide.

Flow cytometry drug discovery targets

HyperCyt at UNMCMD has already been used to undertake a variety of discovery projects on cell and bead-based targets, all described in the PubChem database[19]. Overall, we have worked on more than 50 targets (including multiplexes). The majority of the targets take advantage of the discrimination of bound versus free fluorescence in flow cytometry with roughly equal numbers of cell and bead targets. Cell-based analysis in flow cytometry can take advantage of changes in target

morphology (and topography in special cases discussed below), variations in complex target populations, as well as target interactions with infectious agents. Relatively few adherent cell assays have been performed for two reasons: 1) resuspension of adherent cells is typically cumbersome; and 2) if cells are cultured in the wells, the density of resuspended cells is limited by the surface area at the bottom of the well. In the following section, we will describe how individual modes of analysis have been applied to a diverse collection of targets. Description of projects funded by NIH can be found at the NIH Reporter website[27] by querying based on the name of the target provider (Principal Investigator as identified below).

Fluorescent ligands and other fluorophores which accumulate on or inside cells provide useful detection strategies in flow cytometry. As the binding of ligands to cell surface receptors may typically be discriminated in homogeneous fashion, competitive binding assays have been attractive targets for both GPCRs and intracellular receptors as well as antibodies for ligand-induced binding sites in integrins. The analysis of binding events can also be extended to cell interactions that include host-pathogen (virus and bacteria[28]) interactions in cell suspensions. Moreover, molecules that accumulate within cells such as transporter substrates are also useful in screens for transporter inhibitors. When evaluating multiple cellular targets (**Figure 2**), the cells displaying individual targets are colour-coded using colours distinct from the ligands or the substrates used to measure the biological response[20,24,29].

The expression of fluorescent proteins is also a suitable way for measuring cellular response in HTS flow cytometry with UNMCMD collaborators. Expressed transcript fluorescence has already been applied to screens for quorum sensing in S. Aureus (with P. Gresham), analysis of the branch-points of the TOR pathway (with M. Werner-Washburne), expression of Candida virulence factors (with S. Lee), and the measurement of intracellular pH with pHLourin (with K. Parra). Both TOR pathway and Candida virulence factor targets have been multiplexed by colour-coding cells with cell stains excited by lasers at 405nm and 633nm (analogous to 29) while the GFP fluorescence was excited by a 488nm laser. We will discuss in more detail below how the expression of proteins with novel tagging schemes allows the detection of protein transport as it may be applied to GPCRs.

Endogenous cell features have also been successfully applied to HTS flow cytometry. These include

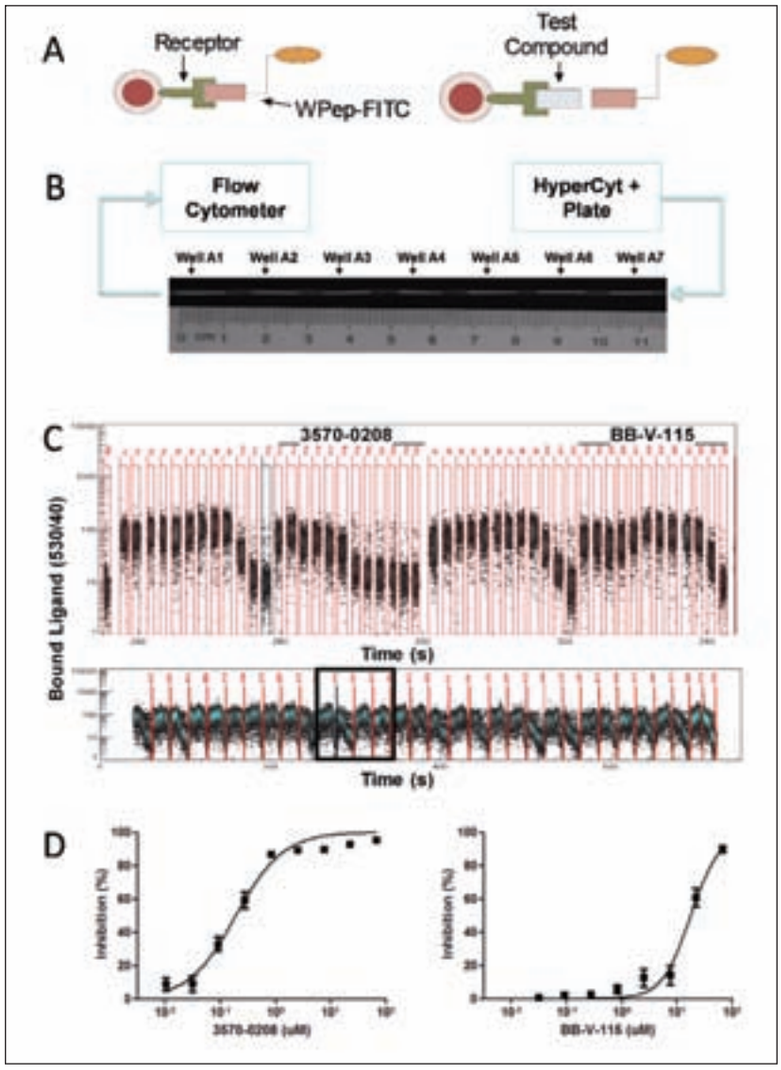

Figure 2: HyperCyt method for flow cytometry high throughput screening and dose response analysis. **A:** The illustrated assay assesses the ability of test compounds to displace fluorescent ligand (WPEP-FITC) from the formylpeptide receptor expressed on intact cells. **B:** Samples are aspirated from microplate wells and delivered to a flow cytometer as a series of fluid volumes (~2µl each) separated by air bubbles. Illustrated are fluid samples (white) as they appear in the tubing used for sample transport. **C:** The samples are detected in the flow cytometer as discrete clusters of events that appear at uniform time intervals. Each cluster represents ~2,000 cells sampled from an individual well. In this example, each test compound was tested in a 12-well sequence in which, from left to right, the first three wells contained no compound and the last nine wells contained three-fold increasing concentrations of compound. The bottom panel shows the fluorescence intensity profile for all 384 wells and the top panel shows a zoomed in view of a 48-well subset. **D:** Dose response profile of two test compounds expressed as % inhibition of WPEP-FITC binding. The 12-well fluorescence intensity sequences for each are indicated in the top panel of C. The IC_{50}s for 3570-0208 and BB-V-115 were 189nM and 17.8µM, corresponding to inhibition constants of 37nM and 3.5µM, respectively[22]

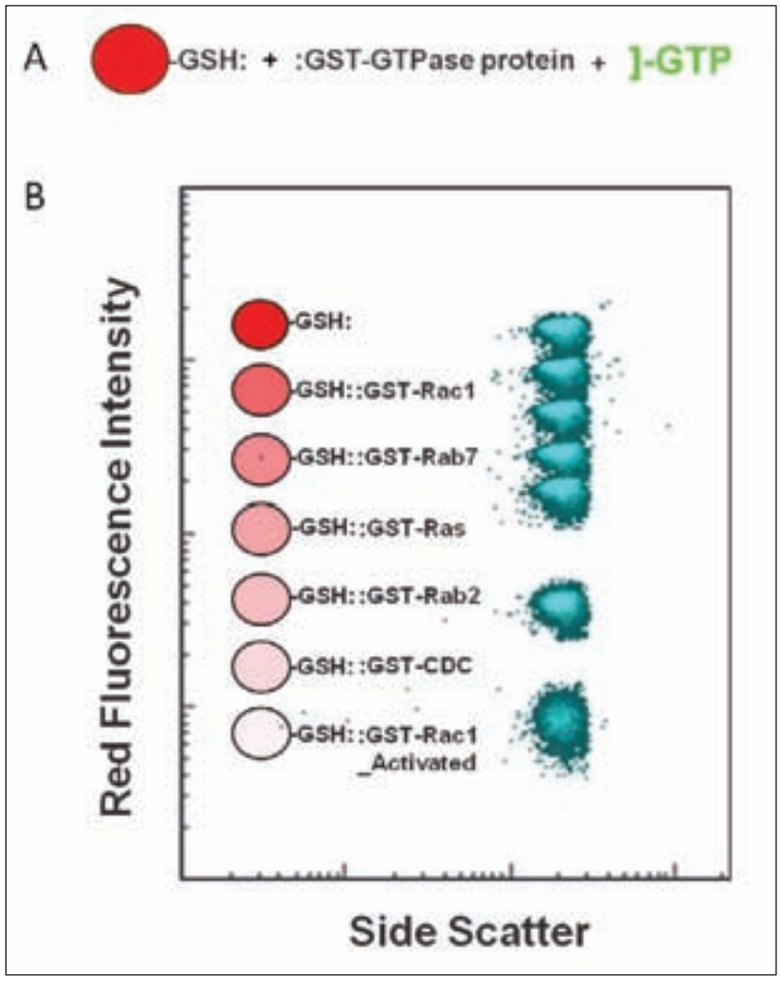

Figure 3: Multiplexed screen to detect potential regulators of signalling by Ras and related low molecular weight GTPases. **A:** General assay method. Microspheres with covalently-linked glutathione were coated with GTPase fusion proteins and incubated with test compounds in the presence of green fluorescent GTP. Hit compounds were detected by a decrease in microsphere green fluorescence intensity resulting from competitive displacement of fluorescent GTP. **B:** Six sets of microspheres with distinct levels of red fluorescence intensity were coated with six different GTPases. All sets were incubated with test compound in the same well and subsequently analysed in parallel. Changes in green fluorescence intensity of each set were monitored independently by electronically gating the analysis of individual colour-coded microsphere populations. A seventh microsphere set with glutathione alone was used to detect non-specific changes in fluorescence intensity resulting, for example, from innate fluorescence of a test compound

changes in cell granularity in prostate cancer cells (T. Thompson[30]) measured by light scatter as well as the expression of fluorescent protoporphyrin IX under regulation of the transporter ABCB6 (P. Krishnamurthy[31]). As flow cytometry excels at analysis of cell populations, heterogeneous populations may also be identified, such as in the case of cell senescence (W. Burhans[32]) where senescent cells differentially produce free radicals of oxygen) or immunological assays such as the T cell immunological synapse (I. Hwang[33]). In the latter case, T-cells bind fluorescent vesicles expressing synapse proteins. The cell populations are detected without a wash step.

Transporters

Transporters are widely distributed in nature to protect cells from toxic molecules. They contribute to the drug resistance of pathogens (R. Cannon) as well as cancer to therapeutics drugs. Moreover, a fungal infection might develop in a cancer patient because of his or her weakened immune system. Transporters also function in the blood-brain barrier. High throughput flow cytometry with fluorescent substrates provides the opportunity to readily evaluate inhibitors for selectivity across diverse transporter families. In addition, with the many fluorescent colours to develop assays, flow cytometry can evaluate multiple substrate interactions to probe multiple sites on a pump. UNMCMD has a focus on opportunities provided by multiplex screening.

GPCR

While it has been a challenge in the flow cytometry field to get information about protein topography, new technology is available for using the flow cytometer as a tool for protein localisation in conjugation with new tagging technology called fluoragen activating peptide (FAP). With Yang Wu, PhD at UNMCMD, we have been working with the Carnegie Mellon University's Technology Center for Networks and Pathways (Molecular Biology and Imaging Center). These fluoragens have rotatable bonds and only become fluorescent, like DNA intercalating dyes, when bound to the tag[34]. Using impermeable and permeable fluoragens allows discrimination of protein location[35]. Thus, when an activated G-protein coupled receptor (GPCR) is transported from the outside to the inside of a cell, the protein tag disappears from the cell surface). This technology, analogous to the B-Arrestin translocation microscopy assay, is appropriate for identifying canonical ligands of GPCRs. The FAP technology readily discriminates cell surface receptors and is suitable for multiplexing[36].

Personalised medicine

The approaches described above are compatible with strategies for personalised medicine, particularly for blood cancers[37-41]. Blood cancer cells are often identified by specific combinations of antibodies or other properties that can be measured in a flow cytometer. Thus 10 million white cells from 10mL of blood would provide 10,000 cells in each of 1,000 wells, potentially to identify approved

drugs, to alter the course of chemotherapy. As blood cancers tend to survive in niches, such as bone marrow, their survival may be impacted by their interactions with other cells. High throughput flow cytometry is also an excellent tool for biomarkers analysis in suspension, soluble analytes with bead-based detection and compound profiling in early phase toxicology.

Bead-based targets and molecular assemblies

A particularly attractive application for flow cytometry involves ligand binding and protein-protein interactions that can be studied in homogeneous assays. Typically these involve the use of molecular capture and display strategies to minimise reagent cost on the order of a penny per target per well. The HyperCyt platform has already been applied to several collaborations for analysis of molecular assemblies, ranging from small molecule protein interactions (GTPase binding of fluorescent GTP[41]) with A. Wandinger-Ness, protein-protein interactions (MEKK-MEK with Nakamura[42]; Rab7-RILP with A. Wandinger-Ness and RGS-Go with R. Neubig[43]), protein-peptide interactions (Bcl-2-family proteins with SH3 peptides with J. Reed/L. Sklar[44]), protein-RNA interactions (GRK-fluorescent aptamer binding with J. Tesmer), disassembly of the intact 30S proteasome with D. Skowyra and antibody-antigen or receptor interaction (Desmoglein with its pathogenic antibody with J Stanley or IgE to the FcE receptor with G. MacKay), and protease-substrate interactions on multiplexed beads (with S. Graves[45,46]). These approaches have proven particularly valuable when other approaches may be expensive due to reagent costs or concentrations, fail, or are improved by multiplexing. Nakamura TR-FRET assay started as a singleplex which was converted to a triplex bead-based assay.

GTPases represent one target that has uniquely leveraged high throughput flow cytometry. Low molecular weight GTPases represents a family of more than 100 proteins that are involved in many different aspects of cell physiology, topography, differentiation and protein trafficking. To identify small molecules that could regulate the ability of GTPases to interact with GTP, we developed an assay in collaboration with Angela Wandinger-Ness, PhD in the pathology department at UNM, and Zurab Surviladze, PhD, who led development of that target at UNMCMD. We had observed previously that while glutathione has low affinity for Glutathione-S-Transferase (GST), the attachment is stable when GSH is coupled to beads at high surface density[47]. We used a set of colour-coded

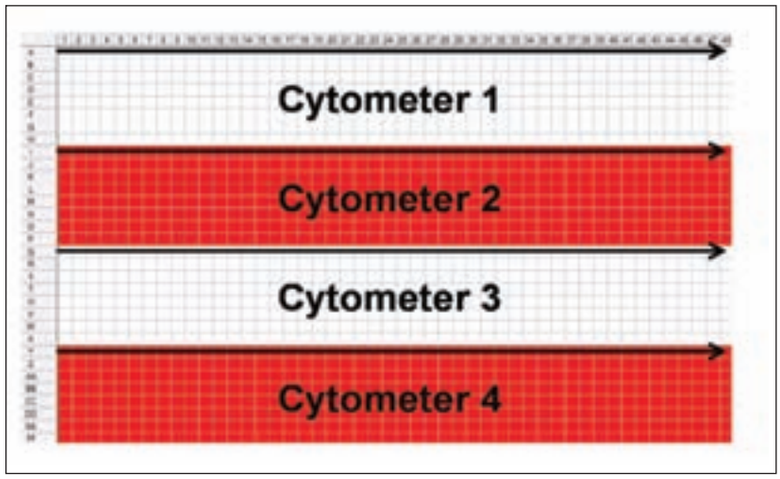

Figure 4
Quad-cytometer approach for high throughput flow cytometry analysis of a 1536-well plate. Four HyperCyt sampling probes are positioned in parallel by an autosampler so that each moves across a 384-well quadrant of a 1536-well plate (alternating colours) and delivers samples to a separate cytometer. The sampling probes move from left to right across each row (arrows), advancing from one row to the next in sequence. This approach allows automated analysis of a 1536-well plate in 10-12min

microspheres displaying glutathione, each having different intensities of red fluorescence which have been commercially available from Duke Scientific (**Figure 3**). A different low molecular weight GTPase fusion protein with GST was captured on each bead set. A green fluorescent GTP was used to identify molecules that could regulate the binding of GTP to members of the family. From a pharmacological perspective many possible activities would be anticipated: activators or inhibitors that hit one, several or many GTPase. As low molecular weight GTPases regulate protein trafficking in cells, UNMCMD collaborators Angela Wandinger-Ness and Laurie Hudson are evaluating molecules from these studies in cancer models.

Technology evolution

With six colour flow cytometers available for as little as $40,000 and tens of thousands of flow cytometers operational worldwide, access to individualised flow cytometry-based discovery is within reach. As with plate readers, individual laboratories are in a position to pilot their own discovery pipeline and to develop suspension targets at their discretion. UNMCMD is in a position to advance and implement flow cytometry collaborations at the same time. The instrumentation leader for the UNMCMD Bruce Edwards now has an NIH funded grant for improved sample handling and automation. (1R01HG005066-01, Advancing High Throughput Flow Cytometry). The work is co-ordinated with the ongoing overall automation of the HyperCyt platform, through the ARRA supplement, to improve sample detection, transition to 1536-well sampling, and integrate a direct sampling system for the flow cytometer. Projects include:

1 Adaptation of HyperCyt to 1536-well sampling by segmenting a 1536-well plate for delivery into four Accuri flow cytometers in 10 minutes (**Figure 4**).
2 Direct sample delivery without use of an intervening peristaltic pump or tubing into flow cytometers that sip or pull rather than push sample, by using an automated xyz stage to deliver wells to the input port of the flow cytometer.
3 Platform integration for complex sample handling to include staining, fixation, permeabilisation, centrifugation, wash and resuspension, as necessary. Unique opportunities for complex sample handling and complex cell suspension for the flow cytometry platform at UNMCMD are likely to include: haematopoeitic stem cells as well as blood cells in leukaemia, immunology, inflammation and infection disease.
4 To take advantage of the multitude of opportunities for complex targets. Customisation of the data analysis software will allow access to multiplexed targets as well as complex cell suspension that rely on genetic colour-coding of cells with fluorescent proteins, antibody staining and novel dye combinations.
5 HyperCyt bubble detection for improving sample resolution.

Academic drug discovery

UNMCMD and HyperCyt reflect and facilitate a growing trend toward academic discovery science[48]. With tens of thousands of flow cytometers available around the world, HT flow cytometry provides a versatile platform for prototyping new targets. The UNMCMD currently evaluates 20 or more targets per year.

The growing translational mission of the NIH is reflected in part by the ongoing funding of 60 NCI designated Cancer Centers and NCRR (Research Resources) funding of 60 Clinical and Translational Science Centers. Technology such as HyperCyt has the potential to benefit from partnerships for repurposing drugs that span the public sector, government, industry and venture philanthropy. Some of the molecules in UNMCMD studies are approved drugs that give academic and clinical colleagues the potential to take existing molecules into clinical trials This direction is complementary to the efforts of the Molecular Libraries Initiative to identify new chemical entities.

For high throughput screening the UNMCMD screening target is 200 wells/minute (96,000 wells/8h day). Although somewhat slow relative to other technologies, it has the advantages of homogeneous resolution of free and bound signals from cellular and molecular targets, as well as

multiplexing and the capability to measure multiple targets or multiple responses of a single target. Among others, these could include phosphoproteins in cell populations[49], epitope expression on the outside of a cell, or intracellular markers. These already available approaches can be essentially mixed and matched with complex sample handling. UNMCMD is intent on exploiting all the intrinsic capabilities of the platform, and to overcome the current limitations of sample handling. We see as an essential part of the UNMCMD mission discovery on targets uniquely suited to flow cytometry and not readily available currently in the discovery community.

Acknowledgements

Work at the UNMCMD was supported by NIH Grants U54MH074425, U54MH084690 and R01HG005066. The authors would like to acknowledge the contributions of collaborators and the grant support to their projects, as well as to all team members of the UNMCMD, current, past and future. DDW

Dr Larry A. Sklar *received his BS in Chemistry at the University of Chicago and PhD in Chemistry at Stanford University. He was a Helen Whitney Postdoctoral Fellow at UCSC and the Methodist Hospital at the Baylor College of Medicine. He was an Established Investigator of the American Heart Association and Associate Member at Scripps before taking a joint appointment as Director the National Flow Cytometry Resource at Los Alamos National Laboratory and the University of New Mexico where he is Regents Professor of Pathology and Distinguished University Professor. He also serves as Associate Director of Basic Research and Co-Director of the Program in Cancer Biology and Biotechnology in the NCI Designated University of New Mexico Cancer Center and the Director of Translational Technology in UNM's Clinical and Translational Science Center. He has more than 300 publications and patents in leukocyte biology, molecular assembly in signal transduction and cell adhesion, and high throughput flow cytometry for drug discovery. He is PI and Director of the University of New Mexico Center for Molecular Discovery for the NIH Roadmap Molecular Libraries Initiative. He is a founder of IntelliCyt and was UNM's 53rd Annual Research Lecturer.*

Dr Bruce S. Edwards *obtained his PhD in Biology at the University of Colorado, Boulder. He was an NIH Postdoctoral Research Fellow and Cetus/Shell Triton Postdoctoral Research Fellow at the University of Wisconsin Clinical Cancer Center in Madison. He came to New Mexico as an Associate Scientist and then Scientist at the Lovelace Medical Foundation in Albuquerque. He founded and directed the flow cytometry core facility at the LMF. He subsequently moved to the University of New Mexico where he is currently Research Professor of Pathology, Director of the Cancer Research and Treatment Center Shared Flow Cytometry Resource and co-leader of the High Throughput Screening Core of the NIH-designated New Mexico Center for Molecular Discovery. He is an inventor of novel technologies developed and patented at UNM that have led to the successful implementation of high throughput flow cytometry. He is a founder of IntelliCyt Corporation, a company whose mission is to commercialise these technologies and develop novel flow cytometry applications.*

References

1 Sklar, LA. Flow Cytometry for Biotehcnology, Oxford University Press, 2005.
2 Sklar, LA, Carter, MB, Edwards, BS. Flow Cytometry in Drug Discovery, Receptor Pharmacology, and High Throughput Screening. Current Opin Pharmacol 7: 527-34, 2007.
3 Edwards, BS, Young, SM, Saunders, MJ, Bologa, CG, Oprea, TI, Ye, RD, Prossnitz, ER, Graves, SW and Sklar, LA. High Throughput Flow Cytometry for Drug Discovery. Expert Opin Drug Discov.2:685-696, 2007.
4 Edwards, BE, Kuckuck, F and Sklar, LA. Plug-flow flow cytometry: an automated coupling device for rapid sequential flow cytometric sample analysis. Cytometry. 37: 156-159, 1999.
5 Kuckuck, F, Edwards, BS and Sklar, LA. High Throughput Flow Cytometry. Cytometry 44: 83-90, 2001.
6 Intellicyt: http://www.intellicyt.com/.
7 Viviabiotech: http://www.viviabiotech.com/equipo.html.
8 Rigel Automation of HyperCyt: http://www.intellicyt.com/download/RigelSBSAutomationPoster.pdf.
9 Intellicyt-Beckman Coulter Agreement: http://www.beckmancoulter.com/HR/PressRoom/oc_pressReleases_detail.asp?Key=8962.
10 Molecular Libraries home: http://mli.nih.gov/mli/mlpcn/.
11 Molecular Libraries Fund Opportunities: http://mli.nih.gov/mli/funding-opportunities/.
12 Molecular Libraries funded research: http://nihroadmap.nih.gov/molecularlibraries/fundedresearch.asp.
13 Solicitation of Assays for High Throughput Screening (HTS) in the Molecular Libraries Probe Production Centers Network (MLPCN) (R03): http://grants.nih.gov/grants/guide/pa-files/par-09-129.html.

Continued on page 46

Continued from page 45

14 Assay Development for High Throughput Molecular Screening (R21): http://grants.nih.gov/grants/guide/pa-files/par-10-182.html.

15 Development of Assays for High-Throughput screening for use in Probe and Pre-therapeutic Discovery (R01) http://grants.nih.gov/grants/guide/pa-files/PA-10-213.html.

16 Fast Track Notice of Opportunity for R01; http://grants.nih.gov/grants/guide/notice-files/NOT-RM-09-011.html.

17 Fast Track Notice of Opportunity for R21: http://grants.nih.gov/grants/guide/notice-files/NOT-RM-09-008.html.

18 Molecular Libraries probes: http://mli.nih.gov/mli/mlp-probes/.

19 National Center for Biotechnology Information: http://www.pubchem.ncbi.nlm.nih.gov/.

20 Young, SM, Bologa, C, Prossnitz, ER, Oprea, TI, Sklar, LA, Edwards, BS. High throughput screening with HyperCyt flow cytometry to detect small molecule formylpeptide receptor ligands. J. Biomol. Screen. 10:374-382, 2005.

21 Edwards, BS, Bologa, C, Young, SM, Balakin, KV, Prossnitz, E, Savchuck, NP, Sklar, LA and Oprea, TI. Integration of virtual screening with high throughput flow cytometry to identify novel small molecule formylpeptide receptor antagonists. Mol. Pharmacol. 68:1301-1310, 2005.

22 Young, SM, Bologa, CM, Fara, D, Bryant, BK, Strouse, JJ, Arterburn, JB, Ye, RD, Oprea, TI, Prossnitz, E, Sklar, LA, Edwards, BS. Duplex High Throughput Flow Cytometry Screen Identifies Two Novel Formylpeptide Receptor Family Probes. Cytometry 75A: 253-263, 2009.

23 Strouse, JJ, Young, SM, Mitchell, H, Ye, RD, Prossnitz, ER, Sklar, LA, Edwards, BS. A Novel Fluorescent Cross-Reactive Formylpeptide Receptor/Formylpeptide Receptor-Like 1 Hexapeptide Ligand. Cytometry 75A: 264-270, 2009.

24 Ivnitski-Steele, I, Sklar, LA, Larson, RS, Lovato, DM, Oprea, TI, Edwards, BS. High throughput flow cytometry to detect selective inhibitors of ABCB1, ABCC1 and ABCG2 transporters. Assay Drug Dev Technol 6:263-76, 2008.

25 Winter, SS, Lovato, DM, Khawaja, HM, Edwards, BS, Ivnitski-Steele, ID, Young, SM, Oprea, TI, Sklar, LA, Larson, RS. High Throughput Screening for Daunorubicin-mediated Drug Resistance Identifies Mometasone Furoate as a Novel ABCB1 Reversal Agent. J Biomol Screen 13: 185-193, 2008.

26 Ivnitski-Steele, I, Holmes, AR, Lamping, E, Monk, BC, Cannon, R, Sklar, LA. Identification of Nile red as a fluorescent substrate of the Candida albicans ATP-binding cassette transporters Cdr1p and Cdr2p and the major facilitator superfamily transporter Mdr1p. Anal Biochem. 2009:394:87-91.

27 NIH Reporter database: http://projectreporter.nih.gov/reporter.cfm.

28 Haynes, MK, Strouse, JJ, Waller, A, Leitao, A, Curpan, RF, Bologa, C, Oprea, TI, Prossnitz, ER, Edwards, BS, Sklar, LA, Thompson, TA. Detection of intracellular granularity induction in prostate cancer cell lines by small molecules using the HyperCyt high throughput flow cytometry system. J Biomol Screen 14:596-609, 2009.

29 Krutzik, PO and Nolan, GP. Fluorescent cell barcoding in flow cytometry allows high-throughput drug screening and signaling profiling, Nature Methods 3: 361-368, 2006.

30 Buranda, T, Wu, Y, Perez, D, Jett, SD, BonduHawkins, V, Ye, C, Edwards, B, Hall, P, Larson, RS, Lopez, GP, Sklar, LA, Hjelle, B. Recognition of decay accelerating factor and alpha(v)beta(3) by inactivated hantaviruses: Toward the development of high-throughput screening flow cytometry assays. Anal Biochem. 402:151-60, 2010.

31 Krishnamurthy, PC, Du, G, Fukuda, Y, Sun, D, Sampath, J, Mercer, KE, Wang, J, Sosa-Pineda, B, Murti, KG, Schuetz, JD (2006). Identification of a mammalian mitochondrial porphyrin transporter. Nature 443 (7111): 586–9, 2006.

32 Mesquita, A, Weinberger, M, Silva, A, Sampaio-Marques, B, Almeida, B, Leão, C, Costa, V, Rodrigues, F, Burhans, WC and Ludovico, P. Caloric restriction or catalase inactivation extends yeast chronological lifespan by inducing H2O2 and superoxide dismutase activity. Proc Natl Acad Sci U S A. 2010 107:15123-8, 2010.

33 Kim, K, Wang, L and Hwang, I. A novel flow cytometric high throughput assay for a systematic study on molecular mechanisms underlying T cell receptor-mediated integrin activation. PLoS One 4:e6044, 2009.

34 Szent-Gyorgyi, C, Schmidt, BA, Creeger, Y, Fisher, GW, Zakel, KL, Adler, S, Fitzpatrick, JA, Woolford, CA, Yan, Q, Vasilev, KV, Berget, PB, Bruchez, MP, Jarvik, JW and Waggoner, AS. Fluorogen-activating single-chain antibodies for imaging cell surface proteins. Nat Biotechnol. Feb;26(2):235-40, 2008.

35 Fisher, GW, Adler, SA, Fuhrman, MH, Waggoner, AS, Bruchez, MP and Jarvik, JW. Detection and quantification of B2AR Internalization in Living Cells using FAP-based biosensor technology. J. Biomolecular Screening, 15: 703-709, 2010.

36 Holleran, J, Brown, D, Fuhrman, MH, Adler, SA, Fisher, GW and Jarvik, JW. Fluorogen-activating proteins as biosensors of cell-surface proteins in living cells. Cytometry A, 77: 776-782, 2010.

37 Rickles, RJ, Pierce, LT, Giordano, TP 3rd, Tam, WF, McMillin, DW, Delmore, J, Laubach, JP, Borisy, AA, Richardson, PG and Lee, MS. Adenosine A2A receptor agonists and PDE inhibitors: a synergistic multitarget mechanism discovered through systematic combination screening in B-cell malignancies. Blood. 116(4):593-602, 2010.

38 Lehár, J, Krueger, AS, Avery, W, Heilbut, AM, Johansen, LM, Price, ER, Rickles, RJ, Short, GF 3rd, Staunton, JE, Jin, X, Lee, MS, Zimmermann, GR, Borisy, AA. Synergistic drug combinations tend to improve therapeutically relevant selectivity. Nat Biotechnol. Jul;27(7):659-66, 2009. Erratum in: Nat Biotechnol. Sep;27(9):864, 2009.

39 Skribek, H. A New in-vivo Like High Throughput and High Content Method for Anti-cancer and Antibiotics Library Screening. Screening Europe. http://www.selectbiosciences.com/conferences/SE2010/Agenda.aspx.

40 Spagnuolo, PA, Hu, J, Hurren, R, Wang, X, Gronda, M, Sukhai, MA, Di Meo, A, Boss, J, Ashali, I, Beheshti Zavareh, R, Fine, N, Simpson, CD, Sharmeen, S, Rottapel, R and Schimmer, AD. The antihelmintic flubendazole inhibits microtubule function through a mechanism distinct from Vinca alkaloids and displays preclinical activity in leukemia and myeloma. Blood. 115(23):4824-33, 2010.

41 Surviladze, Z, Waller, A, Wu, Y, Romero, E, Edwards, BS, Wandinger-Ness, A, Sklar, LA. Identification of a small GTPase inhibitor using a high-throughput flow cytometry bead-based multiplex assay. J Biomol Screen 15:10-20, 2010.

42 Nakamura, K, Zawistowski, JS, Hughes, MA, Sexton, JZ, Yeh, LA, Johnson, GL, Scott, JE. Homogeneous time-resolved fluorescence resonance energy transfer assay for measurement of Phox/Bem1p (PB1) domain heterodimerization. J Biomol Screen. 2008 Jun;13(5):396-405, 2008.

43 Blazer, LL, Roman, DL, Muxlow, MR and Neubig, RR. Use of flow cytometric methods to quantify protein-protein interactions. Curr Protoc Cytom. 2 Jan;Chapter 13:Unit 13.11.1-15 2010.

44 Zhai, D, Jin, C, Huang, Z, Satterthwait, AC, Reed, JC. Differential regulation of Bax and Bak by anti-apoptotic Bcl-2 family proteins Bcl-B and Mcl-1. J Biol Chem. 283(15):9580-6, 2008.

45 Saunders, MJ, Kim, H, Woods, TA, Nolan, JP, Sklar, LA, Edwards, BS and Graves, SW. Microsphere-based protease assays and screening application for lethal factor and factor Xa. Cytometry A, 69A: 342-352, 2006.

46 Saunders, MJ, Graves, SW, Oprea, TI, Sklar, LA and Edwards, BS. High throughput multiplex protease screening for Botulinum Neurotoxin Type A light-chain protease inhibitors. Assay Drug Dev Technol 8:37-46, 2010.

47 Tessema, M, Simons, PC, Cimino, DF, Sanchez, L, Waller, A, Posner, RG, Wandinger-Ness, A, Prossnitz, ER and Sklar, LA: Glutathione-S-transferase-Green Fluorescent Protein Fusion Protein Reveals Slow Dissociation from High Site Density Beads and Measures Free GSH. Cytometry A 69A: 326-334, 2006.

48 Academic Screening Center Directory: http://www.sbsonline.org/i4a/member_directory/feResultsListing.cfm?directory_id=7&viewAll=1.

49 Krutzik, PO, Nolan, GP. Intracellular phospho-protein staining techniques for flow cytometry: monitoring single cell signaling events. Cytometry A. 55(2):61-70, 2003.