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PII: S2472-5552(23)00068-0
DOI: https://doi.org/10.1016/j.slasd.2023.10.001
Reference: SLASD 117

To appear in: *SLAS Discovery*

Please cite this article as: Neil O. Carragher , Judi Wardwell-Swanson , Gregory P. Way , High-Content Imaging 2023: A Joint Special Collection with the Society for Biomolecular Imaging, *SLAS Discovery* (2023), doi: https://doi.org/10.1016/j.slasd.2023.10.001

High-Content Imaging 2023: A Joint Special Collection with the Society for Biomolecular Imaging and Informatics and SLAS

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From October 31st to November 2nd, 2023, the Society of Biomolecular Imaging and Informatics (SBI^2) will hold its 10th annual meeting in Boston, Massachusetts USA. SBI^2 was established in 2011 to serve a burgeoning community of research scientists, instrument manufacturers, and data analysis experts who specialize in automated microscopic image acquisition and analysis. Our community spans academia and industry, and benefits from an active and open exchange of ideas. Over the past decade, SBI^2 has closely adhered to its mission statement: "*The Society of Biomolecular Imaging and Informatics is an international community of leaders, scientists, and students promoting technological advancement, discovery, and education to quantitatively interrogate biological models to provide high context information at the cellular level".* Since the beginning of SBI^2, the field of high content imaging, which measures phenomic information from microscopy images, has undergone a remarkable evolution. Our field has achieved advances in imaging hardware, increasingly more sophisticated cell and tissue based assays (including 3-Dimensional [3D] spheroid and organoid screening formats), increased computing power, and novel application of artificial intelligence methods. As a result of such developments, high content imaging now represents a core capability in many academic institutions and biopharmaceutical organizations, and has contributed to the development of more robust, disease-relevant, and mechanistically-informative biological models supporting new knowledge creation and innovation of therapeutic discovery.

In recognition of the significant impact that high content imaging has and continues to provide in basic and translational research, SLAS Discovery and SBi^2 have put together this special collection of articles to coincide with the 10th anniversary of the annual SBI^2 meeting. This special collection of SLAS Discovery contains four original research article, one short communication and a perspective.

The opening article by Way et al, [1] provides a historical perspective of high content imaging developments and impact since its inception from the early pioneering work of Lansing Taylor and others to the development of open-source image analysis software and recent development of Cell

Painting high content phenotypic profiling by Anne Carpenter, Shantanu Singh, and colleagues. The article also highlights the important role that academic-industry collaborations have in advancing the field and making high impact contributions to drug discovery. The article discusses current gaps and challenges and provides a prospective view on future developments. Key topics which are discussed in depth include: Evolution of high content image analysis; Evolution of high content data analysis pipelines; The role of data integration and multiomics; The role and evolution of image data repositories and sharing standards; and future perspective of high content imaging hardware and software.

We also include five other research articles, which we feel represent the spirit and mission of SBI^2 in our 10th anniversary. Winchell et al, present FocA (Focal Analysis), a Machine Learning (ML) tool designed for the near-real-time checking of in-focus and out-of-focus images in highly-automated high content imaging pipelines[2]. FocA is capable of identifying 100% of out-of-focus and 98% of in-focus images in under 4 seconds per 96-well plate. While the authors acknowledge that several methods for focus evaluation in cell culture images are available including many ML algorithms that have been widely used with great success, improvements in seamless scalability and automation are needed. FocA's downsampling and patch-based model makes it easy to deploy and scale and its simple code structure supports potential repurposing of the same architecture to create models for a variety of image quality tasks, including over-exposure, empty well detection, and contamination detection. Although designed for highly-automated laboratories to enable rapid re-imaging and analysis of out of focus assay plates the authors believe that FocA will also serve useful to the wider community in both automated and non-automated setups. With the rapidly accelerating pace of data collection, automated and scalable tools for image quality that are also modifiable and portable will become increasingly important.

Next, McCarty et al, describe the development of an intact *ex vivo* pancreatic islet bioassay in 384-well high content imaging format that can detect multiple diabetes-relevant endpoints including beta-cell proliferation, chemoprotection, and islet spatial morphometrics. Diabetes poses a global health crisis affecting individuals across age groups and backgrounds, with a predicted global prevalence of 1 in 10 adults by 2040. Loss of beta-cell function and mass is the central event in the etiology of both Type 1 and Type 2 diabetes. Therefore, physiologically relevant assays which can identify new molecular targets or therapeutic agents which promote beta cell expansion or protect from beta cell loss represents a critical unmet need. Established assays include dissociated islet cells plated in 2D monolayer cultures or reconstituted pseudo-islets formed in ultra-low attachment plates. However, these methods have limitations due to the islet cell dispersion process, which can confound results.

To combat these limitations, the authors present an assay workflow for high content imaging of intact islets; First, the authors harvest islets from mice and place them in 384-well plates. After fixation, the authors stain islets with Hoechst, Ki67, Nkx6.1, and Glucagon antibodies and acquire images using a two-pass imaging protocol on a Yokogawa CV8000 (initially capturing a 4X overview image of the entire well followed by a centered 40 × 3D-imaging of each islet). The authors then segment islets with Cellpose and use CellProfiler to extract features, including spatial features from multiple islets per well with single-cell resolution. The authors achieve their aim to develop an assay system that maintains the throughput of 2D dispersed islet assays while capturing the complexity of whole intact islets. The authors apply this pipeline and develop a machine learning based classification of phenotypic response, which they show detects selective beta-cell proliferation induced by the DYRK1A inhibitor, Harmine. This article demonstrates an innovative, end-to-end pipeline pairing accurate disease modelling with advanced computational approaches, which will increasingly become a standard approach targeting a variety of diseases.

The article by Fancher et al, describes the outcome of a high-throughput high content image-based screening campaign supporting novel drug discovery for metastatic castrate-resistant prostate cancer (mCRPC)[4]. While several treatment options are available for mCRPC, most patients develop drug resistance and patients' median overall survival is typically extended by only 3-5 months. At castration testosterone levels, mCRPC tumors still rely upon the androgen receptor (AR) which contributes to tumour progression and drug resistance mechanisms. A major challenge in developing targets for the AR gene, is that transactivation is modulated by >300 coregulatory proteins. Nevertheless, Transcriptional Intermediary Factor 2 (TIF2) is a co-activator (CoA) that is commonly increased in relapsed prostate cancer patients. TIF2 plays an important role; it facilitates AR amino terminal to carboxy terminal (N/C) interactions, stabilizes AR-ligand binding, enhances AR stability, promotes chromatin remodelling, and recruits and assembles the transcriptional machinery on AR target genes. The authors recently completed an AR-TIF2 protein-protein-interaction (PPI) positional biosensor high-content screening campaign of 143,535 compounds to identify small molecules that inhibited the formation of and/or disrupted existing AR-TIF2 PPI complexes. Three hit series, the hydrobenzo-oxazepins (S1), thiadiazol-5-piperidine-carboxamides (S2), and phenyl-methyl-indoles (S3) were selected for further characterization studies that are described in the article. S3 compounds were predicted to bind to a previously described BF-3 allosteric modulator site of AR, while S1 and S2 molecules bind to a binding pocket 1 site adjacent to the orthosteric dihydrotestosterone (DHT) binding site. The authors hypothesize that to overcome drug resistance in mCRPC, we could use small molecule allosteric modulators that prevent and/or disrupt AR PPIs with CoAs like TIF2 to alter AR gene transactivation in combination with the orthosteric agonist DHT. Thus, this article represents a

follow up characterization of hits from an initial high content screening campaign, which has uncovered the molecular mechanisms-of-action of three lead series small molecule compounds. This effort may provide chemical starting points to guide the development of novel and improved mCRPC therapies.

Naito et al, describe a high throughput high content screen to identify lysosomal beta-glucocerebrosidase (GCase) activators/chaperones for the treatment of Gaucher disease, which is the most prevalent lysosomal storage disease[5]. The beta-glucocerebrosidase (GBA1) gene encodes GCase that metabolizes the lipids glucosylceramide (GlcCer) and glucosylsphingosine (GlcSpH). Biallelic loss-of-function mutations in GBA1 cause Gaucher disease. Despite significant efforts to screen potent GCase activators/chaperones, cell-free assays using recombinant GCase protein have yielded compounds with limited potency and only marginal efficacy. The authors utilize a fluorescence labeled GCase suicide inhibitor to directly monitor lysosomal GCase activity and performed high content cell-based screening of 17,317 small molecules in fibroblasts from a Gaucher disease patient with homozygotic mutations in GBA1. To eliminate compound hits that inhibit lysosomal GCase at higher doses and select out those that increase active lysosomal GCase, the authors also utilized a fluorescence-quenched substrate-based probe (GBA1-FQ2) for high-content live-cell imaging as a secondary assay. Importantly, the identified compound hits progressed from the screen also reduce GlcSph in iPSC-derived dopaminergic neurons with a GBA1 mutation, confirming that the compound-mediated increase in lysosomal GCase leads to the clearance of pathologically accumulated GlcSph. This innovative high content screen and results pave the way for developing potent and efficacious GCase chaperone compounds as a potential therapeutic approach for neurological Gaucher disease.

Finally, Huang et al, describe an ex-vivo tumor assay incorporating engineered bone marrow mesenchymal stromal cells (MSCs) expressing a synthetic fluorescent or bioluminescent CD19-sensor receptor to monitor the viability and interaction of primary CD19+ leukemia cells with MSCs in coculture[6]. Ex-vivo drug testing of primary tumor cells isolated from individual leukemia patients is being evaluated in clinical trials to guide therapeutic decisions for patients with resistant disease. However, primary tumor cells are generally difficult to maintain viable ex-vivo in monoculture. Coculture of primary tumor cells with representative components of the in vivo microenvironment such as MSCs can maintain metabolic homeostasis and viability of primary tumor cell cultures for extended periods. Conventional viability assays based on metabolic activity (such as presence of ATP) or cytotoxicity (such as lactate dehydrogenase release) however do not distinguish tumor cells from stromal cells. To specifically monitor the viability of primary acute lymphoblastic leukemia (ALL) cells that interact with MSCs in coculture, the authors engineered MSC cells to express a CD19 binding

synthetic Notch receptor that controls expression of an integrated reporter cassette. Thus, measurement of the reporter signal from MSC.sensor provides a way to monitor the viable ALL cells in contact with cocultured stromal cells in situ. The authors demonstrate the application of the MSC.sensor platform to facilitate the exploration of drug combination therapies including validation of clinically effective combinations in high throughput multiwell plate assay formats.

The articles included in this special collection represent concrete, real-world examples of the technical evolution and positive impact that our field hopes to continue pursuing in the next decade.

Declaration of Conflicting Interests

J.W-S is a consultant for InSphero AG. The authors declared no other potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

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