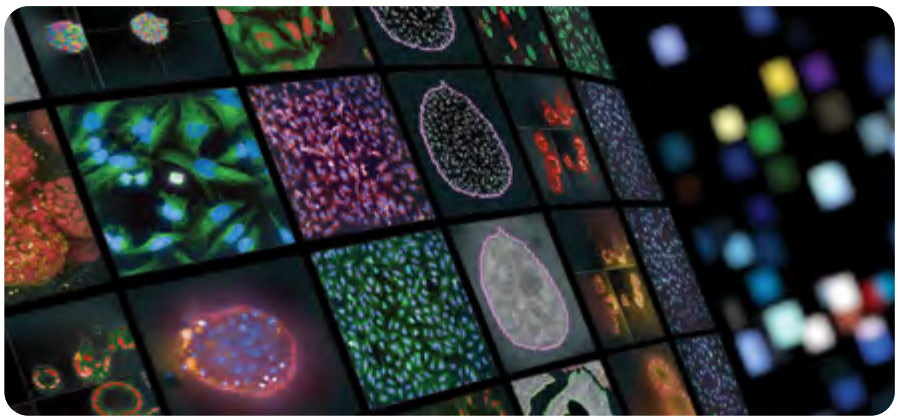
Revvity high-content screening systems in action - 2022/2023 publications



Over 5000 articles were published between 2010 and 2023 using images and data from Revvity's Operetta® CLS™ or Opera Phenix® high-content screening and analysis systems. A selection of these publications from 2022 and 2023 are featured here and categorized by research area.

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Cancer research

Comparison of Two Supporting Matrices for Patient-Derived Cancer Cells in 3D Drug Sensitivity and Resistance Testing Assay (3D-DSRT). Feodoroff, M.; Mikkonen, P.; Turunen, L.; Hassinen, A.; Paasonen, L.; Paavolainen, L.; Potdar, S.; Murumägi, A.; Kallioniemi, O.; Pietiäinen, V., *SLAS Discovery* **2023**, 28 (4), 138–148. DOI: 10.1016/j.slasd.2023.03.002.

Patient-derived cancer cells were embedded in GrowDex® matrices to mimic the growth environment of solid tumors. 3D Cultures were optimized for drug sensitivity and resistance testing. Images were captured on an Opera Phenix, segmented and quantified in 3D using Harmony® software to access cell viability and morphology.

Multifunctional mRNA-Based CAR T Cells Display Promising Antitumor Activity Against Glioblastoma. Meister, H.; Look, T.; Roth, P.; Pascolo, S.; Sahin, U.; Lee, S.; Hale, B. D.; Snijder, B.; Regli, L.; Ravi, V. M.; Heiland, D. H.; Sentman, C. L.; Weller, M.; Weiss, T., *Clin Cancer Res* **2022**, 28 (21). DOI: 10.1158/1078-0432.CCR-21-4384.

mRNA-based multifunctional CART-cells that expressed a multi-targeting chimeric antigen receptor (CAR) and proinflammatory cytokines were generated. These multifunctional cells demonstrated an increase in anti-glioma activity compared to single-expression systems. Images from an Opera Phenix were used for *in vitro* studies of the interaction between the engineered CART-cells cultured glioblastoma cells. Additionally, a convolutional neural network was trained to identify the morphology of activated T-cells.

Inhibition of Exosome Biogenesis Affects Cell Motility in Heterogeneous Sub-Populations of Paediatric-Type Diffuse High-Grade Gliomas. Pericoli, G.; Galardi, A.; Paolini, A.; Petrilli, L. L.; Pepe, G.; Palma, A.; Colletti, M.; Ferretti, R.; Giorda, E.; Levi Mortera, S.; Burford, A.; Carai, A.; Mastronuzzi, A.; Mackay, A.; Putignani, L.; Jones, C.; Pascucci, L.; Peinado, H.; Helmer-Citterich, M.; de Billy, E.; Masotti, A.; Locatelli, F.; Di Giannatale, A.; Vinci, M., *Cell Biosci* **2023**, 13 (1), 207. DOI: 10.1186/s13578-023-01166-5.

Single-cell, optically barcoded clones from paediatric-type diffuse high-grade gliomas were shown to be heterogeneous in their genetic, transcriptional, phenotypic, and motility profiles. The Operetta CLS was used to capture images from live migration and invasion assays, and the Harmony software was used to determine single-cell tracking profiles. Single-cell-derived clones exhibited higher migration speeds and traveled longer distances when co-cultured with other clones than in mono-clone cultures. The Operetta CLS and Harmony software were also used to visualize and quantify the uptake of clone-specific exosomes. When the biosynthesis of these exosomes was blocked, cancer cell motility was reduced, showing that the exchange of exosomes may play an important role in inter-clonal crosstalk.

A Functional Analysis of 180 Cancer Cell Lines Reveals Conserved Intrinsic Metabolic Programs. Cherkaoui, S.; Durot, S.; Bradley, J.; Critchlow, S.; Dubuis, S.; Masiero, M. M.; Wegmann, R.; Snijder, B.; Othman, A.; Bendtsen, C.; Zamboni, N., *Molecular Systems Biology* **2022**, 18 (11), e11033. DOI: 10.15252/msb.202211033.

Two distinct metabolic phenotypes were identified by analyzing 49 metabolic pathways in 180 cancer cell lines grown under identical conditions. The identified metabolic phenotypes correlated with the epithelial-mesenchymal transition rather than with distinct genetic mutations. The authors used third-party software to analyze images captured on an Opera Phenix. Cell shape analysis was combined with untargeted metabolomics and lipidomics to describe the two phenotypes. These two phenotypes could be therapeutically targeted due to their differences in sensitivity to gene knock-out and drug response.

Dual IGF1R/IR Inhibitors in Combination with GD2-CAR T-Cells Display a Potent Anti-Tumor Activity in Diffuse Midline Glioma H3K27M-Mutant. de Billy, E.; Pellegrino, M.; Orlando, D.; Pericoli, G.; Ferretti, R.; Businaro, P.; Ajmone-Cat, M. A.; Rossi, S.; Petrilli, L. L.; Maestro, N.; Diomedi-Camassei, F.; Pezzullo, M.; Stefanis, C. D.; Bencivenga, P.; Palma, A.; Rota, R.; Bufalo, F. D.; Massimi, L.; Weber, G.; Jones, C.; Carai, A.; Caruso, S.; Angelis, B. D.; Caruana, I.; Quintarelli, C.; Mastronuzzi, A.; Locatelli, F.; Vinci, M., *Neuro-Oncology* **2022**, 24 (7). DOI: 10.1093/neuonc/noab300.

The disialoganglioside GD2 was shown to be heterogeneously expressed in diffuse midline glioma tumors. The Operetta CLS and the PhenoLOGIC machine learning built into the Harmony software were used to analyze live-cell, brightfield images of monolayer cultures. The Operetta CLS and Harmony were also used to analyze maximum intensity projections of 3D neurosphere cultures to quantify T-cell invasion. High-throughput cell-based screening assays and *in vivo* imaging were used to identify small molecules that potentiate GD2-CAR-T cells and may facilitate improved therapeutic options.

Refined High-Content Imaging-Based Phenotypic Drug Screening in Zebrafish Xenografts. Sturtzel, C.; Grissenberger, S.; Bozatzi, P.; Scheuringer, E.; Wenninger-Weinzierl, A.; Zajec, Z.; Dernovšek, J.; Pascoal, S.; Gehl, V.; Kutsch, A.; Granig, A.; Rifatbegovic, F.; Carre, M.; Lang, A.; Valtingojer, I.; Moll, J.; Lötsch, D.; Erhart, F.; Widhalm, G.; Surdez, D.; Delattre, O.; André, N.; Stampfl, J.; Tomašič, T.; Taschner-Mandl, S.; Distel, M., *npj Precis. Onc.* **2023**, 7 (1), 1–16. DOI: 10.1038/s41698-023-00386-9.

The authors describe automated HCS imaging with the Operetta CLS and analysis of larval zebrafish xenografts including the quantification of the number of tumor cells and tumor size over time. This assay allows for fast and cost-efficient screening of small compounds and their anti-tumor efficacy in an *in vivo* model. The Prescan/Rescan PreciScan in Harmony significantly speeds up the imaging by identifying the position of the larvae prior to high magnification analysis.

Deep learning

CellDeathPred: A Deep Learning Framework for Ferroptosis and Apoptosis Prediction Based on Cell Painting. Schorpp, K.; Bessadok, A.; Biibosunov, A.; Rothenaigner, I.; Strasser, S.; Peng, T.; Hadian, K., *Cell Death Discovery* **2023**, 9, 277. DOI: 10.1038/s41420-023-01559-y.

CellDeathPred is a deep learning framework that predicts two cell death pathways - ferroptosis and apoptosis – from cell painting data, allowing for additional data extraction during phenotypic screening experiments. The Operetta and Columbus image analysis tools were used to generate the data to train and test the network.

Prediction of Mechanistic Subtypes of Parkinson's Using Patient-Derived Stem Cell Models. D'Sa, K.; Evans, J. R.; Virdi, G. S.; Adam, A.; Bertolli, O.; Fleming, J.; Chang, H.; Leighton, C.; Horrocks, M. H.; Athauda, D.; Choi, M. L.; Gandhi, S., *Nature Machine Intelligence* **2023**, 5, 933-946. DOI: 10.1038/s42256-023-00702-9.

The authors created a predictive model to indicate the presence of Parkinson's disease and a mechanistic subtype in patient-derived neurons with induced mutations. Live cell imaging on the Opera Phenix and Columbus data analysis tools were used to generate the data to train and test the network.

Integrating Inflammatory Biomarker Analysis and Artificial-Intelligence-Enabled Image-Based Profiling to Identify Drug Targets for Intestinal Fibrosis. Yu, S.; Kalinin, A. A.; Paraskevopoulou, M. D.; Maruggi, M.; Cheng, J.; Tang, J.; Icke, I.; Luo, Y.; Wei, Q.; Scheibe, D.; Hunter, J.; Singh, S.; Nguyen, D.; Carpenter, A. E.; Horman, S. R., Cell Chemical Biology **2023**, 30 (9), 1169-1182.e8. DOI: 10.1016/j.chembiol.2023.06.014.

The authors perform a chemogenomic library screen to study intestinal fibrotic activation using both cell painting captured with the Operetta CLS and disease-relevant biomarker. Harmony PhenoLOGIC machine learning was used to extract 860 features per cell from the cell painting images. The artificial intelligence enabled morphological profiling reveals information about tissue plasticity, remodelling, fibrosis, and angiogenesis signalling while the biochemical readouts are used to identify inflammatory regulators.

Multiplexed High-Throughput Immune Cell Imaging Reveals Molecular Health-Associated Phenotypes. Severin, Y.; Hale, B. D.; Mena, J.; Goslings, D.; Frey, B. M.; Snijder, B., Science Advances **2022**, 8 (44), eabn5631. DOI: 10.1126/sciadv.abn5631.

Eight distinct subtypes of human immune cells are found using multiplexed automated fluorescence on the Opera Phenix and convolutional neural networks. The subtypes are influenced by donor age, gender, and blood pressure while the T cell morphology can be linked to transcriptional state and age-associated loss of mitochondria.

Immunology

Application of Human iPSC-Derived Macrophages in a Miniaturized High-Content-Imaging-Based Efferocytosis Assay. Bitzer, S.; Harati, M. D.; El Kasmi, K. C.; Schloesser, D.; Sauer, J.; Olbrich, H.; Schuler, M.; Gantner, F.; Heilker, R., *SLAS Discovery* **2023**, 28 (4), 149-162. DOI: 10.1016/j.slasd.2023.04.002.

Induced pluripotent stem cell-derived macrophages (IDM) are presented as a tool to overcome limited availability and doner variation to facilitate disease modelling and drug discovery. The IDMs are shown to resemble monocyte-derived macrophages in both their surface marker expression and phago-/efferocytosis rate. The Opera Phenix allows the live cell tracking of phago-/efferocytosis events by prey cells and to quantify their rates using Columbus image analysis.

Green and Roasted Coffee Extracts Inhibit Interferon-β Release in LPS-Stimulated Human Macrophages. Artusa, V.; Ciaramelli, C.; Gotri, N.; Bruno, A.; Costa, B.; Palmioli, A.; Airoldi, C.; Peri, F., *Frontiers in Pharmacology* **2022**, 13. DOI: 10.3389/fphar.2022.806010.

Coffee extracts cause an anti-inflammatory immunomodulation by decreasing the release of type I interferons and cytokines. Intercellularly, coffee extracts diminish the nuclear translocation of p-IRF-3, the main transcription factor for interferon-beta synthesis. The cellular location of p-IRF-3 was images with the Operetta CLS using PhenoVue reagents and quantified in Harmony.

New Glucosamine-Based TLR4 Agonists: Design, Synthesis, Mechanism of Action, and *In Vivo* Activity as Vaccine Adjuvants. Romerio, A.; Gotri, N.; Franco, A. R.; Artusa, V.; Shaik, M. M.; Pasco, S. T.; Atxabal, U.; Matamoros-Recio, A.; Mínguez-Toral, M.; Zalamea, J. D.; Franconetti, A.; Abrescia, N. G. A.; Jimenez-Barbero, J.; Anguita, J.; Martín-Santamaría, S.; Peri, F., *J. Med. Chem.* **2023**, 66 (4), 3010-3029. DOI: 10.1021/acs.jmedchem.2c01998.

A series of TLR4 Agonists was synthesized as vaccine adjuvants and their *in vitro* and *in vivo* properties were quantified. The Operetta CLS and Phenovue reagents were used to monitor immune responses in macrophages. The rate of nuclear translocation of NF-kappaB p65 and p-IRF-3 was quantified in Harmony.

Infectious disease

Pharmacological Modulators of Epithelial Immunity Uncovered by Synthetic Genetic Tracing of SARS-CoV-2 Infection Responses. Jiang, B.; Schmitt, M. J.; Rand, U.; Company, C.; Dramaretska, Y.; Grossmann, M.; Serresi, M.; Čičin-Šain, L.; Gargiulo, G., *Science Advances* **2023**. DOI: 10.1126/sciadv.adf4975.

The authors reverse-engineered reporter cells to study SARS-CoV2 pathways using live cell image based phenotypic studies to identify modulators of the cellular antiviral response and DNA damage inducers. The Operetta CLS and Harmony software were used to quantify fluorescent signal in reporter cell lines to identify pharmacological modulators.

ATG7 and ATG14 Restrict Cytosolic and Phagosomal *Mycobacterium Tuberculosis* Replication in Human Macrophages. Aylan, B.; Bernard, E. M.; Pellegrino, E.; Botella, L.; Fearns, A.; Athanasiadi, N.; Bussi, C.; Santucci, P.; Gutierrez, M. G., *Nat Microbiol* **2023**, 8 (5), 803–818. DOI: 10.1038/s41564-023-01335-9.

Autophagy in macrophages is part of the innate-immune defence against microorganisms, including *Mycobactrium tuberculosis* (Mtb). To study the genetic implication of two autophagy-related knockouts, high-content live cell imaging with the Opera Phenix and image analysis in Harmony were used to quantify subcellular bacterial replication and cell death. It was found that the two proteins play an important role in different stages of the infection control by macrophages.

High-Content Imaging as a Tool to Quantify and Characterize Malaria Parasites. Rosenthal, M. R.; Ng, C. L., *Cell Reports Methods* 2023, 3, 100516. DOI: 10.1016/j.crmeth.2023.100516.

Imaging with the Operetta CLS for phenotypic properties of single cells, machine learning, and automated classification and clustering are combined to enable screening of novel antimalaria drugs. Red blood cells infected with *Plasmodium falciparum* are automatically identified and the parasite number, developmental stage, and schizont nuclei are quantified. A Harmony PhenoLOGIC classifier was trained to identify six distinct subpopulations: single rings, multiple rings, single trophozoites, multiple trophozoites, single schizonts, and multiple schizonts.

Metabolic disease

Human Loss-of-Function Variants in the Serotonin 2C Receptor Associated with Obesity and Maladaptive Behavior. He, Y.; Brouwers, B.; Liu, H.; Liu, H.; Lawler, K.; Mendes de Oliveira, E.; Lee, D.-K.; Yang, Y.; Cox, A. R.; Keogh, J. M.; Henning, E.; Bounds, R.; Perdikari, A.; Ayinampudi, V.; Wang, C.; Yu, M.; Tu, L.; Zhang, N.; Yin, N.; Han, J.; Scarcelli, N. A.; Yan, Z.; Conde, K. M.; Potts, C.; Bean, J. C.; Wang, M.; Hartig, S. M.; Liao, L.; Xu, J.; Barroso, I.; Mokrosinski, J.; Xu, Y.; Sadaf Farooqi, I., *Nat Med* **2022**, 28 (12), 2537–2546. DOI: 10.1038/s41591-022-02106-5.

Drugs altering serotonin levels are both prescribed for the treatment of obesity and neuropsychiatric disorders with frequent adverse effects caused by a lack of receptor specificity. The authors identify 13 rare mutations in the serotonin 2C receptor in 19 out of 2,548 obese and 1,117 control individuals using exome sequencing. The Opera Phenix and the Harmony software were used to further quantify the effect of these mutations onto the subcellular location of the receptor in transfected human cells. Cells were automatically plated, treated, and stained for high resolution synapse detection using HCS.

SLC35D3 Promotes White Adipose Tissue Browning to Ameliorate Obesity by NOTCH Signaling. Wang, H.; Yu, L.; Wang, J.; Zhang, Y.; Xu, M.; Lv, C.; Cui, B.; Yuan, M.; Zhang, Y.; Yan, Y.; Hui, R.; Wang, Y., Nat Commun **2023**, 14 (1), 7643. DOI: 10.1038/s41467-023-43418-5.

Knockout of the *Slc35d3* (solute carrier family 35, D3) gene in mice increases susceptibility to obesity and impaired glucose tolerance, while a knock-in is indicated to promote browning of white adipose tissue (a metabolically favourable state). The authors found that the regulation is achieved via NOTCH1 signalling. Using the Opera Phenix to study the intracellular location of NOTCH1 in live cells, shows that SLC35D3 overexpression significantly increase ER accumulation.

Neuroscience

Mitochondrial Dysfunction and Mitophagy Defects in LRRK2-R1441C Parkinson's Disease Models. Williamson, M. G.; Madureira, M.; McGuinness, W.; Heon-Roberts, R.; Mock, E. D.; Naidoo, K.; Cramb, K. M. L.; Caiazza, M.-C.; Malpartida, A. B.; Lavelle, M.; Savory, K.; Humble, S. W.; Patterson, R.; Davis, J. B.; Connor-Robson, N.; Ryan, B. J.; Wade-Martins, R., *Human Molecular Genetics* **2023**, 32 (18), 2808–2821. DOI: 10.1093/hmg/ddad102.

The authors use the Opera Phenix to study mitochondrial membrane function, morphology, and mitophagy in a common genetic cause of Parkinson's disease in patient-derived iPSC dopamine neuronal cultures.

Higher Throughput Workflow with Sensitive, Reliable and Automatic Quantification of Myelination *In Vitro* Suitable for Drug Screening. Seiler, S.; Wälti, C. M.; Barros, V. de; Barbash, S.; Foo, L. C., *Scientific Reports* **2023**, 13 (2883). DOI: 10.1038/s41598-023-29333-1.

Demyelination is commonly occurring in multiple sclerosis; the authors developed a miniaturized co-culture system with compact myelination and a machine vision algorithm to accurately quantify myelination at a single cell level to allow for remyelination compound screening. The Operetta CLS/Opera Phenix were used to monitor cell differentiation and myelination status. Neurites were segmented and characterized using the Harmony software.

Lysophosphatidylcholine Acyltransferase 1 Promotes Pathology and Toxicity in Two Distinct Cell-Based Alpha-Synuclein Models. Nicholatos, J. W.; Tran, D.; Liu, Y.; Hirst, W. D.; Weihofen, A., *Neuroscience Letters* **2022**, 772, 136491. DOI: 10.1016/j.neulet.2022.136491.

The authors show Lysophosphatidylcholine Acyltransferase 1 as a regulator for alpha-synuclein aggregation and degradation using the Opera Phenix and Columbus image analysis to study alpha-synuclein aggregate intensity and quantity in live cells.

Neuroscience *continued...*

A High-Content Neuron Imaging Assay Demonstrates Inhibition of Prion Disease-Associated Neurotoxicity by an Anti-Prion Protein Antibody. Reilly, M.; Benilova, I.; Khalili-Shirazi, A.; Schmidt, C.; Ahmed, P.; Yip, D.; Jat, P. S.; Collinge, J., *Sci Rep* **2022**, 12 (1), 9493. DOI: 10.1038/s41598-022-13455-z.

To study prion-associated neurotoxicity and the protective properties of an anti-prion protein mouse monoclonal antibody ICSM18, this study uses the Opera Phenix for imaging and the Harmony software for detection of healthy neurons, neurite segmentation, and to identify spinophillin-positive dendritic spines. The colocalization of pre- and post-synaptic markers in differentiated primary hippocampal neurons coupled with neurite length is used to establish maturation of the differentiated cells. ICSM18 prevents RML prion-induced neuro- and synaptotoxicity that usually causes branch degeneration, loss of neuron root density, neurite shortening, and increase of dendric spines.

High-Content Synaptic Phenotyping in Human Cellular Models Reveals a Role for BET Proteins in Synapse Assembly. Berryer, M. H.; Rizki, G.; Nathanson, A.; Klein, J. A.; Trendafilova, D.; Susco, S. G.; Lam, D.; Messana, A.; Holton, K. M.; Karhohs, K. W.; Cimini, B. A.; Pfaff, K.; Carpenter, A. E.; Rubin, L. L.; Barrett, L. E., *eLife* **2023**, 12, e80168. DOI: 10.7554/eLife.80168.

This study presents an automated, quantitative high content synaptic phenotyping platform with the Opera Phenix allowing the study of human synaptic development. The effect of small molecules on presynaptic density, neurite outgrowth, and cell viability can be screened using human neurons and astrocytes.

Mutant Huntingtin Confers Cell-Autonomous Phenotypes on Huntington's Disease iPSC-Derived Microglia. Stöberl, N.; Donaldson, J.; Binda, C. S.; McAllister, B.; Hall-Roberts, H.; Jones, L.; Massey, T. H.; Allen, N. D., *Sci Rep* **2023**, 13 (1), 20477. DOI: 10.1038/s41598-023-46852-z.

A mutation in the huntingtin gene *(HTT)* could be linked to cell autonomous disfunction in microglia as part of pathological changes in Huntington's disease. Images collected with the Opera Phenix were scored for cell roundness in Harmony to show microglia activation in absence of immune stimulation which correlated with elevated pro-inflammatory cytokine production. Further, live cell imaging with the Opera Phenix revealed impaired phagocytosis and endocytosis capacity which might indicate that mutations in *HTT* contribute to insufficient clearance of dying neurons and in turn lead to additional microglia activation.

Human iPSC-Derived Astrocytes Generated from Donors with Globoid Cell Leukodystrophy Display Phenotypes Associated with Disease. Lieberman, R.; Cortes, L. K.; Gao, G.; Park, H.; Wang, B.; Jones, P. L.; Hunter, R. B.; Leonard, J. P.; Barker, R. H., PLOS ONE **2022**, 17 (8). DOI: 10.1371/journal.pone.0271360.

Globoid cell leukodystrophy (Krabbe disease) astrocytes were generated from iPSC derived from donors with infantile onset of the disease. These cells display pathological changes in lipid biosynthesis and promote microglia survival while negatively impacting neurons in co-culture as monitored with the Opera Phenix and image analysis in Harmony to quantify cell viability, differentiation marker, and cell distribution in co-cultures.

A *TREM2*-Activating Antibody with a Blood–Brain Barrier Transport Vehicle Enhances Microglial Metabolism in Alzheimer's Disease Models. van Lengerich, B.; Zhan, L.; Xia, D.; Chan, D.; Joy, D.; Park, J. I.; Tatarakis, D.; Calvert, M.; Hummel, S.; Lianoglou, S.; Pizzo, M. E.; Prorok, R.; Thomsen, E.; Bartos, L. M.; Beumers, P.; Capell, A.; Davis, S. S.; de Weerd, L.; Dugas, J. C.; Duque, J.; Earr, T.; Gadkar, K.; Giese, T.; Gill, A.; Gnörich, J.; Ha, C.; Kannuswamy, M.; Kim, D. J.; Kunte, S. T.; Kunze, L. H.; Lac, D.; Lechtenberg, K.; Leung, A. W.-S.; Liang, C.-C.; Lopez, I.; McQuade, P.; Modi, A.; Torres, V. O.; Nguyen, H. N.; Pesämaa, I.; Propson, N.; Reich, M.; Robles-Colmenares, Y.; Schlepckow, K.; Slemann, L.; Solanoy, H.; Suh, J. H.; Thorne, R. G.; Vieira, C.; Wind-Mark, K.; Xiong, K.; Zuchero, Y. J. Y.; Diaz, D.; Dennis, M. S.; Huang, F.; Scearce-Levie, K.; Watts, R. J.; Haass, C.; Lewcock, J. W.; Di Paolo, G.; Brendel, M.; Sanchez, P. E.; Monroe, K. M., *Nat Neurosci* **2023**, 26 (3), 416–429. DOI: 10.1038/s41593-022-01240-0.

TREM2 is a key gene in microglia that modulates the risk of late-onset Alzheimer's disease. Antibody transfer vehicles (ATV) facilitate blood-brain barrier transcytosis of a high-affinity human TREM2-activting antibody that improves microglia proliferation and mitochondria metabolism *in vitro* and boost brain microglia activity and glucose metabolism in an *in vivo* mouse Alzheimer's disease model. The Opera Phenix and Harmony software were used to quantify the TREM2 receptor and ATV:TREM2 trafficking endosomal signalling as well as microglia proliferation and lipid storage.

Physiological cell models

Dynamic Matrices with DNA-Encoded Viscoelasticity for Cell and Organoid Culture. Peng, Y.-H.; Hsiao, S. K.; Gupta, K.; Ruland, A.; Auernhammer, G. K.; Maitz, M. F.; Boye, S.; Lattner, J.; Gerri, C.; Honigmann, A.; Werner, C.; Krieg, E., Nature Nanotechnology **2023**. DOI: 10.1038/s41565-023-01483-3.

The authors generated dynamic DNA-crosslinked matrices with systematic tunability of viscoelastic, thermodynamic, and kinetic properties for single cell and 3D organoid culture. The Opera Phenix was used to visualize the dispersion of live cells and organoids throughout bioprinted structures and characterize cell differentiation in 3D.

Performance Assessment and Economic Analysis of a Human Liver-Chip for Predictive Toxicology. Ewart, L.; Apostolou, A.; Briggs, S. A.; Carman, C. V.; Chaff, J. T.; Heng, A. R.; Jadalannagari, S.; Janardhanan, J.; Jang, K.-J.; Joshipura, S. R.; Kadam, M. M.; Kanellias, M.; Kujala, V. J.; Kulkarni, G.; Le, C. Y.; Lucchesi, C.; Manatakis, D. V.; Maniar, K. K.; Quinn, M. E.; Ravan, J. S.; Rizos, A. C.; Sauld, J. F. K.; Sliz, J. D.; Tien-Street, W.; Trinidad, D. R.; Velez, J.; Wendell, M.; Irrechukwu, O.; Mahalingaiah, P. K.; Ingber, D. E.; Scannell, J. W.; Levner, D., *Commun Med* **2022**, 2 (1), 1–16. DOI: 10.1038/s43856-022-00209-1.

Organ-on-a-Chip liver models offer the potential to predict drug-induced liver injuries earlier in the drug discovery pipeline. For this purpose, liver structure and function were replicated on a microfluidic chip using the Opera Phenix and Harmony image analysis to quantify the expression of cellular genetic markers and membrane markers in live mitochondria, as well as to track drug induced apoptosis. Imaging with the Opera Phenix was performed in the bottom and top channel of whole chips with live or fixed cells.

An in Silico Model of T Cell Infiltration Dynamics Based on an Advanced in Vitro System to Enhance Preclinical Decision Making in Cancer Immunotherapy. Lewin, T. D.; Avignon, B.; Tovaglieri, A.; Cabon, L.; Gjorevski, N.; Hutchinson, L. G., *Frontiers in Pharmacology* **2022**, 13, 837261. DOI: 10.3389/fphar.2022.837261.

To study the infiltration of cancer-targeted T cells in a microphysiological organ-on-a-chip model, the authors developed a mathematical model describing the spatiotemporal dynamics in response to chemotactic cytokine signalling. Live cell imaging with the Opera Phenix provides dynamic data of T cell infiltration on the chip and targeted apoptosis to the model for optimization of key parameters.

A Scalable 3D High-Content Imaging Protocol for Measuring a Drug Induced DNA Damage Response Using Immunofluorescent Subnuclear γH2AX Spots in Patient Derived Ovarian Cancer Organoids. Keles, H.; Schofield, C. A.; Rannikmae, H.; Edwards, E. E.; Mohamet, L., *ACS Pharmacol. Transl. Sci.* **2023**, 6 (1), 12–21. DOI: 10.1021/acsptsci.2c00200.

Defective DNA damage detection and repair is one hallmark of cancer cells as for example in ovarian cancer. To better understand the mode of action of drugs and genetic perturbagens, the authors developed a HCS 3D imaging assay with the Opera Phenix and Harmony software using subnuclear staining and organoid clearing. The Opera Phenix was also used to monitor organoid formation and growth.

3D Microtissues Mimic the Architecture, Estradiol Synthesis, and Gap Junction Intercellular Communication of the Avascular Granulosa. Ip, B. C.; Leary, E.; Knorlein, B.; Reich, D.; Van, V.; Manning, J.; Morgan, J. R., *Toxicol Sci* **2021**, 186 (1), 29–42. DOI: 10.1093/toxsci/kfab153.

3D microtissues of human granulosa cells, the functional unit of ovarian follicles, present in vitro functionality similar to mature tissues such as gap junction intracellular communication. Combining live cell imaging on the Opera Phenix, the quantification of single cell volume within spheroids in Harmony, and automated data analysis, these microtissues offer a novel in vitro drug screening platform to identify modulators of follicular function.

High Throughput 3D Gel-Based Neural Organotypic Model for Cellular Assays Using Fluorescence Biosensors. Kundu, S.; Boutin, M. E.; Strong, C. E.; Voss, T.; Ferrer, M., *Communications Biology* **2022**, 5 (1236). DOI: 10.1038/s42003-022-04177-z.

This study presents 3D fibrin-gel based dopaminergic or glutamatergic neuron and astrocyte co-cultures with fluorescence biosensors for optogenetic activation that allow real-time functional HCS of intracellular calcium signaling and dopamine and glutamate levels using the Opera Phenix.

3D Human Renal Proximal Tubule (RPTEC-TERT1) Organoids 'Tubuloids' for Translatable Evaluation of Nephrotoxins in High-Throughput. Yucha, S. E. V.; Quackenbush, D.; Chu, T.; Lo, F.; Sutherland, J. J.; Kuzu, G.; Roberts, C.; Luna, F.; Barnes, S. W.; Walker, J.; Kuss, P., *PLOS ONE* **2022**, 17 (11), e0277937. DOI: 10.1371/journal.pone.0277937.

Kidney cells grown in physiological relevant 3D morphology, characterized with the Opera Pehnix, show a high degree of similarity to native kidney tissue compared to 2D monolayer cultures. These highly reproducible 'tubuloids' show increased sensitivity to nephrotoxins and demonstrate altered Na+/K+-ATPase signal intensity as could be utilized in high-throughput nephrotoxin assays.

Bisphenol A Impairs Renal Function by Reducing Na+/K+-ATPase and F-Actin Expression, Kidney Tubule Formation *in Vitro* and *in Vivo*. Yoo, M. H.; Lee, S.-J.; Kim, W.; Kim, Y.; Kim, Y.-B.; Moon, K.; Lee, B.-S., *Ecotoxicology and Environmental Safety* **2022**, 246. DOI: 10.1016/j.ecoenv.2022.114141.

Bisphenol A commonly found in household products interferes with kidney straight tubule formation by lowering filamentous actin formation and reducing Na+/K+-ATPase expression even at the current no-observed-adverse-effect-level (NOAEL)-dose. The Operetta CLS was used to characterize actin filament formation in 2D and 3D and to quantify expression levels of relevant markers. F-actin spots and holes were quantified with texture analysis in Harmony.

Other research areas

Pharmacological Perturbation of the Phase-Separating Protein SMNDC1. Enders, L.; Siklos, M.; Borggräfe, J.; Gaussmann, S.; Koren, A.; Malik, M.; Tomek, T.; Schuster, M.; Reiniš, J.; Hahn, E.; Rukavina, A.; Reicher, A.; Casteels, T.; Bock, C.; Winter, G. E.; Hannich, J. T.; Sattler, M.; Kubicek, S., *Nature Communications* **2023**, 14, 4504. DOI: 10.1038/s41467-023-40124-0.

Survival motor neuron domain-containing protein 1 (SMNDC1) is involved in the assembly of the spliceosome in the endocrine pancreas and appears in sub-nuclear Cajal bodies and nuclear speckles. The Opera Phenix was used to study live cell intracellular liquid-liquid phase separation of SMNDC1 in the nucleus and to evaluate the effect of pharmacological perturbations of its Tudor domain.

High-Content Phenotypic Screening Identifies Novel Chemistries That Disrupt Mosquito Activity and Development. Murgia, M. V.; Sharan, S.; Kaur, J.; Austin, W.; Hagen, L.; Wu, L.; Chen, L.; Scott, J. A.; Flaherty, D. P.; Scharf, M. E.; Watts, V. J.; Hill, C. A., *Pesticide Biochemistry and Physiology* **2022**, 182, 105037. DOI: 10.1016/j.pestbp.2022.105037.

A screening platform to discover novel vector insecticides was developed using the Opera Phenix to monitor mosquito larvae activity and phenotypes. Individual larvae were automatically identified in Harmony for image analysis and scored for their phenotypes. Overall mortality was inferred from larvae movement in live imaging.

Optimized High-Content Imaging Screening Quantifying Micronuclei Formation in Polymer-Treated HaCaT Keratinocytes. Saadati, F.; da Silva Brito, W. A.; Emmert, S.; Bekeschus, S., *Nanomaterials* **2022**, 12 (24), 4463. DOI: 10.3390/nano12244463.

A computer-driven, unsupervised quantitative image analysis algorithm was developed in Harmony based on images collected with the Operetta CLS to identify the frequency of micronuclei as a measurement for toxicity and genotoxicity in cell models according to OECD guidelines for analysis. Using this method, the acute and chronic effects of nano- and micro-plastic particles were quantified in non-malignant keratinocytes.

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