High-Throughput Single-Cell Metabolomics for Mechanism-Resolved Toxicology

Shawn Owens[1], Jeany Delafiori[1], Katja Ovchinnikova[1], Theodore Alexandrov[12]

[1]DeepCyte, Inc. [2]Department of Pharmacology, University of California San Diego

Standard in vitro toxicology assays fall short in diverse ways. Cytotoxicity readouts like ATP-Glo detect cell death but miss the sub-lethal metabolic disruptions that precede it. Morphological profiling methods such as Cell Painting capture late phenotypic changes after toxicity is already established, without molecular-level mechanistic information. Targeted mechanistic assays, mitochondrial function, BSEP inhibition, each cover only one endpoint, require a prior hypothesis, and offer no discovery capability across the broader metabolic space. None of these approaches provide the early, hypothesis-free, mechanism-resolved readout that drug safety decisions increasingly demand, particularly as regulatory agencies push toward mechanistic New Approach Methodologies (NAMs) to replace animal models.

MetaCore addresses this gap. Built on HT SpaceM (Delafiori et al., Cell 2025), it combines MALDI imaging mass spectrometry with computational methods to profile the metabolic state of individual cells after compound exposure, mapping perturbation to mechanism to risk at single-cell resolution. The platform detects >100 small-molecule metabolites per dataset across amino acid, nucleotide, carbohydrate, and fatty acid classes, with 20–80 metabolites measured per individual cell. It processes 3,000–5,000 cells per hour and screens 50+ conditions per day, requiring only ~2,500 seeded cells per replicate, a 400-fold reduction versus bulk LC-MS/MS. MetaCore has been validated across cancer, immune, and hepatic cell lines, primary immune cells, and organoid models.

To evaluate MetaCore against standard toxicology readouts, we conducted a benchmarking study in Jurkat T cells, running MetaCore alongside ATP-Glo viability across dose-response panels of compounds spanning genotoxic, oxidative, mitochondrial, and metabolic mechanisms. MetaCore detected metabolic perturbations at sub-cytotoxic

concentrations, produced mechanism-specific signatures, and distinguished between compounds that viability assays scored identically. A second tier compared MetaCore against a functional immunosuppression readout, IL-2 suppression following T-cell activation, to assess whether metabolic profiling adds mechanistic context beyond what functional assays capture.

These capabilities position MetaCore as a screening-compatible, NAM-aligned platform for mechanism-resolved toxicology across diverse cell types. By generating high-throughput, single-cell-resolution mechanistic data, MetaCore provides the foundation for large-scale predictive toxicology, including the immune-specific applications addressed by our DeeTox program.