MEHTRICS aims to challenge the current limits of High-Throughput (HT) cell-based screening by combining HT-RNAi with an emerging new technology for normalizing cultured cell behaviors, namely micropatterns. The current CYTOO micropattern technology is optimized for short-term studies, i.e., within 1 cell cycle. To study longer-lasting biological processes, such as RNAi mediated silencing which typically requires at least 2-4 days for efficient knock-down, new micropattern networks are designed and characterized for their ability to normalize cell behavior over several cell cycles. To improve and predict the designs of these networks, mathematical models that recapitulate cell organization on extended micropattern networks are developed. Since experimental designs required to run RNAi screens are among the most demanding of all HTMC studies and encompass virtually all technical challenges also encountered in compound screens, we expect the proposed scope of activities to deliver the maximal potential for impactful innovation, widespread adoption and clear relevance for all major applications of HT/HCC cell screening. The resulting new tools and methodologies are now incorporated into the respective commercial offerings of CYTOO and CENIX.

**DESIGN OF MICROPATTERN NETWORKS USING COMPUTER ASSISTED MODELING**

**CELL BEHAVIOUR CHARACTERISATION ON MICROPATTERN NETWORKS**

**BENEFITS OF MICROPATTERN NETWORKS FOR siRNA APPLICATIONS**

**CENIX O-MPI™ Assay Overview**

**Networks Benefits on O-MPI™ assay**

**Networks Benefits on O-MPI™ assay**

**The Oncology MultiPlex™ (O-MPI™), monitors cellular proliferation plus key underlying processes, necrosis, apoptosis, and cell cycle progression within the same experimental run.**

**The Oncology MultiPlex™ test**

**Figure 8: Micropattern networks improve the O-MPI™ assay window in various cancer cell lines: cell proliferation (evaluated by nucleic segmentation), apoptosis (Ann A staining), exotoxin (EdU staining), other phenotypes (multiple nuclear morphology parameters), siRNA efficiency.** 6 different networks geometries were tested. Benefits on assay sensitivity are summarized as high (Green), good (Light Green), no benefit (White), and less sensitive (Red). Independent results showed that Micropattern Networks improve the sensitivity of more than 40% of endpoints.

**Figure 9: Example of micropattern networks effects on siRNA mediated knockdown. Three siRNAs with varying potency (A), (B), (C) were tested in MCF7 breast adenocarcinoma cell line. Colours represent 6 different types of micropattern network designs. All data were normalized to samples treated with negative control siRNA (100%). As an example, siRNA efficiency can be increased up to 20% - 70% compared to classical 2D cell culture.**