MEDICAL COLLEGE OF WISCONSIN
CANCER CENTER

Cell Therapy Shared Resource Instrumentation

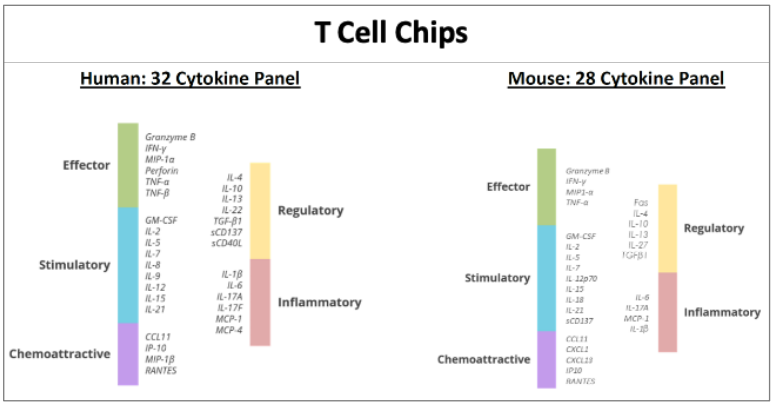
The Cell Therapy Shared Resource (CTSR) provides state-of-the-art production and processing of cell therapy products, innovative on-site CAR-T cell manufacturing and processing, and immuno-oncology consulting services, access to instrumentation for immune profiling and monitoring, and educational workshops. Descriptions of instrumentation are shown below. However, the lab also provides a Promega Glomax Multi Detection System plate reader for performing standard ELISAs.

Isoplexis

This chip-based platform analyzes approximately 2000 single cells (T cell chip) for the production of 32 human cytokines or 28 mouse cytokines and effector molecules including effector, stimulatory, chemo-attractive, regulatory and inflammatory mediators. The analysis process generates a 'polyfunctional stimulation index' (PSI), defined as the percentage of polyfunctional single cells (i.e., secreting two or more proteins) in a sample, multiplied by the average signal intensity of the secreted proteins in individual functional groups (effector, stimulatory, chemo-attractive, regulatory, inflammatory) from each cell.

High frequencies of polyfunctional T cells have been previously associated with cancer vaccine-induced efficacy in murine cancer models (Aurisicchio et al., 2019). Notably, it has recently been shown that the PSI of pre-infusion CD19 chimeric antigen receptor (CAR)-engineered T-cells is associated with post-infusion clinical response in non-Hodgkin lymphoma patients (Rossi et al., 2018), highlighting the power of the PSI analysis.

Briefly, for CAR T-cell assays total CD3 cells or separate CD4 and CD8 subsets, are stimulated in culture overnight with target antigen-expressing cells (e.g., K562-CD19, K562-CD20, Raji (CD19/20+)), and then loaded onto the chips. The chips are then read for cytokine production over a period of 20-24 additional hours. In addition to generating PSI values,



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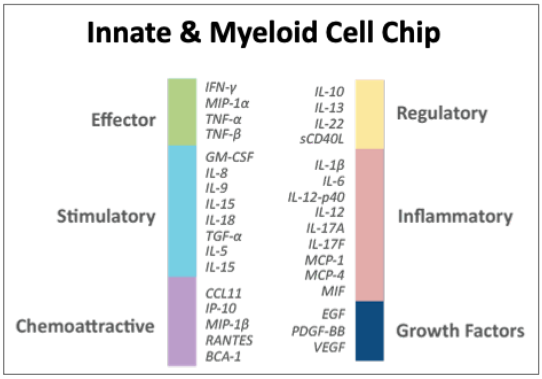
another advantage of the Isoplexis system is its ability to provide information on the secretion of each individual cytokine at the single cell level. Isoplexis also has a 'Single-Cell Innate Immune' cell chip (see figures), and several other chips are in development (see updates and protocols at Isoplexis.com).



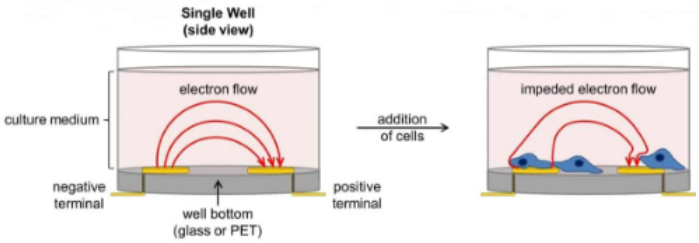
Luminex Flexmap 3D

The FLEXMAP 3D® system combines differentially dyed fluorescent microsphere sets with an innovative instrument design to enable precise, flexible, rapid multiplexing of up to 500 unique assays within a single sample. This instrument can take a 96 or 384-well plate format. More than 40 analytes can be examined in a single sample using a maximum of 25 ul. Several analyte panels are available or custom panels can be designed, and the instrument has 4.5 logs dynamic range.

xCelligence RTCA 6-Plate System

This instrument facilitates measurement of immune cell cytotoxicity or cell death in a 96-well format (maximum of 6 plates at one time). The system is label free, real-time, and assays can be performed over several hours to days.

Target cells need to either be plastic adherent or can be tethered to the well surface using plate-bound antibodies against a cell-surface antigen present on the target cells. The system is based on electrical impedance. Adhesion of cells to gold microelectrodes in each well impedes the flow of electric current between electrodes (see figure at right). This impedance value, plotted as a unitless parameter called "Cell Index," increases as cells proliferate and then plateaus as cells approach 100% confluence. If immune effector cells or a drug induces the destruction of the target adherent cells, the corresponding cytolytic activity can be sensitively and precisely detected. The continuous acquisition of impedance data for each well enables the generation of real-time killing curves for multiple conditions simultaneously.



The system contains an integrated software package that includes a powerful immunotherapy module for running and analyzing real time cell analysis data.

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Bio-Rad droplet digital PCR (ddPCR) System

This instrumentation enables precise, highly sensitive quantification of nucleic acids. It builds on traditional PCR amplification and fluorescent-probe-based detection methods to provide highly sensitive absolute quantification of nucleic acids without the need for standard curves.

The system contains 3 pieces of equipment: 1) droplet generator, 2) standard PCR thermocycler, and 3) droplet reader. The PCR sample is partitioned into 20,000 droplets. After amplification, droplets containing target sequence are detected by fluorescence and scored as positive, and droplets without fluorescence are scored as negative. Poisson statistical analysis of the numbers of positive and negative droplets yields absolute quantitation of the target sequence. The process allows higher precision than traditional PCR and qPCR methods. The CTSR is utilizing this system to assess lentiviral vector copy number in final CAR T-cell products and to track in vivo CAR T-cell persistence.

MACSQuant 10 & 16 Flow Cytometers

These are 10/16-parameter, 8/14-color flow cytometry analyzers manufactured by Miltenyi Biotec. The CTSR is using these instruments for immunophenotyping of CAR T-cell products and patient biospecimens following CAR T-cell infusion.

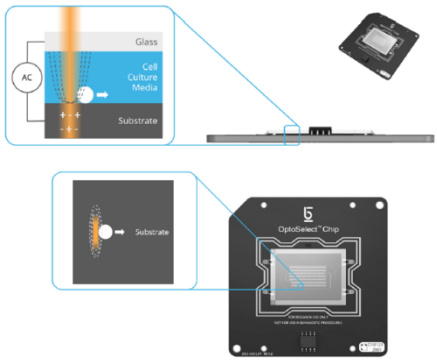
Users can design their own flow cytometry panels or use a CAR T Cell Express Mode Package that contains several multi-color flow panels. Express Modes are a unique add-on for the MACSQuantify Software, developed to simplify flow cytometric analysis. They automate the measurement and analysis of flow experiments via predefined experiment settings as well as acquisition and automated analysis.

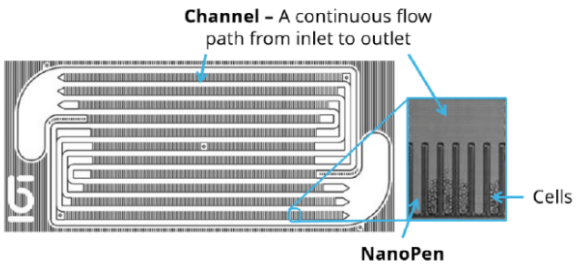
A gating of flow cytometry results will be automatically adjusted for each data file individually to achieve optimal results. Using the Express Modes simplifies flow cytometric analysis and ensures reproducible data analysis.

Berkeley Lights Lightning Opto-fluidics Platform

This novel instrumentation allows users to visualize the phenotype and function of individual cells on a microchip that contains 1,500 nano-chambers. Optical tweezers manipulate cells and place them in selected nano chambers. Depending on cell size, the chambers can hold up to 30 cells each.

There are workflows that allow users to assess single cells for both cytokine production and cell killing capacity. A major strength of the system is that users can recover the cells of interest for further characterization. In addition, users can upload any single nano chamber on the chip for export into a single well for downstream analysis (genomic analysis, additional functional analyses, etc.). The system has a large number of capabilities and workflows, including a Cell Therapy Development workflow, and the company is continuing to develop new ones.





Equipment information is quoted from the manufacturers' websites.

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