Background
Autologous T cells that express engineered antigen receptors (CAR-T cells) represent a promising new cancer therapy tool. The evaluation of quality, specificity, and killing efficiency (potency) of CAR-T cell populations is crucial for the development of potent and safe patient-specific CAR-T cell therapies.

In contrast to classical T cell potency assays (e.g., Chromium-51), live cell imaging allows to analyze T cell/cancer cell interaction in real time with single cell resolution. However, analysis of confluent cell layers is very time-consuming and therefore not possible in high throughput screens. Additionally, variations in cell size and density require fluorescent labeling for automated T cell and cancer cell registration, which might alter the cell behavior.

To facilitate high throughput label-free analysis of T cell potency in a live cell imaging setup, we generated arrays of homogenously distributed single cancer cells or spheroids. By combining optical analysis and advanced image processing, we were able to evaluate cytotoxic T cell activity over time on a single cell level, without the use of any labeling. Additionally, using matrix-embedded 3D arrays, physiological T cell migration conditions could be mimicked.

Micro patterning on the Bioinert Surface

**The Bioinert Principle**
- Thin polyol hydrogel layer, covalently bound to the ibidi Polymer Coverglass #1.5

**Features**
- Biologically inert—no cell or protein adhesion
- Long-term stable
- Ready-to-use
- Highest optical quality for imaging

**Functionalization**
- Specific cell adhesion/tethering for weeks.
- Unspecific cell and molecule immobilization
- Custom-specific adhesion via click chemistry

**Optics**
- Very low autofluorescence
- No visibility of μ-Patterns in brightfield
- Optional μ-Pattern fluorescence

Defined Cancer Cell Arrays on a Micropattern

Using the micropatterning technology, target cells (cancer cells) are immobilized (adhered/tethered) on pads offering either homogenous single cell, or multi cell/cell aggregate distribution.

In contrast to a confluent cell layer, this allows for detailed optical analysis on a single cell level.

T Cell Potency Assays in 2D and 3D

In order to observe T cell/cancer cell interaction, T cells can either be applied in solution (2D assay), or embedded in a more physiological biological 3D matrix (e.g., collagen).

High Throughput T Cell Killing Efficiency Analysis on a Single Cancer Cell Array

Arrays of single cancer cells were obtained by seeding RCC-26 cancer cells on small adhesive micropads (red). Probabilities were highest for capturing single cells on each adhesion structure (Graph).

Conclusion
- We analyze T cell/cancer cell interaction in high throughput using artificial intelligence-based cell recognition (“occupied position” approach).
- We use micropatterning of adhesion ligands to immobilize adherent cancer cells in a single cell or multi-cell manner.
- We observe T cell/cancer cell interaction (2D and physiologically relevant 3D environment) using live cell imaging without the necessity of fluorescent markers.
- We analyze T cell/cancer cell interaction in high throughput using artificial intelligence-based cell recognition (“occupied position” approach).

Outlook
- We aim at applying the micropatterning approach to soluble cancer cells (e.g., B cells via tethering antibodies).
- We aim at analyzing T cell/cancer cell interaction in detail (“T cell tracking”) using higher magnification objectives.
- ACAS (MetaVi Labs and ibidi) Chemotaxis analysis can be used “on top” of cell recognition in order to identify “secondary T cell effects”.

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