

Claims:

1. A method of assaying for antigen-specific cytotoxicity of a T lymphocyte (T cell) in a microfluidic device, the method comprising:

disposing the T cell within the microfluidic device; wherein the microfluidic device comprises a flow region for containing a flow of a first fluidic medium and a chamber opening to the flow region;

disposing a target cell in proximity to the T cell; ~~wherein the microfluidic device comprises a flow region for containing a flow of a first fluidic medium and a chamber opening to the flow region and~~

determining a viability of the target cell after a period of exposure in proximity to the T cell;

wherein determining a viability of the target cell comprises:

receiving a first image of a region of interest having the target cell and receiving a second image of the region of interest having the target cell;

identifying a boundary of the target cell in the second image by aligning the second image with the first image;

detecting a signal located within the boundary of the target cell in the second image; and

determining the viability of the target cell based on the signal located within the boundary.
2. The method of claim 1, wherein the target cell expresses an antigen for which the T cell is specific.
3. The method of claim 1 or 2, wherein assaying is a co-assay and further comprises: disposing a first capture object in proximity to the T lymphocyte, wherein the capture micro-object is configured to capture a first secreted biomolecule released from the T cell; and detecting the first secreted biomolecule captured to the first capture object.
4. The method of claim 3, wherein the first secreted biomolecule is a protein.
5. The method of claim 3 or 4, wherein the first secreted protein released from the antigen-specific T cell is a cytokine.
6. The method of claim 5, wherein the cytokine is Tumor Necrosis Factor alpha (TNF alpha), Transforming Growth Factor beta (TGF beta), Interferon gamma (IFN gamma), Interleukin-1 beta (IL1 beta), Interleukin-2 (IL2), Interleukin-4 (IL4), Interleukin-5 (IL5),

Interleukin -6 (IL6), Interleukin -10 (IL10), Interleukin-12 (IL12), Interleukin-13 (IL13), Interleukin-17A (IL17A), or Interleukin-22 (IL22).

7. The method of any one of claims 1 to 6, wherein the target cell is a cancer cell.
8. The method of claim 7, wherein the target cell expresses an antigen associated with melanoma, breast cancer, or lung cancer.
9. The method of any one of claims 1 to 8, wherein the T cell is a mammalian T cell.
10. The method of any one of claims 1 to 9, wherein the T cell is antigen-specific for a tumor associated antigen and/or expresses a chimeric antigen receptor.
11. The method of any one of claims 1 to 10, wherein the T cell does not express a chimeric antigen receptor.
- ~~12. The method of any one of claims 1 to 11, wherein the chamber comprises a sequestration pen comprising an isolation region for containing a second fluidic medium and a connection region fluidically connecting the isolation region to the flow region; and further wherein the isolation region comprises a single opening and is an unswept region of the microfluidic device.~~
- ~~13~~12. The method of any one of claims 1 to ~~12~~11, wherein the T cell and the target cell are each disposed in the chamber.
13. The method of any one of claims 1 to 12, wherein the chamber comprises a sequestration pen comprising an isolation region for containing a second fluidic medium and a connection region fluidically connecting the isolation region to the flow region; and further wherein the isolation region comprises a single opening and is an unswept region of the microfluidic device.
14. The method of ~~claim 12 or~~ claim 13, wherein the T cell, ~~the first capture object and the~~ target cell are each disposed in the isolation region of the sequestration pen.
15. The method of any one of claims 1 to 14, wherein determining the viability of the target cell comprises contacting the target cell with a detectable marker configured to label a non-viable cell, and detecting a signal located within the boundary of the target cell in the second image comprises detecting a signal associated with the detectable marker.
16. The method of claim 15, wherein the detectable marker configured to label a non-viable cell is configured to label apoptotic cells.
17. The method of claim 15, wherein the detectable marker configured to label a non-viable cell is configured to label calcium flux or mitochondrial membrane potential.

18. The method of any one of claims 1 to 17, wherein determining the viability of the target cell after the period of exposure to the T cell is repeated over a plurality of periods of exposure to the T cell.
19. The method of any one of claims 1 to 18, further comprising labelling the T cell for the presence of one or more cell surface markers associated with proliferation, activation, metabolic activity, memory, exhaustion, and/or lineage.
- ~~20. — A kit for assaying antigen-specific cytotoxicity by a T lymphocyte (T cell) in a microfluidic device, the kit comprising: a microfluidic device comprising a flow region for containing a flow of a first fluidic medium and a chamber opening to the flow region; and a cytotoxicity detection reagent configured to detect viability of a target cell.~~
- ~~21. — The kit of claim 20, wherein the cytotoxicity detection reagent comprises a reagent configured to label an apoptotic cell.~~
- ~~22. — The kit of claim 20, wherein the cytotoxicity detection reagent comprises a reagent configured to detect calcium flux or mitochondrial membrane potential.~~
- ~~23. — The kit of any one of claims 20 to 22, further comprising a first capture object configured to capture a first secreted biomolecule of a T cell.~~
- ~~24. — The kit of any one of claims 20 to 23, wherein the chamber comprises a sequestration pen, wherein the sequestration pen comprises an isolation region for containing a second fluidic medium and a connection region fluidically connecting the isolation region to the flow region; and further wherein the isolation region comprises a single opening and is an unswept region of the microfluidic device.~~

20. The method of any one of claims 1 to 19, wherein

the first image is an illuminated image, and the second image is a non-illuminated image;

or

the first image is a non-illuminated image, and the second image is an illuminated image
or a non-illuminated image.

21. The method of any one of claims 1 to 20, wherein receiving the second image of the region of interest comprises receiving more than one second images, wherein at least one of the more than one second images is a fluorescent image.

22. The method of claim 21, wherein each fluorescent image represents a fluorescent signal from a unique portion of the visible light spectrum.

23. The method of claim 21 or 22, wherein each fluorescent image represents fluorescent signal from a non-overlapping portion of the visible light spectrum.

24. The method of any one of claims 1 to 23, further comprising pre-processing the first image and the second image to reduce anomalies in the image data, and wherein pre-processing comprises reducing noise and/or optical distortion introduced during generation of the first image and the second image.

25. The method of any one of claims 1 to 24, wherein determining a viability of the target cell further comprises processing pixel data in the first image using a machine learning algorithm and detecting the target cell present in the region of interest thereby identifying the boundary of the target cell.

26. The method of claim 25, wherein processing pixel data in the first image comprises using the machine learning algorithm to generate a plurality of pixel masks from the first image for a corresponding plurality of target cell characteristics, wherein each pixel mask comprises a set of pixel annotations, each pixel annotation of the set representing a probability that a corresponding pixel in the image represents the corresponding target cell characteristic.

27. The method of claim 26, wherein the corresponding plurality of target cell characteristics comprises at least: (i) target cell center; (ii) target cell edge; and (iii) non-target cell, and wherein detecting the target cell is based upon the pixel mask corresponding to the target cell center characteristic or a combination of pixel masks that includes the pixel mask corresponding to the target cell center characteristic.

28. The method of any one of claims 1 to 27, wherein disposing the T cell within the microfluidic device comprising disposing a single T cell into the chamber; and wherein the microfluidic device further comprises a plurality of chambers, disposing the T cell within the microfluidic device comprises: disposing a plurality of T cells within the microfluidic device and disposing a single T cell of the plurality of T cells into corresponding chamber of the plurality of chambers.