Identifying and isolating single immune cells based on their function using an Xdrop® workflow

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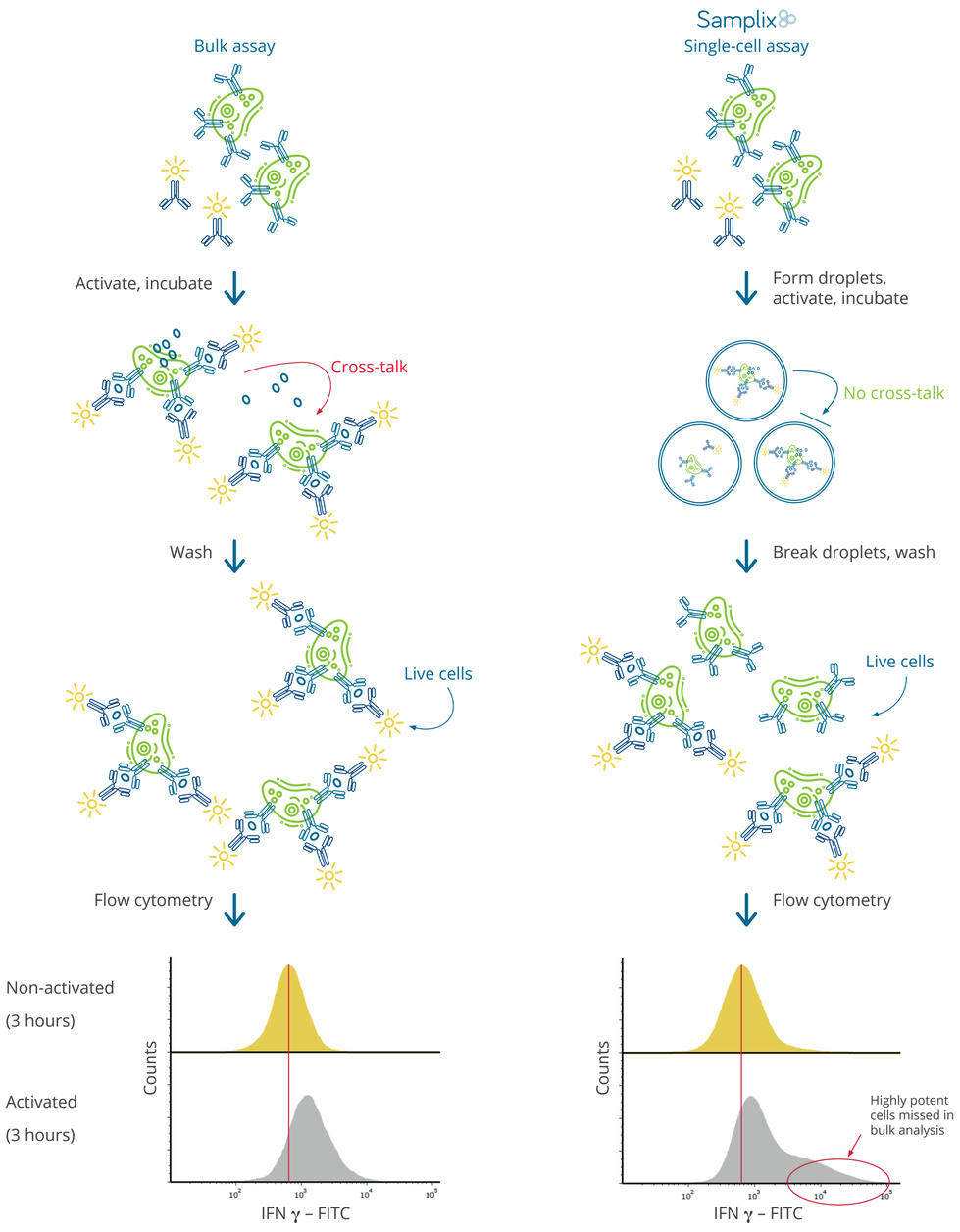
Testing cell functionality on bulk populations of cells cannot reveal heterogeneity in the cell population. It also precludes isolation of individual cells with the desired functionalities. Here, we show how Xdrop facilitates a single-cell cytokine secretion assay followed by the isolation of viable cells with high cytokine production, which we expand and reanalyze. We also show a cell–cell interaction assay where we identify the individual cytotoxic natural killer (NK) cells that can kill co-encapsulated target K562 cells.

These single-cell functional analyses are performed within double-emulsion droplets, which can support cell viability for days in standard CO_2 incubators. We can encapsulate and generate up to 750,000 single-cell assays per sample in ~8 minutes with 1 to 8 parallel sample encapsulations. Flow cytometry analysis is performed on the droplets containing single cells. It is possible to isolate cells in their droplets on a standard cell sorter. The droplets are highly robust, but cells can easily be recovered for further analysis or expansion.

Droplet-based cytokine secretion assay reveals highly potent cells missed in bulk assays

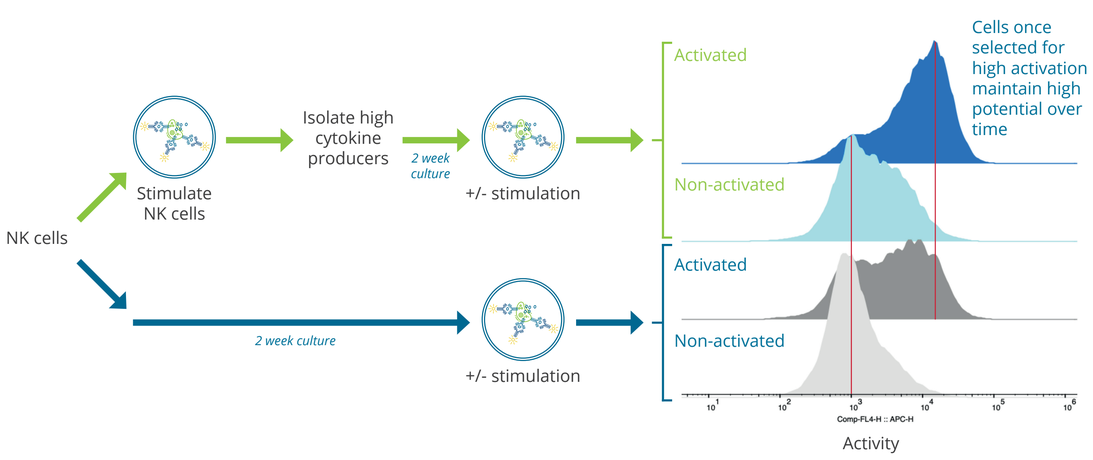
- Cell encapsulation in double-emulsion droplets prevents cross-talk.
- Cells with high and low cytokine secretion are easily discriminated.
- Retrieval of living cells with desired secretion activity is possible.

For the single-cell assay, NK cells were washed and labelled with INF-γ catch reagent according to the Miltenyi® assay protocol, resuspended in MEM α with 100 ng/ml IL-2 and FITC-IFN-γ antibody, and encapsulated. Non-activated cells received the same treatment without exposure to IL-2. Cells from both experiments were analyzed using flow cytometry.



Recovery and expansion of cells with specific cytokine profiles

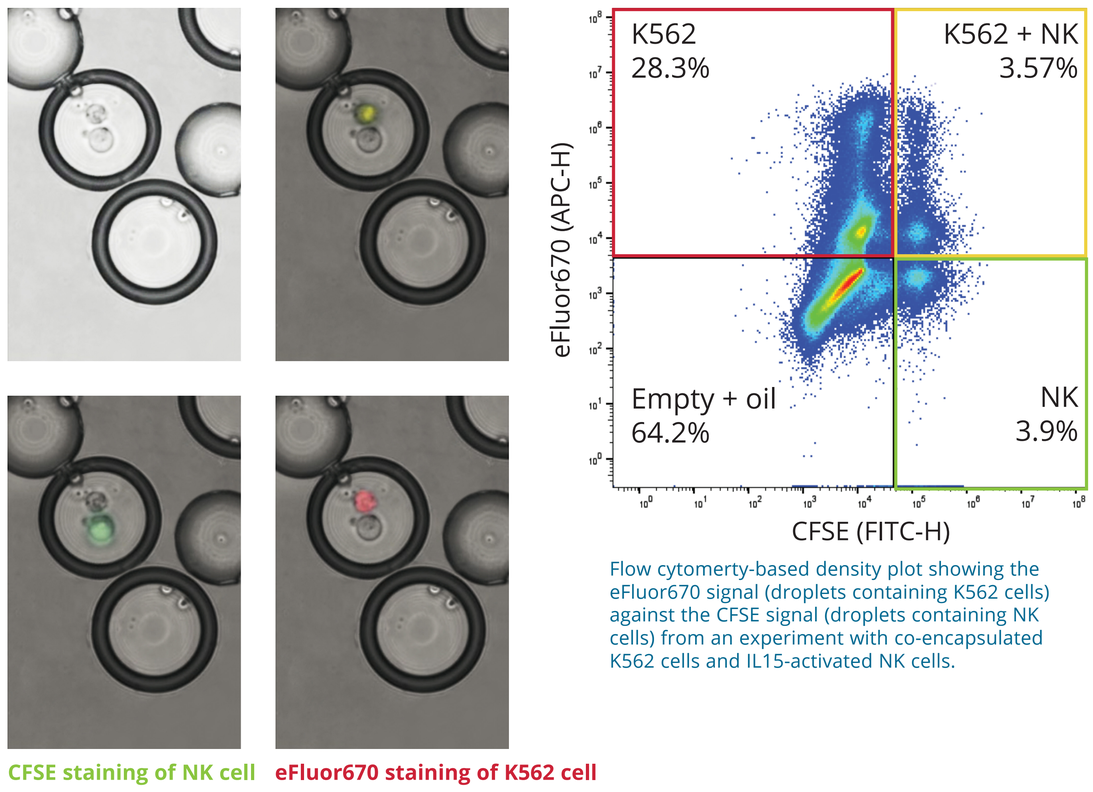
NK cells were labelled and stimulated with IL-2 as above followed by encapsulation with FITC-IFN-γ antibody and PI in MEM α. Cells with a high IFN-γ secretion profile were enriched via sorting on a standard cell sorter (SONY® SH800S) and cultured for 2 weeks. In parallel, non–stimulated cells were cultured in the same medium. Enriched and non-enriched cells were divided and stimulated or not followed by reanalysis of their IFN-γ secretion profile in droplets as described above.



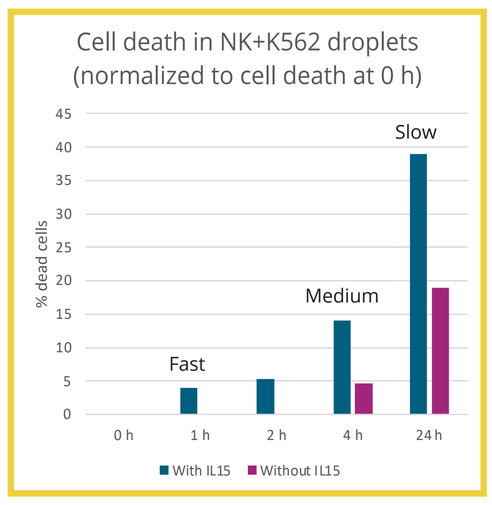
Droplet-based assay rapidly reveals the active killers

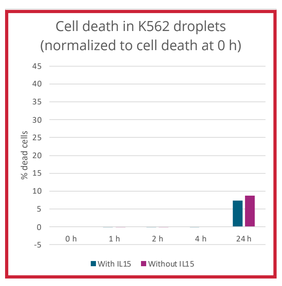
- Small droplet volume (~100 pl) means co-encapsulated cells remain close together and interact quickly, with results in a few hours.
- Cells with killing activity are easily discriminated from cells without this activity.
- Retrieval of living cells with killing capability is possible.
- The assay includes controls in the form of droplets containing only one cell type.

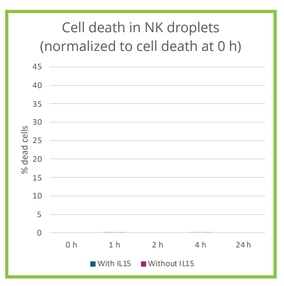
NK cells with or without activation using IL15 and lymphoblasts (K562 cells) were respectively stained with CFSE and eFluor670, and PI was added to both. Droplets were loaded with NK and K562 cells in a 1:3 ratio (NK:K562) using a Xdrop DE50 Cartridge. The droplets were incubated for 1, 2, 4 and 24 h at 37°C, 5% CO_2. Droplets were analyzed using flow cytometry. In the photos below, green indicates an NK cell, red indicates a K562 cell, and yellow shows a dead cell (in this case, a K562 cell). The flow cytomerty-based density plot shows the eFluor670 signal (droplets containing K562 cells) against the CFSE signal (droplets containing NK cells) from an experiment with IL15-activated NK cells. Based on the PI signal for each gate of the density plot, the percentage cell death for that droplet type (K562 cells alone, NK cells alone, K652 cells + NK cells) was calculated, normalized to the dead cells at 0 h.



Flow cytomerty-based density plot showing the eFluor670 signal (droplets containing K562 cells) against the CFSE signal (droplets containing NK cells) from an experiment with co-encapsulated K562 cells and IL15-activated NK cells.



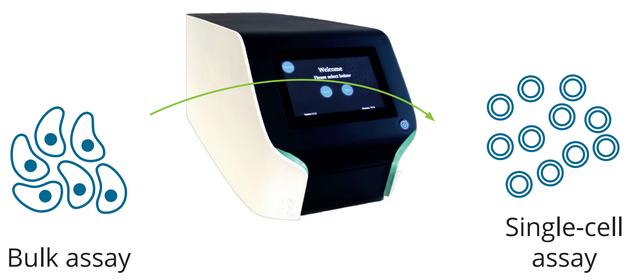




- 4% of the NK cells were identified as “fast killers” (killing the co-encapsulated K562 cell within 1 h)
- 14% were identified as “medium killers”
- 21% were identified as “slow killers”
- Activation with IL15 increased NK cell activity

Xdrop transforms bulk cell assays into single-cell assays.

- Accelerate assay times thanks to picoliter-volume reaction spaces that force faster cell–cell interactions and cell secretion buildup
- Process up to 8 samples in parallel
- Generate ~750,000 single-cell assays per sample in 8 minutes
- Incubate cells within droplets in a CO_2 incubator
- Analyze single cells in droplets on a flow cytometer
- Isolate droplets on a cell sorter
- Recover selected cells for expansion or end-point analyses



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