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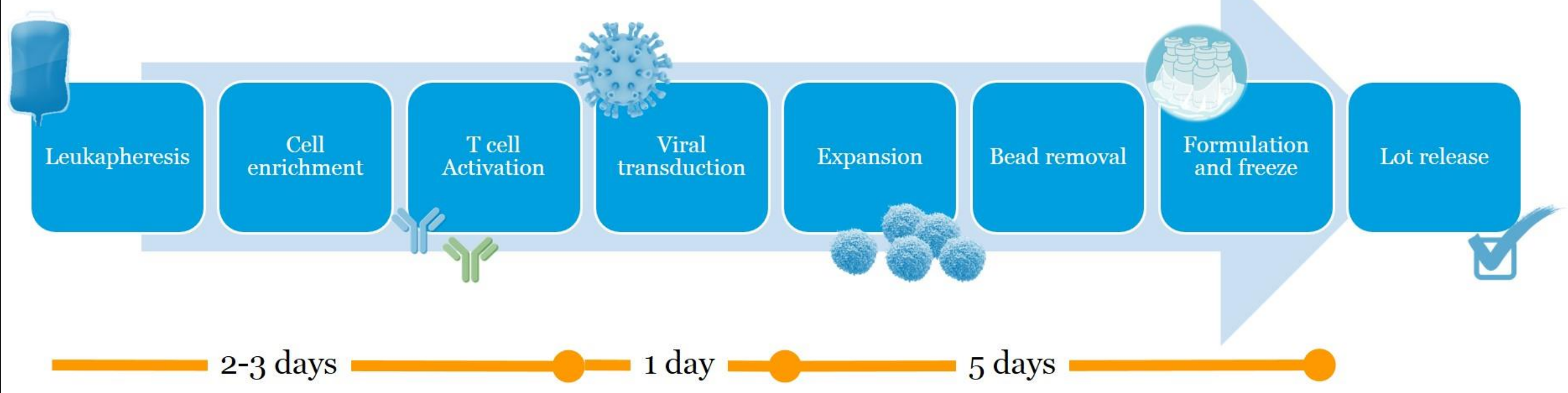
## Introduction:

T-cell immunotherapies show considerable promise in treating various challenging diseases such as blood cancers. These therapies utilise the body's immune system to destroy tumour cells by collecting a patient's own T-cells and modifying them to target cancer cells, by transferring DNA into the cells using viral vectors.

The vector transfer efficiency and its consistency are critical to the final (cell) product quality and cost.

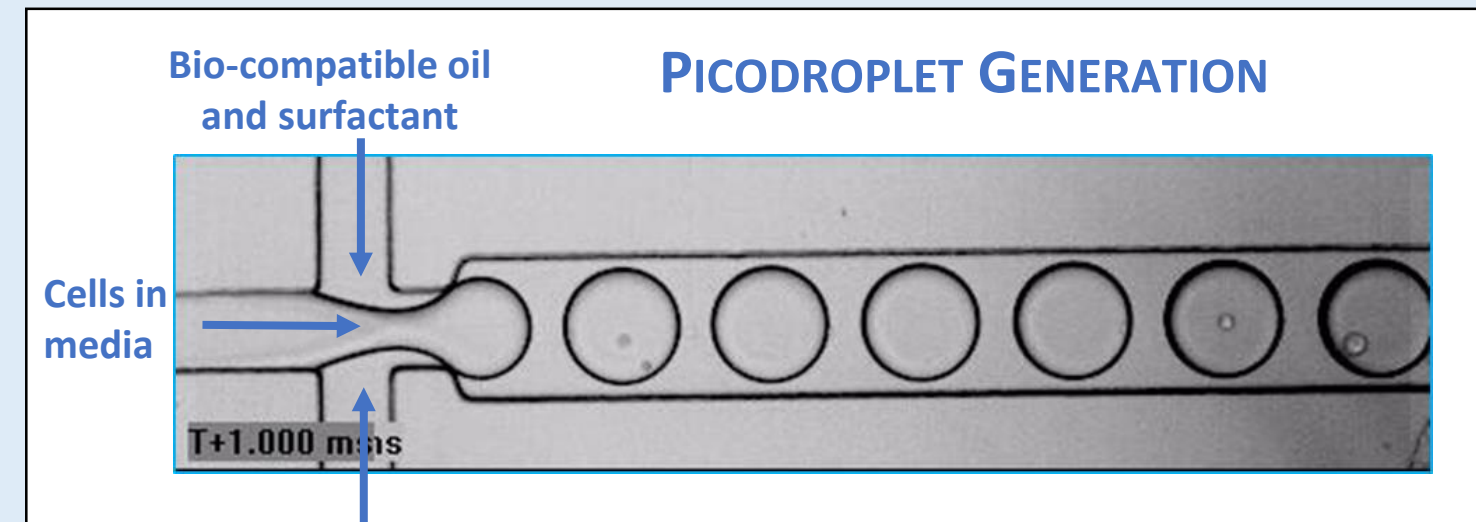
We have engineered new microfluidic-based technologies to improve the efficiency of transferring DNA into cells.

### Conventional T-Cell Therapy Manufacturing Workflow



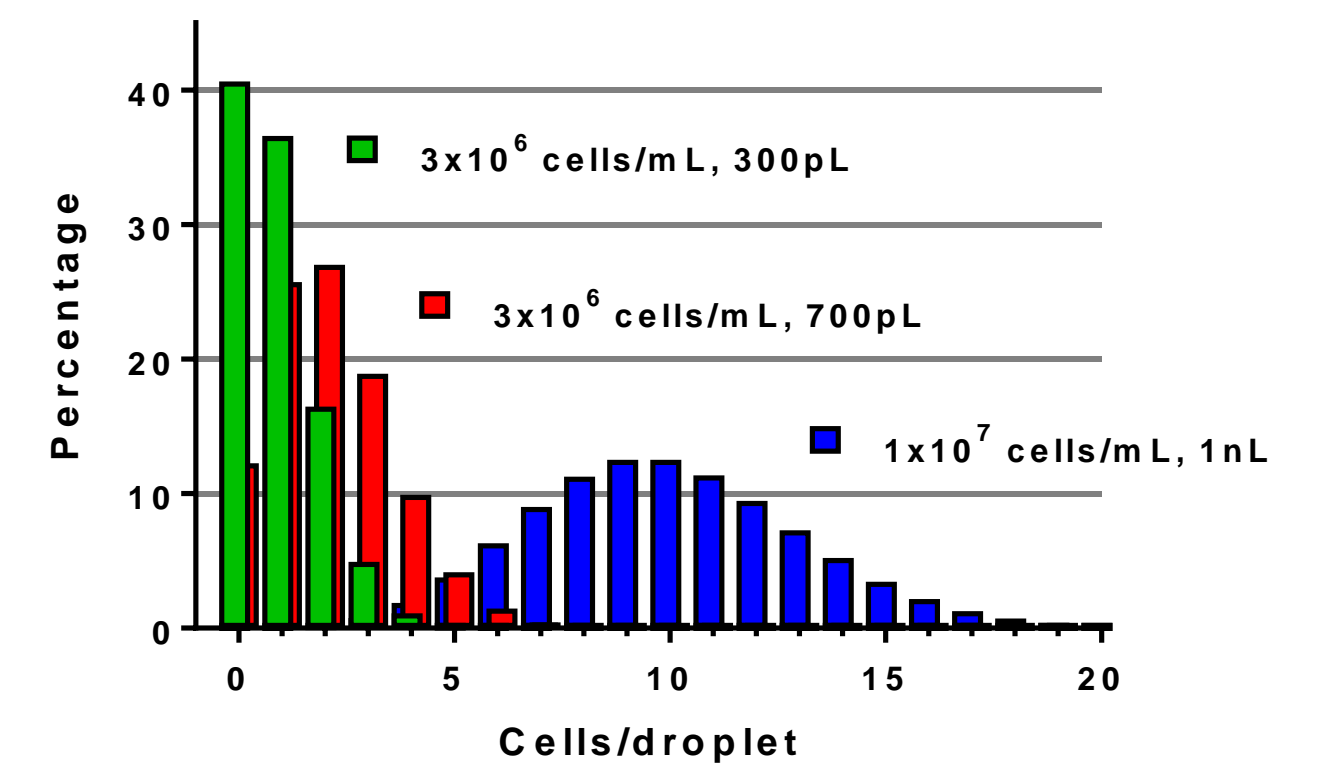
## Picodroplet technology:

The aim of this study was to evaluate the potential of Sphere Fluidics' picodroplet technology in a model workflow for cell therapy. Encapsulating cells and viral vectors in microfluidic picodroplets allows a high degree of control over cell and virus numbers for transduction. This potentially provides an advantage over conventional bulk transduction in terms of efficiency and viral copy integration number.



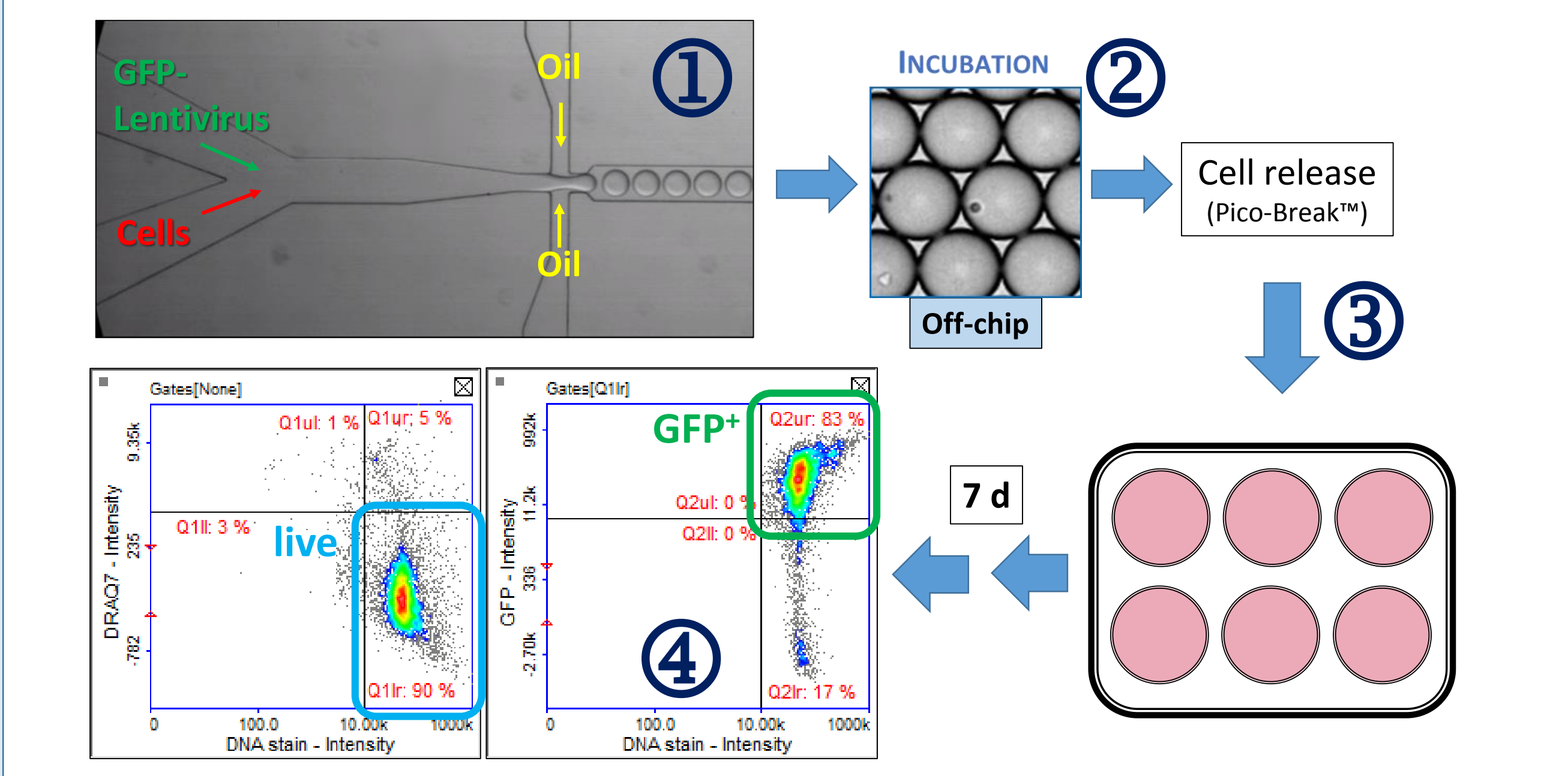
- Picodroplets are **uniform** aqueous droplets in oil.
- Picodroplets are very **small in size**, typically 300 pL, i.e. 5-6 orders of magnitude smaller than the standard liquid volumes in a micro titre plate.
- Picodroplets are generated very **rapidly** at rates of up to 100,000 per min.
- Water-in-oil picodroplets are mechanically and chemically **stable**, through the use of a novel surfactant allowing them to be incubated for extended periods of time without coalescence.
- All components are **bio-compatible** and gas permeable.

Cell distribution in different sized picodroplets



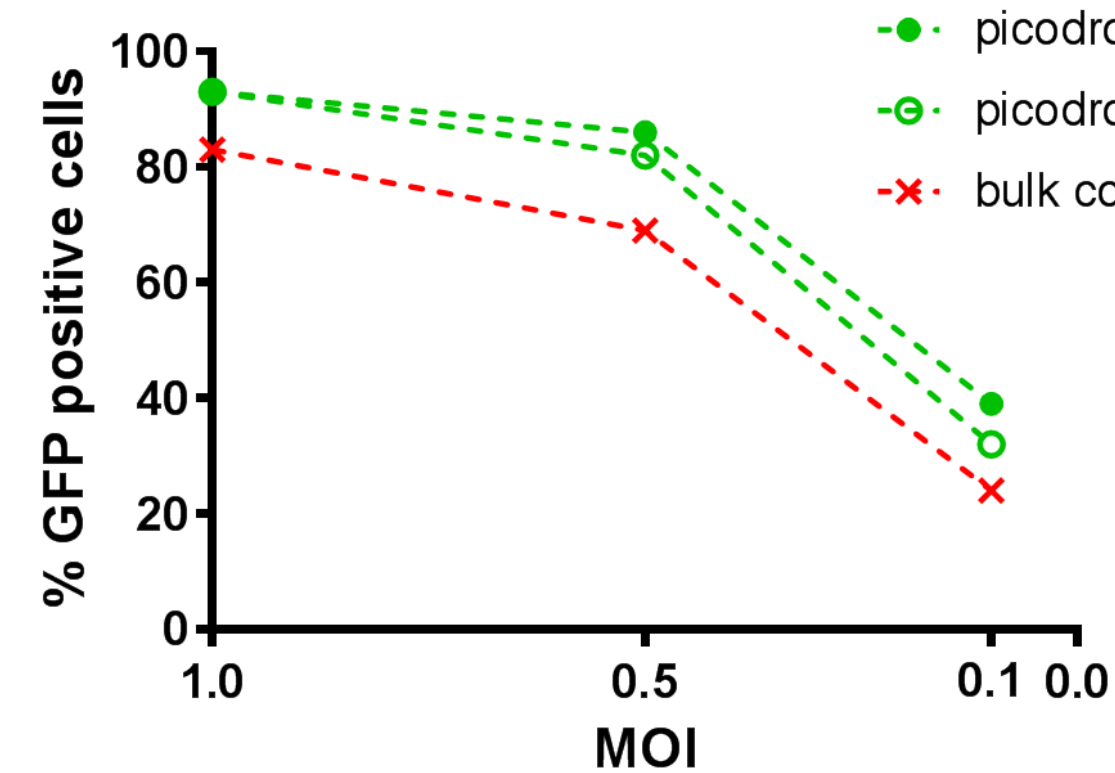
## Results 1: Jurkat cells are efficiently transduced in picodroplets

### Workflow: Lentiviral transduction in picodroplets



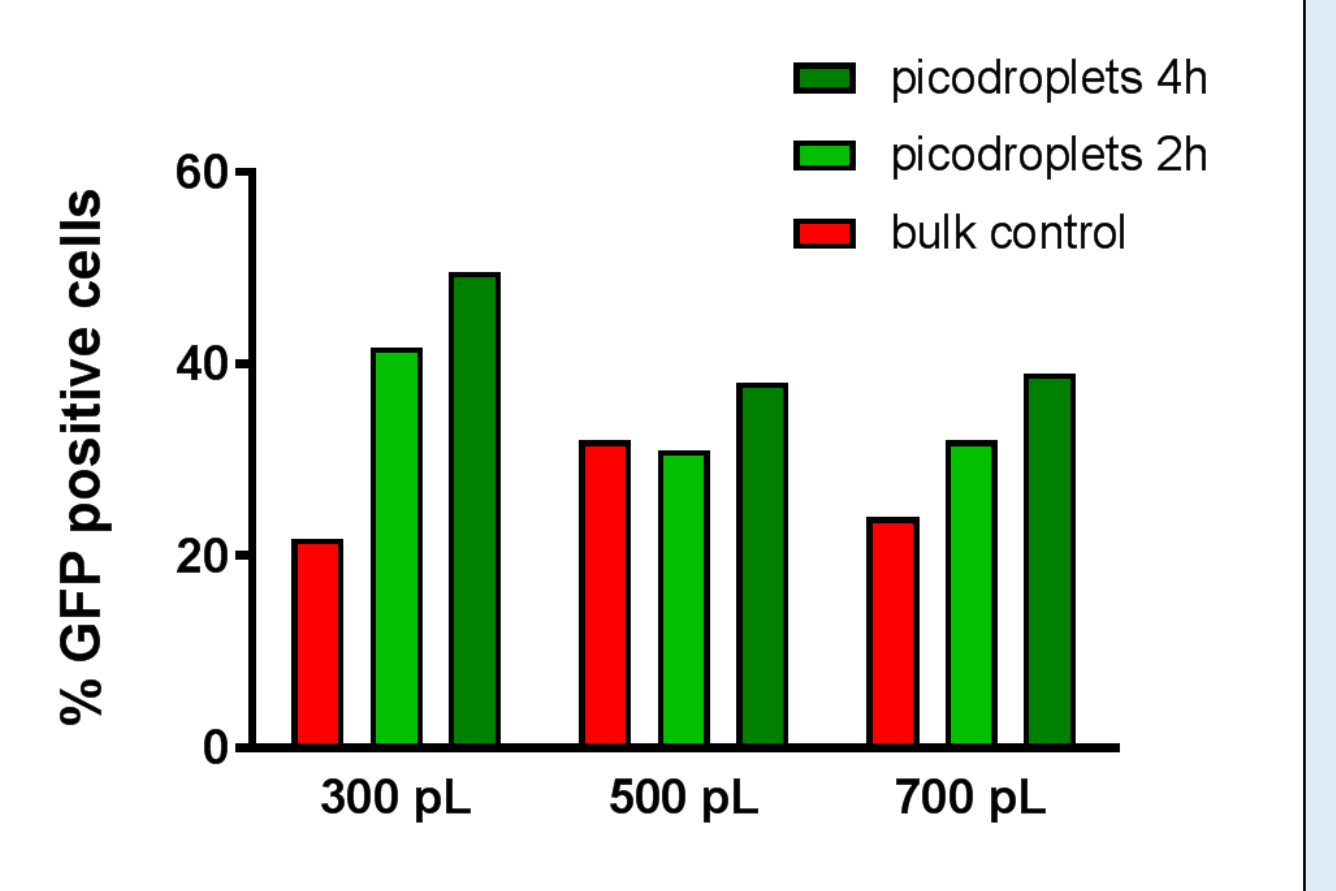
- Cells and a GFP-encoding lentivirus are encapsulated together in picodroplets.
- The resulting emulsion is collected and stored off-chip at 37°C for 2-4h, during which transduction occurs.
- Encapsulated cells are released from picodroplets and incubated in cell culture plates for 7 days.
- Transduction efficiency is assessed by determining GFP expression on a Nucleocounter® NC-3000™ Image Cytometer and compared to conventional transduction performed in bulk.

### Transduction efficiency vs MOI 3x10<sup>6</sup> cells/mL, 700 pL picodroplets



Transduction efficiency was assessed at decreasing virus concentration (MOI) while keeping picodroplet size constant. Transduction efficiency in picodroplets was higher than controls at all MOIs tested. Longer incubation in picodroplets resulted in increased transduction efficiencies. At low MOIs the beneficial effect of picodroplets was more pronounced.

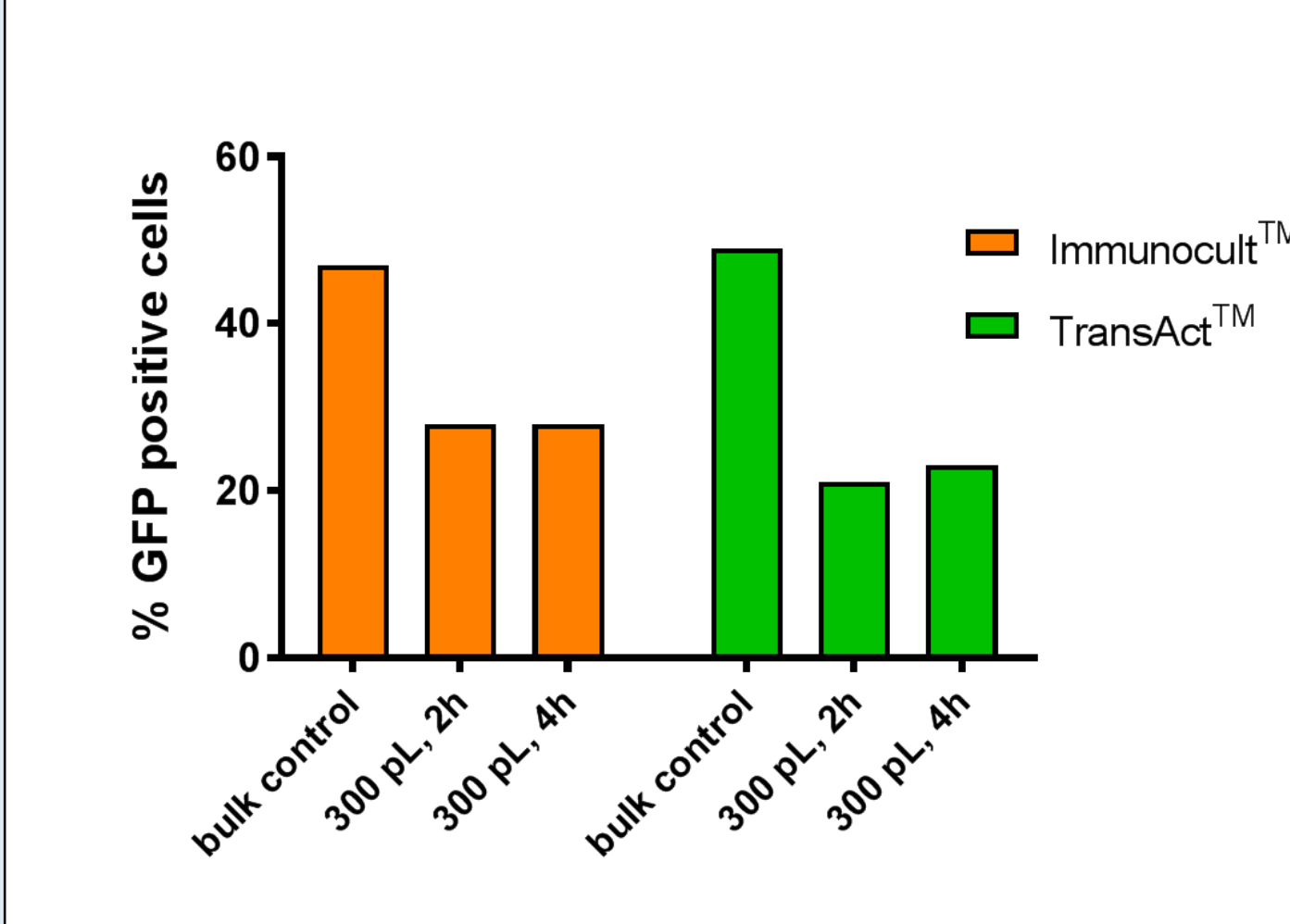
### Effect of picodroplet volume at MOI 0.1



The effect of picodroplet size was investigated at a constant MOI of 0.1. At the lowest picodroplet volume tested (300 pL), stimulation of transduction was strongest (>2-fold), probably due to a closer confinement of cells and viral particles inside the picodroplet.

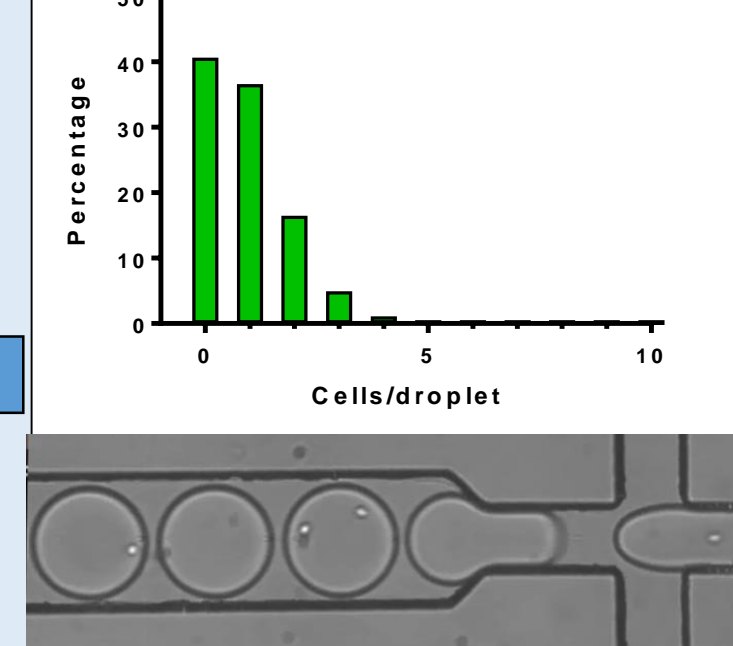
## Results 2: Human T-cells require small cell populations for efficient transduction in picodroplets

### T-Cell Transduction Efficiency in 300 pL picodroplets, MOI=1, 3x10<sup>6</sup> cells/mL

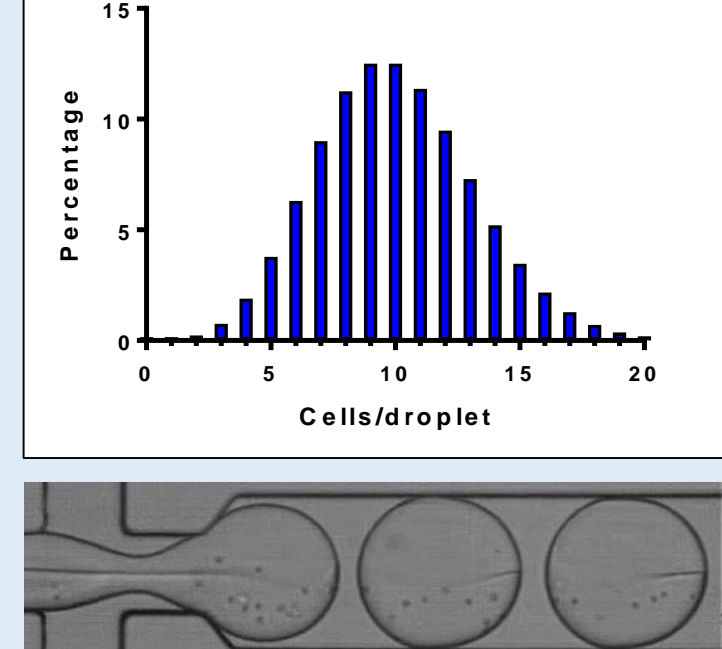


Transduction efficiency in human T-cells was assessed under conditions optimised for Jurkat cells. Two different T-cell activation reagents (Immunocult™ and TransAct™) were tested and co-encapsulated with cells and virus. Unlike with Jurkat cells, we observed considerably lower transduction efficiencies in picodroplets, possibly caused by inefficient T-cell activation in the single cell scenario.

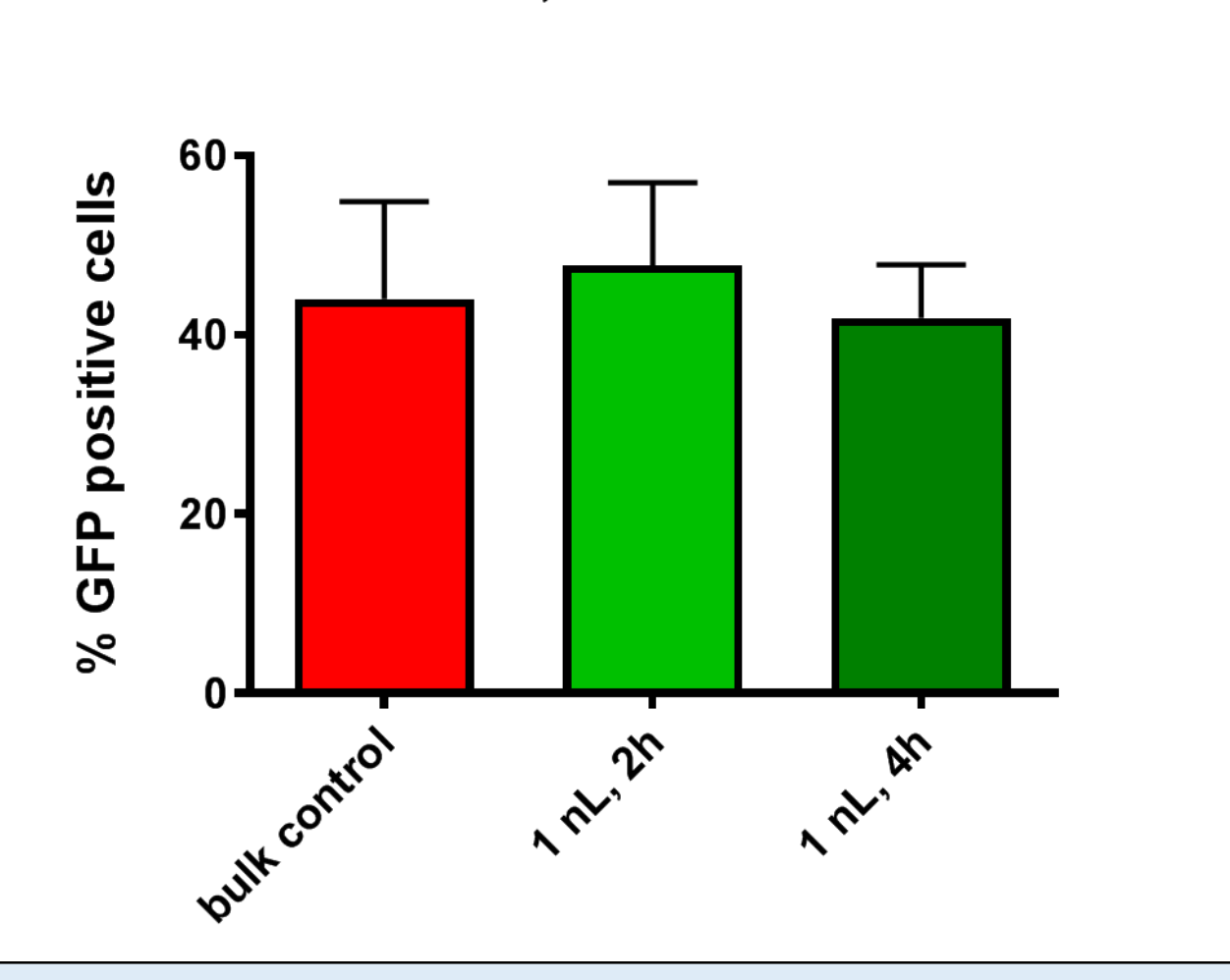
### Cell distribution in 300 pL droplets at 3x10<sup>6</sup> cells/mL



### Cell distribution in 1 nL droplets at 1x10<sup>7</sup> cells/mL

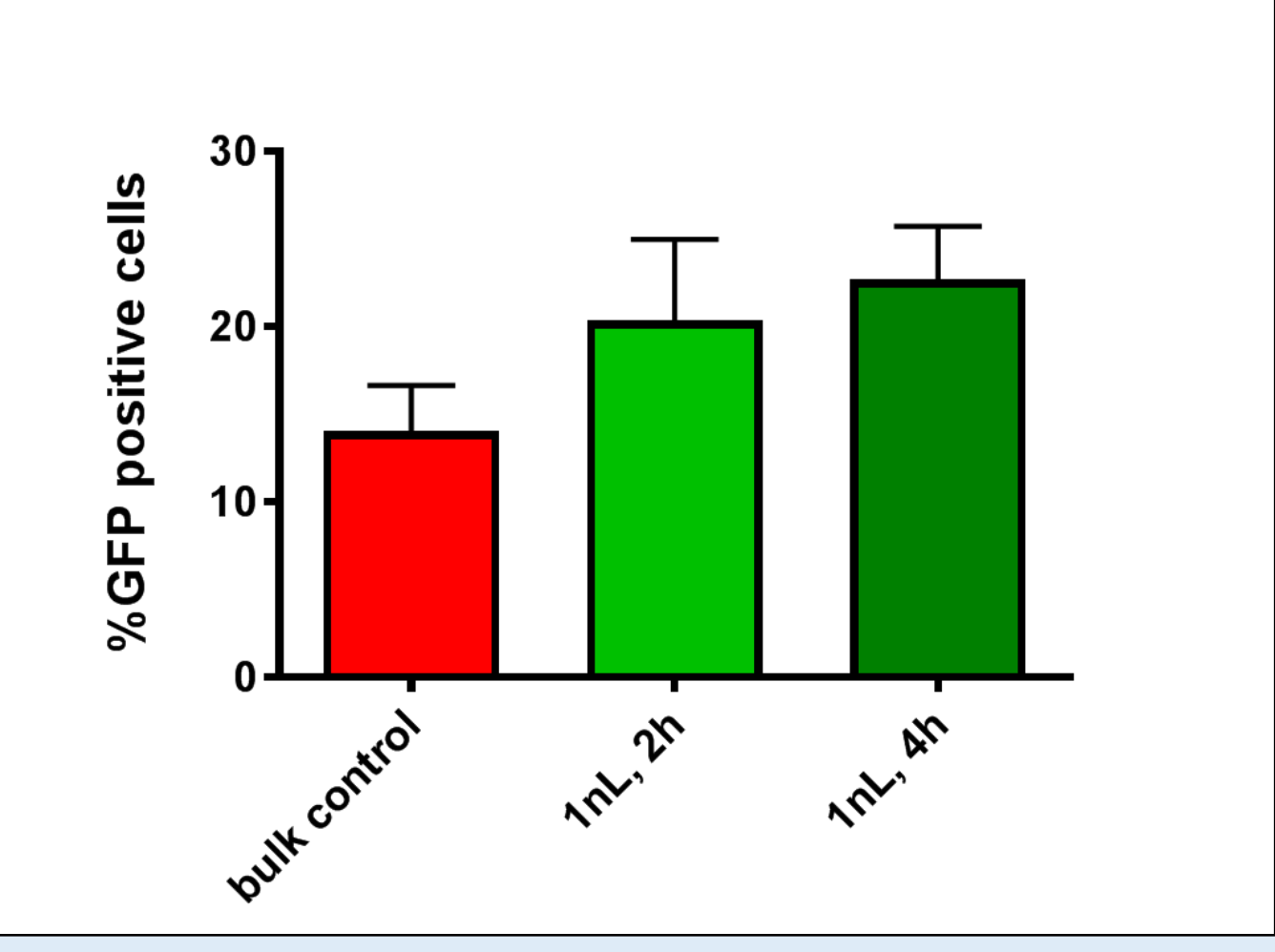


### T cell transduction efficiency in 1 nL droplets, MOI=1, 1x10<sup>7</sup> cells/mL



In larger picodroplets, at higher cell occupancy, transduction efficiencies in picodroplets are comparable to levels obtained with transduction in bulk, indicating efficient activation of T-cells (TransAct™ used for activation in all cases).

### Transduction efficiencies at MOI=0.3-0.4, 1 nL droplets, 1x10<sup>7</sup> cells/mL



At lower MOI, transduction in picodroplets appears to perform better than bulk transduction again, suggesting a plateau effect at MOI=1.

## Conclusion:

Two picodroplet microfluidic-based approaches were tested for viral transduction of Jurkat T-lymphoma and primary human T-cells - at the single cell level in pL volumes of fluid, or with small population of cells using larger volumes (up to nL size). While the model Jurkat cell line was efficiently transduced at the single cell level, primary human T-cells required small cell populations in picodroplets to achieve levels of transduction similar to or better than conventional methods. These new technologies will provide users and manufacturers with useful information and precision needed to develop better cell therapy products.