# Centrifuge Free Cell Radiolabeling Achieved Using Acoustophoresis Methods

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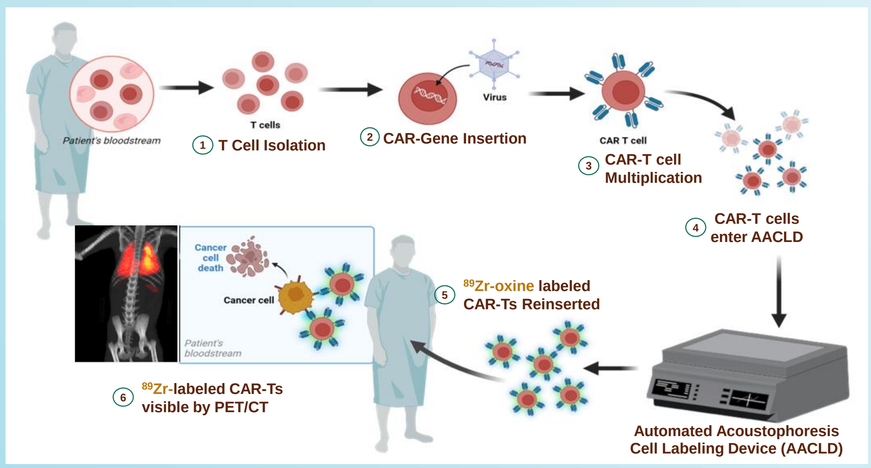
## Abstract

Centrifuge-free radiolabeling was achieved using the AcouWash 2 developed by AcouSort AB (Lund, Sweden)(1). The AcouWash 2 can increase the suspension cell density by a factor of 5 which allows one to reach the target incubation density required by the cell radiolabeling procedure. The labeling procedure was tuned to work with the flow parameters of the AcouWash 2 which resulted in labeling cells with equivalent labeling metrics of % labeling efficiency, specific activity, % free $^{89}$Zr-oxine in the suspension buffer and cell viability between the centrifuge and acoustophoresis cell radiolabeling methods.

## Introduction

- The study of immune cell therapies will be aided by invivo imaging of the immune cells used to treat cancers.
- Labeling cells with $^{89}$Zr-oxine has been developed by NCI which allows imaging of immune cells with PET/CT scanners(2).
- This allows one to assess the efficacy of the immune cell treatment by tracking the cells inside the human subject.



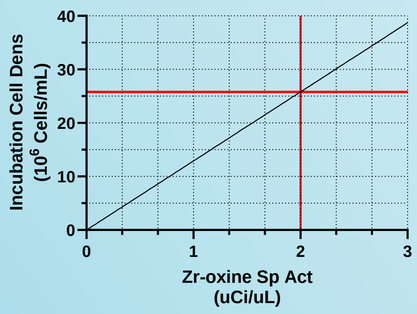

- To bring this tool to the clinic, an automated GMP cell labeling device would greatly simply the procedure standardizing the critical radiolabeling step.
- This study explores the use of acoustophoresis as the cornerstone technology which would be used to develop this automated cell radiolabeling device.
- This work builds from the original studies done with the AcouWash 1 which tested the viability of acoustophoresis as a substitute for a centrifuge in the radiolabeling process(3).

## Material And Methods

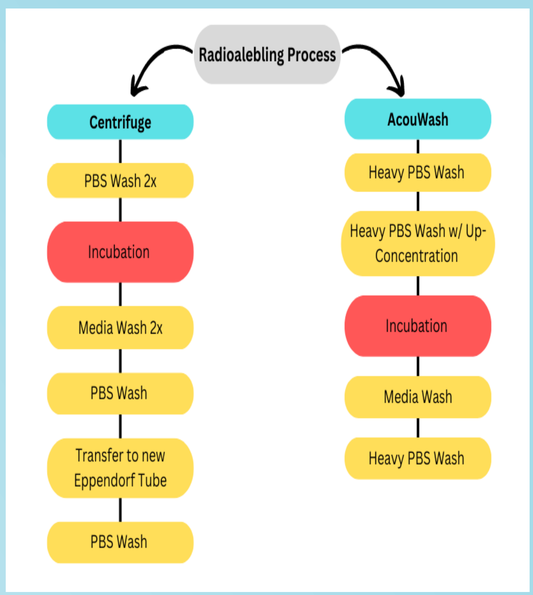
- The current radiolabeling procedure was developed by Dr. Sato at the National Cancer Institute(2).
- The procedure requires a centrifuge for cell washing and suspension density preparation required for the radiolabeling incubation process.

$$\text{CellDens} = \frac{\text{ZrOx}_{SpAct}\ \text{LabEff}}{\text{IncVolRatio}\ \text{Label}_{SpAct}}$$

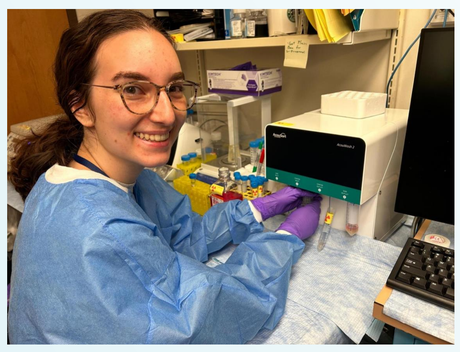
CellDens is typically is about 25x10$^6$ cells/mL for standard labeling conditions



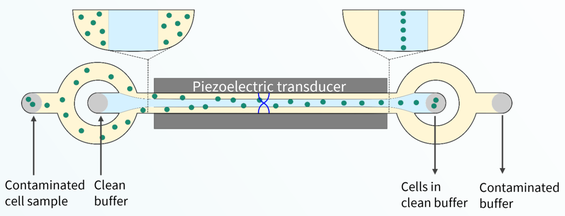

- The equation which determines the incubation cell density required for the incubation step is a function of the $^{89}$Zr-Oxine specific activity, the labeling efficiency, the concentration of $^{89}$Zr-Oxine in the incubation buffer and the target labeled specific activity.
- The procedure requires a centrifuge for cell washing and suspension density preparation required for the radiolabeling incubation process.
- Using the AcouWash 2 for cell washing and incubation density preparation an equivalent radiolabeling procedure was developed.



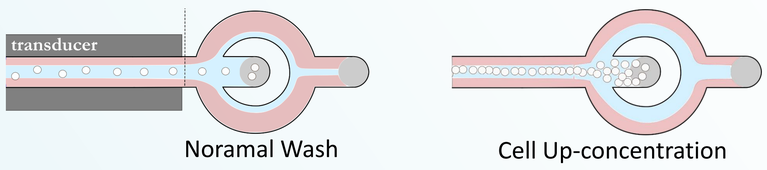

Flow chart showing equivalent radiolabeling steps between the centrifuge method and the acoustophoresis method which uses the AcouWash 2.0



The AcouWash 2 system being used during the acoustophoresis radiolabeling tests by co-author Emma Stevenson.



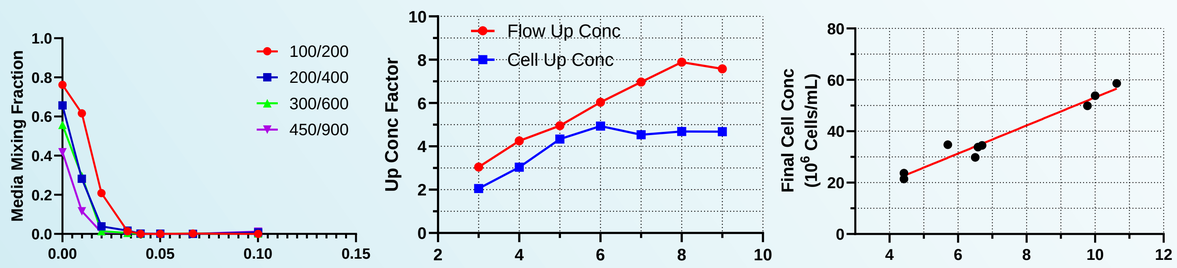

At the heart of the AcouWash 2 is the washing microfluidic chip which transfers cells from one suspension solution to another using sound waves induced by an ultrasound transducer.



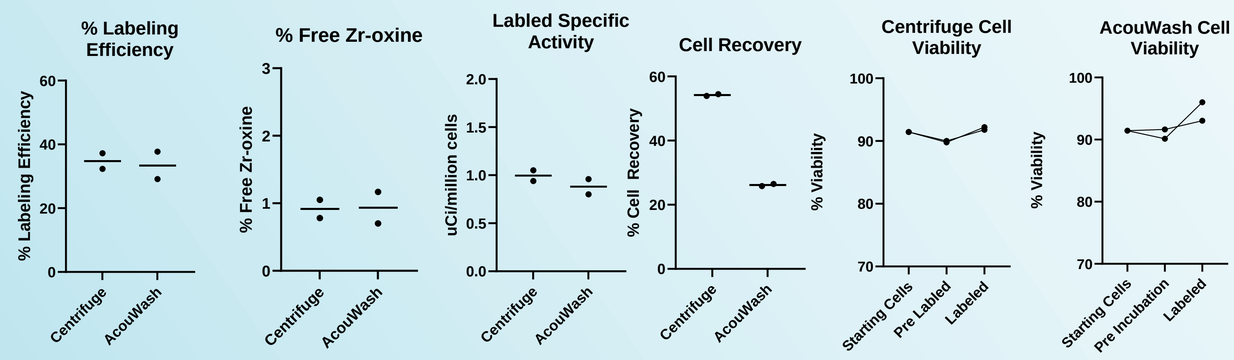

By changing the flow rates between the cell inlet and outlet one can switch between standard cell washing and cell up concentration.

## Results

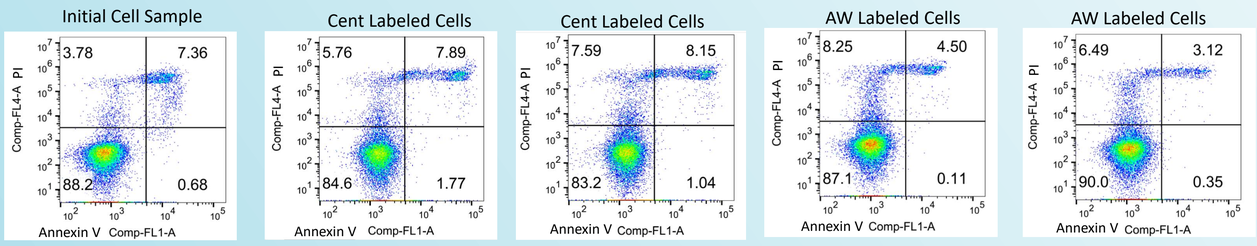
- To prevent fluid mixing between the input and wash buffers, the wash buffer needs to have a higher density than the input buffer which is achieved by adding a small fraction of lymphocyte separation medium to the wash buffer.
- Media mixing fraction was measured as a function of the added fractional amount of lymphocyte separation media and flow rates demonstrating the advantage of operating at the highest flowrates.
- Using EL4 cells (mouse T cell lymphoma), the AcouWash 2 was able to increase the cell density up to a factor of 5 using a 6 to 1 input vs output flow rate ratio.
- The up-concentration factor of 5 was independent of input cell density up to input densities of $10^7$ cells/mL.




- Side by side cell labeling tests were performed between the centrifuge method and the acoustophoresis method using the AcouWash 2.
- Starting cell concentrations were 4.5x10$^6$ Cells/mL resulting in incubation densities of 22.5, the required density needed for incubation.
- Centrifuge vs acoustophoresis labeling metrics were % labeling efficiency of 34% ± 3% vs 33% ± 6%, labeled specific activity of 0.99 ± 0.07 vs 0.88 ± 0.11 uCi/10$^6$ cells and % free $^{89}$Zr-oxine of 0.91% ± 0.19% vs 0.93% ± 0.33%.
- Cell viability was measured using acridine orange fluorescent stain with the Luna FX7 cell counter and Annexin V and PI staining with flow cytometry showing negligible cell viability effects.




Labeling metrics for an N of 2 show very similar results between centrifuge and acoustophoresis cell radiolabeling methods. Because of the small N sample, proper statistical analysis is being deferred until a large sample of radiolabeling runs has been performed.




Flow cytometry results measuring cell death and apoptosis show negligible effect compared to initial cell sample.

## Conclusion

The final acoustophoresis cell washing protocol was finalized a few days before the conference thus allowing only two redundant measures of cell labeling metrics to be presented. Although N of 2 is small, the data show the up-concentration function of the AcouWash 2 does work allowing one to eliminate the centrifuge when radiolabeling cells. This will pave the way to designing a fully automated radiolabeling device based on acoustophoresis technology.

### References

1. AcouWash 2 Product Sheet. In: acousourt.com, editor.
2. Sato N, Wu H, Asiedu KO, Szajek LP, Griffiths GL, Choyke PL. 89Zr-oxine complex PET cell imaging in monitoring cell-based therapies. Radiology. 2015;275(2):490-500.
3. Adler SS, Nyong EC, Glabman RA, Choyke PL, Sato N. Cell radiolabeling with acoustophoresis cell washing. Scientific Reports. 2022;12(1):9125.

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