

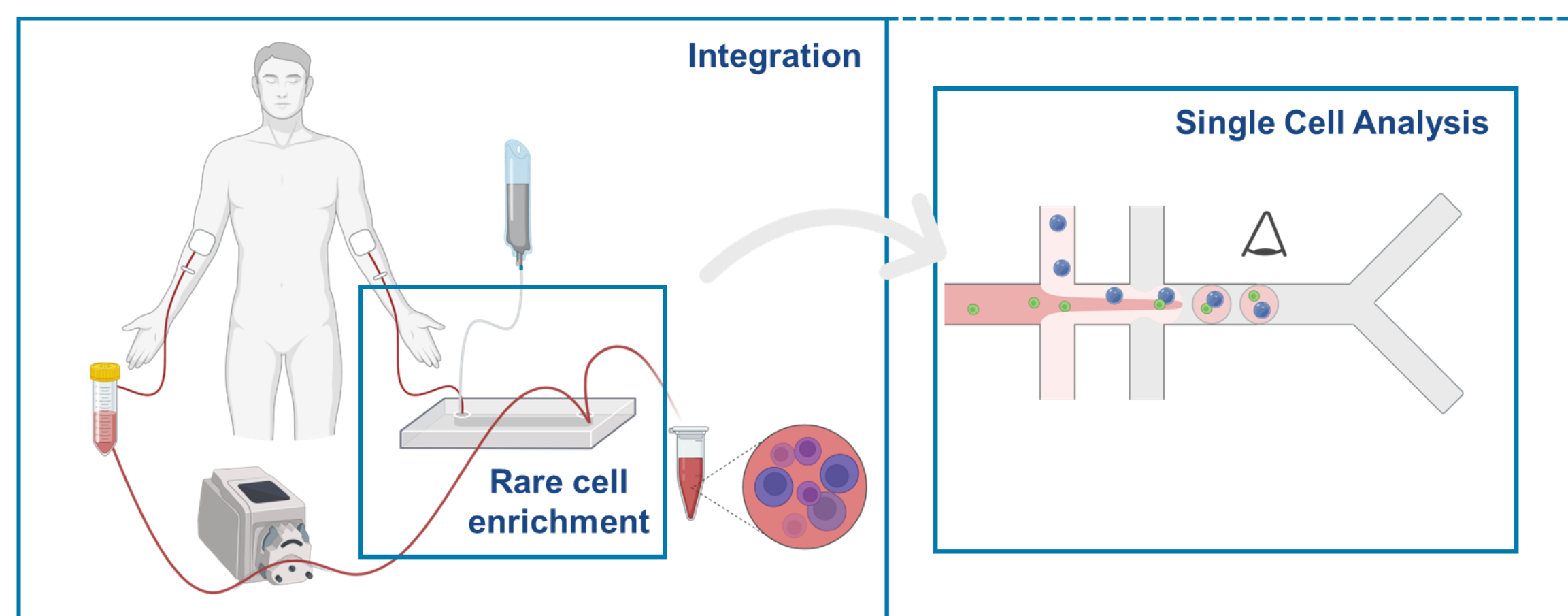
Continuous Flow Immunomagnetic Isolation of Circulating Rare Cells from Undiluted Whole Blood for Single-Cell Analysis

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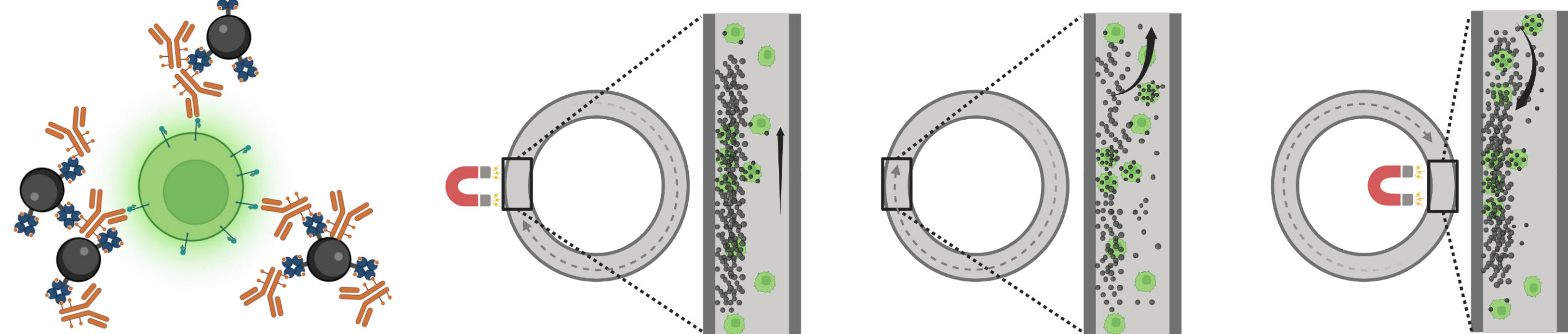
Abstract

Blood offers minimally invasive access to biomarkers, including rare circulating cells like Hematopoietic Stem Cells or Circulating Tumor Cells. However, their very low abundance (<0.01% of total blood cells) makes them difficult to isolate. The system presented here uses continuous positive immunomagnetic enrichment to process undiluted whole blood without clogging, achieving 80% sensitivity and 95% purity at 100 $\mu\text{L}/\text{min}$. Isolated cells can then be compartmentalized using droplet microfluidics for high-resolution single-cell analysis. The workflow is highly specific, adaptable to various targets, and applicable to liquid biopsies, prenatal testing, and endometriosis detection. It supports efficient rare cell isolation and multiomics analysis, advancing precision diagnostics and therapeutic development.



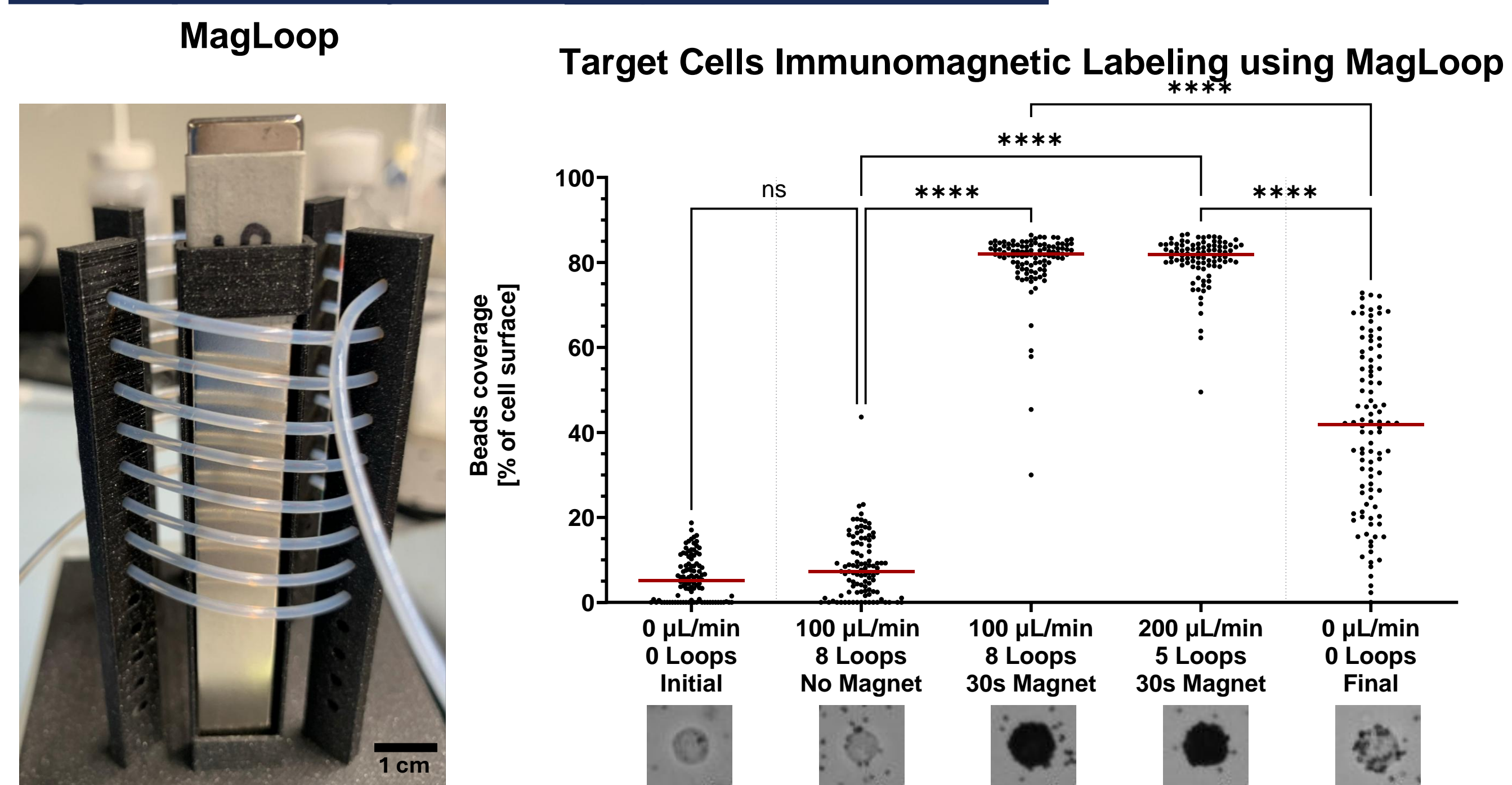
Immunomagnetic Labeling

Immunomagnetic Labeling and Local Bead Concentration Increase:



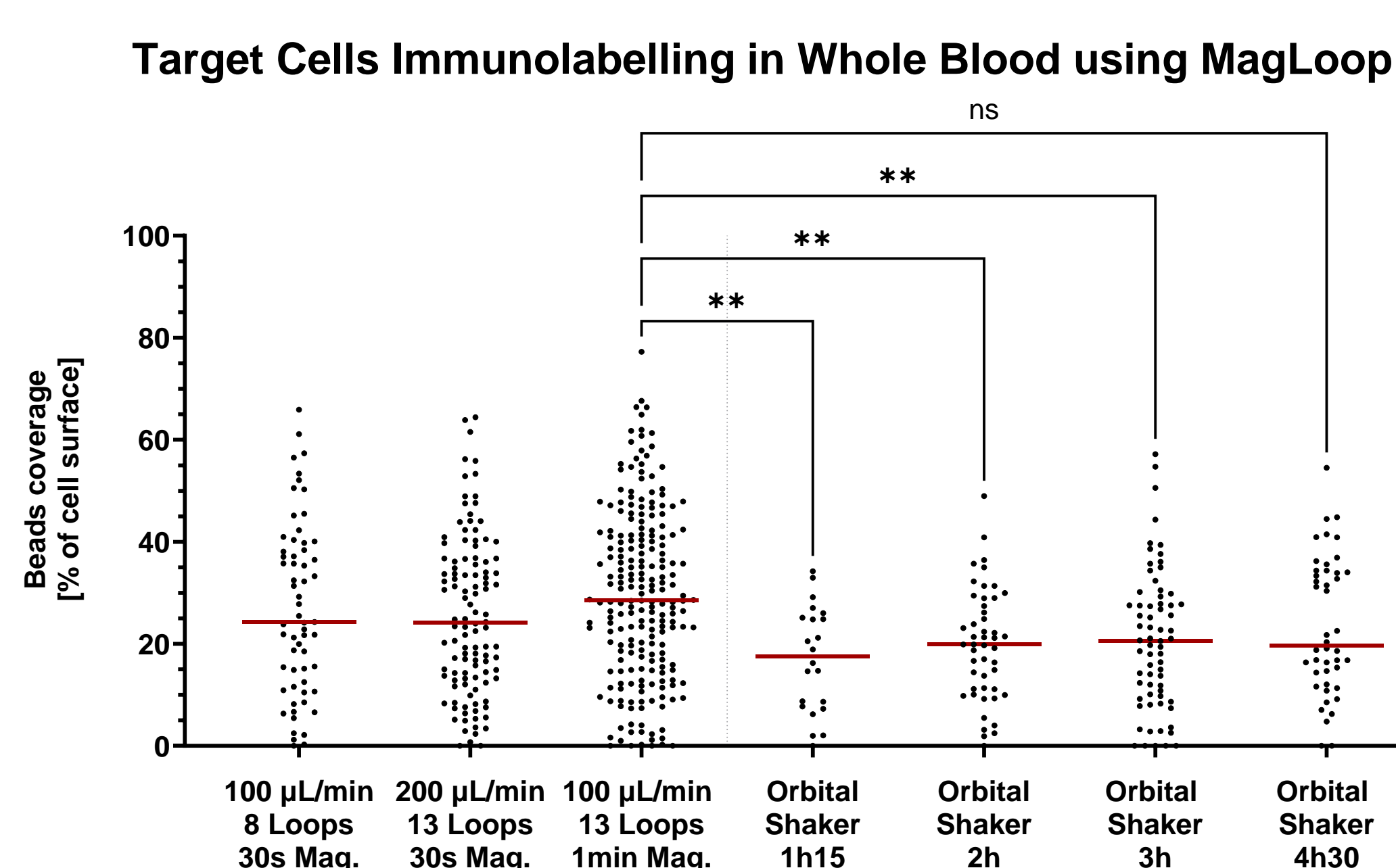
(Left) Immunomagnetic Labeling. Target cell and beads conjugated with anti-EpCAM antibodies through streptavidin-biotin binding. (Right) Local bead concentration increase happening in the MagLoop tower, with alternating magnet positions.

MagLoop Efficiently Labels Target Cells in Solution:



(Left) The MagLoop Tower is composed of 4 pillars to hold tubing in a spiral shape, and 2 opposed magnet holders. (Right) Cells spiked in cell growth medium, at 50'000 cells/mL with 1.7×10^8 magnetic beads/mL. Immunomagnetic labelling efficiency is measured as the ratio of brightfield intensities (sum of pixel intensities over cell surface) of a labelled cell compared to control non-labelled cells. One-Way ANOVA, followed by Tukey's multiple comparison test (**** $p < 0.0001$). (Bottom Right) Representative images of cell coverage. Scale bar = 10 μm .

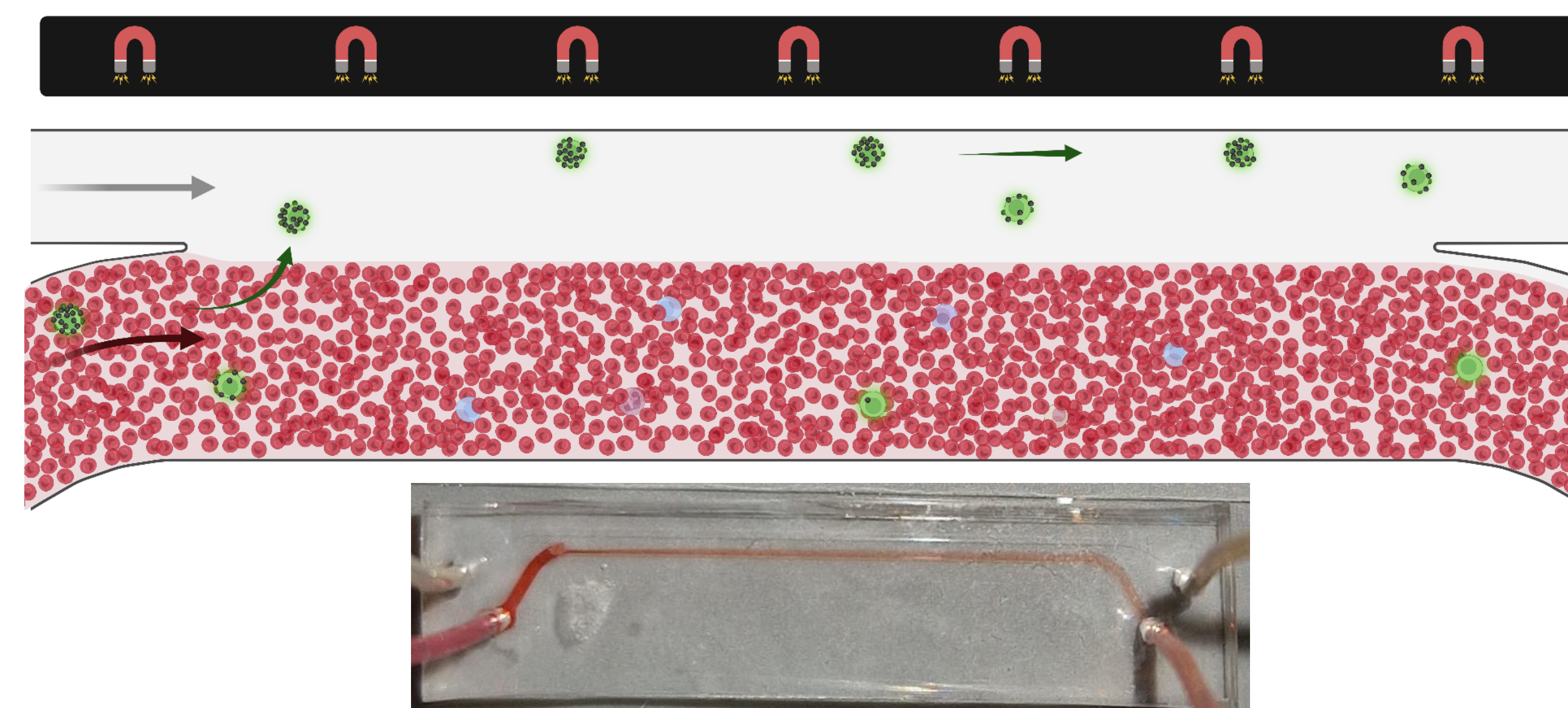
MagLoop Improves Labeling of Target Cells in Undiluted Whole Blood:



Cells spiked in undiluted whole blood, at 500'000 cells/mL, with 1.7×10^8 magnetic beads/mL. To be able to image and analyze cell coverage in brightfield, samples are diluted 1:100 prior imaging. One-Way ANOVA, followed by Tukey's multiple comparison test (** $p < 0.01$).

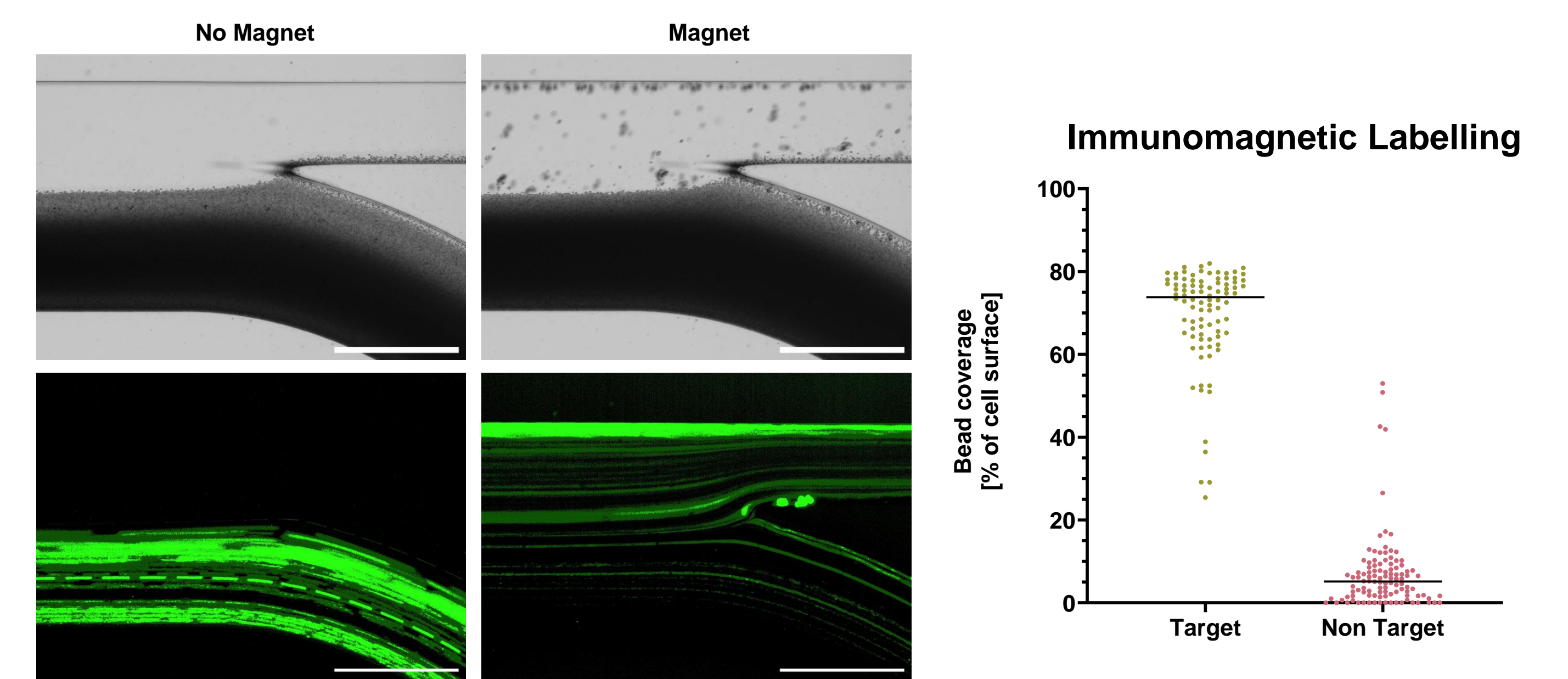
Magnetophoresis

Magnetophoresis chip / H-Filter:



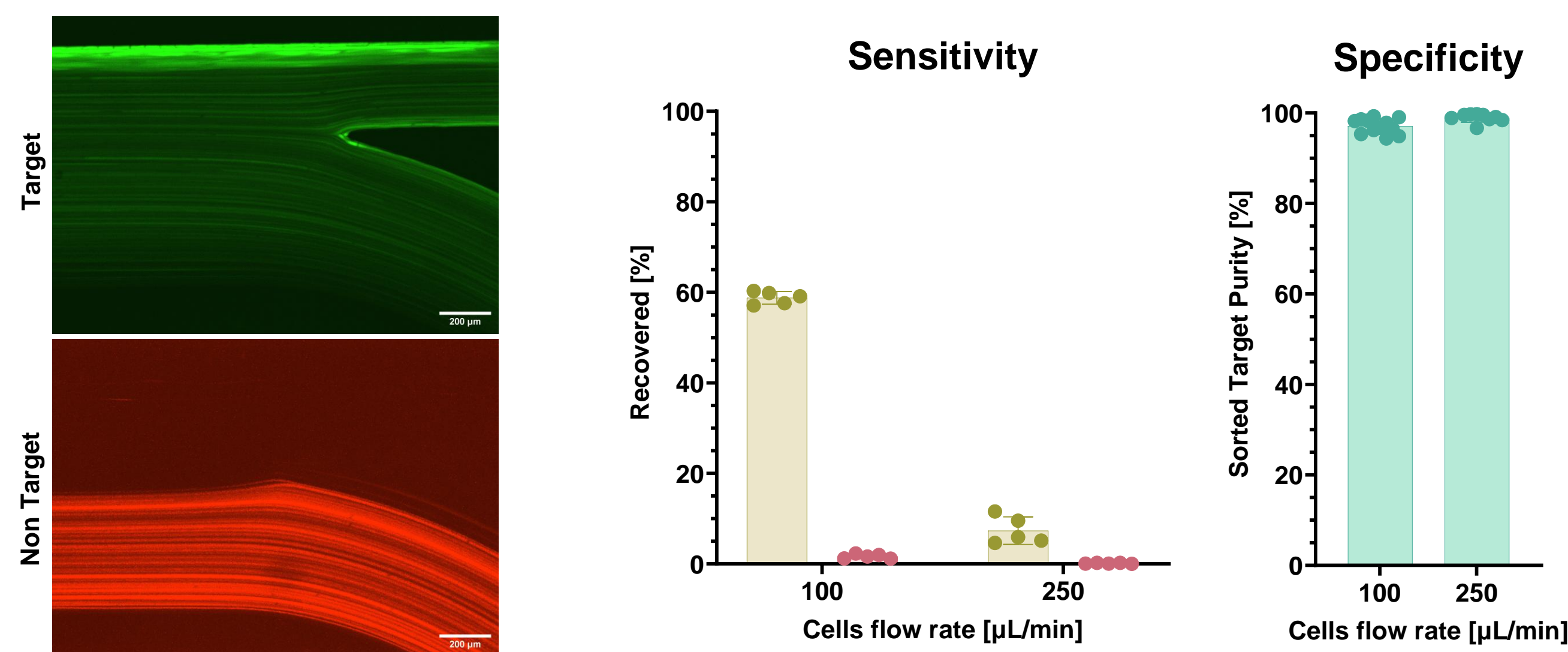
(Top) In order to isolate the immuno-magnetically labeled target cells in fluids, the magnetophoresis chip is formed of two channels (saline solution and whole blood) that merge into one where the solutions can co-flow and let the attracted cells flow up and into a sorting channel, while the blood cells flow untouched towards a waste/recirculation channel. The co-flow area is 25 mm long, 940 μm wide, and 200 μm high. Ratio saline: blood channels is 340:600. (Bottom) Picture of the magnetophoresis chip running with whole blood.

Target Isolation from Undiluted Whole Blood:

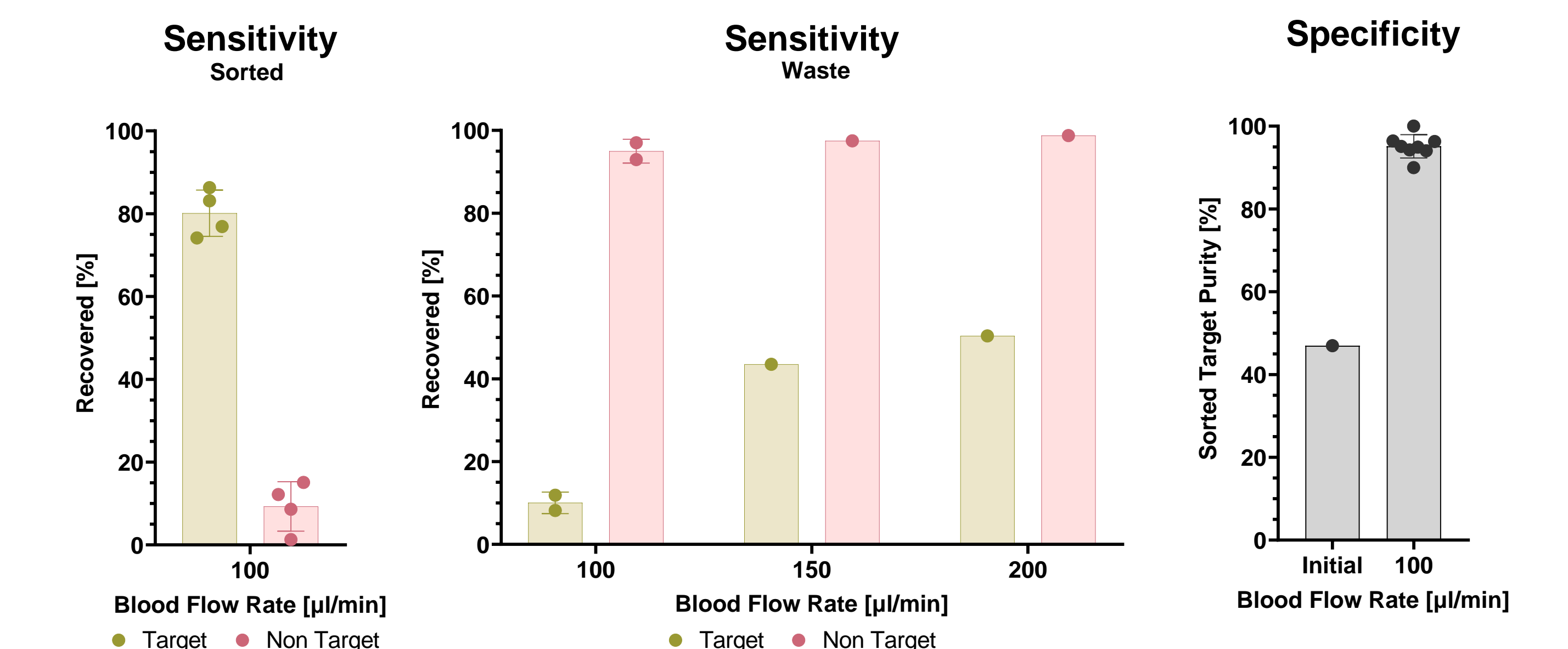


(Right) Representative pictures from magnetophoresis in whole blood. Each picture is a stack of >200 frames from brightfield and fluorescence (target cells) recorded videos. Scale bar = 500 μm . (Left) Immunomagnetic labeling of cells spiked-in whole blood. Each population was spiked-in at a concentration of 50'000 cells/mL, 1:1 ratio.

Target Isolation from a Population of Non-target Cells in Solution:

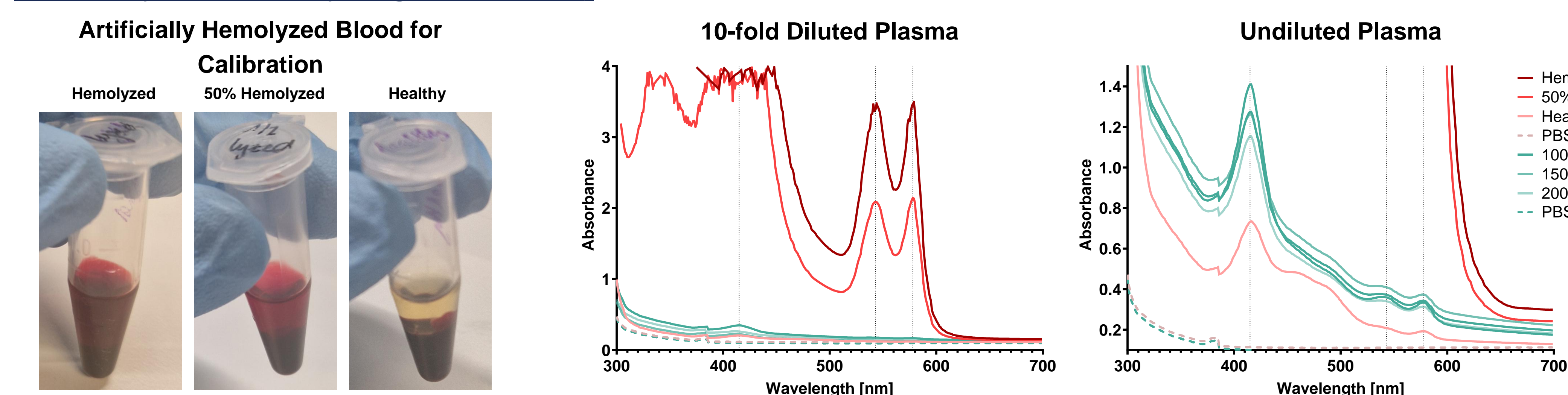


(Left) Representative pictures from magnetophoresis in solution. Each picture is a stack of >200 frames from fluorescence recorded videos. Scale bar = 200 μm . (Middle) Recovered cells in sorted output over total cells (sorted + waste). Cells spiked-in at a concentration of 50'000 cells/mL, 1:1 ratio. Mean \pm Std. (Right) Target cells in sorted outlet over total cells in sorted outlet. Mean \pm Std.



(Left) Recovered cells is sorted output over total cells (sorted + waste). (Middle) Depleted cells: recovered cells in waste outlet. (Right) Target:Non-Target ratio in initial sample, as well as sorted target cells purity in sorted sample. Target cell purity is ratio of target over total cells sorted in outlet sample.

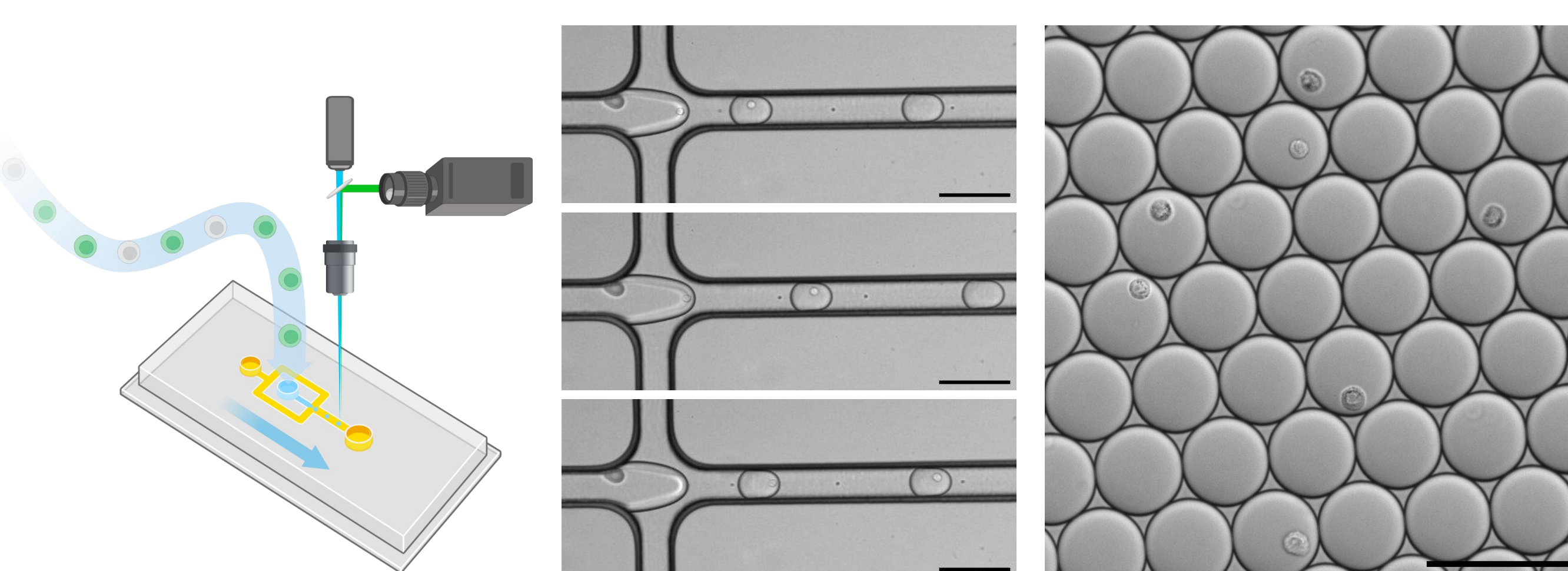
No hemolysis caused by magnetophoresis:



(Left) Representative pictures of artificially hemolyzed blood sample, after centrifugation to separate plasma. (Middle) Spectrometry results of 10-fold diluted plasma samples, showing the hemoglobin absorbance peaks. (Right) Spectrometry of undiluted plasma samples, showing the hemoglobin absorbance peaks, but no hemolysis.

Droplets, Analysis & Outlook

Single-cell encapsulation in droplets for single cell analysis:



(Left) Droplet production chip schematic. (Middle) Single-cell encapsulation during droplet production. Top to bottom images are consecutive frames showing 2 cells being encapsulated into picoliter-sized droplets. Scale = 100 μm . (Right) Droplet emulsion produced. Single cells are compartmentalized. Scale bar = 100 μm .

Summary

In summary, here is presented a robust system for immunomagnetically label cells in flowing solution and whole blood, as well isolating rare circulating cells with high sensitivity and specificity using continuous magnetophoresis, which can then be encapsulated in droplets. The adaptable workflow supports different types of analyses, from fluorescence to preparation for single-cell sequencing, as well as different applications in liquid biopsies, prenatal testing or disease monitoring. It offers significant potential to advance diagnostic and personalized medicine.

Acknowledgements

