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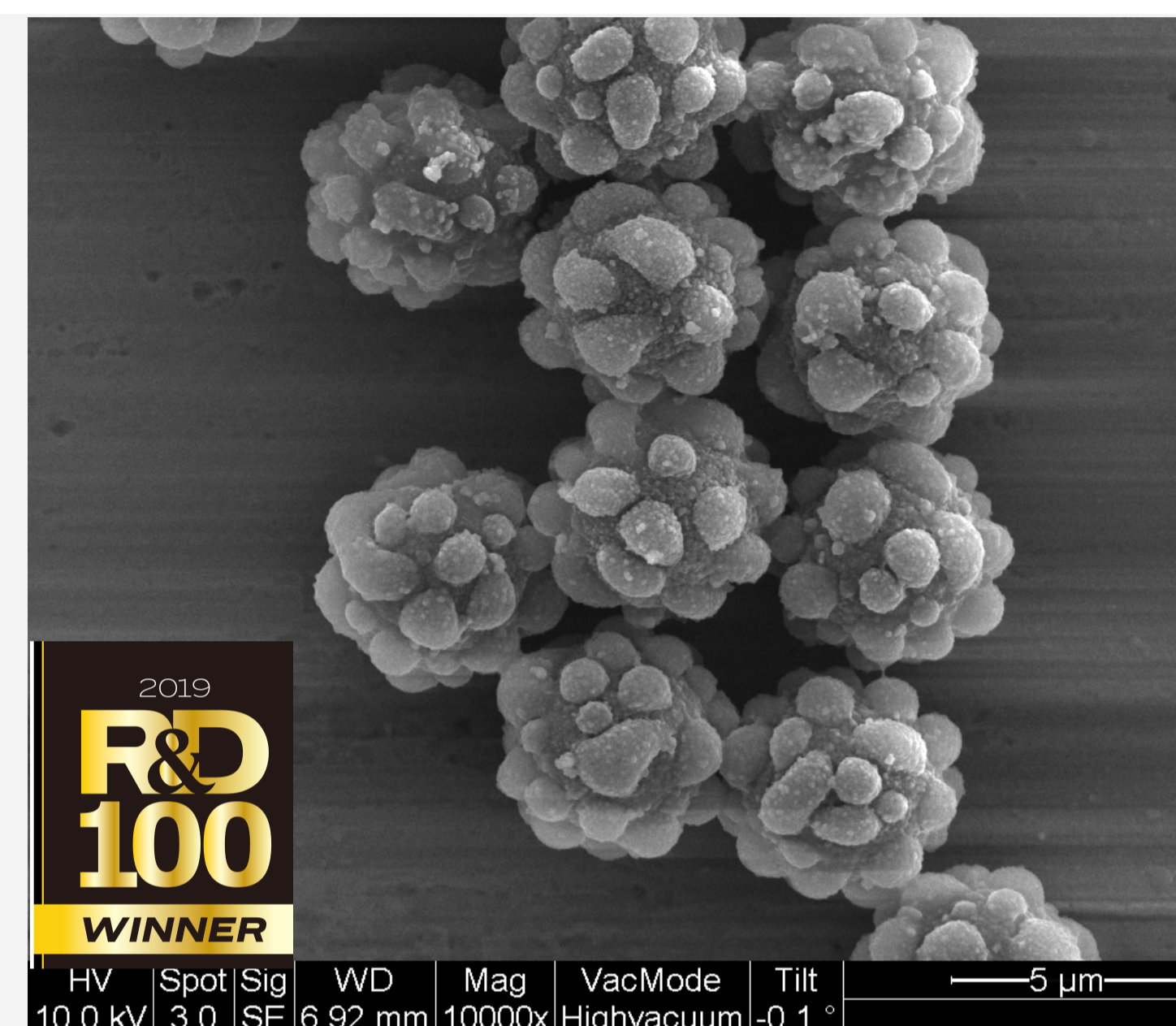
The use of OVIZIO's holographic camera qMod and its associated OsOne software allows for fast & extensive T cells characterization of Critical Processes Parameters (CPPs) such as cell viability, total cell density, beads counting and Critical Quality Attributes (CQAs) such as cell phenotype (morphology-related) e.g. activation-like state in a dye-free set-up.



## Technology Landscape OVIZIO qMod

Adding qMod on a regular brightfield microscope turned it into a versatile 3D quantitative imaging platform to monitor T cells process development.

For each cell on a recorded image, the OVIZIO software OsOne computes a large set of T cells features that can be classified into 3 categories: morphological, optical, texture features. The calculated features can then be used to identify beads and T cells at various levels of activation. In this experiment microscope brand is Leica (DM IRB) and T cells were cultivated on 24-well plates.



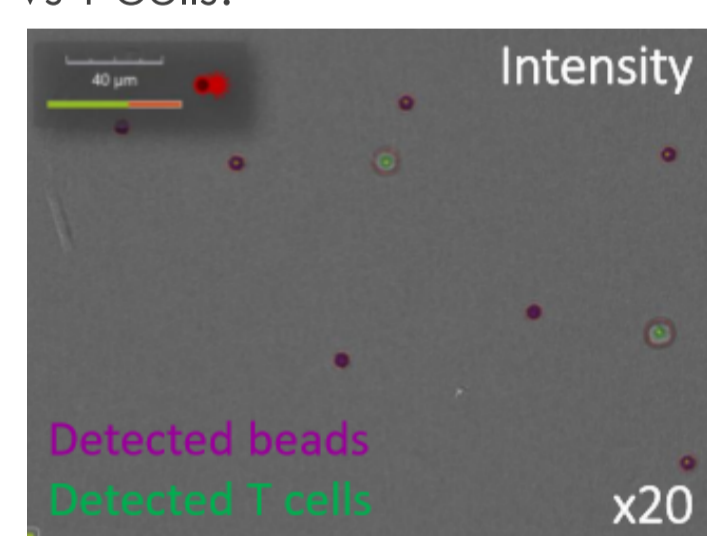
## Technology Landscape ITRI iKNOBEADS



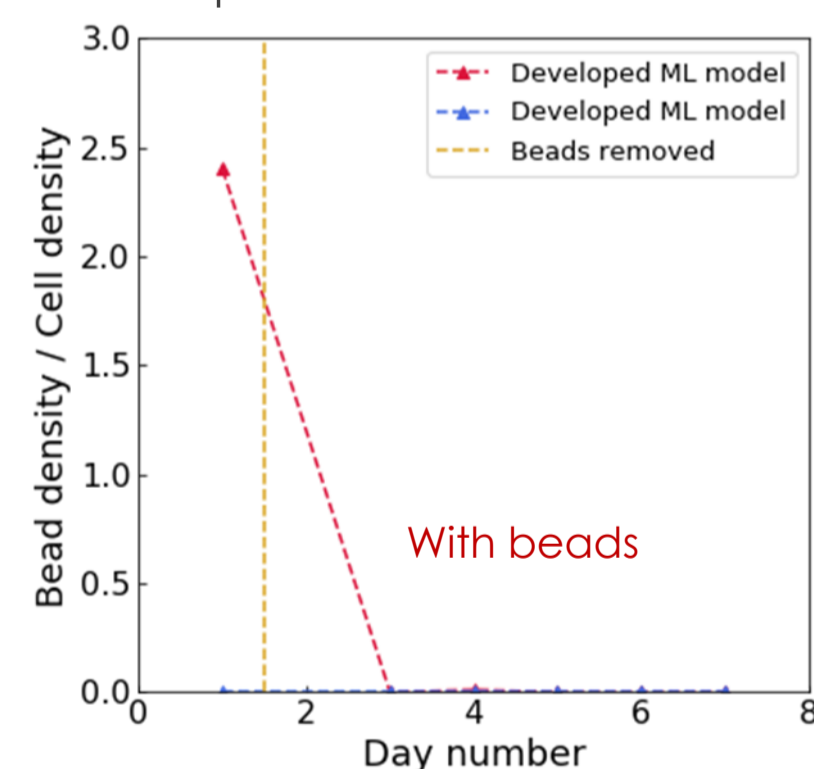
iKNOBEADS are the world's first magnetic microparticles with tailorable sizes and shapes. Their unique morphology with bioinspired knobs and rough surface provides a potential for biochemical applications. iKNOBEADS are the high efficient materials for ex vivo activation and expansion of immune cells for immunotherapy.

## iKNOBEADS Detection

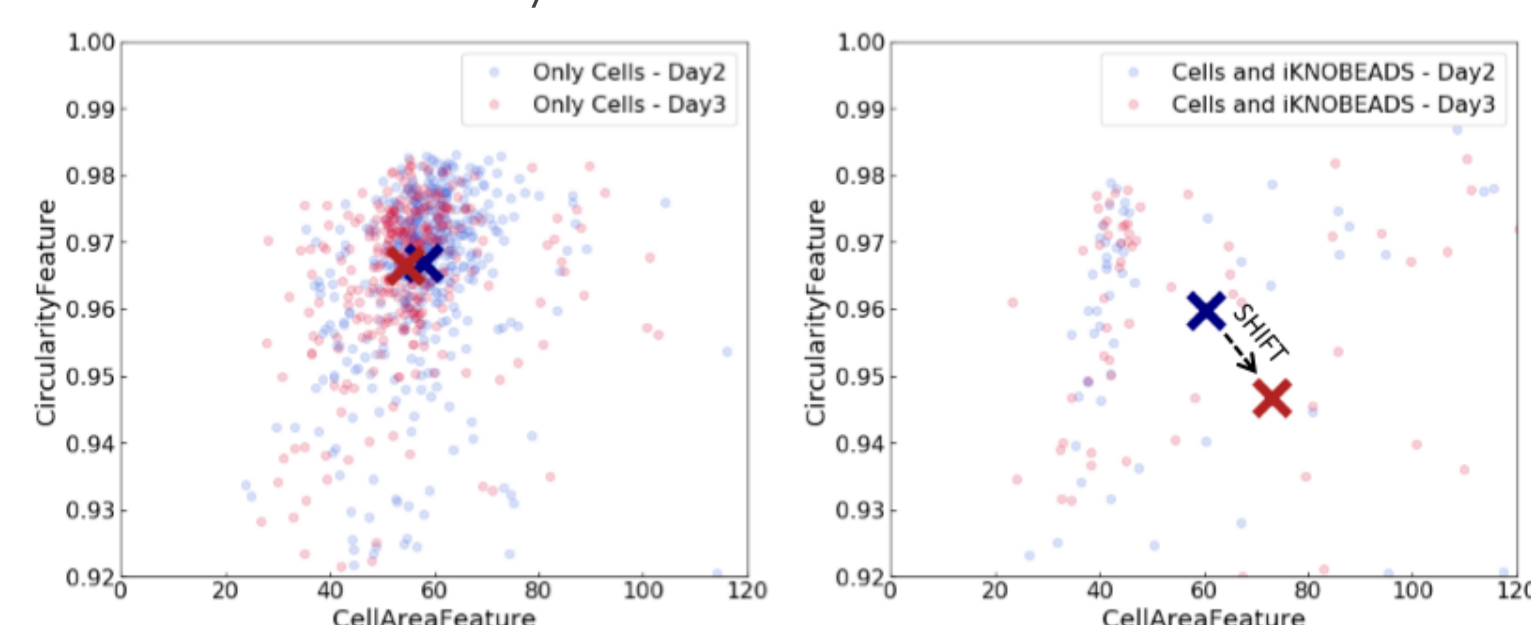
A machine learning model, combination of Neural Networks, has been developed to detect the iKNOBEADS and discriminate vs T cells.



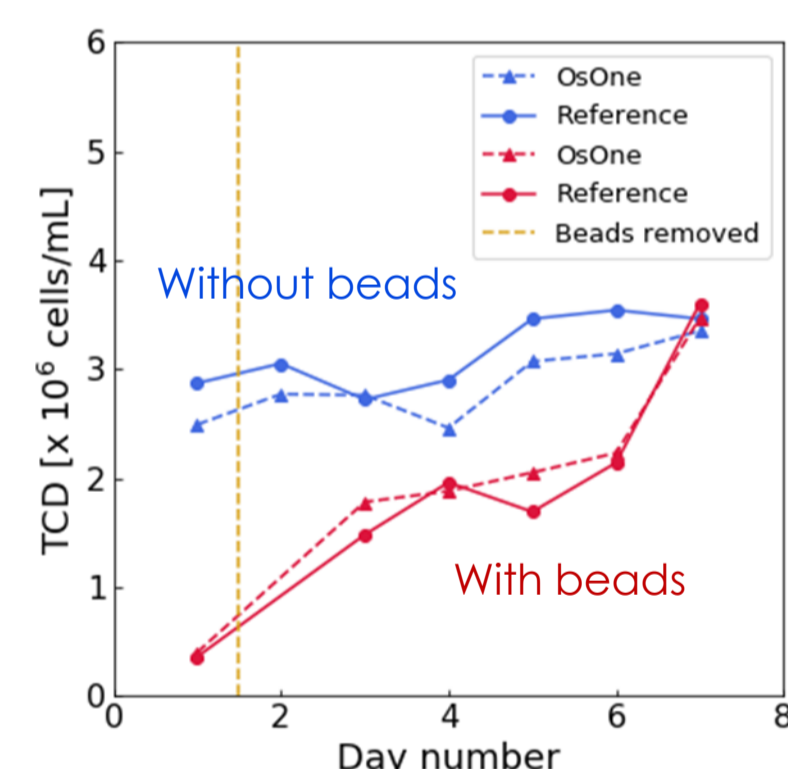
iKNOBEADS can be detected over time to QC the absence of beads at the process end.



When iKNOBEADS are added, the mean cell circularity decreases while the mean cell area increases.

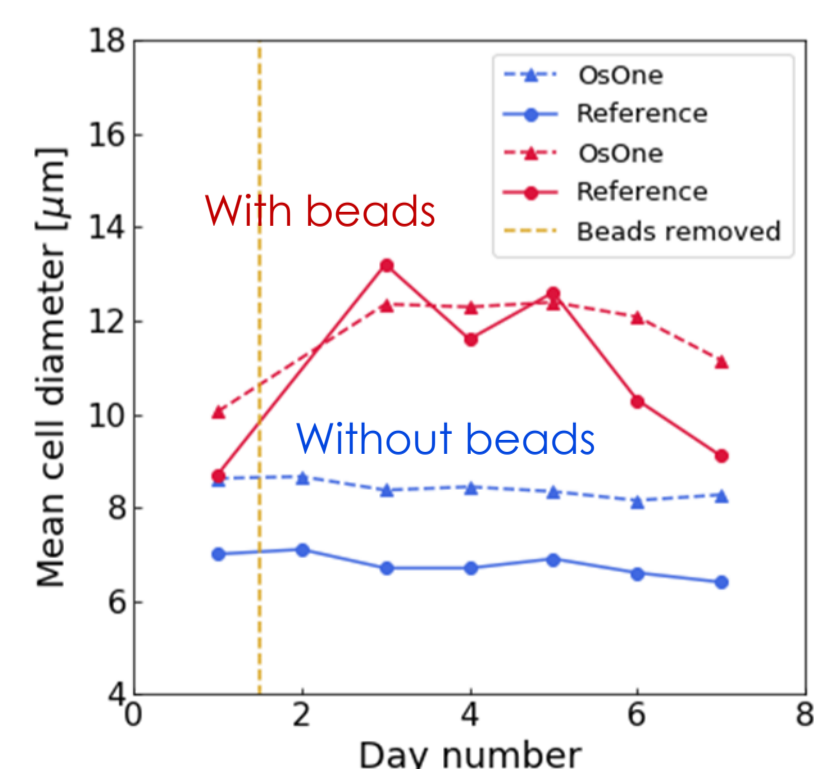


## Total T Cell Density



The Total Cell Density (TCD) behavior and values obtained by OsOne are very close to the references (different techniques between Ovizio and commercial cell counter). This was repeated for several donors.

## T Cell Diameter

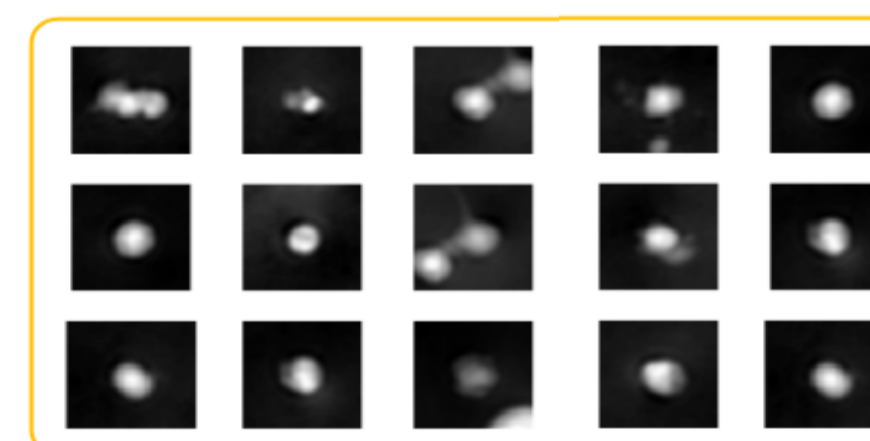


The mean cell diameter values obtained with OsOne follow the same trend as references\*. This was repeated for several donors.

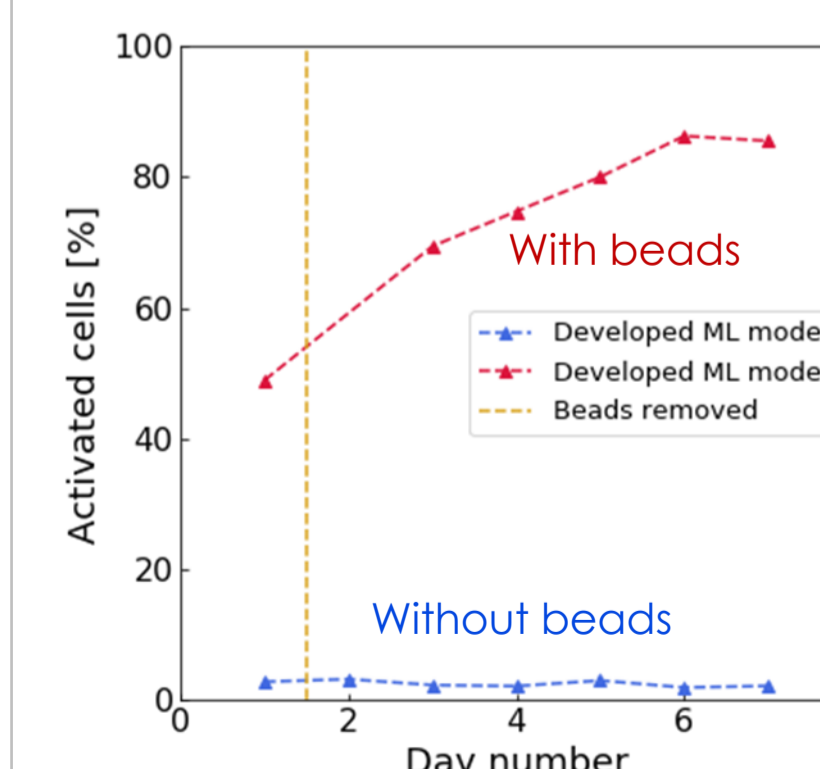
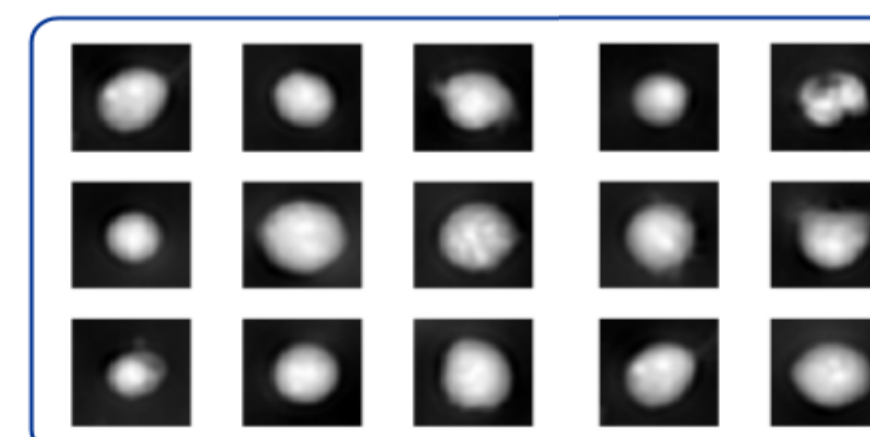
\*For cells with smaller diameters, a constant divergence of 2 µm has been observed between the reference and OsOne as the analysis methods rely on different techniques. At greater cell densities, this constant divergence is lessened or removed.

## T Cell Activation

Non-activated T cells



Activated-like T cells



A machine learning model, Neural Network, has been developed to detect activated-like T-cells. There is a striking change of the cell size over time.

The OsOne developed algorithms can give a % of activation over time. This was repeated for several donors.

The Ovizio's qMod holographic camera and its associated OsOne software can (i) detect, classify and discriminate iKNOBEADS versus T cells and, (ii) track the striking change of the T cell size over time, reflect of the activation stage; without having to dilute the sample nor to add dyes. Applicable also within CAR-T, TCR-T, NK cells processes and CD3/CD28 beads.