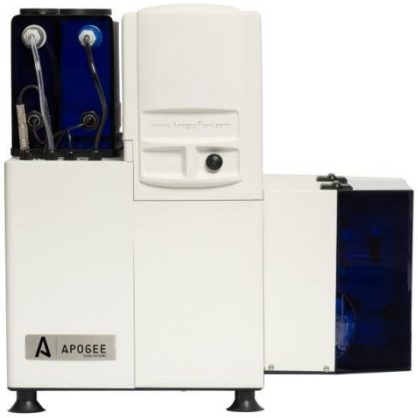
APOGEE FLOW SYSTEMS

Proof of Concept

Centripetal Liquid Dielectrophoretic (cLDEP) Sorting of E. coli



The application of centripetal liquid dielectrophoretic (cLDEP) force to flow cytometry particle sorting allows chosen particles to be plucked from a stream at precisely the right moment. The novel technology has been implemented in the 'Kairos' module for ApogeeFlow cytometers and offers the following features

- High stability: cLDEP force is applied close to the laser for high timing precision
- Fast: 10x higher speed than other methods of enclosed (non-droplet forming) particle sorting methods.
- No moving parts
- Gentle on cells (avoids the high pressures required by droplet sorters)
- Avoids droplet sorter breakoff instability and nozzle problems
- Avoids the sample heating caused by thermal bubble actuator methods

User defined regions of interest on the flow cytometer's histograms allow selection of populations of particles to be sorted based on combinations of any optical parameter.

Method

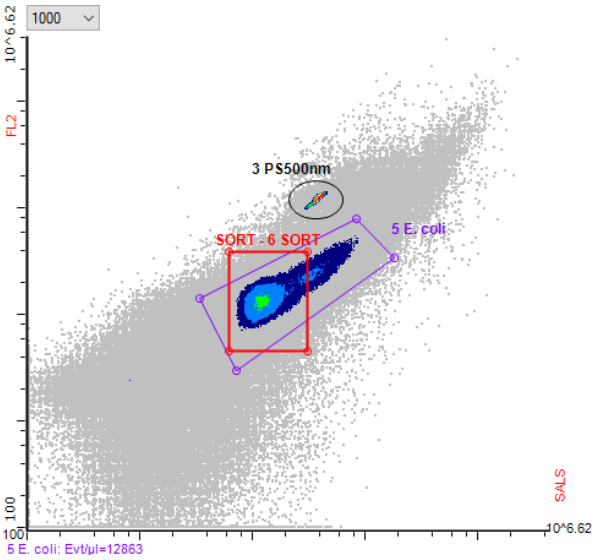
500nm polystyrene test beads were added to a sample of DAPI stained E. coli giving a concentration of 1090 beads/µl as measured by an ApogeeFlow 'Micro-PLUS' cytometer.

The E. coli population consisted of approximately 8,500 B-period cells per microlitre (cells before replication) and 4,000 D-period cells per microlitre (larger cells in the cell cycle period shortly before division).

A sorting region of interest ("ROI" #6) was placed around the B-period E.coli, excluding test beads and D-period E.coli. The sort ROI included 1650 cells per second which were diverted based on their light scatter and DAPI fluorescence characteristics by cLDEP force to a microcentrifuge tube.

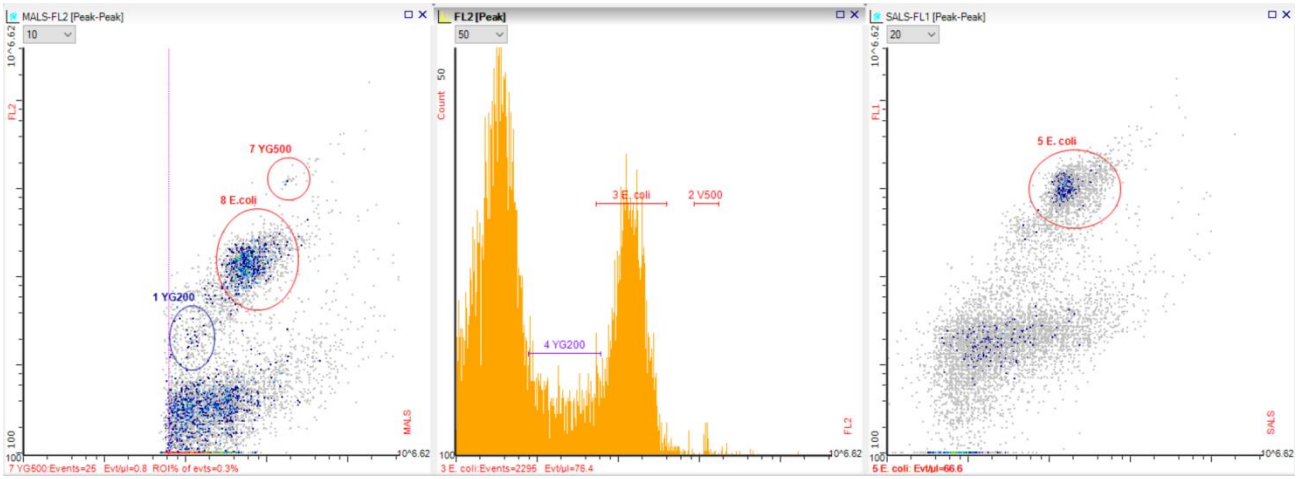
As a control, a second sort was performed after moving the sort ROI to enclose only the 500nm beads.

The resultant 'sorted' samples were re-analysed on the flow cytometer.

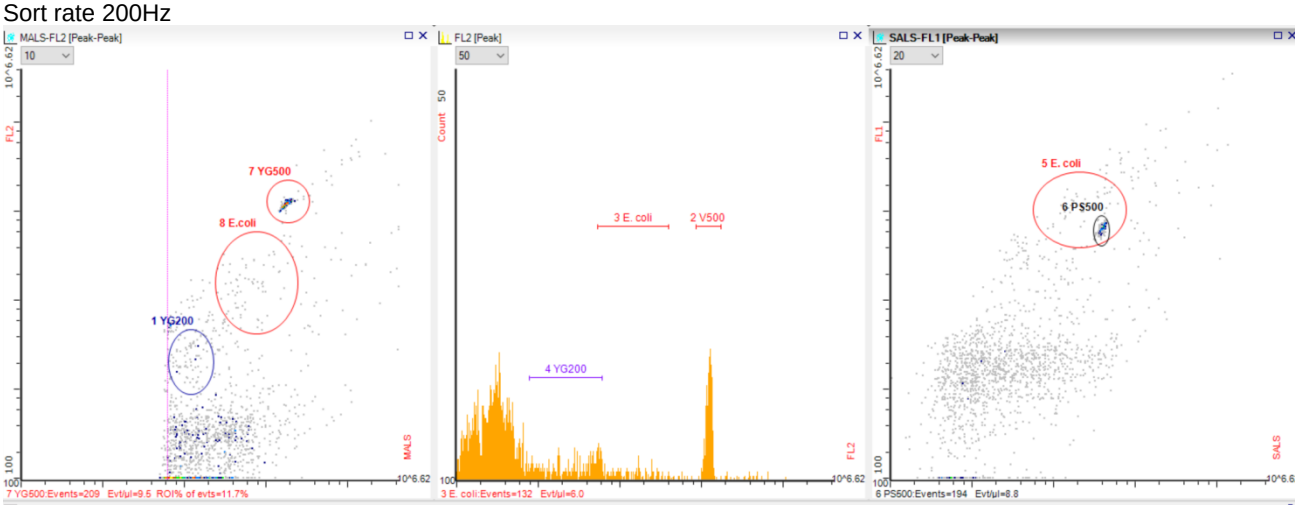


Results

Sort on B-period E. coli
Sort rate 1650Hz



Sort on polystyrene 500nm beads
Sort rate 200Hz



Conclusion

The speed, purity and yield obtained from the *Kairos* cLDEP sorter indicates this new particle sorting method could be usefully applied to a wide range of applications including prokaryotic cells, eukaryotic cells, virions and extracellular vesicles.