**Docket #:** S15-009

# Quantification of Antigen Molecules Without Calibrators Using Dynamic Flow Cytometry

Researchers in the Herzenberg laboratory at Stanford University have patented a method to quantify antigens during flow cytometry without the use of calibrators. Traditional methods for estimating the number of antigens on a cell are based on the detection of target antigens bound with fluorescently labeled antibodies. A calibration procedure is then used to convert the intensity of the fluorescence signal to the number of target antigens. Current commercial calibration methods are limited. Results can vary based on the choice of calibrator, fluorochrome conjugates and sample handling. In addition, these methods are not applicable to labeling with lower affinity antibodies or labeling under non-equilibrium conditions. To overcome these limitations the inventors developed this method for antigen quantification which, instead of using a static calibration system, quantifies the antigen molecules per cell by determining the binding rate constant for each antibody-antigen reaction. This method can be applied to both low and high affinity antibodies, under saturating and non-saturating conditions independent of the conjugated fluorochrome.

**Stage of Development**
Proof of Concept: The inventors demonstrated the applicability of this method by quantifying the CD8α antigen concentration on human T cells.

## Applications

- Quantitative flow cytometry analysis, including use in:
  - Immunology
  - Clinical diagnostics

## Advantages

- Does not require calibrators- approach is independent of specially prepared calibration beads, antibody regents and dyes
- Can be applied to both high and low affinity antibodies
- Can be used under both saturating and non-saturating conditions
- Allows comparison across flow cytometry instruments and experiments

## Publications


- Moskalensky, A. E., Chernyshev, A. V., Yurkin, M. A., Nekrasov, V. M., Polshchitsin, A. A., Parks, D. R., Moore, W.A., Filatenkov, A., Maltsev, V.P. & Orlova, D. Y. (2015). Dynamic quantification of antigen molecules with flow cytometry. *Journal of immunological methods, 418,* 66-74. doi: 10.1016/j.jim.2015.02.001.


## Patents

- Published Application: 20160238597
- Issued: 10,379,118 (USA)

## Innovators

- Darya Orlova
- Andrei Chernyshev
- Alexander Moskalensky
- David Parks
- Leonore Herzenberg

## Licensing Contact


### Mona Wan

Senior Licensing Manager for Special Projects

Email