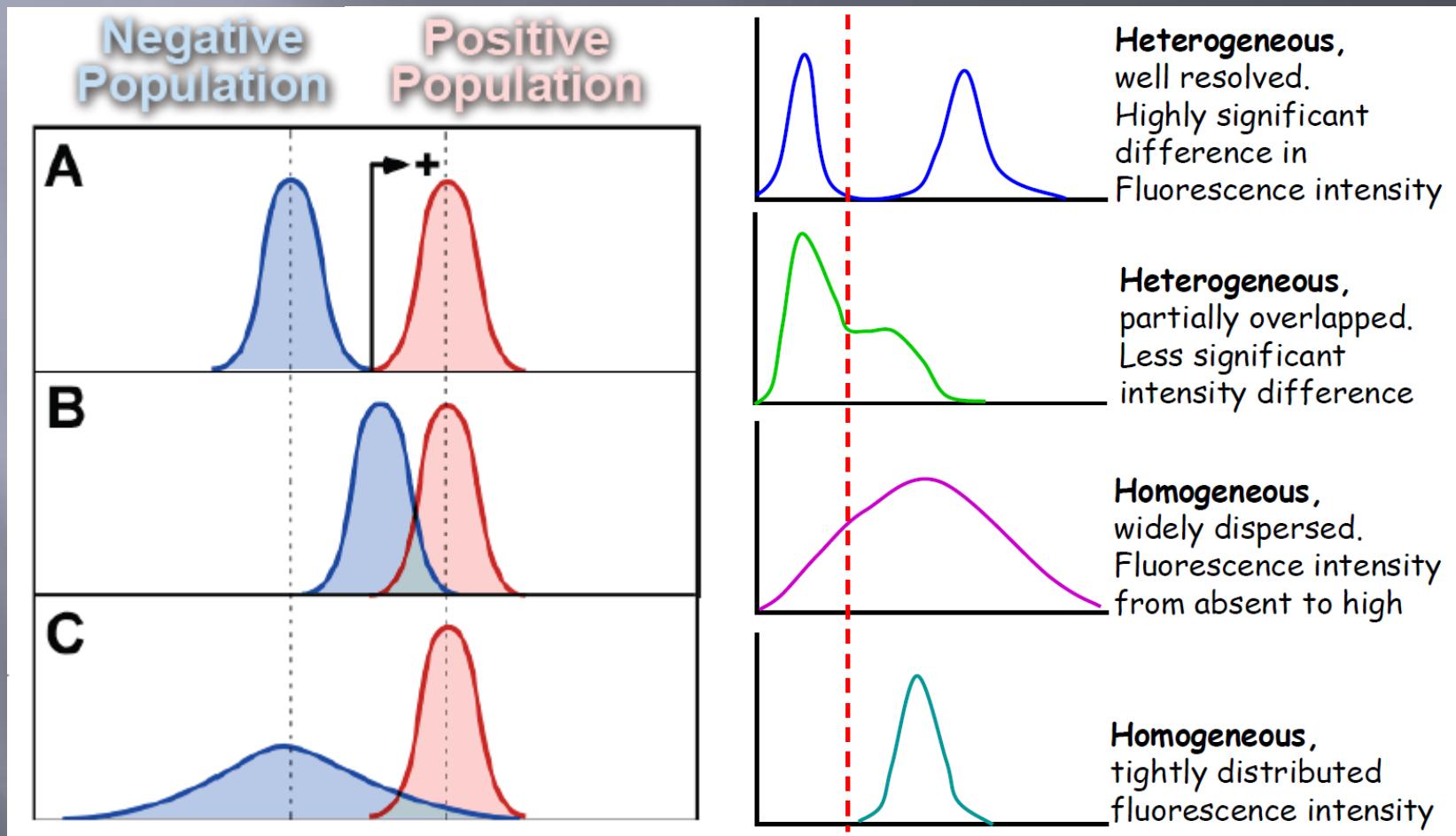


ΔΕΙΓΜΑΤΑ ΕΛΕΓΧΟΥ – ΣΗΜΑ ΥΠΟΒΑΘΡΟΥ ΚΑΙ ΤΕΧΝΙΚΕΣ ΒΕΛΤΙΣΤΟΠΟΙΗΣΗΣ

ΕΚΠΑΙΔΕΥΤΙΚΟ ΣΕΜΙΝΑΡΙΟ
2 ΦΕΒΡΟΥΑΡΙΟΥ 2013

ΣΠΥΡΟΣ ΣΠΥΡΙΔΑΚΗΣ Χημικός, MSc

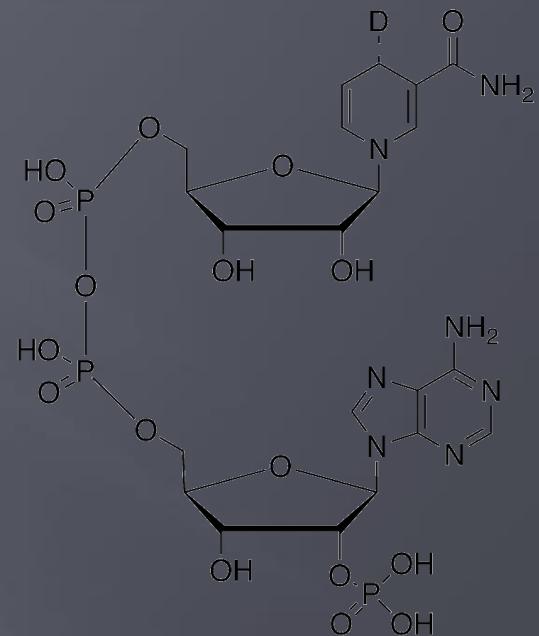
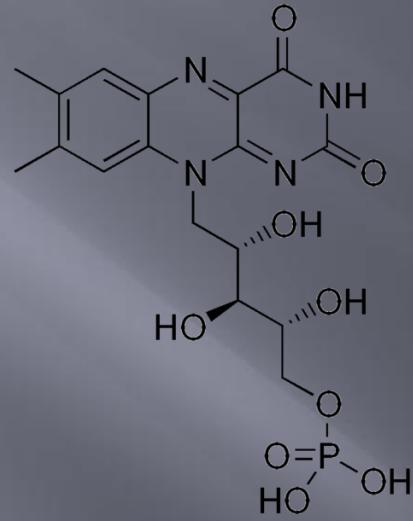
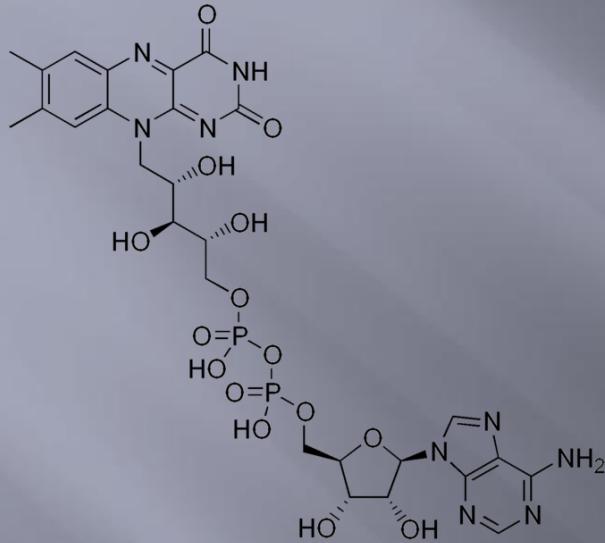
ΘΕΤΙΚΟ ή ΑΠΝΗΤΙΚΟ



ΣΗΜΑ ΥΠΟΒΑΘΡΟΥ

- Αυτοφθορισμός Κυττάρων
- Χρωματική αλληλοεπικάλυψη
(επίδραση compensation)
- Μη επιθυμητή σύνδεση αντισώματος
- Debris

ΑΥΤΟΦΘΟΡΙΣΜΟΣ



FAD

Flavin Adenine Dinucleotide

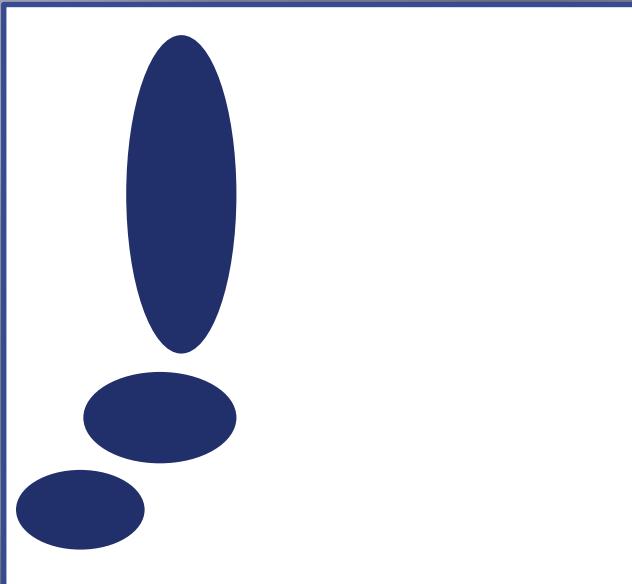
FMN

Flavin Mononucleotide

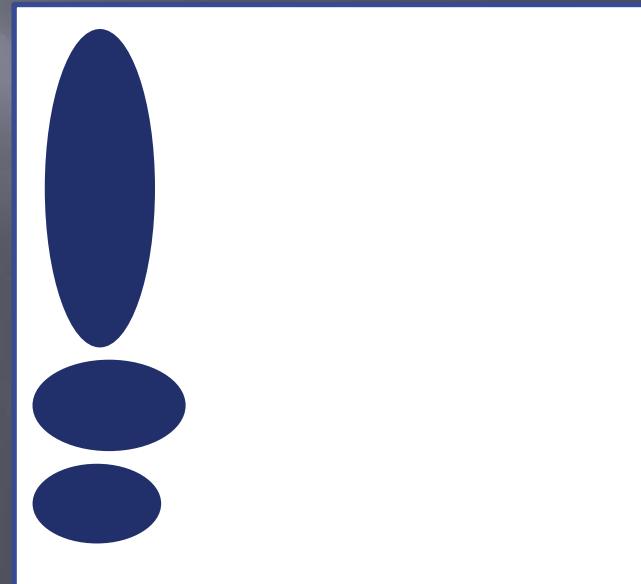
NADPH

ΑΥΤΟΦΘΟΡΙΣΜΟΣ

- Μήκος Κύματος LASER (488nm / 532nm)
- Είδος και μέγεθος κυττάρων
- Κατεργασία δείγματος
- Κανάλι ανίχνευσης

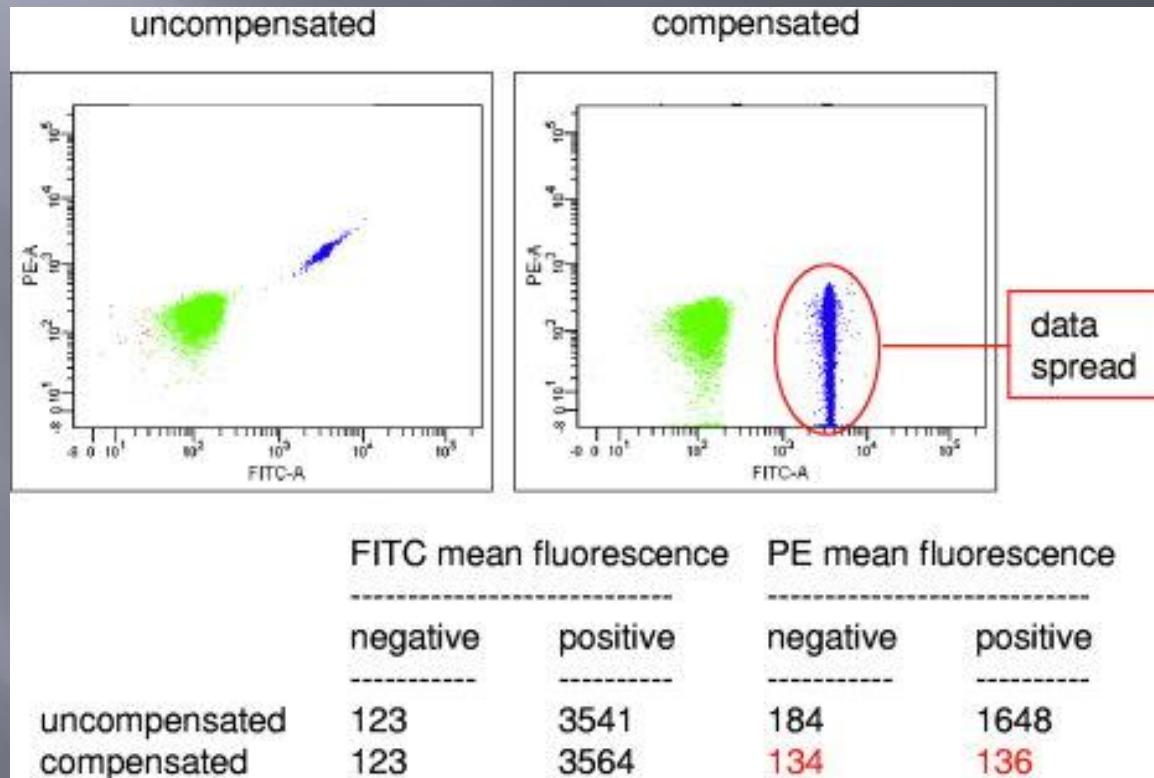


FITC

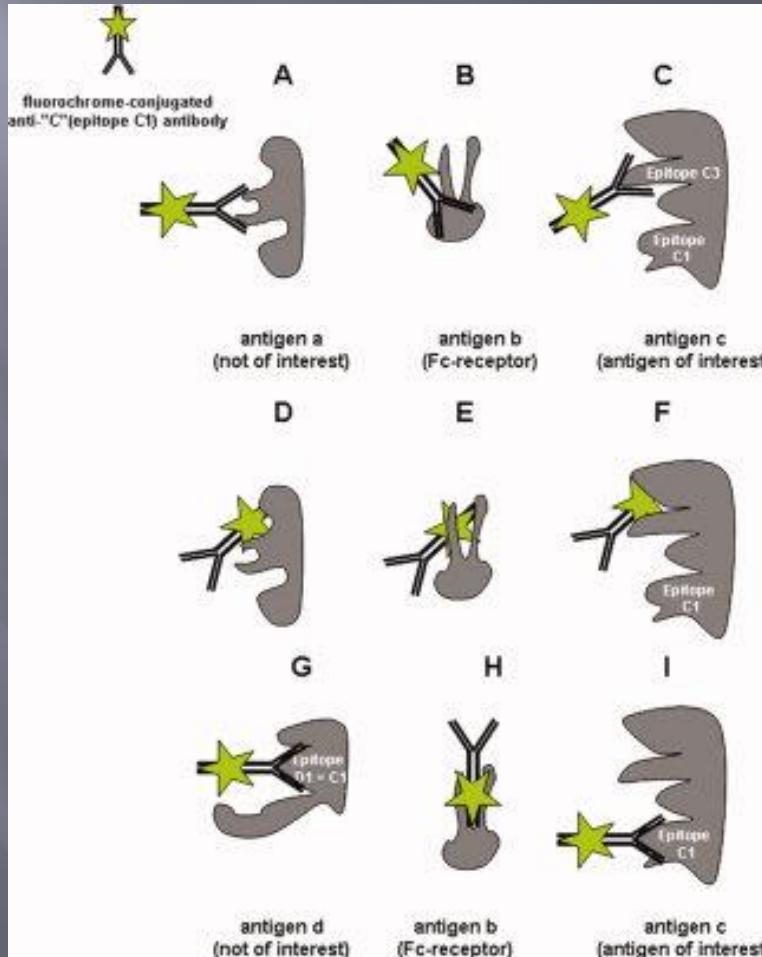


PE-CY5

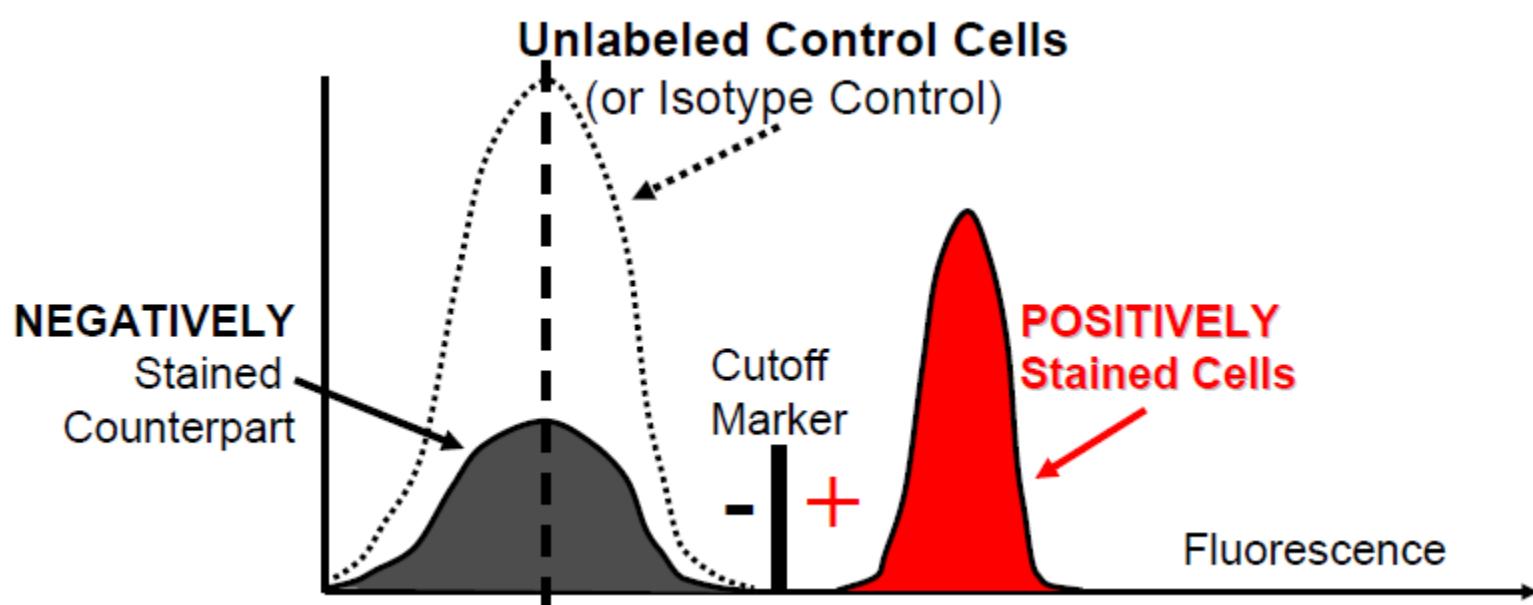
ΕΠΙΔΡΑΣΗ COMPENSATION



ΜΗ ΕΙΔΙΚΗ ΣΥΝΔΕΣΗ

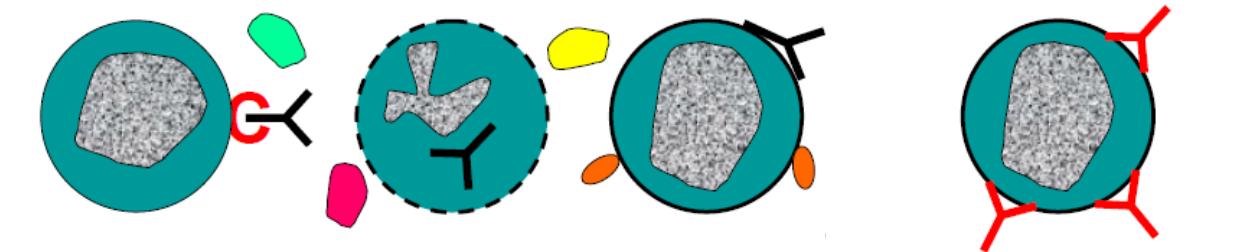


ΘΕΤΙΚΟ ή ΑΠΝΗΤΙΚΟ



ΙΣΟΤΥΠΙΚΟ ΚΟΝΤΡΟΛ

The Isotype Control



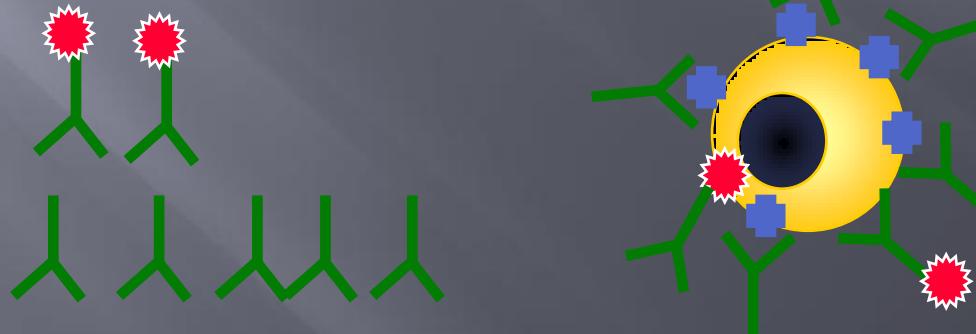
Ιδια υποτάξη

Ιδια χρωστική

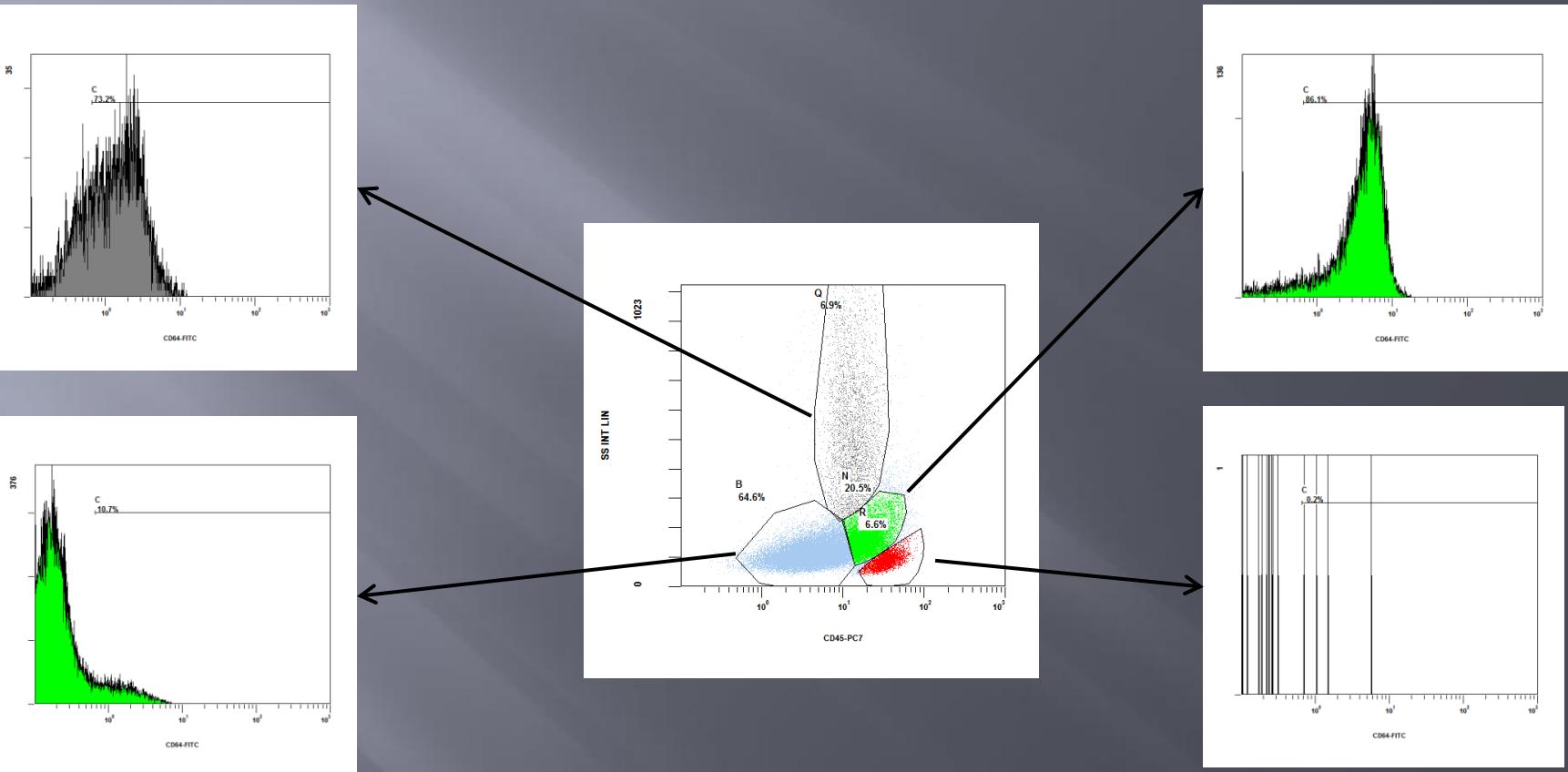
Ιδια F/P

Ιδια συγκέντρωση πρωτεΐνης

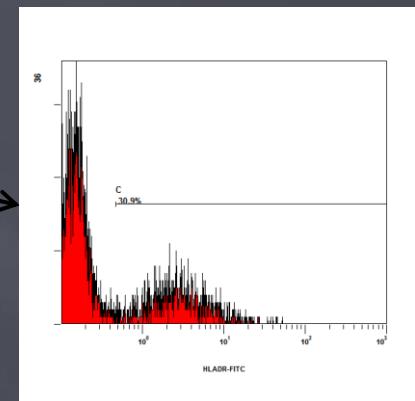
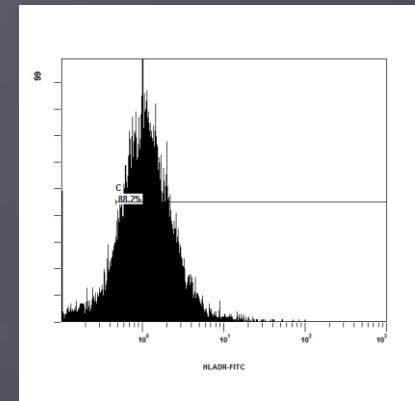
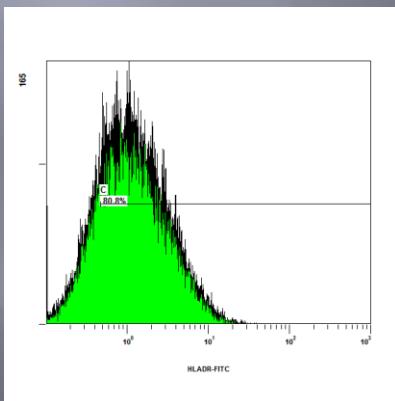
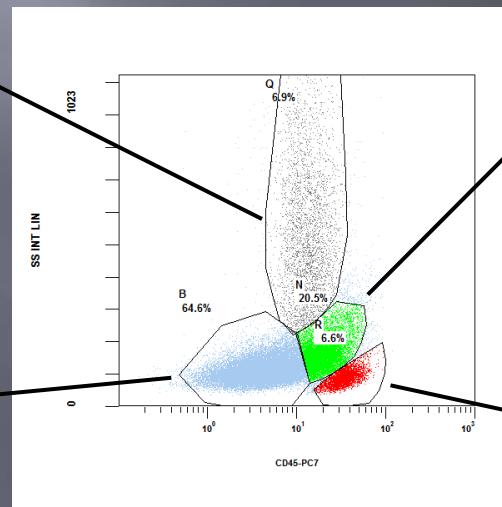
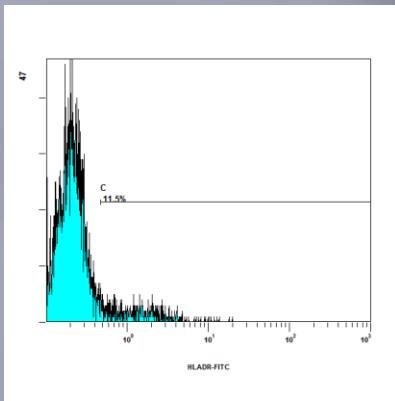
ΙΣΟΚΛΩΝΙΚΟ ΚΟΝΤΡΟΛ



ΕΣΩΤΕΡΙΚΟ ΚΟΝΤΡΟΛ



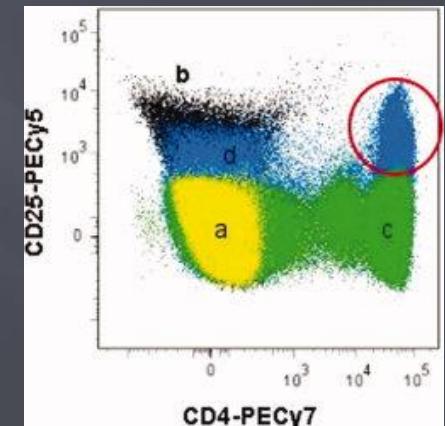
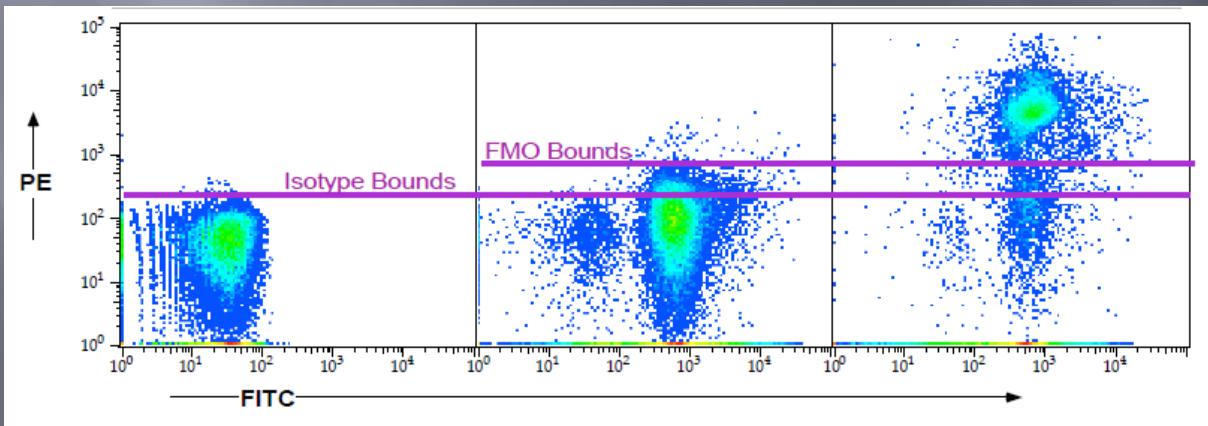
ΕΣΩΤΕΡΙΚΟ ΚΟΝΤΡΟΛ



FMO CONTROL

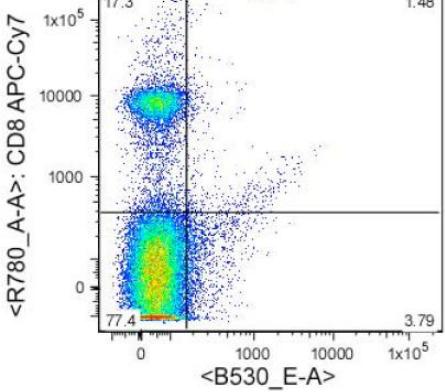
Fluorescence Minus One

	FITC	PE	PE-CY5	PE-CY7
1	CD3	CD4	CD8	CD45RO
2		CD4	CD8	CD45RO
3	CD3		CD8	CD45RO
4	CD3	CD4		CD45RO
5	CD3	CD4	CD8	

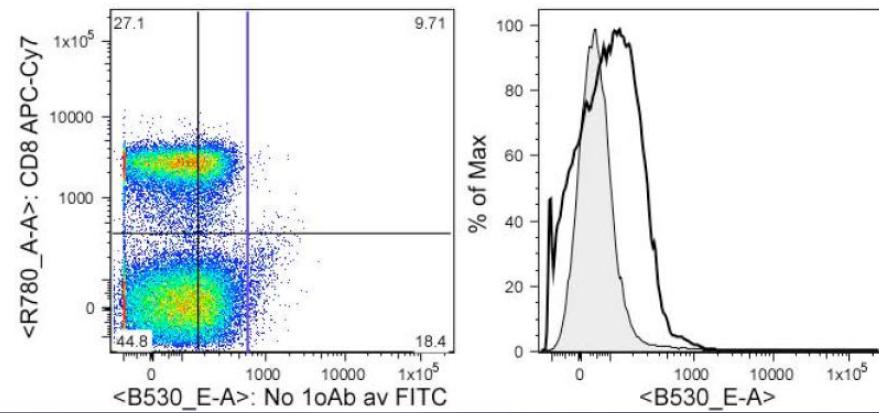


FMO CONTROL

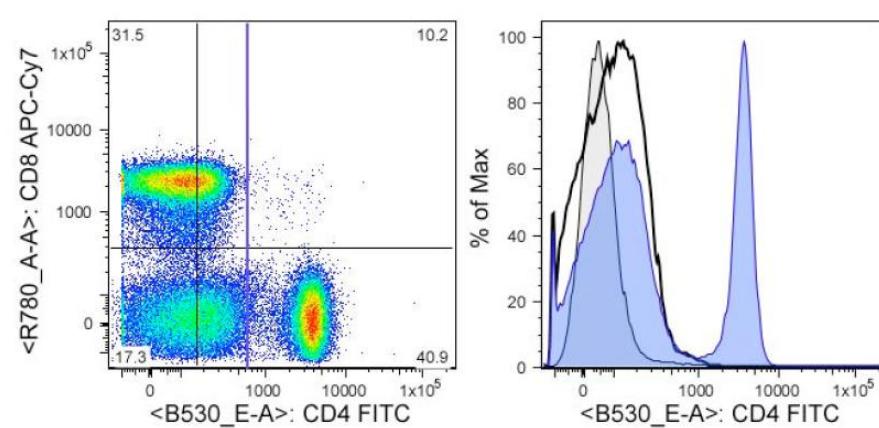
CD8 APC-Cy7 Only



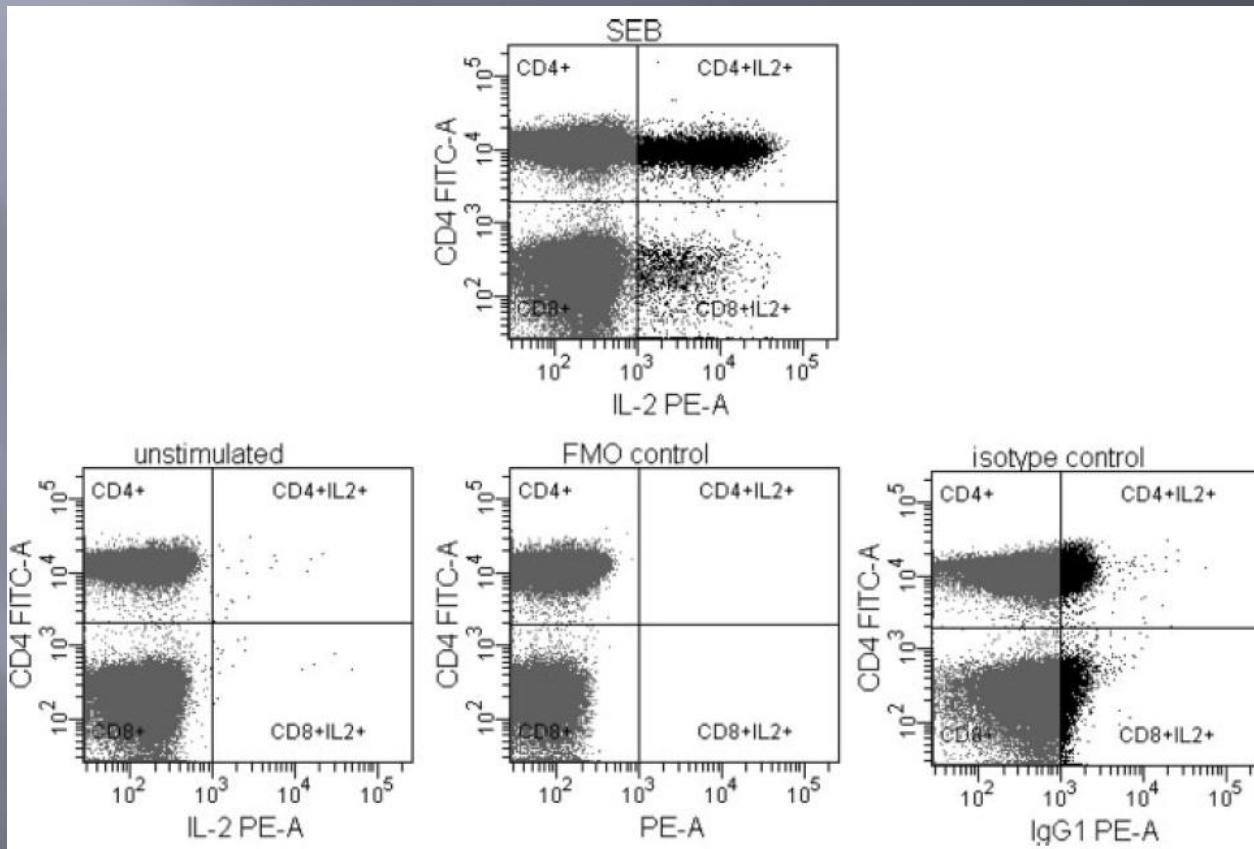
Fluorescence minus one



Fluorescence minus one

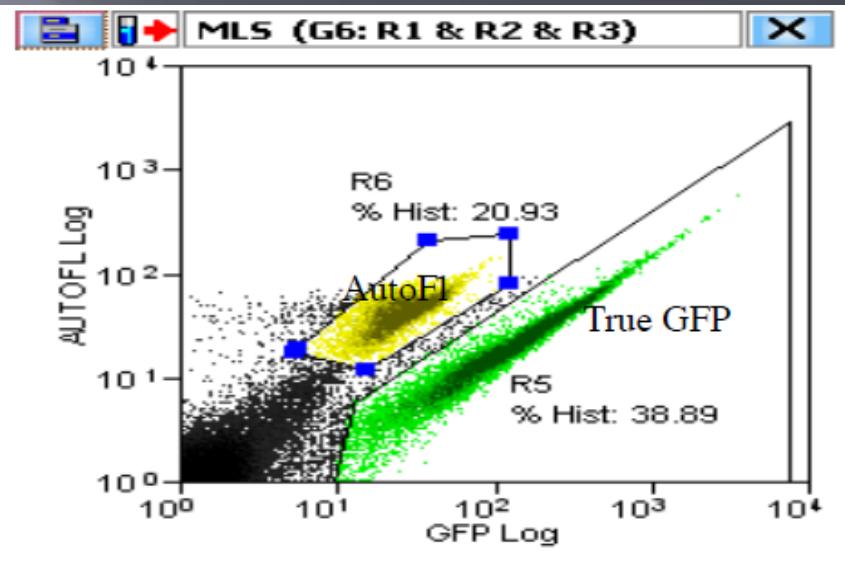
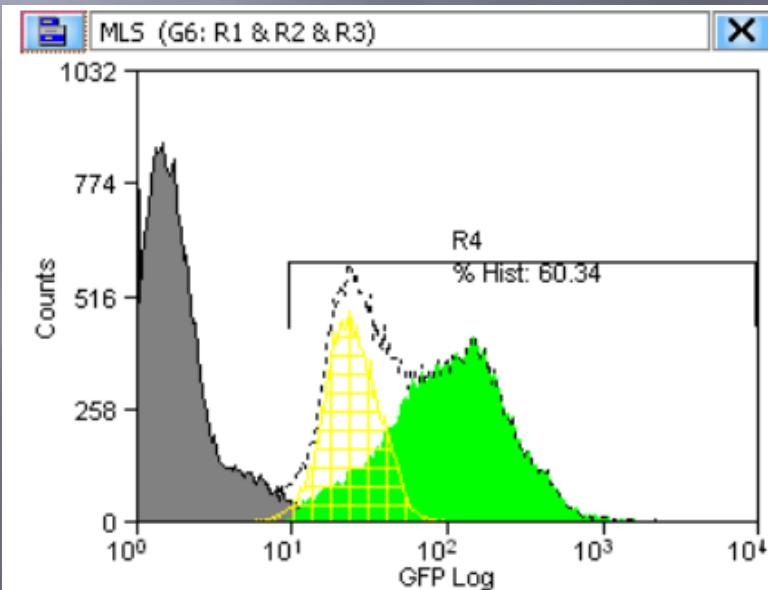


FMO CONTROL

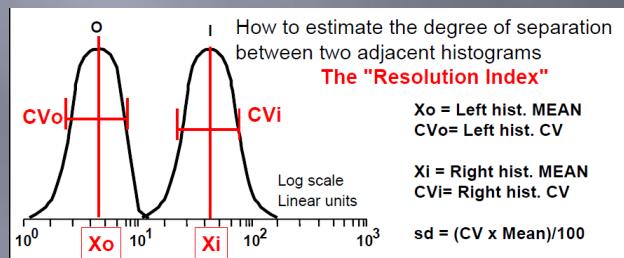
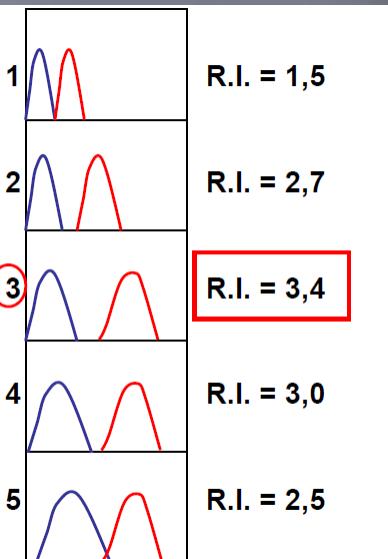
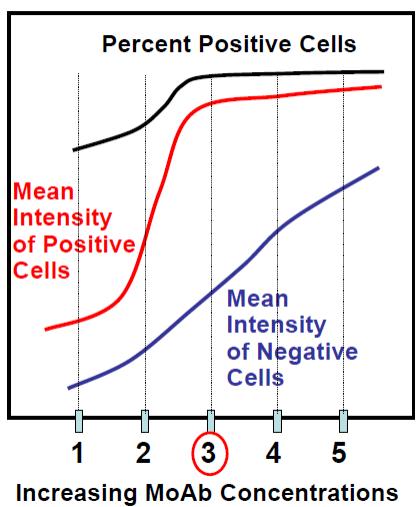


ΤΕΧΝΙΚΕΣ ΒΕΛΤΙΣΤΟΠΟΙΗΣΗΣ

- Απόσβεση Φθορισμού (Quenching)
- Εναλλακτικό Gating



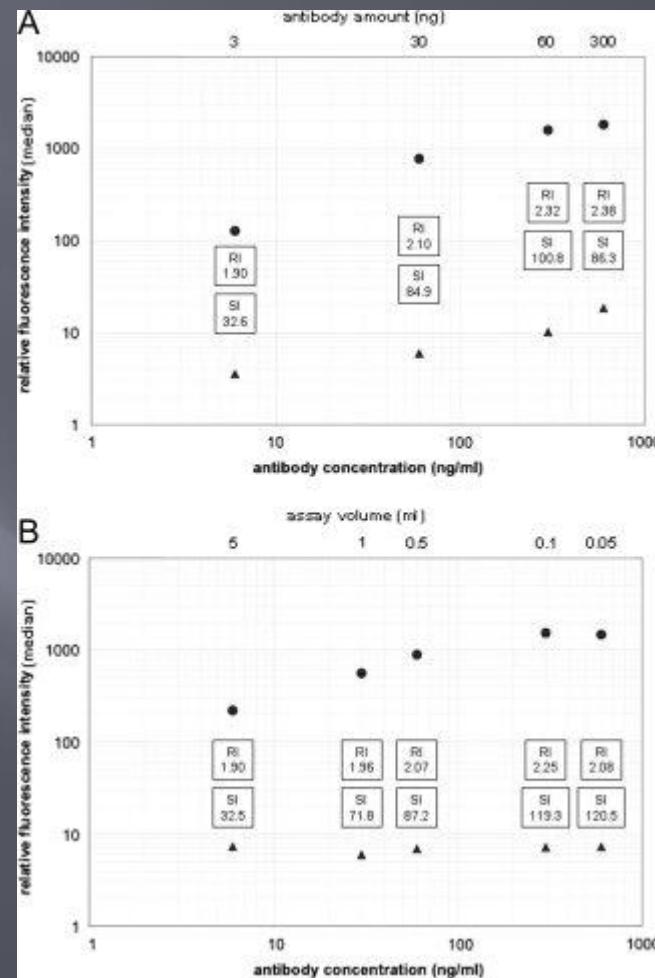
ΤΕΧΝΙΚΕΣ ΒΕΛΤΙΣΤΟΠΟΙΗΣΗΣ



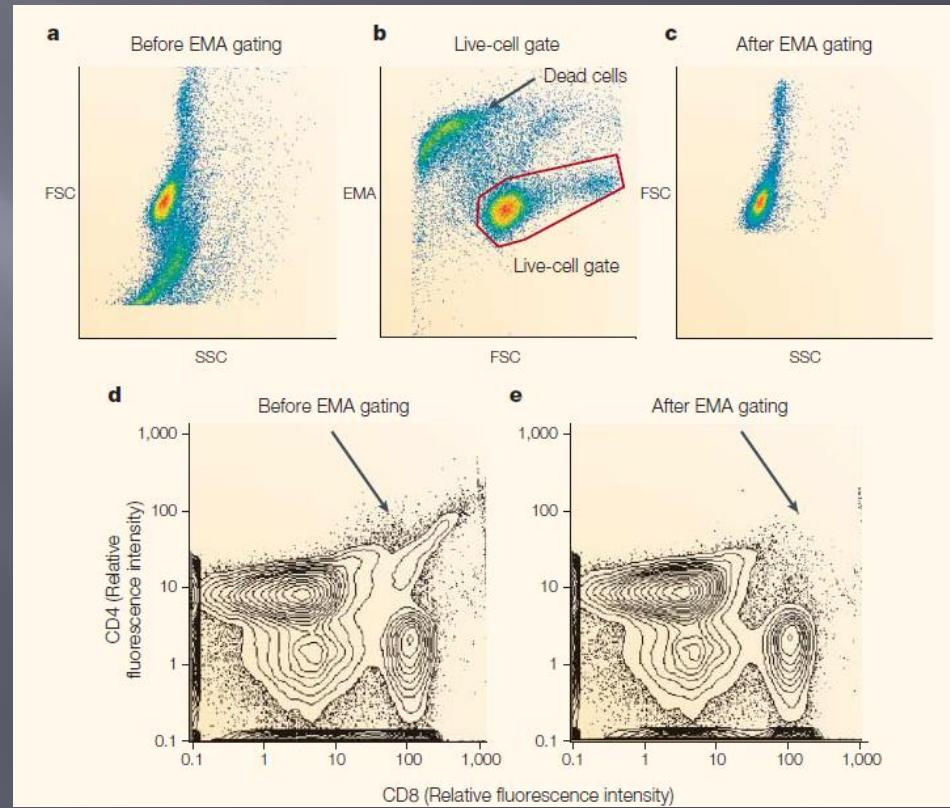
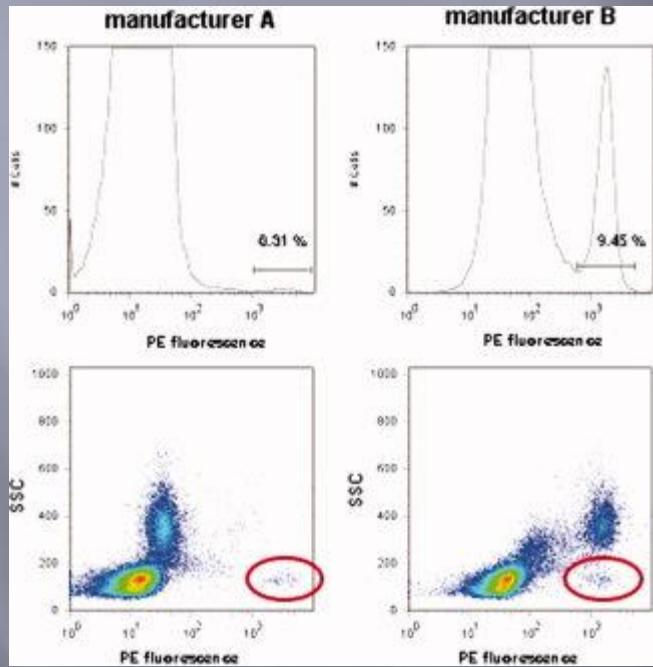
Difference between mean values

$$\text{Resolution Index} = \frac{Xi - Xo}{\sqrt{sdi^2 + sdo^2}}$$

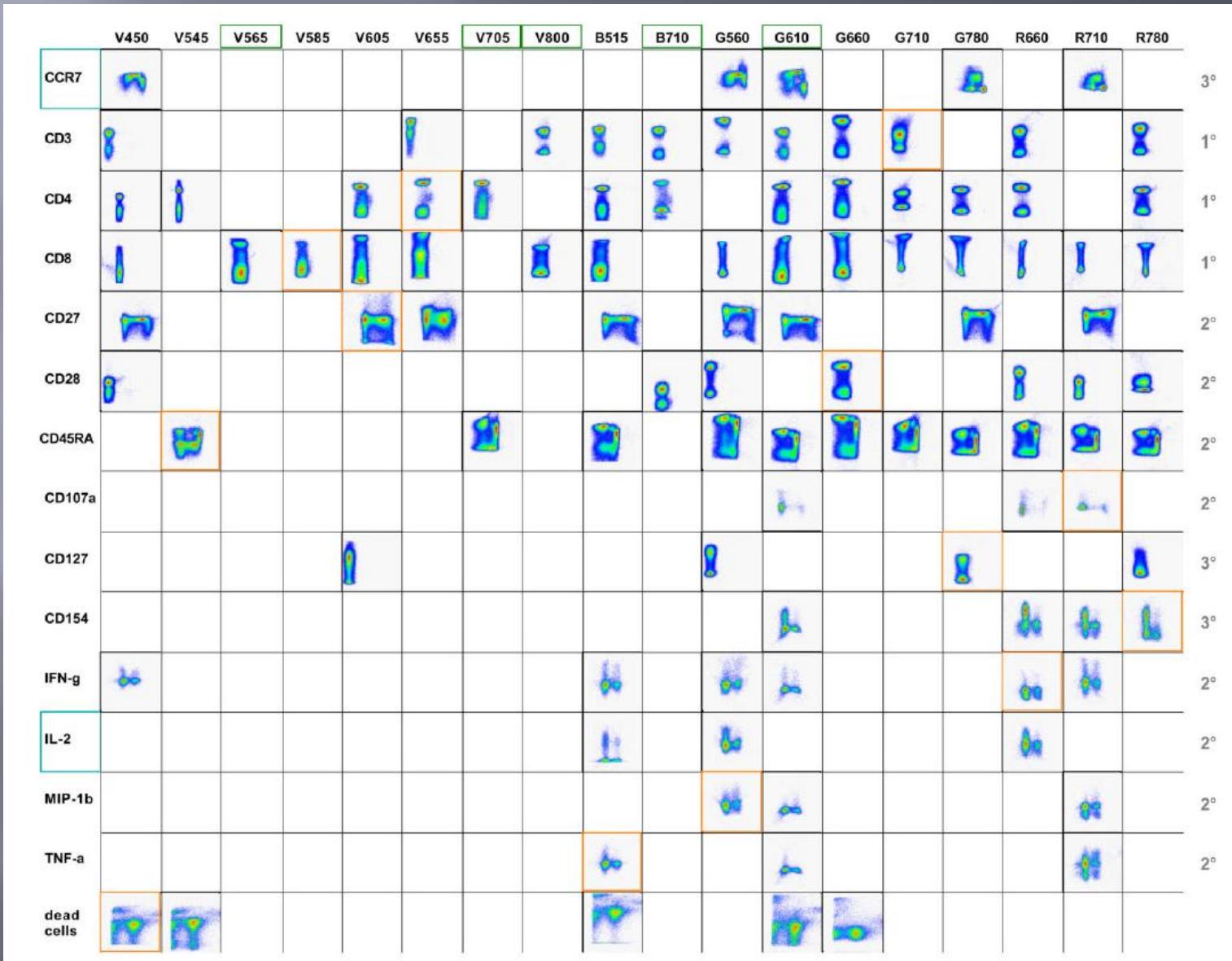
Global variance



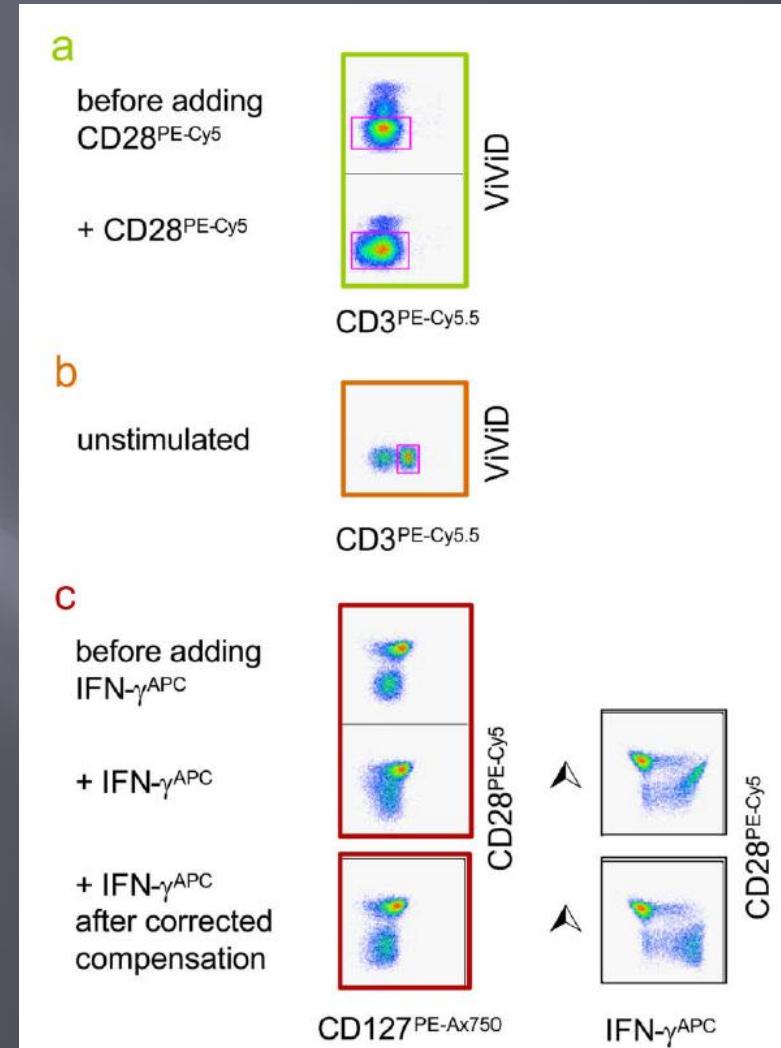
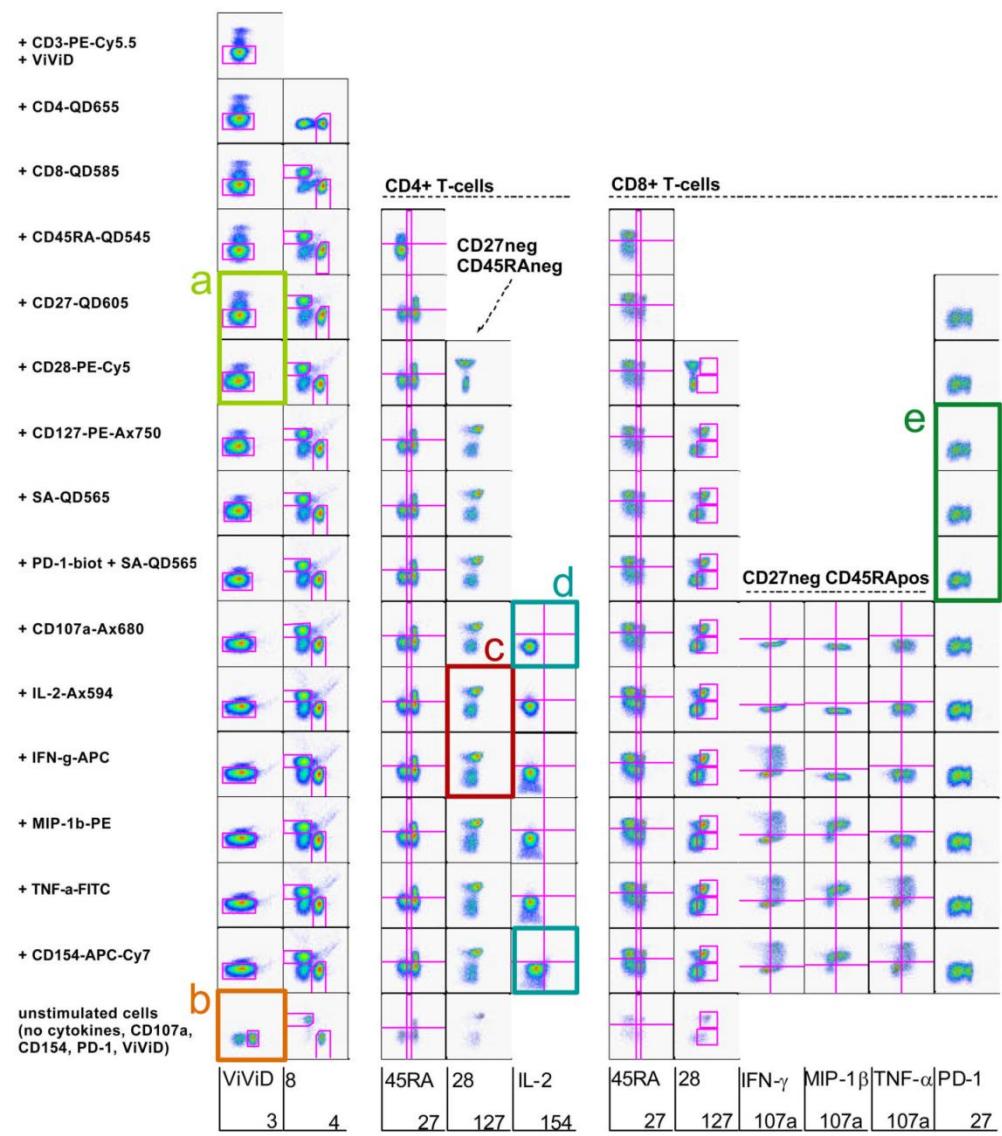
ΤΕΧΝΙΚΕΣ ΒΕΛΤΙΣΤΟΠΟΙΗΣΗΣ



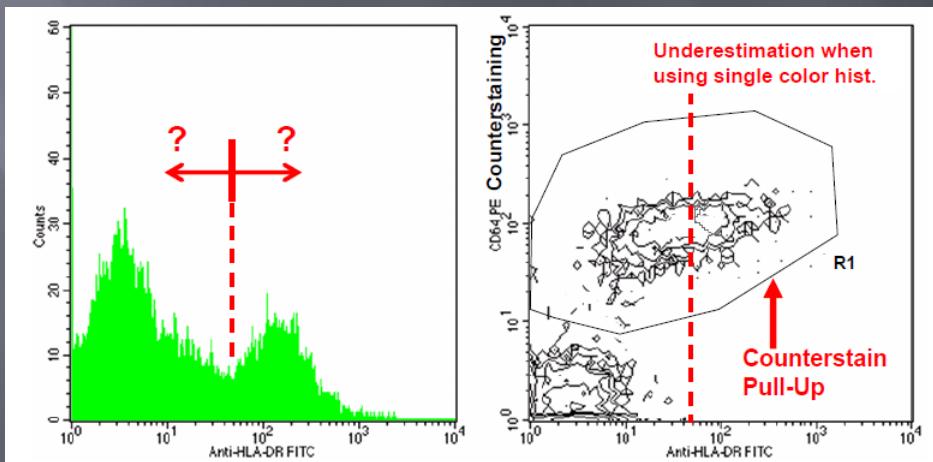
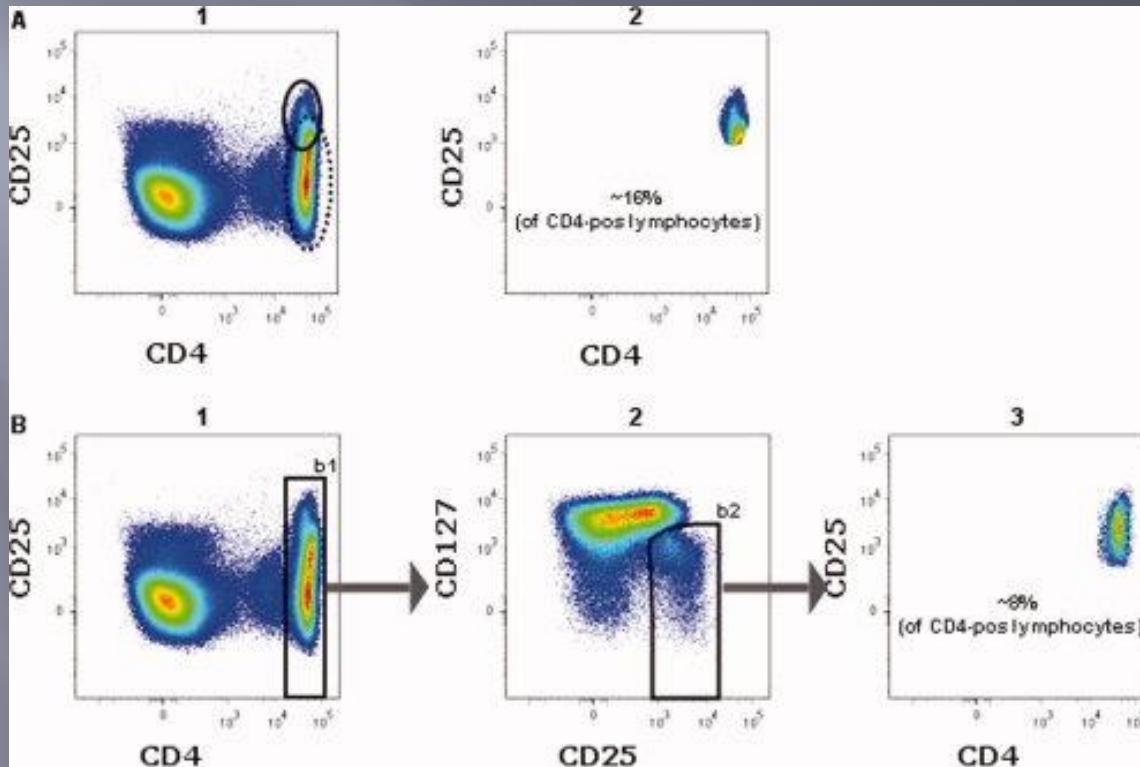
ΤΕΧΝΙΚΕΣ ΒΕΛΤΙΣΤΟΠΟΙΗΣΗΣ



ΤΕΧΝΙΚΕΣ ΒΕΛΤΙΣΤΟΠΟΙΗΣΗΣ



ΤΕΧΝΙΚΕΣ ΒΕΛΤΙΣΤΟΠΟΙΗΣΗΣ



BΙΒΛΙΟΓΡΑΦΙΑ

Considerations for the Control of Background Fluorescence in Clinical Flow Cytometry

Ruud Hulspas, Maurice R.G. O'Gorman, Brent L. Wood, Jan W. Gratama, and D. Robert Sutherland Cytometry Part B 2009; 76B: 355-364.

Isotype Controls in the Analysis of Lymphocytes and CD34 Stem and Progenitor Cells by Flow Cytometry—Time to Let Go!

M. Keeney, J.W. Gratama, I.H. Chin-Yee, D.R. Sutherland Cytometry (Communications in Clinical Cytometry) 34:280-283 (1998)

Isotype Controls – Time to Let Go?

Maurice R.G. O'Gorman^{1*} and Joanne Thomas² Cytometry (Communications in Clinical Cytometry) 38:78-80 (1999)

Positive or Negative ? That is the question

Bruno Brando 3rd European Course on Clinical Cytometry, Antwerp, Belgium, 4-5 September 2007

Technical Note: Flow Cytometry Controls, Instrument Setup and the Determination of Positivity

Holden T. Maecker* and Joseph Trotter Cytometry Part A 69A:1037-1042 (2006)

Seventeen-colour flow cytometry: unravelling the immune system

Stephen P. Perfetto, Pratip K. Chattopadhyay and Mario Roederer Nature (August 2004) vol4 p:648-655

Selecting Fluorochrome Conjugates for Maximum Sensitivity

Holden T. Maecker,* Tom Frey, Laurel E. Nomura, and Joe Trotter Cytometry Part A 62A:169-173 (2004)

Publication of Optimized Multicolor Immunofluorescence Panels

Yolanda Mahnke, Pratip Chattopadhyay,Mario Roederer Cytometry Part A • 77A: 814818, 2010

A practical approach to multicolor flow cytometry for immunophenotyping

Nicole Baumgartha , Mario Roedererb Journal of Immunological Methods 243 (2000) 77-97

New Approaches to Multicolor Compensation & Data Visualization In Flow Cytometry

Dr. Cynthia Guidos, Flow Cytometry Workshop for the Department of Immunology June 2, 2008 MSB 4279

Flow Cytometry Data: how to make sense of it?

Adrian Smith Advanced Cytometry Facility Centenary Institute, The University of Sydney, Bosch Institute

Optimizing a Multi-colour Immunophenotyping Assay

Yolanda D. Mahnke, Mario Roederer Clin Lab Med. 2007 September ; 27(3): 469-v

Nine-Color Flow Cytometry for Accurate Measurement of T Cell Subsets and Cytokine Responses. Part I: Panel Design by an Empiric Approach

Bridget E. McLaughlin,¹ Nicole Baumgarth,² Martin Bigos,³ Mario Roederer, Stephen C. De Rosa,⁵ John D. Altman,⁶ Douglas F. Nixon,⁷ Janet Ottinger, Carol Oxford,⁹ Thomas G. Evans,¹⁰ David M. Asmuth Cytometry Part A 73A: 400410, 2008