



ESCCA

European Society
for Clinical Cell Analysis

EUROPEAN CERTIFICATE FOR CYTOMETRY OPERATORS
GUIDELINES AND SUGGESTED EDUCATIONAL RESOURCES

www.escca.eu

1. BACKGROUND

The European Society for Clinical Cell Analysis (ESCCA) is committed to promote high quality education in Cytometry. Since its foundation, ESCCA has fostered its own continuous Educational Program, coordinated by ESCCA Board and Scientific Committees. In addition, ESCCA collaborates with other institutions in the diffusion of educational activities of relevance in Cytometry and related fields.

The ESCCA Education, Certification & Quality Assessment Committee is aware of the technical complexity and constant evolution of cytometry instrumentation and its applications, requiring constant training and education. These issues have been covered traditionally by the Education Activities incorporated in the ESCCA Conferences and, by the ESCCA International and Local Schools on Cytometry.

The final goal of the educational efforts of ESCCA is to help ESCCA members, and especially the young ones, to attain excellence in their work in cytometry, be it in the clinical or in the basic fields. In order to provide our members with a means to certify their knowledge and skill in clinical flow cytometry, the ESCCA Education, Certification & Quality Assessment Committee provides the European Cytometry Certificate.

Each year, the ESCCA Board will award the best performing candidate who achieved a score of 90% or higher for the examination. The awardee will receive **an outstanding achievement recognition and a free registration** for the upcoming ESCCA Conference.

2. THE EUROPEAN CYTOMETRY CERTIFICATE

The European Cytometry Certificate has the following certifications:

- **The European Certificate for Cytometry Operators:**

This first level of certification may be attained after evaluation of the candidate's knowledge about basic flow cytometry as further described in this document. Applicants should demonstrate a minimum of three years of experience in flow cytometry. The first level of ESCCA Certificate Examination is available online and open for applications.

- **The European Certificate for Cytometry Specialists - hemato-oncology:**

This second level of certification may be achieved after evaluation of the candidate's skills and knowledge about clinical flow cytometry-hemato-oncology. Eligible candidates should satisfy the following requirements:

- a. Baccalaureate and/or Master degree and/or Doctorate from an accredited/approved educational institution in biological/life science, physics, engineering or an appropriately related field;

- b. Minimum of three years acceptable laboratory experience in cytometry (clinical, research, industry or veterinary) in an ISO 15189, JCI, CAP accredited laboratory or laboratory authorized by a governing regulatory association or Ministry.

Certification consists of an online examination and revision of the candidate's CV by the ESCCA Education, Certification & Quality Assessment Committee.

3. THE EUROPEAN CERTIFICATE FOR CYTOMETRY OPERATORS: RULES

1. The European Certificate for Cytometry Operators is also available for non-members.
2. The examination language is English.
3. The fee for the examination and certification is € 100 (members) or € 250 (non-members, including a 1 year- ESCCA membership).
4. Candidates should demonstrate a minimum of three years of experience in flow cytometry. The candidate's experience should be attested by the director of the employing facility or other entitled authority.
5. The examination consists of 100 multiple choice questions and will last a maximum of 2 hours.
6. The examination will be considered as passed if at least 60 questions are correctly answered.
7. The content of the examination and examples of questions are described later in this document.
8. Candidates who successfully pass the examination will receive a certificate and will be named on a dedicated page of ESCCA website and, when convenient, in the site of ESCCA-affiliated society.
9. Certification will expire after three years.
10. Reinstatement of certification before the date of expiration is free of charge upon proof of continuous education and practice in flow cytometry from an approved educational institution or other entitled authority. The proof should be addressed to the ESCCA Exam Committee and emailed to membership@escca.eu
11. For reinstatement of certification after the date of expiration but no more than 5 years, submission of an application for certificate reinstatement and a completed declaration form documenting all continuing education earned within the previous years is required. The reinstatement fee is 50 euros, for which an invoice will be sent. Reinstatement will take effect after receipt of the amount due
12. Reinstatement of expired certificate for more than 5 years is not possible. In such cases individuals will be required to retake and pass the certification examination in order to reinstate their certification.

4. THE EUROPEAN CERTIFICATE FOR CYTOMETRY OPERATORS: PRACTICAL ASPECTS

1. The examination for the European Certificate for Cytometry Operators will be proceeded online through the Moodle platform of the ESCCA website anytime during the year, except during public holidays.
2. The application process is as follows:
 - The candidate should apply for the examination via the [ESCCA membership section](#) by completing the online registration form in the section 'ESCCA Certification Exam'.
 - An official attestation of the number of years of experience of the candidate, issued by the director of the employing facility or other entitled authority, should be uploaded in the online registration form.
 - The exam can take place from Monday - Friday between 09.00 - 16.00 hrs. CET. All international public holidays are excluded.

Two dates must be selected in the registration form: the preferred date and a back-up date. The exam can take place from 1 month after the submission of the application. Confirmation of the date is subject to the availability of the exam supervisors. The final date will be confirmed in the notification of acceptance.
 - The registration fee of €100 (members) or € 250 (non-members) can be paid by iDeal (Dutch candidates only) or credit card.
 - After submission of the registration your application will be reviewed by the Exam Committee. The candidate will receive a notification of acceptance or rejection by email within 2 weeks after submission of the registration form.
 - In case the application is rejected, the registration fee will be reimbursed.
 - If a candidate fails the exam, the registration fee cannot be reimbursed.

5. THE EUROPEAN CERTIFICATE FOR CYTOMETRY OPERATORS: GUIDELINES

A. Examination content, relative weight of enlisted topics:

- Basic flow cytometry: 40%
- Sample preparation: 10%
- Data acquisition and analysis: 10%
- Validation and quality control: 10%
- Haematology: 10%
- Immunology: 10%
- Stem cell transplantation: 5%
- Cell cycle analysis: 5%
- Functional analysis: 5%
- Apoptosis: 5%

B. Examples of questions:

Basic flow cytometry

A 605 BP is an optical filter that:

- A) Allows passage of light with a wavelength longer than 605 nm
- B) Allows passage of light with a wavelength shorter than 605 nm
- C) Allows passage of a narrow range of wavelengths centred around 605 nm**
- D) Blocks a narrow range of wavelengths centred around 605 nm

Sample preparation

Fluorescently-labelled antibodies are sensitive to:

- A) Temperature and light**
- B) pH and vibrations
- C) All of the above
- D) None of the above

Data acquisition and analysis

According to Boolean criteria, the gate “A or (B and C)” encompasses:

- A) The events which are in the gate A, B, and C
- B) The events which are in the gate A and B, but not in C
- C) The events in the gate A, plus the events common to gate B and C**
- D) The events in the gate A, with the exception of the events common to gate B and C

Validation and quality control

Clinical specificity measures:

- A) The proportion of positives that are correctly identified as such
- B) The proportion of negatives that are correctly identified as such**
- C) The repeatability, or reproducibility of the measurement
- D) The proximity of measurement results to the true value

Haematology

The recommended markers to confirm the myeloid lineage in acute leukemias are:

- A) CD13, CD33
- B) CD34, CD117
- C) CD15, CD65
- D) MPO and ≥ 2 of the following markers: CD11c, CD14, CD64, lysozyme**

Immunology

Which phenotype is correlated with cytotoxic T cells:

- A) CD3+, CD8+, CD5+
- B) CD3+, CD4+, Granzyme B+**
- C) CD3+, CD8+, CD2+(dim)
- D) CD3+, CD4+, CD2+(dim)

Stem cell transplantation

Which of the parameters are used to enumerate viable CD34+ cells in a stem cell product?

- A) SSC and FSC
- B) 7-AAD and CD45
- C) All of these**
- D) None of these

Functional analysis

Which of the following fluorochromes is used to detect intracellular superoxide ion:

- A) Dihydrodichlorofluorescein diacetate
- B) 4-amino-5-methylamino-2', 7'-difluorofluorescein diacetate (DAF)
- C) Dihydrorhodamine 123
- D) Dihydroetidine**

Apoptosis

The monoclonal antibodies against the BCL-2 protein used in studies of apoptosis:

- A) Bind to a surface molecule expressed in apoptosis-resistant cells
- B) Bind to a surface molecule expressed in apoptosis-sensitive cells
- C) Bind to an intracellular molecule expressed in apoptosis-resistant cells
- D) Bind to an intracellular molecule expressed in apoptosis-sensitive cells**

6. SUGGESTED BIBLIOGRAPHY AND RESOURCES AVAILABLE IN THE INTERNET

A. BOOKS

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<http://www.beckman.com/coulter-flow-cytometry/practical>

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<http://flowbook.denovosoftware.com/>

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<http://www.beckman.com/coulter-flow-cytometry/first-principles>

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<http://www.intechopen.com/books/editor/flow-cytometry-recent-perspectives>

Schmid, I, Ed. (2012) Clinical Flow Cytometry-Emerging Applications. InTech Open Science

<http://www.intechopen.com/books/clinical-flow-cytometry-emerging-applications>

Schmid, I, Ed. (2016) Flow Cytometry - Select Topics. InTech Open Science
<http://www.intechopen.com/books/editor/flow-cytometry-select-topics>

B. BASIC GUIDES

GENERAL ASPECTS

MDBioproducts, Flow Cytometry Guide
www.mdbiosciences.com

Chromocyte, A Beginners Guide to Flow Cytometry
www.chromocyte.com

Rahman M, Introduction to Flow Cytometry. AbD-Serotec
<https://www.abdserotec.com/introduction-to-flow-cytometry.html>

FLUORESCENCE

Principles of fluorescence, Imperial College London
<http://www.imperial.ac.uk/media/imperial-college/medicine/facilities/film/Fluorophores-website.pdf>

Chapter 2 - Principles of Fluorescence- AbD Serotec
<https://www.abdserotec.com/introduction-to-flow-cytometry.html#chapter2>

AbCam Fluorochrome chart – a complete guide
<http://docs.abcam.com/pdf/secondary-antibodies/abcam-fluorochrome-chart.pdf>

The Fluorescent Protein Color Palette
<http://www.microscopyu.com/pdfs/FPCOLORpalette.pdf>

Tandem Dyes-Biolegend
http://www.biolegend.com/tandem_dyes

Introduction to Click Chemistry
<http://www.lumiprobe.com/click-chemistry>

PANEL DESIGN, SETUP AND COMPENSATION

The Stain Index: What Is It and What Does It Tell You?

<http://www.biolegend.com/newsdetail/1245/>

Biolegend Panel Selector

<https://www.biolegend.com/panelselector>

Biolegend Panel Construction

https://www.biolegend.com/custom_panel_construction

C. REVIEWS

GENERAL ASPECTS

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http://iti.stanford.edu/content/dam/sm/iti/documents/himc/Bendall_etal_Trends-Immunology_2012.pdf

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David A. Basiji, William E. Ortyrn, Luchuan Liang, Vidya Venkatachalam, Philip Morrissey. Cellular Image Analysis and Imaging by Flow Cytometry Clin Lab Med. Author manuscript; available in PMC 2008 September 1. doi: 10.1016/j.cll.2007.05.008
<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2034394/pdf/nihms29623.pdf>

Bendall, SC; Simonds EF, Qiu P, Amir ED, Krutzik PO, Finck R, Bruggner RV, Melamed R, Trejo A, Ornatsky OI, Balderas RS, Plevritis SK, Sachs K, Pe'er D, Tanner SD, Nolan GP (6 May 2011). "Single-Cell Mass Cytometry of Differential Immune and Drug Responses Across a Human Hematopoietic Continuum". Science 332 (6030): 687–696
doi:10.1126/science.1198704

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doi:10.1016/j.copbio.2014.07.004

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Erika A O'Donnell, David N Ernst, Ravi Hingorani. Multiparameter Flow Cytometry: Advances in High Resolution Analysis. Immune Netw. 2013 April; 13(2): 43–54. Published online 2013 April 30. doi: 10.4110/in.2013.13.2.43
<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3659255/pdf/in-13-43.pdf>

FLUORESCENCE

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<http://www.intechopen.com/books/biosensors-for-health-environment-and-biosecurity/fluorescent-biosensors-for-protein-interactions-and-drug-discovery>

Nathir A. F. Al-Rawashdeh (2012). Current Achievement and Future Potential of Fluorescence Spectroscopy, Macro To Nano Spectroscopy, Dr. Jamal Uddin (Ed.), ISBN: 978-953-51-0664-7, InTech, DOI: 10.5772/48034.

<http://www.intechopen.com/books/macro-to-nano-spectroscopy/current-achievement-and-future-potential-of-fluorescence-spectroscopy>

Steven Ripp, Gary Sayler and Dan Close (2011). Mammalian-Based Bioreporter Targets: Protein Expression for Bioluminescent and Fluorescent Detection in the Mammalian Cellular Background, Biosensors for Health, Environment and Biosecurity, Prof. Pier Andrea Serra (Ed.), ISBN: 978-953-307-443-6, InTech, DOI: 10.5772/17028.

<http://www.intechopen.com/books/biosensors-for-health-environment-and-biosecurity/mammalian-based-bioreporter-targets-protein-expression-for-bioluminescent-and-fluorescent-detection>

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PANEL DESIGN, SETUP AND COMPENSATION

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Mahnke Y, Chattopadhyay P, Roederer M. Publication of optimized multicolor immunofluorescence panels. Cytometry, 77A: 814–818. doi: 10.1002/cyto.a.20916.

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DATA ANALYSIS

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<http://www.nature.com/nmeth/journal/v10/n3/pdf/nmeth.2365.pdf>

BIOSAFETY

Flow cytometry: Biosafety recommendations and protective measures
<http://www.biosafety.be/CU/FlowCytometry/FCMMenu.html>

Biosafety in Flow Cytometry – To Be or Not to Be...
<http://bitesizebio.com/21608/biosafety-in-flow-cytometry-to-be-or-not-to-be/>

D. WEBINARS, VIDEOS AND ANIMATIONS

GENERAL ASPECTS

History of Flow Cytometry-BioLegend
<https://www.biolegend.com/historyofflow>

Molecular Probes—Introduction to Flow Cytometry

<https://youtu.be/sfWWxFBltPQ>

Introduction to Flow Cytometry Webinar

<https://youtu.be/o2joszUiVhM>

MCBC Flow Cytometry Training Course - Session 1

<https://youtu.be/fMNNXlh4OkQ>

Flow Cytometry-Genesync

<https://youtu.be/6j-AzBocWKw>

Flow Cytometry lecture part 1

<https://youtu.be/YPb9Pfp66c?list=PL1DA0F59A86AFCE59>

Flow Cytometry lecture part 2

<https://youtu.be/YEi96A7L1rU?list=PL1DA0F59A86AFCE59>

Flow Cytometry lecture part 3

<https://youtu.be/JXovmJJOjs8?list=PL1DA0F59A86AFCE59>

FLUORESCENCE

Fluorescence Tutorials-Thermofisher

<http://www.thermofisher.com/es/en/home/support/tutorials.html>

Chemwiki

http://chemwiki.ucdavis.edu/Core/Physical_Chemistry/Spectroscopy/Electronic_Spectroscopy/Fluorescence

Molecular Probes Tutorial Series—Introduction to Fluorescence

<https://youtu.be/SGFlr1jFNBM>

Lecture 4 part 1 (fluorescence, Jablonski diagram):

<https://youtu.be/5KLBrnaulg>

Lecture 4 part 2 (fluorescence spectral distribution, parameters)

https://youtu.be/PYmjrl_8OY0

Lecture 4 part 3 (fluorescence microscope, applications of fluorescence, photobleaching)

<https://youtu.be/ywE6VaVm5kg>

Lecture 4 part 4 (FRET)

https://youtu.be/JH2LIffu_7I

Microscopy: Introduction to Fluorescence Microscopy (Nico Stuurman)

<https://youtu.be/AhzhOzgYogw>

Microscopy: Fluorescent Proteins (Roger Tsien)

<https://youtu.be/gK9aYnklr3w>

The expanding palette of fluorescent proteins

<https://youtu.be/n7f1-PttVcs>

Nobel Laureate Martin Chalfie - "Green Fluorescent Protein: Lighting up Life"

<https://youtu.be/YCY0Inhb4oI>

What are Quantum Dots?

<https://youtu.be/LIPDyl53rZA>

SAMPLE PREPARATION

Techniques of Breast Biopsy - Manipal Hospital

<https://youtu.be/ZcWOPmyPj68>

Dividing the FNA aspirate sample for ancillary testing

<https://youtu.be/J3gBCqAu3GM>

DNA content cell cycle analysis using flow cytometry

<https://youtu.be/MIE0Xnr9oz>

DATA ANALYSIS

Molecular Probes Tutorial Series—Analyzing Flow Cytometry Data

<https://youtu.be/ccR5snuCE80>

Basics of flow cytometry, Part I: Gating and data analysis

https://youtu.be/y9-mojIXU_I

INSTRUMENTATION

Beckman Coulter Flow Cytometry

<https://www.youtube.com/playlist?list=PL1DA0F59A86AFCE59>

Gallios Flow Cytometer

<https://youtu.be/5TtOpfYwqoQ>

Gallios Cytometer Tour Guides

<http://beckman.eu/assets/training/flowcytometry/flowcytometer/index.html>

FC500 Flow Cytometer

<https://youtu.be/kLcf7QsSfrQ>

Modifying the FC500 Flow Cytometer with Multiple Lasers

<https://youtu.be/XygiLak2BUM>

Flow Cytometry -Beckman Coulter Life Sciences

https://www.youtube.com/playlist?list=PLzfAZrs5hqGdSCbWAv5Sjzm_J7761PoyA

"The BD Accuri C6 Flow Cytometer"-David Lee, BD Biosciences

<https://youtu.be/k0QhLWk3RO4>

Accuri C6 Flow Cytometer.mp4

<https://youtu.be/gz09Oi3ci8A>

Accuri Cytometers

<https://youtu.be/6lqvpykoqil>

Displaying Accuri CFlow Data and Using the CFlow Importer in FCS Express

<https://youtu.be/9HTSgzBJ4v4>

Becton Dickinson Flow cytometry

<https://www.youtube.com/playlist?list=PLrTm-FBR3jxT9sJOH8BTakbhokek1X2fc>

Attune-Thermo Fisher Scientific Flow Cytometry

https://www.youtube.com/playlist?list=PLGlVFEwL2wDHYu3pyBrrkClt_0jRuRcao

Milteny Biotec Flow Cytometry

<https://www.youtube.com/playlist?list=PL5EpKG-c5XfrPax8A-Oh3sHy4CLuJRkQ>

ADVANCES IN CYTOMETRY

ACOUSTIC PRE-FOCUSING CYTOMETRY

The Discovery of Acoustic Focusing & the Attune® Flow Cytometer

<https://youtu.be/b2ilHENugE0>

Attune® Acoustic Focusing Cytometer Tutorial

<https://youtu.be/kpkL2EEJDsU>

The Next Generation in Acoustic Cytometry

<https://youtu.be/Q1PIICS5VnM>

HyperCyt revisited

<https://youtu.be/jf-1Q3QZ6Oc>

MASS-SPECTROMETRY CYTOMETRY

National CyTOF Meeting 2014: Scott Tanner, PhD, Fluidigm Corp

<https://youtu.be/HnUVWIhKA3k>

SPECTRAVIEWERS

eBioscience

<http://www.ebioscience.com/resources/fluorplan-spectra-viewer.htm>

ThermoFisher

<https://www.thermoFisher.com/es/en/home/life-science/cell-analysis/labeling-chemistry/fluorescence-spectraviewer.html>

BioLegend

<http://www.biolegend.com/spectraanalyzer>

Becton Dickinson

<http://m.bdbiosciences.com/us/s/spectrumviewer>

E. OTHER RECOMMENDED SITES FOR EDUCATIONAL RESOURCES

Chromocyte

<https://www.chromocyte.com/>

Purdue University Cytometry Labs (PUCL)

<http://www.cyto.purdue.edu/>

Cytobank

www.cytobank.org

FlowRepository

www.flowrepository.org

Bitesize Bio

<http://bitesizebio.com/category/technical-channels/flow-cytometry/>

Work-Flow

<https://work-flow.tech/flowpublic/>

Websites of Cytometer-Manufacturing Companies

ACEA Biosciences, Inc.

<http://www.aceabio.com/>

Apogee Flow Systems

<http://www.apogeeflow.com/>

Beckman Coulter

<http://beckman.es/coulter-flow-cytometry>

Becton Dickinson Biosciences

<http://www.bdbiosciences.com/eu/applications/s/flowcytometry?WT.srch=1&gclid=CP6gi-ql8swCFU4o0wodSOsLxg>

Bio-Rad

<http://www.bio-rad.com/es-es/category/flow-cytometry>

Standard BioTools

<https://www.standardbio.com/products/instruments/helios-a-cytof-system>

Merck-Millipore

http://www.merckmillipore.com/ES/es/products/life-science-research/cell-analysis/yjSb.qB.uBwAAAE_3S53.M6W,nav

Miltenyi Biotec

<http://www.miltenyibiotec.com/en/products-and-services/mac-flow-cytometry.aspx>
[Propel Labs](#)

Sony Biotechnology

<http://www.apogee-flow.com/>

Stratedigm

<https://stratedigm.com/>

Sysmex

<http://www.sysmex-europe.com/products/flow-cytometry.html>

Thermo-Fisher

<https://www.thermofisher.com/es/es/home/life-science/cell-analysis/flow-cytometry.html>

Websites of Companies Manufacturing Antibodies and Fluorescent Probes

Abcam

<http://www.abcam.com/>

Antibody BCN

<http://www.antibodybcn.com/>

Beckman Coulter

<http://beckman.es/coulter-flow-cytometry/reagents>

Becton Dickinson

<http://www.bdbiosciences.com/eu/reagents/research/antibodies-buffers/immunology-reagents/c/744843>

BioLegend

<http://www.biolegend.com/>

Bio-Rad

<https://www.bio-rad-antibodies.com/>

Cell Signaling Technology

<http://www.cellsignal.com/>

Cytognos

<http://www.cytognos.com/index.php/es>

Agilent

https://www.agilent.com/?gclid=Cj0KCQiAxbefBhDfARIsAL4XLRodJy034rMWT7f7VaBRPHCYcqvPzC1RNUfgM35YZqbbdPDYwTl10MaApCPEALw_wcB&gclsrc=aw.ds

Enzo Life Sciences

<http://www.enzolifesciences.com/>

ExBio

<http://www.exbio.cz/>

Hycult Biotech

<http://www.hycultbiotech.com/>

Immunostep

<http://immunostep.com/>

Labclinics

<http://www.labclinics.com/>

Miltenyi Biotec

<http://www.miltenyibiotec.com/en/products-and-services/mac-flow-cytometry/reagents.aspx>

Santa Cruz Biotechnology

<http://www.scbt.com/>

Thermo-Fisher

<https://www.thermofisher.com/es/es/home/life-science/antibodies.html>

Tonbo Biosciences

<http://www.tonbobio.com/>

Websites of Companies Producing Cytometry Software

De Novo Software

<https://www.denovosoftware.com/>

FlowLogic Software

<http://www.inivai.com/flowlogic>

FlowJo Software

<http://www.flowjo.com/>

Infinicyt Software

<http://www.infinicyt.com/>

Kaluza Software

<http://beckman.es/coulter-flow-cytometry/software/kaluza-analysis-software>

Phoenix Flow Systems

<http://www.phnxflow.com/>

Verity Software House

<http://www.vsh.com/>

F. OTHER BOOKS

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<http://www.springer.com/gp/book/9783642548260>

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Springer, 2013

In Living Color: Protocols in Flow Cytometry and Cell Sorting. RA Diamond y S DeMaggio
Springer, 2013

Flow Cytometry: Principles, Methodology and Applications. S Papandreou, Ed. Nova Science
Publishers, 2013

Recent Advances in Cytometry, Part B: Advances in Applications: 103 (Methods in Cell
Biology), Z Darzynkiewicz, E Holden, W Telford y D Wlodkowic, Eds. Academic Press, 2011

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Cytometry. U Sack, A Tárnok, Eds. Karger Publishers, 2008

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Clinical Flow Cytometry: Principles & Application. K.D. Bauer, R.E. Duque, T. V. Shankey, editors. Williams & Wilkins, Baltimore, 1993