



Application of next generation cytomics in AI semen quality control

Dr. Szabolcs T. Nagy

Hungarian University of Agriculture and Life Sciences

Institute of Animal Sciences

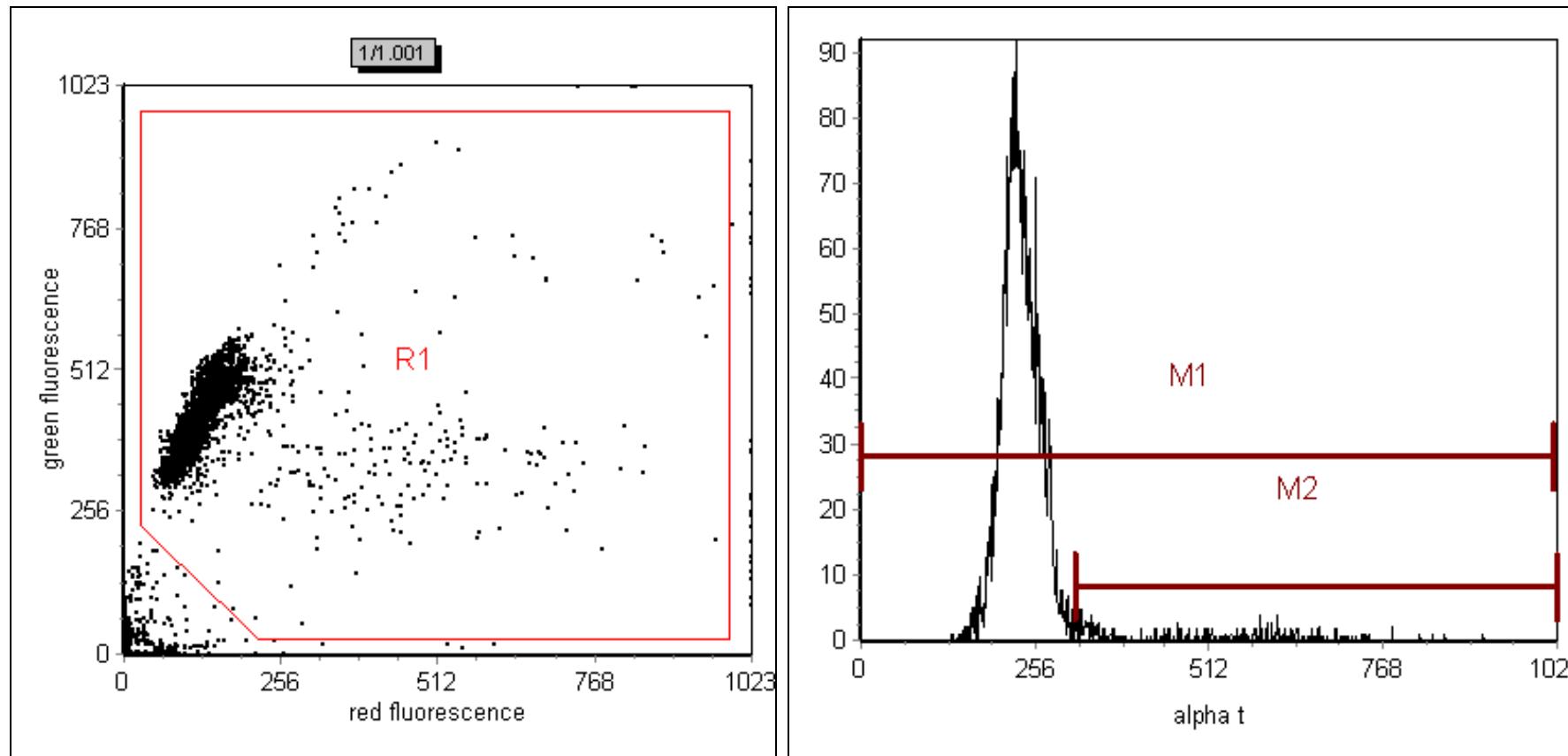
Department of Precision Livestock Farming and Animal

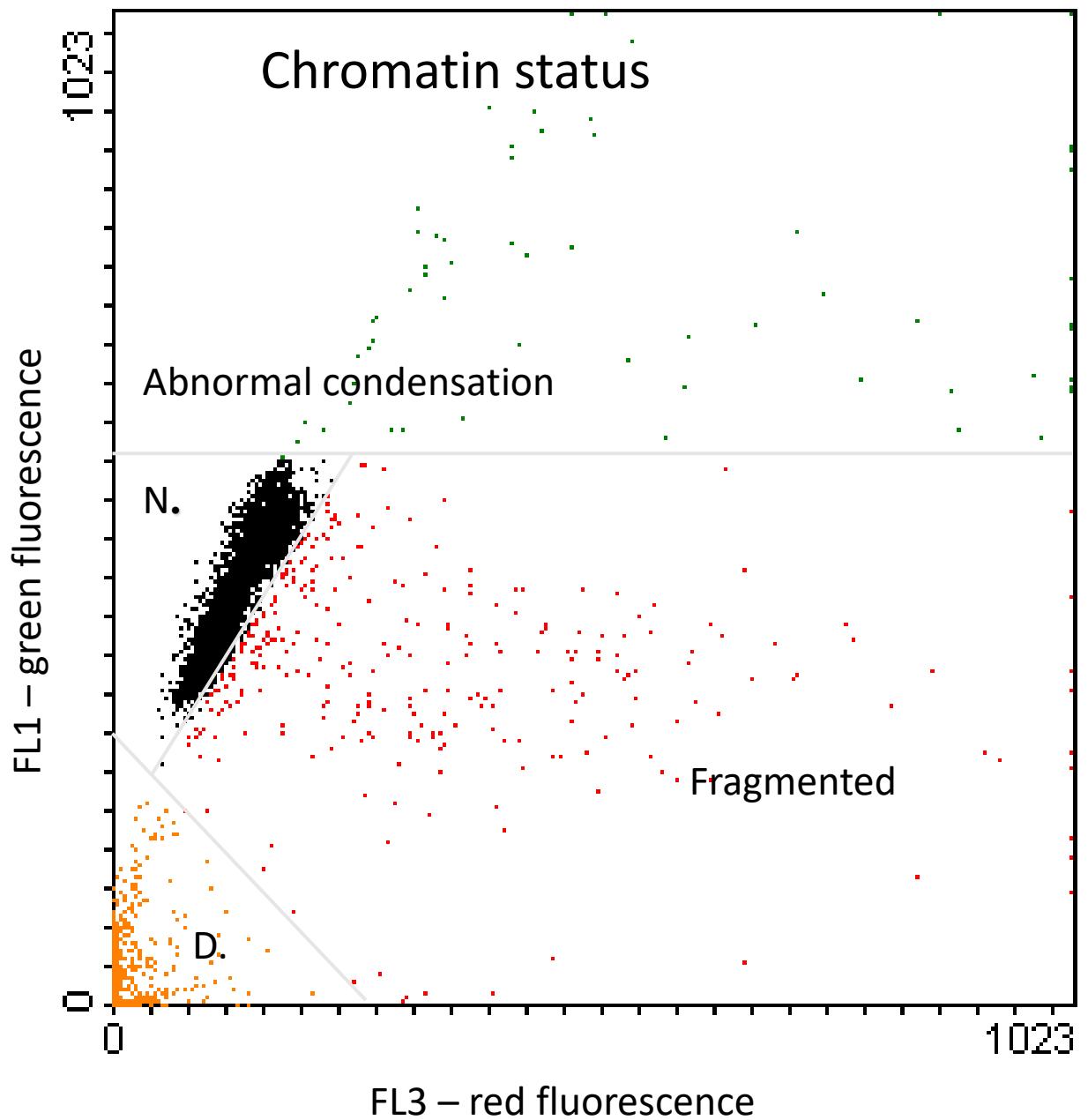
Biotechnics

Keszthely, Hungary



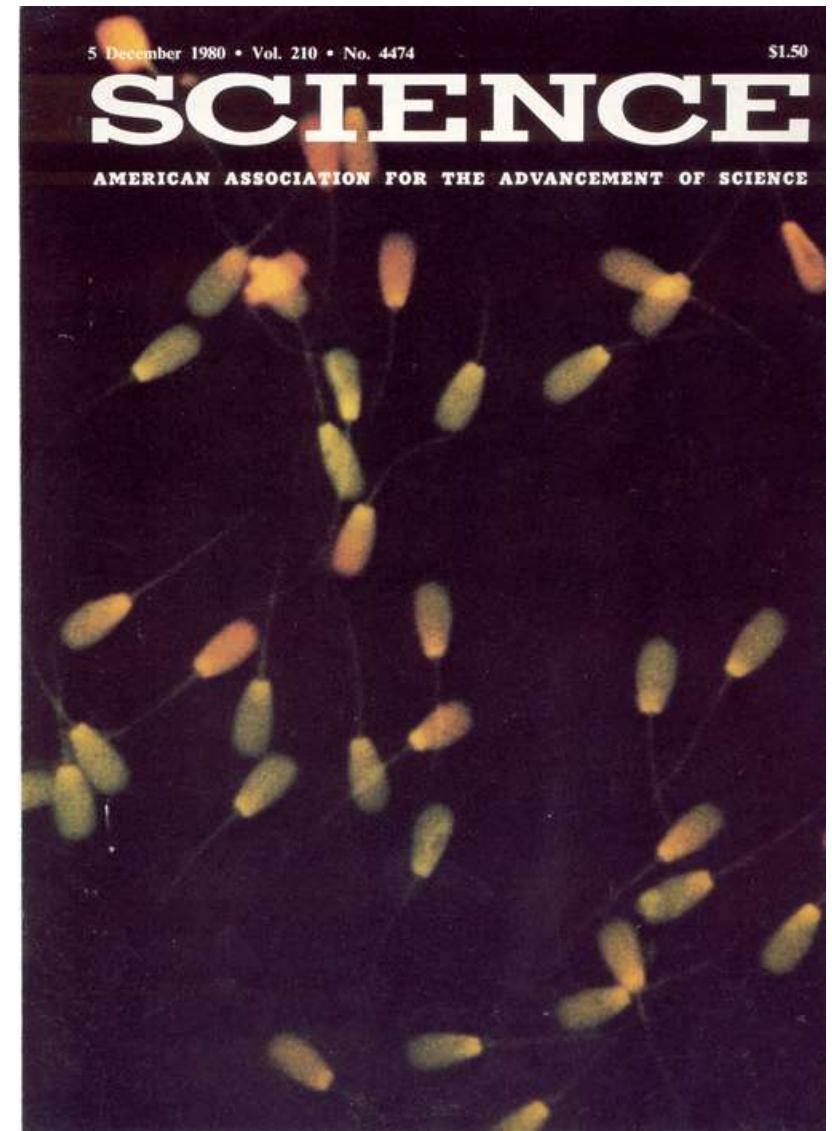
SPERM CHROMATIN STRUCTURE ASSAY™





QUANTAL vs. QUANTITATIVE

- Yes-or-no (quantal): viability
- Quantitative: mitochondria, DNA-fragmentation, etc.





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Theriogenology 63 (2005) 1752–1763

www.journals.elsevierhealth.com/periodicals/the

Theriogenology

Sperm chromatin stability in frozen-thawed semen is maintained over age in AI bulls

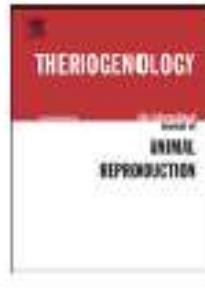
Triin Hallap^{a,c,*}, Szabolcs Nagy^{a,b,d}, Margareta Håård^e,
Ülle Jaakma^c, Anders Johansson^b,
Heriberto Rodriguez-Martinez^a



Contents lists available at SciVerse ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com



Sperm chromatin structure and sperm morphology: Their association with fertility in AI-dairy Ayrshire sires

S. Nagy^{a,*}, A. Johannisson^b, T. Wahlsten^c, R. Ijäs^d, M. Andersson^e, H. Rodriguez-Martinez^f

^aGeorgikon Faculty, Department of Animal Sciences and Animal Husbandry, University of Pannonia, Keszthely, Hungary

^bDepartment of Anatomy, Physiology and Biochemistry, Swedish University of Agricultural Sciences (SLU), Ultuna, Uppsala, Sweden

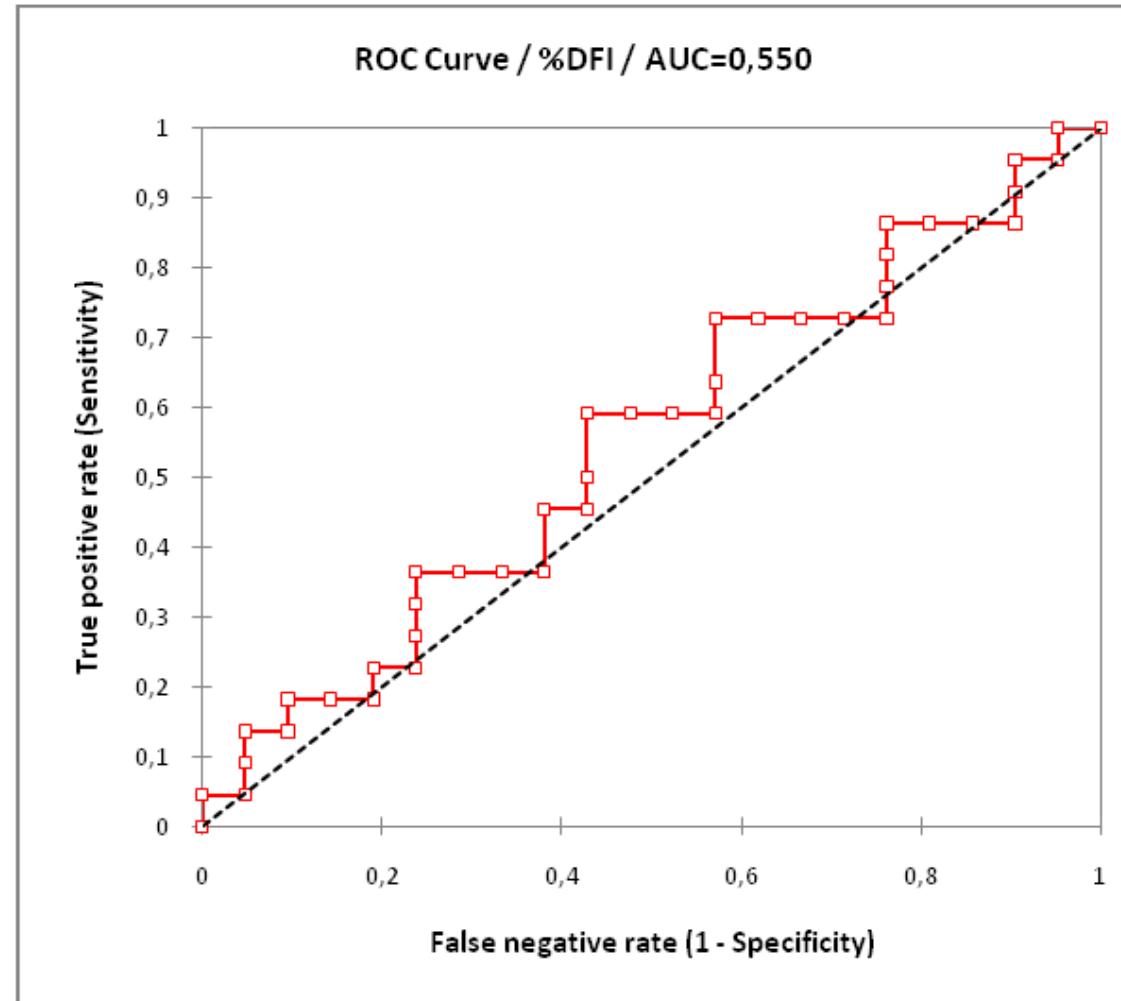
^cFaba co-op, Vantaa, Finland

^dViking Genetics Finland, Hollola, Finland

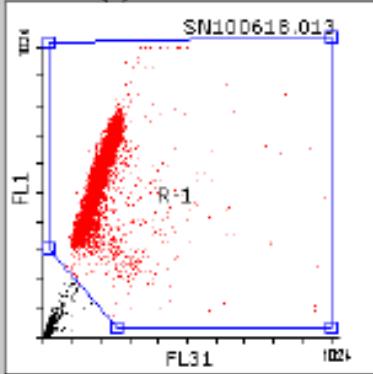
^eFaculty of Veterinary Medicine, Department of Production Animal Medicine, University of Helsinki, Saarentaus, Finland

^fDepartment of Clinical and Experimental Medicine, Linköping University, Linköping, Sweden

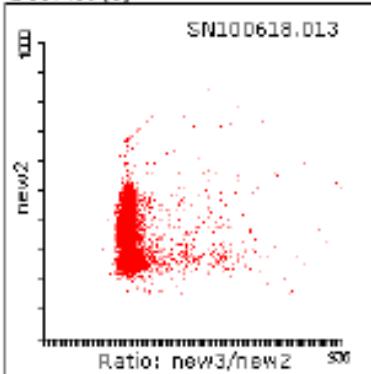
SCSA diagnostic value...



Dot Plot (1)



Dot Plot (8)



CellCalculator(2)

File Name SN100618.013
 Parameter Name new1
 Parameter Max 2000
 Visualization Lin
 Raw Formula $\{FL1\} + \{FL31\}$
 Formula $\{FL1\} + \{FL31\}$

CellCalculator(3)

File Name SN100618.013
 Parameter Name new2
 Parameter Max 1000
 Visualization Lin
 Raw Formula $\{NEW1\}/2$
 Formula $\{NEW1\}/2$

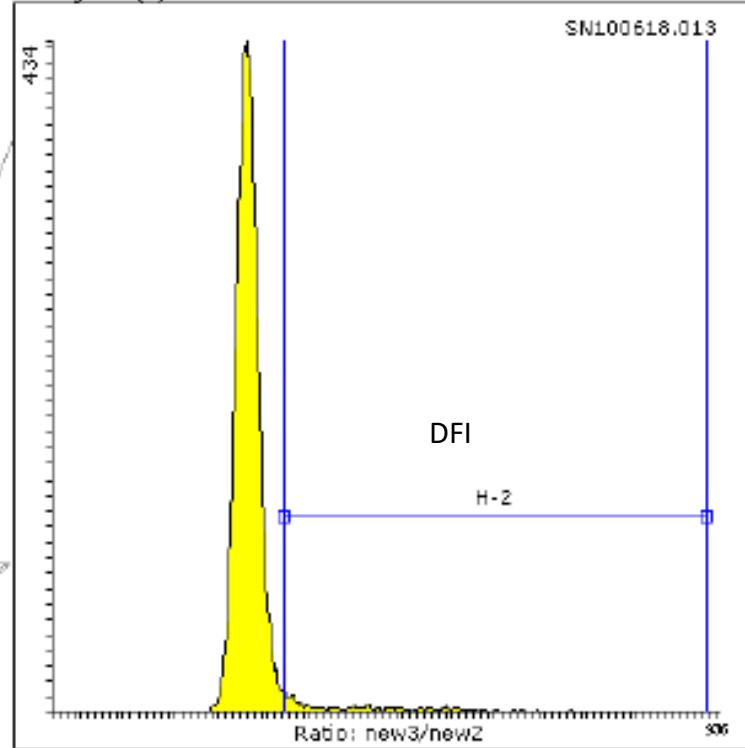
CellCalculator(4)

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 Parameter Name new3
 Parameter Max 512000
 Visualization Lin
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 Formula $\{FL31\} * 512$

Ratio Calculator(5)

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 Denominator new2
 Multiplier 1
 Parameter max 936 (automatic)
 Parameter name Ratio: new3/new2
 Visualization Lin

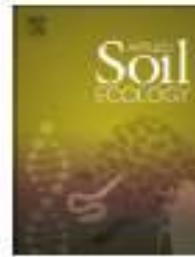
Histogram (6)



Statistics (7)

	Events	% of Vis	Mean	GeoMean	Median	CU
All events	9 106	100,00	279,20	276,63	271,54	16,74
H-2	421	4,62	441,79	429,17	418,91	25,36





Short communication

Rapid analysis of photoautotroph microbial communities in soils by flow cytometric barcoding and fingerprinting



László Menyhárt^a, Szabolcs Nagy^b, Anita Lepossa^{c,*}

^a University of Pannonia, Georgikon Faculty, Department of Economic Methodology, H8360 Keszthely, Dék F. u. 16., Hungary

^b University of Pannonia, Georgikon Faculty, Department of Animal Sciences, H8360 Keszthely, Dék F. u. 16., Hungary

^c University of Pannonia, Georgikon Faculty, Department of Crop Production and Land Use, H8360 Keszthely, Dék F. u. 16., Hungary

R-environment



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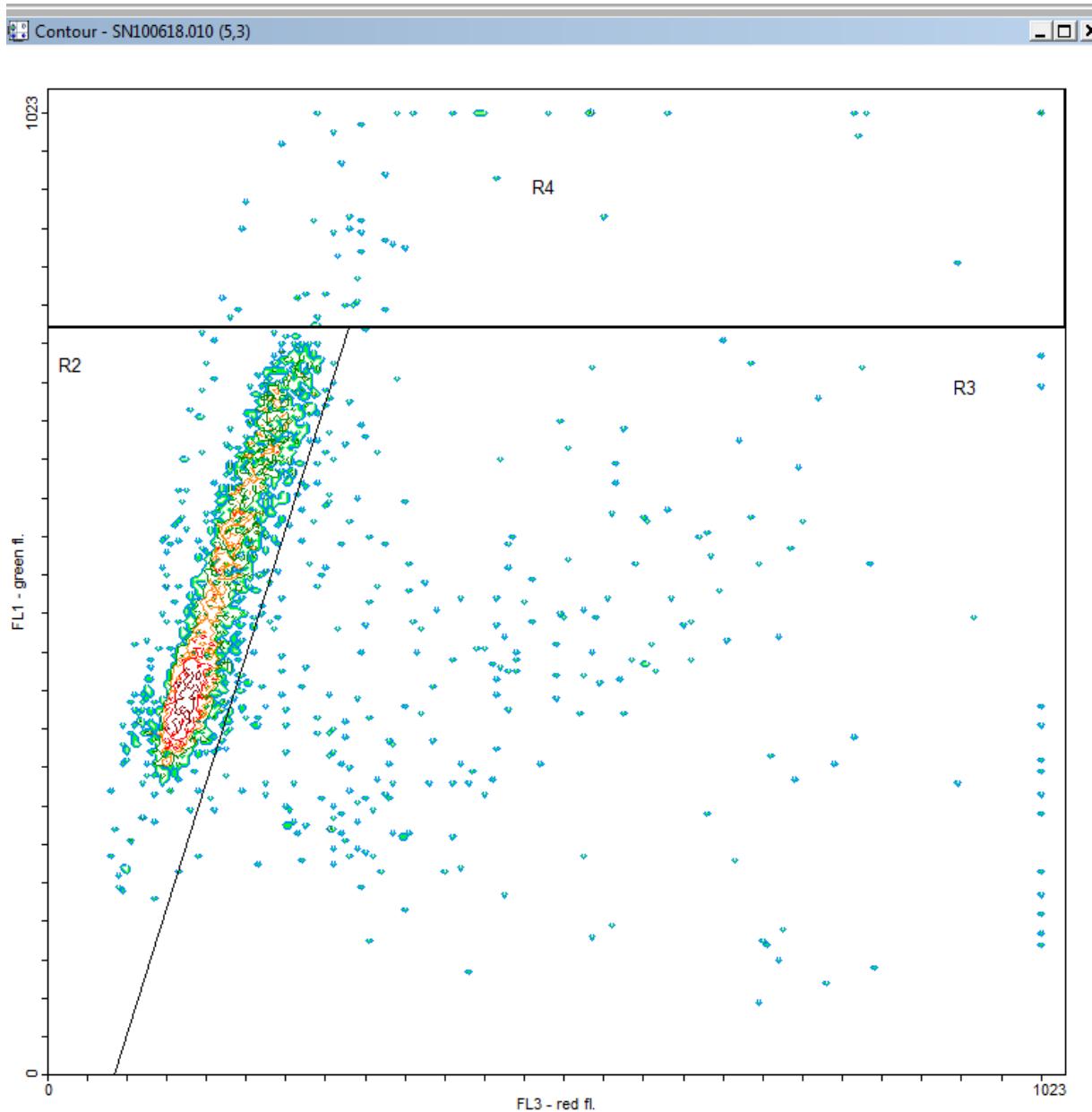
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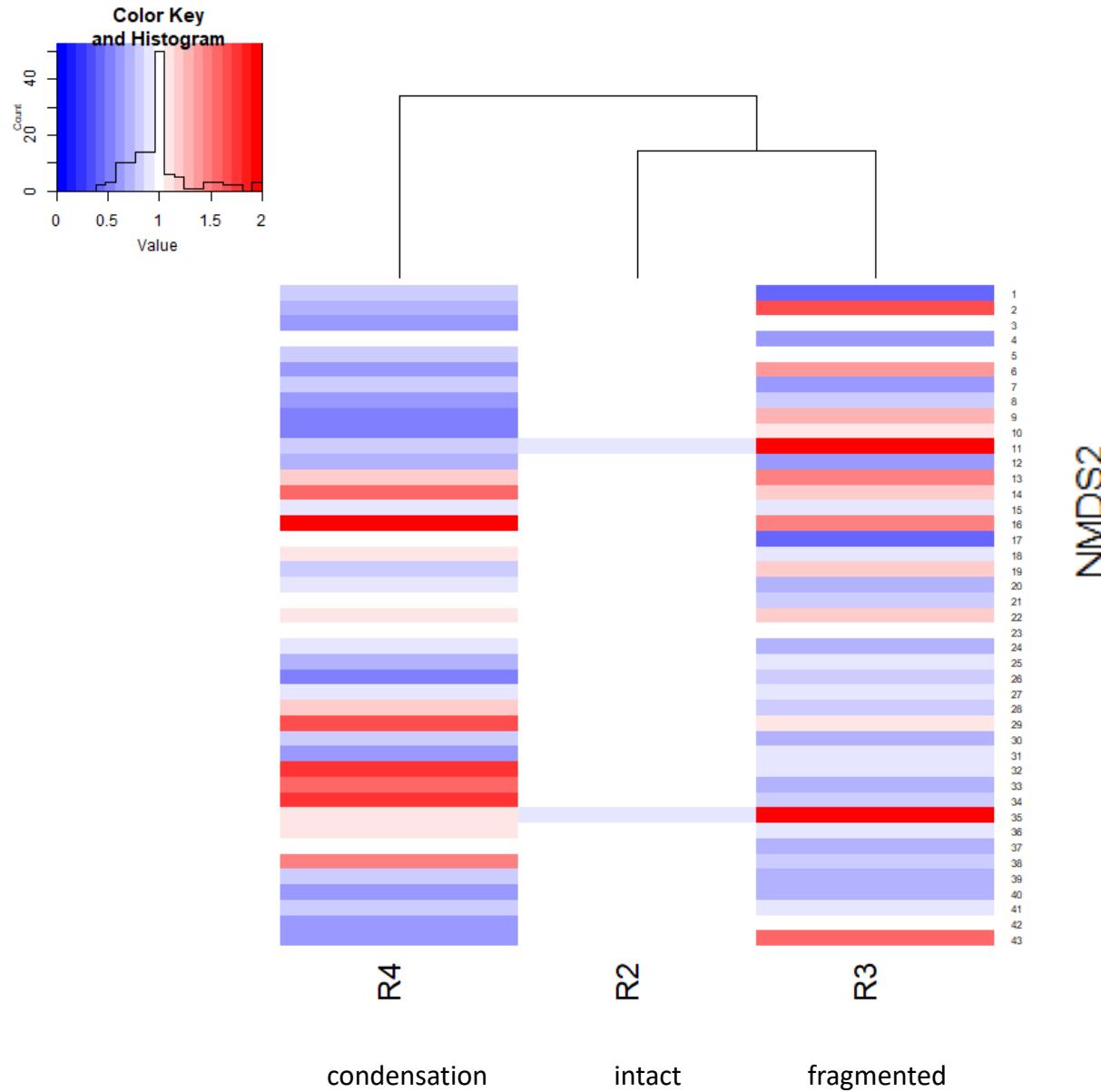
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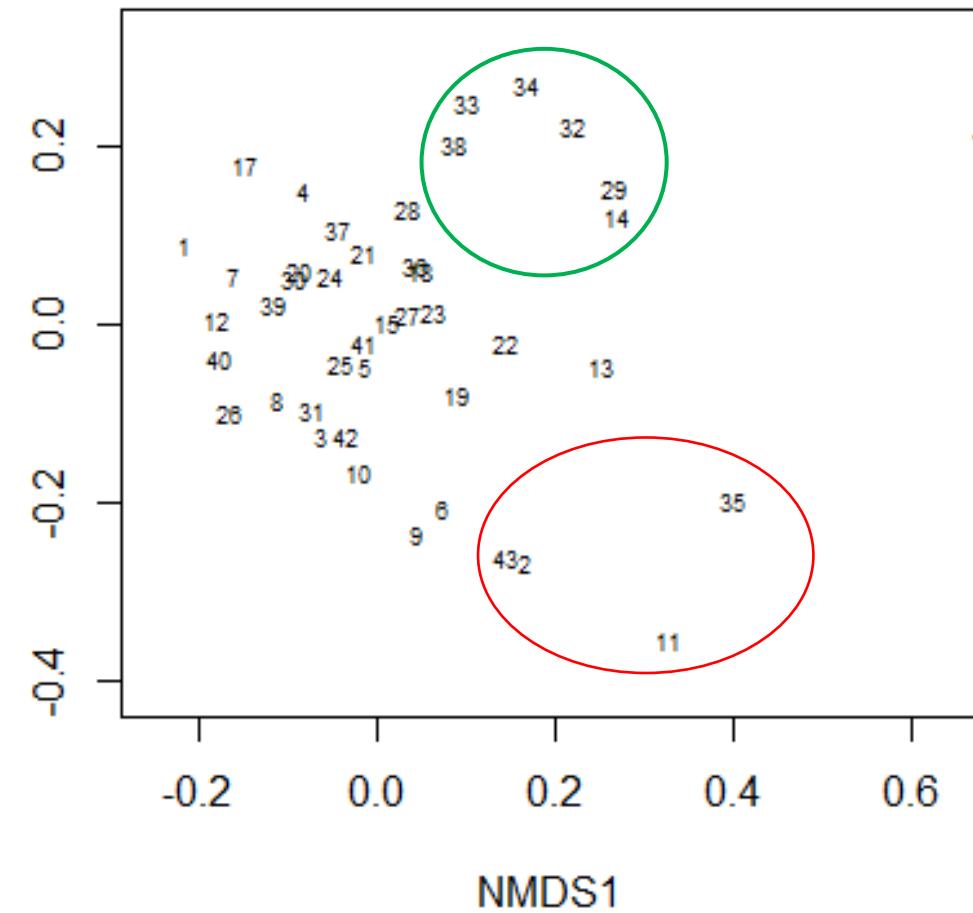
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cydar	Aaron Lun	Using Mass Cytometry for Differential Abundance Analyses	766
CytoDx	Zicheng Hu	Robust prediction of clinical outcomes using cytometry data without cell gating	1524
cytofkit	Jinmiao Chen, Matthew Myint	cytofkit: an integrated mass cytometry data analysis pipeline	206
cytolib	Mike Jiang	C++ infrastructure for representing and interacting with the gated cytometry	201
CytoML	Mike Jiang	GatingML interface for openCyto	665
diffcyt	Lukas M. Weber	Differential discovery in high-dimensional cytometry via high-resolution clustering	1473

Flow barcoding (CyBAR) - SCSA

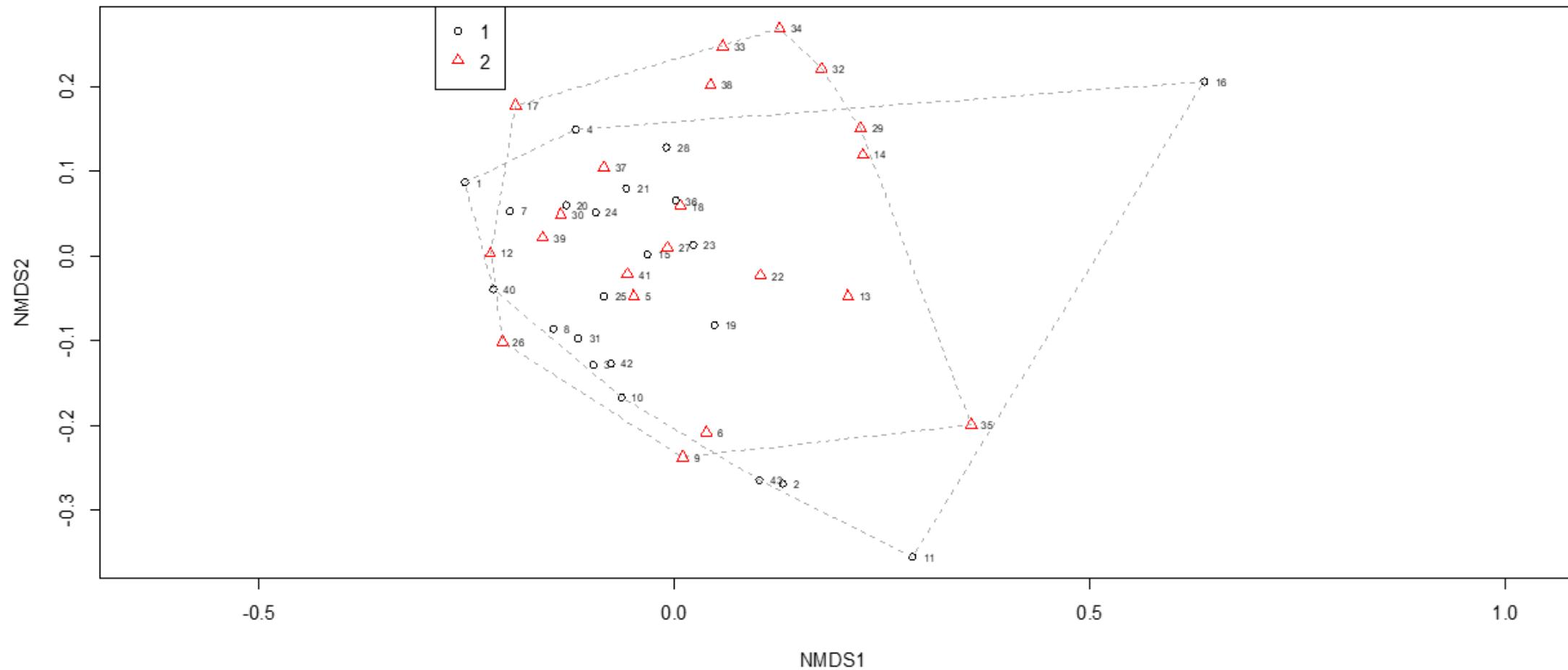




NMDS plot of normalized cell numbers



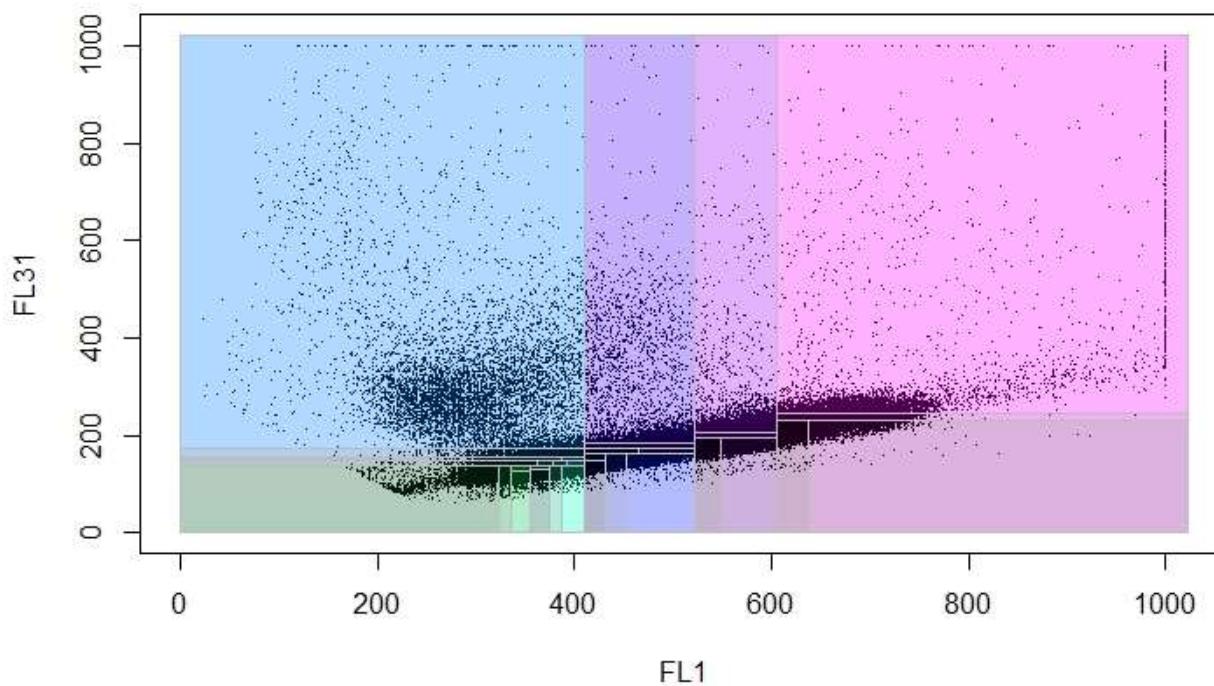
NMDS plot of
SCSA_CYBAR; NRR



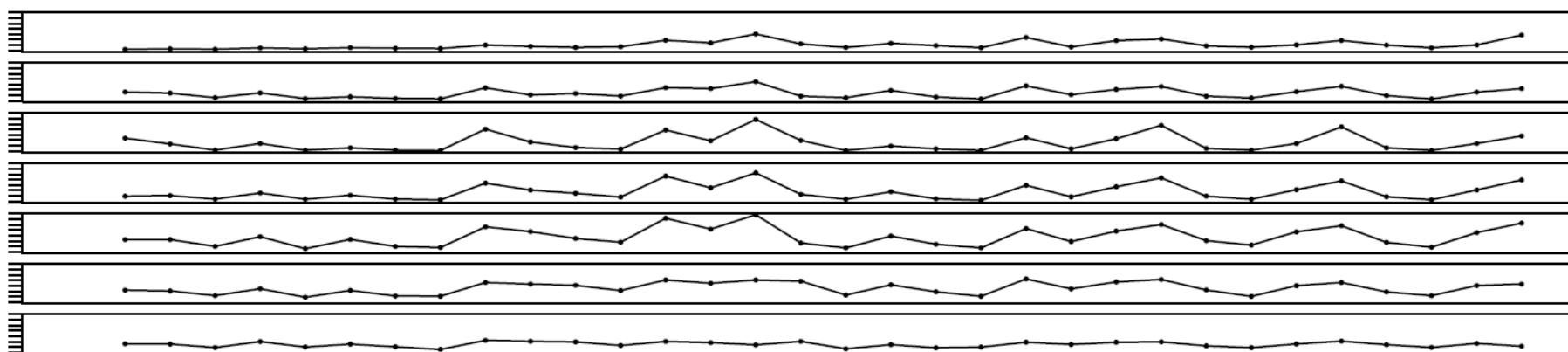
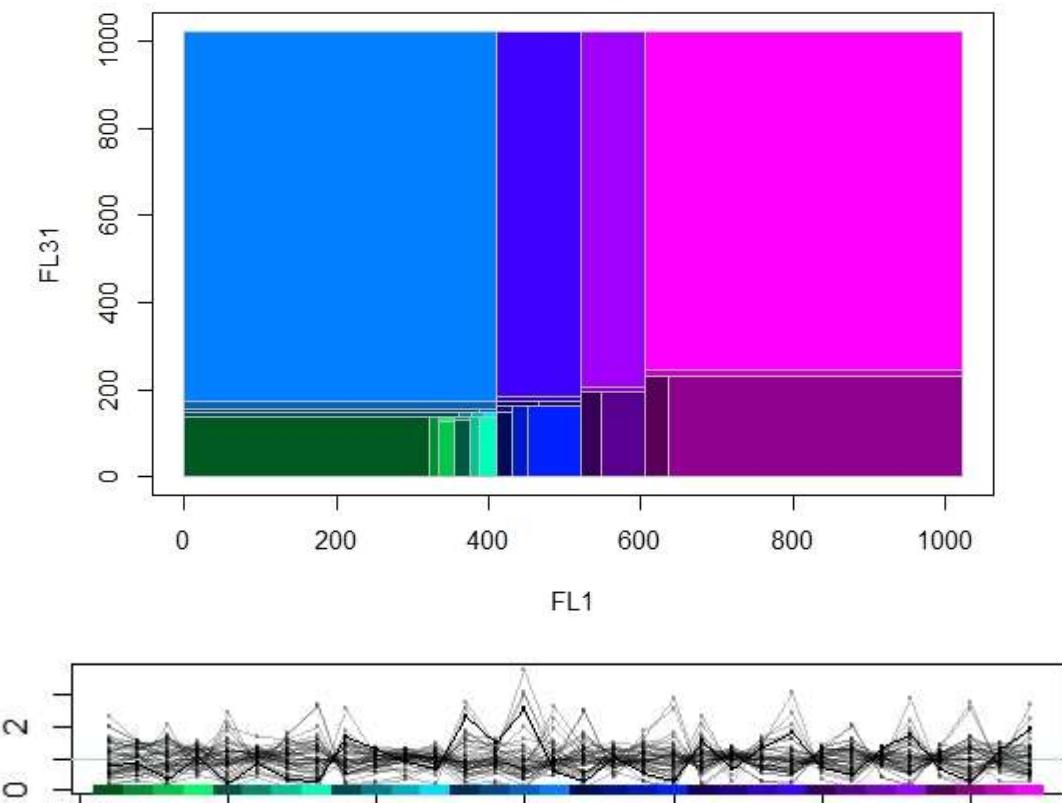
Legend: 1=low NRR; 2=normal NRR

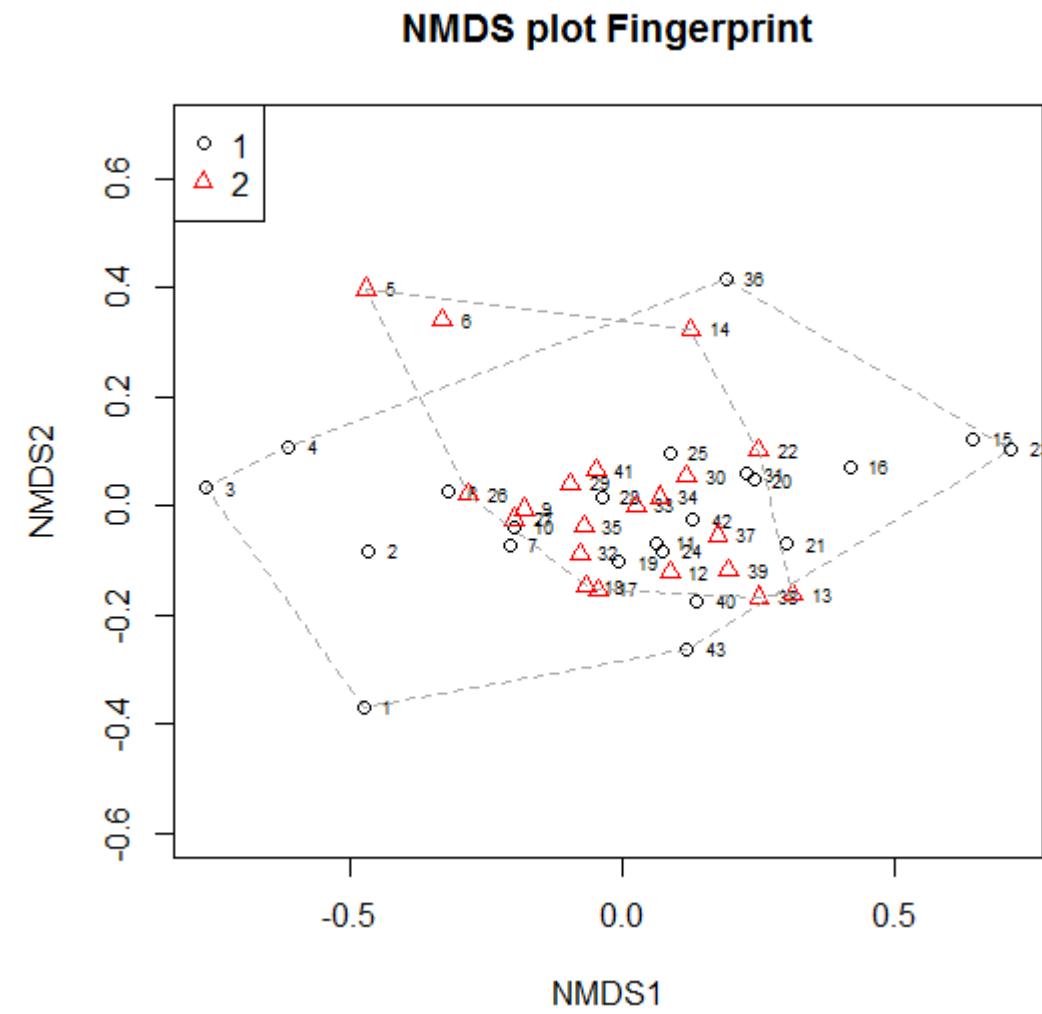
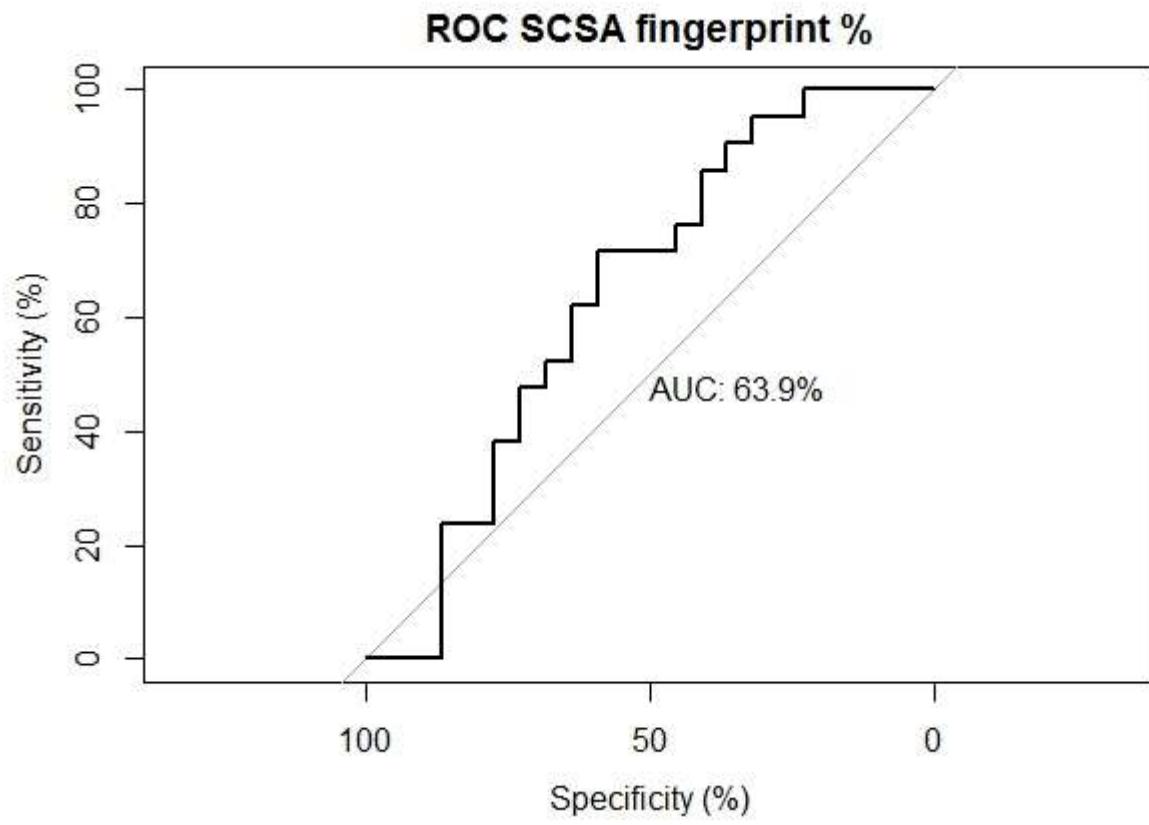
Flow fingerprinting (FlowFP) - SCSA

Model

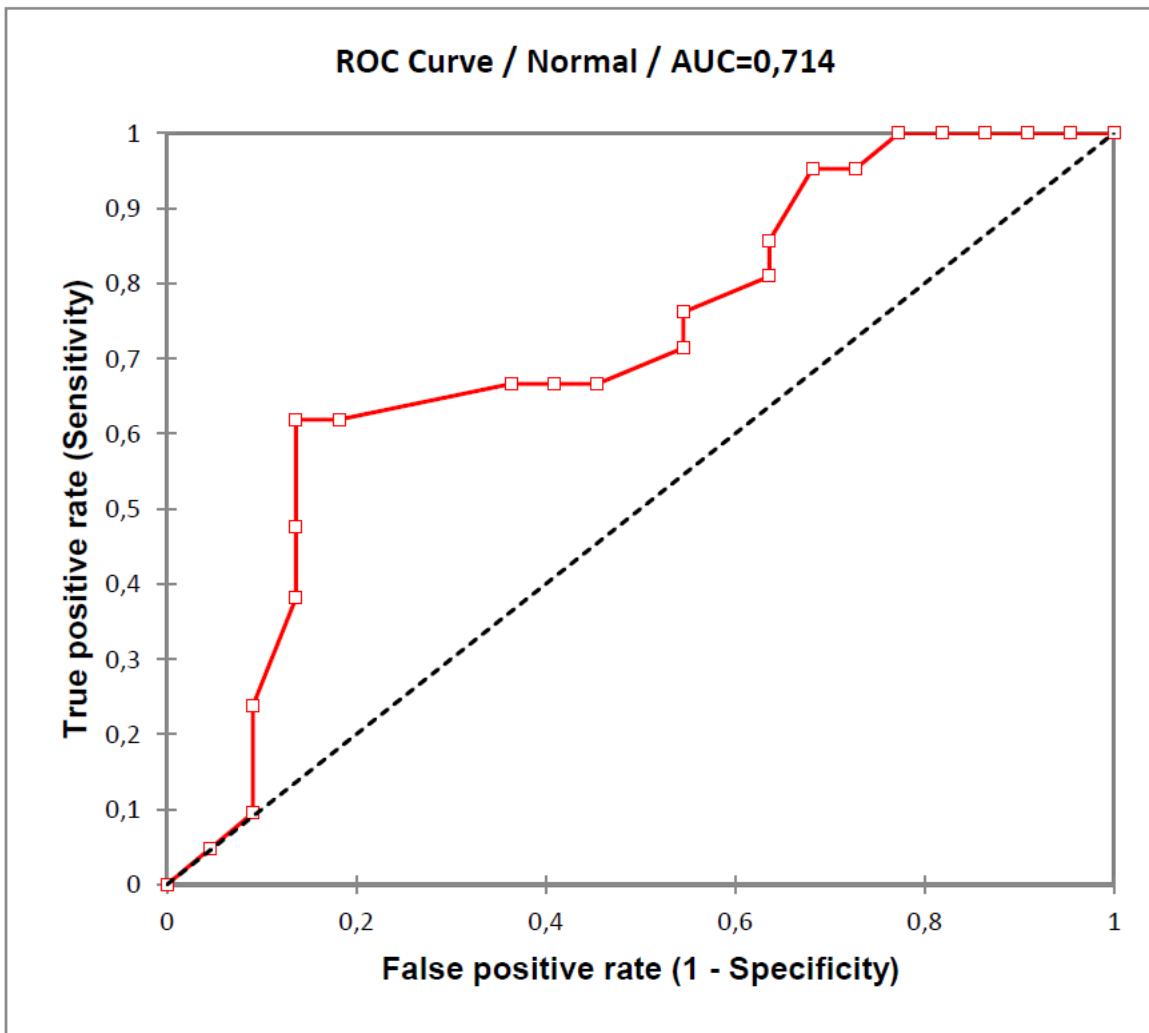


Model





Normal morphology!



0196-4763/82/0205-0344\$00.00/0

CYTOMETRY

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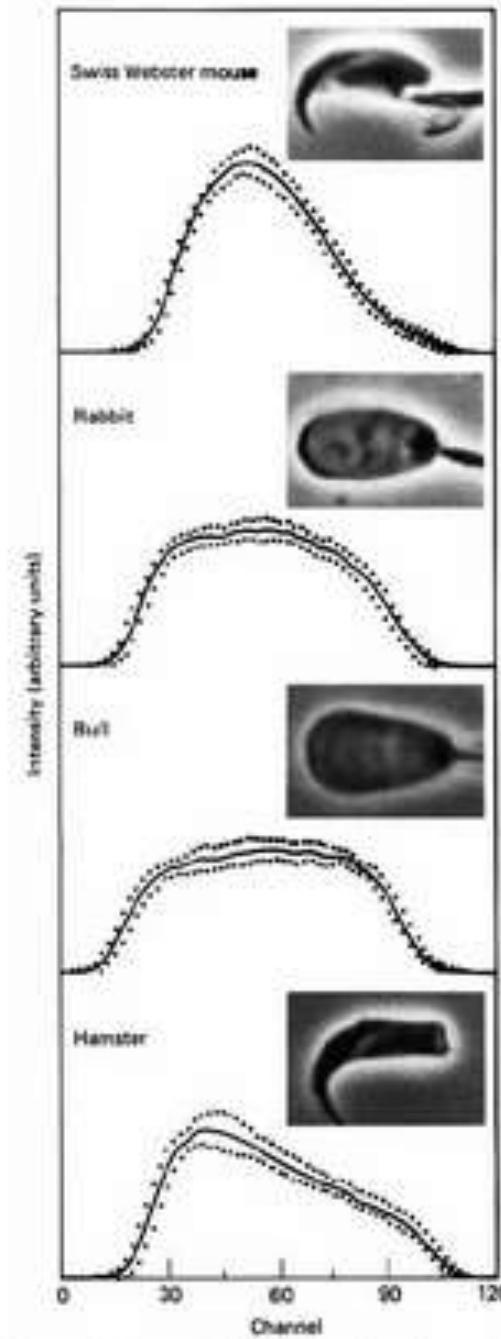
Vol. 2, No. 5, 1982
Printed in U.S.A.

Quantification of Mammalian Sperm Morphology by Slit-Scan Flow Cytometry¹

David A. Benaron, Joe W. Gray,² Barton L. Gledhill, Suzanne Lake, Andrew J. Wyrobek, and Ian T. Young

Lawrence Livermore National Laboratory, Biomedical Sciences Division, L-452, University of California, Livermore, California 94550

Received for publication August 18, 1981; accepted November 19, 1981





Published: 18 March 2012

Tracking protein aggregation and mislocalization in cells with flow cytometry

[Yasmin M Ramdzan](#), [Saskia Polling](#), [Cheryl P Z Chia](#), [Ivan H W Ng](#), [Angelique R Ormsby](#), [Nathan P Croft](#),
[Anthony W Purcell](#), [Marie A Bogoyevitch](#), [Dominic C H Ng](#), [Paul A Gleeson](#) & [Danny M Hatters](#) 

[Nature Methods](#) 9, 467–470 (2012) | [Cite this article](#)

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Pulse Width for Particle Sizing

Robert A. Hoffman¹

¹BD Biosciences, San Jose, California

ABSTRACT

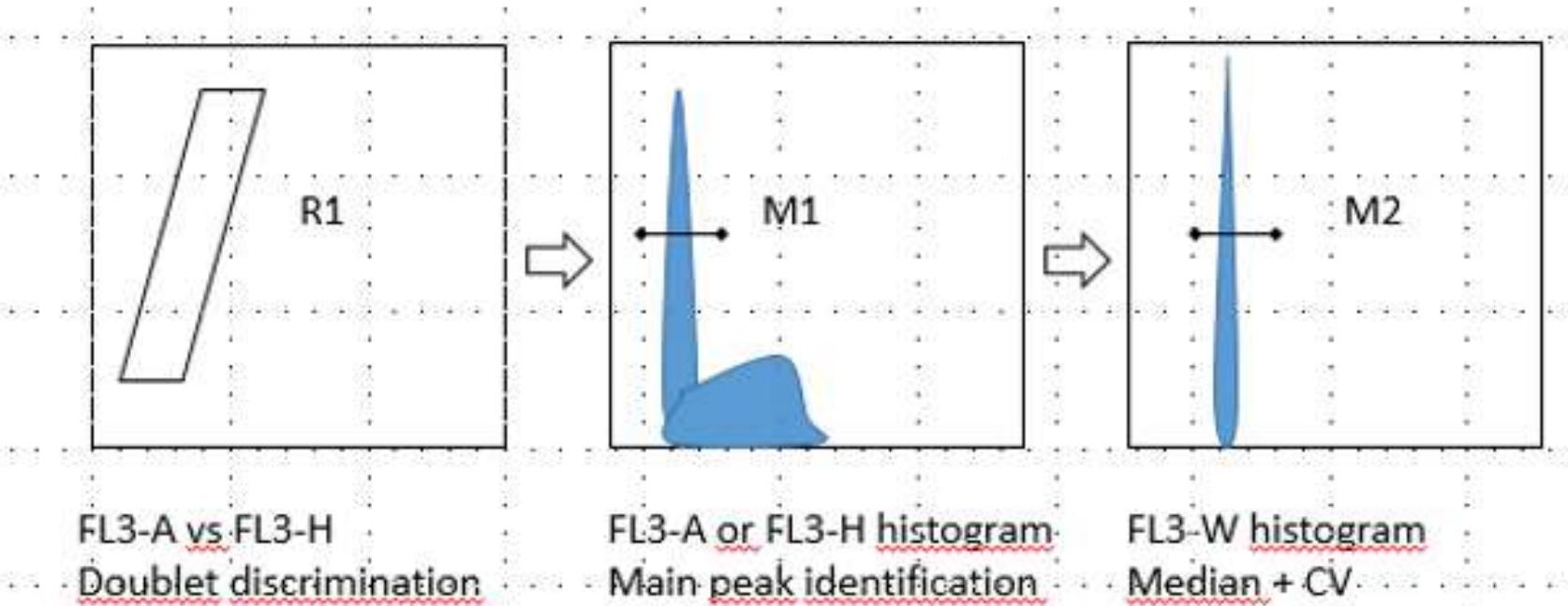
The widths of optical pulses in flow cytometry contain information about the size of particles. This size information is independent of many of the factors that affect light scatter as a measure of particle size, and any light scatter or fluorescence signal can be used to measure pulse width. For fluorescence signals, the pulse width can be predicted theoretically for many particle shapes, and quantitative size calibration is possible. To be a meaningful independent parameter, the pulse-width measurement must be independent of the pulse amplitude. This unit provides protocols for determining the signal range over which amplitude independent pulse-width measurements can be made and methods for calibrating the pulse-width measurements to particle diameter. Calibration and application examples are provided and briefly discussed. *Curr. Protoc. Cytom.* 50:1.23.1-1.23.17.

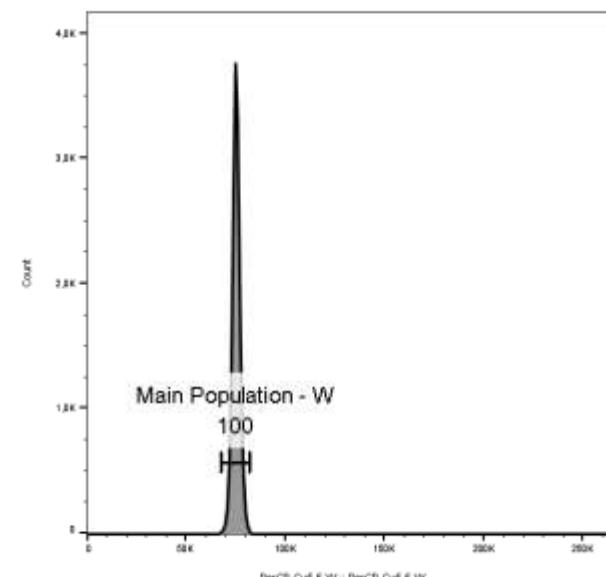
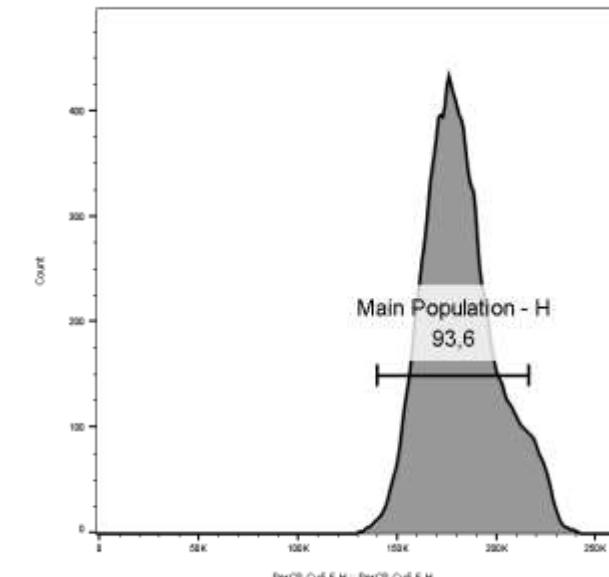
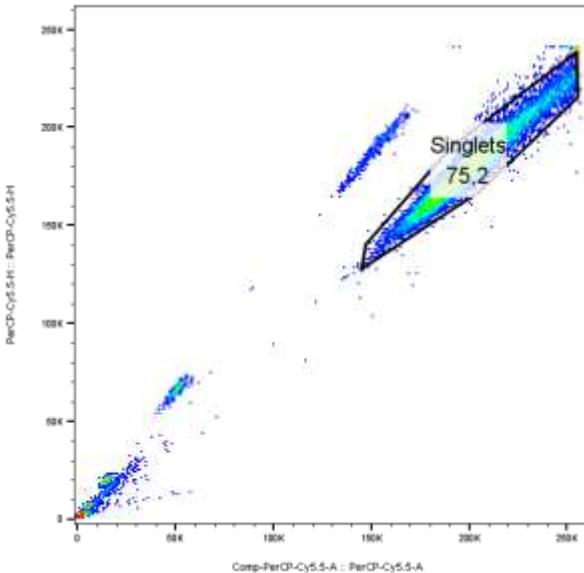
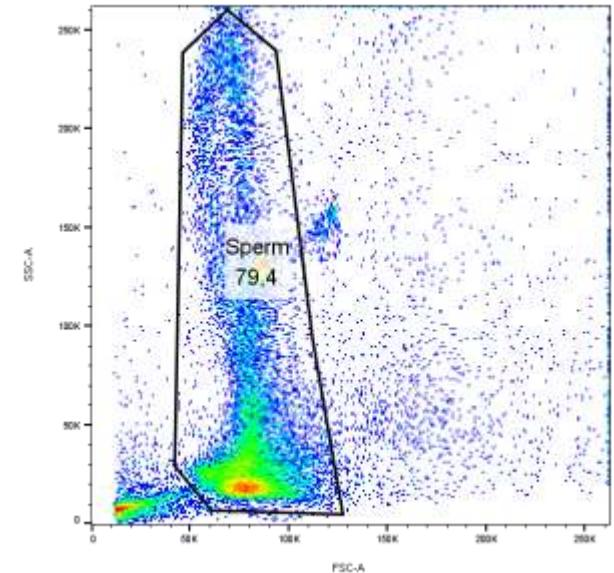
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Keywords: pulse width • flow cytometry • size • fluorescence • light scatter

PulSA approach – gating plan

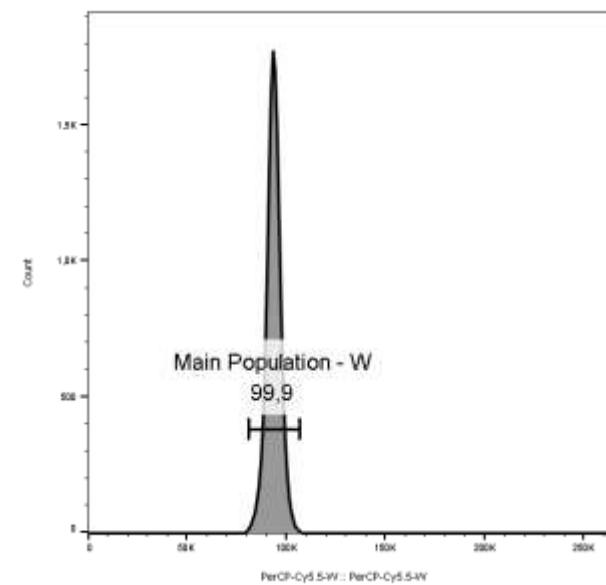
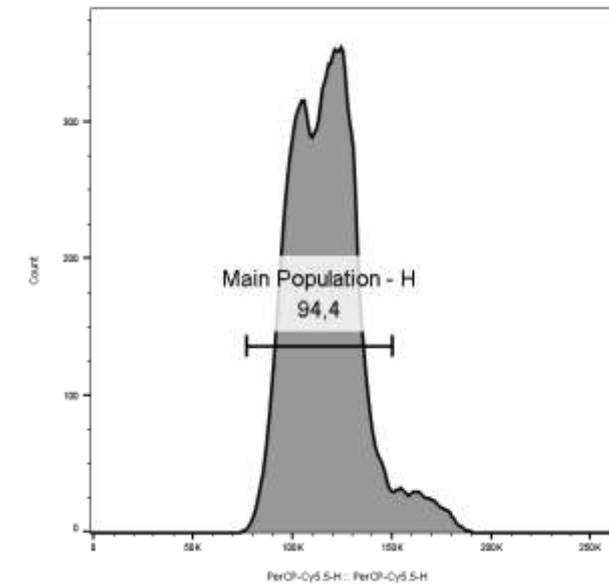
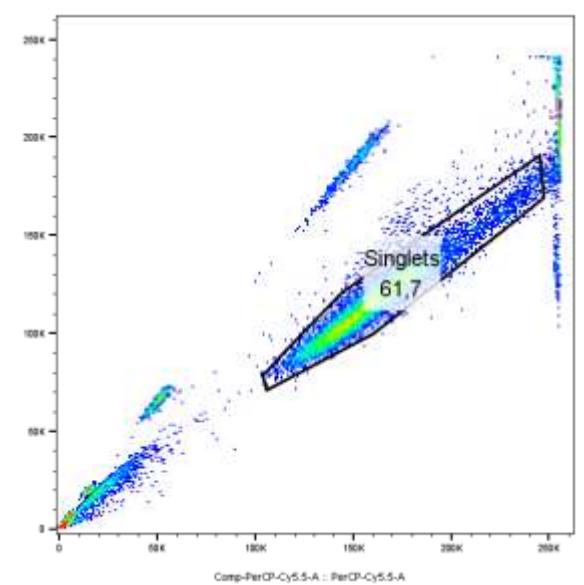
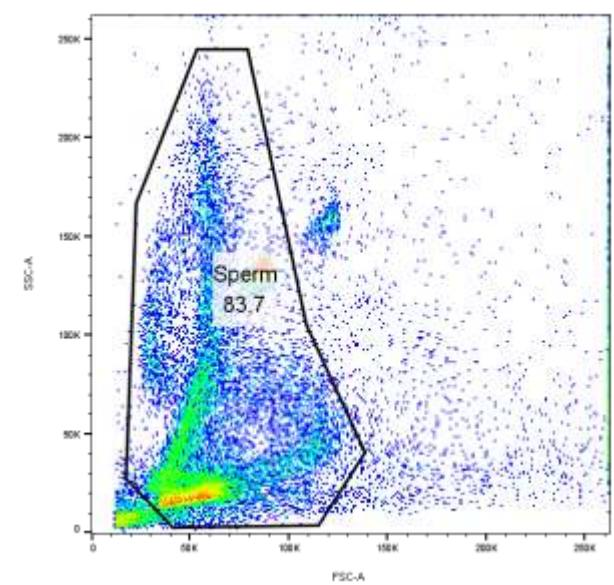
A two-step logical gating would be applied to do pulse shape analyses: doublet discrimination (R1), main peak identification (M1), then using R1 AND M1 logical gating, the FL3 Width histogram values (median or mean, CV) would be recorded.





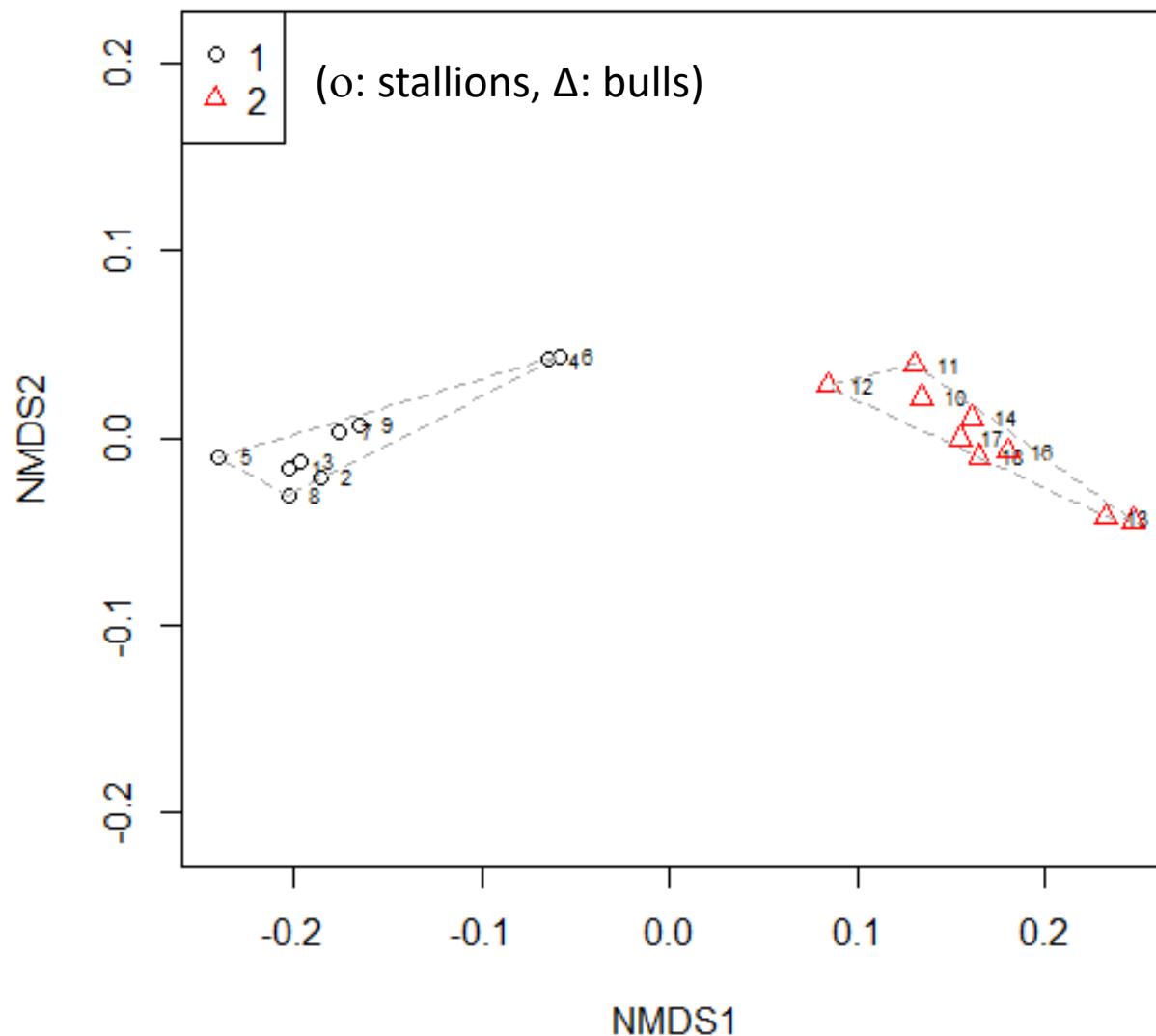
Stallion

Bull

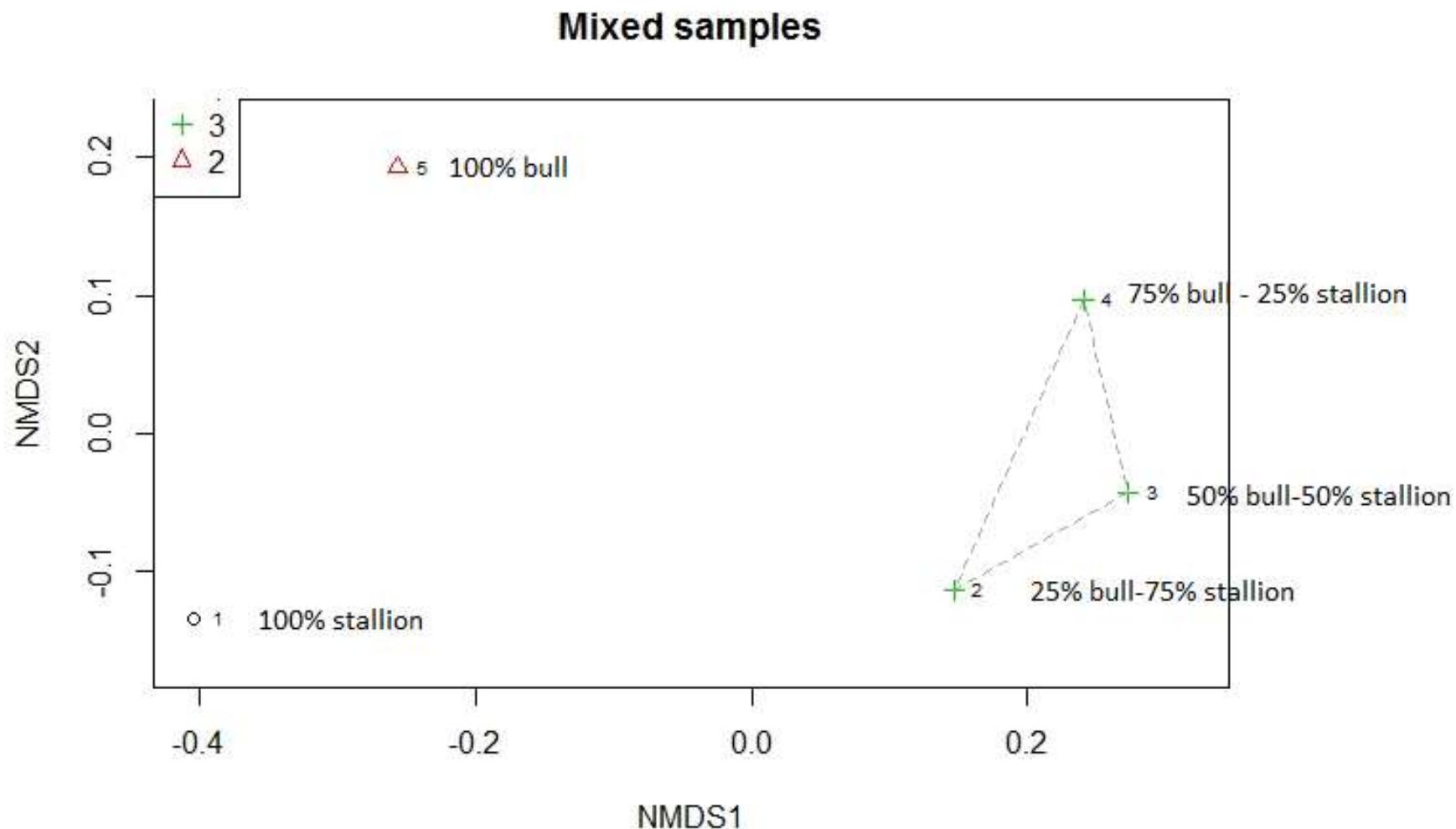


Bulls vs stallions

NMDS plot of Fxcycle with beads adjusted



Bulls vs stallions – mixed samples („abnormals”)



QUICK CYTOGENETIC SCREENING OF BREEDING BULLS USING FLOW CYTOMETRIC SPERM DNA HISTOGRAM ANALYSIS

Szabolcs NAGY^{1*}, Péter J. POLGÁR¹, Magnus ANDERSSON² and András KOVÁCS³

¹Department of Animal Sciences and Animal Husbandry, Georgikon Faculty, University of Pannonia, Deák F. u. 16, H-8360 Keszthely, Hungary; ²Department of Production Animal Medicine, Faculty of Veterinary Medicine, University of Helsinki, Saari Unit, Saarentaus, Finland; ³Institute of Animal Science, Biotechnology and Nature Conservation, Faculty of Agricultural and Food Sciences and Environmental Management, University of Debrecen, Debrecen, Hungary

(Received 7 December 2015; accepted 4 May 2016)

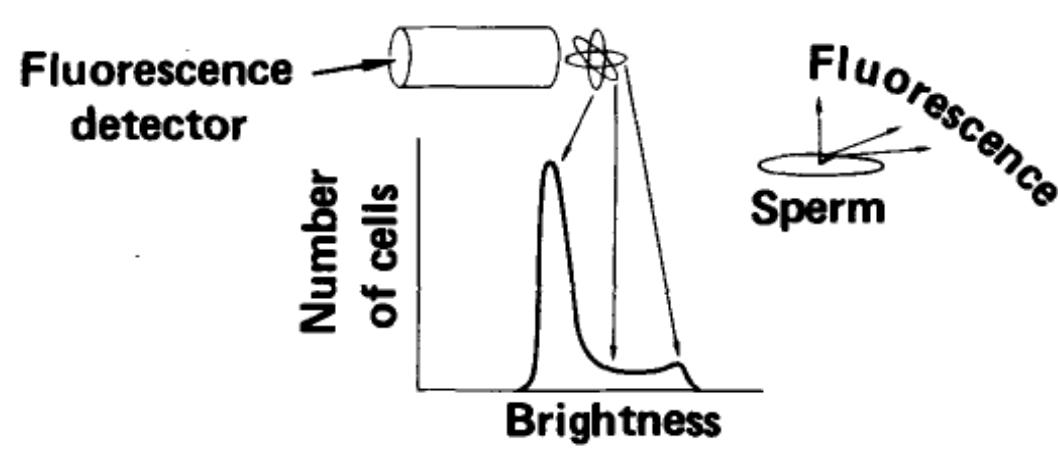
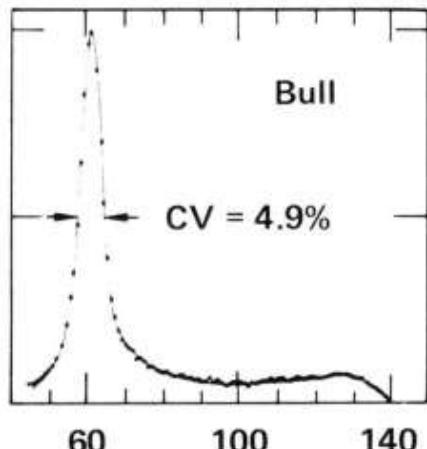
Cytometry of Deoxyribonucleic Acid Content
and Morphology of Mammalian Sperm^{1,2}

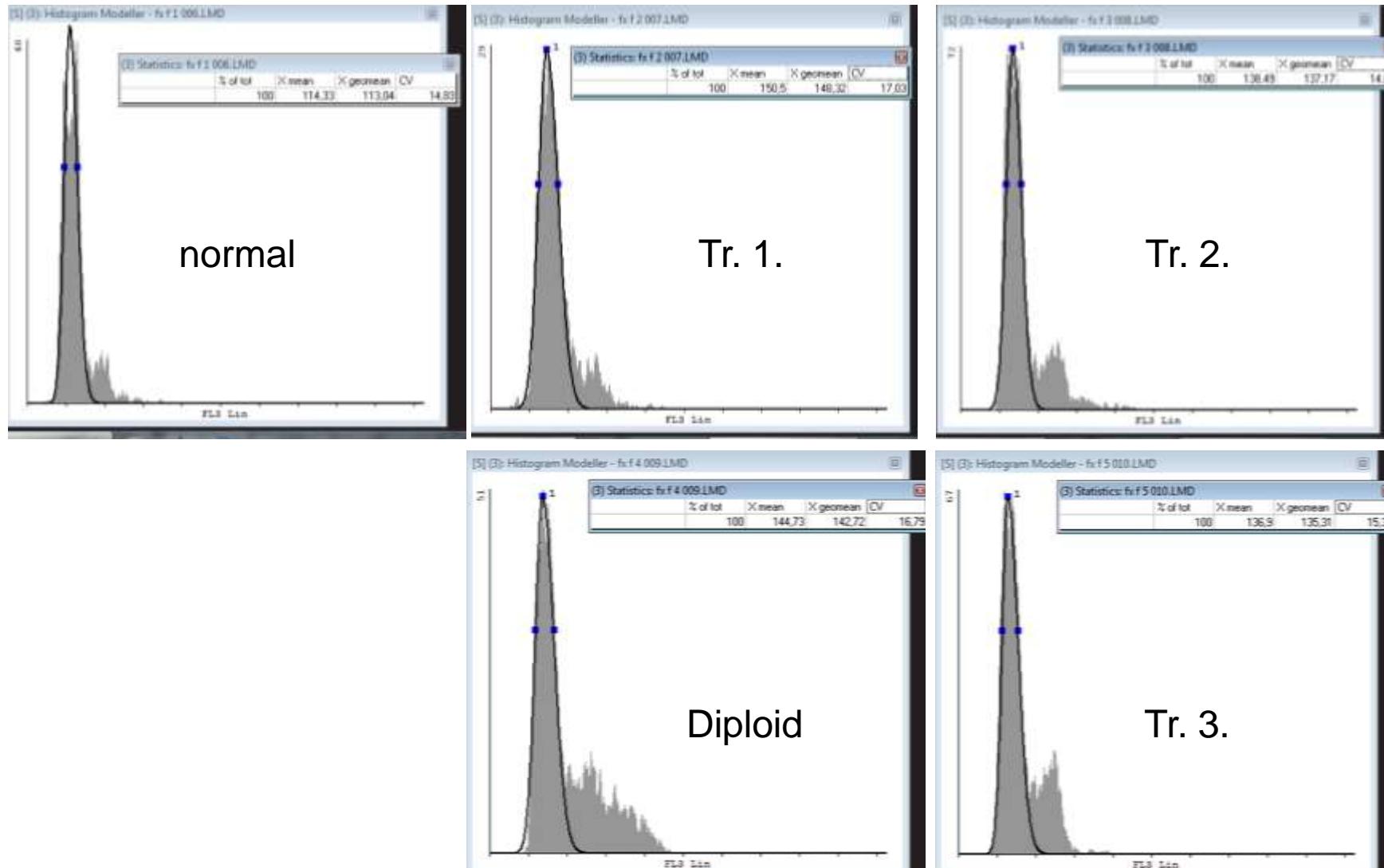
1983 J Dairy Sci 66:2623-2634

BARTON L. GLEDHILL

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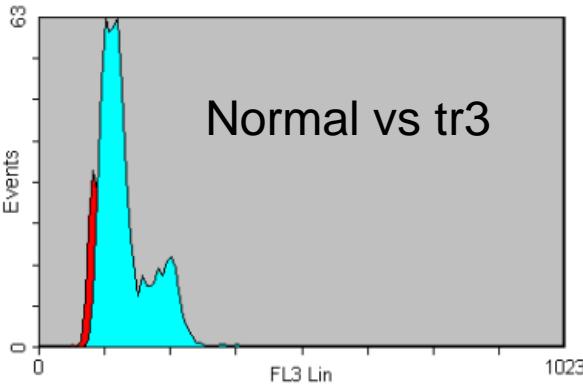
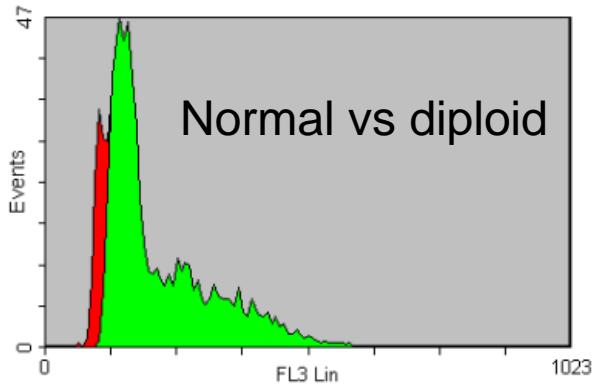
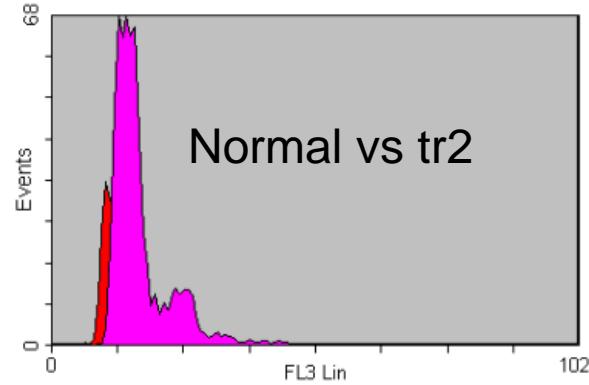
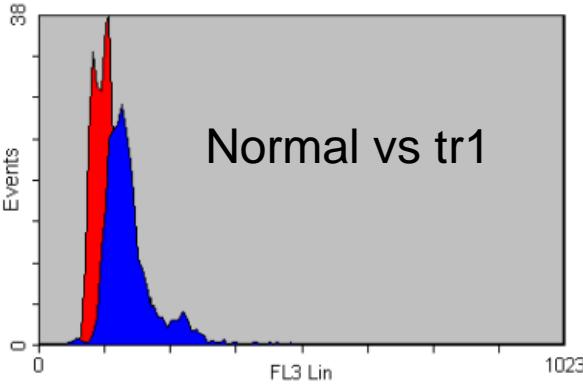
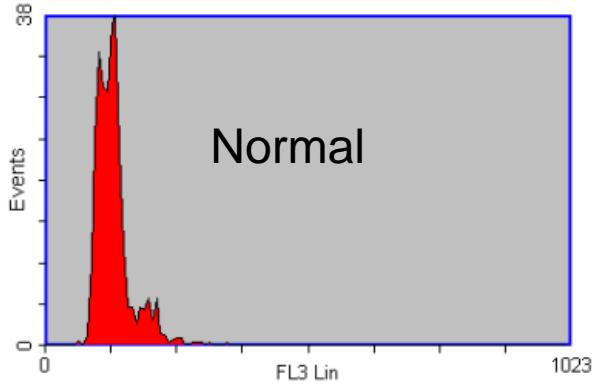
Effect of random sperm head orientation on the DNA-histogram shape





Histogram modelling:
Assuming normal distribution and adding a gaussian curve to the main peak

Histogram overlapping for Kolmogorov-Smirnov analysis



Cellular Symphony Flow Cytometry Software



Visit the website

The Cellular Symphony

The Cellular Symphony is an audio representation of flow cytometric data. Each cell is represented by musical notes of timbre, pitch and volume dependent on its FCM parameters.

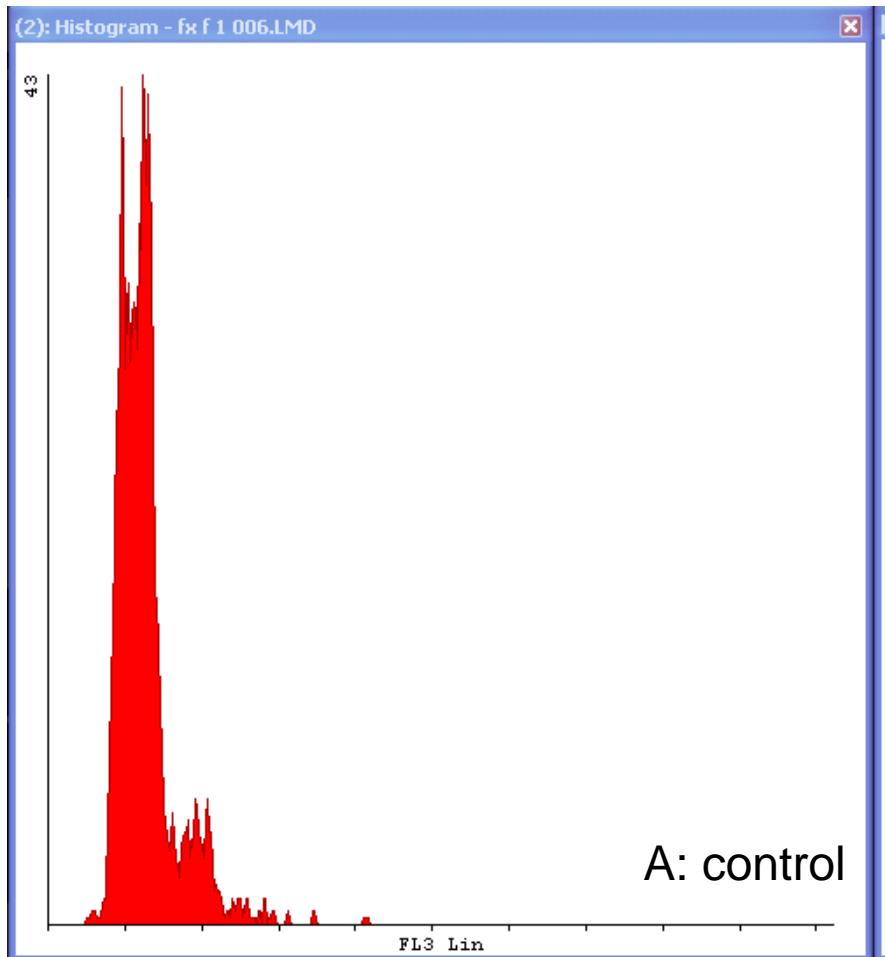
The program correlates the audio output with 2D and 3D graphical display. It may be that only a musical savant can detect the intricacies in a 3-instrument cellular symphony or it may be that the average cytometrist also can do so with practice. Your comments are invited.

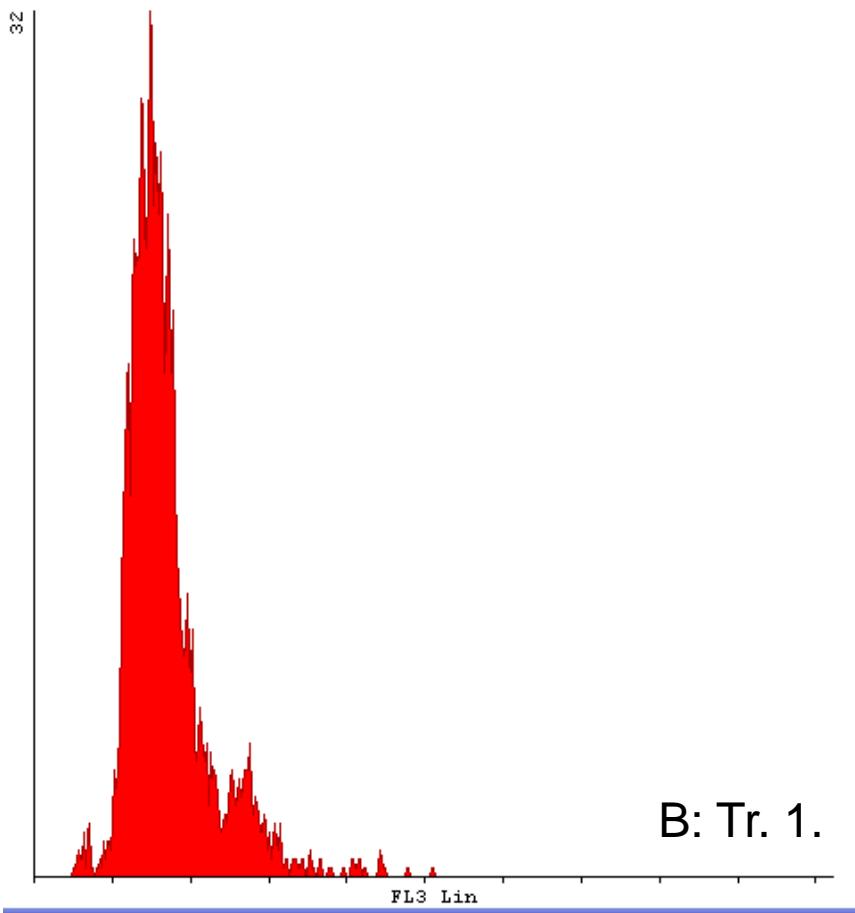
The screenshot shows a web page from the Walter + Eliza Hall Institute. At the top, there's a navigation bar with links like 'About Us', 'Research', 'Education', 'Technology', 'Institute', 'Contact Us', 'Support Our Research', 'News & Events', and 'Search'. Below the navigation, a green banner reads 'Free Flow Cytometry Software'. The main content area has a heading 'The Cellular Symphony' with a sub-section 'WARNING: Do not subject primary data to any of these programs. All files should be backed up prior to processing.' Below this, there are two download links: one for Macintosh (114,173 bytes) and another for other systems (429,226 bytes). To the right of the main content, there's a sidebar titled 'INSTITUTE' with various links such as 'Administration', 'Research & Discovery', 'Education', 'Technology', 'Institute', 'Contact Us', and 'Support Our Research'. There's also a 'RECENT NEWS' section with links to 'New research highlights', 'Research breakthroughs', 'Institute news', 'Publications', 'Press releases', 'Media coverage', 'Publications', 'Press releases', and 'Institute news'.

This software has been developed and used at The Walter and Eliza Institute and is provided in a spirit of collegiality for testing by interested parties. It is provided without warranty of any kind and no claims are made regarding its suitability for any task. All are Java programs and will run on systems with Java 1.4.2 or later installed. Unpack the downloaded file to extract the application and launch by double-clicking.

WARNING: Do not subject primary data to any of these programs. All files should be backed up prior to processing.

- [Download Cellular Symphony for Macintosh \[114,173 bytes\]](#).
- [Download Cellular Symphony for other systems \[429,226 bytes\]](#).





Cytogenet Genome Res 121:245–248 (2008)
(DOI:10.1159/000138891)

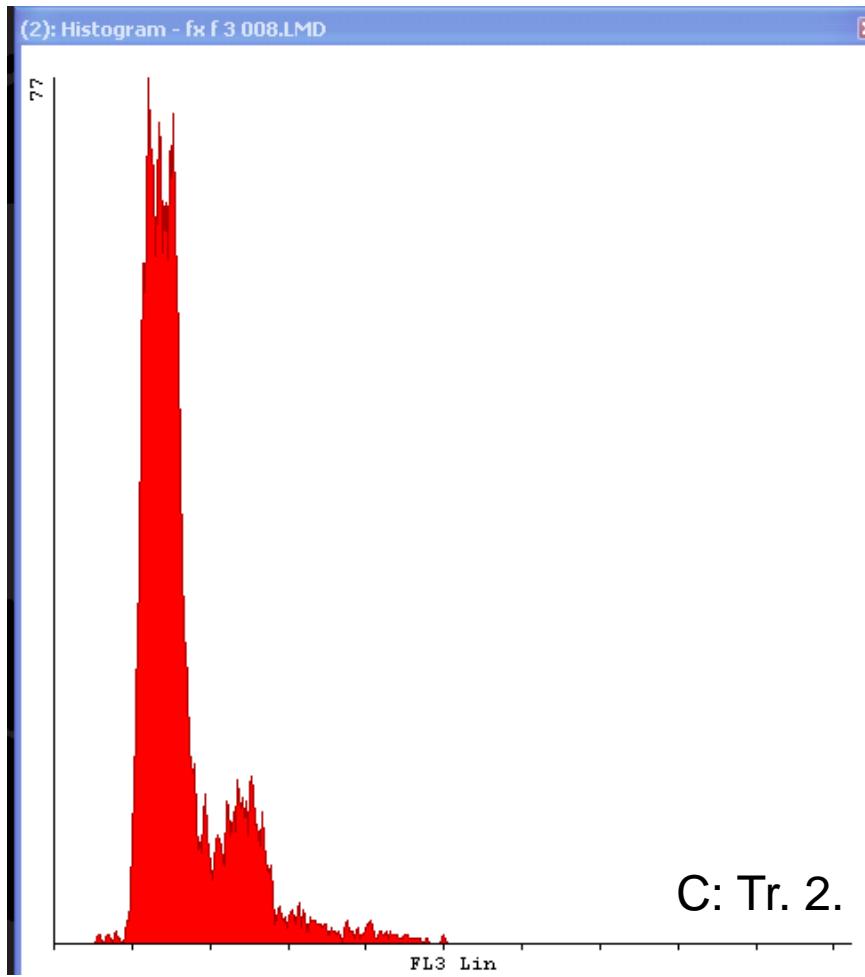
Identification of a new reciprocal translocation in an AI bull by synaptonemal complex analysis, followed by chromosome painting

Switalski M.^a · Andersson M.^b · Nowacka-Woszuk J.^a · Szczerba I.^a · Sosnowski J.^a ·
Kopp C.^b · Cernohorska H.^c · Rubes J.^c

^aUniversity of Life Sciences, Poznan (Poland); ^bUniversity of Helsinki, Helsinki (Finland); ^cVeterinary Research Institute, Brno (Czech Republic)



B: Tr. 1.



C: Tr. 2.



Theriogenology

Volume 38, Issue 4, October 1992, Pages 623–631



Embryo quality and andrological study of two subfertile bulls versus five control bulls with normal fertility

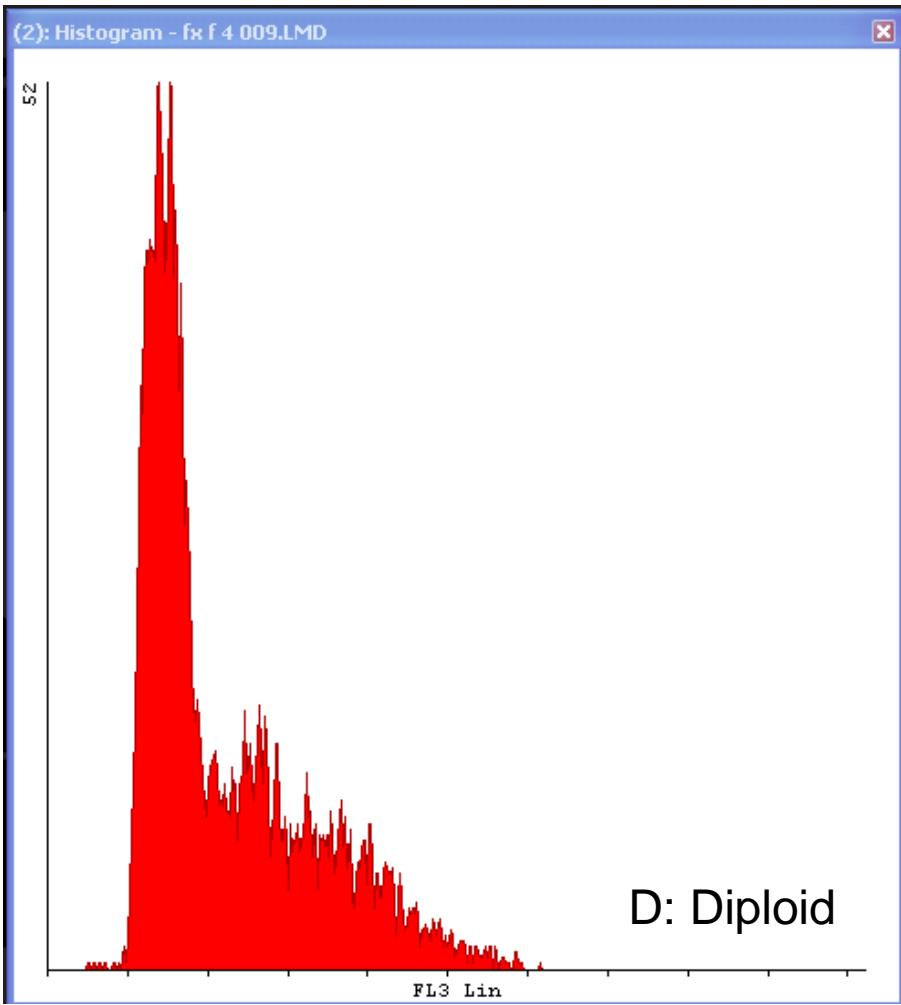
M. Andersson¹, J. Aalto², I. Gustavsson³

¹ Department of Obstetrics and Gynaecology College of Veterinary Medicine, Hautjärvi 04840, Finland

² Osuuskunta Itäjalostus, Pieksämäki 76101, Finland

³ Swedish University of Agricultural Sciences Department of Animal Breeding and Genetics S-75007 Uppsala, Sweden





Theriogenology

Volume 73, Issue 4, 1 March 2010, Pages 421–428



Diploid spermatozoa caused by failure of the second meiotic division in a bull

T. Revay^a, C. Kopp^b, A. Flyckt^b, J. Taponen^b, R. Ijäs^c, S. Nagy^d, A. Kovacs^e, W. Rens^f, D. Rath^g, A. Hidas^a, J.F. Taylor^h, M. Andersson^b,  

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^d Institute of Analytics, Environmental Sciences and Limnology, University of Pannonia, Veszprem, Hungary

^e Centre for Agricultural Sciences and Engineering, Institute of Animal Breeding Sciences, University of Debrecen, Debrecen, Hungary

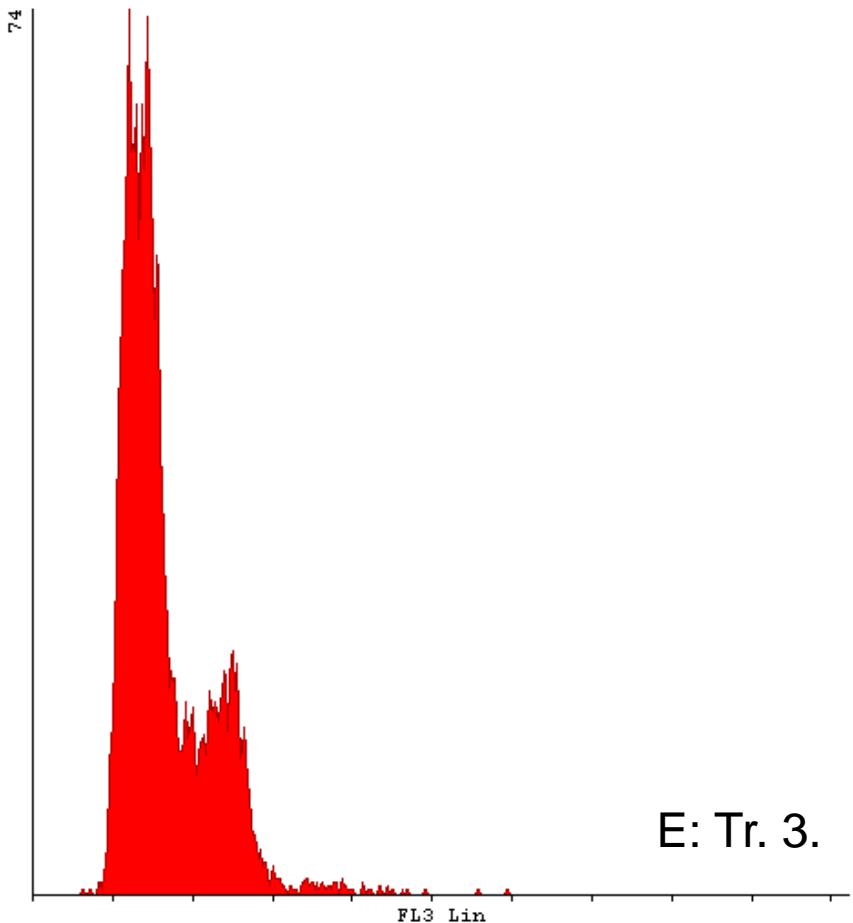
^f Department of Veterinary Medicine, University of Cambridge, Cambridge, United Kingdom

^g Federal Agricultural Research Centre, Institute for Animal Breeding, Neustadt, Germany

^h Division of Animal Sciences, University of Missouri, Columbia, Missouri, USA



(2): Histogram - fx f 5 010.lmd



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PLoS One. 2013; 8(9): e75659.

Published online 2013 Sep 26. doi: [10.1371/journal.pone.0075659](https://doi.org/10.1371/journal.pone.0075659)

PMCID: PMC3784456

Ectopic *KIT* Copy Number Variation Underlies Impaired Migration of Primordial Germ Cells Associated with Gonadal Hypoplasia in Cattle (*Bos taurus*)

Heli Venhoranta,^{#1,*} Hubert Pausch,^{#2} Michał Wysoczański,² Izabela Szczerbał,³ Reetta Hänninen,⁴ Juhani Taponen,¹ Pekka Uimari,⁵ Krzysztof Flisikowski,⁶ Hannes Loh,⁴ Ruedi Fries,² Marek Switonski,³ and Magnus Andersson¹

Toshi Shiota, Editor

¹Department of Production Animal Medicine, University of Helsinki, Saarentaus, Finland

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⁵AgriFood Research Finland, MTT, Biotechnology and Food Research, Jokioinen, Finland

⁶Chair of Livestock Biotechnology, Technische Universität München, Freising, Germany

Massachusetts General Hospital, United States of America

#Contributed equally.

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Competing Interests: The authors have declared that no competing interests exist.



E: Tr. 3.

Acknowledgements

- National Scientific Research Fund, Hungary (NKFI OTKA K139145)
- Anders Johannisson (SWE)
- Magnus Andersson (FIN)
- Andy Kovacs + (HUN)

THANKS!

