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# FLOW

## Clinical Flow Cytometry e -Newsletter

# focus

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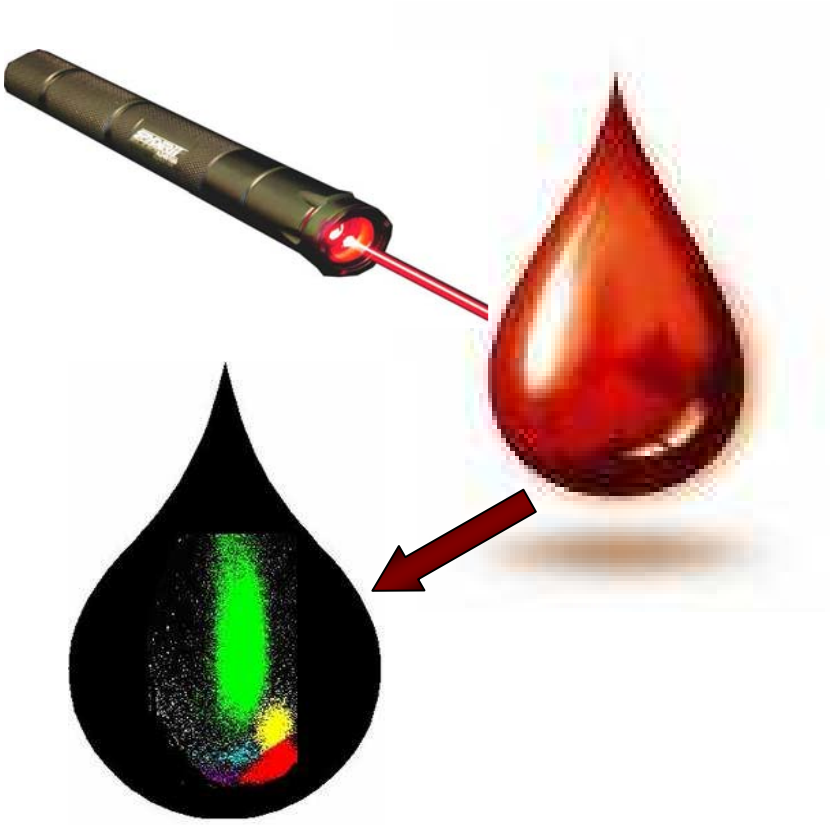
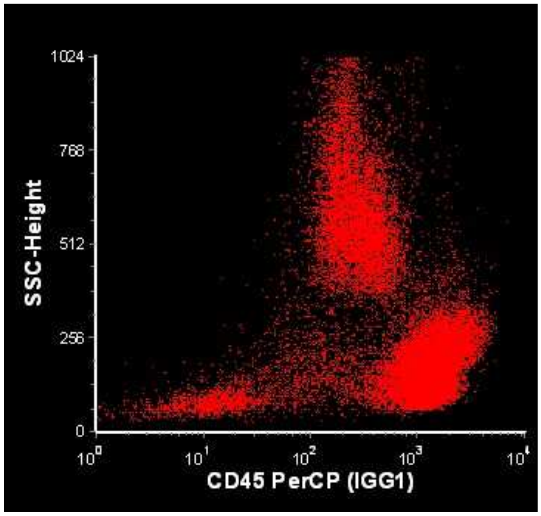


# FLOW FOCUS

**VOL.01, ISSUE 2, JUNE 2009**

**CLINICAL FLOW CYTOMETRY e-NEWSLETTER**




**It gives us immense pleasure in writing the editorial for this e-newsletter in its second issue. When it was launched 2 months back there was definite hope to continue this endeavor further, however, we were also skeptical as to how it would be taken by the flow community at large. We now feel encouraged by the overwhelming response from our esteemed readers who have acknowledged it well.**

**While there have been words of encouragement, there has been some criticism too and we have taken it in our stride. We understand that it was difficult to open the newsletter last time since there were a lot of graphics involved. This time around we have tried to keep the graphics to bare minimum. However, I am sure that all will agree to the fact that flow without colors and graphics will be totally out of taste and, therefore, the requisite graphic data has been included.**

**I hope that you all will enjoy reading this issue of "Flow Focus" as well!!**

**Editors**

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Immunophenotyping, as we know, is dependent on the fluorochromes tagged antibodies excited by LASER technology. LASER technology at its inception was one of the most controversial technologies for which patenting saw many court battles between Gordon Gould and LASER manufacturers. **Gordon Gould** [A] (July 17, 1920 – September 16, 2005) was an American physicist who is widely, but not universally, credited with the invention of the laser. Gould recorded his analysis and suggested applications in a laboratory notebook under the heading "Some rough calculations on the feasibility of a LASER: Light Amplification by Stimulated Emission of Radiation"—the first recorded use of this acronym. Even though his role in the actual invention of the laser was disputed over decades in the courts, Gould was elected to the National Inventors Hall of Fame in 1991.

A

B

Arthur Schawlow and Charles Townes independently discovered the importance of the Fabry-Pérot cavity—about three months later—and called the resulting proposed device an "optical maser". **Charles Hard Townes** [B] (born July 28, 1915) an American Nobel Prize-winning physicist and educator is known for his work on the theory and application of the maser, on which he got the fundamental patent, and other work in quantum electronics connected with both maser and laser devices. He received the Nobel Prize in Physics in 1964.

**Dr. Arthur Leonard Schawlow** [C], brother-in-law of Charles Townes, a professor for some time at Stanford University, together with Townes showed theoretically that masers could be made to operate in the optical and infrared region and proposed how this could be accomplished in particular systems. This work resulted in their joint paper on optical and infrared masers, or lasers (light amplification by stimulated emission of radiation).

C

D

**Theodore Harold "Ted" Maiman** [D] (July 11, 1927 - May 5, 2007) an American physicist is credited with the invention of the first laser. Maiman received the Japan Prize in 1987. He developed, demonstrated and patented the world's first laser in 1960. Maiman's laser, based on a synthetic ruby crystal grown by Dr. Ralph L. Hutcheson, was first operated on 16 May 1960 at Hughes Research Laboratories in Malibu, California. Due to his work on the laser, he was twice nominated for a Nobel Prize and was given membership in both the National Academies of Science and Engineering. He received the Oliver E. Buckley Prize in 1966. He was the recipient of the 1983/84 Wolf Prize in Physics, and was inducted into the National Inventors Hall of Fame that same year. Besides, he received F&J. Hertz and Japan Prizes. Japan Prize is considered equivalent to Nobel Prize. Many world universities awarded him Honorary Degrees. The last Honorary Degree he received in 2002 from Simon Fraser University, Vancouver, Canada.

# FLOW CYTOMETRY: A BEGINNER'S VIEW-POINT!*

[*Statutory Warning: Article meant only for newbies ☺]

Flow cytometry is a technique which every hematology trainee aspires to learn and master. Though we all know working on the instrument is the only way to learn the skill, a basic understanding of the procedure before we take the plunge would certainly help us in going a long way.

To the beginner flow cytometry appears valuable albeit complex, as it is usually more informative as compared to microscopy. We expect and hope that our seniors would be able to do magic and yield definite answers regarding difficult lesions which draw a blank on morphology alone.

An idea of how flow cytometry is done and what the basic idea behind it is expected of us; as we better know what we are actually out to achieve. ; For eg: before we begin we must have some clue as to what the basic principles of flow cytometry are. What is meant by fluidics, what is the role of illumination system, optics, and electronic system in cytometry? What the clinical utility of flow cytometry is? How immune-phenotyping of cells would help us distinguish between benign and malignant lesions, how to classify leukemias/lymphomas, how flow cytometry can be useful to detect minimal residual disease, for DNA cell cycle analysis…….etc…etc.

Practically, first things we learn are - terminologies such as *Data analysis*, *Data acquisition* and *Data interpretation.* Other new words that take prominence in our vocabulary are *gating, list-mode, scatters* and *fluorescence and dot plots*.

But our problems do not end here – suppose we do manage to acquire, decipher and interpret the data, we realize that actually, the real challenge does not merely lie in production of results - but in producing results which are not a consequence of inadvertent or unintentionally produced artefacts… and they better be reproducible.

Another enemy of the untrained eye is debris, which mingles with the normal cells on the screen, looks like positive cells as and when it pleases, and bewilders us as to who would save us from this mess!

The idea is to train the eye in identifying the normal cell populations so as to recognize the aberrant ones as and when they pop up.

Skill is also to be acquired in ascertaining whether a cell population is actually positive or not. A practical and systematic approach is required to analyze the visual pattern seen, as to our dismay, being to the right side of a line drawn along the first log is not sufficient to call them positive and merely calculating percentage positives for each antibody is not the right technique.

To add to our distress, pre-analytical work-up such as specimen handling and processing is also equally important. Try running a sample, more than 48 hrs after collection after you have nicely put it aside and forgotten about it. Exposure to extreme temperatures, presence of blood clots or gross hemolysis will also do the sample no good!

Flowcytometric immunophenotyping of hematopoietic disorders is a complex and demanding exercise that requires a good understanding of cell lineages - which obviously we don't have. If we manage to get the hang of the basic technique, we tend to realize that the elite and learned people use complicated jargon such as the dreaded term – *compensation,* which leaves us flummoxed.

We must also know that total cell counts are important and must be able to decide on important and intelligent questions; for eg: How much dilution is to be made? How much antibody is to be added? - Which our technicians will ask - and in order to avoid looking like fools at that inopportune moment, we better have an answer. Talking of antibodies, we should have an idea as to which antibody is to be tagged to which flurochrome. Which fluorochromes are used on your flow cytometer? Which flow cytometer do you use? How many lasers are there in your flow cytometer? How is it different from other flow cytometers? What are the antibody panels you are going to use?.....the list goes on…….!!

Quality control, quality assurance and inter-laboratory comparison is another aspect of flow cytometry, which we better not touch upon as of now and leave it to the knowledgeable.

So my dear fellow strugglers……once you put your foot into this quagmire, you realize that the questions are endless……*and you have miles to go before you reap*☺!! But don't lose heart!

All said and done; *Best of luck* in your endeavors…. may you have as much fun with your plots and scatters as I am having. Enjoy it while you can; as all your escapades will definitely be under someone's able guidance and hopefully no harm will come to the patient!

**P.S:** This article showcases my experience with flow cytometry and is in no way an attempt to improve anyone's general knowledge or be educative*!!*

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# Role of flow cytometric immunophenotyping in mature T- and NK-cell neoplasms

Assessment of immunophenotypically abnormal mature T/NK-cell populations is more challenging than clonal mature B-cell populations which are much more common and better characterized. In addition, mature B-cell clonal processes are readily identifiable by demonstration of monotypic immunoglobulin light chain expression. However, flow cytometry does offer a robust technique for assessing expression pattern and intensity of multiple antigens on T/NK-cell populations of interest. It is imperative to interpret this information in the context of the clinical presentation, geographical context (some lesions are endemic), physical location of the lesion, morphology of the neoplastic cells, tissue immunohistochemistry (e.g., cytotoxic molecules like TIA-1, granzyme B, etc), virology studies [e.g. in situ hybridization for Epstein-Barr virus encoded RNA (EBER), serology for HTLV-1, etc], and molecular studies, such as T-cell receptor (TCR) gene rearrangement (GR) through polymerase chain reaction or Southern blot. Perhaps the most critical of the above mentioned parameters is the interpretation in light of clinical findings, e.g., is the patient asymptomatic, are there any cytopenias or B symptoms, etc. site of involvement- skin, nose/paranasal sinuses, blood/bone marrow, liver/spleen, lymph nodes, small intestine, etc, because of specific clinico-pathologic presentations in multiple subtypes of mature T/NK-cell neoplasms. Flow cytometry, therefore, can aid in the identification of aberrant T-cell populations, but the specific diagnosis is heavily dependent on evaluation of all the above mentioned parameters.

Amongst the commonly employed flow cytometric reagents, non-neoplastic mature T-cells express CD2, CD3 (surface and cytoplasmic), CD5, CD7, along with either CD4 or CD8. A subset of T-cells can express NK-cell associated antigens, viz. CD16, CD56, and CD57. Most mature T-cells express TCR-alpha beta (a/b) while a smaller subset are TCR-gamma delta (g/d). Normal subsets of g/d T-cells lack CD5, and are double negative for CD4 and CD8. Non-neoplastic NK cells express CD2, CD7, CD16, CD56 and CD57 and lack surface CD3, CD4 and CD5. CD8 is variable, but may be normally expressed in a small subset. NK-cells also express **cytoplasmic** CD3 epsilon, which is also detectable by immunohistochemistry.

Aberrancies of antigenic expression in neoplastic T-cells have to be distinguished from variations in normal/reactive situations. Absence or decreased expression of surface CD3 and/or CD5 is highly suggestive of an aberrant T-cell population. CD7 diminution or loss may again indicate an aberrant T-cell population, but has to be interpreted in the right context, as a subset of normal T-cells in the blood and skin as well as in certain reactive situations, can be CD7 negative. Abnormal T/NK-cell populations can also occasionally express myeloid antigens such as CD13, CD15 and CD33. T-cell processes are CD4 (mostly) or CD8 positive, or rarely, be double negative for CD4 and CD8 (CD4-/CD8-), or double positive for CD4 and CD8 (CD4+/CD8+).

Here is a summary of the more frequently encountered mature T-cell processes submitted for flow cytometry:

**BLOOD:**

**T-large granular lymphocytic leukemia** is a clonal expansion of predominantly CD8-positive T-cells that may co-express NK-cell associated antigens such as CD16, CD56 or CD57, and with frequent aberrant loss/decreased expression of CD5 and/or CD7. This indolent process may be associated with neutropenia, rheumatoid arthritis, splenomegaly and pure red cell aplasia. Rare forms of this disorder express CD4 instead of CD8.

**Sezary cells** in the blood of a patient with cutaneous T-cell lymphoma are often CD4+ and frequently lack CD7. These cells also show variable presence and intensity of CD25 expression. The diagnosis needs to be made in the appropriate clinical context of advanced CTCL and with characteristic morphology of Sezary cells.

**Adult T-cell leukemia/lymphoma (ATLL)** has a CD4+, CD7 negative phenotype similar to CTCL/Sezary syndrome but in contrast frequently show strong uniform expression of CD25. This entity has to be entertained in the appropriate epidemiologic setting [i.e. in zones endemic for human T-cell leukemia virus-1 (HTLV-1)], and should be confirmed by pertinent serologic and molecular testing for HTLV-1.

**T-prolymphocytic leukemia (T-PLL)** is most frequently CD4+/CD8-, sometimes double positive for CD4 andCD8, and less commonly, CD8+/CD4-. Unlike CTCL/Sezary or ATLL, it frequently retains the expression of CD7, is negative for CD25 and lacks aberrant loss of expression of other pan T-cell antigens. There is usually no expression of NK-associated antigens nor cytotoxic molecules such as TIA-1, granzyme B or perforin. The disease often presents with marked lymphocytosis, with prolymphocytic or small cell morphology.

**Hepatosplenic T-cell lymphoma** is almost always a TCR-g/d process that is frequently negative for CD5, double negative for CD4 and CD8, and for CD57; often positive for CD3, CD7, CD56, and variably positive for CD16. The disease is hepato-splenic in distribution with frequent blood and bone marrow involvement and an aggressive course.

**LYMPH NODES:**

**Peripheral T-cell lymphoma, NOS** or **angioimmunoblastic T-cell lymphoma (AITL)** in a lymph node or sometimes in extranodal tissues or blood/bone marrow, are frequently CD4-positive, TCR a/b processes, and may show variable aberrancies of CD3, CD5 or CD7 expression. CD10 expression on T-cells may be seen in a large subset of AITL. For AITL, characteristic constellation of histopathologic findings includes neoplastic CD10-coexpressing T-cells with clear cytoplasm, scattered EBER-positive B-immunoblasts, all set in a backdrop of proliferated capillaries enmeshed in a expanded CD21 or CD23-positive follicular dendritic cell meshwork.

**EXTRANODAL SOLID TISSUES:**

One NK-cell neoplasm of note is the nasal type extranodal NK/T-cell process (previously called lethal midline granuloma), that may also present outside of head and neck locations. This lymphoma is commoner in Asia and parts of Latin America, but rare elsewhere. The immunophenotype is

characteristic of NK cells, with no expression of surface CD3 by flow cytometry but positivity for cytoplasmic CD3 by immunohistochemistry; frequent lack of CD4, CD5, CD8 expression, positivity for CD2, CD7, CD56, cytotoxic molecules (TIA-1, granzyme B or perforin) and diffuse EBER expression by ISH in the neoplastic cells, that are often present in an angiocentric/angiodestructive infiltrate associated with marked necrosis.

Other specific T-cell processes affecting the skin (e.g. CTCL, subcutaneous panniculitis-like T-cell lymphoma) or the small intestine (enteropathy associated T-cell lymphoma, which expresses cytotoxic molecules, CD3, CD7, and CD103 with usual lack of CD4, CD5 and CD8 expression) are generally not available fresh for flow cytometric assessment but can be diagnosed with characteristic morphologic features and immunohistochemistry.

To conclude, clonal mature T/NK-cell processes can be suspected on flow cytometric immunophenotypic features, but the confirmation of a neoplastic process and further subclassification requires a rigorous correlation with clinical features, site of involvement, morphology, immunohistochemistry, molecular, and pertinent viral studies

**Suggested Reading:**

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# CD45 GATING IN LEUKEMIA/LYMPHOMA PHENOTYPING - CAN WE DO WITHOUT IT?

**"Not everything that counts can be counted and not everything that can be counted counts" Albert Einstein**

## Introduction to gating

In flow cytometry, cell suspensions stained with monoclonal antibodies tagged to immunofluorescent dyes are directed in a fluid stream into a flow cell where they are hit with a laser beam. The light is scattered from the cells and is also partly absorbed. The scattered light reflects the physical attributes of the cell (measured as forward and side scatter) and absorbed light is re-emitted as fluorescence. This light is sensed by detectors and an electrical impulse generated is converted to a digital signal by a digital-analogue converter. All events of a sample are stored as "list-mode files". Usually, all events of a particular tube are stored as a single file. This file is accessed later for editing and events of interest are analyzed and results are generated.

Unless specified otherwise, gating is a technique of defining cells to be interrogated after physical and fluorescent properties of the entire sample have been acquired on the basis of gated parameters. In simple words it is the denominator of your question. It is crucial to understand that the parameter upon which the sample needs to be gated is present in every tube. Forward and side scatter parameters (FSC/SSC) are present in every analysis. Similarly, if an additional parameter for gating, for example, CD45 is to be used, then CD45 should be present in every tube. This ensures that "we are talking of the same population"

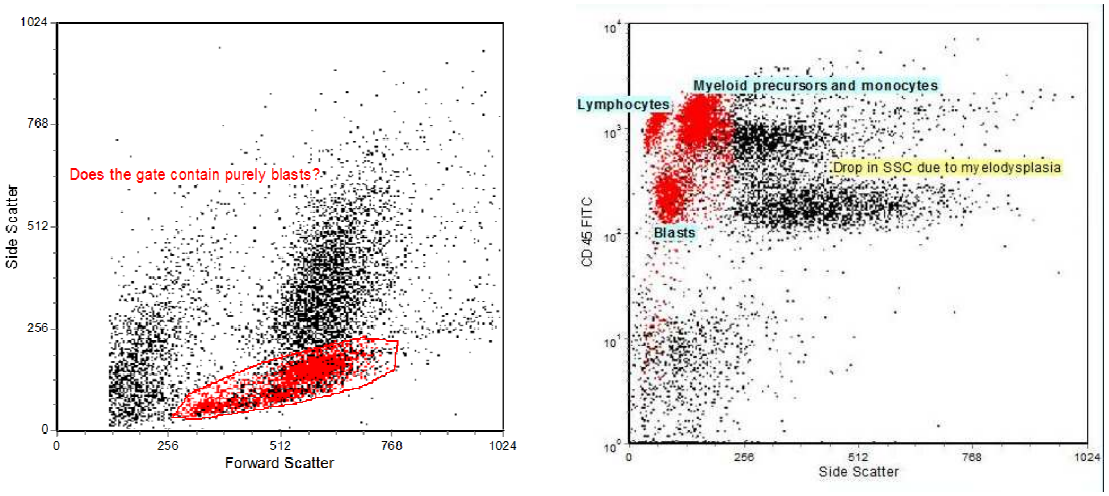


Figure 1: A 56 year old male diagnosed to have AML with myelodysplasia related changes. A gate (in red) on the FSC/SSC plot (left picture) is made for evaluation of blast phenotype. The events coloured in red (right picture) reflected in the CD45 (Y-axis) and SSC (x-axis) plot, include blasts, lymphocytes, myeloid precursors and debris. All of these events have been included in the gate on FSC/SSC.

**Why is gating based on FSC/SSC not optimal for leukaemia and lymphoma?**

To understand why traditional FSC/SSC fails as a primary gating strategy, one has to look into the evolution of clinical flow cytometry (FCM) as a science. The demand for diagnostic FCM increased with the onset of HIV epidemic. Flow cytometers were required for rapidlymphocyte subset analysis. FSC/SSC gating was done for evaluating lymphocytes and not other lineage of cells. For analysis of haematolymphoid neoplasms, the submitted sample (usually bone marrow or peripheral blood) contains multiple cell populations with overlapping size and morphologic complexity. As blast population gets mixed with normal "resident" cells (monocytes, myeloid precursors and lymphocytes) falling in the same region, the accuracy of the analysis drops. This is highlighted in Figure 1. This problem was recognized as early as 1988.[1]

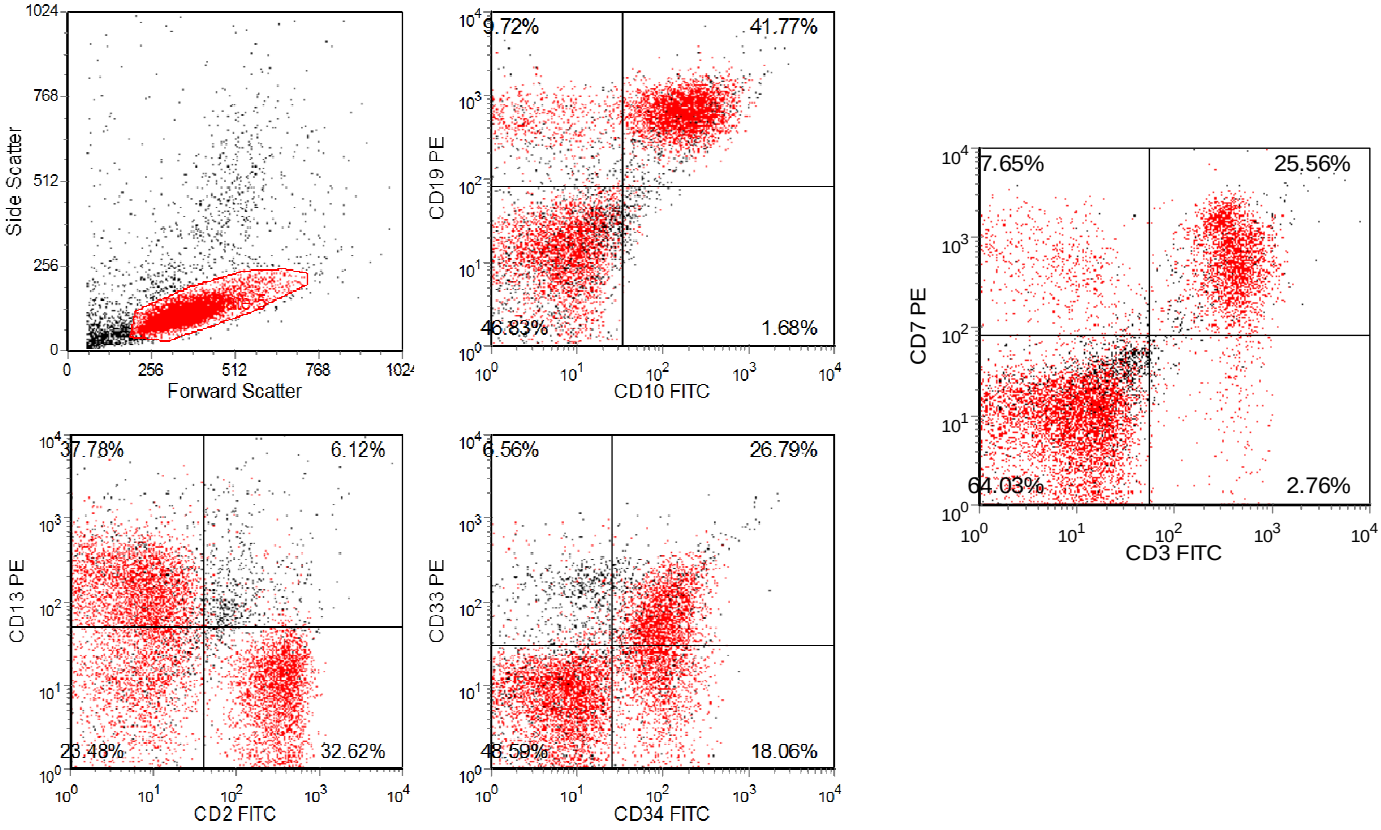


Figure 2: A 10 year old male with 45% blasts in peripheral blood. A gate (in red) on the FSC/SSC plot (left picture) is made for evaluation of blast phenotype. A diagnosis of Pre-B ALL with aberrant CD33 is made on a CD45/SSC plot. Normal T lymphocytes contaminating the FSC/SSC gate give false CD2, CD3 and CD7 positivity.

Figure 2 also demonstrates this problem. Gating on FSC/SSC fails to exclude resident T lymphocytes, which give incorrect positive values of CD2, CD3 and CD7. This case also highlights the hazards of concentrating on percent positive values and not on abnormal phenotype.

Inability to define an accurate denominator of interest for analysis is the main drawback of gating on FSC/SSC. As a result, it is difficult to track a neoplastic population. Utilizing the property that, CD45 expression is usually of lower intensity on blasts than it is on normal lymphocytes or monocytes, a study done by Lacombe et al in 1997 analyzed 74 cases of AML by immunophenotyping. Blast cells were first identified by CD45/SSC and those results were compared to FSC/SSC gating procedure and morphology. The percentages of blast cells on morphology correlated better with the values determined by CD45/SSC gating as compared to FSC/SSC gating. Also, the CD45/SSC gating procedure improved phenotypic determination by discriminating between leukemic blast cells and residual normal cells by excluding normal cells from the phenotypic analysis of leukemic blast. CD45/SSC enabled efficient discrimination between the various cell lineages and facilitated the analysis of leukemic blasts present in low proportions. They elegantly demonstrated that FSC/SSC resulted in false positive (%) values of CD5, CD3, CD7, CD10, CD14 &CD11b and false negative (%) values of CD34, CD33, CD13 and HLA-DR.[2] The false positive results are explained by inclusion of mature lymphocytes, granulocytes and monocytes in the analysis. As these values increase, other values will involuntarily decrease, thus explaining the false negatives. Distinct advantages of CD45/SSC gating are described in Table1. Another advantage is that, it usually helps in identifying a blast population

**Evolution of CD45 and Side Scatter as a gating strategy**

One of the first studies which utilized CD45/SSC as a gating strategy was by Stelzer et al.[3] The authors stated that the amount of CD45 antigen was different in separate lineages of cells and it changed during the maturation of cells within a single lineage. Flow cytometric quantitation of CD45 binding capacity, in combination with right-angle light scatter (SSC) was used to quickly identify eight major components of normal bone marrow. They rightly stated that these groups of cells could then be independently assessed for other cell surface markers. This can be demonstrated in Figure 3.

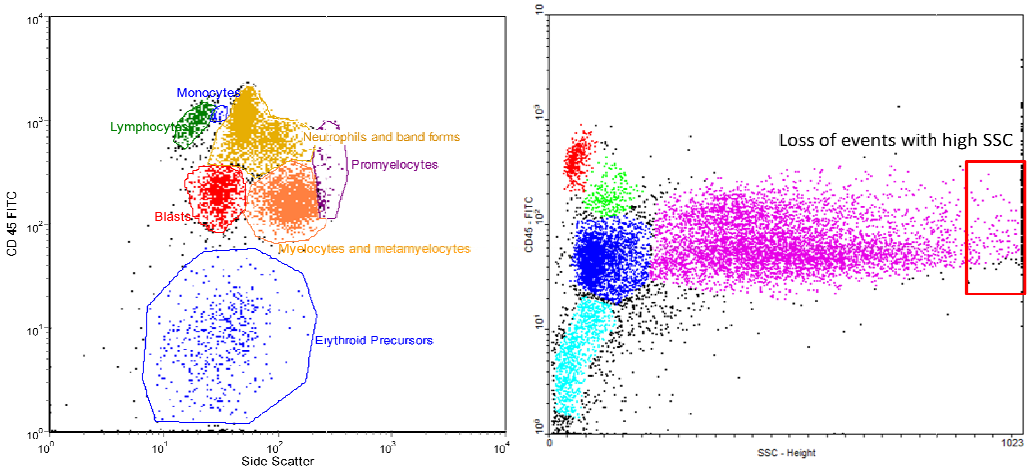


Figure 3: Components of the bone marrow (Left). It should be noted that SSC here has been acquired on the Log scale as opposed to a linear scale (Compare with figure on right hand side). This permits better quantification of SSC and prevents loss of events with very high side scatter such as promyelocytes.

This strategy was used on neoplastic haematolymphoid samples by Borowitz et al.[4] In this oft quoted paper, the authors utilized CD45 to gate on blasts in haematolymphoid neoplasms. They discovered different patterns seen in different FAB subtypes of AML. Found this strategy superior to FSC/SSC in isolating and analyzing leukemic cells.

**Recommendations around the globe**

US-Canadian Consensus Recommendations (1997)[5]

"Gating, is defined as the selection of a specific cell population for examination of parameter expression....In general, the best gating strategies will be different for different diseases....A universal gating strategy for all samples that employs forward vs. right angle light scatter (as was popularized for lymphocyte phenotyping) is not appropriate. In acute leukaemia, display of CD45 and right angle light scatter is a simple approach for the identification of blast populations." The authors also suggested a few strategies such as "Gating on B cells to look at clonality in preparations using simultaneously a B-cell marker and anti-kappa and anti-lambda antibodies, B-cell antibody (eg CD19 or CD20)as a common marker in all tubes used to characterize the neoplastic population in cases of B lymphomas, Utilizing a pan T-cell antibody (eg CD3)to characterize the phenotype of T-cell lymphoproliferative disorders."

2006 Bethesda International Consensus[6]

"At least one common reagent should be present in each of the tubes used for the evaluation of a particular lineage to allow correlation or tracking of populations between tubes. They suggested use any of the following gating strategies

1. CD45 versus side scatter
2. Lineage-associated antigens: e.g. CD19 for B cells and CD3 for T cells.
3. Maturity associated antigens: e.g. CD34 for gating specifically on blasts.

Their comments on FSC/SSC as a gating strategy were "This most basic method requires no additional reagents, but has a limited ability to discriminate between populations.....should not be used as the primary method for population identification in light of the more powerful approaches outlined earlier. One exception is in the recognition of neoplasms composed of large cells where it may provide the best method for tracking populations between tubes."

NCCLS Guidelines (2005)[7]

"A precise gate ensures both that the same cells are evaluated in different tubes, and that irrelevant cells with overlapping light scatter properties (e.g., erythroid precursors, lymphocytes, and monocytes) do not contaminate the blast gate, confounding interpretation. Although the immunophenotypic heterogeneity of acute leukemias necessitates modifying the location of the blast gate in multiparametric space in individual cases, a display of CD45 expression vs. SSC typically resolves blast populations well from other contaminating mononuclear cells. In designing antibody panels for the analysis of acute leukemias, therefore, it is useful to include CD45 in each tube."

Other supporters and a sole opposition[8]

Guidelines from Spain, Italy, Latin America and India recommended the utility of this strategy. A sole opposition to this strategy came from the British committee for standards in haematology (BCSH), which rather emphatically stated that this strategy was not required for routine diagnosis. Strangely, no explanation was offered for this approach.

**CD45/SSC gating in the diagnosis of lymphoma.**

This is a more difficult proposition. Does one need a CD45 gating in regular cases of CLL? It is left to user discretion. It definitely helps to focus exclusively on the lymphocytes. CD45 in most cases can confirm that they are lymphoma cells based on expression of bright CD45. However, once an aberrant phenotype is identified then the gating should be more specific for e.g. Using CD19 or CD3 for B-cell and T-cell NHLs respectively.

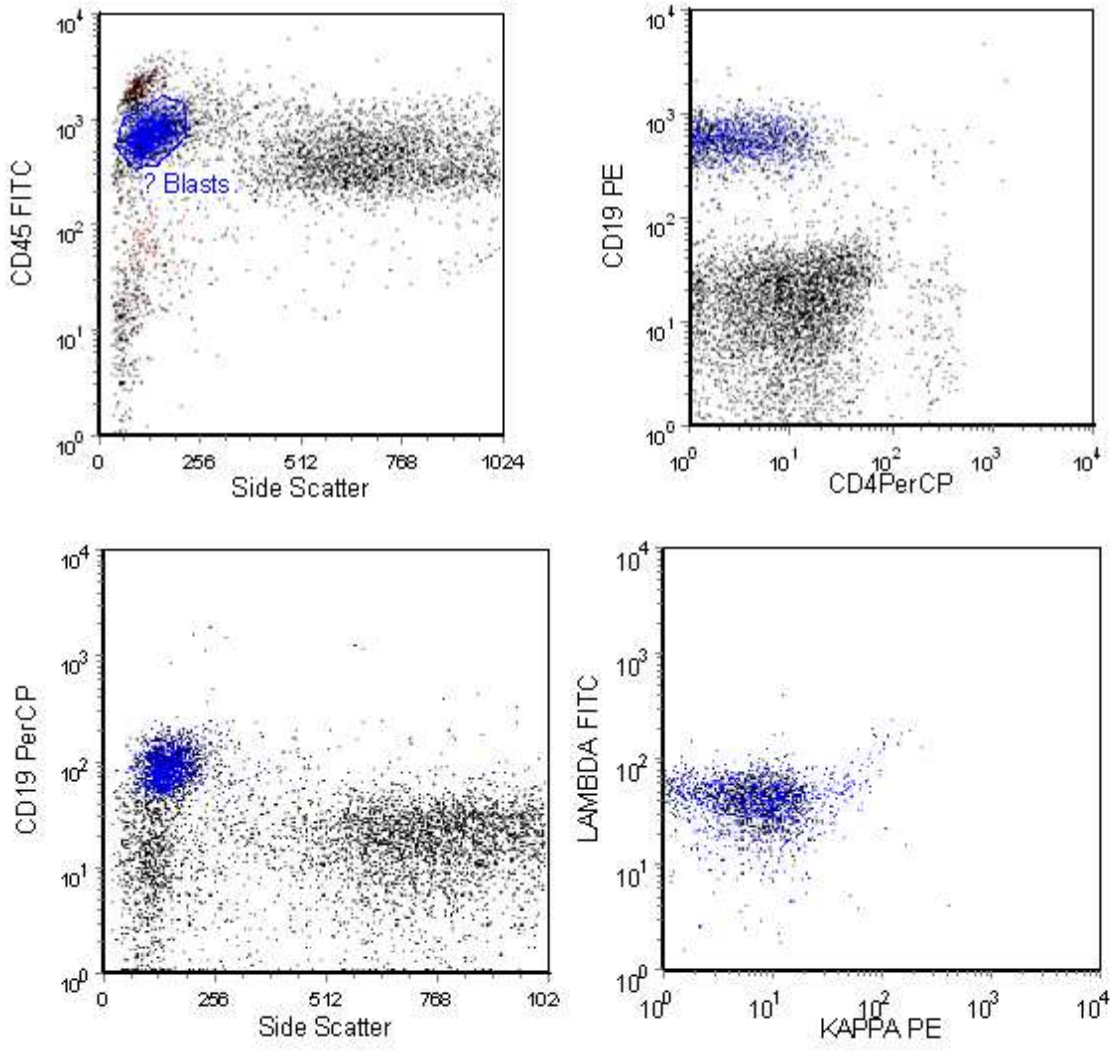


Figure 4: A case of CLL with possible transformation to Richter's syndrome. The neoplastic population in this case expressed dim CD45 in addition to other markers suggestive of CLL.

**A few caveats**

One should be aware that a dim CD45 is not specific for blasts. Rarely, lymphomas especially large cell lymphomas express dim CD45.(See figure 4) Bertram et al have documented dim to negative CD45 up to 19% of large cell NHLs.[11] These cases have a potential to be misdiagnosed as an acute leukaemia. Care must be taken to correlate with trephine and lymph node biopsy. Similarly T-cell ALLs can occasionally express high levels of CD45, close to or adjacent to the region of lymphocytes.

**Technical Notes**

The CD45 antigen is expressed in abundance on cells. Therefore it would be appropriate to tag it with a weak fluorochrome (eg Per-CP). It should also be present in analysis tube containing the isotype controls (e.g. FITC Isotype/PE Isotype/CD45-Per-CP/APC Isotype). Any newly purchased antibody (of a specific lot) should be titrated and the volume determined by the titration experiment be used.

**The future of CD45 gating controversy?**

The chief objection to CD45/SSC gating is that it adds to the cost and it uses an extra channel. As multicolour FCM (>6 colours) becomes prevalent the number of tubes for analysis will decrease. Adding CD45 to 3 or 4 tubes may not really make a difference. These debates will become irrelevant and will die of natural causes. The concept of gating using commonly expressed antibodies is here to stay.

| | **Advantages of CD45/SSC gating** |
|---|---|
| **1** | Effective analysis of only the population of interest |
| **2** | Rules out contaminant populations |
| **3** | Enables small population analysis (e.g. Lymphoblastic lymphoma infiltration in marrow) |

Table 1: Advantages of CD45/SSC gating

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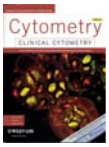
# JOURNAL SCAN

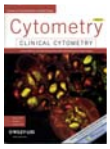
Ryan J, et al. Minimal residual disease detection in childhood acute lymphoblastic leukaemia patients at multiple time-points reveals high levels of concordance between molecular and immunophenotypic approaches **BJ H 2009; 144(1): 107-115**

Robert W. Allan, M.A. Ansari-Lari and Sandra Jordan. DRAQ5-Based, No-Lyse, No-Wash Bone Marrow Aspirate Evaluation by Flow Cytometry. **AJCP 2008 129:706-713**

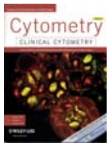
Meganathan Kannan, Firdos Ahmad, Birendra Kumar Yadav, Pratik Kumar, Paresh Jain, Rajive Kumar and Renu Saxena. Carrier Detection in Glanzmann Thrombasthenia. **AJCP 2008 130:93-98**



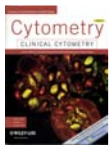
D'Souza E, Ghosh K, Colah R. A Comparison of the choice of monoclonal antibodies for recovery of fetal cells from maternal blood using FACS for noninvasive prenatal diagnosis of hemoglobinopathies. **Cytometry Part B 2009; 76B: 175-180**.



Gujral S, Badrinath Y, Kumar A, Subramanian PG, Raje G, Jain H, Pais A, Kadam PSA, Banavali SD, Arora B, Kumar P, Hari Menon VG, Kurkure PA, Parikh PM, Mahadik S, Chogule AB, Shinde SC, Nair CN. Immunophenotypic profile of acute leukemia: critical analysis and insights gained at a tertiary care center in India. **Cytometry Part B 2009; 76B: 199-205**.



Coetzee LM, Tay SS, Lawrie D, Janossy G, Glencross DK. From research tool to routine test: CD38 monitoring in HIV patients. **Cytometry Part B 2009 – [on line early]**



Mantei K, Wood BL. Flow cytometric evaluation of CD38 expression assists in distinguishing follicular hyperplasia from follicular lymphoma. **Cytometry Part B 2009 – [on line early]**

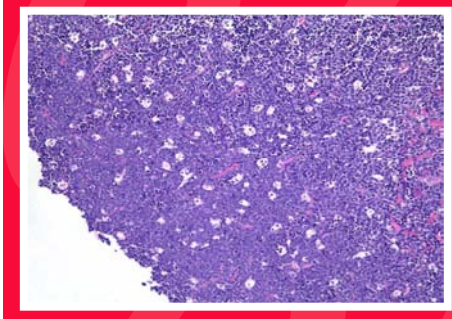
Motwani J, et al. Predictive value of flow cytometric minimal residual disease analysis in childhood acute lymphoblastic leukaemia at the end of remission induction therapy – results from a single UK centre **BJH 2009; 144(1): 133-135**.

# QUIZ TIME

***10 year old boy with***

***Intestinal obstruction***

**1**



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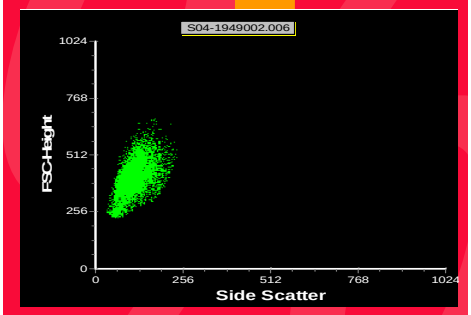


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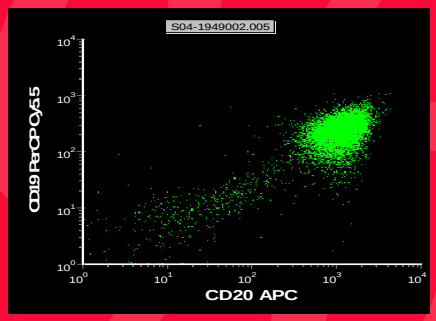


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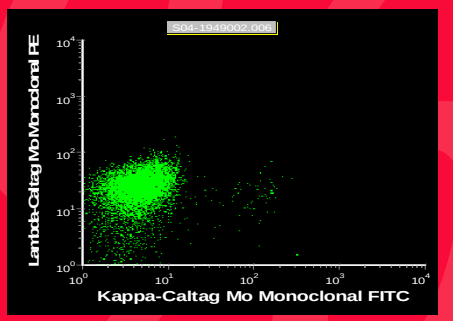


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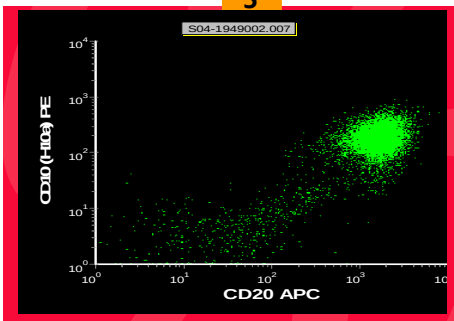


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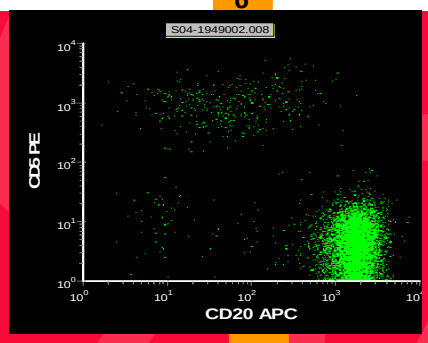


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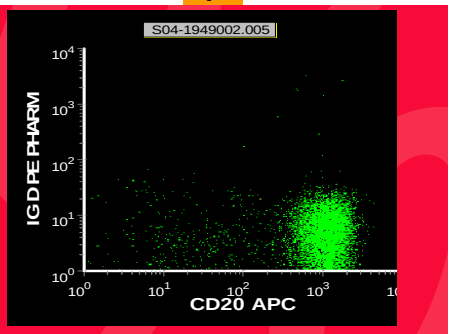


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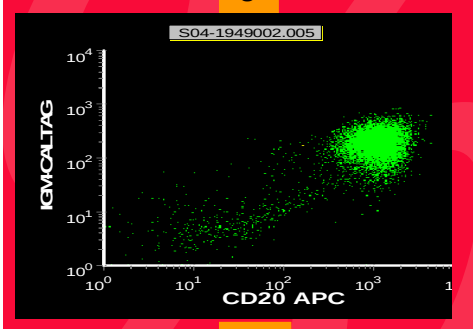


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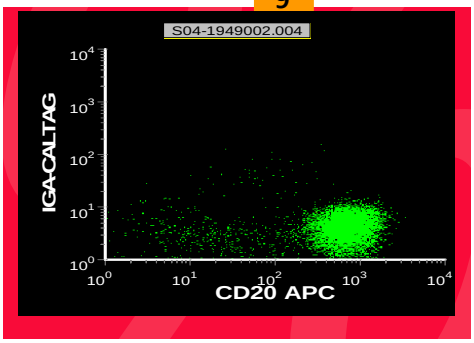


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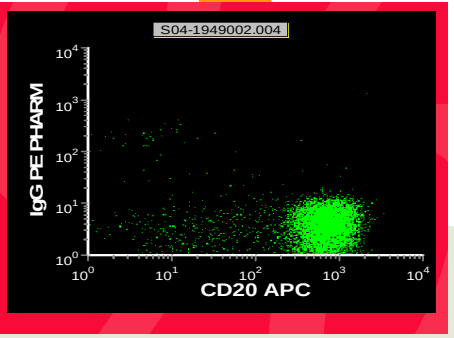


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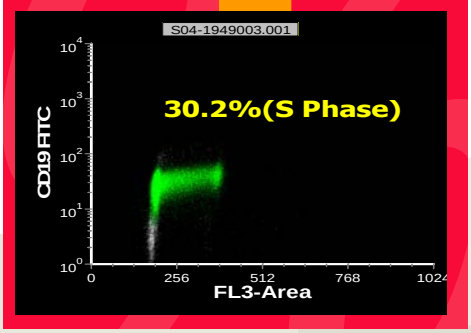


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http://flowfocus.webs.com/

***Contributed by:***
***Dr Tina Dadu***

# REWARDS & BRICKBATS

"Your news letter is very impressive and will fulfill a great need. I am glad you have taken it upon you to foster flow cytometry education"……**Dr Awtar Krishan,** Professor, Dept of Pathology, Miller School of Medicine, University of Miami, Miami (USA).

"I would suggest this would be an excellent item to post on the CCS website, with appropriate acknowledgements, etc, since TCS is the sister society of CCS"……**J Philip McCoy, Jr.**, PhD, Head, Flow Cytometry Core, NHLBI – NIH, 10 Center Dr, MSC 1357, Bldg 10, Rm 8C103D, Bethesda, MD 20892

"This is fantastic! Many kudos to the people involved in getting this job done!"……**H. Krishnamurthy**, Central Image-Flow Facility, National Centre for Biological Sciences, Tata Institute for, Fundamental Research, UAS-GKVK campus, Bellary Road, Bangalore 560 065 Karnataka, INDIA

"Kudos! It is a super duper effort to start something like this, a newsletter for clinical cytometry. Once again congratulations to you, Tina and Dr Sood. Keep up the good work"……**Dr Sumeet Gujral,** Associate Professor, Department of Pathology, Tata Memorial Hospital, Mumbai

"Congratulations!" ……**Dr Tejinder Singh,** Director Professor & Head of Dept. of Pathology Maulana Azad Medical College, New Delhi

"Just great to read the Newsletter - very sophisticated and of a great standard (both academically and publishing wise). Congrats to both Tina and you and keep it up"…….. **Dr Shirish Kumar,** Sr Consultant Hematologist, Sir Ganga Ram Hospital, New Delhi, currently with WHO, Geneva.

"This is absolutely fantabulous ! What an Idea, Sir Ji !!! and what a neat execution of the idea. Three cheers to you, Tina and your team"……**Dr Paresh Jain,** Scientific Advisor, BD Biosciences, India.

"Excellent newsletter. Only one suggestion. If background graphics could be cut down, then people like me who have slower comps will be able to open it faster. And sure we all would benefit from these newsletters and if forthcoming issue is known in advance then can contribute also"……**Dr Rajan Kapoor**, SR Clinical Hematology, AIIMS, New Delhi

"Thanks a lot. I must say I am highly impressed with the very professional layout and copyediting!" …**Dr Prashant Sharma,** DM Fellow Hemato-pathology, AIIMS, New Delhi

"That is very nice. I am really glad you are taking this incentive. FCM needs new faces and fresh perspective in India"…….. **Dr Nikhil Patkar**, Post Doctoral Fellow, Molecular Haematology and Flow Cytometry Facility, Department of Clinical Haematology, Christian Medical College, Vellore, Tamil Nadu, India

"This mail is not for 'constructive criticism', but in appreciation of the effort and enthusiasm your group is showing. Please keep up the good work!"……**Dr Shivali Ahlawat,** Oncquest Laboratories, New Delhi, India.

"A commendable effort and I wish it all success!"……**Dr Shashi Narayan**, Senior Consultant Haematologist, Sir Ganga Ram Hospital, New Delhi.

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