Designing Multicolor Flow Cytometry Panels in Hematolymphoid Neoplasms

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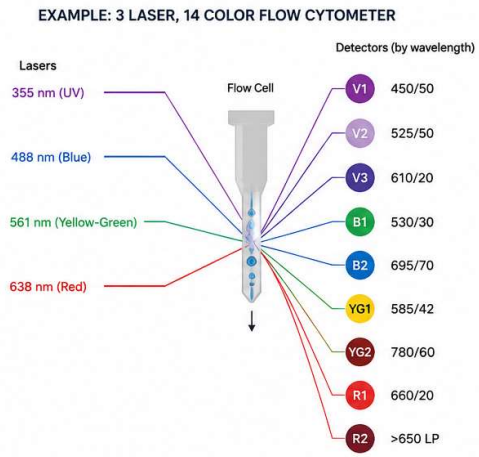
Flow cytometry panel designation

1. know your instrument
2. Sample preparation
3. Marker expression
4. Antigen density
5. Cell frequency
6. Choosing a Fluorophore

FLOW CYTOMETRY PANEL DESIGNATION – EXAMPLE

Marker	Purpose	Fluorochrome	Laser (nm)	Detector / Filter	Color (Channel)
CD45	Leukocyte identification	BUV395	355	450/50	Violet
CD3	T cell identification	PerCP-Cy5.5	488	695/70	Blue
CD4	T helper cells	FITC	488	530/30	Green
CD8	Cytotoxic T cells	APC	633	660/20	Red
CD19	B cells	PE	561	585/42	Yellow
CD56	NK cells	PE-Cy7	561	780/60	Far Red
CD14	Monocytes	BV510	405	510/50	Blue-Green
CD16	Granulocytes / Monocytes	BV605	405	610/20	Blue
CD25	Activation marker (IL-2Rα)	BV711	405	720/40	Indigo
CD127	IL-7 receptor (naïve T cells)	BV786	405	780/60	Deep Red
Viability Dye	Live / Dead discrimination	7-AAD	638	>650 LP	Near IR



KEY DESIGN PRINCIPLES

- Define the purpose and populations of interest.
- Choose fluorochromes based on brightness, antigen density, and spectral compatibility.
- Minimize spillover and ensure proper compensation.
- Include viability dye and appropriate controls.

FLUOROCHROME GROUPS

UV (355 nm): BUV395
Blue (488 nm): FITC, PerCP-Cy5.5
Yellow-Green (561 nm): PE, PE-Cy7
Red (633/638 nm): APC, 7-AAD
Violet/Blue Dyes (405 nm): BV510, BV605, BV711, BV786

TIP

Always validate the panel experimentally and adjust based on your instrument and sample type.

know your instrument

- The first step in designing a flow cytometry panel is understanding the instrument in detail, because the panel must be built around the cytometer's optical capabilities rather than around the markers alone. Even an excellent antibody combination can perform poorly if it is not matched to the instrument.

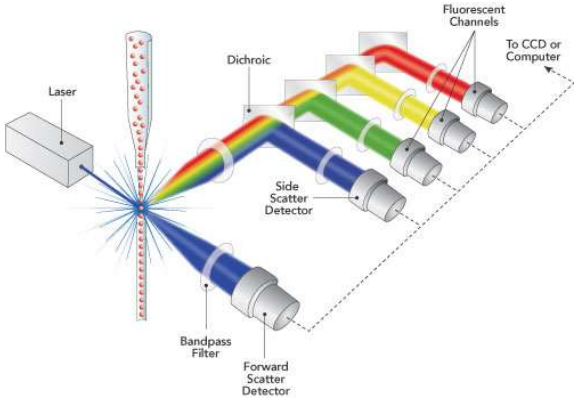
know your instrument

- This step usually includes several components:

1- Know the available lasers

Determine the excitation lasers on the cytometer (for example 405 nm violet, 488 nm blue, 561 nm yellow-green, 638–640 nm red). Different fluorochromes are excited optimally by different lasers.

- For example on your 10-color Beckman Coulter Navios system, fluorochrome selection depends on what channels are present and which lasers are installed



know your instrument

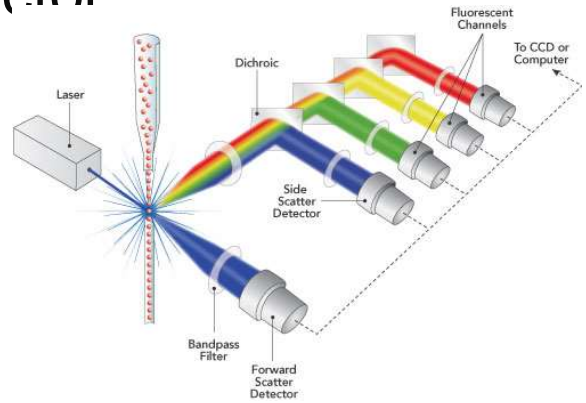
2- Know detector and filter configuration

Each detector collects light within a specific wavelength range determined by optical filters. You need to know:

– Number of fluorescence channels

– Band-pass filters used for each channel

– Which fluorochrome is assigned to each detector



know your instrument

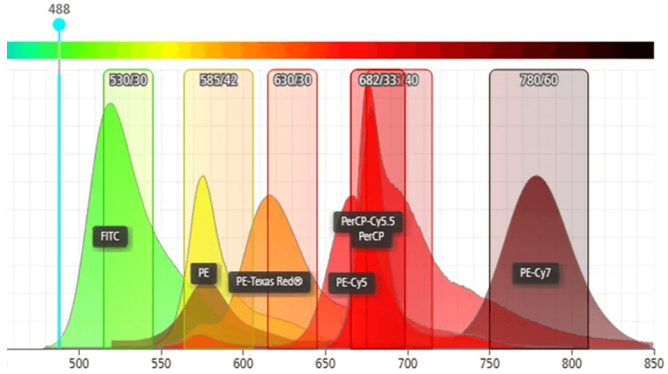
3- Know instrument sensitivity and brightness performance
Not all channels perform equally. Some channels have:
– higher signal-to-noise ratio
– better sensitivity for dim markers
– more background noise

- بنابراین در طراحی پانل فقط روشنایی فلوروکروم مهم نیست بلکه باید بدانیم دستگاه ما در هر کانال چه عملکردی دارد

Bright fluorochromes should generally be assigned to low-expression antigens, while highly expressed antigens can be paired with dimmer fluorochromes.
Example:
CD19 (bright antigen) → FITC may be acceptable
MRD marker with dim expression (CD81, CD58 abnormalities, CD200 weak expression) → PE or APC may be preferable

know your instrument

4- Understand spectral overlap and compensation requirements
Fluorochromes emit across broad wavelength ranges and may spill into adjacent detectors.



Examples:

- FITC → spills into PE
- PE → spills into ECD
- APC → spills into APC-A700 or APC-A750

Knowing expected spillover helps avoid problematic fluorochrome combination

know your instrument

5- Review validated laboratory templates

For clinical laboratories, especially in hematopathology and MRD work, review existing validated panels and instrument-specific templates before building a new panel.

For example, with B-ALL EuroFlow-style panels, marker selection is optimized specifically around instrument optical configurations.

COG(Children Oncology Group) panel

MD Anderson panel

【10-color-MFC】

FITC	PE	PerCP-Cy5.5	PE-Cy7	APC	APC-R700	APC-H7	V450	V500-C	BV605
CD38ME	CD56	Ig-λ	CD117	Ig-κ	CD27	CD19	CD138	CD45	CD81

【EuroFlow-NGF】

	FITC	PE	PerCP-Cy5.5	PE-Cy7	APC	APC-C750	BV421	BV510
Tube 1	CD38ME	CD56	CD45	CD19	CD117	CD81	CD138	CD27
Tube 2	CD38ME	CD56	CD45	CD19	Ig-κ	Ig-λ	CD138	CD27

know your instrument

6-Understand practical instrument limitations
Examples:
•Maximum number of colors measurable simultaneously
•Availability of compensation matrices
•Daily QC performance
•Stability of PMT settings
•Detector linearity
"Know your cytometer before choosing antibodies. The instrument determines which fluorochromes can be used, how well they are detected, and how much interference will occur between them."
بنابراین یک پانل خوب برای یک دستگاه ممکنست برای دستگاه دیگر قابل اجرا نباشد

Sample preparation

- In flow cytometry panel design, the “sample preparation” step means planning how the specimen will be processed before staining and acquisition, because preparation directly affects antigen preservation, cell viability, background staining, and data quality.
- For a laboratory workflow, this step typically includes:

Sample preparation

1. حساسیت آنتی‌ژن‌ها به لیز یا فیکساسیون

بعضی آنتی‌ژن‌ها در اثر لیز RBCیا فیکساسیون کاهش می‌یابند.

مثال:

- CD16
- CD11b

اگر چنین مارکری در پانل وجود دارد، بهتر است روی فلوروکروم‌های روشن‌تر قرار گیرد

Sample preparation

۲- Surface stainingیا Intracellular staining

اگر قرار است:

- TdT
- MPO
- Cytoplasmic CD3
- Cytoplasmic CD79a

بررسی شوند، باید سلول‌ها permeabilizeشوند.

این مرحله می‌تواند شدت برخی مارکرهای سطحی را تغییر دهد، بنابراین باید آنتی‌بادی‌ها و فلوروکروم‌ها متناسب با پروتکل انتخاب شوند.

Sample preparation

Cell loss during preparation-3

در برخی روش‌های preparation، به‌ویژه:

- Bone marrow with low cellularity
- CSF
- MRD samples

مقداری از سلول‌ها از دست می‌روند.

در این شرایط مارکرهای حیاتی برای گیتینگ باید روی فلوروکروم‌های بسیار روشن قرار گیرند

Sample preparation

Viability-4

اگر نمونه قدیمی یا با کیفیت پایین باشد:

- Autofluorescenceافزایش می‌یابد.
- Non-specific bindingبیشتر می‌شود.

در چنین مواردی ممکن است نیاز به:

- Viability dye

در طراحی پانل داشته باشید.

Sample preparation

5. نوع نمونه

پانلی که برای

Peripheral bloodطراحی شده لزوماً برای:

- Bone marrow
- Lymph node
- Body fluids

مناسب نیست.زیرا ترکیب سلولی و اتوفلورسانس متفاوت است

Sample preparation

6-Different anticoagulants can influence staining:

•EDTA: common for routine immunophenotyping

•Heparin: sometimes better for functional assays

•Some markers may be affected by certain anticoagulants

- بیان این مارکرها با ضدانعقاد EDTA کاهش می یابد CD11b,CD16, CD14, CD64
- برای MRD تا ۲۴ ساعت ضدانعقاد EDTA قابل قبول است . بیشتر از آن بیان CD10, CD19, CD34, CD45 کاهش می یابد

Sample preparation

Sample preparation directly influences antigen preservation, cell viability, fluorescence intensity, and the number of analyzable cells. Therefore, the specimen type and processing protocol must be considered before selecting markers and fluorochromes during flow cytometry panel design.

Marker expression and Ag density

- The third step, **marker expression and antigen density** , focuses on understanding how each antigen behaves biologically before assigning fluorochromes. This step is critical because panel performance depends not only on instrument configuration but also on the density and distribution of target markers.

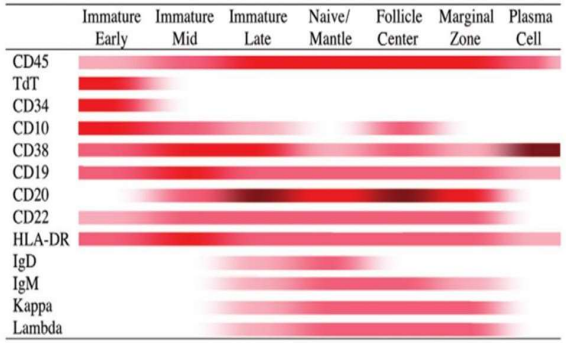
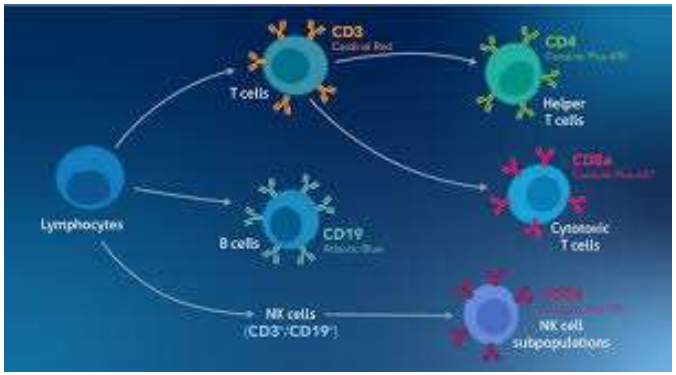


Figure 34-6 B cell differentiation. The intensity of antigen expression throughout B cell maturation is depicted for antigens commonly used in the clinical laboratory. Normal mature B cells express κ or λ light chains, never both. Darker colors represent higher levels of antigen expression.



Key aspects of marker expression include:

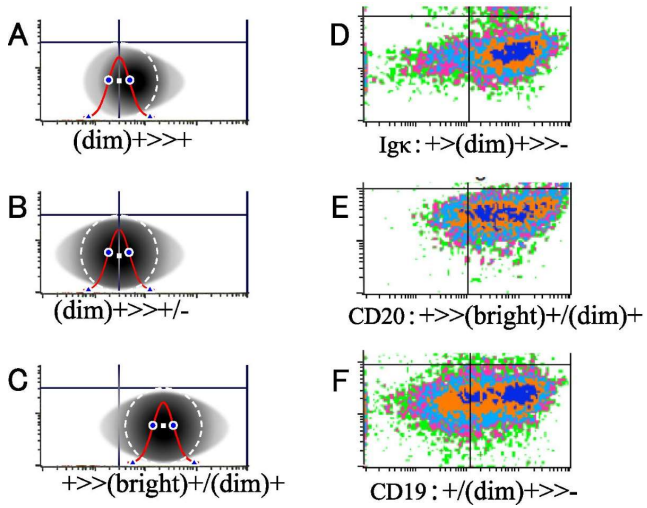
•**Antigen density (bright vs dim expression):**
Determine whether a marker is highly expressed or weakly expressed on the target cells. Dim markers should generally be paired with brighter fluorochromes (for example PE or APC), whereas strongly expressed markers can be assigned to dimmer fluorochromes. A weak antigen placed on a weak fluorochrome may become difficult to distinguish from background.
Examples:
•CD45 on mature leukocytes → usually bright
•CD19 on B cells → often moderate to bright
•CD34 on blasts → often relatively dim
•Aberrant markers in leukemias may be weak or partial

Key aspects of marker expression include:

- **Expression pattern:**

Assess whether expression is:

- Positive versus negative
- Dim or bright
- Heterogeneous versus homogeneous
- Partial versus complete



Key aspects of marker expression include:

•**Co-expression characteristics:**
Determine which markers are likely to be present on the same cell population. Co-expressed markers placed on fluorochromes with high spectral overlap can create spreading error and reduce resolution.

• **مثال:**
در یک پانل B-ALL مارکرهای **CD19**و **CD10**معمولاً روی یک سلول به‌طور همزمان بیان می‌شوند. اگر این دو مارکر به فلوروکروم‌هایی با همپوشانی طیفی زیاد (مثلاً PEو PE-Texas Red) متصل شوند، سیگنال قوی یکی از آنها می‌تواند باعث افزایش نویز در کانال دیگر شود و تشخیص دقیق شدت بیان CD10یا CD19را دشوار کند.

Marker expression and Ag density

This marker-expression assessment becomes the basis for the next step: **matching markers with fluorochrome brightness and minimizing spectral interference.**

Cell frequency

•**Determine whether the target population is common or rare**

Examples:

•Mature T cells in peripheral blood → very common

•Plasma cells in bone marrow → relatively uncommon

•Hematogones → low frequency

•Minimal residual disease (MRD) cells → very rare

•Paroxysmal Nocturnal Hemoglobinuria clone populations → may be extremely rare

Rare populations require greater assay sensitivity than abundant populations

Cell frequency

•**Adjust panel design according to rarity**

For rare cells:

•Use brighter fluorochromes for essential markers

•Minimize spreading error into critical channels

•Optimize gating markers

•Increase total events acquired

For common populations, fluorochrome assignment may be more flexible.

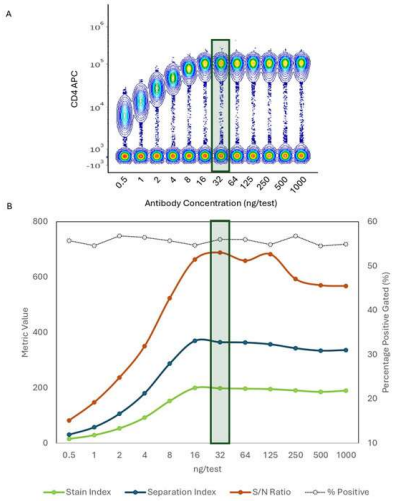
برای مارکرهای مهم در جمعیت های نادر باید فلوروکروم هایی با تداخل کم انتخاب شوند

Cell frequency

نهایتا اینکه در طراحی پانل هر چه فراوانی جمعیت هدف کمتر باشد باید برای مارکرهای تشخیصی آن جمعیت از فلوروکرومهای روشن تر باspillover کمتر و resolution بالاتر استفاده شود تا سلولهای نادر از نویز زمینه قابل تفکیک باقی بمانند

Cell frequency

نسبت آنتی‌ژن به آنتی‌بادی (Antigen-Antibody Ratio) در فلوسایتومتری اهمیت بسیار زیادی دارد



1. کمبود آنتی‌بادی (Antibody Under-Titration)

اگر تعداد آنتی‌ژن‌ها زیاد باشد ولی آنتی‌بادی کافی اضافه نشود:

- همه اپی‌توپ‌ها اشغال نمی‌شوند.
- شدت فلورسانس کاهش می‌یابد.
- مارکرهای dim ممکن است منفی کاذب شوند.
- تفکیک جمعیت‌ها ضعیف می‌شود.

مثال:

در یک نمونه CLL با لنفوسیتوز شدید، اگر همان حجم آنتی‌بادی که برای 10^6 سلول استفاده می‌شود روی 10^7 سلول استفاده شود، CD20 یا CD79b ممکن است ضعیف‌تر از مقدار واقعی دیده شوند

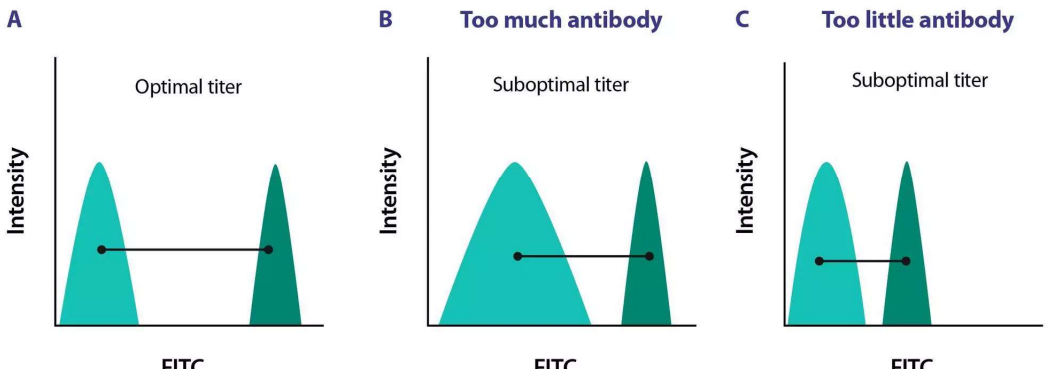
Cell frequency

2. آنتی‌بادی بیش از حد (Antibody Over-Titration)

اضافه کردن آنتی‌بادی بیش از مقدار بهینه نیز مشکل‌ساز است:

- افزایش اتصال غیر اختصاصی
- افزایش Background fluorescence
- کاهش نسبت Signal-to-Noise
- افزایش Spilloverبه کانال‌های دیگر

در نتیجه ممکن است جمعیت‌های ضعیف به خوبی تفکیک نشوند.



Choosing a Fluorophore

- The final step in flow cytometry panel design, “choosing a fluorophore,” means matching each antibody marker with an appropriate fluorescent dye so that all markers can be detected clearly with minimal interference. This step strongly affects sensitivity, resolution of populations, and compensation requirements.
- Major considerations include:
- Match fluorophore brightness to antigen density

This is one of the most important rules:

•Dimly expressed antigens → assign bright fluorophores

•Brightly expressed antigens → assign dim fluorophores

Choosing a Fluorophore

Examples:

•CD34, CD117, CD25, CD200, MRD markers → often placed on brighter fluorophores

•CD45, CD3, CD19, CD20 → usually tolerate dimmer fluorophores because expression is strong

For example:

•CD34 → PE or APC (bright)

•CD45 → PerCP or FITC (less bright)

Using a weak fluorophore for a dim antigen may make the population disappear

Choosing a Fluorophore

- Consider co-expression patterns

Markers expressed on the same cells should ideally not be assigned to highly overlapping fluorophores.

For example, if blast cells co-express:

- CD34
- CD117
- HLA-DR

Avoid assigning these to fluorophores that heavily interfere with one another.

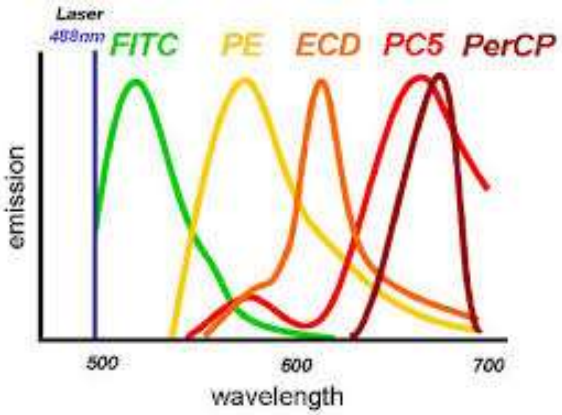
Instead, distribute them across different laser lines when possible

Choosing a Fluorophore

A common practical rule:
Low-density antigen → bright fluorophore
High-density antigen → dim fluorophore
Co-expressed markers → separate fluorophores with minimal overlap
This final fluorophore-selection step integrates all previous steps: knowledge of the instrument, sample preparation, marker expression, antigen density, and cell frequency. It converts the marker list into a functional multicolor panel.

Fluorophore Separation

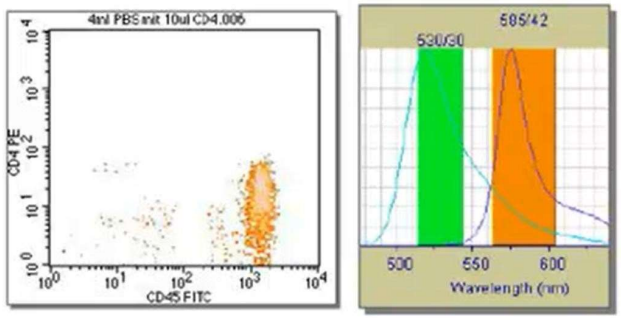
- Ideally, when building multicolor panels, **it is best to separate fluorophore excitation across lasers**, and where possible, the emission across the detectors.
- This will **minimize the amount of spillover** and therefore compensation you will need to do.
- **However as you increase the number of fluorophores in your panel, this will not always be possible.**
- Therefore other considerations need to be included in your design



Avoid overlap of strong positive populations into detectors, that should analyze dim signals.

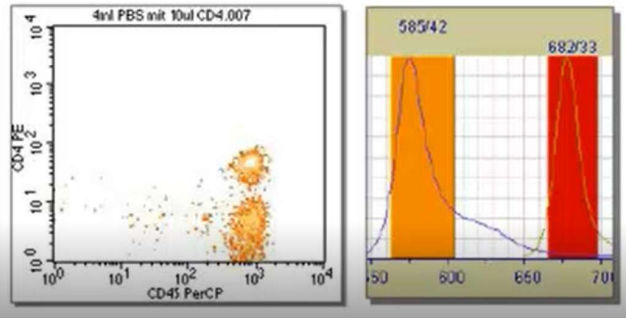
Strong Spillover of FITC into FL2

⇨ **You cannot longer separate dim Fl2-positive signals**



No Spillover of PerCP into FL2:

⇨ **Separation of dim PE and bright PerCP double positive signals is possible**



Fluorophore Separation

یکی از روش‌های رایج برای کاهش اثر **spillover**این است که فلوروکروم‌ها را بر اساس **الگوی بیان آنتی‌ژن‌ها** با دقت انتخاب کنیم.
یعنی قبل از اختصاص فلوروکروم به هر مارکر، باید بدانیم کدام مارکرها روی یک سلول با هم بیان می‌شوند و **کدام مارکرها هرگز روی یک سلول دیده نمی‌شوند.**

Fluorophore Separation

- این کار را می‌توان با اختصاص فلوروکروم‌هایی که spillover قابل توجهی دارند به مارکرهای **متقابلاً انحصاری (Mutually Exclusive)**انجام داد؛ مانند **CD3و CD19**.
- CD3روی سلول‌های Tبیان می‌شود.
- CD19روی سلول‌های Bبیان می‌شود.
- یک سلول طبیعی همزمان CD3و CD19را بیان نمی‌کند.

بنابراین اگر فلوروکروم CD3مقداری سیگنال به کانال CD19نشت کند، معمولاً مشکلی ایجاد نمی‌شود؛ زیرا سلول +CD3واقعی اصولاً CD19ندارد و برعکس.

Fluorophore Separation

برعکس، برای مارکرهایی که روی یک سلول با هم بیان می‌شوند (**co-expression**) باید فلوروکروم‌هایی انتخاب شوند که حداقل spilloverرا به یکدیگر داشته باشند.

مثال‌ها:

- CD19 و CD10 در B-ALL
- CD34 و CD117 در AML

Fluorophore Separation

- **Parent–Descendant Rule قانون والد–فرزند**

گاهی نمی‌توان از ایجاد مقداری **spillover(نشت سیگنال فلورسنت)** بین دو کانال جلوگیری کرد. در چنین شرایطی می‌توان مارکرها را طوری انتخاب کرد که این spillover مشکلی در تفسیر نتایج ایجاد نکند.

در قانون Parent–Descendant ، دو مارکر روی یک جمعیت سلولی به‌صورت سلسله‌مراتبی بیان می‌شوند:

- **Parent marker مارکر والد :** روی جمعیت بزرگ‌تر وجود دارد.
- **Descendant marker مارکر فرزند:** روی زیرمجموعه‌ای از همان جمعیت بیان می‌شود.

اگر سیگنال مارکر فرزند به کانال مارکر والد نشت کند، معمولاً مشکلی ایجاد نمی‌شود؛ زیرا سلول‌های فرزند ذاتاً مارکر والد را نیز بیان می‌کنند.

Fluorophore Separation

مثال CD3و CD4

• CD3 روی تمام سلول‌های Tمثبت است.

• CD4 فقط روی بخشی از سلول‌های Tمثبت است.

فرض کنید فلوروکروم CD4 مقداری spilloverبه کانال CD3 داشته باشد.

در این حالت:

•سلول‌های CD4+ از قبل CD3+ هستند.

•بنابراین افزایش جزئی سیگنال CD3 ناشی از spillover ، تفسیر CD3 را تغییر نمی‌دهد.

•هنوز می‌توان سلول‌های Tرا به‌درستی شناسایی کرد.

به همین دلیل این ترکیب از نظر طراحی پنل قابل قبول است.

کاربرد عملی در طراحی پنل

قانون Parent–Descendant می‌گوید اگر spillover از مارکری به کانال مارکری رخ دهد که همان سلول‌ها به‌طور طبیعی آن را بیان می‌کنند، این spillover معمولاً اهمیت بالینی یا تحلیلی ندارد و می‌توان آن را در طراحی پنل پذیرفت

PARENT–DESCENDANT RULE

in Flow Cytometry Panel Design

Rule: Assign brighter fluorochromes to descendant markers, and dimmer fluorochromes to parent (backbone) markers.

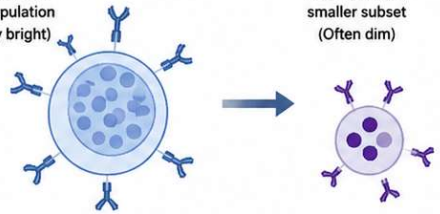
1. CONCEPT

PARENT
(Backbone marker)

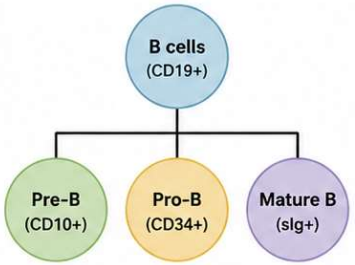
Expressed on a large population (Usually bright)

DESCENDANT
(Subpopulation marker)

Expressed on a smaller subset (Often dim)



2. EXAMPLE: B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA (B-ALL)



CD19 is a PARENT marker:

- Present on all B cells
- Usually bright
- Not the limiting factor for detection

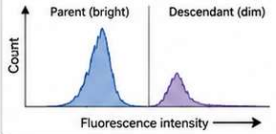
CD10, CD34, slg are DESCENDANT markers:

- Present on smaller subsets
- Often dim
- Need brighter fluorochromes for better resolution

3. WHY IT MATTERS

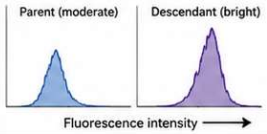
If you put BRIGHT fluorochrome on PARENT marker ✗

- Parent is already easy to detect
- Wasted bright channel
- Descendant (dim) may be missed or poorly resolved
- Reduced sensitivity for MRD



If you put BRIGHT fluorochrome on DESCENDANT marker ✓

- Parent still clearly detected even with dimmer dye
- Descendant (dim) becomes clearly visible
- Better separation & sensitivity (important for MRD)



4. PRACTICAL EXAMPLE (10-COLOR PANEL)

Marker	Role	Typical Expression	Fluorochrome Choice	Reason
CD19	Parent (Backbone)	Bright, on most B cells	FITC / ECD (dimmer)	Easy to detect, save bright dyes
CD10	Descendant	Dim–moderate, subset	PE (bright)	Needs sensitivity for detection
CD34	Descendant	Dim, subset	APC (bright)	Important for identification
CD22	Descendant	Dim–moderate	PC5.5	Better separation
CD45	Parent	Bright	Krome Orange (moderate)	Backbone, not limiting

KEY TAKEAWAY: Do not waste your brightest fluorochromes on markers that are already easy to detect. Use them for the markers that are dim and critical for defining your target population.

Medical indication of flowcytometry for hematolymphoid malignancy:

1. The diagnosis and classification of hematopoietic neoplasms.
2. Assessment of biological parameters associated with prognosis.
3. Detection of antigens used as therapeutic targets.
4. Detection of residual neoplastic cells following therapy.

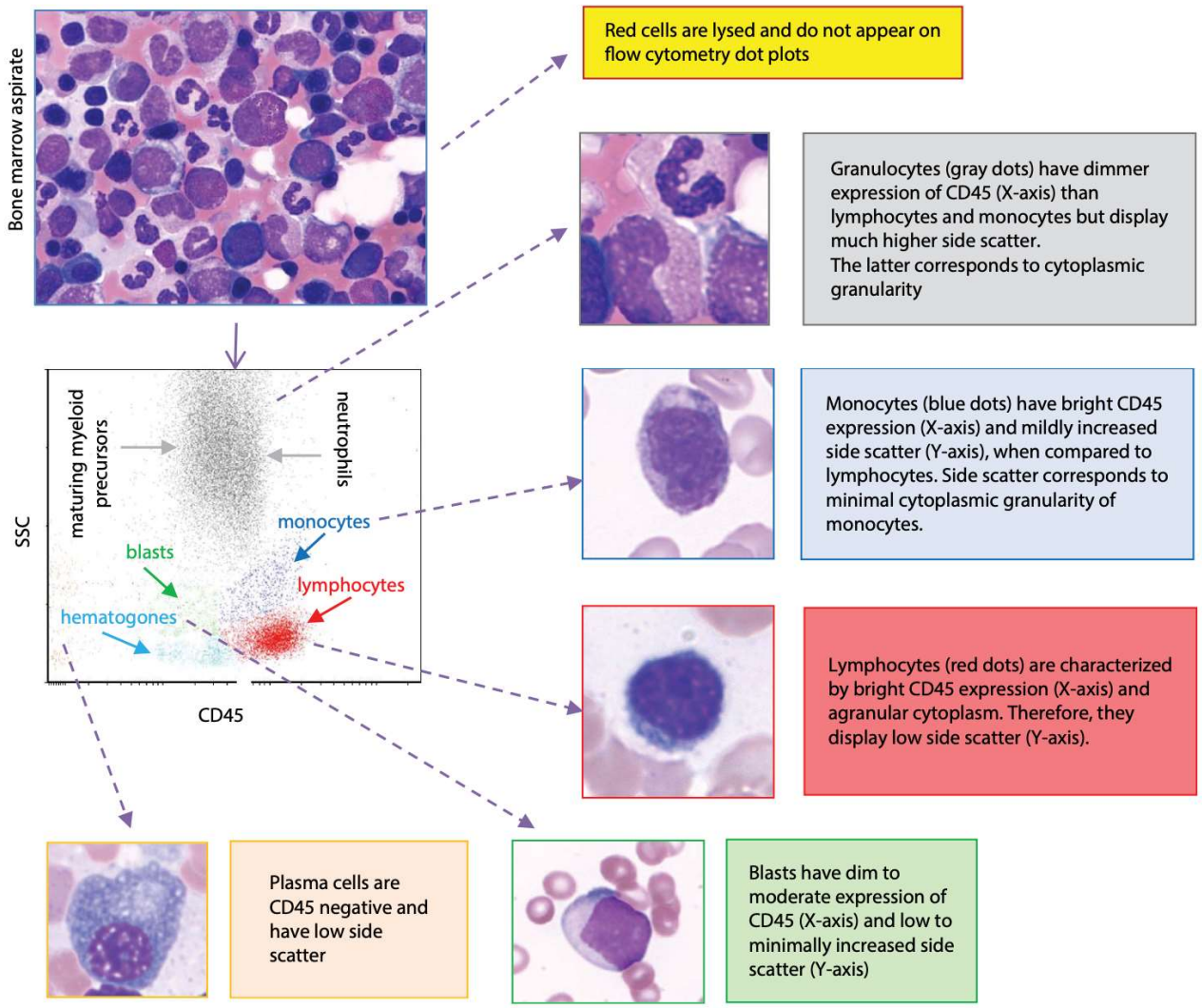


FIGURE 2.10 Gating strategy. Part of the sample from the bone marrow aspirate or blood (tube) is smeared on the microscope glass slide for morphologic correlation, while the rest is incubated with antibodies, lysed, fixed, and submitted for flow cytometry analysis; see text for details.

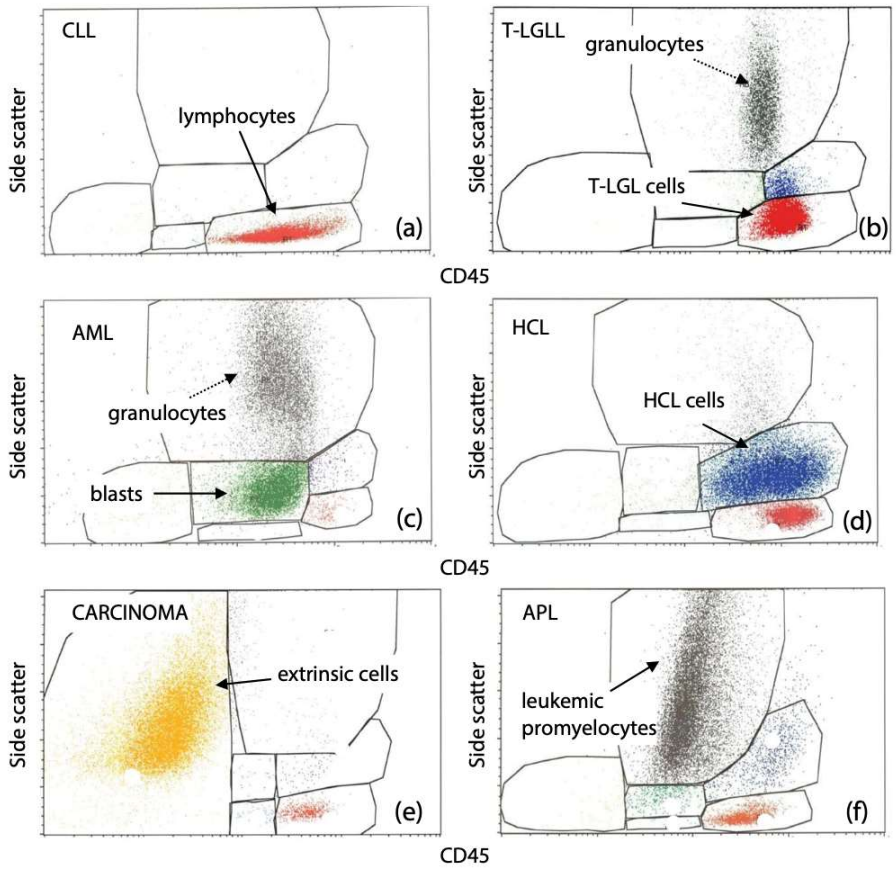


FIGURE 1.22 Side scatter (SSC; orthogonal, right angle scatter) on Y axis corresponds to granularity of the cytoplasm (X axis presents CD45 expression). Lymphocytes (a through f; red dots) have low SSC, whereas neutrophils (b–c), cancer cells (e) and atypical promyelocytes (f) have high SSC. Monocytes (b; blue dots), blasts (c; green dots) and HCL cells (d; blue dots) have higher SSC than lymphocytes (compare with red dots).

Lymphocytic gate

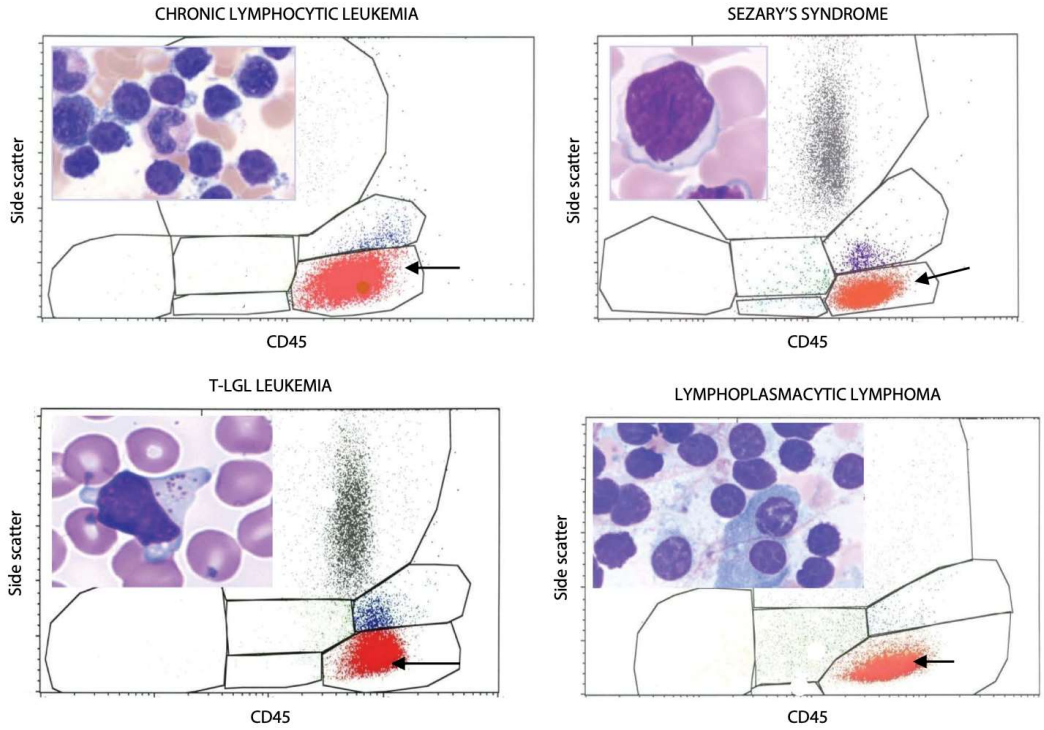
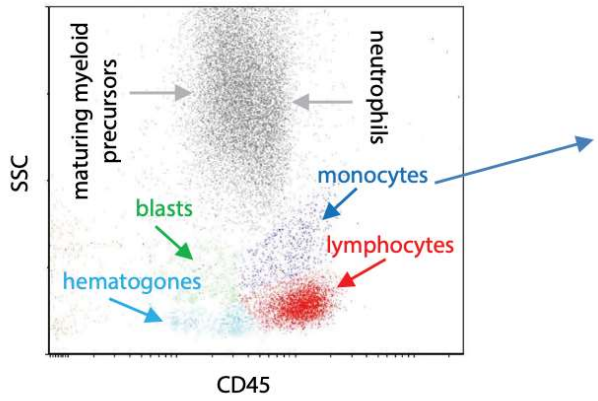


FIGURE 2.11 "Lymphocytic" gate. Benign lymphocytes and the majority of mature lymphoproliferative disorders are characterized by low side scatter (SSC) and bright expression of CD45 (red dots; arrow).

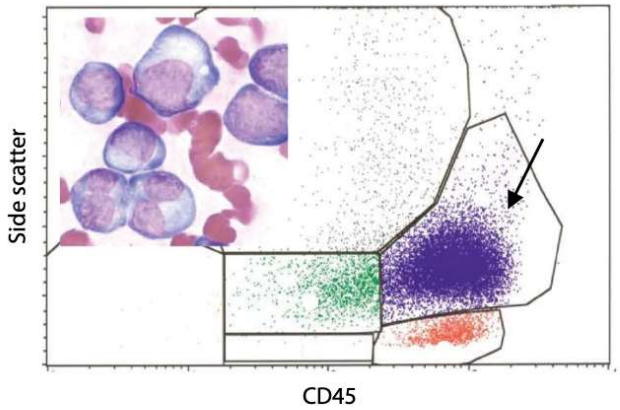
Monocytic gate



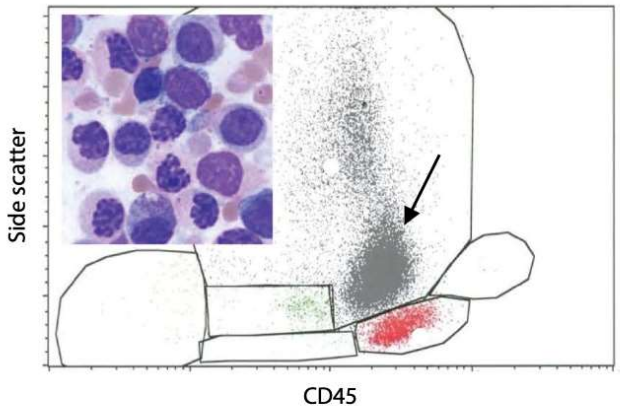
"Monocytic" gate

monocytes, monoblasts
hairy cell leukemia
occasional T/NK-LGL leukemias
mast cell leukemia
rare plasma cell neoplasms
granulocytes in MDS

ACUTE MONOBLASTIC LEUKEMIA



MYELODYSPLASTIC SYNDROME



Monocytic gate

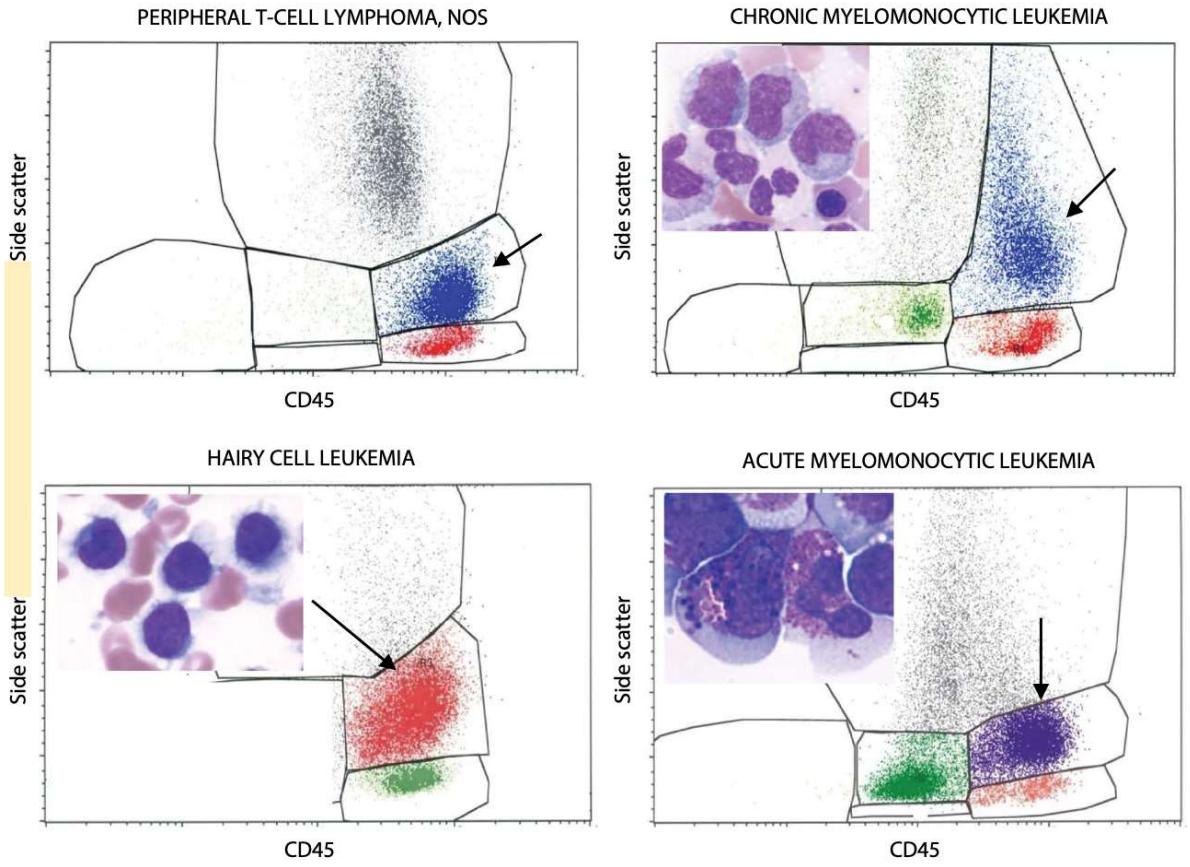
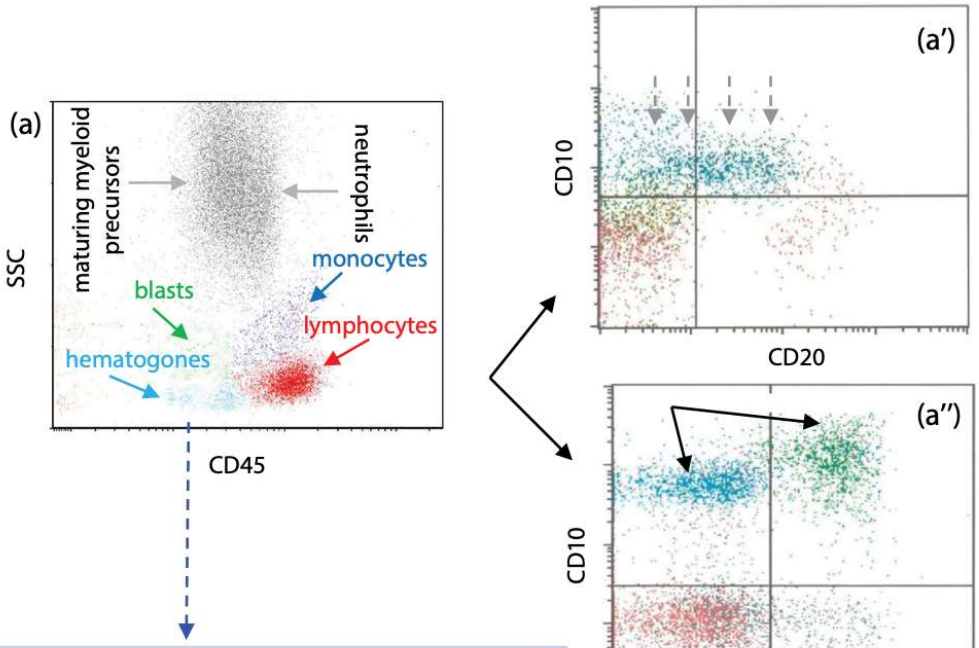


FIGURE 2.12 "Monocytic" gate. Benign monocytes and majority of neoplastic monocytic proliferations are characterized by bright CD45 expression and slightly increased side scatter (SSC). They appear above lymphocytic gate on CD45 versus SSC display (arrow). Apart from monocytic cells, similar SSC and CD45 properties are often seen in hairy cell leukemia and occasional lymphoproliferations, mast cell leukemia (unusual and rare form of mast cell disease), and markedly dysplastic granulocytes.

Hematogone gate



Hematogones gate

hematogones
B-lymphoblastic leukemia (B-ALL)
T-lymphoblastic leukemia (T-ALL)
occasional AML (non-M3)
occasional B-and T cell lymphoproliferative disorders

Hematogone gate

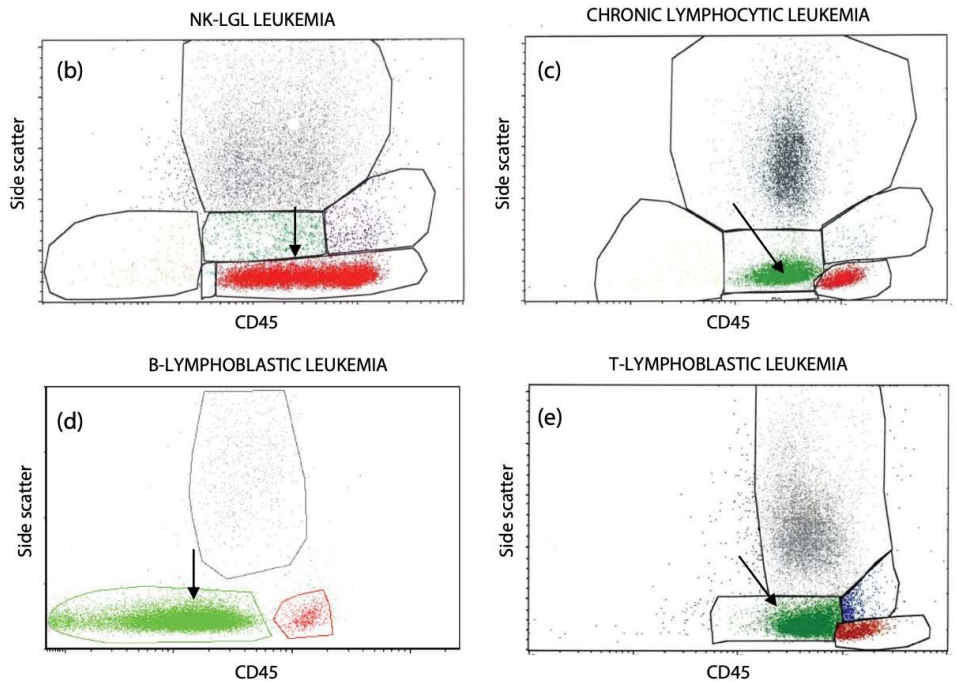
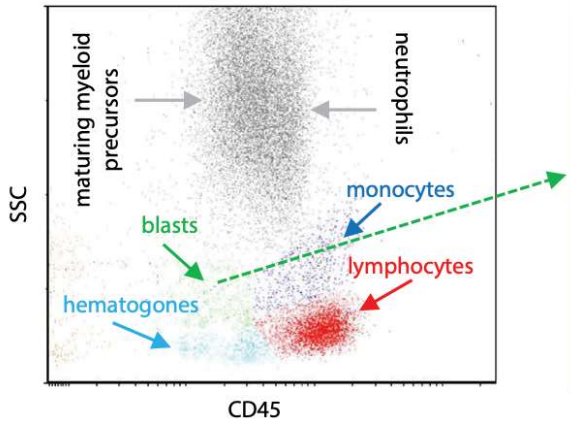


FIGURE 2.13 "Hematogones" gate (a). Hematogones are characterized by very low side scatter (SSC) and dimmer expression of CD45 when compared to lymphocytes (red dots); they have variable ("smeared") expression of CD20 (a′; broken arrows), and are positive for CD10 and partially for CD34 (a″). Occasional lymphoproliferative disorders and blasts may have similar to hematogones SSC and CD45 properties (b–e).

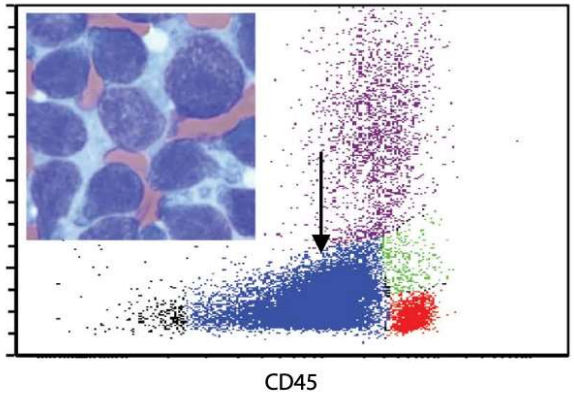
Blast gate



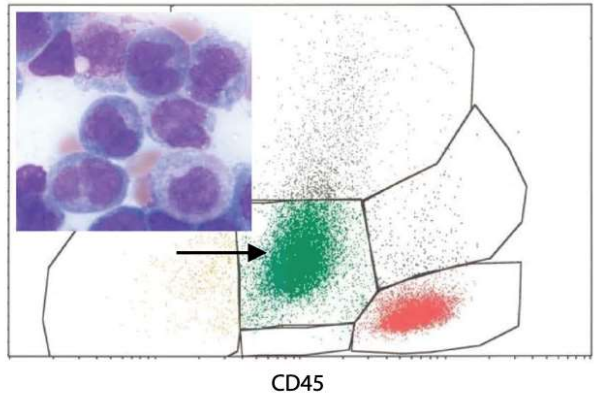
"Blast" gate

acute myeloid leukemia (AML), blast phase of MPN
acute promyelocytic leukemia, microgranular variant
precursor B-cell leukemia/lymphoma (B-ALL)
precursor T-cell leukemia/lymphoma (T-ALL)
blastic plasmacytoid dendritic cell neoplasm (BPDCN)
occasional B-cell lymphomas e.g., diffuse large B-cell lymphoma (DLBCL) or follicular lymphoma (FL)
occasional plasma cell myelomas (PCM) and plasma cell leukemia (PCL)

B-ALL



MICROGRANULAR APL (APLv)



Blast gate

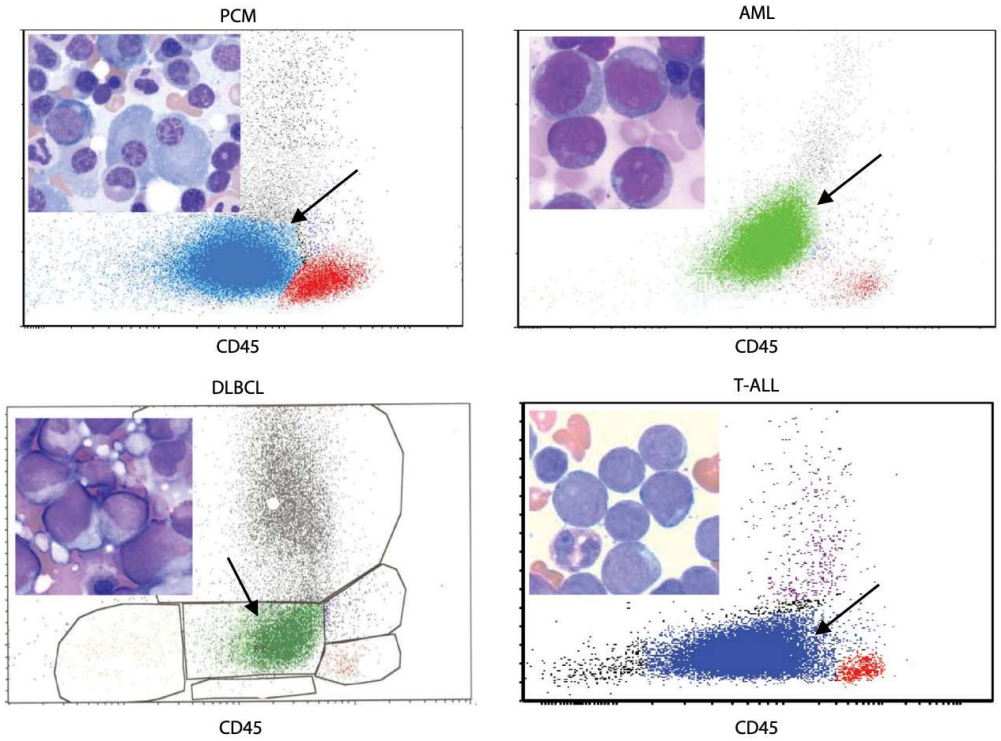
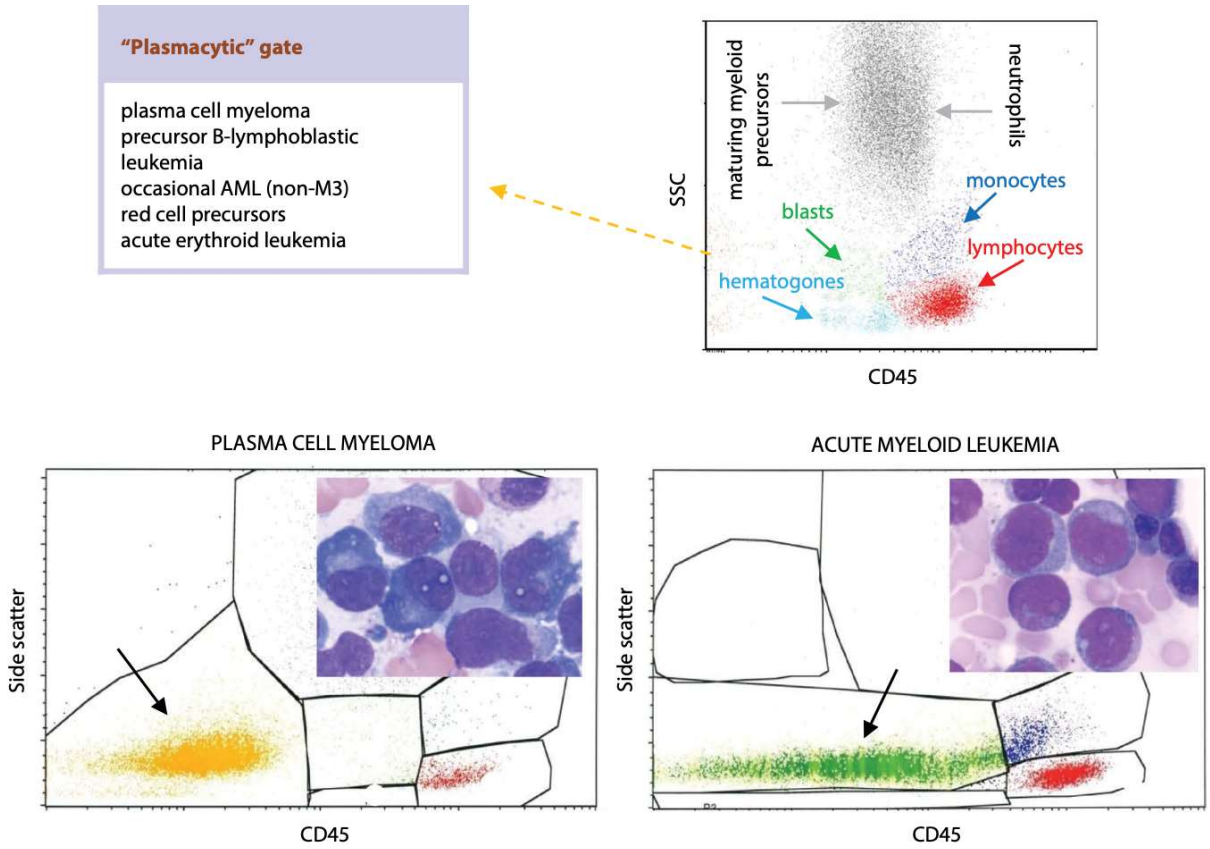


FIGURE 2.14 "Blast" gate. Myeloid precursors (blasts) have moderate CD45 and low side scatter (SSC). Occasional lymphoproliferative disorders, plasma cell tumors, as well as granulocytes with decreased granularity and microgranular APL have similar CD45 versus SSC characteristics.

Plasmacytic gate



Plasmacytic gate

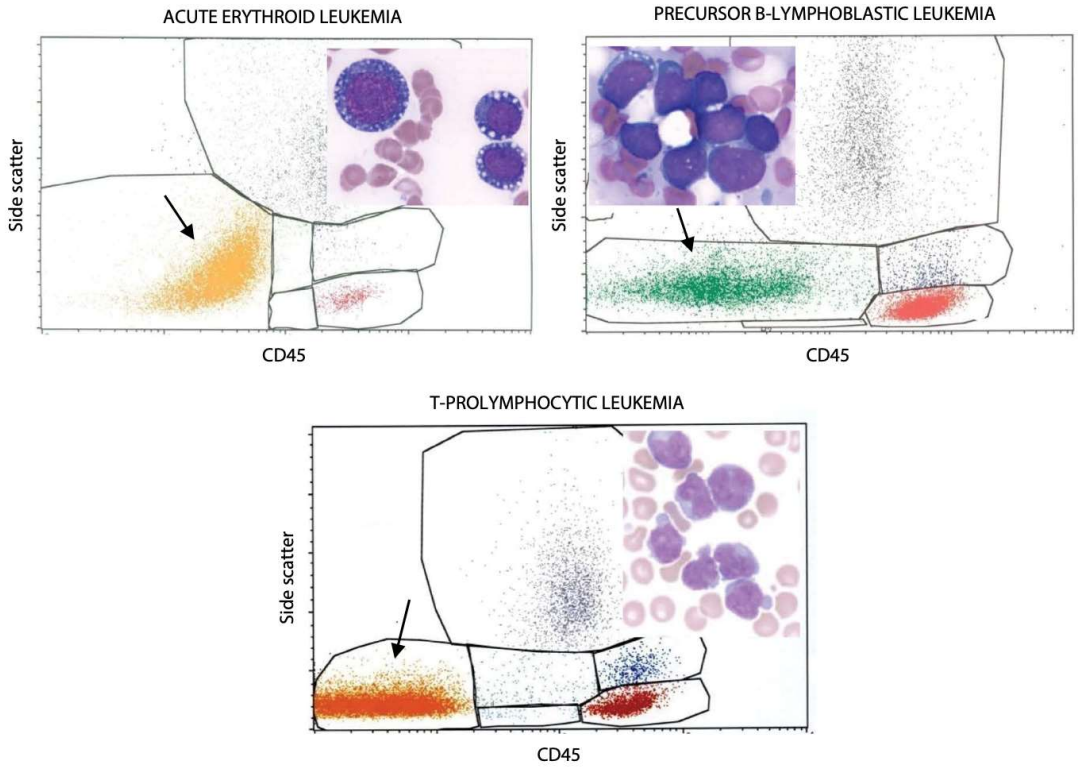
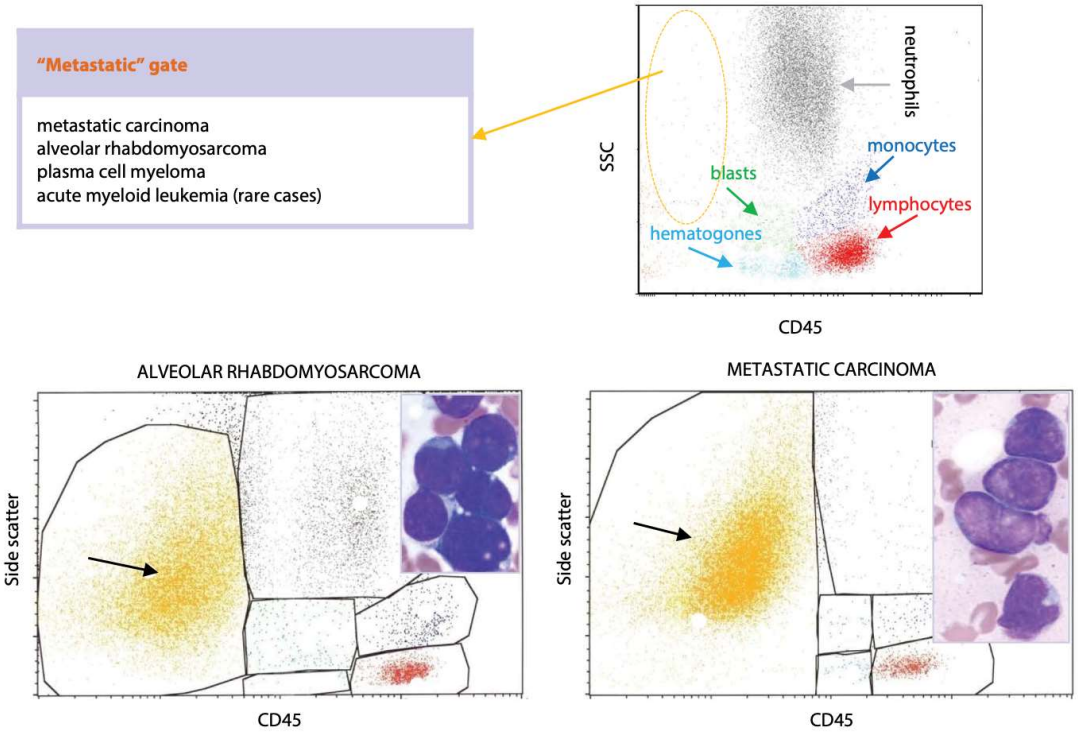


FIGURE 2.15 "Plasmacytic" gate. Apart from plasma cell neoplasms, erythroid precursors, occasional AML and B-ALL are characterized by negative CD45 and low side scatter. Rare T-PLL may be CD45-negative.

Metastatic gate



Metastatic gate

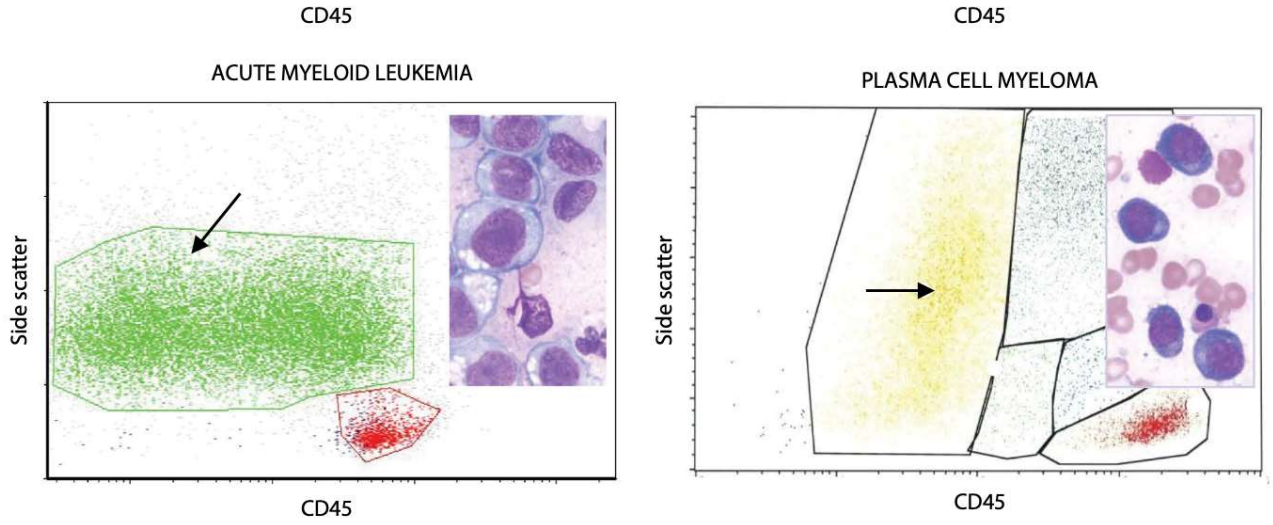
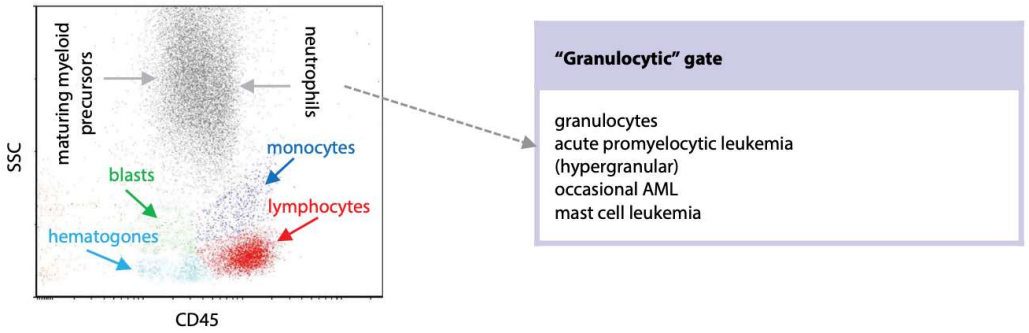
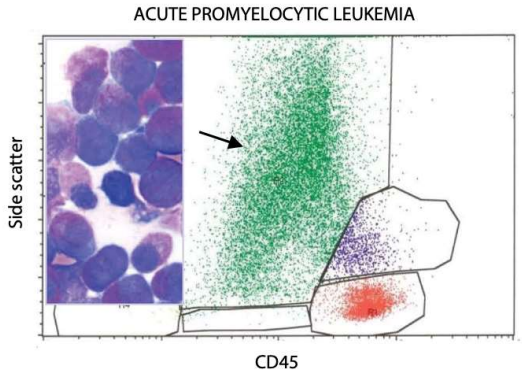
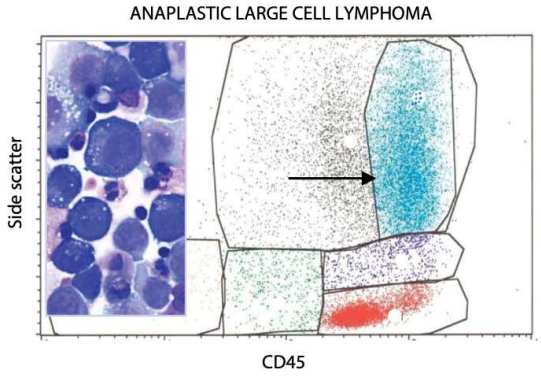


FIGURE 2.16 "Metastatic" gate. High side scatter and negative CD45 is typical for non-hematopoietic tumors and rare plasma cell neoplasms.

Granulocytic gate







Granulocytic gate

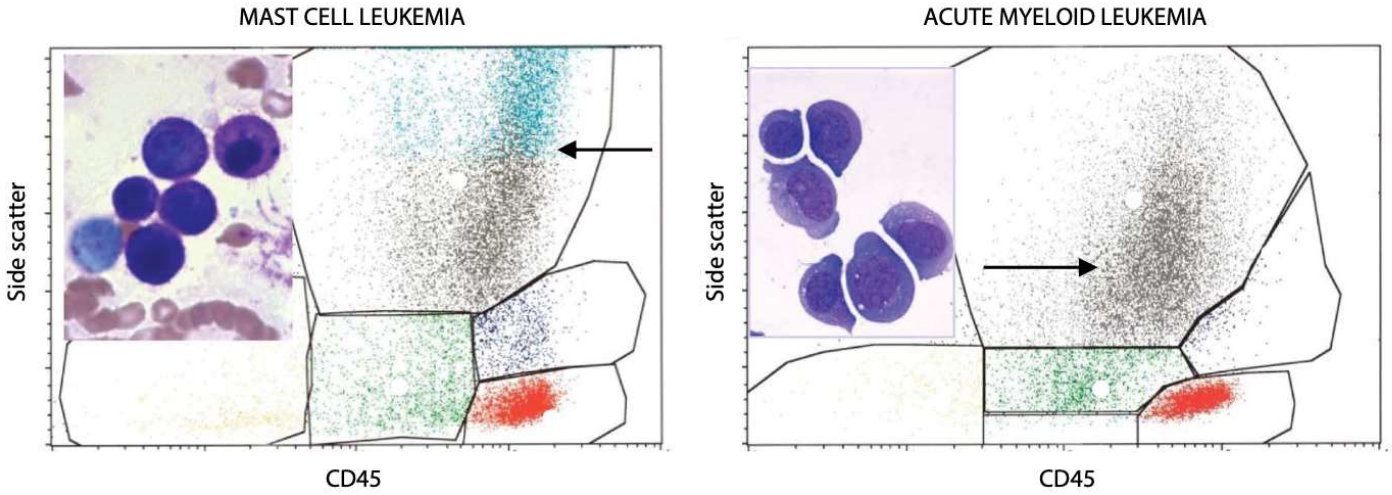


FIGURE 2.17 "Granulocytic" gate. Granulocytes are characterized by high side scatter (SSC) and moderate expression of CD45. Hematopoietic tumors with similar characteristics include mast cell proliferations, acute promyelocytic leukemia (hypergranular variant), and occasional large cell lymphomas and rare acute myeloid leukemias.

The End