



# Clinical Flow Cytometry in Hematologic Malignancies Workshop

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Office of In Vitro Diagnostics and Radiological Health  
Center for Devices and Radiological Health

FDA

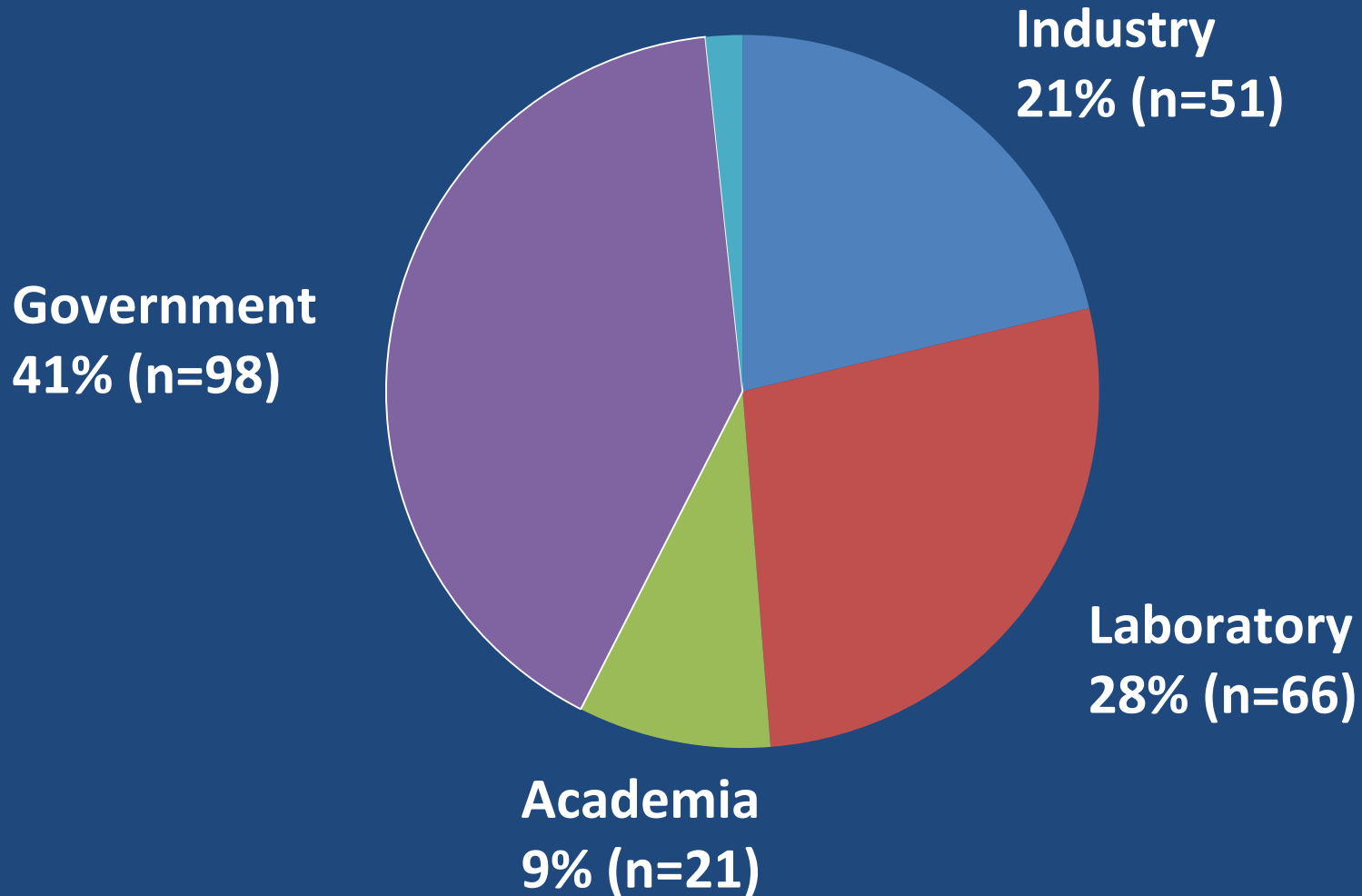


# Purpose:

...to seek public input from academia, Government, laboratorians, industry, clinicians, patients and other stakeholders on the role of clinical flow cytometry in hematologic malignancies, in order to develop a specific regulatory policy for this class of in vitro diagnostic devices.

# Workshop Participants

## 134 On-site; 108 Website



# WWW.FDA.GOV



U.S. Department of Health & Human Services

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## It's Food Allergy Awareness Week

Learn what you need to know to reduce the risk of allergic reactions to foods.

1 2 3



### For Consumers & Patients

Updates and information for staying safe and healthy



### For Health Professionals

Medical product safety information, adverse event/problem reporting and more



### For Scientists & Researchers

NCTR, pediatrics, clinical trials, Critical Path Initiative and more



### For Industry

Guidance, registration and listing, import programs and more

## Recalls & Alerts

## [Approvals & Clearances](#)

## [Report a Problem](#)

[Recalls & Safety Alerts](#)

[Enforcement Report](#)

[Warning Letters](#)

## FDA Initiatives

- [Innovation](#)
- [Globalization](#)

# Workshop Overview

## Introduction

Gerald Marti	Welcome
Maria Chan	Introduction
Kevin Maher	Overview of FDA Device Regulation

## Session 1: Analytical Challenges in Standardization & Validation of FCM

Lili Wang	Joint NIST ISAC Standardization Framework for Quantitative Flow Cytometry
Stephen Perfetto	NIAID NIH Nature Protocol for Quality Assurance for Multicolor FCM Using a Suite of Calibration Beads
Heba Degheidy	Multicolor Bead Flow Cytometry Standardization
David Barnett	UK NEQAS Proficiency Testing in Acute and Chronic Leukemias
Eric Hsi	Background on College of American Pathologists (CAP) Proficiency Testing for Leukemia/Lymphoma

## **Session 2: Transitioning from Analytical to Clinical QC in FCM**

Virginia Litwin	The Role of Biomarkers in Clinical Trials and the Fit-for-Purpose Method Validation Approach
Cherie Green	Flow Cytometry Validation for Drug Development Instruments
Teri Oldaker	LDTs in Flow Cytometry: ICSH/ICCS Guidelines for Validation of Fluorescent Cell-based Diagnostic Testing
M Stetler-Stevenson	Flow Cytometric Diagnosis of Low Grade B-cell Leukemia/Lymphoma

**Public Comment Session**

**Panel Discussion**

## **Session 3: Clinical Diagnostic Flow Cytometry**

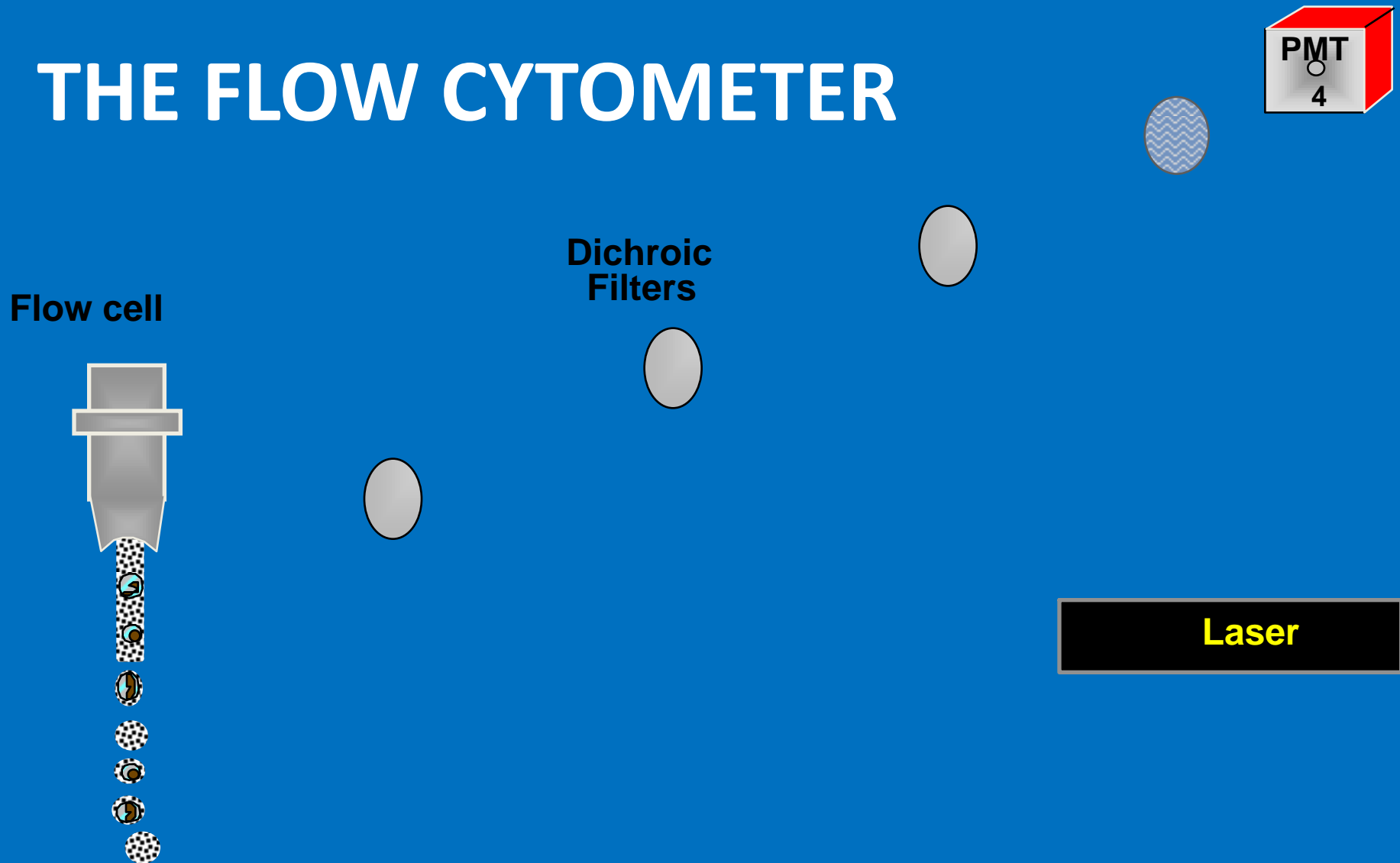
Brent Wood	Bethesda Consensus Conference on Clinical Flow Cytometry of Hematolymphoid Malignancies
Tomas Kalina	Euroflow: Standardization, Monoclonal Antibody Selection and Panel Configuration
Andy Rawstron	Different Approaches to and Standardisation - Experience from ERIC, EMN, Euroflow and ESCCA
Phillip McCoy	A Model for Harmonizing Flow Cytometry in Clinical Trials – The HIPC Lyoplate Experience

Panel Discussion (Robert Becker, Raul Braylan)

## **Session 4: Flow Cytometry Data Analysis Software**

Brent Wood	Perspective on Software Pitfalls and Challenges Public Comment Session Panel Discussion (Michael Borowitz, Raul Braylan, Maryalice Stetler-Stevenson, Brent Wood)
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# THE FLOW CYTOMETER

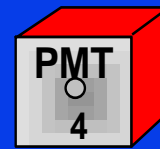




Flow cell

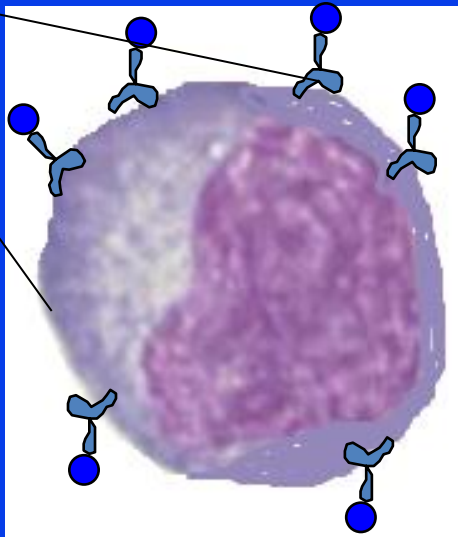


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Filters

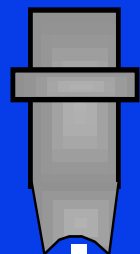


Laser

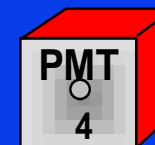
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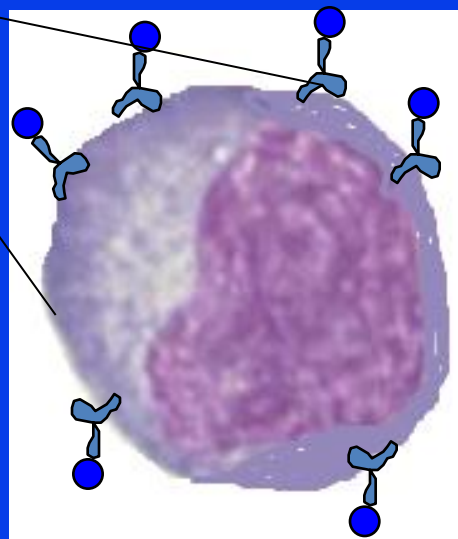
Flow cell



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Filters



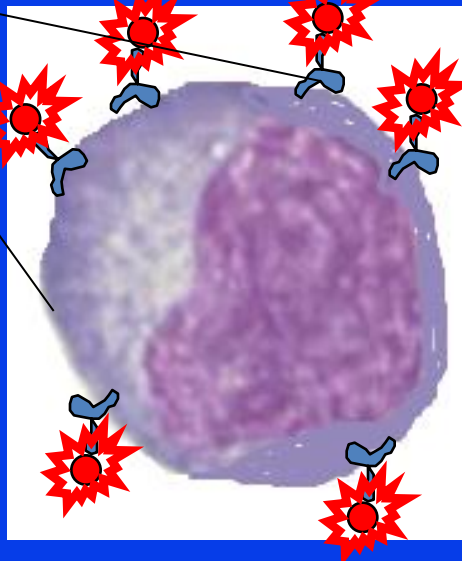
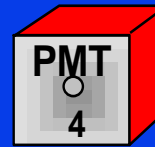
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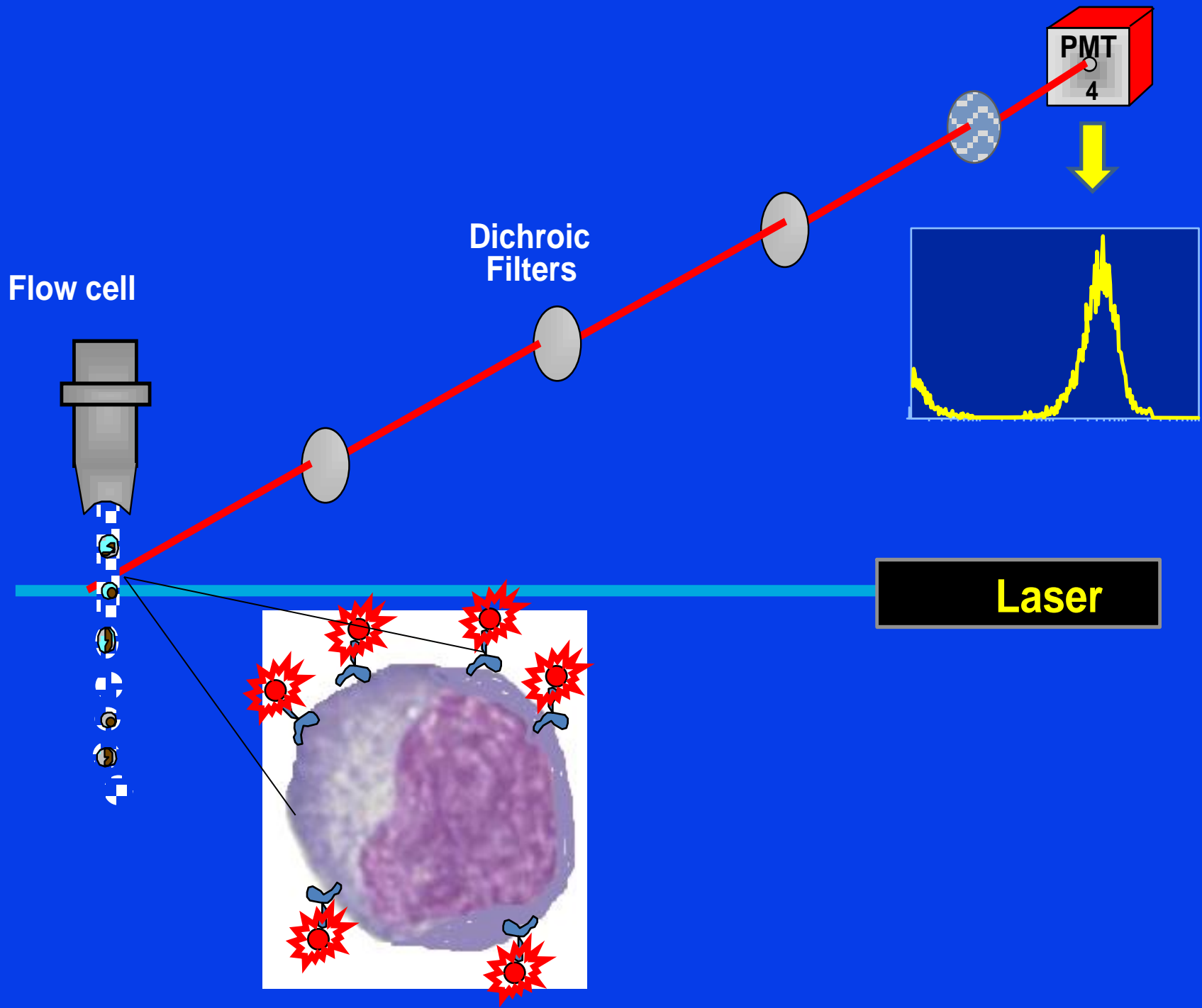


Flow cell

Dichroic  
Filters

Laser





# IVD Cleared Multi-Analyte Assays

Lymphocyte Subset Analysis

Stem Cell Analysis

MHC expression

Tetramer

# Hematologic Malignancy

**Lineage Assessment**

**Maturational Stage**

**Phenotype**

**Diagnostic (CLL, Hairy Cell)**

**Adjunctive**

**Tumor Burden**

**Companion Diagnostic**

**Prognosis (MRD)**

# Barriers to Clearance of Devices for Hematologic Malignancy

Complex Biology

# WHO Classification of Hematologic Malignancy

## I. Myeloid Neoplasms

### A. Acute myeloid leukemias/ myeloid sarcoma

CD13, CD33, CD15, CD4, CD64, CD14, CD11b, CD117, CD34, myeloperoxidase

### B. Other myeloid neoplasms

#### i. Myeloproliferative neoplasms

#### ii. Myelodysplastic syndromes

## II. Lymphoid Neoplasms

### A. Non-Hodgkin lymphomas

#### i. Precursor lymphoid neoplasms

##### a. B lymphoblastic leukemia/lymphoma

CD19, CD22, CD10, TdT, CD34

##### b. T lymphoblastic leukemia/lymphoma

CD3 (cytoplasmic), CD2, CD5, CD7, usually either CD4/CD8 double positive or double negative, CD1a, TdT



# WHO Classification of Hematologic Malignancy

## ii. Mature B cell lymphomas

### 1. Small B cell lymphomas

#### a. Chronic lymphocytic leukemia

CD19, CD20 (dim), CD5, CD23

#### b. Follicular lymphoma

CD19, CD20, CD10, BCL-2, BCL-6

#### c. Mantle cell lymphoma

CD19, CD20, CD5, Cyclin D1

#### d. Marginal zone lymphoma

CD19, CD20 (negative for CD5, CD10, and CD23)

### 2. More aggressive B cell lymphomas

#### a. Diffuse large B cell lymphoma

CD19, CD20

#### b. Burkitt lymphoma

CD19, CD20, CD10, BCL6

### 3. Plasma cell neoplasms

CD19, CD38, CD138, CD20+/-, kappa or lambda, CD56

# WHO Classification of Hematologic Malignancy

## iii. Mature T/NK cell lymphomas

### 1. Peripheral T cell lymphoma, NOS

CD3, CD2, CD5, CD7, CD4 or CD8

### 2. Anaplastic large cell lymphoma

CD3, CD2, CD4, CD30, EMA

## B. Hodgkin lymphomas

CD15, CD30, and PAX5 positive (CD20 +/-) and negative for CD45

## III. Histiocytic and Dendritic cell neoplasms

CD1a, S100, CD207 (Langerin)

## IV. Hematologic neoplasms with a propensity for bony involvement (forming tumor masses)

### A. Plasma cell neoplasms (plasmacytoma, plasma cell myeloma)

### B. Diffuse large B cell lymphoma

### C. Langerhans cell histiocytosis

### D. Less common

#### i. Anaplastic large cell lymphoma

#### ii. Myeloid sarcoma

#### iii. Burkitt lymphoma

#### iv. Hodgkin lymphoma

#### v. Plasmablastic lymphoma (EBV+ large cell lymphoma seen in HIV+)

# Barriers to Clearance of Devices for Hematologic Malignancy

## Complex Biology

- No Consensus on appropriate panel composition / design

## Complex Technology

- Numerous Variables to Control

# Complex Technology

- Specimen Type

  - Anticoagulant

- Monoclonal Antibody

  - Specificity

  - Affinity

  - Fluorochrome Label

  - Concentration used

  - Panel constituents

- Instrument Make

- Instrument Model

  - Number of fluorescence channels

  - Optical Systems for Fluorescence Detection

- Instrument Standardization

  - Voltage

  - Spectral Compensation

- Data Acquisition and Analysis

  - Software

  - Gating Strategies

- Data Reporting

# Barriers to Clearance of Devices for Hematologic Malignancy

## Complex Biology

- No Consensus on appropriate panel composition / design

## Complex Technology

- Numerous Variables to Control

- Not a Chemistry Test (e.g. Automated)

## Complex Market

# Testing Environment

**In-House Lab**

**Draw**



**Screening panel**



**Specific Panel**

**Reference Lab**

**Draw**



**Ship**



**Comprehensive  
Panel**

# Barriers to Clearance of Devices for Hematologic Malignancy

## Complex Biology

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## Complex Market

- No consensus on best practices

- No One-Size-Fits-All solution

## Complex Interpretation

- Practice of Medicine

- Advances in Software may Challenge This Concept

## Complex Validation

- No FDA Guidance

## Complex and Rapid Evolution

- Reluctance to Manufacture Products That May Soon Be Obsolete

## Complex Manufacturing

- Errors and Inefficiencies are Problematic

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# Acknowledgements

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Ms. Angela De Claro

Ms. Christine Lincoln

## **ASCO**

## **Workshop Planning Committee**

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... and all workshop  
participants



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# Thank You

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