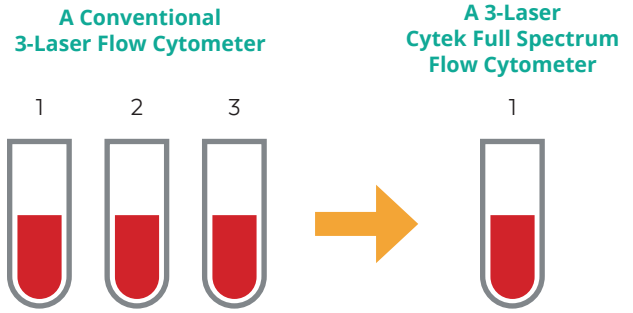
White Paper | Cytek Northern Lights

Creating Efficiency in the Lab with Full Spectrum Flow Cytometry

Introduction

When working with conventional flow cytometers, researchers are often limited by the number of reagents they can combine into a single tube. To gather the desired amount of information from each sample, they need multiple tubes, often with some redundant markers across them, to fully characterize cells of interest. With Cytek's full spectrum flow cytometers, researchers can consolidate a larger number of parameters into a 1-tube assay. With more reagents combined per tube, less sample is needed, and the need for redundant markers is eliminated.

To demonstrate these sample-to-answer workflow efficiencies, Cytek's scientists designed and optimized a 1-tube acute myeloid leukemia (AML) minimum residual disease (MRD) detection assay based on a published 3-tube assay[1]. Data from the original 3-tube assay and the consolidated 1-tube assay were generated using a Cytek 3-laser full spectrum flow cytometer and compared.



Streamlining the 3-Tube MRD Assay

Transferring an existing conventional flow cytometer assay to Cytek's full spectrum flow cytometers was simple. The Cytek 3-laser system is equipped with violet, blue, and red excitation lasers just like the conventional system, which meant that the reagents used in the original 3-tube panel could be used. Instrument set-up was quick as the Cytek system does not need optical filter changes. An unstained reference control as well as single stained reference control tubes were prepared and acquired using the system's pre-defined CytekAssaySetting - an optimized set of detector gains that works well without adjustment for most immunophenotyping applications. The experiment was unmixed by following the steps of the unmixing wizard to enable live unmixing of the multicolor tubes as they were acquired.

To combine the 3-tube assay into a 1-tube assay, panel design best practices discussed in **this Cytek webinar** were applied to choose a combination of reagents to purchase and test. After an initial test of the assay in a normal donor bone marrow sample (data not shown), the panel went through several iterations to ensure optimal resolution of each marker in the assay. The final 1-tube assay design is shown in table 1.

Both the original 3-tube and the new 1-tube assays were tested on the same healthy bone marrow sample to ensure that the 1-tube assay provided comparable results to the original 3-tube assay. These results are shown in figure 1 using the same gating strategy published for the original 3-tube assay.

After confirming that the panels were performing equivalently for healthy bone marrow, the next step was to compare the results for patient bone marrow samples. Multicolor samples were prepared again for both assays, this time for 3 different bone marrow samples: one fresh sample from a healthy donor; one cryopreserved sample from a newly diagnosed patient with AML; and one cryopreserved sample from a patient in remission from AML. The already recorded single stained reference controls from the first test of the assay were re-used to acquire and live unmix these additional samples. The results in figure 2 show that detection and characterization of abnormal cell populations was straightforward with the 1-tube assay.

MRD ASSAY DATA

1 Wood, B. L. (2020). Acute Myeloid Leukemia Minimal Residual Disease Detection: The Difference from Normal Approach. Current Protocols in Cytometry, 93(1). doi:10.1002/cpcy.73

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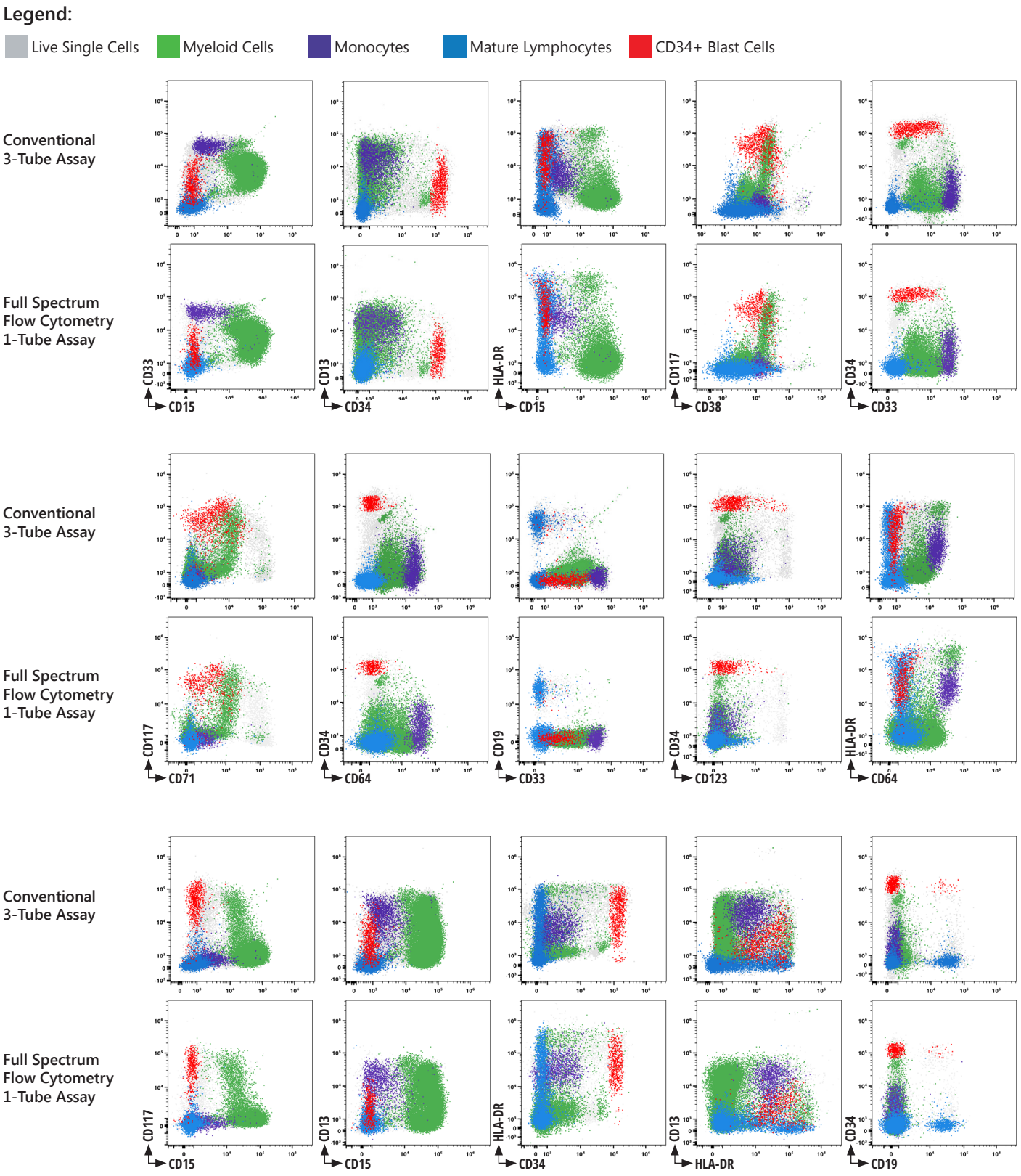

Figure 1. Comparable Performance Between 1-Tube and 3-Tube Assays. Both assays were evaluated in a fresh bone marrow sample from a healthy donor. Data was generated with a 3-laser Cytek full spectrum flow cytometer. For every pair of markers displayed, the resolution of the different cellular subsets is similar between the 2 assays.

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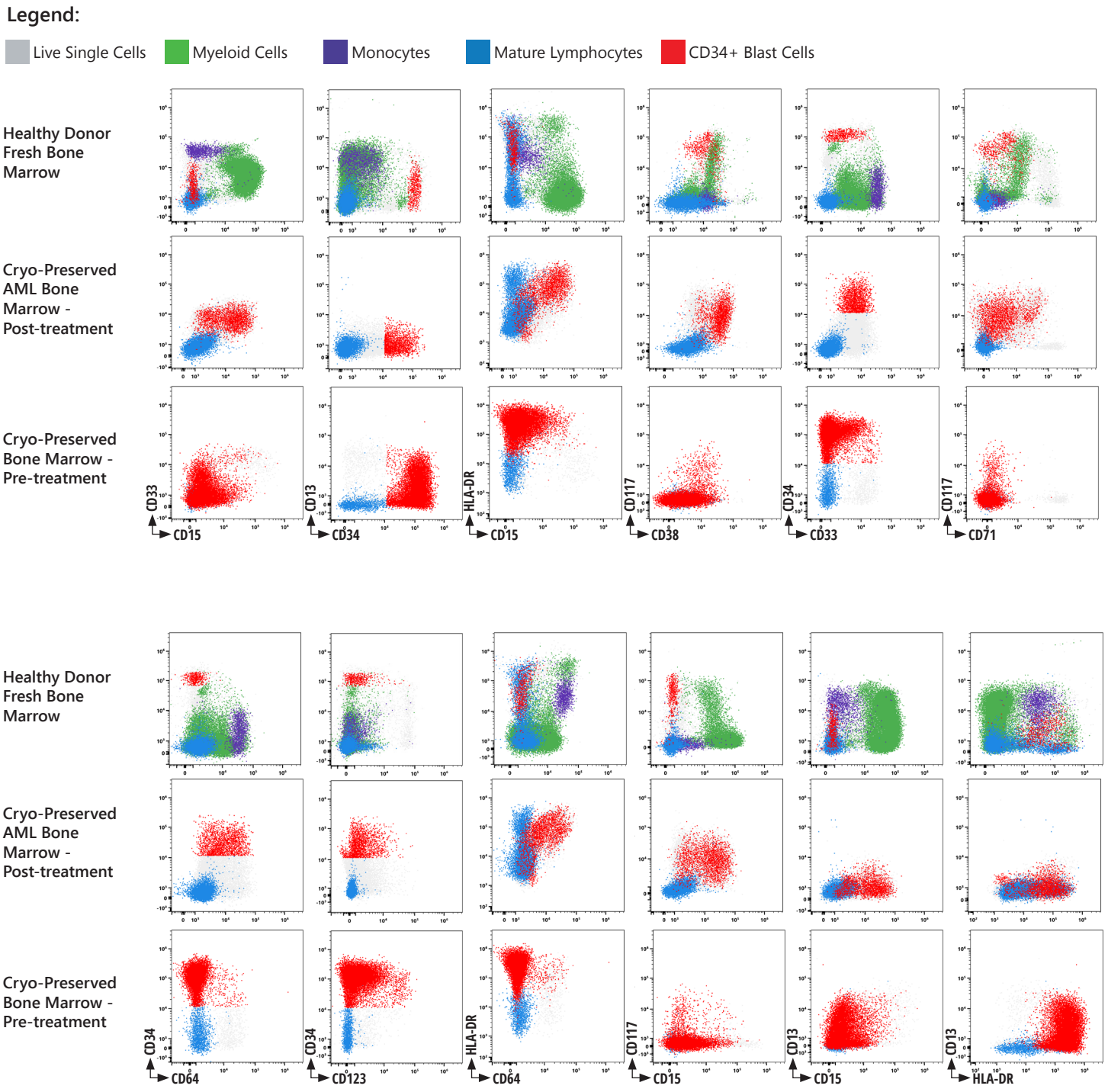

Figure 2. High quality full spectrum flow cytometry 1-tube assay patient data. The 1-tube MRD assay was tested on three different bone marrow samples: one fresh sample from a healthy donor; one cryopreserved sample from a newly diagnosed patient with AML; and one cryopreserved sample from a patient in remission from AML.

Summary

With Cytek's full spectrum flow cytometers, scientists can build high quality 1-tube assays and create efficiency in their labs by reducing sample and reagent use, sample preparation time, and acquisition time. Cytek's team of experienced technical application specialists are available to train, support, and guide researchers to ensure each researcher's assay goals are met.

To learn more about Cytek's cell analysis solutions, visit us at www.cytekbio.com or contact our technical sales representatives at sales@cytekbio.com.

3-Tube Assay*			1-Tube Assay	
Tube	Specificity	Fluorochrome	Specificity	Fluorochrome
1	CD13	PC7	CD13	PE-Cy7
1	CD15	FITC	CD15	FITC
1	CD19	PE-CF594	CD19	Brilliant Violet 750
1	CD33	PE	CD33	PE
1	CD34	APC	CD34	APC
1	CD38	A594	CD38	Brilliant Violet 510
1	CD45	APC-H7	CD45	APC-H7
1	CD71	APC-A700	CD71	APC-A700
1	CD117	PC5	CD117	PE-Cy5
1	HLA-DR	Pacific Blue	HLA-DR	Brilliant Violet 480
2	CD4	ECD	CD4	Brilliant Violet 570
2	CD13	PC7		
2	CD14	PC5.5	CD14	PE-Cy5.5
2	CD16	APC-A700	CD16	Pacific Blue
2	CD34	APC		
2	CD38	A594		
2	CD45	APC-H7		
2	CD64	FITC	CD64	Brilliant Violet 605
2	CD123	PE	CD123	Brilliant Violet 421
2	HLA-DR	Pacific Blue		
3	CD5	PC5	CD5	PE/Dazzle 594
3	CD7	PE	CD7	Brilliant Violet 786
3	CD33	PC7		
3	CD34	APC		
3	CD38	A594		
3	CD45	APC-H7		
3	CD56	A488	CD56	Brilliant Violet 711
3	HLA-DR	Pacific Blue		

Table 1. Panel designs for the 3-tube conventional flow cytometer and the 1-tube full spectrum flow cytometer AML MRD assays. Fluorochrome names in the 3-tube assay are copied verbatim from the reference publication for consistency. For non-fresh samples shown in figure 2, Zombie NIR was added to all multicolor tubes to exclude dead cells from the analyses.

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