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Benchmarking Compensation-Free Multiparameter Flow Cytometry

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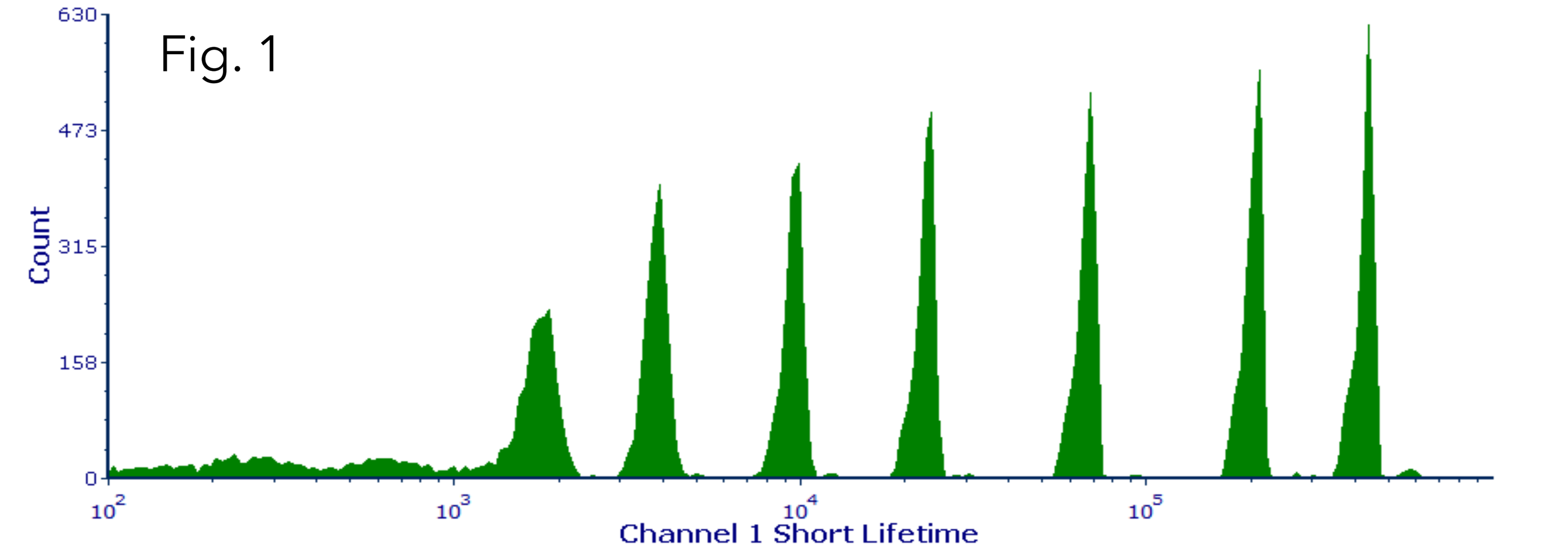
BACKGROUND

At Kinetic River we have been using fluorescence lifetime to distinguish fluorophores with otherwise identical emission characteristics. This approach enables compensation-free flow cytometry on a compact platform with fewer components than a traditional analyzer. While this unique technology is very attractive, going from proof of concept to a reliable instrument delivering reproducible results requires overcoming several challenges, including some that have no parallel in conventional flow cytometry.

METHODS

The compensation-free technology we developed has been previously reported (CYTO 2016–2018). Recently we have completely overhauled the *Arno* platform to achieve better stability, uniformity, sensitivity, dynamic range, and reproducibility. Fluidic control was improved using higher-resolution regulators, resulting in sample delivery with exceptional stability. Optical performance was improved by implementing stable, modular excitation and collection. Electrical noise reduction was achieved through extensive isolation of the signal paths. The scope-based DAQ module was replaced with a multi-GHz PXI platform capable of streaming raw data at high event rates. Algorithm improvements led to more robust separation of different lifetime components, resulting in an expanded dynamic range. And refinements in fluorophore selection and panel design produced better cell population resolution.

Fig. 1



RESULTS

We measured 8-peak Rainbow beads (Spherotech) on the *Arno* system to provide a baseline comparison with traditional flow cytometers. With all 8 peaks resolved in our measurements, system CVs of ~2–3% in FSC and ~5% in FL, and a dynamic range of at least 3 decades (**Fig. 1**), the *Arno* system proves to have comparable sensitivity to the best conventional instruments.

Fig. 2a

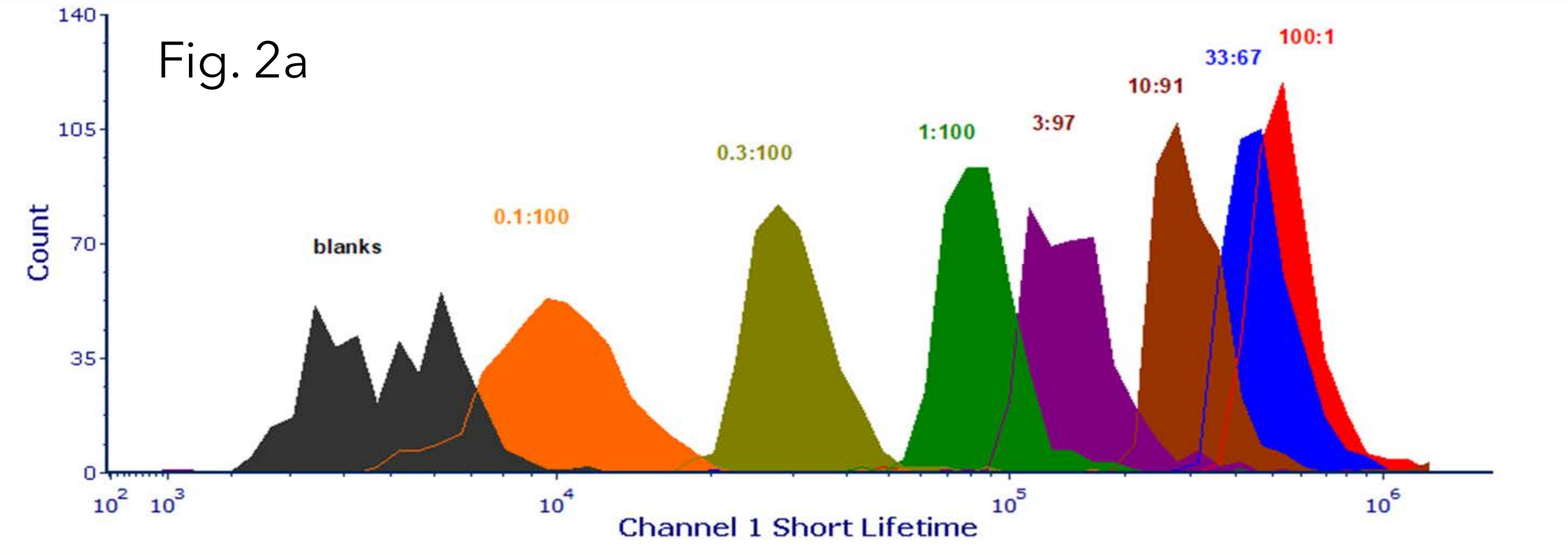


Fig. 2b

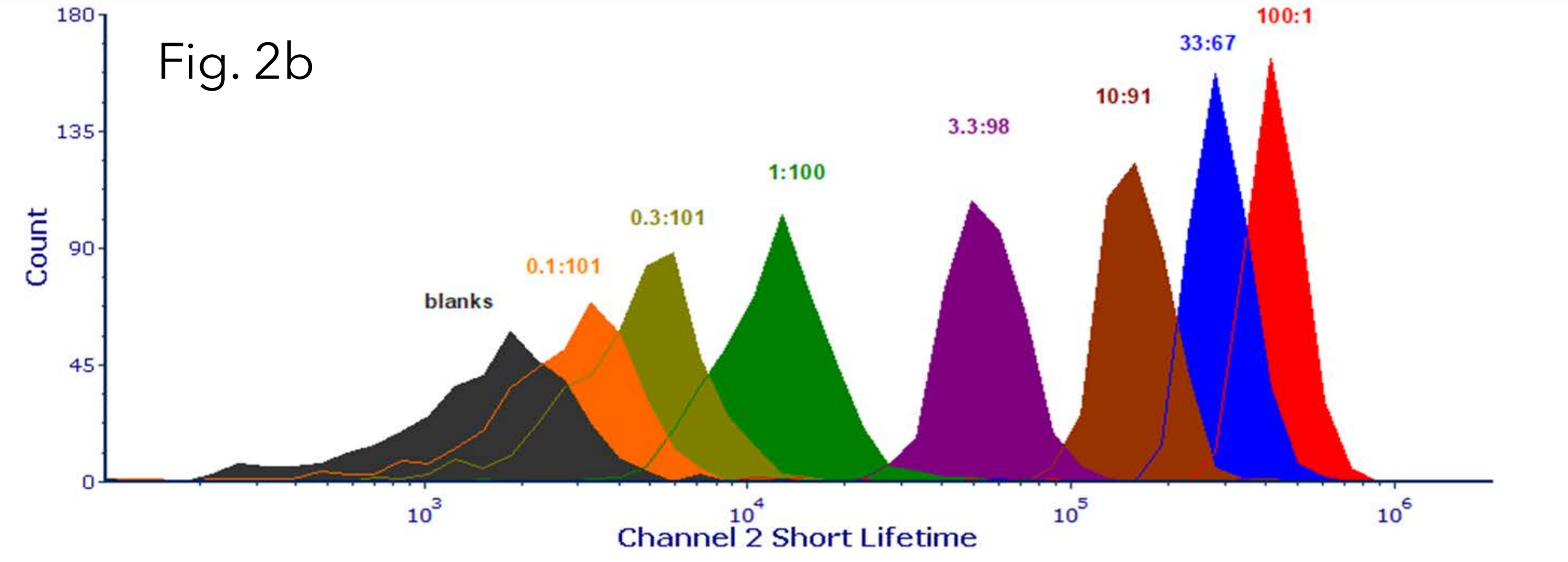


Fig. 2c

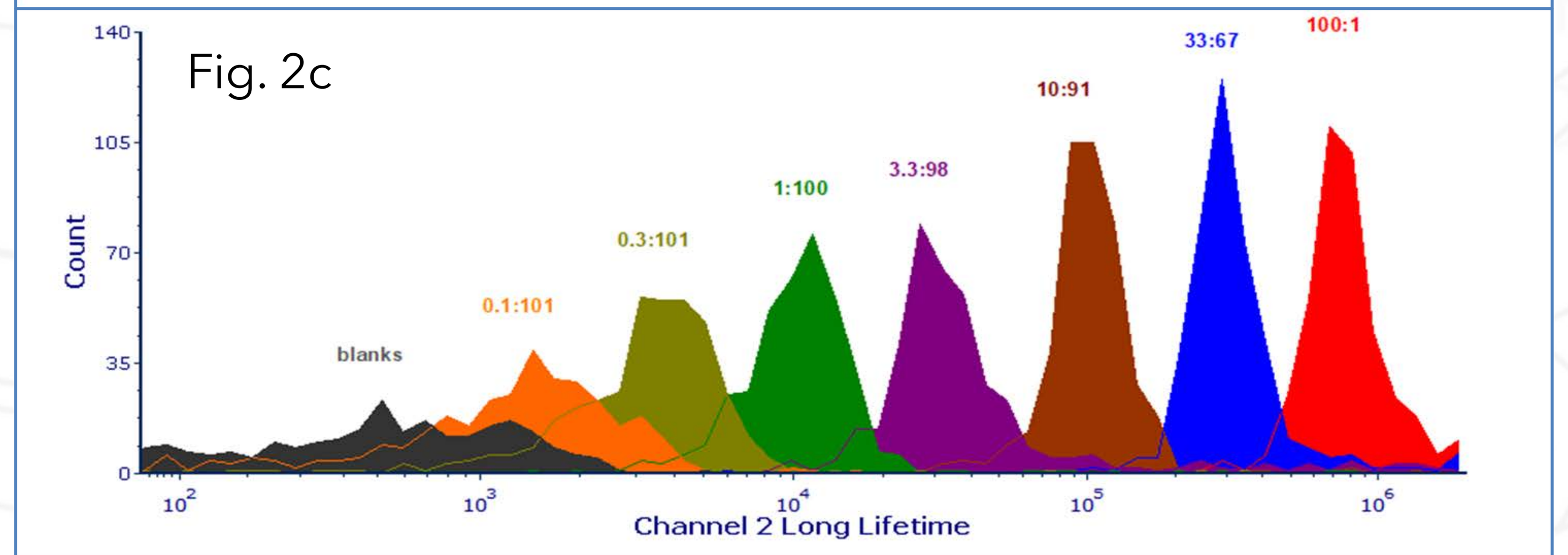
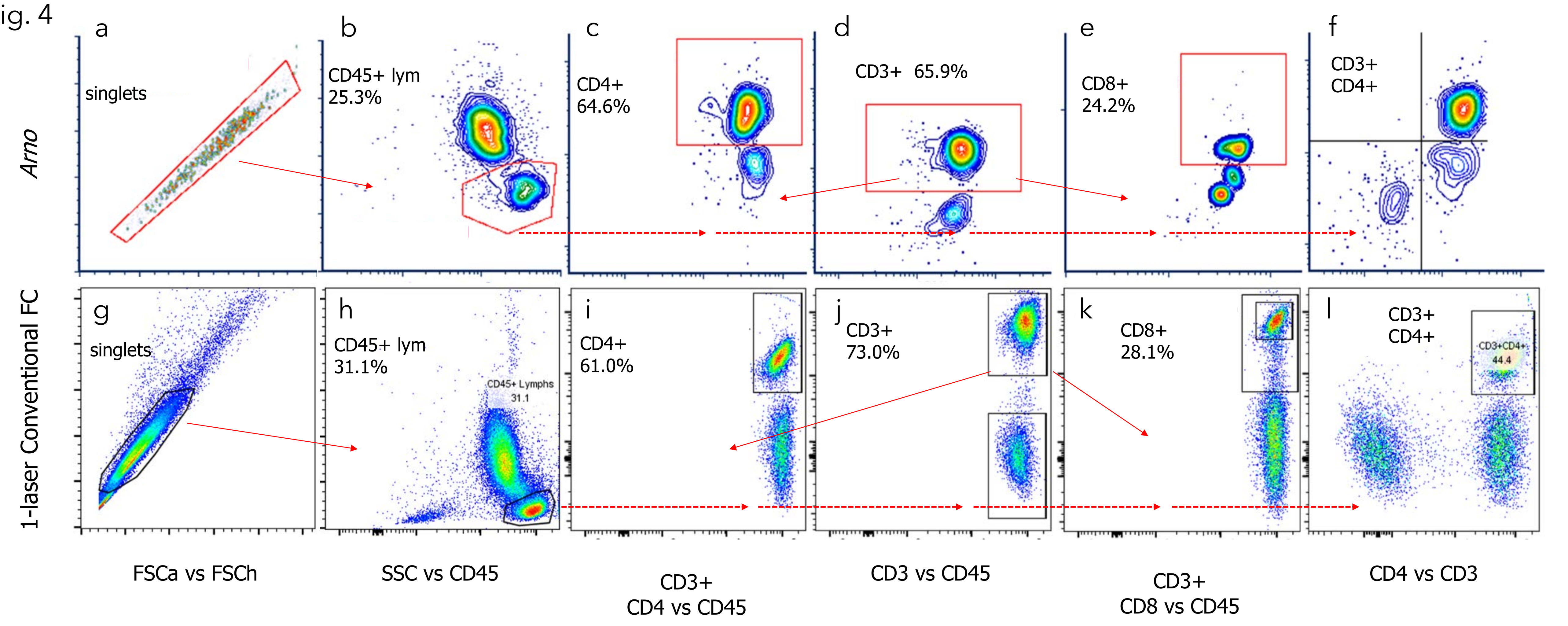
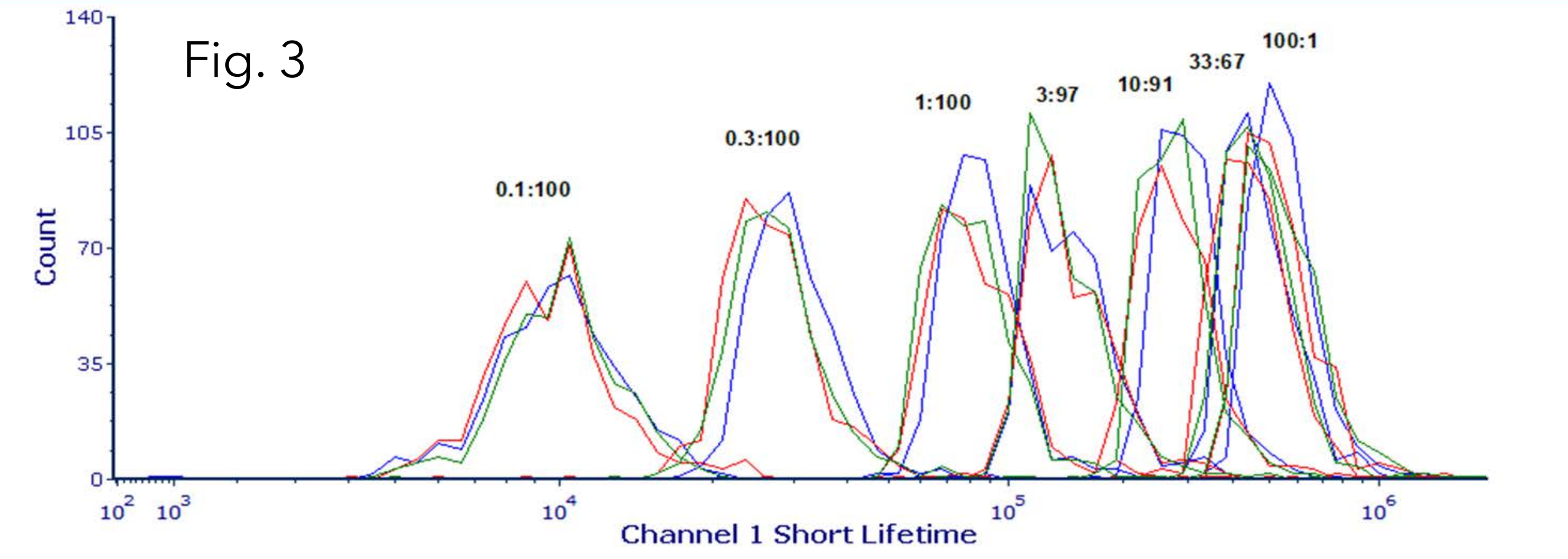


Fig. 4



Currently available bead sets, however, are not designed to allow full characterization of a lifetime-based flow cytometer. In order to perform comprehensive tests on the *Arno*, we used universal capture beads (ThermoFisher Scientific, Miltenyi Biotec) in conjunction with a variety of antibody conjugates chosen to span our instrument's detection range both spectrally and lifetime-wise. We performed titration series using three fluorophores (ThermoFisher Scientific, Miltenyi Biotec) individually against biotin (from 100:1 to 0.1:100), and simultaneously (distinguished by color and by lifetime). The titration results indicate a dynamic range of at least 3 decades in all lifetime and spectral channels, with room to expand (**Figs. 2a–c, Fig. 3**).

Fig. 3



We additionally repeated a standard benchmarking cell-based assay: CD45/CD3/CD4/CD8 immunophenotyping of lyophilized leukocytes (BD Horizon Dri Leukocytes), performed after selecting fluorophore conjugates with improved performance (ThermoFisher Scientific, BioLegend, BD Biosciences) and optimizing Staining Indices (SI) by titration. The results (**Fig. 4**) show very good agreement in population percentages with those obtained on a traditional flow cytometer (BD Biosciences LSR II). The *Arno* system, however, achieved that without any spillover compensation.

CONCLUSION

After a major overhaul of our flow cytometry development platform, the *Arno* compensation-free flow cytometry technology is now operating at levels of stability, uniformity, sensitivity, dynamic range, and robustness comparable to those found in mature commercial instruments, while offering the benefits of fewer components, smaller footprint, and compensation-free operation.

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