Sputum-Derived Cellular Profiles Produced by Flow Cytometric Analysis

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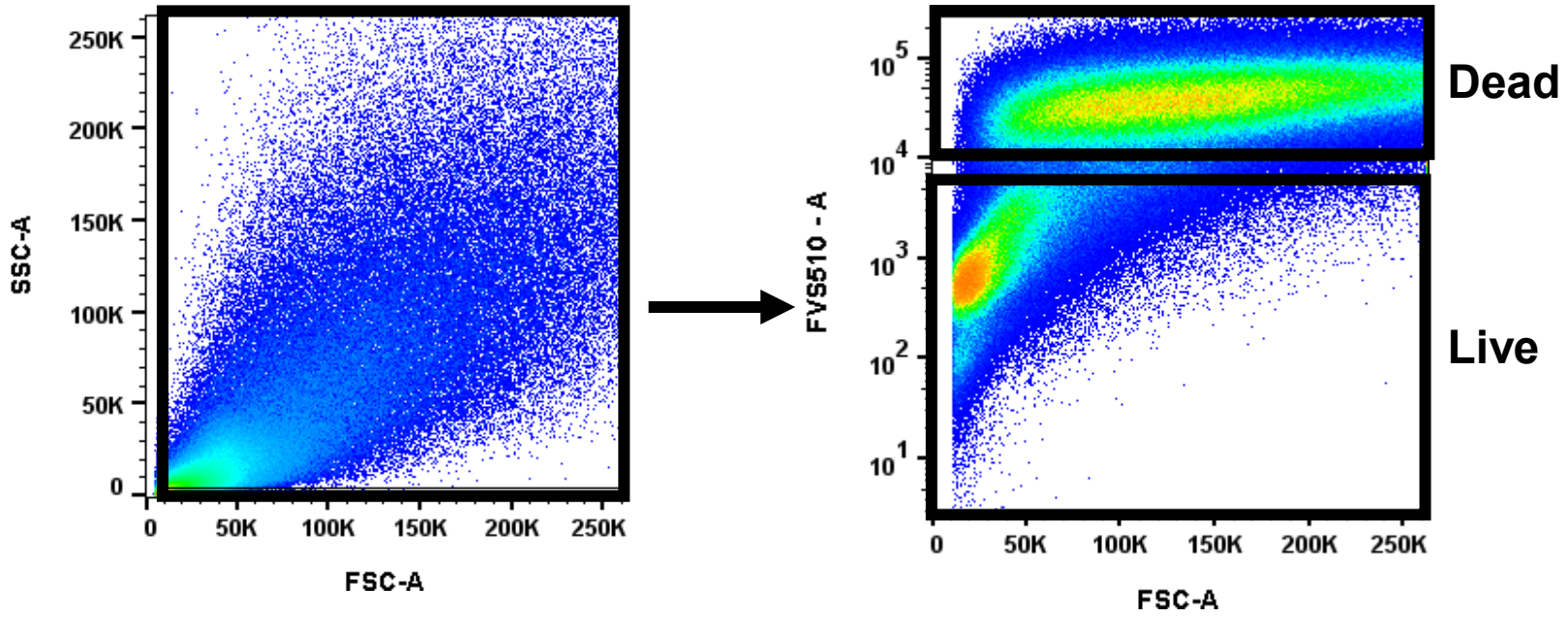
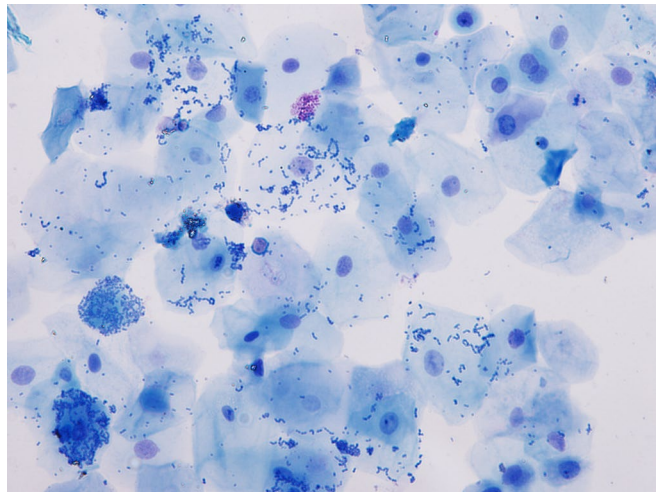
Squamous epithelial cells excluded from analysis via viability dye staining

- ❑ Sputum liquefaction using warmed NAC and DTT
- ❑ Nylon strainer filtration to collect single cell suspension
- ❑ Average cell yield per sputum sample: **20 x 10^6 total cells**
- ❑ Average cell viability per sample: **65%**
- ❑ Average squamous epithelial cell (SEC) contamination: **20%**

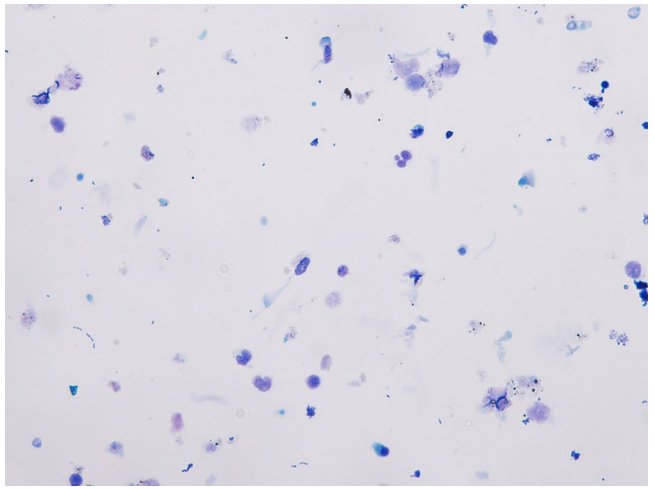


Sorted Dead Cells



40X mag

Sorted Live Cells



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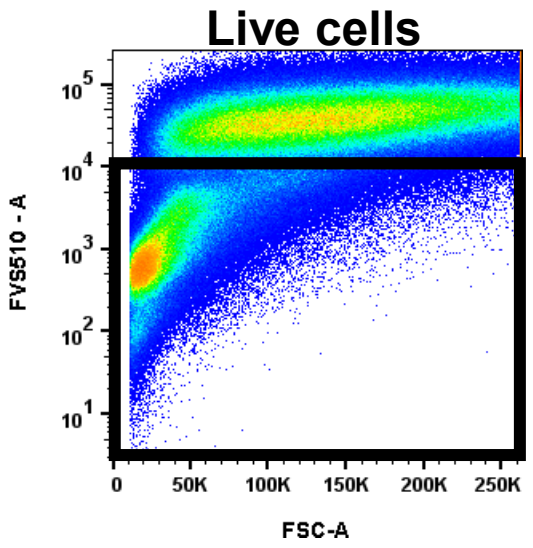
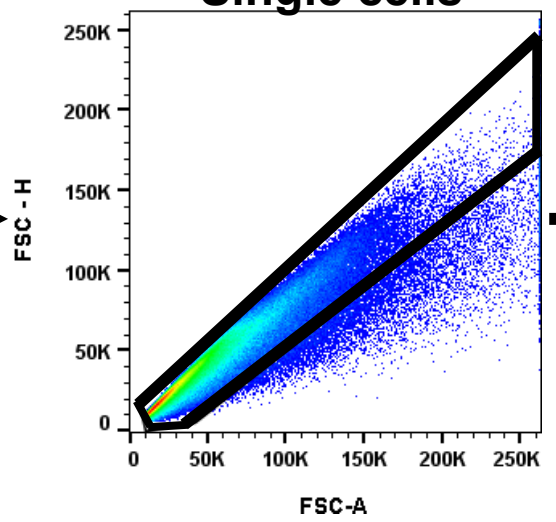
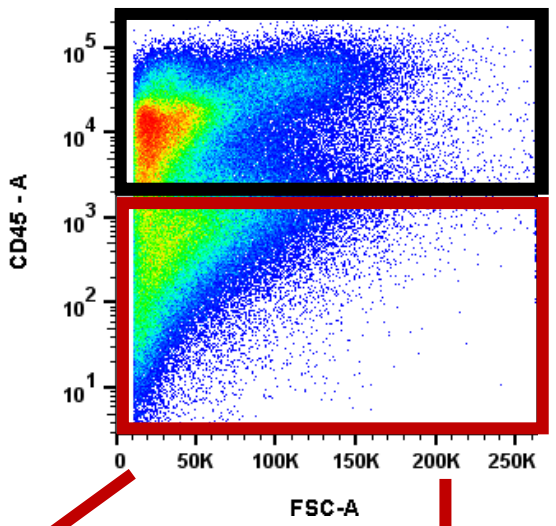
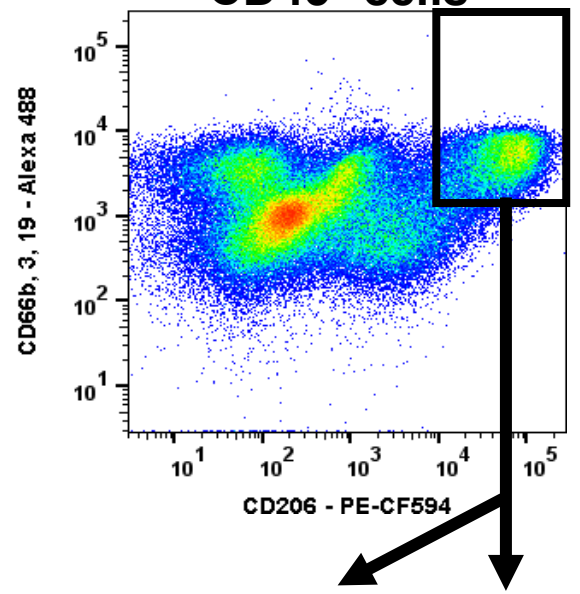
Sputum-derived cellular profiles split into two lineages: leukocytes ($CD45^+$) and non-leukocytes ($CD45^-$)

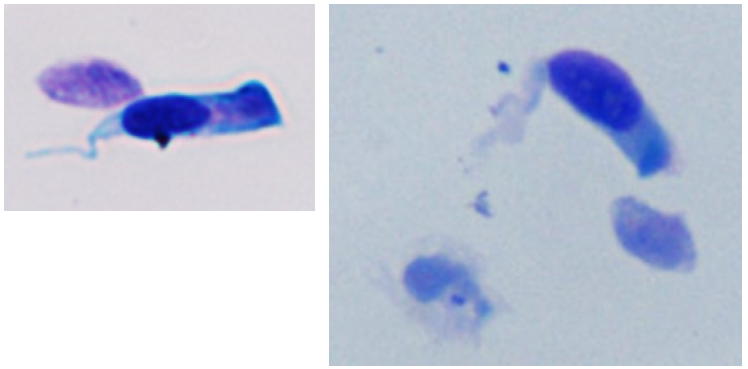




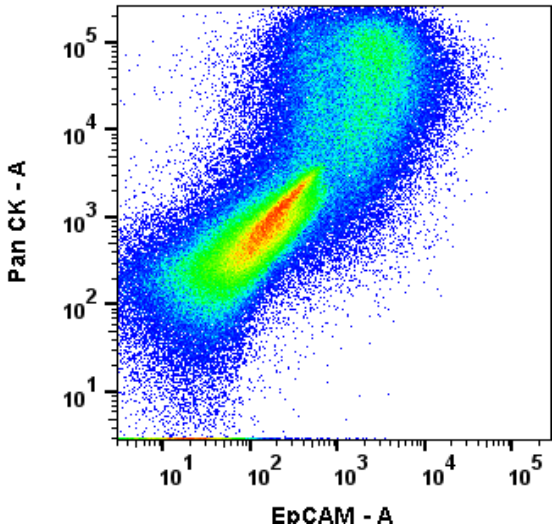




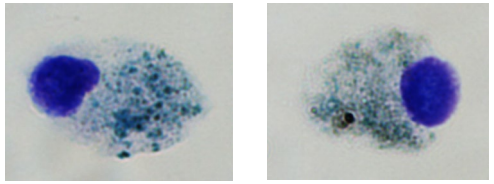
Sorted $CD45^-$ population



Bronchial epithelial cells



Sorted $CD45^+$ population



Alveolar macrophages

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High-throughput sputum analysis for clinical diagnosis

- We chose to investigate whether sputum can be analyzed on a flow cytometry platform analogous to its use for the diagnosis of hematologic malignancies.
- Sputum donors used the acapella® airway assist device (Smiths Medical) to collect sputum over a 3-day period.
- Samples were processed into a single cell suspension and analyzed efficiently by flow cytometry.
- Reproducible profiles of sputum for both the leukocyte and non-leukocyte lineages were obtained.
- The presence of both alveolar macrophages and bronchial epithelial cells indicate that the sputum sample represented the environment of the lung.
- Our data reveals that flow cytometers can analyze samples isolated from sputum in a high-throughput manner that can be developed for diagnostics of lung health.

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