Flow Post-its
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Nuclei Sorting for Multi-omics Profiling

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Recent advances in the understanding of genome complexity has led to growing interest in the role of DNA and its impact on health and disease. **Isolation of nuclei** via flow cytometry based cell sorting is a crucial step to improve purity of the nuclei (especially, when focusing on tissues that are difficult to extract intact cells from, e.g.: brain or frozen tissue), which is further used as input for **Single-nucleus Sequencing (SNS)** and **multi-omics profiling**.

Gating strategy for nuclei sorting

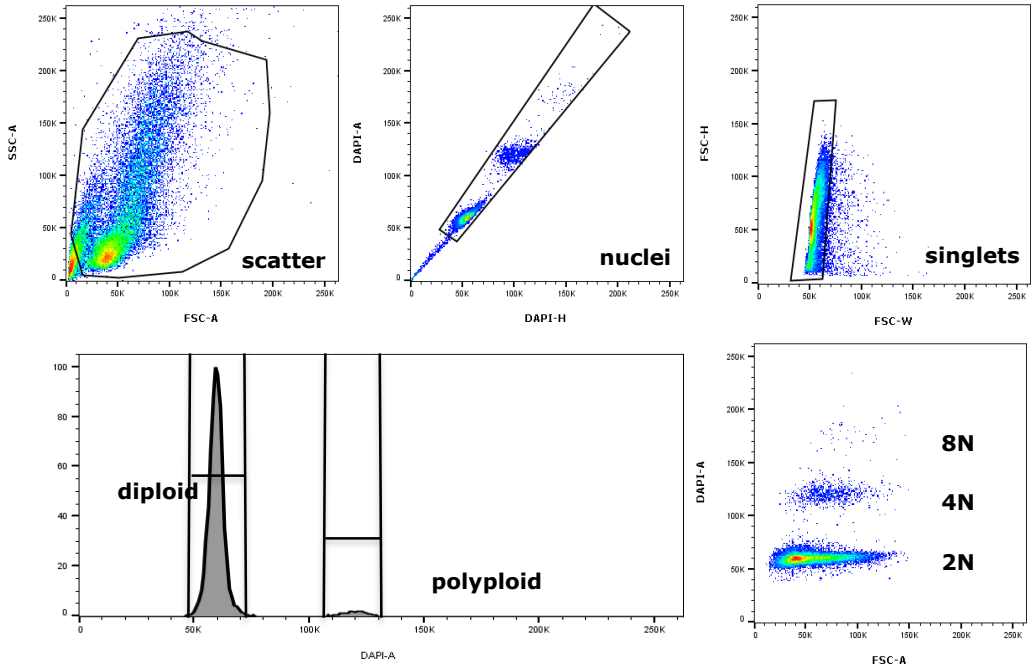

Figure. Gating strategy for sorting of single nuclei based on the DNA content. **a.** FSC vs. SSC plot to exclude debris **b.** DAPI – height vs. DAPI – area plot allows to distinguish nuclei subpopulations **c.** FCS – width vs. FCS height plot for doublet discrimination **d.** DAPI dot plot and histogram showing nuclei ploidy profile.

Important Notes

- Record at least 10,000 **DAPI+ nuclei** to make sure gates are set properly
- **Keep Flow Rate = 1 -** Higher pressure differentials can result in wider peaks and consequently loss of resolution, making it difficult to identify ploidy
- **Do not vortex,** as it can irreversibly damage the nuclei
- Keep samples/reagents **on ice** and try to work as fast as possible (during sample prep of frozen tissue, keep samples on dry ice)
- To achieve better visualization - set up DAPI in **linear scale.**
- Accurate determination of nuclei concentration is absolutely critical. Always count and stain the **same amount** of nuclei.
- **Suggested reading for detailed protocol:** Baslan et al. (2016) , doi: 10.1038/nprot.2012.039