An automated workflow for optical pooled screens with massively multiplexed phenotypic readouts on AVITI24

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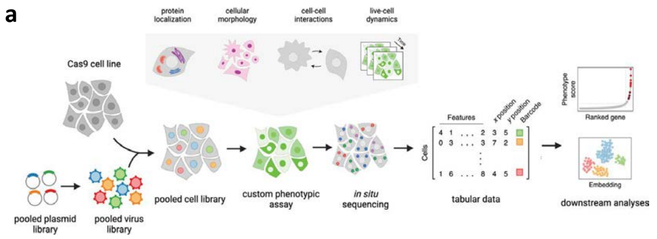
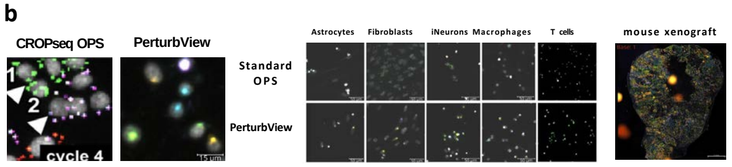
Optical pooled screens

a



b



c

Current limitations

- Most OPS workflows are still manual or only semi-automated
- Challenging to perform multimodal readouts (RNA + protein), limited plexity

Figure 1: Optical pooled screens and PerturbView
a) Optical pooled screens (OPS) use targeted in situ sequencing of perturbations to link phenotypes and genotypes from a pooled library, making it practical to perform genome-wide imaging screens b) PerturbView leverages in vitro transcription to substantially amplify ISS signal, enabling screens in a wide variety of model systems with highly multiplexed phenotypic readouts c) Currently, it is fairly laborious to perform OPS experiments and challenging to integrate multimodal readouts with ISS.

Multimodal profiling with AVITI24

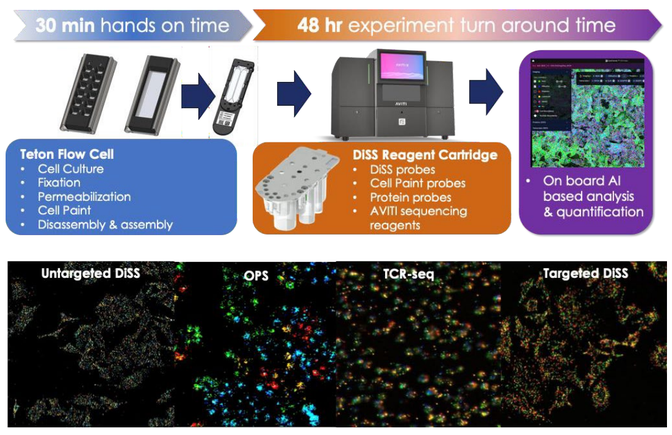
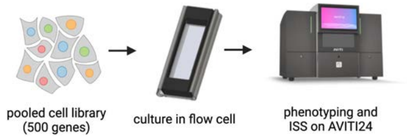


Figure 2: Cytoprofiling on Element AVITI24
The AVITI24 platform enables the simultaneous detection of high-plex protein expression, morphological analysis using Cell Paint, and DiSS of reads exceeding 100 bases. This approach supports both untargeted total transcriptome analysis and targeted probe panels designed for specific applications, including Optical Pooled Screening (OPS), T-cell and B-cell receptor sequencing, and the analysis of specific genes of interest.

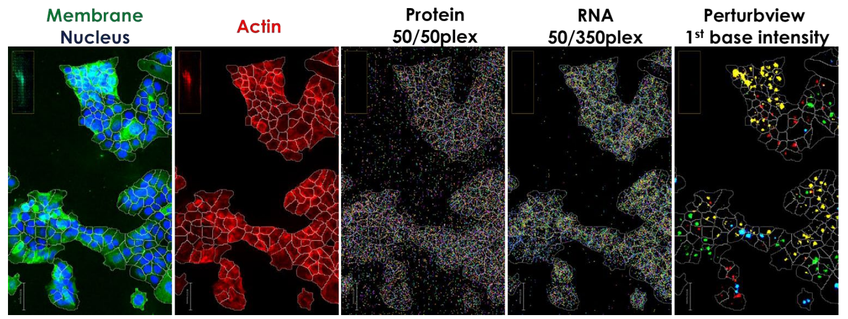
A highly multiplexed optical screen in breast cancer cells

a



Cell line	MCF-7
IF markers	nucleus, actin, wheat germ agglutinin (cell membrane)
RNA markers	350 markers: apoptosis, cell cycle, MAPK
Protein markers	50 markers: apoptosis, cell cycle, MAPK

b



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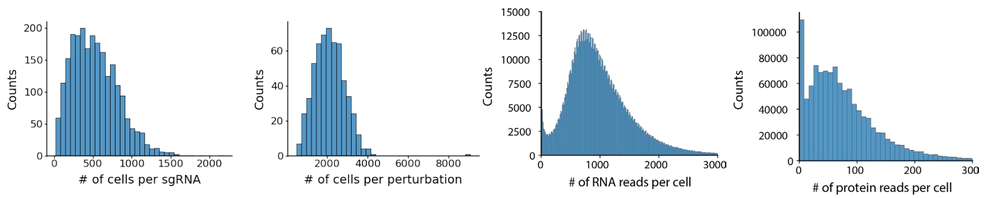

Figure 3: A 500-gene screen leveraging morphological, RNA, and protein readouts
a) Schematic of the screening workflow and table summarizing the phenotypic panel b) Example images from the screen c) The screen sampled 490 cells/guide and 2074 cells/gene (median) d) Most cells captured ~900 RNA counts and ~70 protein counts across 350 RNA and 50 protein markers.

Validation of phenotypic readouts with control perturbations

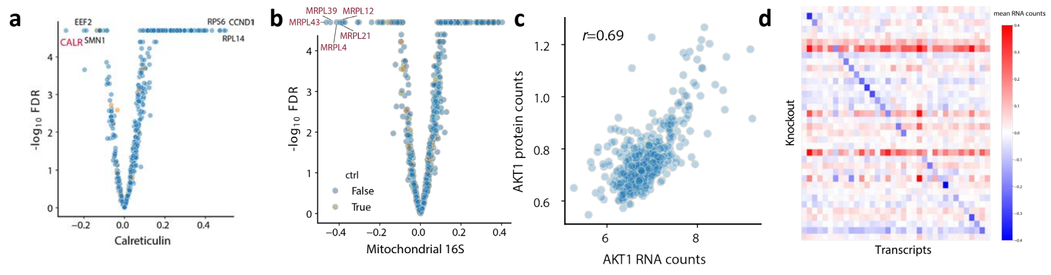

Figure 4: Morphological, RNA, and protein readouts perform as expected for control perturbations
a) CALR KO is the top hit for calreticulin immunofluorescence (endoplasmic reticulum channel for Cell Painting) b) MPRL genes are the top hits for 16s mitochondrial marker. c) protein and RNA levels of AKT1 show concordance across different perturbations. d) For 19/30 RNA markers, the corresponding gene is among the top 3 down hits.

High-content morphological profiling

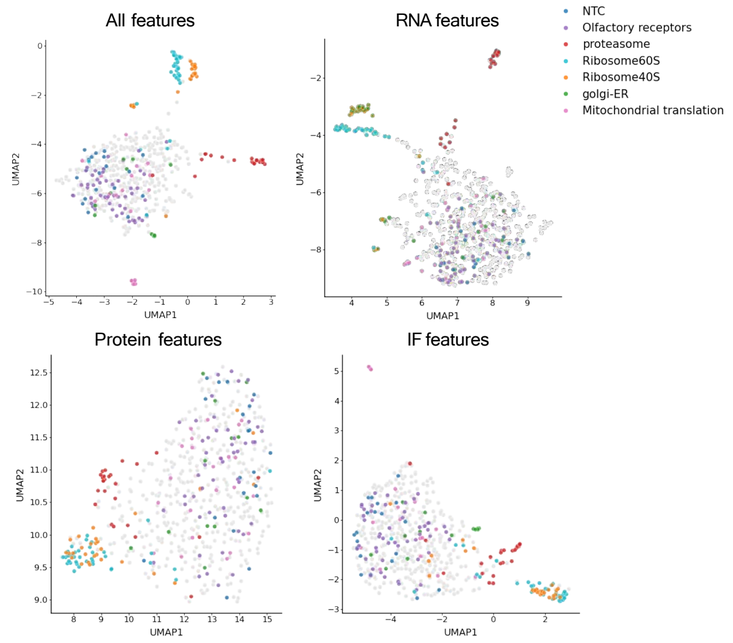

Figure 5: Cytoprofiling on Element AVITI24
Phenotypic embeddings of perturbations using all features, morphology, RNA, or protein.

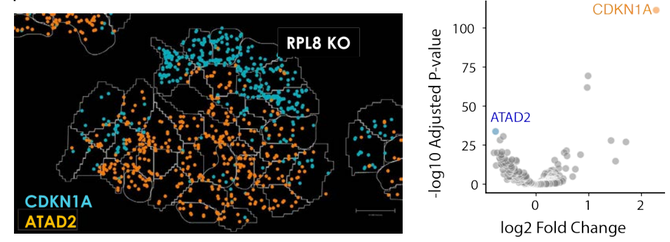

Figure 6: Visualization of ribosomal gene RPL8 perturbation impact on RNA
Perturbation of ribosomal gene RPL8 dramatically upregulates CDKN1A and downregulates ATAD2

Conclusions and next steps

- We completed a fully-automated 500-gene optical pooled screen with highly multiplexed and multimodal phenotypic panels, validated the performance of individual markers, and demonstrated recovery of canonical biology using image-based profiling.
- We aim to perform genome-wide imaging screens with rich phenotypic readouts across a variety of cell types to better understand disease progression and identify novel targets.