Highly sensitive transcriptomic-based pooled CRISPR screening enabled by Spatial Molecular Imager (SMI)

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Summary

Spatial molecular imager (SMI) uses nucleic acid hybridization cycles of fluorescent molecular barcodes to enable in-situ measurement of biological targets on intact sample with subcellular resolution. Using SMI, we demonstrate an imaging based pooled CRISPR screens by simultaneously visualizing guide RNA (gRNA) and accurately measuring transcripts of interest within the same cell.

Main Objectives and Method:

- Designed flexible and robust SMI assay capable of simultaneously detecting gRNAs and quantifying RNA expression in the same cell that can scale in target plex (up to 1000) and number of cells interrogated (>100K)
- Using LNCaP prostate cancer cell line, applied epigenetic perturbations to an androgen receptor (AR) enhancer to validate impact on the AR gene and its downstream targets (55 gRNAs + 37 genes + controls)

Key Results:

- **High Resolution** – Unambiguous visualization and digital detection of gRNAs in single cells
- **High Sensitivity** – Demonstrated by:
  - Detection of key AR related target genes that are typically low expressor (TPM ~ 1-10)
  - Quantitation of AR related target genes on over 90% of the cells analyzed (~10% drop-out rate)
  - High detection sensitivity of rare modified cells (below 1%) was observed
- **Good concordance** – Gene expression changes measured by SMI vs. Z-scores from RT-PCR drop-out assays

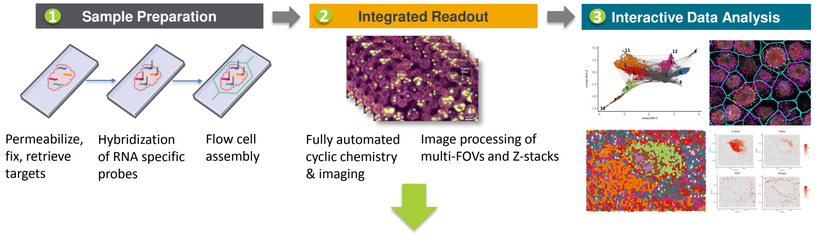
Key advantages:

- High plex – Flexible panel design to visualize and measure up to 1000 gRNAs and genes within single cells
- Accurate quantitation – Decreased 3'-end transcript bias and use of both endogenous and exogenous controls
- High throughput with simple workflow – Tens to hundreds of thousands of cells analyzed per SMI run

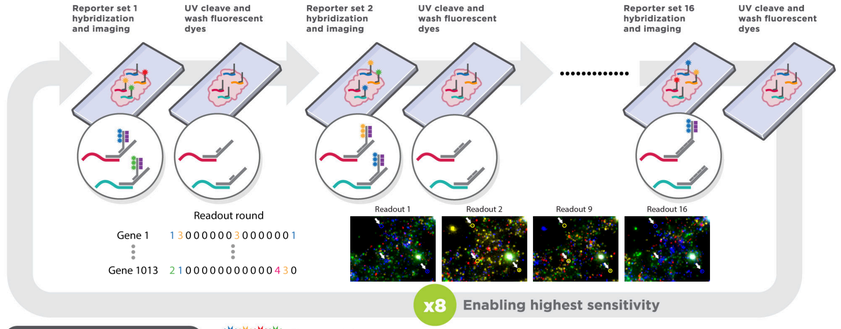
For research use only. Not for use in diagnostic procedures.

Introduction: Spatial Molecular Imager

SMI is a single instrument solution for subcellular spatial analysis



SMI's cyclic readout enables high-plex detection



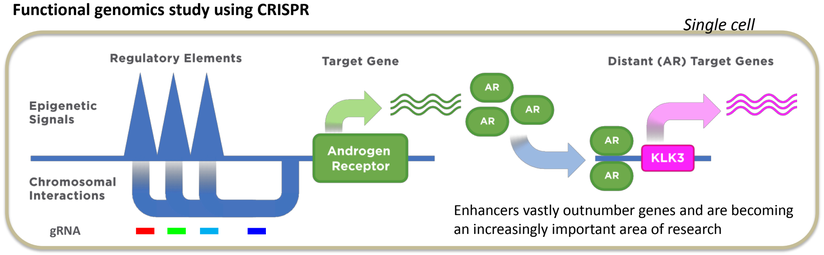
Barcode encoding scheme: 64 bits (4-colors x 16 cycles)

Fluorescent dye
PC-Linker

* More technical details on SMI on Beechem et al. AGBT 2021 poster

Fundamental Challenge – Connecting regulatory elements to their genes

Functional genomics study using CRISPR

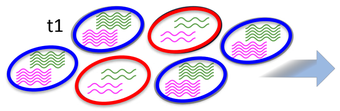


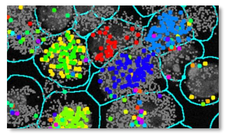
Enhancers vastly outnumber genes and are becoming an increasingly important area of research

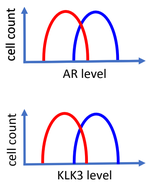
Method Overview: Pooled CRISPR screening using transcriptomic readout on SMI

1. SMI compatible gRNA library design
2. Transduction & cell passage
3. Cell/sample prep onto glass slide for SMI
4. SMI run and analysis

High-throughput image based approach that simultaneously visualizes gRNA and measures transcripts of interest within the same cell



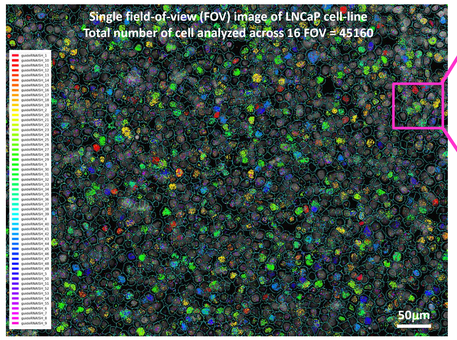


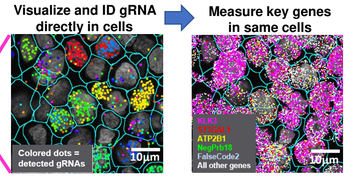


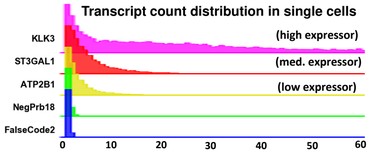
Quantitative transcript measurement and gRNA identification at single cell level

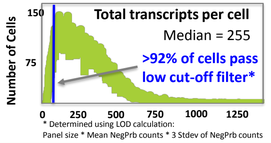
SMI produces high data density in virtually every cell

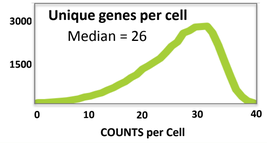
SMI sensitivity enables detection of both low and high expression genes and gRNAs

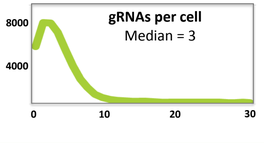






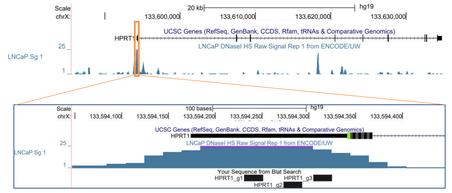






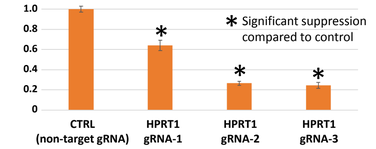
Proof-of-concept: Measuring effect of genomic perturbation by RNA readout using SMI

Positive control: gRNA designed to HPRT1 locus

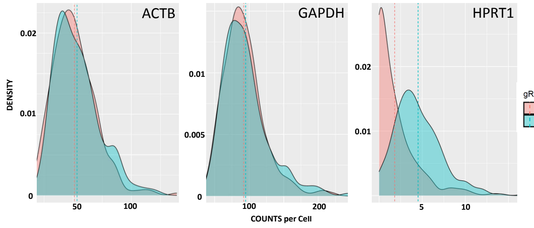


Validated gRNA design and function

Relative HPRT1 gene expression across different cell populations modified by a single gRNA type (measured by qRT-PCR)



SMI data: Statistical analysis of transcript distribution between two gRNA designated cells [cells detected with HPRT1 gRNA vs. non-target gRNA (CTRL)]



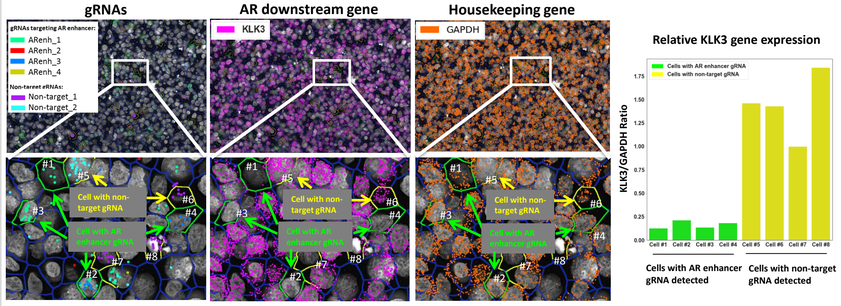
Count distributions were analyzed using 500 selected cells, where total transcript counts were > 100 based on the summary of all hybridized transcripts (n=37). Non-human genome targeting CTRL (n=217) and HPRT1_g2 targeting (n=283) gRNAs were designated to particular cells, if ≥3 counts observed.

No change between HPRT1 and CTRL cells

No change between HPRT1 and CTRL cells

Significant difference between HPRT1 and CTRL cells

Visualize and quantify genomic perturbation at single cell resolution on tens of thousands of cells using SMI



Conclusions

Using SMI technology, we demonstrated the ability to conduct highly multiplexed gRNA pooled screening, which seamlessly allows simultaneous identification of gRNA and accurate digital measurement of transcripts of interest at single cell resolution.

Concordance between this approach with existing RT-PCR based drop out assay validates the ability to perform CRISPR screens using gene expression as a readout. This approach, with further refinement would be transformative for understanding regulatory element biology because it allows a more direct and accurate way to functionally connect regulatory element-to-target gene relationships.