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 – Hundreds of thousands of cells analyzed per SMI run

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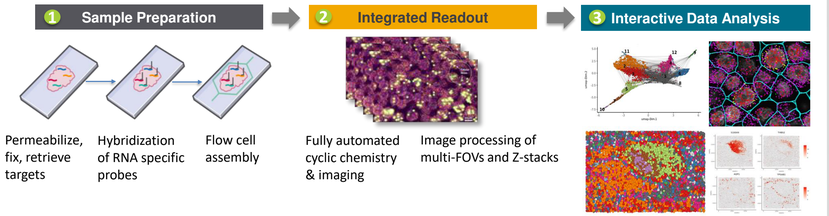
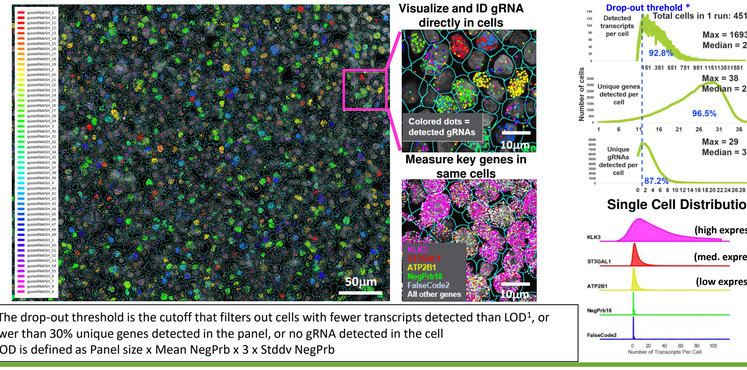
Introduction: Spatial Molecular Imager

SMI is a single instrument solution for subcellular spatial analysis



SMI produces high data density in virtually every cell

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



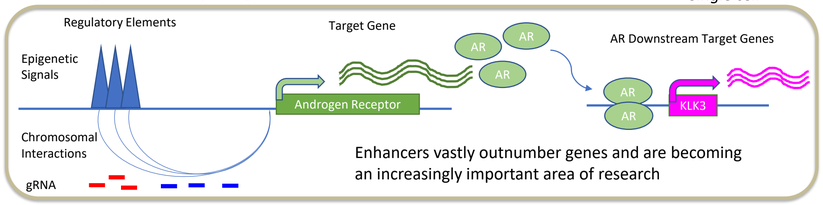
* The drop-out threshold is the cutoff that filters out cells with fewer transcripts detected than LOD[1], or fewer than 30% unique genes detected in the panel, or no gRNA detected in the cell

[1] LOD is defined as Panel size x Mean NegPrb x 3 x Stddv NegPrb

Fundamental Challenge – Connecting regulatory elements to their genes

Functional genomics study using CRISPR

Single cell

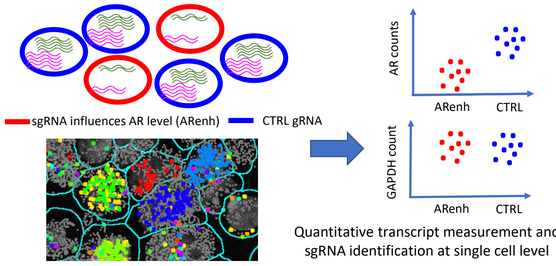


Epigenetic regulation of gene expression is a complex biological proses. Different techniques were developed to connect regulatory elements to their target gene. However, to understand this dynamic and complex process is still challenging. CRISPR technology made possible to create genetic and epigenetic perturbations in a pooled fashion manner which is a powerful approach to connect regulatory elements to biological procedures, like cell proliferation and drug resistance or sensitivity.

Method Overview: Pooled CRISPR screening using transcriptomic readout on SMI

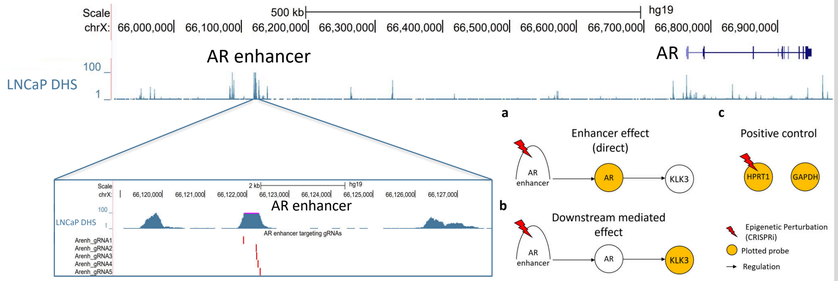
1. **SMI compatible gRNA library design**
2. **Transduction & cell passage**
3. **Cell/sample prep onto glass slide for SMI**
4. **SMI run and analysis**

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



Single Molecular Imaging (SMI) is a new approach to precisely quantitate expressions of variety of gene of interest. This platform also enables to detect sgRNA by specific probe based direct or indirect (added 50 bp tag) way. In this work, we are combining these advantageous features of the SMI platform: (a) identification of genetic perturbation by specific detection of the gRNA and (b) accurate quantitation of gene expression at single cell level to connect regulatory element to their target genes.

Experimental Set up: Epigenetic perturbation and expected outcomes

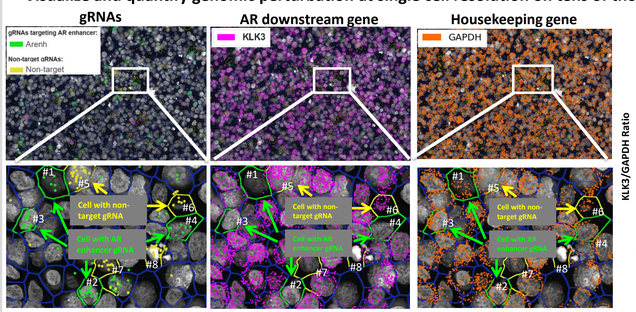


Previously, we identified a somatically acquired enhancer of the androgen receptor (AR) in ~650 kb distance, which is active only in metastatic castration resistant prostate cancer (mCRPC). We proved in metastatic prostate cancer model (LNCaP cell line), that this enhancer is critical for cell growing via the transcriptional regulation of the AR. We used 5 AR enhancer targeting gRNA (Arenh gRNA1-5, red stripes) in LNCaP CRISPRi model to show regulatory effect at gene expression level of the AR enhancer.

In this work, using a pooled gRNA screening approach combined with the SMI platform, we are showing (**a**) what are the critical positions of the AR enhancer directly influences the AR gene level. (**b**) We are demonstrating the AR mediated (indirect) effect interrogating the level of KLK3, which is an AR regulated downstream target gene. (**c**) As for positive control, we are targeting the HPRT1 gene promoter, showing specific suppression of the HPRT1 gene, but not in other genes including GAPDH.

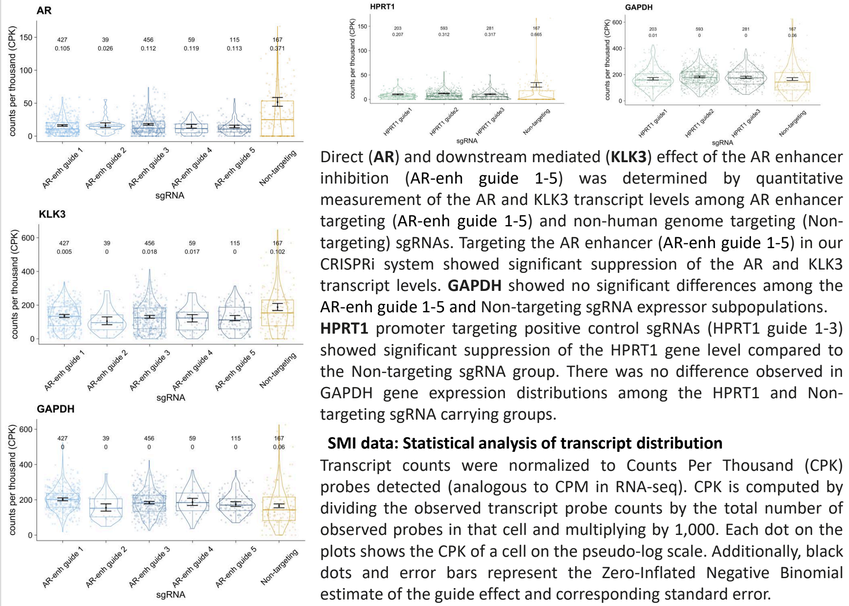
Proof-of-concept: Measuring effect of genetic perturbation by RNA readout using SMI using direct and downstream target genes

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



Single cell analysis demonstrate unambiguous relationship between gRNA and AR regulated downstream target gene (KLK3). Cells with gRNA targeting the AR enhancer show suppressed level of KLK3 compared to cells which are carrying non-targeting gRNAs.

Results: Pooled CRISPR screening using transcriptomic readout on SMI



Direct (**AR**) and downstream mediated (**KLK3**) effect of the AR enhancer inhibition (**AR-enh guide 1-5**) was determined by quantitative measurement of the AR and KLK3 transcript levels among AR enhancer targeting (**AR-enh guide 1-5**) and non-human genome targeting (Non-targeting) sgRNAs. Targeting the AR enhancer (**AR-enh guide 1-5**) in our CRISPRi system showed significant suppression of the AR and KLK3 transcript levels. **GAPDH** showed no significant differences among the **AR-enh guide 1-5** and Non-targeting sgRNA expressor subpopulations. **HPRT1** promoter targeting positive control sgRNAs (HPRT1 guide 1-3) showed significant suppression of the HPRT1 gene level compared to the Non-targeting sgRNA group. There was no difference observed in GAPDH gene expression distributions among the HPRT1 and Non-targeting sgRNA carrying groups.

SMI data: Statistical analysis of transcript distribution

Transcript counts were normalized to Counts Per Thousand (CPK) probes detected (analogous to CPM in RNA-seq). CPK is computed by dividing the observed transcript probe counts by the total number of observed probes in that cell and multiplying by 1,000. Each dot on the plots shows the CPK of a cell on the pseudo-log scale. Additionally, black dots and error bars represent the Zero-Inflated Negative Binomial estimate of the guide effect and corresponding standard error.

Conclusions

The SMI platform accurately and robustly measures transcripts of interest as well as gRNAs in single cells. In this proof-of-concept study, we performed a pooled CRISPR screen against an enhancer of the AR gene and used gene expression as a readout. We showed that the SMI platform could detect both gRNA identities and transcripts of interest (both low-moderate and high expressors) in single cells with minimal dropout. This transformative and flexible technology enables the dramatic expansion of experimental possibilities by employing gene expression as a readout.

QR Code