In situ mutational mapping of tumor cells and tissues at subcellular resolution

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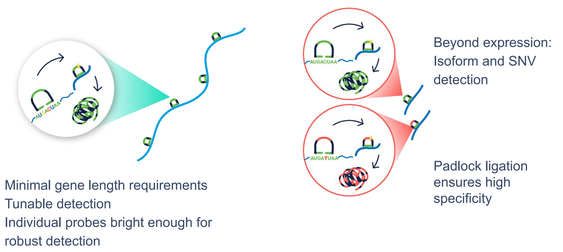
1. Introduction

In situ techniques enable studying cells and tissues within their cellular context. This is of great importance in cancer, which often displays genetic and phenotypic heterogeneity with distinct subclones harboring different capacity to grow and spread. It was recently shown that by spatially mapping dozens of mutations within tumor tissues, tumor evolution and microenvironmental impact could be studied at the subclonal level[1].

2. Methods

We are developing an *in situ* technique that spatially maps multiple expressed mutations simultaneously at subcellular resolution. The technique was validated in well characterized cell lines, targeting single nucleotide variants of the *KRAS* codon 12. The specificity was further validated in a mixed cell population, targeting multiple cell-specific mutations. As a proof of concept, we applied the technique to FFPE human breast tumor tissue sections, previously characterized by Illumina TruSight Oncology 500 on extracted tumor DNA. In addition to the 280 plex Xenium Human Breast Gene Expression Panel, we targeted several mutations *in situ* and mapped their spatial distribution in the tumor tissue sections.

The Xenium chemistry



- Minimal gene length requirements
- Tunable detection
- Individual probes bright enough for robust detection

References

1. Lomakin, A., Svedlund, J., Strell, C. *et al.* Spatial genomics maps the structure, nature and evolution of cancer clones. *Nature* (2022). https://www.nature.com/articles/s41586-022-05425-2

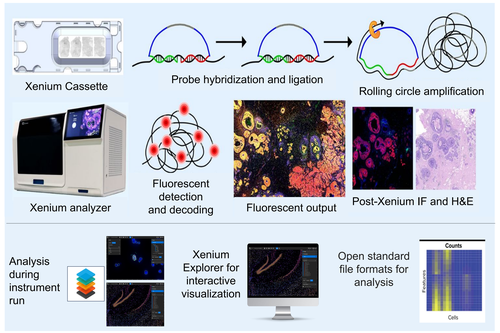


Figure 1. The Xenium workflow. The Xenium In Situ platform uses a microscopy based read-out. A FFPE tissue section of 5 µm was sectioned onto a Xenium slide, followed by hybridization and ligation of specific DNA probes to target mRNA and rolling circle amplification. The slide was placed in the Xenium Analyzer instrument for multiple cycles of fluorescent probe hybridization and imaging. Unique optical signatures allowed for decoding of the target genes, from which a spatial transcriptomic map was constructed across the entire tissue section. The Xenium data can be further registered with post-Xenium IF / H&E images.

3. Single nucleotide variant calling of *KRAS* in cell lines

Two well characterized cell lines, wild type and mutant for *KRAS* codon 12, were genotyped using the Xenium chemistry. Probes targeting either wild type or mutant transcripts were applied to both cell lines and detected with different fluorophores.

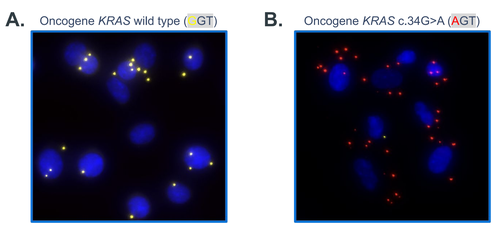


Figure 2. *In situ* genotyping of *KRAS* codon 12 in cell lines. (A) ME180 cell line that is wild type for *KRAS* codon 12, detected in yellow. (B) A549 cell line that is mutant for *KRAS* codon 12 (c.34G>A), detected in red. Nuclei was detected in blue.

4. Multiplexed mutation detection identifies different cells

A 50:50 mixture of ME180 and A549 cell lines was used to decode wild type and mutant *KRAS* c.34G>A, *KEAP1* c.997G>T, *CBL* c.1753C>T and *STK11* c.109C>T transcripts, also match/mismatch probes against *GAPDH*.

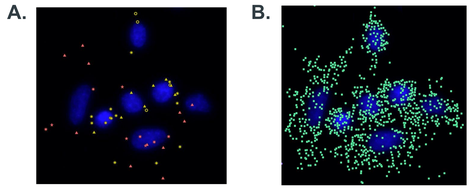
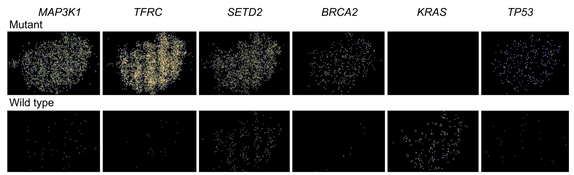


Figure 3. Multiplexed mutation detection in mixed cell population. (A) Decoded wild type transcripts are illustrated in yellow and mutant in red. (B) Decoded *GAPDH* match probes in green and mismatch probes in purple. Nuclei in blue.

5. Spatially resolved mutational mapping of FFPE human breast cancer

Multiple probes targeting wild type and mutant transcripts were applied to a 5 µm FFPE section and decoded using a prototype Xenium instrument.



Detected gene codon	NGS data
MAP3K1 c.2716G>A	mutant (VAF:99.73)
TFRC c.424G>A	mutant (VAF:66.67)
SETD2 c.5885C>T	mutant (VAF:98.76)
BRCA2 c.1114A>C	mutant (VAF:99.51)
KRAS c.38G>A	wild type
TP53 c.215C>G	mutant (VAF:63.54)

Figure 4. Multiplexed mutation detection in FFPE human breast cancer. Spatial distribution of wild type and mutant transcripts in the tissue. The FFPE block used was previously characterized by NGS on extracted tumor DNA as *MAP3K1* c.2716G>A mutant, *TFRC* c.424G>A mutant, *SETD2* c.5885C>T mutant, *BRCA2* c.1114A>C mutant, *KRAS* c.38G>A wild type and *TP53* c.215C>G mutant.

6. Cell typing of FFPE human breast cancer

Cell typing was done using the 280 plex Xenium Human Breast Gene Expression Panel. Transcriptional profiles of cells were analysed with K-mean clustering.

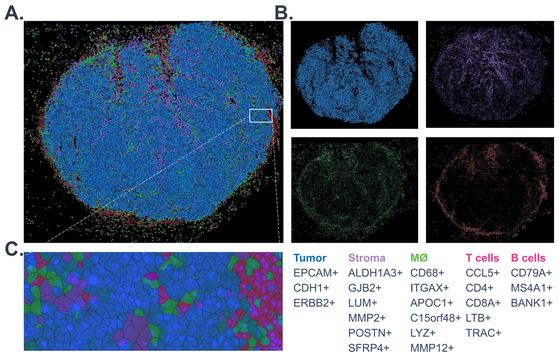


Figure 5. Spatial distribution of cell types in FFPE human breast cancer. (A) Spatial distribution of identified cell clusters in the tissue. (B) Four cell clusters corresponding to tumor cells (blue), stroma (purple), macrophages (green) and T/B cells (pink). (C) Zoomed image of the clustered cell types.
The tumor cell cluster was EPCAM+/CDH1+/ERBB2+, the stroma cell cluster ALDH1A3+/GJB2+/LUM+/MMP2+/POSTN+/SFRP4+, the macrophage cell cluster CD68+/ITGAX+/APOC1+/C15orf48+/LYZ+/MMP12+ and the T and B cell clusters were CCL5+/CD4+/CD8A+/LTB+/TRAC+ and CD79A+/MS4A1+/BANK1+, respectively.

7. Conclusion

In summary, we have shown that the Xenium In Situ technology has the capability to identify different genotypes of cells by simultaneous detection of wild type and mutant transcripts. We further imply the feasibility of the technique to spatially resolve the genetic and phenotypic landscape of tumors by spatially mapping multiple mutations and performing gene expression profiling in FFPE breast cancer tissue sections.

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