# Using artificial intelligence to perform spatial gene expression profiling of colorectal carcinoma using a novel, high resolution, ink-based on-slide printing technology from Quantumcyte, QCPRECISE!™

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## Background

We recently developed a quantitative segmentation algorithm (QuantCRC[1]) to identify regions of interest and objects that correspond to well-described morphologic features in colorectal carcinoma (CRC). We subsequently applied QuantCRC[1] to 6,648 CRCs and developed a prognostic image-based biomarker of tumor recurrence (Gastroenterology. 2022 Dec;163(6):1531-1546). QuantCRC[1] segmentation masks also allow for spatial localization of the various regions of interest (ROI) in the digitized image. In this study we utilize the spatial localization of ROIs to test QuantumCyte's QCPRECISE!™ novel platform to extract material from complex ROIs using semi-conductor print masking technology integrated with artificial intelligence (AI)-based digital pathology. (**Figure 1.** The QCPRECISE!™ platform. **Figure 2.** The QCPRECISE!™ workflow. The digital images are used to guide the extraction of RNA from ROIs with 5 micron accuracy. **Figure 3.** A. Tumor bud/poorly differentiated cluster (TB/PDC, Red) and non-budding carcinoma (green) ROIs as identified by the QuantCRC[1] algorithm. B. QuantCRC[1] TB/PDC ROIs. C. Postlysis image after TB/PDC RNA lysis using the QCPRECISE!™ process.

## Study Design

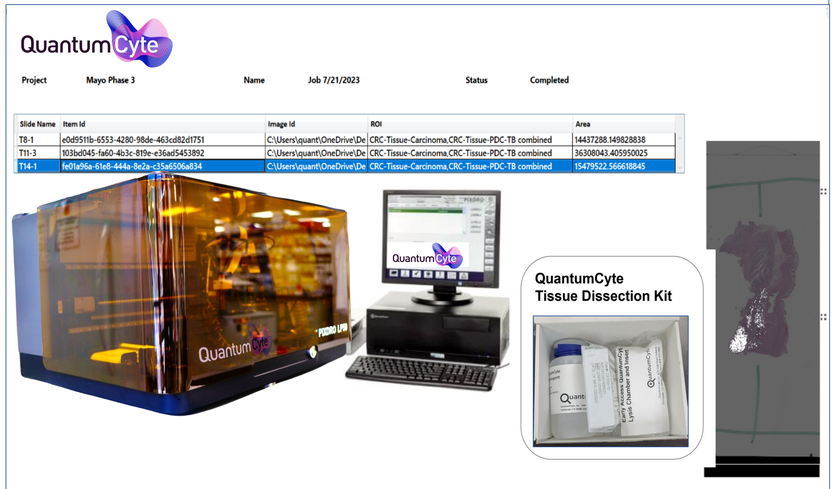
We applied QuantCRC[1] and the QCPRECISE!™ process to FFPE H&E stained slides from 4 CRCs resected. Using our novel print masking technology, we isolated RNA from 4 QuantCRC[1]-derived ROIs for RNAseq analysis including total tumor bed, carcinoma, TB/PDC (thought to represent epithelial-mesenchymal transition (EMT), and immature stroma (16 total ROIs). A bioinformatics pipeline was developed to analyze transcriptomic data using this methodology.

## Materials and Methods

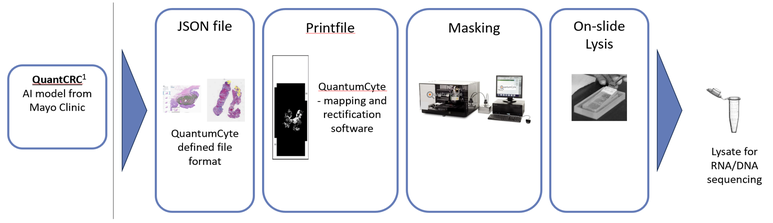
FFPE colorectal carcinoma (CRC) tissue blocks were sectioned at 5 microns thick on to QuantumCyte's premarked microscope slides. Alternate slides from each CRC sample were designated for TB/PDC or Carcinoma for the QCPRECISE!™ workflow **(Figure 2).** All slides were H&E stained, scanned using a Leica Aperio scanner, and ROIs were identified using the QuantCRC AI-algorithm[1]. Total RNA was purified using the Qiagen All Prep purification kit protocol. For the QCPRECISE™ process, printfiles were generated using the QCPRECISE!™ software and the masks were printed onto the tissue mounted slides **(Figure 2).** The mask inhibits access of Proteinase K (PK) digestion. Following the PK formulation and protocol as described in the Allprep purification kit, PK buffer was added directly to the slide using a Grace Bio-labs Hybriwell placed directly over the ROI. Lysis was then performed by placing the slide into a humidity chamber and incubating at 56°C for 60 minutes. The crude lysate was recovered from the slide and RNA was purified following the Allprep protocol, quantified using Qubit, and RIN and DV200 values calculated by using the Bioanalyzer. RNA was prepped for sequencing using the Roche KAPA RNA HyperPer kit. Data was analyzed at Mayo Clinic and results were reported as discussed in this poster.

**Disclosures**
This study was partially funded by Mayo Clinic. BK, SM, JV, BJ, SS, CK and RP are full time employees of Mayo Clinic. JB,BC, BL, KK, DD are full-time employees of QuantumCyte Inc. QCPRECISE!™ is a trademark of QuantumCyte Inc.

## Figure 1: The QCPRECISE!™ platform.

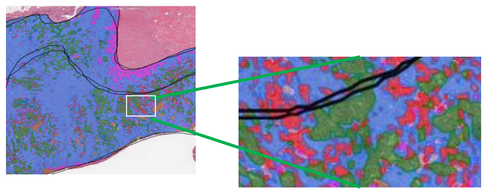



## Figure 2: The QCPRECISE!™ Platform workflow.




## Figure 3: (A) TB/PDC (Red) and non-budding carcinoma (Green) ROIs as identified by the QuantCRC[1] algorithm. (B) Pre- and (C)post-lysis images of spatially targeted micro dissection of clusters of tumor bud/poorly differentiated clusters (TB/PDC) using the QuantCRC[1] AI model with the QCPRECISE!™ platform.

Figure 3A



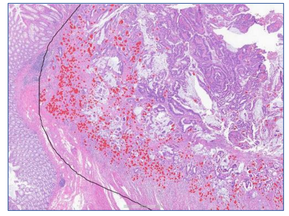


Figure 3B

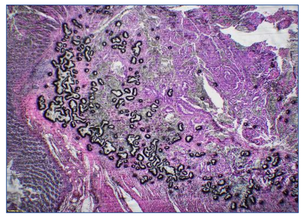


Figure 3C

## Results

RNA was isolated from all 4 ROIs from each tumor (16 total ROIs). Compared to the non-budding carcinoma ROIs, 431 genes (370 upregulated) were differentially expressed in the TB/PDC ROIs and 550 genes (545 upregulated) were differentially expressed in the immature stroma ROIs (**Figure 4A.** Heatmap of gene expression in one CRC). The top 10 upregulated genes in the TB/PDC ROIs were *APOE, COL6A2, LGALS1, BGN, COL1A2, COL3A1, SPARC, TIMP2, TAGLN,* and *VIM*. Many of the upregulated genes have been implicated in EMT. The top 10 upregulated genes in immature stroma ROIs were *FNDC1, C3, DES, CHRDL1, TNX2, CCL21, ANK2, SLIT3, STAB1,* and *PTGIS*. Pathway analysis (**Figure 4B**) revealed that cell migration and metastasis pathways were activated in TB/PDC ROIs compared to non-budding carcinoma areas.

## Figure 4: (4A) Heatmap of TB/PDC, carcinoma, immature stroma, and total tumor gene expression in one CRC, and (4B) TB/PDC pathway analysis.

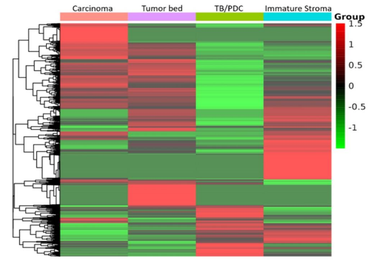



Figure 4A

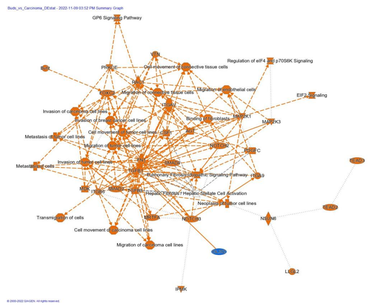


Figure 4B

## Conclusions

QuantumCyte's methodology allows for integration of spatial, molecular, and AI-derived quantitative pathologic data. In contrast to other technologies, this methodology allows for multi-omic spatial profiling at scale and is agnostic to downstream analysis method.

## Discussion

The QCPRECISE!™ platform is currently being integrated into standard clinical workflows. This study demonstrates the potential to use the platform to differentially isolate nucleic acid from TB/PDC and non-budding carcinoma from the same tumor tissue to evaluate cancer heterogeneity in a clinical setting at scale.

## References

1. Pai RK, Banerjee I, Shivji S, et al. Quantitative Pathologic Analysis of Digitized Images of Colorectal Carcinoma Improves Prediction of Recurrence-Free Survival. Gastroenterology. 2022;163(6):1531-1546.

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