Molecular Fingerprint™ for CDx

Spatial, multi-omic molecular maps for predictive analytics

Abstract | Single biomarkers are no longer sufficient to describe complex biological systems such as the tumor microenvironment or to predict response to targeted therapies. Advances in molecular analysis have led to the understanding that tumor growth is driven by a network of factors and that spatially resolved, multi-omic measurements are the key to understanding this complex network. The vast interdependencies between mutations, expression, and locale collectively create a **Molecular Fingerprint** for the biological system. Combined with artificial intelligence, assessing Molecular Fingerprints is the next frontier in discovery and diagnostics.

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Introduction

600,000 cancer patients die each year as their disease fails to respond to treatment. Billions of dollars are spent to care for and treat patients that will ultimately develop resistance to standard and targeted therapies, and billions more are spent by drug companies developing new treatments for cancer, such as targeted therapies and immuno-oncology regimens. Resistance and tumor escape are well documented in both targeted and immuno-oncology treatments and occurs due to tumor heterogeneity[1]. New tools are needed to help researchers and scientists discover the causes and implications of tumor heterogeneity. Diagnostic tools are needed to aid in clinical trial selection as well as to accurately profile heterogeneity or clonality in a patient's tumor specimen in order to precisely determine therapeutic treatments. Scientific research, drug discovery, translational medicine, and diagnostics have different needs and require different tools to address the fundamental problem of single and bulk cell analysis from tissue specimens routinely obtained for analysis.

Molecular Fingerprint (mPrint)

The Molecular Fingerprint platform spatially maps heterogeneity in tissues. It includes a novel sample prep method that allows any standard downstream molecular analysis to produce spatially resolved results. This enables spatial, multi-omic studies that are compatible with any commercially available kits.

Formalin-fixed (FFPE) or frozen-fixed tissues are bonded to a microfluidic cartridge where buffers are applied to the tissue while high resolution images are captured. Target cells/regions are digitally located, marked, and then physically removed from the tissue and transported to a collection plate for molecular analysis. A laser removes regions of interest (ROI) and the cells are transported via microfluidic away from the tissue and indexed. Indexing is managed such that each collection well is correlated with a location in the high-resolution image of the tissue so that molecular maps can be digitally reconstructed after downstream analysis. These maps are the multi-variable inputs required to train AI to make predictions about treatment options and acquired drug resistance in cancer patients.

Figure 1 Mechanism of acquired resistance due to clonal evolution after treatment, leading to treatment failure and relapse.

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Mapping heterogeneity for predictive analytics

Acquired and pre-existing resistance to targeted therapies inhibits successful personalized medicine. Most positive patient responses to these treatments are temporary, as resistance inevitably leads to disease progression or relapse[2]. The presence of sub-clonal populations with high heterogeneity is the leading cause of drug resistance and treatment failure[3,4].

While heterogeneity can be teased out bioinformatically, this process fails to convey the spatial context of the tissue microenvironment[5]. This context is crucial as the distribution of immune cells, tumor mutational burden, and expression profiles collectively create the complex biomarker network of a cancer[6].

The mPrint platform can visualize this complex network across the entire tissue or selectively from morphologically determined Regions of Interest (ROI), shown in **Figure 2**.

Beyond ROI's, heterogeneity can be mapped across the entire tissue in a process called 'tiling' (**Figure 3**) A tissue mounted on a standard pathology slide is imaged and then physically separated into small 'tiles' that are indexed and transported to a microplate. Samples can be analyzed with any commercially available method (PCR, sequencing, mass spec, etc) and the results correlated back to the original imagery to make a spatially resolved heat map. Because the raw tissue material from each 'tile' is transported to a microwell on a plate, multi-omic studies are feasible in order to create spatial maps of RNA, DNA, protein, etc.

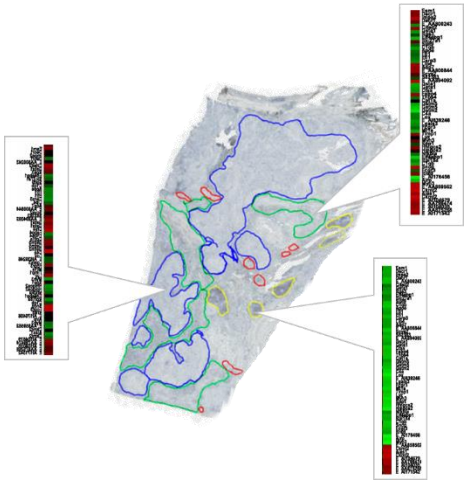

Figure 2 Morphologically distinct regions of interest in lung cancer identified by a Pathologist that are then isolated and analyzed by PCR. mPrint software supports custom annotations, automated collection of annotated regions, and visualization of molecular results through an online portal.

Performing analysis on small 'tiles' increases the equivalent purity of each sample and reveals heterogeneity that would otherwise

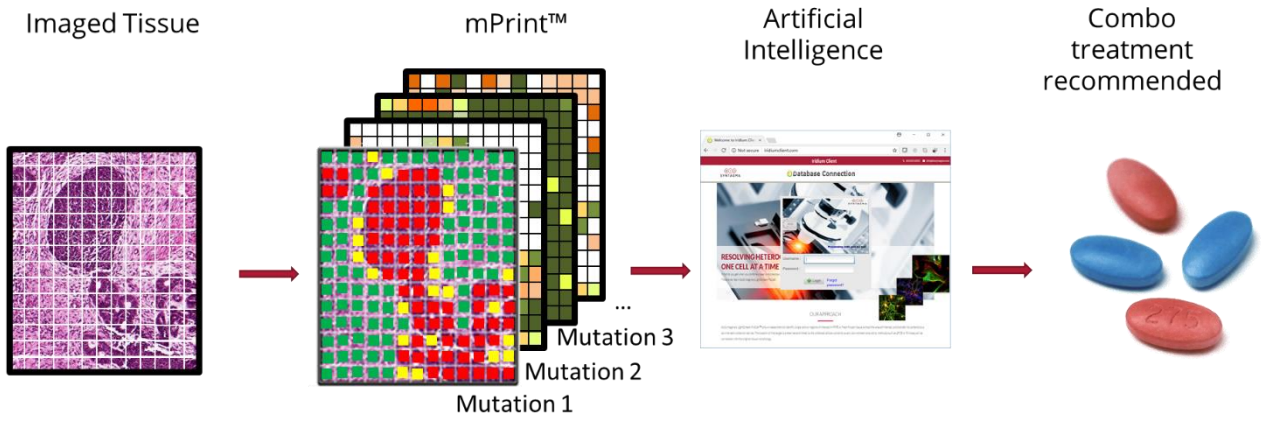

Figure 3 Using AI to mine comprehensive molecular maps to guide treatment for cancer patients. The mPrint first images tissue and then physically segments the tissue into "tiles" (FAR LEFT). Then each tile is sent for an independent molecular analysis and the results are digitally reconstructed into a map (LEFT). This multiplexed, spatial map is used for predictive analytics (RIGHT) to screen patients for CDx and combo treatment recommendations preventing acquired drug resistance.

be missed due to dilution when using standard methods. Datasets of these multiplexed, multi-omic maps can be classified by a trained artificial intelligence algorithm to code patients as likely responders or non-responders to targeted therapies. For those that are unlikely to respond, the algorithm can predict combo treatments based on the clonal cell populations identified within the tumor correlated to spatial markers.

Visualizing Gene Expression in Colorectal Cancer

IHC provides in-situ molecular information but is limited in multiplexing capabilities and by the availability of antibodies. In **Figure 4**, the mPrint platform produced a spatial RNA expression map for a metastatic colorectal cancer patient. Morphologically distinct regions were identified by Pathologist and were individually isolated for qPCR.

ROI's were collected from serosa, stroma, interphase, and cancerous regions along strips spanning the tumor cross-section (core to outside). Preliminary results in CRC and Melanoma both showed gradients in PD-1 expression, such as can be found by IHC, but extended gene panels illustrated other non-obvious trends.

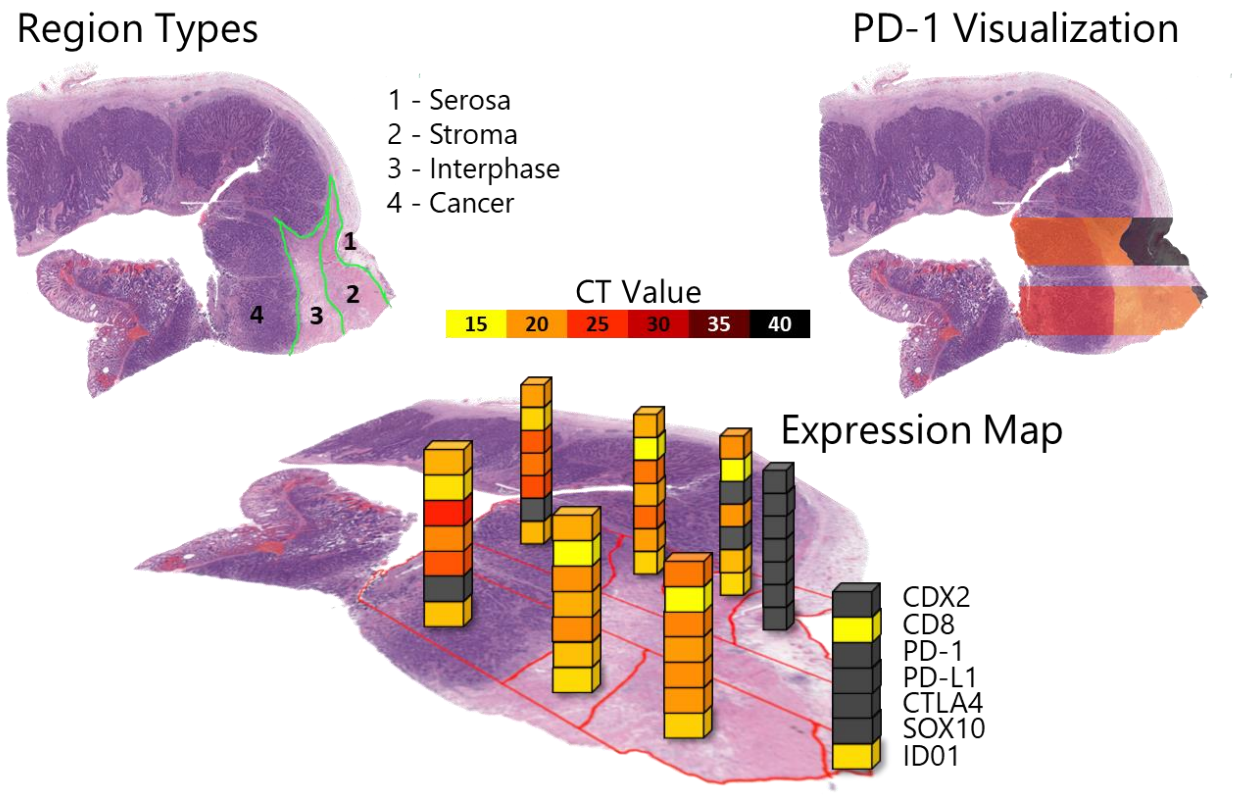

Figure 4 Gene expression measured by qPCR in metastatic colorectal cancer. Morphologically distinct regions were annotated by Pathologist (TOP LEFT) and then collected on the mPrint platform. A 2-D heatmap of PD-1 expression in different regions shows an expression gradient in the tumor (TOP RIGHT). Multiplexed results are shown over each region by a panel of 7 genes (BOTTOM)

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Spatial Analysis Uncovers Hidden Immune Response

Heterogeneity and low tumor purity have been shown to bias molecular analysis[7]. In-situ technologies are necessary to compensate for this bias and reveal the accurate status of the tumor microenvironment. Here, the mPrint was used to measure an immune response that was masked due the pitfalls of standard methods.

Targeted RNA-seq was performed on human breast ductal carcinoma using Illumina's AmpliSeq Immune Response Panel. Tissue sections from the same patient were sequenced with a portion of the sections going through standard processing techniques (scraping of the glass slide) and a portion going through the mPrint method. The mPrint tissues were annotated to distinguish cancerous and healthy ROI's, then each type was collected and enriched such that there was a collection tube for cancerous cells, healthy cells, and a mixed control tube (the scrape).

Sequencing results (**Figure 5**) show overexpression of several genes in enriched cancerous ROI's and low expression from corresponding healthy cells from the same tissue. However, scraped tissues that included both cancer and healthy cells showed similar expression as the healthy samples. The immune response was masked due to heterogeneity and dilution.

This tissue had highly complex ROI's and high tumor purity with approximately 70% cancer content. Therefore, traditional macrodissection techniques would not have been able to reproduce this work. And because of high tumor purity, a reference laboratory would have likely hand scraped this tissue without macrodissection, which would have produced skewed results.

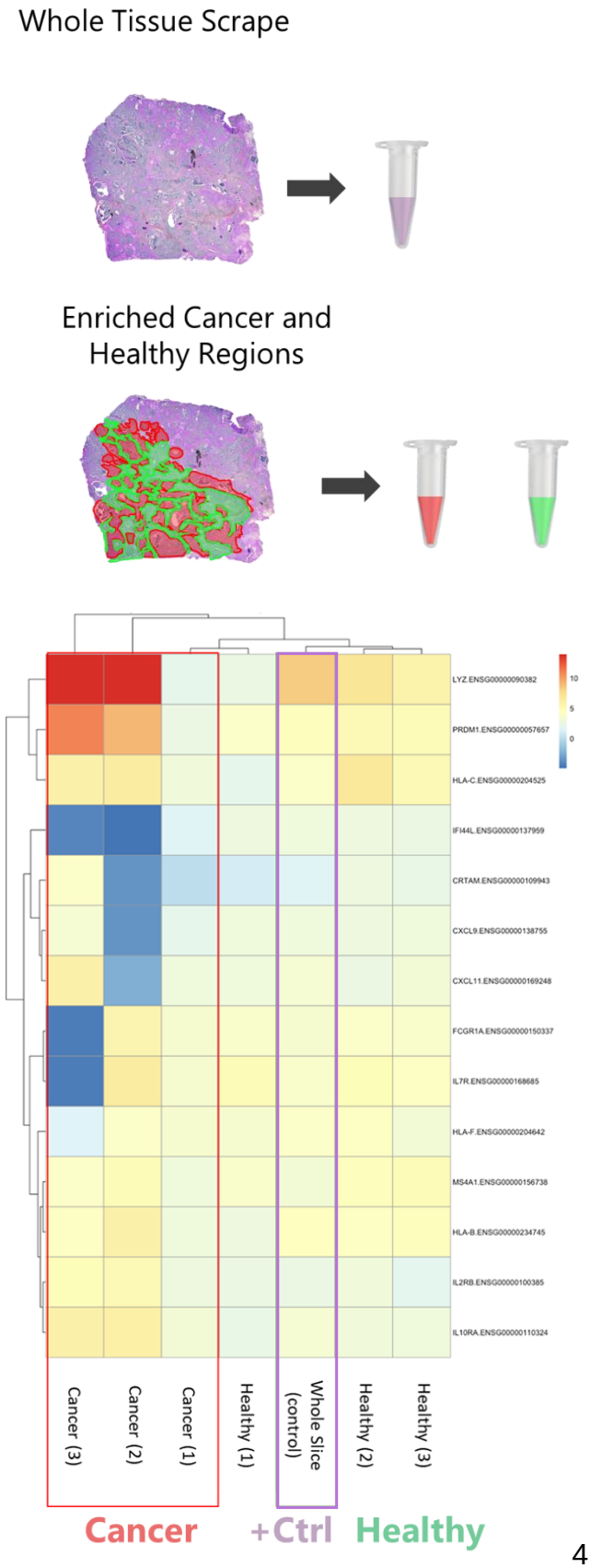

Figure 5 Targeted RNA-seq from breast cancer tissues collected via scraping (TOP) and enrichment of cancer and healthy ROI's (MIDDLE). Seq results show overexpression in cancer ROI's which is masked in scraped tissues which appear incorrectly similar to healthy tissues (BOTTOM)

Resolving Heterogeneity Enables Discovery

As the importance of the immune response is increasingly recognized for developments in immuno-oncology, sequencing is a crucial discovery tool. However, as demonstrated in **Figure 5** above, heterogeneity and a lack of spatial resolution skews seq results. Here, total RNA-seq was performed using Illumina's Whole Transcriptome on breast ductal carcinoma samples collected using the mPrint. Cancerous and healthy ROI's were collected from the same tissue sections and compared to total tissue scrapes from the same patient.

The results in **Figure 6** show groupings of alternating over and under-expressing genes when comparing cancer and healthy regions. However, the scraped tissues appear to show expression along the lines of the "sum of the parts" in terms of numbers of reads since the scrapes include both cancerous and healthy tissues. Trends in gene expression in the tumor microenvironment are hidden by traditional sequencing methods but elucidated by spatial sample prep prior to analysis.

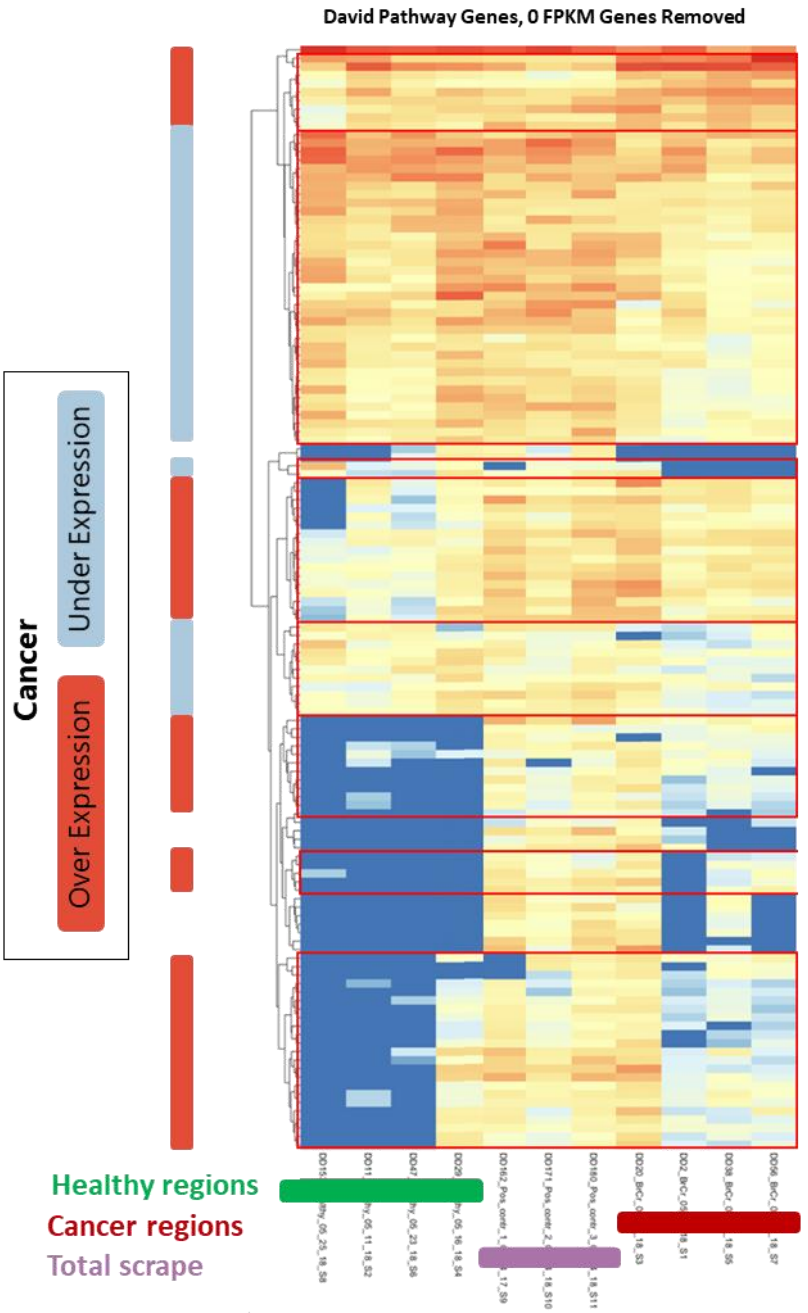

Figure 6 Total RNA-seq results collected from cancerous ROI's, healthy ROI's, and total tissue scrapes. Heterogeneity is clearly observed between cancerous and healthy tissues while total scrapes show total reads while overlooking expression trends in the tumor microenvironment

Building Artificial Intelligence to Screen Patients Using Molecular Maps

Molecular maps correlating morphology and multi-omic measurements in-situ are crucial in quantifying the tumor microenvironment. However, this data is massively multiplexed with non-obvious complex spatial variables making mining it a challenge for traditional informatics methods. For this reason, artificial intelligence is being employed to turn these molecular maps into training data sets that will enable a classifier to make recommendations for patients.

bioSyntagma has developed an AI architecture that is capable of correlating multi-omic and spatial data sets with patient outcomes in order to screen patients into likely groups of therapeutic responders and non-responders (**Figure 7**). While this is initially useful as a CDx for immuno-oncology treatments, the architecture is scalable such that it can be applied to recommend combination treatments based on a molecular map. In this way, clonal populations, the immune response, and genetic predispositions can be incorporated into a single test that could overcome the inevitable acquired drug resistance inherent with targeted therapies.

Spatial, multi-omic maps provide key insights into the tumor microenvironment that, when coupled with early detection, could enable predictive analytics to finally implement personalized medicine and end trial-and-error-treatments.

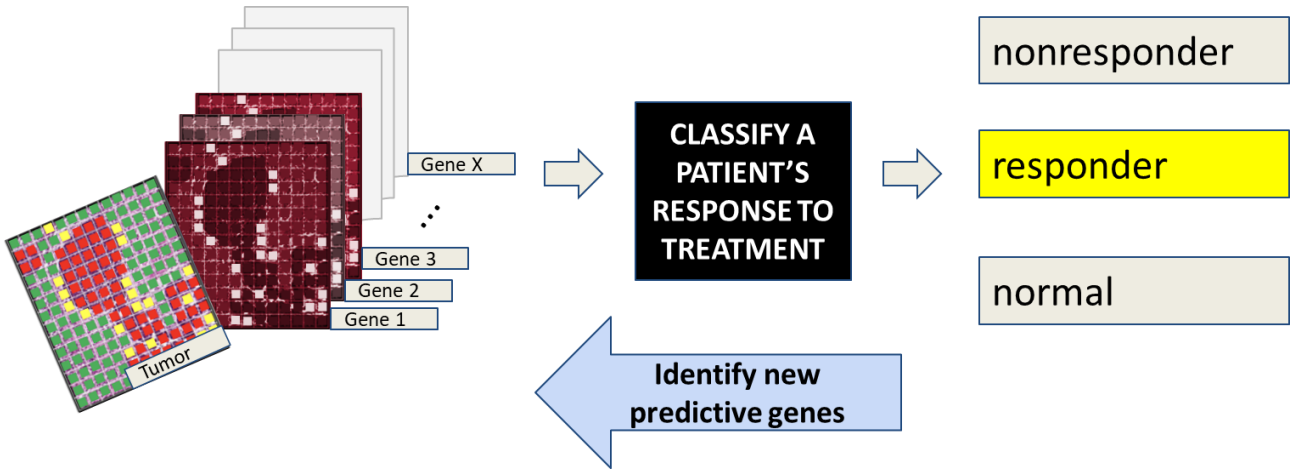

Figure 7 Classifying patients into groups of responder/non-responders using an artificial intelligence classifier. Molecular maps produced by the mPrint platform are fed into the classifier where spatial, multi-omic maps characterize the tumor microenvironment and segment patients into groups.

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Learn More

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