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Spatial Omics: Ready for Clinical Practice?

Authors: **Max Jorgensen[1], Emma Gardecki[2,3], Jochen K. Lennerz[3], Surya D. Nagaraja[3]**
Reviewer: **Lauren Ritterhouse, MD PhD[3]**

Clinical sequencing technologies have seen a massive growth; however, technologies able to collect morphological and spatial data on these samples is still largely emerging, with limited traction in clinical settings to-date. Examples of technologies able to accomplish this include spatial transcriptomics, spatial profiling, and hyper-plexed proteomics approaches in research settings. Here we present the results of a technology assessment including a questionnaire that focused on the analyte, technology, vendor, number of biomarkers a system can identify on a single tissue sample, resolution in µm, sample type, compatibility with formalin-fixed paraffin embedded tissue samples and/or fresh frozen, and whether a separate instrument is required or can be accomplished with existing laboratory equipment. The results are listed and portrayed against traditional light microscopy, and we included an idealized prototype workflow for spatial omics in clinical practice.

Introduction

The advent of high-throughput sequencing (HTS) has changed the field of diagnostics in the last decade. Particularly in the field of cancer diagnostics, HTS has allowed for interrogation of the mutational status of many genes simultaneously from a single specimen, allowing for clinicians to determine the genomic identity of a tumor. The integration of HTS into clinical diagnostics has allowed for subgrouping of tumors in recent years, guiding both diagnosis and therapeutics.

The advent of HTS technologies includes the fast and simultaneous interrogation of many targets in one run. In oncology, genotyping has become an integral part of therapeutic decision-making. Given the increasing clinical demand for a growing number of molecular and mutational findings, the field of HTS has grown to a sizeable segment of the diagnostic market.

Despite progress in HTS, and in addition to regulatory and reimbursement issues, several technological factors currently limit the scope of clinical-scale sequencing: (1) *Limited input material*. Most clinical sequencing assays require a minimum of millimeter-scale tissue samples*. Many initial samples for the diagnosis of cancer are small, minimally invasive biopsies. These small pieces of tissue pose unique challenges in obtaining sufficient tumor DNA for genotyping; (2) *Tumor purity*. Tumor tissue can contain numerous other non-tumor components resulting in different ratios of tumor-to-normal cells. From the perspective of obtaining genetic information of the tumor, this pre-analytical variable represents an impurity. (3) *Loss of spatial information*. While this may not be of large concern in the clinic today, increased precision will likely become warranted as cancer treatment regimens become more personalized and tailored to the unique proteogenomic characteristics of a tumor. Clinical HTS also does not provide any information towards grade or stage in a morphological context, which still relies on traditional microscopy. Altogether, while HTS gives massive throughput, it is limited in resolution when considered alone and relies upon additional spatial diagnostic methods to come to a diagnosis.

While sequencing capabilities have seen large growth and innovation, the technologies used to collect morphological and spatial data on these samples remained relatively stagnant by comparison. Until just a few years ago, researchers had been limited by imaging plex capabilities on available commercialized platforms. While single-plex technologies, like IHC or ISH, are commonly used to resolve an individual protein or nucleic acid marker spatially at high resolution, multiplex offerings were handicapped by limitations in quantitation, inconsistent interpretation, low dynamic range, and required labor-intensive workflows. While clinicians have not yet had a

[1] DeciBio Consulting, Los Angeles, California, USA; [2] Smith College, Northhampton, Massachusetts, USA; [3] Center for Integrated Diagnostics, Department of Pathology, Massachusetts General Hospital/Harvard Medical School, Boston, Massachusetts, USA; Corresponding author: J. Lennerz 55 Fruit Street, Boston, MA, USA. JLennerz@partners.org

need for multiplex technologies as they have not yet been shown to be clinically relevant, investigators in the basic research, translational, and discovery settings have historically been left struggling to implement complex multiplexing workflows which can take years to master[†].

Over time, though, inventors worked to address the unmet need in the basic research, translational, and discovery laboratories of seeking to combine the high resolution of microscopy with the throughput capabilities of HTS to better understand tissue morphology through high-plex approaches. The market segment within tissue analysis in which these technologies are addressed has come to be known as Spatial Omics. Examples of these technologies and platforms include spatial transcriptomics (e.g., 10x Genomics Visium), spatial profiling (e.g., NanoString Technologies GeoMx), and hyper-plexed proteomics approaches (e.g., Akoya Biosciences CODEX). To date, the adoption of these technologies has been driven by the performance of immunotherapies and biopharma companies' belief that these high-plex technologies can help fuel their R&D engines to help develop the next generation of therapies.

The rapid expansion of this market has attracted significant investment activity, with major life science research tools companies like 10x Genomics and Bruker investing heavily in the space[‡]. Only a handful of technologies, though, are fully commercialized. The state-of-the-art methods today are impressive but simultaneously technically challenging. On paper, many of these techniques achieve the same goals—highlighting dozens, hundreds, or even thousands of markers for researchers in a tissue sample. This has thus far made it difficult for researchers to determine which of these (sometimes costly) platforms is best for their research. Further complicating an instrument acquisition is the amount of readily available performance specifications to be able to properly compare the platforms and techniques. No resources have previously been available for researchers to compare these platforms on parameters like cost or plex. Here, we've aggregated data for the commercialized approaches to highlight the spectrum of capabilities. Of note, we recognize that other important factors may be at play when labs select the most appropriate platform for their needs (e.g., sample throughput, clinical utility), especially in clinical settings.

Approach. The primary aim of this project was to create a resource that describes the relationship of plexity, resolution, and cost. While each of these are important metrics to the overall positioning of technologies in this field, they are not the only ones nor agreed upon as the most important. Whole-slide capabilities and throughput, for example, are both important considerations for many researchers and are not included in our analysis.

The authors met and developed a questionnaire to collect relevant information about technology specifications. The questionnaire focused on the analyte, technology, name (e.g., company, location, state and/or country), number of biomarkers a system can identify on a single tissue sample (defined here as plex), resolution in μm (as stated by the company), sample type and compatibility with formalin-fixed paraffin embedded tissue samples and/or fresh frozen (FF), and whether a separate instrument is required or can be accomplished with existing laboratory equipment (e.g., next-generation sequencer). The total cost per run (the combined cost of all consumables and required costs) for a maximum-plex run was divided by the plex and number of samples to achieve an estimate of cost per analyte per sample.

One of the authors (EG) collected this information by contacting companies and laboratories in the field through website reviews and product interviews during the summer of 2021. Each company was contacted through a general representative and was able to pitch their technologies to our author. Website and publication reviews yielded additional and/or missing information. Importantly, the final compiled data was sent to be reviewed by various industry contacts by another one of the authors (MJ).

Results. Most commercialized systems, when compared across factors such as plex, cost, and resolution fall within a narrow grouping. Plex and resolution, two of the most impactful attributes of these platforms, have long existed as opposite forces—researchers could achieve high resolution at low plex, and vice versa. While this tradeoff remains (Fig. 1A) with the platforms capable of the highest plex only offering multi-cellular resolution,

Analyte	Technology	Company	City	State, Country	Plex	Resolution (μm)	Cost	Sample Type	Type
RNA									
	In Situ by 10x	10x Genomics	Pleasanton	CA, USA	> 1,000	Subcellular		FF, FFPE	In Situ
	MERSCOPE	Vizgen	Cambridge	MA, USA	500	0.1	$250K	FF, Fixed Frozen	In Situ
	Pisces	Veranome	Mountain View	CA, USA		Single cell			In Situ
	Rebus Esper	Rebus Biosystems	Santa Clara	CA, USA	30	0.26		FF	In Situ
	SMI	NanoString	Seattle	WA, USA	> 1,000	Single cell		FF, FFPE, organoid	In Situ
	Visium Gene Expression	10x Genomics	Pleasanton	CA, USA	Unbiased	55		FF, FFPE	Oligo Capture
	Visium HD	10x Genomics	Pleasanton	CA, USA	Unbiased	5		FF, FFPE	Oligo Capture
Protein									
	Cell DIVE	Leica Microsystems	Wetzlar	Germany	100	0.3225	$450K	FFPE, TMA	Antibody-based
	ChipCytometry	Canopy Biosciences	St. Louis	MO, USA	200	0.5	$286K	FF, FFPE, cell suspension	Antibody-based
	CODEX	Akoya Biosciences	Marlborough	MA, USA	40	0.2	$85K	FFPE	Antibody-based
	COMET	Lunaphore Technologies	Tolochenaz	Switzerland	40	0.23	$450K	FF, FFPE	Antibody-based
	MACSima	Milteyi Biotec	Auburn	CA, USA	187	42	$825K	FF, FFPE, PFA	Antibody-based
	Orion	RareCyte	Seattle	WA, USA	21	0.2	$550K		Antibody-based
	Phenoptics	Akoya Biosciences	Marlborough	MA, USA	8	0.2	$350K	FF, FFPE	Antibody-based
	Visium Protein	10x Genomics	Pleasanton	CA, USA	10			FF, FFPE	Antibody-based
	Hyperion	Fluidigm	South San Francisco	CA, USA	37	1	$900K	FF, FFPE, cell smear	Antibody-based
	MIBIscope	Ionpath	Menlo Park	CA, USA	40	0.28	$900K	FFPE	Antibody-based
Multi-Analyte									
	GeoMx DSP	NanoString	Seattle	WA, USA	> 1,000	10	$295K	FF, FFPE, TMA	Antibody-based

Table 1. Specifications on spatial omics technologies, by analyte type. Blank spaces represent data that was not available at time of publication. Cost represents the total cost of instrument. Abbreviations: FF, fresh frozen; FFPE, formalin fixed paraffin embedded; TMA, tissue microarray; PFA, paraformaldehyde. Note: While Akoya Biosciences' Phenoptics platform is listed under proteins, it is compatible with ACD's RNAscope assay to allow for spatially resolving RNA transcripts. Data was collected during the summer of 2021 and may not be reflective of the most recent product announcements.

some platforms have been pushing the boundaries of this dichotomy. Vizgen's MERSCOPE is a notable example, and the only platform located in the optimal research quadrant of Fig. 1A when considering plex and resolution. Behind MERSCOPE, Canopy Biosciences' ChipCytometry and Leica Biosystems' Cell DIVE technologies fall just outside of this quadrant and are the closest proteomics platforms to it. We expect that, as methods currently limited to research contexts continue to be developed, commercial technologies will increase in their plex and resolution as cost per analyte reduces. Just in the last few years, several research methods have been published that allow for interrogation of thousands of genes at near cellular resolution. Many such methods require custom-made bead arrays or complex imagining instrumentation that are limited to expert laboratories. When considering the optimal clinical corner of Fig. 1A, Akoya Biosciences' Phenoptics platform is the closest of a very tight group of proteomics platforms. It is only a matter of time until these methods enter the clinical sphere—likely, at a lower plex—as there are multiple ongoing clinical trials looking into the use of multiplex diagnostics for treating cancer[8].

When considering the cost per analyte per sample, which aims to measure the cost efficiency of operating the instrument, and plex, more platforms shift towards the optimal research quadrant, shown in Fig. 1B. These include 10x Genomics' Visium, NanoString Technologies' GeoMx DSP, and Vizgen's MERSCOPE, with Canopy Biosciences' ChipCytometry falling just outside this quadrant. The optimal region for clinical applications, by comparison, includes a number of different proteomics platforms, with Akoya Biosciences' Phenoptics platform again being closest to the optimal corner. An important aspect to consider with the operation of these

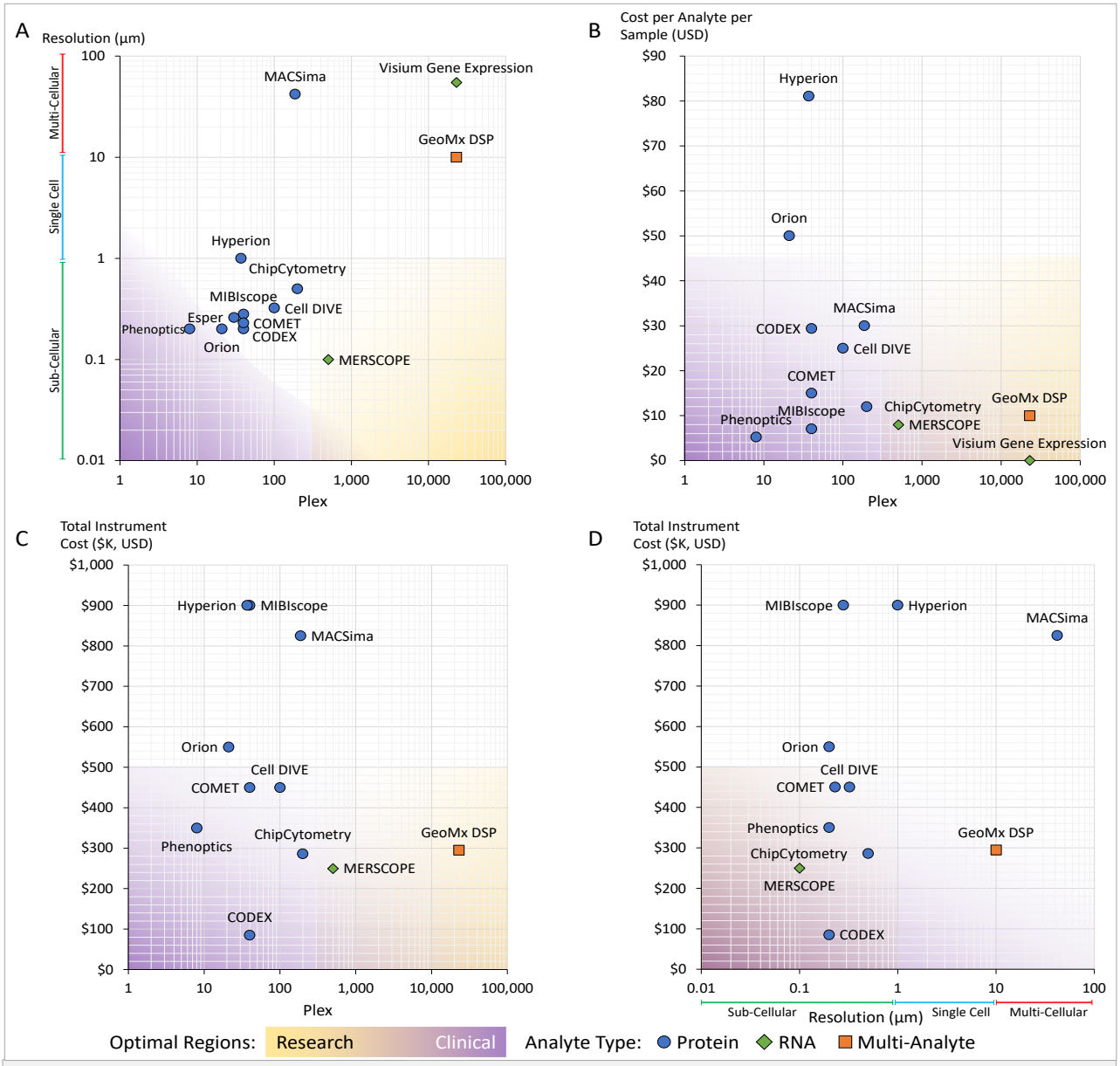

Figure 1. Plots detailing specifications of each technology with the optimal corners for research and clinical purposes are in yellow and purple, respectively. Notes: GeoMx DSP's maximum resolution of 10 µm is shown, but protocols using an ROI resolution of 50 µm are recommended; GeoMx DSP's cost per analyte per sample reflects the cost of protein targets, cost of RNA targets is in-line with the cost of Visium; resolution scale (sub-cellular, single cell, and multi-cellular) is approximate; Visium and the GeoMx DSP are capable of detecting the whole transcriptome, and plex is listed as 23,000 to reflect this capability in the plots.

instruments is the detection method used, as many technologies have historically relied on antibodies. Sequencing-based methods, like Visium, can be operated at a significantly lower cost per RNA target than antibody-based methods. Nowhere is this more prominent than when comparing the cost of detecting proteins with the GeoMx DSP (shown Fig. 1B) and the cost of detecting RNA transcripts (not shown), with the latter being approximately 1% of the cost of the former per target.

When observing the relationship to total instrument cost, increased total instrument cost loosely positively correlates with increased plex (Fig. 1C). Today, however, only MERSCOPE and the

GeoMx DSP are located in the optimal research quadrant. Looking to the clinical region, Akoya Biosciences' CODEX now appears alongside the Phenoptics platforms in the optimal shaded area in addition to other proteomics approaches. Interestingly, when considering total instrument cost and resolution, the two factors appear to negatively correlate (Fig. 1D). This finding was unexpected and goes against expectations but is likely not a causal relationship.

While significant advancements have been made in the past few years to increase plex capabilities, Fig. 1 illustrates that spatial omics technologies are not entirely optimized with only one platform (MERSCOPE) found in all four optimal research quadrants and one vendor routinely coming close to the optimal clinical regional (Akoya Biosciences). The field is looking for high-resolution low-cost instruments, but instead labs are having to choose between their budgets or their research capabilities. Further, the tradeoff between resolution and plex remains, as the highest-plex offers comparatively limited resolution when contrasted with other available platforms and platforms expected to launch soon. Additionally, some metrics which are not included in this analysis, like whole-slide capabilities or throughput, could place additional platforms in the optimal research and clinical regions or remove the previously mentioned platforms from them. A researcher who does not require whole-slide imaging capabilities may, for example, determine the region of interest-based NanoString GeoMx to be sufficient or optimal for their applications, while others may consider the regional approach limiting and prefer imaging entire sections of tissue instead. Reflecting on HTS, instrument and running costs have decreased significantly over the past decade, and as the spatial omics market matures, we anticipate dramatic drops in costs.

Despite the previously mentioned advancements, spatial omics is not ready for wide-scale clinical implementation. Many commercial spatial diagnostic platforms come with considerable overhead, both in instrument cost as well as cost per assay. Importantly, the information gained by spatial omics technologies is not yet clinically actionable due to a lack of high-powered analysis offerings. This drives the need for further understanding of how spatial relationships play a role in basic tumor biology. Technologies have only recently reached the resolution and throughput to allow for interrogation of this biology in the research space. As further work is done understanding how tumor cells relate to one another and non-malignant cells in three-dimensions, we will gain the ability to integrate spatial omics into clinical diagnostic algorithms. We expect spatial omics to follow in the paths of HTS and other modern molecular methods—automated analysis platforms for digital pathology have already paved the way for generating high-impact insights from similar data.

Let us consider what a spatial omics workflow may look like in the context of cancer diagnostics in the future if proved clinically relevant, limiting ourselves to RNA sequencing at this time (though multi-omic methods are certainly on the horizon). Of note, clinical relevancy in this case will require both proven utility of both multiplex spatial methods and transcriptomic biomarkers, as the vast majority of biomarkers today are protein-based. The goal from such an approach would be to perform comprehensive diagnostics, traditional histological analysis to genome-scale molecular characterization, from two slides. A tumor specimen from the operating room may be freshly frozen and sectioned (Fig. 2A), as is done in many clinical settings currently. One slide can be used for standard histology and a preliminary diagnosis can be ascertained in minutes. A second slide would then be submitted for spatial sequencing, where sequencing reads may be paired to specific coordinates on the slide, assisted by the first frozen histological specimen. As spatial sequencing methods increase their capture rate, the data generated from RNA sequencing can be used to computationally recreate many of the standard diagnostic methods used today (Fig. 2B). Immunohistochemistry can be simulated by plotting the expression values for genes and

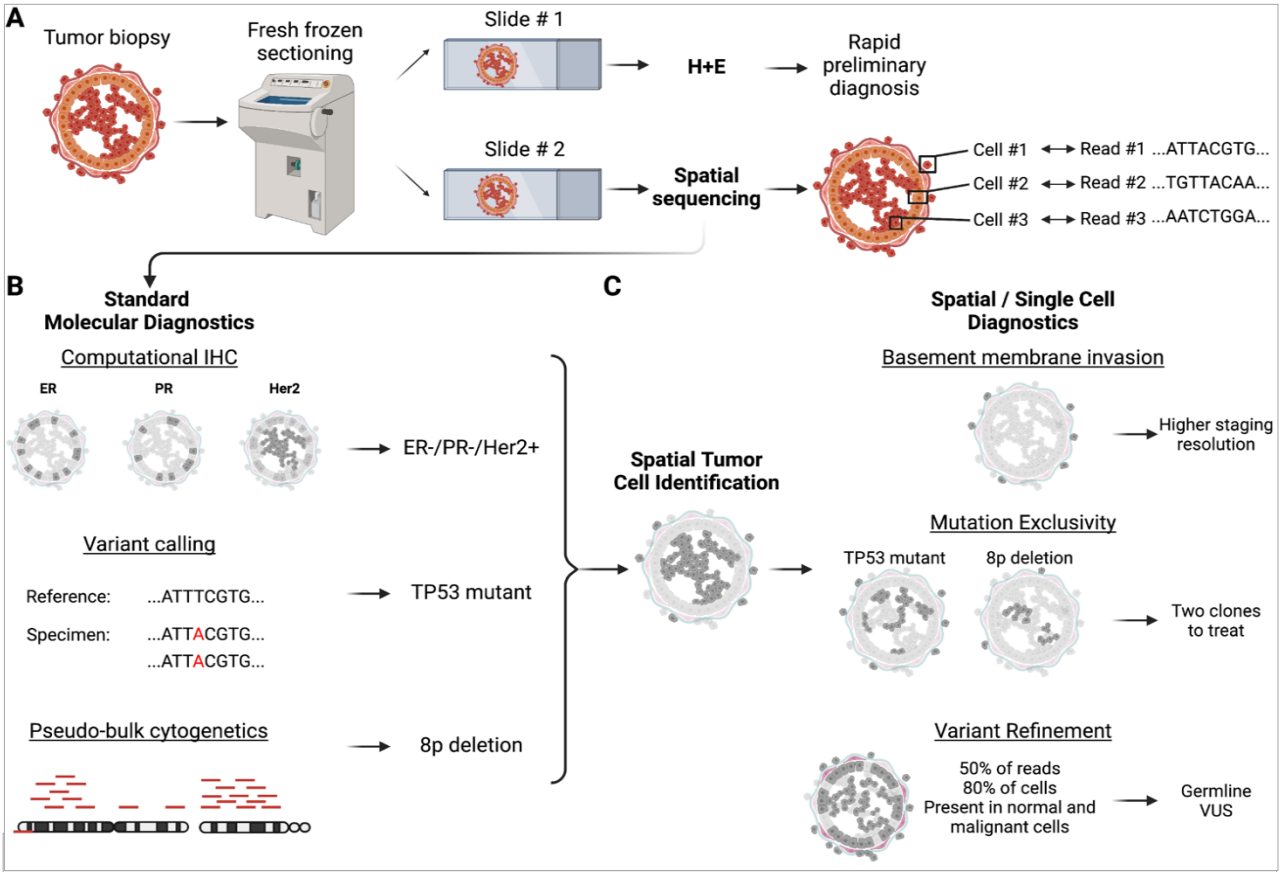

Figure 2. Workflow detailing a future idealized spatial omics workflow for use in the clinic. (A) Initial standard processing methods for a tumor specimen, submitting one for rapid diagnosis and a second for spatial omics. (B) Existing molecular diagnostic data can be obtained from spatial sequencing. (C) Unambiguous tumor cell identification allows for novel diagnostic methods.

markers of interest in the suspected tumor type. The clear advantage here over standard IHC is that the single slide would essentially generate an entire transcriptome's worth of data and IHC could be performed for any marker in the genome. This becomes particularly relevant in the setting of tumors of unknown primary. Similarly, the spatial information associated with the HTS data can simply be ignored and the data can be "pseudo-bulked." The sequencing data could be interpreted through standard informatics pipelines to allow for variant calling and a focused report of relevant hits would be produced. Cytogenetics and structural variant calling could also be performed from this data. However, once the mutational profile of the tumor is identified, individual tumor cells may be identified spatially by mapping mutant reads in space (Figure 2C) and the door opens for novel and higher resolution diagnostics. This would allow unambiguous identification of invading tumor cells, providing substantial support to existing microscopy-based tumor staging methods. Furthermore, assigning mutations to tumor cells in space would determine whether every cell shares the same mutational profile. Identifying clonal populations of tumor cells, each with a distinct pattern of mutations, will prove to be crucial to personalized therapies. For instance, consider a glioblastoma patient with *EGFR* and *PDGFRA* amplifications identified by HTS. It can only be determined through methods with cellular resolution whether these mutations are present in the same cells; should it prove that each tumor cell contains either an EGFR or PDGFRA amplification, but never both, treating for only one target will prove

insufficient. Finally, the interpretation of variants would be clarified, as variants that roughly appear in 50% of reads and spatially map to normal cells would definitively demonstrate a germline origin and considered as noncontributory to tumor biology.

Conclusion. While the notion of "genome-wide IHC from a single slide" may seem expensive and of questionable clinical utility, it is important to consider that the rapidly dropping cost of sequencing will have the expense of these methods soon converge with that of standard diagnostic methods. Soon, performing a dozen IHC stains, a targeted panel of next generation sequencing, and standard cytogenetics may be more expensive than a single slide run of spatial omics platform.

What are the barriers to spatial sequencing being widely used in the clinic? Many of these methods currently require complex instrumentation, relying on microfluidic devices, custom synthesized bead arrays, and high-resolution fluorescent imaging. Perhaps even more daunting are the analytical requirements. Current spatial sequencing pipelines are non-trivial and not standardized, partially because the yield on many spatial sequencing methods is lower than that of standard HTS methods. When these reads are then distributed across spatial coordinates, the data becomes sparse, such that sequencing reads for certain genes only appear at rare coordinates in the image. Overcoming this limitation requires smoothening of data, both in physical space and higher dimensional space.

Yet the advantages of integrating spatial sequencing into diagnostics are clear. Most institutions currently limit their HTS methods to a panel of clinically actionable genes. This becomes an issue when considering tumors of unknown primary source. A genome-wide assay of gene expression would allow for molecular characterization of a tumor without any initial assumption of its classification. As is currently being developed with DNA methylation data in brain tumors, large scale generation of spatial omics data could allow for publicly available classifiers and the notion of "unknown primary" may cease to exist. Additionally, the key diagnostic consideration of tumor invasion—in staging, guiding therapy, determining the efficacy of resection—would benefit substantially from spatial sequencing. For example, if an excised lymph node is found to have lymphoma, the extent of invasion is determined by searching for cells with abnormal morphology extending past the node's capsule. By combining the current microscopy-based diagnostics with spatial sequencing, these invasive cells could be identified more precisely and quantitatively. As spatial sequencing would provide the molecular profile of these invading cells, it may even become possible to use this profile to predict and track the tumor clones responsible for recurrence and metastases.

Overall, spatial omics is a rapidly developing field with many applications. As we consider how to best integrate these new methodologies into the clinical practice, more clinically focused products are being launched (e.g., Akoya Biosciences' Phenoptics portfolio). It is important to identify concrete clinical, value-added applications to identify the best entry point.

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