Spatial Transcriptomics Inferred from Pathology Whole-Slide Images Links Tumor Heterogeneity to Survival in Breast and Lung Cancer

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Abstract

Digital analysis of pathology whole-slide images is fast becoming a game changer in cancer diagnosis and treatment. Specifically, deep learning methods have shown great potential to support pathology analysis, with recent studies identifying molecular traits that were not previously recognized on pathology H&E whole-slide images. Simultaneous to these developments, it is becoming increasingly evident that tumor heterogeneity is an important determinant of cancer prognosis and susceptibility to treatment, and should therefore play a role in the evolving practices of matching treatment protocols to patients. State of the art diagnostic procedures, however, do not provide scalable methods for characterizing and/or quantifying tumor heterogeneity, certainly not in a spatial context. In this paper, we present a scalable approach that accurately and automatically spatially resolves mRNA and miRNA expression levels on pathology whole-slide images. This is the first demonstration of this type of inference from H&E images. We use this method to produce tumor molecular cartographies and to characterize certain aspects of tumor spatial transcriptomics. Specifically, we develop a heterogeneity index (HTI), derived from the molecular cartographies. Applying our methods to breast and lung cancer slides, we show a significant statistical link between heterogeneity and survival. Our results highlight the value of automated analysis of pathology whole slide images. Our methods potentially open a new approach to investigating tumor heterogeneity and other spatial molecular properties and their link to clinical characteristics, including treatment susceptibility and survival.

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1 Introduction

Digital pathology – the automated computer-vision analysis of pathology whole-slide images (WSIs) – is fast becoming a game changer in cancer diagnosis and treatment. Deep learning methods have been studied extensively in this context, and were recently shown to be efficient for certain tasks, such as detecting metastases [1, 2], immune cells [3, 4] and cancer type [5]. In a recent study [6] used deep learning to study immune geospatial variability and how it may affect the emergence of aggressive clinical phenotypes. These achievements led researchers to more recently explore whether such methods could go a step further, and identify molecular traits that are not known to be associated with cell/tissue morphology, such as mutations [5] and cancer cell dependence on hormones [7].

Simultaneous to these technological advances, the importance of tumor heterogeneity is being increasingly recognizes as a major feature associated with resistance to treatments and as a determinant of prognosis [8, 9, 10, 11, 12]. Polyclonality and tumor subclones – sub-populations of tumor cells that differ in molecular characteristics such as mutations, copy number aberrations and gene expression profiles – are a hallmark of cancer and may affect treatment outcome and disease progression [13, 14, 15, 4, 16, 17]. There are currently three main approaches for characterizing tumor heterogeneity: using single-region bulk-measurements, offering high coverage but no spatial resolution; using multi-region bulk-measurements, somewhat increasing spatial resolution; and, more recently, using higher-resolution and higher-coverage techniques such as single-cell. Bulk measurement of tumor molecular properties has greatly developed in the last three decades [18, 19, 20]. The bulk approach, however, provides no spatial context. Recent work showed that clonal estimation from single-region sampling is less accurate than that obtained by multi-region sampling and that single-region clonal composition estimations vary greatly between methods [21]. When multiple regions are considered, important associations between heterogeneity and clinical characteristics arise. In a cohort of 41 gastric cancer patients, [9] discovered an association between heterogeneity and survival using a genome-wide single-nucleotide variation array to estimate the number of clones. This was followed by fluorescence in situ hybridization (FISH) to derive clonal locations for 3 samples across 4 target regions. [22] combined single cell RNA sequencing (scRNA-seq) with spatial transcriptomics to map and characterize the different cell populations in heterogeneous pancreatic tumors. In multiple myeloma, [23] inferred spatial organization from scRNA-seq data, using a clustering-based approach, to characterize immunological alterations occurring in the tumor microenvironment during disease progression. Others have used a computational approach to infer spatial position probabilities for individual cells from scRNA-seq data, enabling the spatial reconstruction of single-cell gene expression [24]. In [11], single-cell pathology subgroups were spatially resolved using mass cytometry imaging, covering an average of $2,246$ cells per image across 381 images, to characterize clonal populations in breast cancer. One of their findings associated a specific single-cell pathology subtype that comprised multiple epithelial cell communities with poor survival. In a similar setting, spatially-derived statistics from single-cell data were shown to improve prognostic predictions [10]. These findings emphasize the importance of characterizing tumor heterogeneity in a spatial context. However, multi-region and especially single-cell techniques are expensive and are still limited in cell coverage compared to WSIs. WSIs hold the potential to spatially resolve molecular measurements using complete cell coverage [6, 25]. They can therefore be leveraged to increase utility in a different dimension, and for a very small price.

In this paper, we present an automated and scalable method to both visualize and quantify tumor heterogeneity in the spatial context of WSIs and, using it, discover a significant link between tumor heterogeneity and survival outcome in breast and lung cancer. Briefly, we train deep neural networks to provide cartographies of mRNA and miRNA expression from WSIs. We then use these to produce a rich spatial representation of tumors (a tensor molecular cartography). Finally, we use the tensor cartographies to both quantify and visualize tumor heterogeneity within each slide. We then use the inferred heterogeneity levels to perform survival analysis. An overview of our method is shown in Figure 1.

Our main contributions are: (1) Models that map from WSIs to gene expression levels, reaching 0.8-0.94 AUC (area under the curve) on a held-out random test set for multiple genes in two independent cohorts; (2) A method for automatically generating molecular cartographies that can be further studied and inspected; (3) A novel method for quantifying tumor heterogeneity based on the spatial co-location of molecular traits, relevant also outside the realm of digital pathology; (4) A statistically significant link between tumor heterogeneity and survival outcome in two separate cancer cohorts.

Our results lead to three important conclusions: first, that, for some genes, it is possible to accurately and automatically spatially resolve gene expression on WSIs; second, that tumor heterogeneity can be derived both visually and quantitatively from WSIs; and third that there may be a significant link between tumor heterogeneity, as measured by our approach, and survival and that this link may differ in nature between tumor types. Our research further highlights the value of digital pathology and the importance of investigating the link between tumor heterogeneity and clinical outcome. Our code is available online (Methods 4.5).

2 Results

2.1 Training models to identify gene expression on pathology whole-slide images

An overview of our methods is shown in Figure 1. We begin by obtaining diagnostic hematoxylin and eosin (H&E) slides for TCGA BRCA and LUAD samples and matching mRNA and miRNA expression data (Methods 4.1). We use diagnostic (FFPE) slides rather than tissue (frozen) slides since the latter lack sufficient morphological detail for a complete pathological diagnosis [26]. Out of the diagnostic slides, we discard any damaged, heavily stained or annotated slides as they can cause the model to focus on artifacts, including clues from pathologists' markings. This results in 761 slides for breast and 469 for lung. We obtain matching normalized mRNA and miRNA expression data for a total of 10 molecular traits for breast and 5 for lung, as listed in Table 1. Breast mRNAs were chosen from the PAM50 genes [27] and lung mRNAs and all miRNAs (breast and lung) were based on the literature (see Methods 4.3 for further details).

Each cohort is processed, trained and evaluated separately (Figure 1 (a) and (b)). We begin by randomly assigning subjects into a held-out test set (10%). We then randomly split the remaining subjects into train (80%) and validation (10%) sets 5 times (bootstrap sampling) to obtain 5 different and randomly selected train-validation sets. We split on subjects rather than slides so that slides from the same subject are assigned to the same set to avoid similarities between test and train/validation. Importantly, the split is performed before any molecular trait is processed to ensure that all traits see the same split. We then assign each slide into one of the sets according to its subject's association and proceed to label the slides and prepare our training set.

Per trait, we label each slide based on its sample's expression percentile for that trait. We then split each slide into non-overlapping tiles of 512×512 at $\times 20$ zoom, resulting in hundreds to thousands of tiles per slide (depending on the slide's size). Following previous methods [5], each tile inherits its labels from its parent slide. We label each slide (and tiles) based on its percentile expression level: high (1) for those above median low (0) for those below. This raises the concern that not all tiles are representative of its slide label (e.g. a tile representing a healthy portion of a tumor sample), leading to noisy labeling. To potentially reduce noise, we set aside complete slides (all corresponding tiles) with expression levels between the 20th and 80th percentiles and use them for out-of-distribution evaluation. These samples do not participate in the model's train/validation/test sets. We proceed to use the labeled tiles to train our models.

We train each trait separately and repeat this process 5 times (once for each of the random train/validation splits of the non-test samples). We use the Inception v3 classifier [28], as in [5]. No transfer learning was used following previous work showing improved performance by fully training Inception v3 [5].

Once trained, we obtain the trait's validation performances for all 5 runs by measuring the slide-level AUC for each of the five models (we evaluate using slide-level AUC following, e.g., [5, 29]). To do so, we first transition from predictions on tiles to predictions on slides by computing the slide's percent positively classified tiles (as in [5]). We then compute the slide-level AUC for each model using these scores as the slide-level prediction. The final model for each of the 5 rounds uses the weights that had the best slide-level AUC on that round's validation set.

Besides providing a better evaluation of our models' performance, a key advantage of training multiple models on different train/validation splits is the option to combine them into an ensemble model [30]. We develop such an ensemble model for each trait by combining its top three models, as measured on their respective validation set, and taking their majority vote. For example, if the last three models from the 5 train/validation repeats yielded the best performances (each on their respective validation set) and their predictions for a given tile are: 0, 0, 1 then the ensemble will predict 0 for that tile by majority vote.

For each trait, we evaluate both its 5 models and its ensemble model on the trait's held-out test set (test slides with expression levels within the 0-20% and 80-100% percentiles for that trait) as well as on its OOD sets (slides within the 20-80% percentiles). Neither of these test sets participated in the trait's model development (train/validation) in any of the 5 rounds.

2.2 Achieving high prediction performance for identifying gene expression on pathology whole-slide images

We obtain high test performance rates across multiple traits. The number of slides in the test set varies across traits, depending on cohort (TCGA-LUAD is roughly half the size of TCGA-BRCA) and on label (expression) availability, and is therefore described per trait in brackets below. In Table 1, we observe that the average test performance measured across all 5 train/validation rounds (first column) obtains high AUCs for over half the traits. Most prominent are miR-17-5p (N=33), MKI67 (N=36) and FOXA1 (N=31) in breast as well as miR-17-5p (N=13) and KRAS (N=17) in lung. Especially noteworthy is miR-17-5p as it performed exceptionally well in both cohorts (for which model

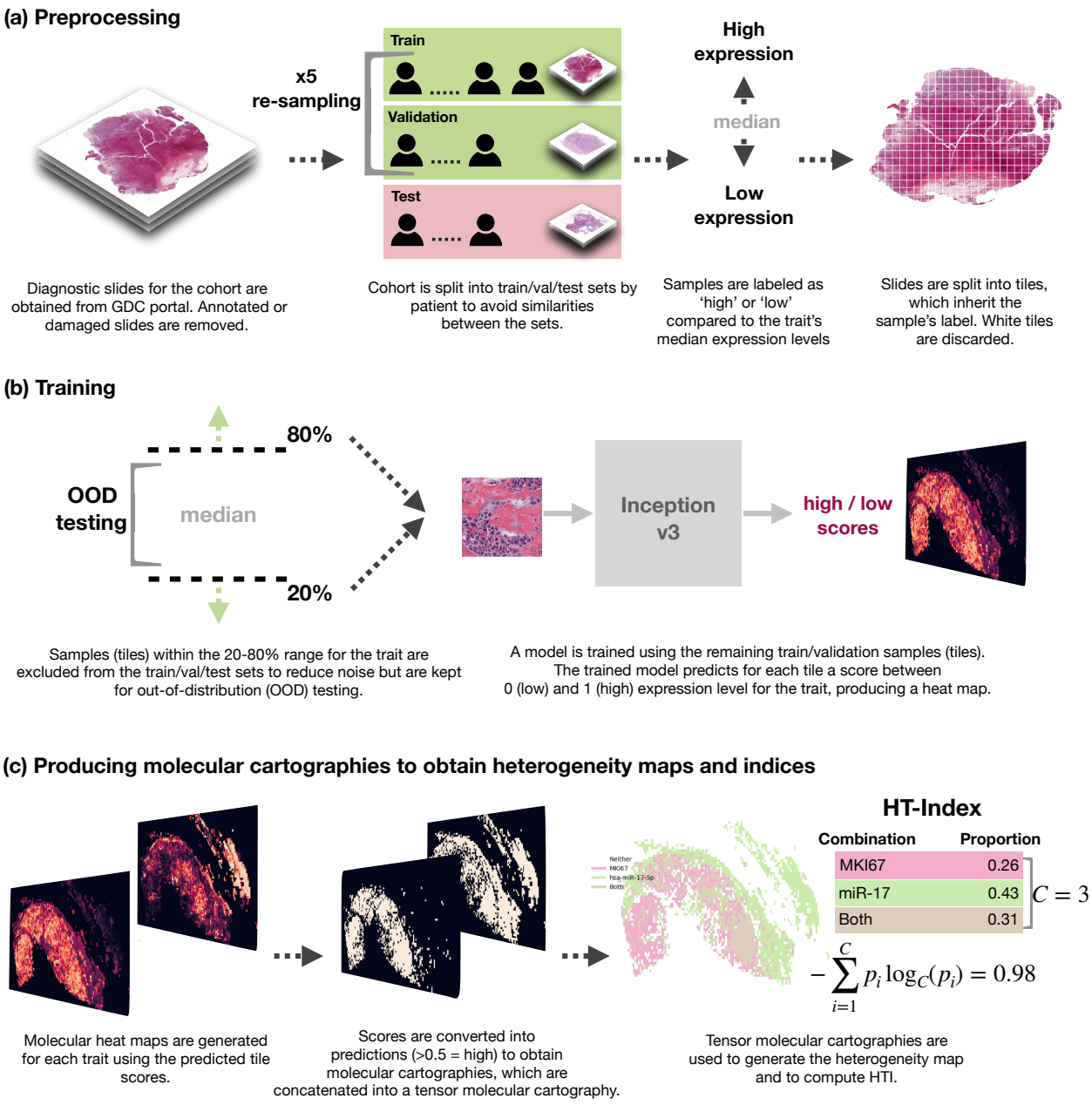

Figure 1: Overview of our methods: (a) prepossessing, (b) model training per trait and (c) producing tensor molecular cartographies per slide from which heterogeneity maps and indices are derived.

development and testing is completely separate), further suggesting that mRNA and miRNA expression can be detected from tissue morphology and that this may be applicable for other cancer types.

The use of ensemble models further improves the performance in nearly all traits. miR-17-5p improves from 0.83 to 0.87 AUC in breast and from 0.85 to 0.95 AUC in lung. We observe similar effects for MKI67 and FOXA1 in breast as well as for KRAS in lung. In Figure 2 a (breast) and d (lung) we can see the test-set distribution of the ensembles' slide scores for the ground-truth labels across the different traits. We clearly observe that the images with bulk label above median have higher slide-level predictions. This is especially true for miR-17-5p (in both cohorts), MKI67, FOXA1, MYC, miR-29a-3p and ESR1 in breast as well as KRAS and CD274 (PDL-1) in lung. We also computed the correlation of our predicted levels to the actual percentiles (Supplementary S1). For example, in breast miR-17 we observe Spearman correlations of 0.63 (FDR p-value $8e^{-04}$), 0.21 (FDR p-value 0.02) and 0.13 (FDR p-value 0.01) in test, OOD-near and OOD-all respectively.

To the best of our knowledge, these are the first models to automatically detect mRNA and miRNA expression levels on H&E whole-slide images.

Table 1: Results for breast and lung cohorts for test and OOD sets. Slides are scored using the percent positively classified tiles. Each of the 5 trained models is evaluated on the held-out test set and the two OOD sets separately. Average AUC results and range (brackets) are shown on the left, followed by ensemble AUC results for test, OOD-near and OOD-all. In bold are AUCs of at least 0.6.

	Trait	**Per-slide AUC**			
		Test 5-run average (range)	**Test ensemble**	**OOD-near ensemble**	**OOD-all ensemble**
TCGA-BRCA	miR-17-5p	**0.83 (0.79-0.88)**	**0.87**	**0.62**	0.56
	MKI67	**0.74 (0.53-0.87)**	**0.87**	**0.72**	**0.63**
	FOXA1	**0.7 (0.6-0.76)**	**0.74**	**0.61**	0.56
	MYC	**0.62 (0.58-0.67)**	**0.63**	0.59	0.58
	miR-29a-3p	**0.62 (0.5-0.69)**	**0.67**	**0.65**	0.55
	ESR1	0.58 (0.55-0.63)	0.56	**0.75**	0.57
	CD24	0.57 (0.47-0.67)	0.56	0.57	0.47
	FOXC1	0.53 (0.46-0.57)	0.52	**0.68**	**0.61**
	ERBB2	0.5 (0.46-0.57)	0.53	0.52	0.5
	EGFR	0.5 (0.35-0.67)	0.42	0.57	0.57
TCGA-LUAD	miR-17-5p	**0.85 (0.72-0.95)**	**0.95**	0.43	0.54
	KRAS	**0.64 (0.33-0.9)**	**0.73**	0.56	0.52
	CD274 (PD-L1)	**0.61 (0.57-0.63)**	**0.6**	**0.64**	0.51
	miR-21-5p	0.55 (0.47-0.62)	0.54	**0.75**	**0.63**
	EGFR	0.44 (0.22-0.78)	0.39	0.48	0.46

2.3 Performance for out-of-distribution (OOD) samples

An added advantage in setting aside the OOD slides is the ability to further challenge our models on out-of-distribution samples. We use the ensemble models for each trait. In Table 1 we report two types of AUC results on the OOD set: one for the next decile tier after the test set's percentiles, i.e. 0.2-0.3 and 0.7-0.8, which we designate as near-distribution (OOD-near) and another for the full OOD set (OOD-all). As before, the number of slides available in the two OOD sets depends on the cohort and the intersection between slide and label availability for the trait. In the OOD-near set, breast has between 140 and 158 and lung has between 79 and 103 slides (roughly 20% from each cohort's slides). The OOD-all set has between 423 and 454 slides for breast, and between 256 and 283 for lung (roughly 60% of each cohort's slides - the remainder after using the top and bottom 20% for model development). We are able to test our models on such large test sets since, by design, none of the slides in their percentile range were included in the trait's train/validation/test sets and therefore they were all held out. Our results demonstrate that the models extend to out-of-distribution samples relatively well. This can also be observed in Figure 2 (middle, right) which depict the distribution of scores per label for the OOD-near and OOD-all sets. This is especially evident in the breast cancer cohort. For example, the score distributions for MKI67 for the "above median" (pink) class tend to be higher than those for the "below median" (gray) class. The overall better breast results are likely due to the larger amount of data available for training, emphasizing the importance of collecting larger data sets.

2.4 Tensor molecular cartographies of gene expression as a window to tumor heterogeneity

After confirming the performance of our models, we proceed towards our goal of analyzing tumor heterogeneiy (Figure 1 c). We do so by producing, for each slide, multiple molecular cartographies, each representing a single molecular trait. We then combine the molecular cartographies into a tensor molecular cartography to obtain a richer spatial representation of the molecular traits detected within the slide. Creating a molecular cartography for a single trait is straight forward: we simply spatially arrange the binary predictions of a slide's tiles in a matrix so that the position of a tile's score in the matrix corresponds to its position in the pre-tiled slide, as illustrated in (Figure 1 c left). Once we obtain several molecular cartographies for a single slide, we stack them into a tensor molecular cartography (Figure 1 c middle) that now represents the slide across several traits.

We use the tensor molecular cartography to visualize heterogeneity – this will later serve us to confirm our method for quantifying heterogeneity. As shown in Figure 1 (c right), we do so by identifying which tiles are positive for each possible combination of traits and assign different colors to each combination. For example, using two traits, A and B, we obtain a tensor of depth 2 (one molecular cartography per trait) and each tile is colored as one of three options: A

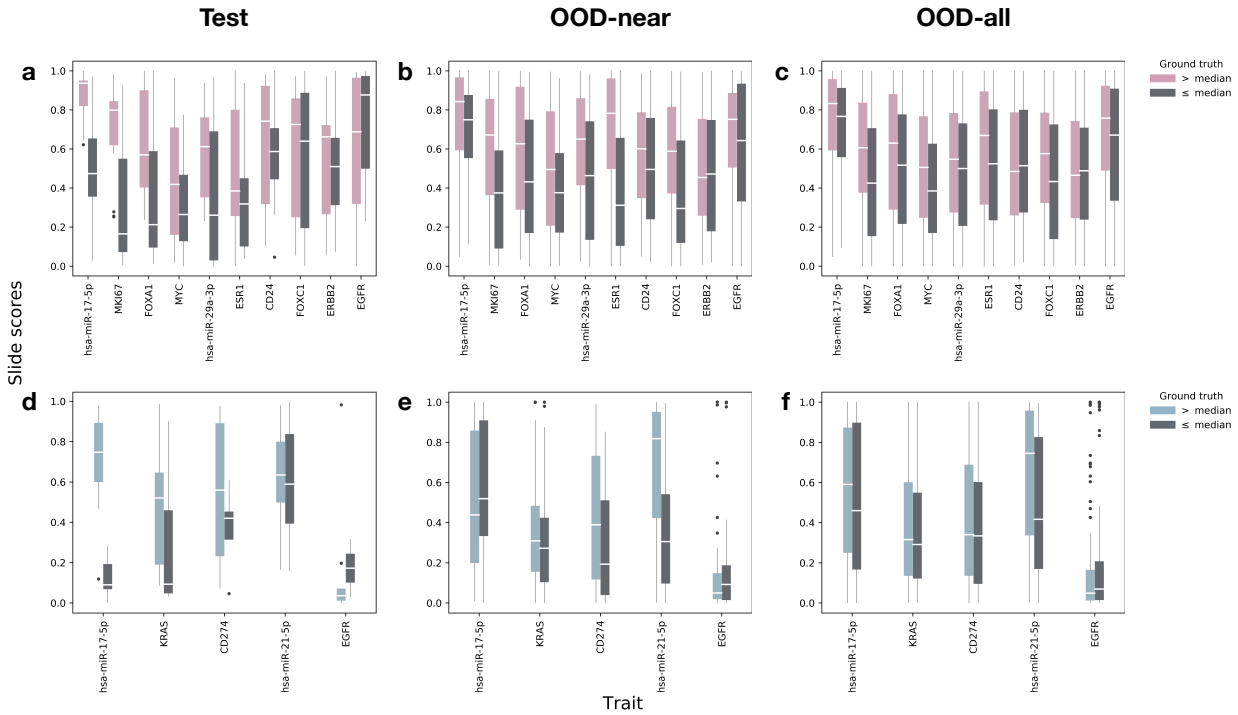

Figure 2: WSI score distribution for test, OOD-near and OOD-all sets compared to the actual bulk-measured values (lower values in gray). Scores are computed using the ensemble model for each trait (percent positive tiles by majority vote of the best 3). Middle white lines reflect the median slide score. Whiskers extend to $1.5 \times IQR$. (a)-(c) breast test, OOD-near and OOD-all respectively; (d)-(f) lung test, OOD-near and OOD-all (respectively). In the test set, especially worth noting are miR-17-5p in both cohorts, MKI67, FOXA1, MYC, mir-29a-3p and ESR1 in breast as well as KRAS and CD274 (PD-L1) in lung. In the OOD sets most notable are MKI67, FOXA1, MYC, ESR1 and FOXC1 in breast as well as miR-17-5p and miR-21-5p in lung.

(only), B (only) and Both. Figure 3 shows examples for such heterogeneity maps, along with their level of heterogeneity, as obtained using HTI described below. Such molecular maps of pathological images could be used by pathologists, in addition to routinely stained diagnostic markers, to further classify and identify cancer subtypes, potentially leading to better informed clinical decisions.

2.5 Quantifying tumor heterogeneity from tensor molecular cartographies

As intra-tumor heterogeneity has been proposed as an obstacle to effective treatment and cancer eradication, we propose an approach to compute and quantify the level of heterogeneity in a given image from its tensor molecular cartography. We used a variation on Shannon's entropy, commonly used to measure diversity and heterogeneity in various settings [31, 11]. Formally, we compute:

$$HTI = -\sum_{i=1}^{C} p_i \log_C(p_i) \tag{1}$$

where C is the maximum number of non-empty trait combinations that may be observed on a slide and equals $2^{|traits|} - 1$ (the number of subsets excluding the empty set), and p_i is the proportion of tiles for which exactly all models in combination i provided a positive prediction.

For example, given a tensor molecular cartography for two traits A and B (e.g. FOXA1 and MKI67), $C = 3$ (3 possible non-empty trait combinations: A (only), B (only) and Both). If the slide is homogeneous with nearly all of its tiles falling into one of these 3 options (say Both), then $p_{Both} \approx 1, p_A \approx 0, p_B \approx 0$ (each of the single-trait molecular cartographies is nearly all 1s), resulting in an HTI of 0. If, however the slide is heterogeneous with $1/3$rd of the tiles

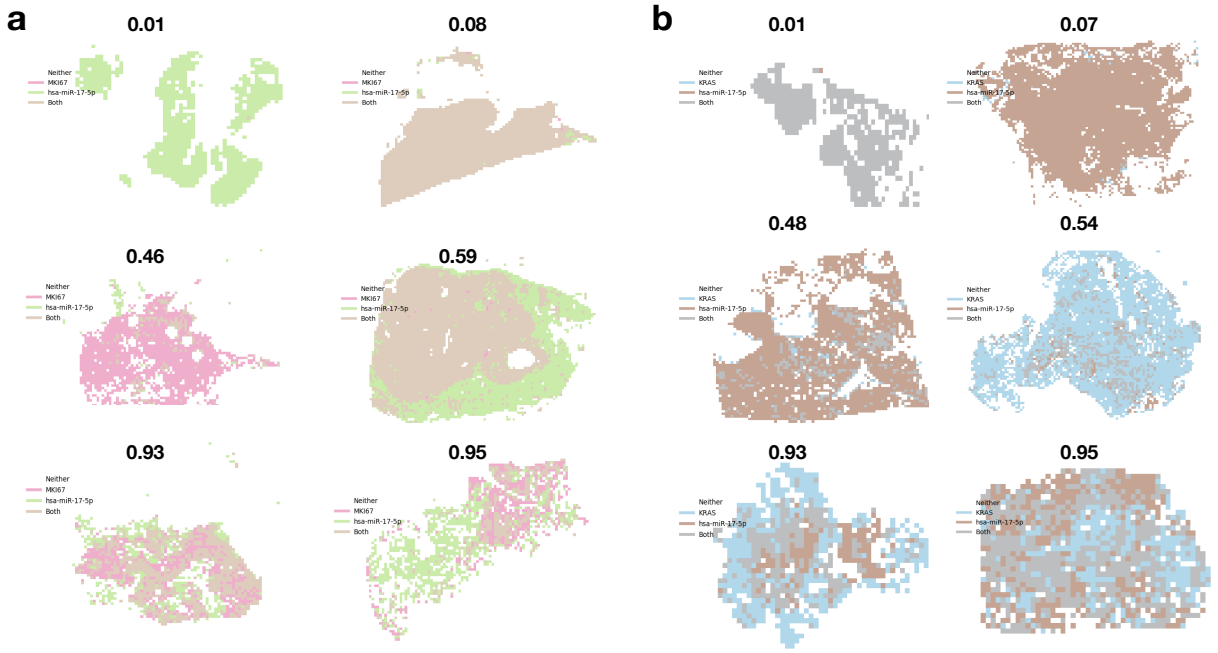

Figure 3: Heterogeneity maps and corresponding HTIs. (a) Breast cancer cohort with traits MKI67 (pink) and miR-17 (green). Brown indicates that both traits manifest (both models predicted positive). (b) Lung cancer cohort with traits KRAS (blue) and miR-17 (brown). Gray indicates both traits manifest. Corresponding HTIs appear directly above. Rows appear in increasing HTI order from top to bottom (top 0-0.1, middle 0.4-0.6, bottom 0.9-1).

falling into each option then: $p_{Both} = 1/3, p_A = 1/3, p_B = 1/3$ and we obtain an HTI of 1. The logarithm base C guarantees that HTI $\in [0, 1]$. If A and B are two molecular traits (e.g. FOXA1 and MKI67), a high HTI would reflect there may be two subclones whereas a low HTI would reflect single clonal dominance.

We note that HTI can be applied to any set of binary matrices (or vectors) of identical shape, each of which describes the presence of a single trait. As such, it can be used in other settings involving the localization of clinically relevant traits.

Figure 3 depicts several heterogeneity maps and their associated HTIs.

2.6 A statistically significant link between tumor heterogeneity and survival

We sought to understand whether tumor heterogeneity is linked to survival outcome and whether the link can be inferred through the spatial analysis of pathology whole-slide images. To do so, we combine the test and OOD slides and split them into high and low heterogeneity groups based on their HTIs. Specifically, for a given cohort, we first generate tensor molecular cartographies per slide using the ensemble models of the top two performing molecular traits for that cohort (from Table 1). We then compute HTI for each slide as described in Section 2.5 and split them into two groups: > 0.5 and ≤ 0.5 (high and low heterogeneity respectively). We perform survival analysis on these groups using Mantel's log-rank test and Kaplan-Meier curves. We use only slides in the combined set of OOD and test slides. Since OOD slides are trait-dependent, we use only slides in the intersection of the two OOD sets.

Figure 4 describes the survival analysis results for each cohort using the top two traits by test performance from Table 1 in each (MKI67 and miR-17 for breast and miR-17 and KRAS for lung). In breast, high heterogeneity (blue) is significantly different from low heterogeneity (orange) with a log-rank p-value of 0.04 (Figure 4 (a)). In lung, we observe significant differences between higher and lower HTIs when heterogeneity differences are more distinct (> 0.7 vs ≤ 0.3), with a log-rank p-value of 0.07 (Figure 4 (c)).

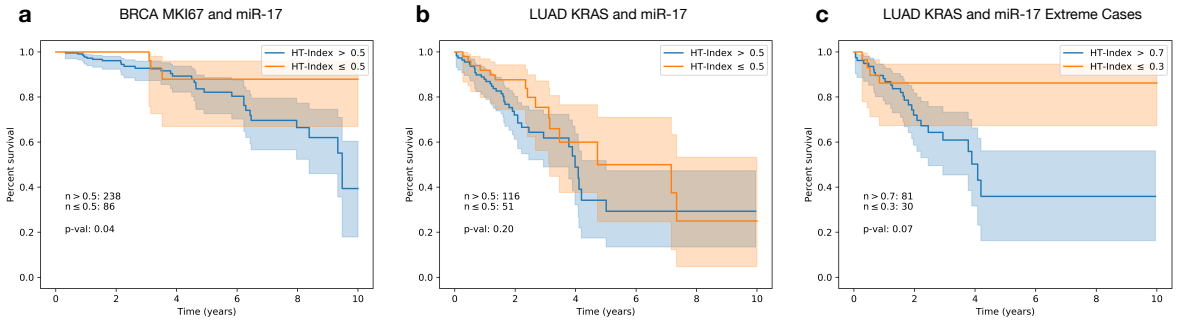

Figure 4: Survival analysis with respect to HTI derived from two traits in breast and lung cancer. For each trait combination in (a) and (b), slides were split into high and low HTI (> 0.5 and ≤ 0.5 or blue and orange respectively). In (c) slides were split into > 0.7 and ≤ 0.3 HTI. Each survival curve is shown with a 95% confidence interval.

3 Discussion

This work investigated the potential of analyzing tumor heterogeneity from the rich spatial data available in H&E WSIs. Using deep learning we created high resolution maps of multiple mRNA and miRNA expression levels within a whole-slide image and combined these maps into a tensor molecular cartography. We then used the cartographies to spatially visualize and quantify tumor heterogeneity. Other methods, such as single-cell profiling, can also be used to infer heterogeneity. These methods are expensive, may lack spatial context, and would only cover a few thousand cells.

We applied our method to both breast and lung cancer pathology whole-slide images (H&E). We trained several models per trait and tested each of these models on both a held-out test set and two large out-of-distribution sets, containing hundreds of WSIs the models have never before encountered. Results showed that several mRNA and miRNA can be identified and localized automatically within whole-slide images with high AUCs. Especially notable are our results for miR-17, which obtained high AUCs in both lung and breast. This is interesting in light of indications that miR-17 is over-expressed across many cancer types [32].

Using ensemble predictions from the top two performing mRNA/miRNA in each cohort we generated a tensor molecular cartography for each slide, enabling us to both visualize the distribution of traits (e.g. as heterogeneity maps) and to compute HTI. By representing each patient with their HTI and performing survival analysis, we showed that the inferred tumor heterogeneity is significantly linked to poor survival, especially in breast cancer. We stress that this link cannot be identified through obvious means by directly using expression levels (Figure 5) or through the PAM50 [27] breast cancer types, which are not associated with HTI (Figure 6). This analysis highlights the potential clinical value in producing tensor molecular cartographies from H&E WSIs.

Our approach has important limitations. While slide-level AUCs provide a measure of performance, commonly used in similar settings [5, 29], there is no guarantee that each individual tile prediction is accurate. Such a tile-based assessment could be made possible in the future if, for example, spatial transcriptomics data is made available alongside H&E slides. Additionally, our models were trained using hundreds of slides per cohort, which may not be readily available in other settings. As H&E slides are becoming increasingly digitized, this concern may be alleviated.

Our results open a window to further analyses. For example, from a molecular biology perspective, comparing molecular cartographies of miRNAs from the same family may be interesting in light of recent findings showing they are context (e.g. tissue) dependent [33]. Similarly, miRNA/mRNA relationships (as explored in [34, 35]) may be analyzed from a spatial perspective. From a technical aspect, it may be interesting to explore whether transfer learning between molecular traits or cohorts is possible and to what extent. Also a multilabel approach may be possible, although it may require larger datasets and careful label-balancing to obtain satisfactory results across all traits.

As heterogeneity plays an increasingly key role in cancer treatment, providing pathologists with a solution to view the distribution of clinically relevant traits that are not currently visible on slides may be of great value. Since H&E slides are a standard component of routine diagnostic protocols, a natural solution may be offered by the fast and automated digital mapping of the molecular landscape within H&E slides. One such solution is offered through our methods of producing tensor molecular cartographies and heterogeneity maps from WSIs.

Our findings, and the methods developed in this study, can be applied to many more traits and in broader contexts. As a future direction we hope to combine our approach with scRNA-seq data to obtain finer resolution mapping, improving the relevance of both H&E and transcriptomics. Molecular cartography from H&E potentially enables a new approach to investigating tumor heterogeneity and other spatial molecular properties and their link to clinical characteristics. An interesting aspect of such endeavors will be to link spatial properties to treatment susceptibility and precision care.

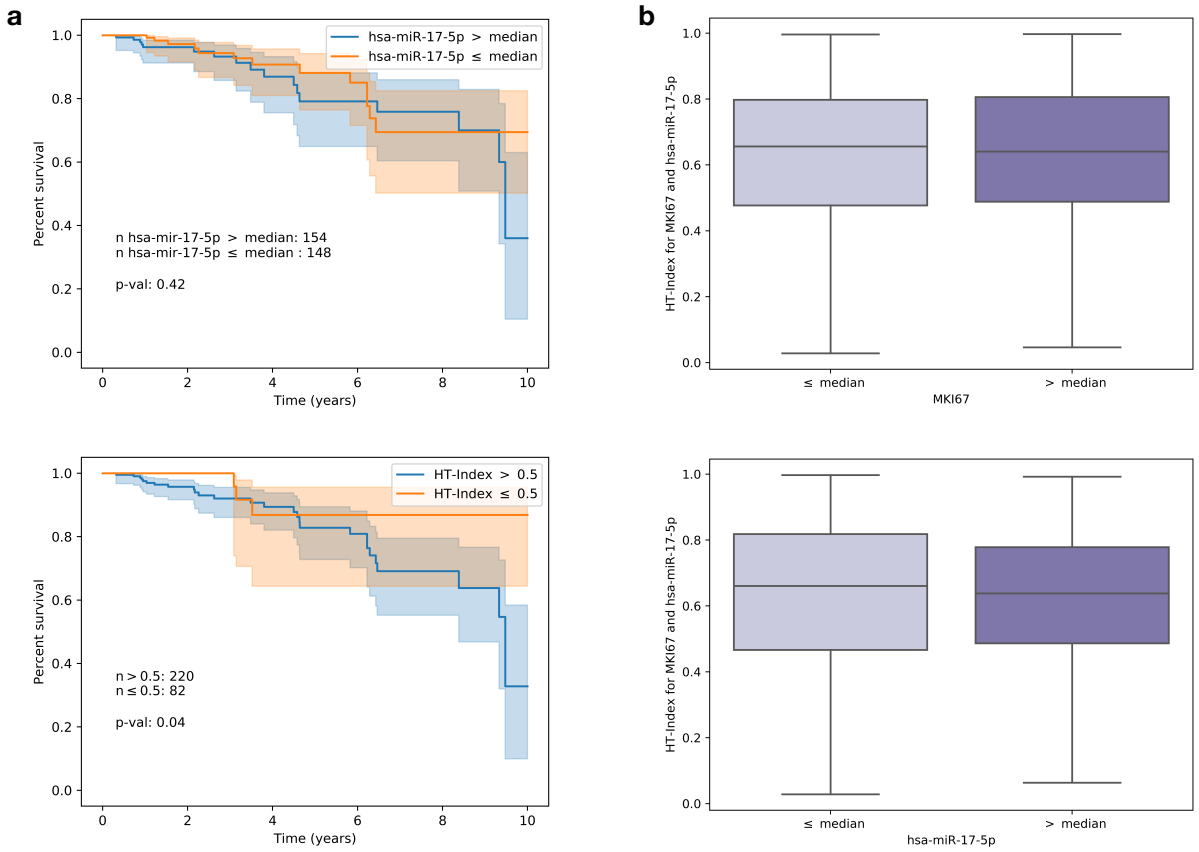

Figure 5: Left panel (a): Survival analysis using bulk-measured expression levels for miR-17 (top) compared to our approach (bottom) when applied to the same patient basis available for both. Right panel (b): distribution of HTI for above and below median ground-truth expression levels for MKI67 (top) and miR-17 (bottom).

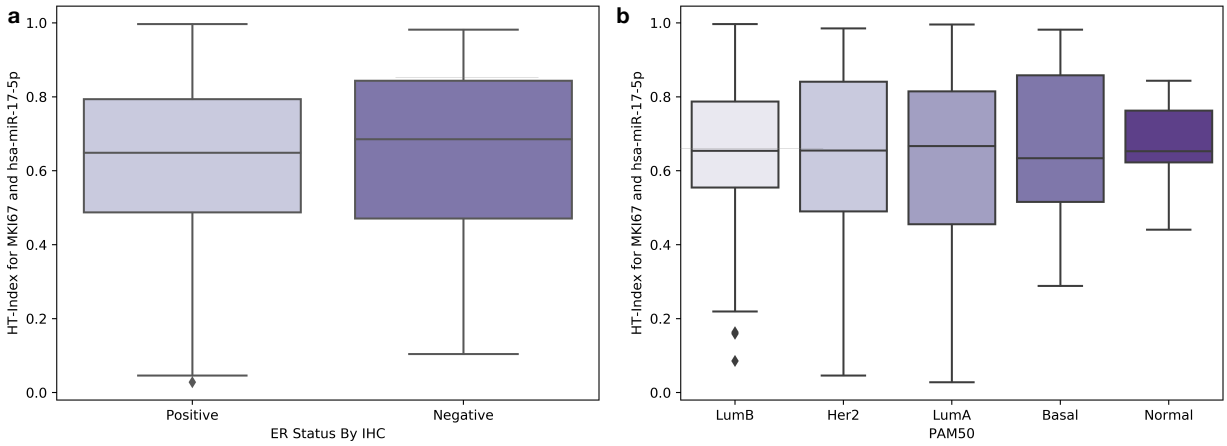

Figure 6: Distribution of HTI per ER status (left) and PAM50 type (right) in the data used for survival analysis.

4 Methods

4.1 Data

All whole-slide images are available online at the GDC repository (`https://portal.gdc.cancer.gov/repository`), by selecting *Diagnostic Slide* under *Experimental Strategy* for the relevant project (e.g. TCGA-BRCA). Matching expression levels were obtained from the GDC's website at: `https://gdc.cancer.gov/about-data/publications/pancanatlas` (see RNA and miRNA). Matching survival data were obtained from cBioportal at: `http://www.cbioportal.org/study/clinicalData` from the following files: "Breast Invasive Carcinoma (TCGA, Firehose Legacy)" and "Lung Adenocarcinoma (TCGA, Firehose Legacy)".

4.2 Training specifications

All of our models were developed in TensorFlow [36] using the Inception v3 classifier [28] with the last layer modified to one output. All models are trained from random initialization, following previous work showing improved performance by fully training Inception v3 [5]. Each of the 5 train/validation rounds is trained on mini-batches of labeled tiles from the training set using the Adam optimizer [37]. Models were evaluated on the labeled validation tiles every 1/16th epoch (full pass on all training tiles) to avoid overfitting caused by tile similarities between mini-batches (each slide contains hundreds to thousands of tiles, many of which are likely to be similar to one another). No data augmentations were performed, except for random horizontal and vertical flips of the training tiles to further reduce overfitting. Learning rate started at 0.001 and was decayed when performance on the validation set plateaued for 10 steps, with early stopping after 30 steps of no validation improvement. Each final trained model used the weights that performed best on its validation set.

Each model was trained on a single Tesla K80 machine with 8 GPUs and took approximately 12 hours until convergence on the validation set (when the aforementioned early stopping was invoked). Mini-batch size per GPU replica was 18, for a total of $18 \times 8 = 144$ tiles per training step.

4.3 mRNA and miRNA selection

mRNAs: For breast, we sorted PAM50 [27] genes by their expression variance in our dataset and selected from the top (highest variance). CD24 is not in PAM50, but is one of the highest expression-varying mRNAs in the cohort and is over-expressed in many cancers [38]. For lung, we based our selection on previous research, e.g. [39, 5, 40, 41].

miRNAs: Selection of miRs is based on previous work identifying miR-17 and miR-29 among the top up-regulated and down-regulated miRNAs (respectively) in breast cancer [35]. Others have also associated miR-17 [42, 43, 44] and miR-29 [45, 46] with breast cancer. miR-17 was also chosen for lung since the miR-17 family was shown to be universally over-expressed in many cancers, including lung [32], and has been directly associated with lung cancer [47, 48, 49] as has miR-21 [50, 51].

4.4 Survival analysis

Each patient is represented by a single HTI to perform survival analysis. Where patients were associated with more than one whole-slide image (e.g. a patient with diagnostic slides DX1 and DX2), the DX1 slide was used to determine HTI. For data used for survival see Methods 4.1. Analysis was performed using the Lifelines package for Python (`https://lifelines.readthedocs.io/en/latest/`).

4.5 Code availability

The code used for this work will be made publicly available upon publication.

4.6 Author contribution

ALJ and ZY designed the study and developed the methods. ALJ performed all data analysis. XT and VK performed follow up analysis. XT curated data and prepared it for analysis. ZY supervised the study. ALJ and ZY wrote the manuscript with contributions from all authors

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5 Supplementary Material

5.1 Supplementary Note S1

Table S1: Spearman correlations and FDR-corrected p-values for breast and lung cohorts across test, OOD-near and OOD-all data sets. Slides are scored using the percent positively classified tiles produced by the ensemble model for each trait. Ground-truth values are the actual slide percentiles (0.1, 0.2 etc.). In bold are significant FDR-corrected p-values ≤ 0.1.

	Gene / Trait	**Test**		**OOD-near**		**OOD-all**	
		Spearman	**p-value (FDR)**	**Spearman**	**p-value (FDR)**	**Spearman**	**p-value (FDR)**
TCGA-BRCA	miR-17-5p	**0.63**	**8e-04**	**0.21**	**0.02**	**0.13**	**0.01**
	MKI67	**0.58**	**1e-03**	**0.38**	**4e-06**	**0.27**	**6e-08**
	FOXA1	**0.44**	**0.04**	**0.19**	**0.03**	**0.12**	**0.01**
	MYC	0.36	0.11	**0.16**	**0.07**	**0.13**	**0.01**
	miR-29a-3p	0.38	0.11	**0.27**	**4e-03**	**0.13**	**0.01**
	ESR1	0	1.00	**0.43**	**5e-07**	**0.21**	**3e-05**
	CD24	0.10	0.93	0.12	0.17	8e-03	0.86
	FOXC1	4e-03	1.00	**0.31**	**3e-04**	**0.21**	**3e-05**
	ERBB2	0.07	0.99	0.03	0.70	0.02	0.72
	EGFR	0.05	1.00	0.12	0.16	**0.12**	**0.02**
TCGA-LUAD	miR-17-5p	0.56	0.22	-1e-01	0.40	1e-03	0.98
	KRAS	0.38	0.32	0.11	0.40	0.03	0.74
	CD274 (PD-L1)	0.39	0.39	**0.23**	**0.10**	0.07	0.43
	miR-21-5p	0.17	0.49	**0.43**	**2e-04**	**0.24**	**4e-04**
	EGFR	0.27	0.49	-3e-02	0.78	-7e-02	0.43

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