Tejas Easwar (the28@cam.ac.uk)

"Integration of digital pathology with multi-omics – the good, bad and the ugly"

Introduction

Pathology is often perceived as a field steeped in tradition with the image of researchers peering down microscopes coming to mind. However, in today's rapidly evolving landscape, this could not be further from the truth; the convergence of technology and biology coupled with the use of artificial intelligence (AI) has led to a new era of innovation. Upcoming technologies have often been combined to yield better results.

The term 'Digital pathology' was born following the use of whole-slide scanners, transforming traditional histopathological slides into a digital format; this evolution has pushed past just digitisation, with increasingly sophisticated computational methodologies that now incorporate AI methods. This digitisation has driven the curation of large libraries, such as the Cancer Genome Atlas (TCGA) [1]. Such ambitious projects have not only established extensive image libraries but have also paved the way for integration with large datasets involving genetic, clinical and outcome information.

The suffix '-omics' suggests large datasets of molecules. It initially referred to genomics – the study of the entire genome. Since then, omic technologies have expanded to integrate entire pools of transcripts, proteins, metabolites, microbiomes and more; the analysis of these various datasets is the basis of multiomics. Spatial multiomic technologies are one

example of how these two fields can be integrated. In this technology, samples are usually fixed fresh frozen or formalin-fixed paraffin-embedded. Following this, computational data integration is undertaken, and tissues undergo next-generation sequencing, RNAseq, FISH, epigenomic analysis and more [2].

This essay will explore the benefits, challenges and ethical considerations when integrating these two fields.

"The Good" - How Integration can be useful

The convergence of digital pathology with multi-omics presents a key advantage in enhancing prognostic prediction and diagnostic precision. An established metric for evaluating the accuracy of diagnostic and predictive assessments is the Area under receiver operator characteristic curves (AUC). This curve plots the relationship between 'sensitivity' and '1-specificity', where an AUC value of 1.0 denotes perfect prediction, while 0.5 represents a completely random prediction [3]. The higher the AUC the better the model is at predicting; for a model to be adopted clinically it will typically have an AUC greater than 0.9 as well as undergoing rigorous testing.

Lung cancer is the most common cancer and the main cause of cancer deaths worldwide with an estimated 2.1 million new cases and 1.8 million deaths annually [4]. Lung adenocarcinoma (LUAD) is the most major histological subtype. In a lung adenocarcinoma

patient cohort, Chen et al. (2021) [5] looked at the potential of integration multiomics with digital histopathology data for survival prediction. The study extracted image features from digital histopathological slides using the CellProfiler 3.0 software [6], and performed systematic analyses to correlate the features from histopathological images and omics profiles. They used histopathological image features alone or integrated them with genomics, transcriptomics and proteomics data. From this, prognostic models were calculated for 1, 3 and 5 year years; the AUC values for 5-year survival are shown in Table 1

Table 1 – Area under the curve values adapted from test sets from Chen et al. (2021). Proteomics model alone without histological features performed comparably, hence was not included.

Test Set	**AUC Value for 5 year survival**
Genomic Model	0.745
Genomic Model + Histological Features	0.832
Transcriptomics model	0.786
Transcriptomics + Histological features	0.840
Proteomics + Histological Features	0.850
Histopathological Features + Multiomics	**0.908**

Interestingly, when histological features were added, AUC values increased in all models highlighting that it improves prediction. Furthermore, the incorporation of genomics, transcriptomics and proteomics into a multomic model yielded the highest AUC. This indicates that an integrative model is more precise than using image features or omics alone; the result show that histological features and omics may be complementary in prognosis prediction. Thus, clinicians can better stratify high-risk and low risk LUAD patients and tailor treatments accordingly. This is of particular relevance to lung cancer since historically it has had poor treatment outcomes, and a more tailored approach may improve

outcomes for patients. Nevertheless, certain limitations marred the study's outcomes. Specifically, some of the test cases had a small number of cases limiting accuracy, therefore larger and more diverse datasets would be required to backup this data. Additionally, the study primarily focused on prognosis implications, thus future directions should explore diagnostic potential of integration.

Breast cancer has the highest morbidity and mortality in women across the world [7], with HR+/HER2- being the most common molecular subtype accounting for 50-79% of cases [8]. Whilst the number of therapies has increased, long-term recurrence still remains a clinical problem and so a targeted precision approach is vital, much like LUAD. Hu et al. (2023) [9] used a deep-learning model to predict histopathological features and multiomic molecular features, in a cohort of HR+/HER2- breast cancer patients. They study used two convolutional neural networks in series, with the second trained to predict clinicopathological features, somatic mutations, important cancer-related pathways, immunotherapy biomarkers and prognosis; common clinicopathological features for prediction included Ki67 and histological grade assigned according to the World Health Organisation. The AUC values for multiomic features and clinicopathological features are shown in Table 2

Table 2 - Area under the curve values adapted from test sets from Hu et al. (2023)

Test Set	**AUC**
Histological Grade	0.68 (grade I); 0.82 (grade II); 0.90 (grade III)
Ki67	0.81 (low Ki67) 0.80 (high Ki67)
Somatic mutations	0.50-0.85 (TP53 = 0.68; GATA3 = 0.68)
Gene set enrichment analysis of cancer pathways	0.63 -0.87

Biomarkers associated with Immunotherapy responses	0.59-0.76

The results found that clinicopathological features that good prediction accuracy, particularly histological grade and Ki67. For somatic mutations, the models achieved high accuracy in predicting TP53 and GATA3 mutations, which have previously been shown to be of prognostic significance. However, a wide range of somatic mutations were predicted and only a few had high prediction accuracy; a cumulative genetic score could be developed. Cancer-related pathways and immunotherapy genes similarly showed promising AUC values; however, similar to somatic mutations, they showed a wide range of predictions. In addition, this study was unable to integrate clinicopathological features with the multiomic datasets to test whether this improves the prediction and future directions can look at this. Nonetheless, the study shows that individually these features have potential to predict HR+/HER2- breast cancer; it shows that multiomics features and histopathological data can lead to a more streamlined approach to managing HR+/HER2- breast cancer. Using this knowledge, clinicians could refine and personalise therapeutic interventions.

The biggest clinical advantage of integration for patients is personalised and precision approaches. By harnessing these datasets, clinicians can steer their diagnosis, treatment choices and prognostic decisions. Yet, potential extends further than this – the generation of risk scores can quantify this risk. Notably, the polygenic risk score (PRS) has shown great promise for precision medicine, finding applications across a diverse range of complex diseases. PRS sums the effects of all common variants of a particular disease using genome-wide association (GWAS) data. However, the clinical utility of PRS has been limited by its inability to capture the role of environment, lifestyle nuances as well as gene-gene interactions (epistasis) [10]. A multi-omic approach, using epigenomic and transcriptional

data, that also includes digital histopathological components could rectify this limitation, thus augmenting the efficacy of PRS within clinical contexts [11]. A recent stride in this field is the advent of the 'poly-omic' risk score. Arehart et al. (2022) developed this score to predict a cohort of inflammatory bowel disease patients by incorporating metagenomics, metatranscriptomics, viromics and metabolomics, yielding an AUC of 0.80 [12]. These studies show the potential of multi-omic datasets to predict risk in complex diseases. Consequently, the integration of digital pathology and multiomic data emerges as a compelling avenue for the quantification of risk, enhancing clinical understanding and decision making. Clinically, this could result in faster diagnosis and stratifying patients into risk groups, freeing up doctors for more difficult cases and prioritisation in waiting lists.

Moreover, the integration of multiomic datasets and digital pathology offers a pivotal advantage through the implementation of automated algorithms. These cutting-edge tools harness AI, coupled with the capabilities of machine learning and deep learning. Machine learning encompasses the process of providing data to the system to refine predictions, while deep learning harnesses artificial neural networks comprising multiple layers to amplify computational power for predictive tasks [13]. Even fundamental tasks like counting objects, such as lymph node metastases in breast cancer cases [14], and biomarkers like Ki-67 [15], stand benefit from AI-driven automation. This technology holds the potential to substantially mitigate human errors and improve overall accuracy. As a result, the integration of digital pathology and multiomic data emerges as an indispensable approach, using automation to improve diagnostic precision and streamline clinical workflows.

The disruptions brough about by the COVID-19 pandemic affected traditional pathology practices, catalysing the development and embrace of digital pathology to ensure the

seamless continuation of clinical and academic research. The integration of digital pathology methods with multiomic datasets stands poised to embrace this new approach. Notably, it would facilitate remote working, reducing the need to transport glass slides as well as reduce logistical and safety concerns. Furthermore, it would allow for centralised teams of pathologists, with specialist hubs, optimizing efficiency and resource allocation, allowing for more collaborative prospects. Beyond this, digital pathology methods align with telepathology which would help diagnosis, education and research [16]. Telepathology is the practice of pathology at long-distance, favouring digital pathology imaging over traditional hand-on light microscopy. This has been likely inspired by the field of teleradiology, whose success has been driven by the implementation of a set of standards for digital radiology imaging. Currently, standards, regulation and legal issues are being addressed acting as a catalyst for further telepathology advances. The integration of multiomic datasets with digital pathology may complement the growing field of telepathology.

Overall, the convergence of multomic datasets with digital pathology will likely be an instrumental tool that will shape future pathology practices, improve efficiency and collaboration.

"The Bad" - Challenges of Integration

Despite the substantial diagnostic and prognostic utility shown by the integration of these two fields, there are certain challenges on the horizon. At an infrastructure level, storage demands of digital biopsy slides can be considerable, often involving several gigabytes per

patient. While the demand for storage grows exponentially, local storage capabilities frequently struggle to keep pace. This disparity is a key challenge that particularly affects economically constrained nations with limited infrastructure. Nevertheless, the challenges remain even in well-developed healthcare systems such as the NHS. The NHS grapples with numerous 'legacy' systems that cannot cope with the escalating digital demands required for the integration of digital pathology with multiomics. In addition there is a lack of interoperability within these storage systems meaning that it will be challenging to integrate datasets from different systems [17]. Therefore, in order for the integration of digital pathology and multiomic datasets to be adopted clinically, NHS computer systems and infrastructure must level up from legacy systems.

A significant challenge when creating AI models for digital pathology and large multiomic datasets is ensuring the models can perform well on new, unseen data; this is known as 'model generalisation' [18]. A common issue is overfitting, where a model becomes too specialized during training and works well on the training data but struggles with new data [19]. For example, deep learning models using histopathological images are often trained on datasets that are split into training and test sets. These models can achieve high accuracy on such test sets, outperforming experienced pathologists. However, these test sets lack the diversity seen in real-world data. This mismatch can result in poor performance in actual clinical scenarios, potentially leading to incorrect diagnoses and treatment choices. Therefore, in for an integrated models to be adopted clinically, it must be developed using appropriately large and diverse training tests, and tested rigorously to ensure that their prediction accuracy is consistent even with new data.

“The Ugly” – Ethical and Practical Considerations

The integration of digital pathology with multiomic datasets is not entirely free of ethical issues. This approach requires collaboration, requiring pooling of sensitive personal data. However, this data-sharing collides with the principles of data ethics and legal regulations like GDPR, which emphasize the restraint in processing personal data. The data-intensive nature of multiomics and AI-driven digital pathology stands in stark contrast to the ethos of data minimization upheld by ethical standards and processing laws [18]. Machine learning algorithms, particularly deep learning, thrive on large datasets of clinical information to identify patterns. However, these large datasets pose various issues, for example they could attract hacking or ransomware attacks jeopardising confidentiality, as shown by the ‘Wannacry’ cyber-attack in 2017. Consequently, a fine balance of data minimisation principles with appropriately large datasets for algorithms to be effective must be found. Furthermore, personal data will be required to be repurposed to form a diagnostic archive and help to improve the machine learning algorithms. Therefore, gaining fully informed consent will be crucial to safeguard ethical considerations and pave the way for sustained research.

In addition, healthcare equity would also need to be addressed when integrating these datasets. AI bias has repeatedly been shown to affect gender and race [20, 21]. While data underrepresentation plays a significant role in generating such bias, the underlying factors contributing to this bias remain multifaceted and not fully understood. Although underrepresentation in data is a common reason, there are still many unknown pathways causing this bias. Before clinical adoption, models must be tested on different races and

genders to see whether prediction accuracy remains. Additionally specific diseases, such as cancers, disproportionally affect certain groups and races [22]; they often have specific genetic mutations, for example EGFR mutations are significantly more common in Asian lung cancer patients. When these conditions are inadequately represented within the training data, it causes bias and skews results. Models must be trained and tested on a wide range of individuals. This will address these issues of bias and underrepresentation, which is vital for integration of multiomic datasets with digital pathology to be adopted clinically.

In conclusion, much like the classic western film “The Good, the Bad and the Ugly” where the three main characters go on a quest to seek buried gold, the integration of multiomics with digital pathology requires a balanced consideration of three aspects: the benefits, challenges and ethical/social considerations. Just like the characters looked for their pot of gold, considering all three aspects will lead to our goal of clinical adoption and utility, with diagnostic and prognostic use. Integration of these two fields holds the potential for more efficient diagnosis of patients, alleviating healthcare burdens, and providing more tailored treatments. Ultimately this could lead to better patient outcomes. However, it's important to use these models as tools that complement human expertise rather than replacing it; human expertise in pathology will be vital to ensure that this information is assimilated and used appropriately. Potential bias and errors must also be promptly addressed to ensure reliability of this approach. Similar to the narrative of the film, we stand on the brink of a transformative advancement in pathology, and addressing the benefits, challenges and ethical/social considerations could lead to clinical use and better outcomes for patients.

References

1. Tomczak, K., P. Czerwinska, and M. Wiznerowicz, *The Cancer Genome Atlas (TCGA): an immeasurable source of knowledge.* Contemp Oncol (Pozn), 2015. **19**(1A): p. A68-77.
2. Vandereyken, K., A. Sifrim, B. Thienpont, and T. Voet, *Methods and applications for single-cell and spatial multi-omics.* Nat Rev Genet, 2023. **24**(8): p. 494-515.
3. Nahm, F.S., *Receiver operating characteristic curve: overview and practical use for clinicians.* Korean J Anesthesiol, 2022. **75**(1): p. 25-36.
4. Bray, F., et al., *Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries.* CA Cancer J Clin, 2018. **68**(6): p. 394-424.
5. Chen, L., et al., *Histopathological Images and Multi-Omics Integration Predict Molecular Characteristics and Survival in Lung Adenocarcinoma.* Front Cell Dev Biol, 2021. **9**: p. 720110.
6. McQuin, C., et al., *CellProfiler 3.0: Next-generation image processing for biology.* PLoS Biol, 2018. **16**(7): p. e2005970.
7. Sung, H., et al., *Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries.* CA Cancer J Clin, 2021. **71**(3): p. 209-249.
8. Kay, C., et al., *Current trends in the treatment of HR+/HER2+ breast cancer.* Future Oncol, 2021. **17**(13): p. 1665-1681.
9. Hu, J., et al., *Prediction of clinicopathological features, multi-omics events and prognosis based on digital pathology and deep learning in HR(+)/HER2(-) breast cancer.* J Thorac Dis, 2023. **15**(5): p. 2528-2543.
10. Koch, S., J. Schmidtke, M. Krawczak, and A. Caliebe, *Clinical utility of polygenic risk scores: a critical 2023 appraisal.* J Community Genet, 2023.
11. Ota, M. and K. Fujio, *Multi-omics approach to precision medicine for immune-mediated diseases.* Inflamm Regen, 2021. **41**(1): p. 23.
12. Arehart, C., et al., *Poly-omic risk scores predict inflammatory bowel disease diagnosis.* bioRxiv, 2022.
13. van der Laak, J., G. Litjens, and F. Ciompi, *Deep learning in histopathology: the path to the clinic.* Nat Med, 2021. **27**(5): p. 775-784.
14. Ehteshami Bejnordi, B., et al., *Diagnostic Assessment of Deep Learning Algorithms for Detection of Lymph Node Metastases in Women With Breast Cancer.* JAMA, 2017. **318**(22): p. 2199-2210.
15. Varga, Z., et al., *How reliable is Ki-67 immunohistochemistry in grade 2 breast carcinomas? A QA study of the Swiss Working Group of Breast- and Gynecopathologists.* PLoS One, 2012. **7**(5): p. e37379.
16. Weinstein, R.S., et al., *Overview of telepathology, virtual microscopy, and whole slide imaging: prospects for the future.* Hum Pathol, 2009. **40**(8): p. 1057-69.
17. Office, N.A., *Digital transformation in government: addressing the barriers to efficiency*. 2023.
18. Goodfellow I, B.Y., Courville A, *Deep Learning*. 2016, Cambridge, Massachusetts: MIT Press.

19. Rice L, W.E., Kolter JZ. , *Overfitting in adversarially robust deep learning. .* arXiv, 2020.
20. Obermeyer, Z., B. Powers, C. Vogeli, and S. Mullainathan, *Dissecting racial bias in an algorithm used to manage the health of populations.* Science, 2019. **366**(6464): p. 447-453.
21. Larrazabal, A.J., et al., *Gender imbalance in medical imaging datasets produces biased classifiers for computer-aided diagnosis.* Proc Natl Acad Sci U S A, 2020. **117**(23): p. 12592-12594.
22. Ozdemir, B.C. and G.P. Dotto, *Racial Differences in Cancer Susceptibility and Survival: More Than the Color of the Skin?* Trends Cancer, 2017. **3**(3): p. 181-197.