Virtual Biopsies: Revolutionizing Non-Invasive Histopathology with AI

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Abstract

The paradigm in oncological diagnostics is shifting toward non-invasive, data-driven tissue characterization—collectively termed virtual biopsies. Although Physical biopsies remain the gold standard, their clinical utility is limited by procedural risks, sampling biases, and an inability to fully capture tumors spatial and temporal heterogeneity.

This chapter traces the rapid evolution of virtual biopsies, from whole – slide imaging (WSI) to advanced radiomics, pathomics, and deep learning frameworks – including convolutional neural networks (CNNs), transformers, and generative models (e.g., GANs, diffusion models). These techniques extract high-dimensional, sub -visual features from clinical images (MRI, CET, PET, OCT), producing digital biomarkers for precise tumor volumetrics and real-time monitoring of progression and treatment response.

We detail the technical workflow—from automated image segmentation to predictive modeling—showcasing AI's >90% concordance with histopathology for cancer subtyping and Gleason grading. Integrating with liquid biopsies (e.g., ctDNA + radiomics) enables robust multimodal diagnostics, potentially reducing invasive procedures by 30% and advancing personalized medicine.

Despite challenges like data standardization, AI's black-box opacity (requiring explainable AI), and rigorous regulatory validation (e.g., FDA approval), virtual biopsies herald a holistic, dynamic, patient – centered shift in precision medicine. This revolution promises to streamline clinical decision – making and enhance long – term outcomes.

Key highlight:

- **Challenges highlighted:** Standardization ensures interoperability; explainable AI builds trust; FDA validation confirms safety/efficacy.
- **Key Benefits:** Holistic (multimodal integration), dynamic (real-time tracking), patient centered (fewer invasive).
- **Impact:** Optimizes decisions, improves prognosis via longitudinal monitoring.

Keywords: Virtual Biopsy, Precision Oncology, Artificial Intelligence, Machine Learning, Non-invasive Diagnostics, Tumor Heterogeneity, Medical Imaging, digital pathology, Explainable AI (XAI), Computational Pathology.

1. Introduction:

Physical biopsy is considered the gold standard for the diagnosis of diseases, such as cancer or cirrhosis. However, it is an invasive procedure that is likely to be biased (cutting a piece of a significantly large organ) and carries the risk of complications. Virtual biopsy may be defined as the use of advanced imaging (MRI, CT, PET) with radiomics and artificial intelligence to extract microscopic data based on macroscopic data. It allows clinicians to observe the cell structure of a tissue without necessarily having to cut the skin. The diagnosis, classification, grading, and prognostic evaluation of disease involves a lot of the role of histopathology.[1] The traditional steps involved ithediagnosticic process includevetissuremovall, by either biopsy or surgical excision, tissue fixation, processing, sectioningstainingnng and microscopic examinatiobybe pathologists. Despite being a highly informative process, it is characterised by several limitations, including the invasiveness of the process, occurrence of complications, sampling error, and delays in making the diagnosis. Digital pathology, together with advanced medical imaging and artificial intelligence, has provided new possibilities for the non-invasive characterisation of tissues. One of these innovations is virtual biopsy, which is considered a revolutionary concept because it aims to reconstruct histopathological information based on the use of computational and imaging methods. The most appropriate terminology that can be used in the process is virtual biopsy when extracting histological, molecular, and microstructural information from radiological images or label-free imaging procedures is performed using advanced computational analysis. [2] With the rapid development of image analysis using AI-based applications, virtual biopsies will be increasingly able to predict the histology of tumours, molecular changes, and treatment response with regard to imaging data. It is an emerging science that overlaps radiology, pathology, genomics, and computational sciences and has become a promising alternative to conventional tissue sampling in several clinical environments. [3]

2. Virtual biopsy and its conceptual overview:

Several technological components are used together in the process of converting traditional medical imaging data into a virtual slide. One of the most important is called radiomics, and it involves the extraction of quantitative imaging features, such as texture, shape, and intensity schemes, which are not visible to the human eye. High-dimensional features enable the characterisation of the microstructure and heterogeneity of tissues. Another important component is deep learning (DL). Medical images are coupled with the histopathological results of biopsies in paired datasets to train convolutional neural networks (CNNs). Under monitored learning, models can learn to recognise imaging patterns related to specific pathological states, and disease signatures are automatically predicted.[4] In addition, multi-parametric imaging analysis is useful in the generation of virtual slides. It is possible to construct a more detailed biological and structural portrait of the tissues using magnetic resonance imaging (MRI) and adding multiple imaging sequences, for example, T1- weighted, T2- weighted, and diffusion-weighted sequences. T1- weighted, T2- weighted, diffusion-weighted sequence, etc. This multifaceted method enables to expand the possibilities of the simultaneous capturing of multiple physiological and morphological data with the opportunities of a single imaging session. Generally, virtual biopsy is a phrase to refer to a form of non-invasive diagnosis, wherein medical imaging data are processed with strong computational algorithms to gain information about a tissue level, which, until recently, has been made available by a histopathological examination of a physical biopsy sample.[5]. Compared to the conventional methods of biopsy, where tissues are extracted either surgically or with a needle, virtual biopsy will use a combination of state-of-the-art imaging technologies and quantitative extraction of visual characteristics in images, machine learning and deep learning algorithms, and combining multimodal data. Such data may be radiological data, histopathological data and even genomic or molecular data, and may be employed in order to gain a more intricate understanding of tissue characteristics. These properties can provide both fine structures and tissue heterogeneity that cannot be easily recognised using conventional visual analysis techniques. Virtual biopsy is a multidisciplinary field involving radiology, digital pathology, computational biology, and artificial intelligence. Virtual biopsy hence a multidisciplinary radiology-digital pathology-computational biology and artificial intelligence flow. The given approach will transform the nature of the way the diagnostic process is performed, as it allows to perform computational analysis of the properties of the tissue with no need to resort to the invasive procedure, and offer the process of the precision medicine evolution.[6]

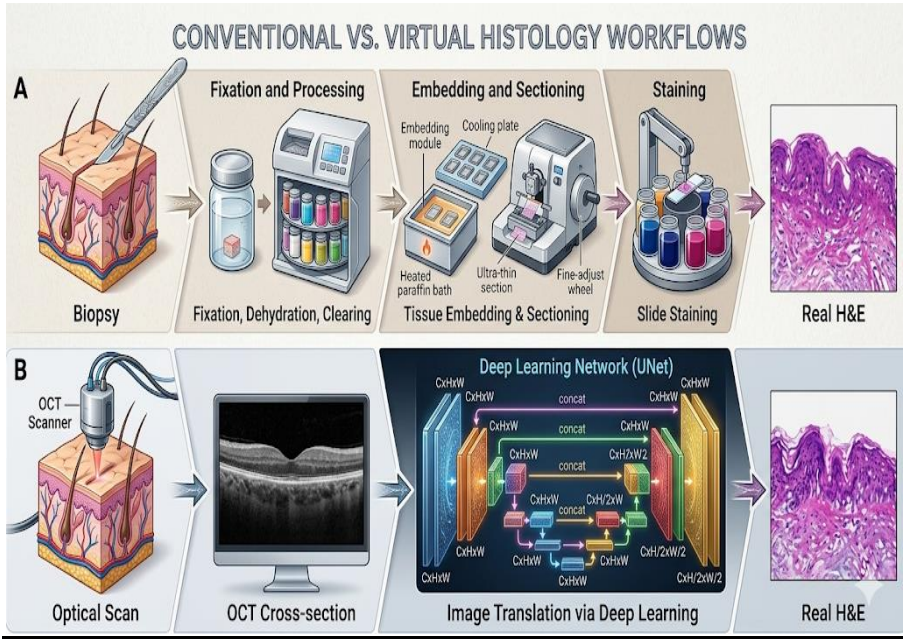

Figure: 1- Traditional biopsy versus virtual biopsy. (A) In a traditional biopsy, tissue is first excised and then undergoes multiple steps including fixation, dehydration, clearing, embedding, sectioning, and staining to yield 2D H&E sections, which can be examined under a microscope. (B) For a virtual biopsy, an OCT scan of tissue is acquired, and a trained neural network transforms the 2D OCT image into a corresponding H&E-like image. Credit: *Science Advances* (2024).

3. Modern applications in different fields

In the field of oncology, virtual biopsy solves the problem of temporal and spatial heterogeneity. A physical needle biopsy only captures approximately 1/1, 000, 000 of the tumour volume. AI-driven imaging is applied to the entire tumour mass. Molecular Subtype Prediction: Models, such as the DNA methylation foundation model (MethylFM), have shown MRI radiomic patterns linked to epigenetic regulatory dynamics in 2025–2026. For example, virtual biopsies are now used to predict the efficacy of PD-1/PD-L1 inhibitors in NSCLC [7]. By monitoring changes in texture over time (so-called delta-radiomics), AI identifies so-called pseudoprogression when a tumour becomes larger on a scan because of the infiltration of immune cells before medical professionals prematurely end an effective treatment.[8]

Eliminating (Non-Alcoholic Steatohepatitis) NASH in Hepatolog Multi-parametric

(a). Ric ultrasound (MPUS): MPUS is an innovative breakthrough in the field of non-invasive diagnostics that combines three unique physical dimensions of tissue into a single, artificial intelligence-driven biological map. Although conventional B-mode imaging can provide the underlying anatomical context, current ensemble models (usually with transformer-based structures) can concurrently process high-dimensional data in shear wave elastography (which measures mechanical stiffness by using acoustic push pulses) and in attenuation imaging (which measures the attenuation of waves passing through lipid-rich cells). [9] The AI can learn from a large number of data, in which such acoustic signatures are associated with the "ground truth" needle biopsy, and thus can disentangle confounding factors such as active inflammation or congestion with chronic scarring. Therefore, with these scores, as of 2026, the sensitivity of a diagnostic gray zone has reached a historic landmark of 0.97, which transcends between early-stage fibrosis (F1-F2) and end-stage cirrhosis, thus enabling clinicians to track disease progression or treatment response with a certain degree of granular accuracy, previously only possible by invasive surgical sampling.[10]

(b). Fragmentome revolution- The Fragmentome Revolution represents a paradigm shift in molecular diagnostics by utilizing Artificial Intelligence to decode the non-random patterns of cell-free DNA (cfDNA) shed into the bloodstream, a technique formally known as "Liquid-Virtual Hybrid Biopsy." Unlike traditional liquid biopsies that focus on specific genetic mutations, fragmentomicsanalyzes the size, distribution, and genomic positioning of DNA fragments is a digital signature dictated by the chromatin structure of the original tissue.[11] A study at Johns Hopkins demonstrated that AI models, specifically deep learning architectures trained on vast genomic landscapes, can identify the unique fragmentomic pattern of liver-derived DNA under the stressof fibrotic remodellingg. By integrating these circulating signals with existing imaging data, this hybrid approach transcends the limitations of standard blood markers or ultrasound alone, achieving unprecedented precision in detecting cirrhosis and mapping the trajectory of liver scarring without the need for a single tissue-penetrating needle.[12]

Nephrology: A significant success in the implementation of virtual day-zero biopsies with its mass application in transplant medicine is to control a long-standing chronic organ shortage problem by limiting the so-called cold

ischaemia time–the time an organ remains outside the body.[13] Traditionally, a wedge biopsy and the presence of a standby pathologist to reportinterstitiallfibrosissandtubularratrophyy (IFTA,)whicht can require hours and has a high inter-observer coefficien,t were used to determine the quality of a donor kidne [14]y. [14]By 2026, global registries, including the United Network for Organ Sharing (UNOS), have been drawn toward certifying AI-based virtual appraisals that bypass such delays. Systems can now predict a history of chronic hypertension by consuming 11 core parameters of a donor, including serum creatinine, age, body mass index (BMI), and a history of chronic hypertension, to generate a predictive histological score with the assistance of ensemble machine learning models, specifically random forests owing to their stability and gradient-boosted accuracy. With these models having a multi-AUC (area under the curve) of 0.83, chronic tissue damage is very well estimated and is, in effect, a surrogate of microscopic analysis. This enables transplant surgeons to make binary choices randomly at the moment of offer and thus reduces the number of rejected organs and provides better quality kidneys to the recipients earlier and in a better physiological condition. [15]

Virtual staining in dermatology: The Generative AI Histology represents a paradigm shift in diagnostic dermatology by literally bridging the gap between non-invasive bedside imaging and the ultimate diagnostic benchmark of pathology. [16] The core aspect of this technology is conditional generative adversarial networks (cGANs) and sophisticated convolutional neural networks (CNNs), which are trained using large sets of paired images and their corresponding physical biopsy slides of grayscale Reflectance Confocal Microscopy (RCM). Although RCM enables clinicians to visualise skin cells in their living conditions at approximately 1000 × magnification, the produced images are black and white and have a steep learning curve for those accustomed to the pink-and-purple colour scheme of a typical haematoxylin and eosin (H&E) stain. This can be solved with AI-based virtual staining, which works by performing a pixel-to-pixel translation: the model identifies certain cellular structures, for example, nested melanocytes in a melanoma or keratinocyte patterns in a carcinoma, and applies digital stains that simulate the affinity of H&E.[17] This means that in vivo histopathology can be conducted with an example, wherein a dermatologist can analyse a suspicious lesion in real-time. Without a scalpel, the process eliminates the risk of scarring and infection, provides immediate diagnostic feedback to the patient, and a patient with lesions that are genuinely malignant only undergoes surgical excision; thus, the number-needed-to-biopsy ratio in clinical practice is greatly reduced.[18]

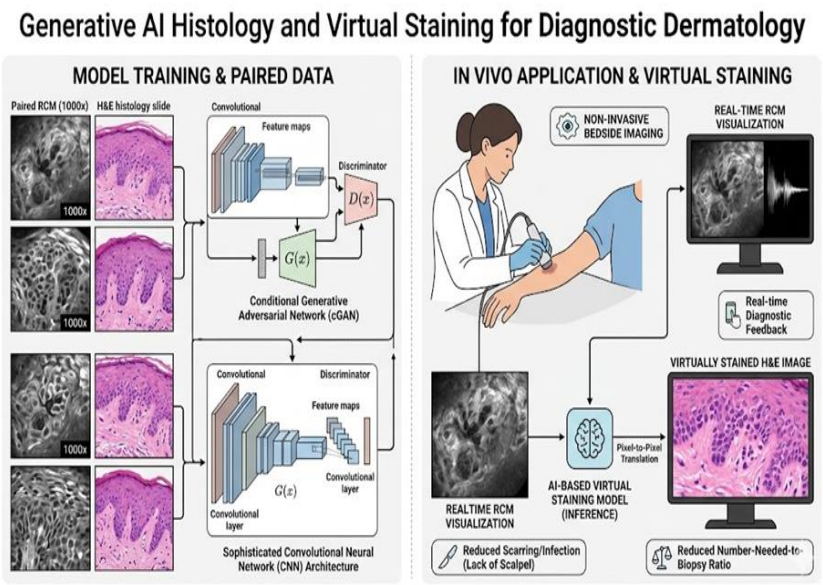

Figure: 2- This diagram illustrates how non-invasive bedside imaging, like Reflectance Confocal Microscopy (RCM), is transformed using deep learning into a diagnostic benchmark. By training Conditional Generative Adversarial Networks (cGANs) on large sets of paired grayscale RCM images and physical H&E biopsy slides, the AI model learns to perform virtual staining.This in an AI generated image.

Conclusion: The transformative trajectory of diagnostic medicine emphasises that virtual biopsy is not merely a replacement for the needle, but a sophisticated evolution toward continuous, non-invasive digital monitoring. By synthesising macroscopic imaging with microscopic cellular architecture through AI-driven digital staining and fragmentomic signatures, the field is successfully addressing long-standing challenges related to sampling bias and tissue heterogeneity. This shift toward real-time clinical decision-making—exemplified by virtual organ triage and in-vivo skin histology—represents the pinnacle of precision medicine, significantly reducing patient risk and cold ischaemia times while increasing diagnostic accuracy. Ultimately, the integration of these AI models into a comprehensive digital framework marks the dawn of a more humane, data-rich era in which the boundaries between radiology and pathology dissolve, allowing for predictive simulations that ensure personalised treatment is delivered with unprecedented speed and granular detail.

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