




# Unveiling tumor invasiveness: enhancing cancer diagnosis with phase-contrast microtomography for 3D virtual histology

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**Abstract** Malignant diseases are characterized by a critical trait known as invasiveness, where tumor cells tend to spread from the primary tissue layer into surrounding healthy tissues and distant organs. Presently, histopathology offers essential insights for diagnosing, classifying, predicting outcomes, and guiding patient-specific treatments. However, histology offers two-dimensional data from chosen cutting planes. Although 3D histological volumes can be generated through serial sectioning or whole slide imaging, this method is laborious, may introduce processing artefacts, and lacks isotropic spatial resolution. These limitations pose a considerable challenge to accurate diagnoses, particularly when dealing with micro-infiltrating carcinomas. These lesions, characterized by minute infiltrations, demand a three-dimensional representation for comprehensive visualization, essential for precise identification and assessment. Emerging X-ray-based virtual histology technology offers three-dimensional visualization of soft-tissue specimens, enabling virtual slicing in any direction or at any point. This approach can assist in guiding tissue sectioning for optimal representation of tumor cross sections during histological analysis. Micro-infiltrating carcinomas from the breast, cervix, and thyroid were imaged using X-ray phase-contrast microtomography (PhC- $\mu$ CT) at the Elettra synchrotron facility in Trieste, Italy. Comparative assessment of histological and CT slices by pathologists revealed that PhC- $\mu$ CT aids in classifying lesions by highlighting distinct tissue components and, notably, identifying tissue invasion. Reviewing a volume image allows pathologists to trace the entire lesion, identifying invasion sites that might be overlooked in individual or serial histological sections. Consequently, this proposed method could complement pathologists' tools, potentially enhancing diagnoses by minimizing under-staging and reducing false negative results.

## 1 Introduction

Epithelial cells form the linings or coverings of various organs and tissues in the body, they are found in the skin, respiratory tract, digestive system, and other body organ, and serve as a protective barrier. The term “carcinoma” specifically denotes cancers that arise from epithelial tissue, comprising the majority, approximately 90%, of all cancer types [1]. These cancers display distinct

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morphological changes during their progression. Notably, as a benign tumor transforms into a malignant and invasive carcinoma, the tumor cells undergo a shift in appearance and behavior [2]. Initially, benign tumors typically consist of well-differentiated cells, maintaining an organized structure similar to the tissue from which they originate. However, as these tumors progress to a malignant state, the cells undergo alterations in their morphology, from a highly differentiated, epithelial morphology to a more migratory and invasive phenotype [3, 4]. This change signifies a critical shift, enabling the tumor cells to become metastatic [5, 6]. Invasive carcinoma cells possess the ability to breach the basal lamina barrier [7, 8], a structure separating epithelial and connective tissues, allowing them to invade neighboring tissues [2]. This invasion into adjacent tissues signifies a pivotal stage in the cancer progression, as it marks the tumor's capacity to spread beyond its original location, significantly impacting the severity of the disease and the related treatment approaches. The progression of carcinoma across organs like the breast, cervix, and thyroid shares similar patterns, highlighting a parallel shift from benign to more aggressive forms despite their different locations within the body.

Breast carcinoma holds a significant place in oncology, being one of the most prevalent cancers worldwide [9, 10]. Globally, breast carcinoma stands as one of the most commonly diagnosed cancers in women, with varying incidence rates based on geographical, socio-economic, and lifestyle factors [11]. According to estimations, millions of new cases are reported annually [9]. Breast carcinoma encompasses various types [12], among which invasive ductal carcinoma (IDC) and invasive lobular carcinoma (ILC) are predominant [13]. Invasive ductal carcinoma, the most common form, involves cancer cells breaking through milk duct walls and invading surrounding breast tissue. Conversely, invasive lobular carcinoma tends to spread in a single-file pattern, making it challenging to detect via imaging techniques. This form of spread does not cause the distinctive mass formation typical of other carcinomas, making it less conspicuous on imaging scans like mammograms. As a result, its diffuse and infiltrative growth pattern can often escape detection or appear as less defined abnormalities on imaging. Cancer cells possess the ability to infiltrate nearby lymph nodes and, in advanced stages, spread to distant organs. Detecting non-invasive forms in breast cancer before they become invasive, is crucial for preventing the spread of cancer cells beyond the breast tissue. This allows for more conservative treatment options and higher survival rates. Worldwide screening programs often use mammography as the primary tool [14], supplemented by additional imaging or tests in some regions. However, the efficiency of mammography can vary based on factors like breast density, posing challenges in accurately identifying invasive forms [15].

Cervical carcinoma is among the most common gynecological malignancy globally [16, 17]. It presents a distinct profile among gynecological cancers and is closely linked to persistent human papillomavirus (HPV) infection [18, 19]. Its incidence rates vary significantly worldwide, with higher prevalence in regions where screening programs are limited or HPV vaccination is less accessible [20]. Cervical cancer staging employs the FIGO (International Federation of Gynecology and Obstetrics) system [21], that categorizes the disease based on factors like tumor size, depth of invasion, and lymph node involvement, guiding treatment strategies and predicting outcomes. Cervical carcinoma's invasive nature refers to its ability to progress from the cervix into deeper layers and adjacent tissues. The malignancy typically begins as pre-cancerous changes (cervical intraepithelial neoplasia - CIN [22]) before developing into invasive carcinoma. These carcinomas can be broadly classified into two main types based on their cellular origin and characteristics: squamous cell carcinoma (SCC) and adenocarcinoma. The first one, accounting around 70–80% of cases, originates in the squamous cells lining the outer surface of the cervix (ectocervix). Chronic HPV infection, particularly with high-risk HPV types, is a significant risk factor for the development of SCC [18]. Adenocarcinoma develops from the glandular cells lining the cervical canal (endocervix), and can also have an HPV-related etiology [23]. Early detection of cervical cancer is crucial for intervention before lesions become invasive carcinomas, or allows effective treatments to prevent progression [24]. Global screening programs typically use pap smears or HPV testing, effective in detecting pre-cancerous changes but may not consistently predict invasive forms, emphasizing the need for more accurate diagnostic approaches.

Thyroid carcinoma [25, 26] is one of the most common endocrine cancers worldwide, though incidence rates vary geographically. For thyroid cancer, there is not a universal screening program due to the absence of a highly effective and practical screening test. Detection often occurs incidentally or during diagnostic evaluations for other medical issues [27], limiting the ability to identify invasive forms in the early stages. Over recent years, there has been an upward trend in the diagnosis of thyroid cancers [28], partly attributed to enhanced detection methods like imaging (e.g., ultrasound, radioactive iodine scanning, MRI) and fine-needle aspiration biopsies. This detection can lead to surgical interventions, potentially preventing the progression to invasive forms and reducing the need for more aggressive treatments. Thyroid carcinomas represent malignancies originating in the thyroid gland, affecting the butterfly-shaped organ located at the base of the neck. Various types of thyroid cancer exist, each exhibiting unique characteristics and behaviors [29, 30]. Papillary thyroid carcinoma is the most common type (80%), often presents well-defined papillary structures when examined under a microscope, and typically carries a relatively favorable prognosis compared to other thyroid cancer types. Follicular thyroid carcinoma (FTC) originates from thyroid follicular cells. Histologically, FTC displays a follicular growth pattern and may invade blood vessels, distinguishing it from benign follicular adenomas. Thyroid carcinomas, when invasive, can breach the thyroid's capsule, invading nearby tissues and structures [30]. In more advanced stages, certain types can metastasize, spreading to lymph nodes or distant organs like the lungs or bones [31].

Distinguishing between invasive and non-invasive forms of carcinomas is crucial for guiding treatment decisions, predicting prognosis, and tailoring therapies to the specific characteristics of the cancer [32–34]. Identifying invasive behavior informs clinicians about the cancer's spreading potential, allowing for more effective management and follow-up. This differentiation ensures that patients receive appropriate treatments, minimizing unnecessary interventions and optimizing overall outcomes. Histopathological evaluation [35, 36] plays a pivotal role in this process, providing essential insights into the behavior of the tumor and shaping a

personalized approach to patient care. However, histological assessment is a complex task as it involves a multi-step procedure including tissue fixation, slicing, staining, imaging, and analysis. To achieve this, highly specialized personnel, costly equipment and a significant amount of time are needed, thus hindering prompt diagnosis and treatment [37]. Resource-intensive processes and limited accessibility to advanced facilities contribute to delays and operational hurdles, straining healthcare resources. Moreover, histology, is intrinsically two dimensional. While serial sectioning, i.e., the analysis of multiple sections of the same sample along its depth, provides partial depth details, it necessitates slicing, preparing, imaging, and reconstructing multiple sections, adding considerable time and expenses. Additionally, the slicing process might distort tissues or cause cutting artefacts [38]. Importantly, a notable limitation is understanding the invasive nature and its diffusion in the surrounding tissue, especially in biopsy analysis due to restricted tissue samples available. This issue is particularly significant for in-situ breast carcinomas. In such situations, the capacity to conduct thorough immunohistochemistry is crucial for delineating the molecular and cellular attributes of the cancer. Nevertheless, the histological processes use a substantial portion of the accessible tissue, reducing the amount available for vital complementary examinations.

The emergence of virtual histology addresses limitations inherent in traditional histopathology. Virtual histology, through techniques such as X-ray Phase-Contrast microtomography (PhC- $\mu$ CT) [39], offers non-destructive, three-dimensional imaging capabilities with high sensitivity to soft tissues [40–43]. This approach allows for detailed visualization without the need for invasive tissue preparation required in serial sectioning or the limitations of two-dimensional analysis, allowing specimens to be virtually sliced at any point and in any direction. In cancer diagnosis and research, virtual histology can provide a more comprehensive understanding of tissue structures, aiding in better-informed assessments and potentially more precise diagnosis [37, 44]. Virtual histology can enable guided sectioning of tissues in histological analysis for selecting the most suitable cutting plane when dissecting specimens and selecting histological sections, to optimally obtain the largest/most representative cross section of the tumor [45].

In this context, infiltrating carcinomas of the breast (invasive ductal carcinoma), cervix (squamous cell carcinoma), and thyroid (follicular thyroid carcinoma), were acquired by means of PhC- $\mu$ CT at the synchrotron facility Elettra (Trieste, Italy) [46]. Through a meticulous one-to-one comparison between histological and co-registered tomographic slices, pathologists could assess the capabilities of PhC- $\mu$ CT in characterizing the morphology of lesions and different tissue components, with a specific focus on tissue invasion.

## 2 Materials and methods

### 2.1 Tissue sample preparation

Post-operative tissue samples of breast, cervix, and thyroid, are processed for histology: fixed in 10% neutral-buffered formalin for 24 h, dehydrated using alcohol and xylene, and embedded in paraffin wax to form tissue blocks measuring 3.0 cm  $\times$  2.5 cm  $\times$   $\sim$ 0.5 cm. Microtome sectioning (performed after the tomographic acquisitions) creates slices of about 4–5  $\mu$ m on glass slides, followed by staining with hematoxylin and eosin (H &E). This staining combination highlights structures by coloring the nuclei blue/purple with hematoxylin and giving a pinkish hue to eosinophilic elements like cytoplasm, collagen, and muscle fibers. All resulting histological images are digitized using a D-Sight F 2.0 slide scanner, ensuring consistent acquisition conditions with a magnification of 20 $\times$  and 0.5  $\mu$ m pixel size.

### 2.2 Comparative histopathology: distinguishing invasive versus non-invasive carcinoma

The distinction between invasive and non-invasive ductal carcinoma lies in the presence or absence of invasion through the basal lamina [47]. Non-invasive ductal carcinoma, also known as ductal carcinoma in situ (DCIS), involves abnormal cells confined within the milk ducts without breaching the basal lamina into the surrounding tissue. These abnormal cells have not invaded the adjacent healthy tissue; and therefore, the basal lamina remains intact. In accordance to the guideline published by the American Joint Committee on Cancer, this cancer is classified as minimally invasive if the infiltrative lesion is less than 1 mm [48]. In contrast, in the invasive ductal carcinoma (IDC) case, cancer cells extend beyond the ducts and infiltrate the surrounding stroma or tissue. This invasion through the basal lamina is a critical factor that differentiates IDC from DCIS in histopathological analysis. Identifying the presence of invasive patterns through detailed microscopic examination of tissue samples helps pathologists distinguish between these two forms of ductal carcinoma, allowing for accurate diagnosis and appropriate treatment planning.

In histopathology, the distinction between invasive and non-invasive forms of squamous cell carcinoma (SCC) of the cervix lies in their penetration of the basement membrane, akin to the basal lamina in breast tissue. Non-invasive squamous cell carcinoma, often referred to as carcinoma in situ or high-grade squamous intraepithelial lesion, indicates abnormal cell growth confined within the epithelial layer of the cervix. Typically, the basement membrane remains intact in non-invasive SCC. Conversely, invasive squamous cell carcinoma is characterized by the presence of cancerous cells extending beyond the epithelial layer and infiltrating the connective tissue.

The differentiation between invasive and non-invasive forms of follicular thyroid carcinoma (FTC) is determined by their invasion of structures beyond the thyroid capsule, including vascular invasion. Due to its tendency to invade blood vessels, FTC is more

likely to metastasize to distant organs rather than to regional lymph nodes, thus resulting in hematogenous dissemination [49]. Non-invasive FTC signifies abnormal cell growth confined within the thyroid gland, enclosed by the fibrous capsule, without penetrating the capsule or invading adjacent structures such as blood vessels. Typically, the fibrous capsule remains intact in non-invasive cases, and there's no evidence of cancerous cells spreading into blood vessels. Conversely, invasive FTC involves the penetration of cancerous cells through the thyroid capsule, extending beyond its boundaries. FTC is generally sub-divided into widely and minimally invasive FTC (WI-FTC and MI-FTC) [50]. MI-FTC is limited to microscopic capsular and/or vascular invasion, are uniformly considered to be low risk and can be treated with local resection alone. On the contrary, in WI-FTC cancer cells penetrate the thyroid capsule and invade surrounding tissues extensively, including adjacent structures or organs, showing a more aggressive behavior. These cases might necessitate complete thyroid removal and additional therapy to mitigate regional recurrence or potential distant spreading, following an assessment of evolving clinical risks [30]. Thyroid nodules are a common occurrence and can frequently grow to substantial sizes. The examination of the nodule's capsule is a crucial step to accurately categorize it clinically. Histopathological assessment plays a crucial role in delineating these invasive patterns, but challenges arise, particularly in larger lesions where evaluating the entire nodule's capsule becomes intricate. This difficulty poses a risk of missing critical prognostic insights crucial for effectively managing the patient's condition and treatment plan. It is imperative to overcome these challenges to ensure comprehensive assessment and informed decision-making in patient care.

### 2.3 Experimental PhC- $\mu$ CT setup

X-ray PhC- $\mu$ CT scans of the paraffin-embedded samples were performed at SYRMEP beamline of Elettra using the propagation-based phase-contrast imaging modality [51]. The polychromatic X-ray beam was produced by a bending magnet and filtered with 1.0 mm of silicon (Si), resulting in an average energy of 20 keV. The imaging system used an sCMOS detector (Hamamatsu ORCA-Flash 4.0 model C11440-22C) with a 6.5  $\mu$ m pixel size and a sensitive area of 2048 $\times$ 2048 pixels. The sensor was paired with magnifying optics, allowing to adjust the effective pixel size from 1 to 6.5  $\mu$ m. X-ray conversion was accomplished through a 45  $\mu$ m thick GGG:Eu scintillator. In this particular study, an effective pixel size of 4  $\mu$ m was selected, generating images with a lateral field-of-view (FOV) of approximately 8 mm in diameter. The resolution of the imaging system at the selected pixel size was measured in a previous study and is approximately equal to 10  $\mu$ m [52]. Each block of paraffin-embedded tissue was mounted vertically, with the longest dimension parallel to the acquisition system's rotation axis. The image acquisition consisted of 1800 evenly spaced projections acquired on a 180 degrees rotation, each with an exposure time of 200 ms per projection. To optimize soft-tissue visibility and spatial resolution, the propagation distance was set at 500 mm, validated through a prior optimization study [52]. The source-to-sample distance was fixed at 22.3 m. Since the detector's FOV was smaller than the sample size, local-area CT datasets of regions of interest delineated by pathologists were performed. Reconstructed tomographic images were then stitched together, yielding a full volume visualization of the region of interest ("Mosaic tomography" [53]). Notably, PhC- $\mu$ CT scanning did not necessitate any specific sample preparation, hence being compatible with subsequent histological investigations. With the set acquisition parameters, no noticeable radiation-induced damage was observed.

The acquired projections underwent initial processing, involving conventional flat-fielding image correction and the removal of ring artefacts. Subsequently, phase retrieval was applied by using the Homogeneous Transport-of-Intensity-Equation (TIE-Hom) algorithm [54] with a  $\delta/\beta$  filter parameter set at 350. The resulting data underwent reconstructions through a Filtered-back Projection algorithm and Shepp-Logan filtering using Syrmep Tomo Project, an open source software suite designed for the beamline [55]. Multiple scans of the same specimens are stitched together using Avizo<sup>®</sup> 9.3 software version.

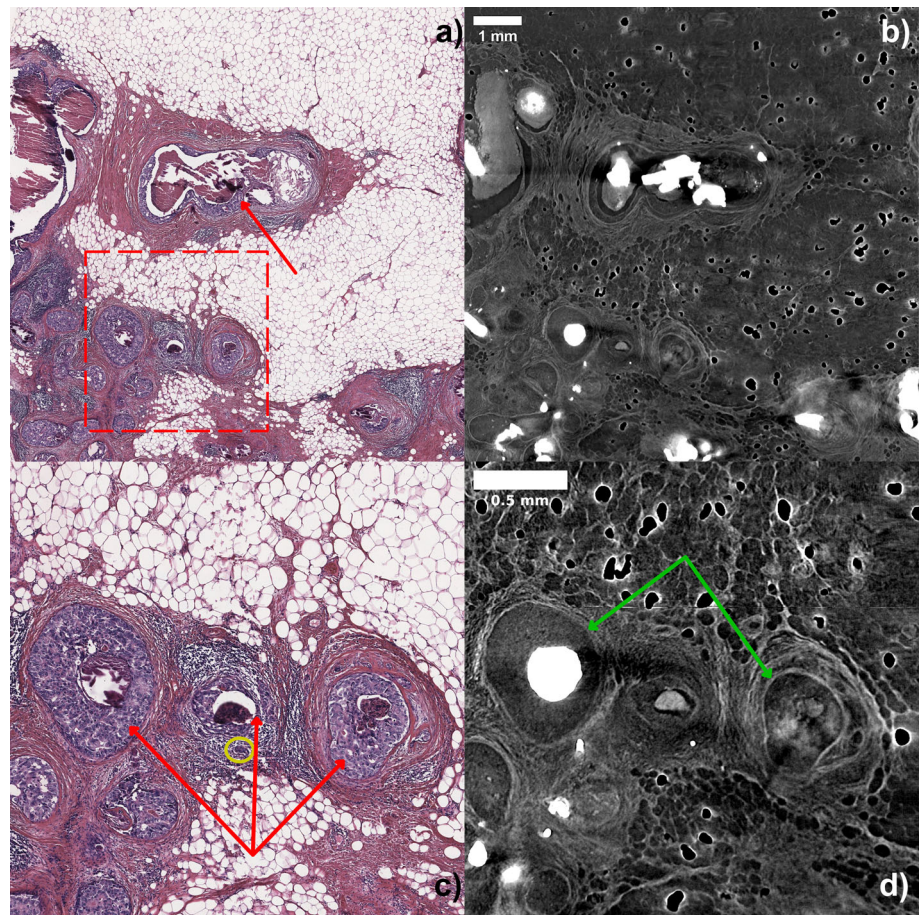
## 3 Results

Results are subdivided in three subsections for breast, cervix, and thyroid tissues, respectively. Each subsection presents a detailed comparison between traditional histopathology and virtual histology. The purpose of the comparison is not to establish the superiority of PhC-CT over histology, but to emphasize its inherent ability to provide morphological details essential for assessing potential invasion and fundamental to diagnostic evaluation, with the added value of a three-dimensional representation of the whole tissue rather than one or a few 2D sections. Through the juxtaposition of PhC-CT images with their histological counterparts, readers are offered a comprehensive understanding of tissue morphology as obtained with both techniques.

### 3.1 Invasive ductal carcinoma of the breast

Figure 1 shows the visual comparisons between a histological section and the corresponding PhC- $\mu$ CT image. This comparison illustrates the distinct features of non-invasive ductal carcinoma (DCIS) and invasive ductal carcinoma (IDC). The image shows a section of breast tissue where the adipocytes (that appear empty with a thin rim of cytoplasm close to the basal lamina) and some milk ducts can be observed (rounded structures in the middle part of the image). The central one (see Fig. 1c) has the characteristics of a micro-invasive carcinoma, where small clusters (inside the yellow ellipse) of tumor cells, accompanied by inflammatory cell infiltration in the stroma, are observed. The surrounding ductal carcinoma in situ (DCIS, as indicated by the red arrows) showcases

**Fig. 1** Comparison between histology (a) and PB- $\mu$ CT (b) of a section of a breast tissue showing three milk ducts. Panels c and d are the zoom of the region contoured with the red dashed lines in (a). The intact basement membrane (indicated by green arrows in panel d surrounds the lateral duct, while is partially broken in the central one). The histological features of a case of micro-invasive carcinoma show small clusters of tumor cells (highlighted in yellow in panel c together with infiltrates of inflammatory cells in the stroma. Nearby, the ductal carcinoma in situ (red arrows) displays high nuclear grade and comedo-type necrosis with dystrophic calcification, visible under H&E staining. Dark rounded objects surrounded by intense bright contours in the PB- $\mu$ CT image correspond to air bubbles within the paraffin

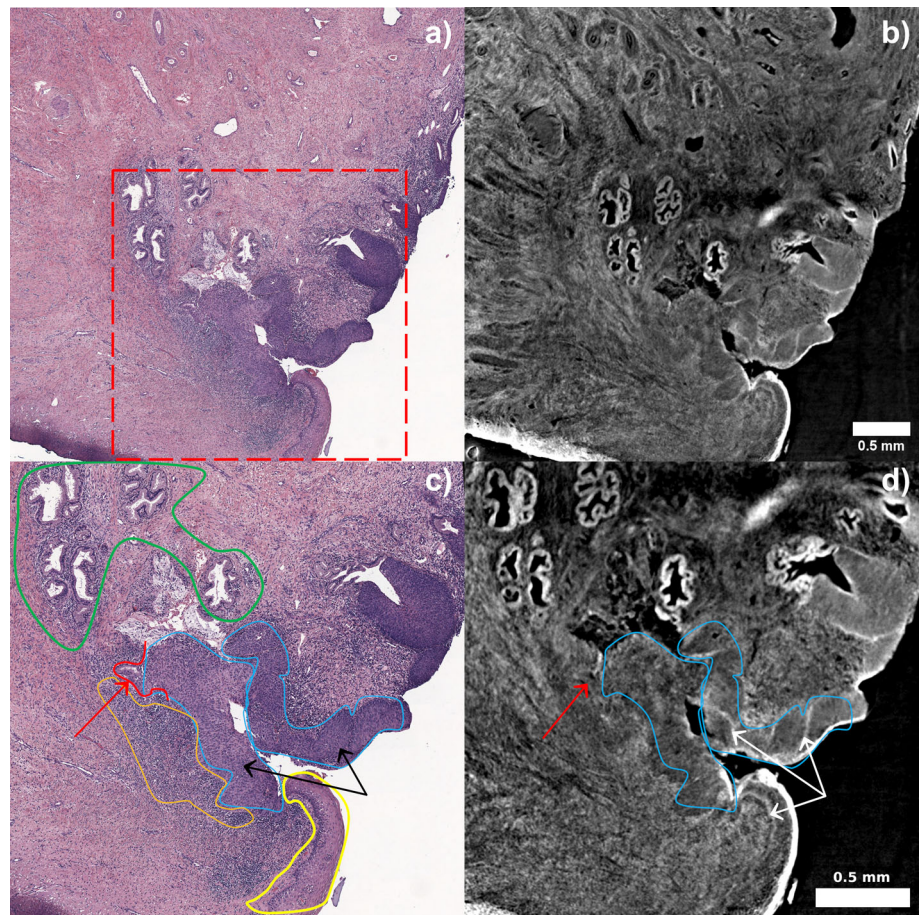


high nuclear grade, comedo-type necrosis, and dystrophic calcification (as observed in the H&E stain). In PhC- $\mu$ CT, while the direct identification of invasive cell clusters might not be feasible, the visualization of the basal lamina remains pivotal. This structure is notably depicted as a brighter filament in the CT image (see green arrows), making it a discernible morphological element for distinguishing between IDC and DCIS, as the presence or absence of disruptions in the basal lamina serves as a critical indicator. This characteristic becomes distinctly visible through PhC- $\mu$ CT. In addition, it is important to note that the histological section, obtained by cutting the tissue post-tomographic acquisition, might occasionally miss the calcifications clearly visible in the CT image (see the very bright spot corresponding to a large calcification that is absent from the histological image). This discrepancy is a common artefact that can arise during the tissue sectioning process using a microtome for histopathological examination. Multiple air bubbles can be observed in tomographic slices, appearing as dark structures encircled by bright contours. These are attributed to phase artefacts resulting from the significant interface between air and paraffin or tissue. Notably, these bubbles are often observed within adipose tissue, which appears as a spongy-like structure. Air trapping in adipose regions during tissue embedding in paraffin poses a common challenge in histology labs due to the porous nature of adipose tissue, leading to significant air content. Immersion of tissues in liquid paraffin can result in the formation of bubbles within the paraffin block. Recent publications have proposed and discussed potential solutions to mitigate this artefact [56, 57].

### 3.2 Squamous cell carcinoma of the cervix

Figure 2 shows the visual comparisons between a histopathological section and the corresponding PhC- $\mu$ CT image of the cervical tissue. The closed line in green shows the glandular component of the cervix (clearly identifiable also in the CT image); while, the closed line in yellow shows the healthy stratified squamous epithelium lining it. The basement membrane's depiction as bright, distinct filaments within the PhC- $\mu$ CT images (white arrows) holds significant diagnostic value. Its visibility provides a crucial histopathological indicator aiding in the differentiation between non-invasive and invasive cervical squamous cell carcinoma. In non-invasive forms, the basement membrane remains intact, presenting as a continuous, uninterrupted structure. Contrastingly, in invasive cases, the breach or irregularity of this membrane signifies the infiltration of cancerous cells beyond the epithelial layer, indicating an advanced, invasive stage of the disease. By juxtaposing these images, we can observe the key histopathological features and how they correspond to the findings in the CT images. The figure illustrates an early stage of invasion (see the epithelial component

**Fig. 2** Comparison between histology (a) and PhC- $\mu$ CT (b) of a portion of cervical tissue. Panels (c) and (d) are the zoom of the region contoured with the red dashed lines in (a). This sample contains a very early stage of invasion (see the epithelial component that enters the stroma as indicated by the red arrows and the red line) of a cervical micro-invasive squamous cell carcinoma with a Grade 3 intraepithelial lesion (inside the closed blue lines). The closed line in yellow shows the healthy stratified squamous epithelium lining the cervix. The closed line in green shows the glandular component of the cervix. The orange line enclose an inflammatory infiltrate. The bright, distinct appearance of the basement membrane in PhC- $\mu$ CT images (indicated by white arrows) holds vital diagnostic significance, aiding the differentiation between non-invasive and invasive cervical squamous cell carcinoma

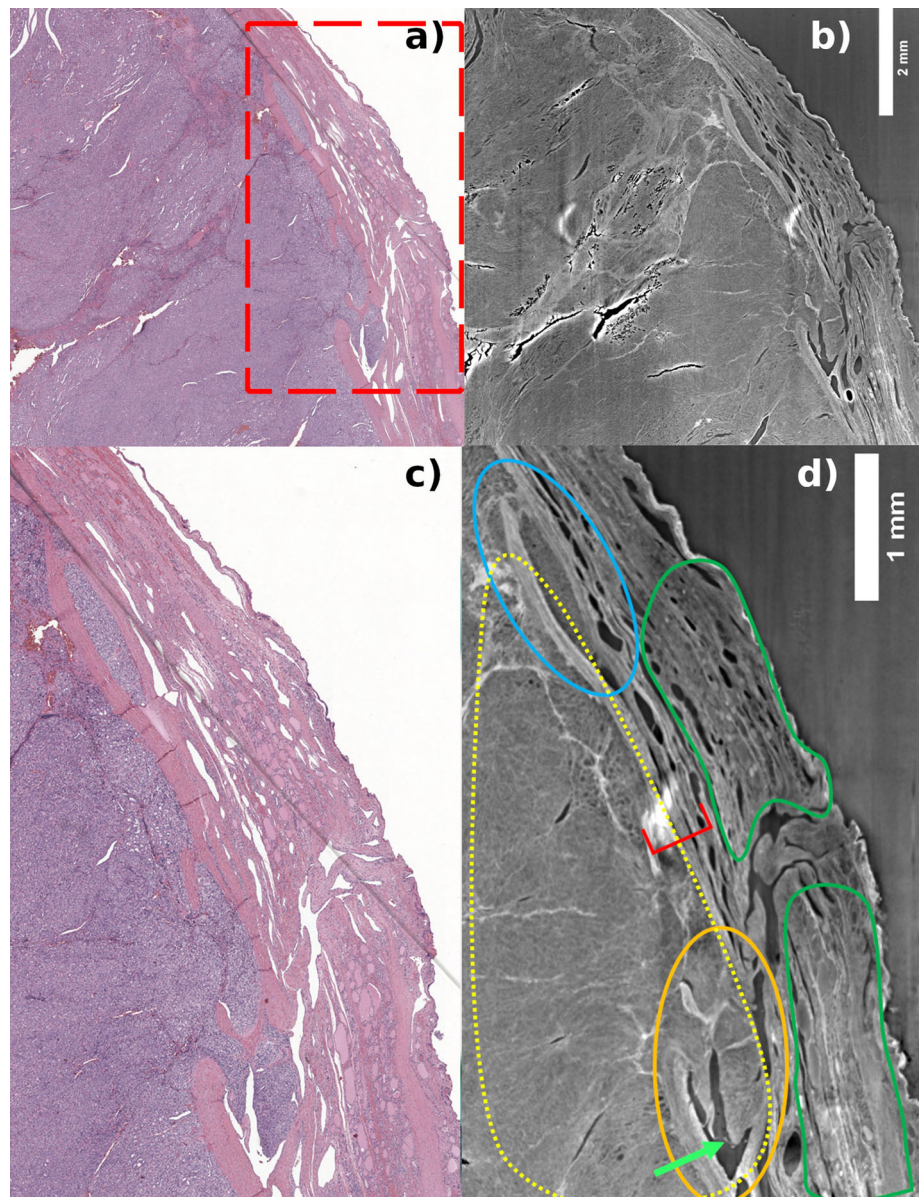


that enters the stroma as indicated by the red arrows and the red line), showcasing the emergence of micro-invasive squamous cell carcinoma within a Grade 3 cervical intraepithelial lesion (inside the closed blue lines). This image captures the transition from pre-cancerous changes to the early invasive phase, offering a detailed view of the evolving pathology. It visually delineates the critical point where abnormal cellular changes progress from remaining confined within the epithelial layer to infiltrating the underlying tissue (an inflammatory infiltrate is countered by the orange line). This presentation highlights the delicate phase of transition, aiding in the understanding of the histological nuances between pre-invasive and invasive states in cervical squamous cell carcinoma. However, unlike the breast tissue, the basement membrane's visibility in cervical tissue is less distinct. Achieving clearer visualization demands an additional acquisition at higher resolution specifically targeting selected regions of interest from the initial image captured at a larger pixel size. This additional step becomes necessary to obtain a more detailed and precise representation, crucial for accurate differentiation between non-invasive and invasive cervical squamous cell carcinoma. This investigation will be part of a future study and will employ a pixel size of  $1 \mu\text{m}$  and the half-acquisition modality [58] to double the field of view.

### 3.3 Follicular thyroid carcinoma

Figure 3 shows the visual comparisons between a histopathological section and the corresponding PhC- $\mu$ CT image of the thyroid tissue characterized by the presence of both capsular and vascular invasions. The comparison between the histological representation Fig. 3a, c with the PhC- $\mu$ CT images Fig. 3b, d illustrates the advantages of PhC- $\mu$ CT in categorizing the lesion. In fact, the PhC- $\mu$ CT imaging distinctly delineates various structures within the thyroid tissue: the parenchyma (marked by a green closed line), the tumor tissue (indicated by a closed yellow dotted line), and the fibrous capsule (highlighted with a red squared bracket). Notably, the orange and blue ellipses in the images correspond to capsular and vascular invasions, respectively, with the vessels visually denoted by the green arrow. Additionally, the PhC- $\mu$ CT images reveal a capsular de-lamination resulting from the neoplasm. Overall, PhC- $\mu$ CT provides an in-depth, 3D view of FTC tissues, enabling comprehensive inspection and analysis. Its ability to visualize structures like the capsule and invaded vasculature in a three-dimensional context aids pathologists in understanding tumor morphology and invasion patterns with unparalleled detail and accuracy (see the supplementary video S3).

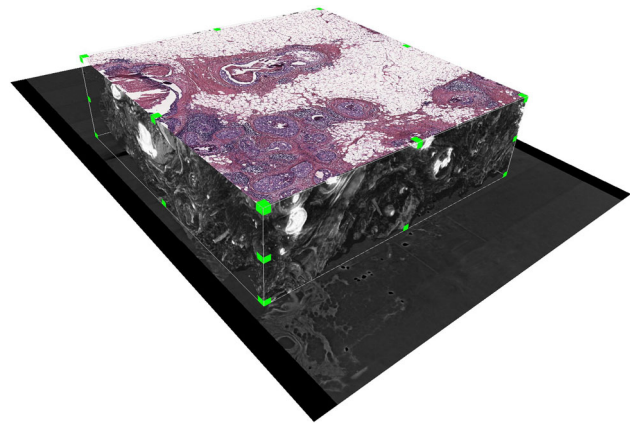
**Fig. 3** Comparison between histology (a) and PB- $\mu$ CT (b) of a sample containing a minimally invasive follicular thyroid carcinoma. Panels c and d are the zoom of the region contoured with the red dashed lines in (a). It can be noted the presence of the parenchyma (dark green closed line), the tumor tissue (closed yellow dotted line), and the capsule (red squared bracket). Orange and blue ellipses show, respectively, capsular and vascular invasions (the vessels are indicated by the green arrow) and a capsular de-lamination due to the neoplasm



### 3.4 Advantages of 3D imaging in lesion tracking

The 3D capability inherent in the proposed technique could play a significant role in facilitating lesion tracking within a volumetric space, as opposed to analyzing only a minute portion of the tissue. This feature is particularly highlighted in Fig. 4 where a histological slice, representing only a fraction of the tissue, is juxtaposed and aligned atop the PhC- $\mu$ CT volumetric rendering (in reference to the ductal carcinoma of the breast sample). Such a comparison illustrates the substantial disparity in scale and coverage between the limited histological slice and the extensive tissue volume captured by tomography. The depth and spatial context provided by 3D data enrich our understanding of the tumor's intricate spatial distribution and its dynamic relationship with neighboring tissues. This deeper insight allows for the identification of subtle patterns, correlations, and anomalies that may elude detection in traditional histological samples (refer to annotations in supplementary videos), given the limited portion of the tissue that can be inspected. In cases where histological examination yields inconclusive results or fails to capture the complexity of pathology, 3D data become indispensable. They offer a complete representation of tissue structure, enabling clinicians and researchers to explore and interpret data along arbitrary planes, leading to more accurate diagnoses and informed treatment decisions. Moreover, CT images serve as a valuable tool for further investigations. For instance, in cases where the status of the basal lamina or the capsule is uncertain or disrupted, additional histological examination may be required (see supplementary video S1). Having 3D data can streamline this process, providing pathologists with a clear understanding of the tissue's spatial distribution.

**Fig. 4** Visual comparison displaying the histological slice ( $\sim 6 \text{ mm} \times \sim 6 \text{ mm} \times \sim 5 \mu\text{m}$ ), representing approximately 1/300th of the tissue, overlaid and magnified on the volumetric rendering obtained through tomography (rendered volume size  $\sim 6 \text{ mm} \times \sim 6 \text{ mm} \times \sim 1.5 \text{ mm}$ ), relative to the breast ductal carcinoma case. The aim of the image is to illustrate the vast difference in scale between the tiny histological sample and the comprehensive 3D volume acquired via tomography



#### 4 Discussion

Early detection is crucial for improved outcomes in the treatment of breast, cervical, and thyroid carcinomas. Detecting these cancers in their early, non-invasive stages significantly increases the chances of successful (and less invasive) treatment and better prognoses. Histopathology plays a critical role in the early detection process. This examination aids in identifying pre-invasive lesions, enabling early intervention before these lesions progress to invasive carcinomas, and also guides the characterization of breast tumors, determining their invasiveness and helping tailor treatment strategies [59]. However, while histopathology can identify invasive carcinomas, it might struggle to detect very early stage lesions, leading to potential underdiagnosis or misinterpretation. In cervical cancer, histopathology through Pap smears or biopsies can miss certain pre-cancerous changes or invasive patterns due to sampling errors or subjective interpretation. This could lead to false negatives or delays in detecting invasive carcinomas. In thyroid cancer, histopathological assessment via fine-needle aspiration biopsy may not always distinguish between benign and malignant nodules accurately. Moreover, histopathology's reliance on tissue samples may limit its ability to assess the full extent of invasion, especially in larger lesions.

While histopathology serves as a cornerstone for diagnosing invasive and non-invasive forms across these carcinomas, its limitations in providing a comprehensive three-dimensional assessment, detecting subtle micro-invasions, and its reliance on tissue sampling, emphasize the need for advanced imaging modalities like PhC- $\mu$ CT to complement traditional histopathological approaches. This combination can offer enhanced diagnostic accuracy, especially in identifying subtle invasion patterns [60], potentially improving early detection strategies for human cancers. In breast cancer, PhC- $\mu$ CT aids in identifying the invasion of cancer cells through the basal lamina, crucial for distinguishing between invasive and non-invasive forms [61]. It provides enhanced visualization of the basal lamina and surrounding structures, offering pathologists a clearer distinction between DCIS and IDC (see supplementary video S1). Similarly, in cervical cancer, our PhC- $\mu$ CT allows for visualization of invasive patterns through the inspection of the basement membrane (see supplementary video S2). While it aids in differentiating between invasive and non-invasive squamous cell carcinoma, achieving precise and clear imaging of micro-invasive foci and their extension into surrounding tissues may require additional acquisitions at higher resolutions for selected regions of interest. For thyroid carcinoma, PhC- $\mu$ CT aids in detecting vascular and capsular invasions (see supplementary video S3), crucial in distinguishing between invasive and non-invasive forms of follicular thyroid carcinoma [62]. Its ability to visualize the capsule and microvascular invasion enhances the evaluation of tumor extension, aiding in the differentiation between minimally invasive and widely invasive subtypes. The integration of PhC- $\mu$ CT into diagnostic and research contexts can offer a multifaceted approach to understanding tumor pathology. The 3D data and continuity provided by PhC- $\mu$ CT can enable pathologists to conduct comprehensive analyses, delving into the intricate details of lesion morphology and invasion patterns. This non-destructive imaging modality allows for thorough exploration without compromising tissue integrity. By providing a complete spatial representation of tissue structures, PhC- $\mu$ CT can enhance the depth of analysis and can facilitate a distinct understanding of pathological features. Additionally, PhC- $\mu$ CT's integration with histology presents a symbiotic relationship, where the strengths of each modality complement the other. PhC- $\mu$ CT data can allow pathologists to precisely identify optimal cutting locations, strategically selecting regions of interest for further histological examination. This process could involve a collaborative effort between radiologists and pathologists, where the former can provide valuable insights into the radiological features of the tissue, guiding pathologists in the interpretation of CT images and the selection of optimal cutting locations. In practice, this streamlined workflow could involve the initial review of CT images to identify areas of interest or potential abnormalities. Subsequently, these insights can be used to guide the selection of specific tissue sections to be cut for histological analysis. By leveraging the spatial context provided by 3D data, pathologists can ensure targeted and precise tissue sampling, maximizing the diagnostic yield while minimizing unnecessary tissue processing.

Our results are based on acquisitions carried out at a synchrotron facility that, as known, are in limited number and pose challenges due to their restricted accessibility. For this reason, many efforts are being devoted to translating phase-contrast  $\mu$ CT to conventional



sources, so that researchers and clinicians can overcome in the future the logistical and financial barriers associated with synchrotron-based imaging. While conventional sources offer broader accessibility, they lack the beam quality and coherence of synchrotron radiation, affecting spatial resolution, contrast, and sensitivity. Despite these challenges, advancements in technology and imaging methodologies have enabled significant improvements in this field. Techniques such as grating-based interferometry [63, 64], edge-illumination [65], and propagation-based phase-contrast imaging [62, 66], have been developed or optimized to enhance contrast and spatial resolution, making them suitable for a wide range of biomedical imaging tasks using virtual histology. The findings from these translational studies show exceptionally promising results, suggesting their potential for future integration into clinical practice. It is worth noting that synchrotron images (like those obtained in this study), with their high quality, can serve also as a standard for benchmarking images obtained with conventional sources.

## 5 Conclusion

Based on our analysis of three pathological specimens of breast, cervical, and thyroid carcinomas, PhC- $\mu$ CT shows promise as a tool that could complement traditional histopathology. This innovative technique appeared to facilitate the identification of crucial elements such as the basal lamina in breast cancer, potentially aiding in distinguishing between invasive and non-invasive forms. It also allowed for the examination of micro-invasive foci in cervical cancer, which may contribute to a better understanding of invasion through the basement membrane. Additionally, in thyroid carcinoma, allows the detection of vascular and capsular invasions, which could assist in subtype classification based on invasion extent. Recent published research has demonstrated that follicular thyroid carcinomas can be identified using PhC- $\mu$ CT even with a compact and laboratory setup.

The technology's potential to capture epithelial tissue lining layers and invasions with clarity suggests a possible improvement in diagnostic accuracy in clinical settings. The availability of 3D data and continuous visualization may empower pathologists, allowing for more thorough lesion inspection and enhancing understanding of tumor morphology and invasion patterns. This capability might offer a strategic advantage in selecting representative cross sections after tomographic acquisition, potentially enriching the depth and accuracy of pathological examination.

Acknowledging that further research is necessary to validate these initial findings, it is important to note that any potential implementation in a clinical context would require compact systems. Our findings may serve as a benchmark in terms of quality, highlighting the technology's potential to enhance diagnostic precision and understanding of complex carcinomas. However, rigorous validation and feasibility studies are essential before widespread clinical adoption can be considered.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1140/epjp/s13360-024-05188-x>.

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**Data Availability Statement** All data supporting the findings of this study are available within the paper.

## Declarations

**Conflict of interest** The authors declare no Conflict of interest.

**Ethical approval** Tissue samples are chosen in accordance with the operative protocol of Trieste University Hospital and the standard procedures of the Anatomy and Histology Department. Before undergoing CT imaging studies, patients provide written informed consent as per the Directive 2004/23/EC of the European Parliament and Council for maintaining quality and safety standards in handling human tissues.

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