



SCHOOL OF COMPUTATION, INFORMATION AND
TECHNOLOGY - INFORMATICS

TECHNISCHE UNIVERSITÄT MÜNCHEN

Master's Thesis in Biomedical Computing

**Deep Learning Based Analysis of
Tumor-infiltrating Lymphocytes in H&E
Stained Histological Sections for Survival
Prediction of Breast Cancer patients**

Margaryta Olenchuk





SCHOOL OF COMPUTATION, INFORMATION AND
TECHNOLOGY - INFORMATICS

TECHNISCHE UNIVERSITÄT MÜNCHEN

Master's Thesis in Biomedical Computing

**Deep Learning Based Analysis of
Tumor-infiltrating Lymphocytes in H&E
Stained Histological Sections for Survival
Prediction of Breast Cancer patients**

**Deep Learning basierte Analyse von
tumorinfiltrierenden Lymphozyten in H&E
gefärbten histologischen Schnitten zur
Überlebensvorhersage von
Brustkrebspatienten**

Author:	Margaryta Olenchuk
Supervisor:	Prof. Dr. Peter Schöffler
Advisor:	Dr. Philipp Wortmann, Ansh Kapil
Submission Date:	15.12.2022

I confirm that this master's thesis in biomedical computing is my own work and I have documented all sources and material used.

Munich, 15.12.2022

Margaryta Olenchuk

Contents

1	Introduction	1
2	Related work	4
2.1	Deep learning-based semantic segmentation	4
2.1.1	Fully convolutional networks (FCNs)	4
2.1.2	Encoder-decoder networks	4
2.1.3	Recurrent neural networks (RNNs)	5
2.1.4	Transformers	6
2.2	TILs as prognostic biomarker	6
	List of Figures	9
	List of Tables	10
	Bibliography	11

1 Introduction

Breast cancer is the most common form of cancer diagnosed worldwide and the leading cause of cancer-related death among women. [1] It is a heterogeneous disease, consisting of several morphological and molecular subtypes. The molecular subtypes are among the prime factors to characterize breast cancer. There are four main clinically used [2] groups defined based on the status of several receptors, namely the Hormonal Receptor (HR, which is positive if either Estrogen Receptor (ER) or Progesterone Receptor (PR) is positive) and the human epidermal growth factor receptor 2 (Her2):

1. Luminal A (HR positive, Her2 negative)
2. Luminal B (HR positive, Her2 positive)
3. Her2 enriched (HR negative, Her2 positive)
4. Triple Negative (HR negative, Her2 negative)

Regardless of the subtype, breast cancer is primarily classified by its histological appearance. Thus for diagnostic confirmation a patient's biopsy or surgical resection samples are sectioned onto microscope slides for staining, often with hematoxylin and eosin (H&E), followed by a visual diagnosis by a pathologist. Pathologists examine tissue for abnormalities that indicate breast cancer. Cancer causes changes in tissue at the sub-cellular scale, hence an analysis of normal and tumor tissue can provide novel insights into tissue characteristics, lead to a better understanding of mechanisms underlying cancer progression and provide valuable information for medical decision-making such as tumor grading and treatment choices. [3]

One of the characteristics of histological images that can be visually assessed by pathologists is lymphocytic infiltration. There are a number of publications that emphasize the prognostic value of tumor-infiltrating lymphocytes (TILs), especially in triple negative (TNBC) and human epidermal growth factor receptor 2 (HER2+) breast cancer [4, 5]. TILs are mononuclear immune cells that infiltrate tumor tissue. They have been detected in almost all solid tumors, including breast cancer. [6] The development and progression of malignant tumors can be characterized by an interaction between the cells in the tumor microenvironment and TILs. In the early stage HER2+ and TNBC, TILs are detectable in up to 75% of tumors. [7] Studies have shown that an increased degree of lymphocytic infiltration is predictive of better long-term control of the disease. The patients with a high proportion of TILs in the tumor tissue and high immunogenicity of the tumor were shown to respond better to the chemotherapy. Accumulating evidence indicates that tumor-infiltrating lymphocytes are clinically useful biomarkers in TNBC and HER2+ and that they play an essential role in cancer progression. [8] Further research and development of TILs related biomarkers would grant clinicians essential prognostic information and promote the research on novel treatments and therapeutics.

For instance, since TILs with exhausted phenotype are associated with loss of antitumor immunity, single-cell RNA-seq of TILs has been already performed to search for new immune checkpoint blockade targets that enable the precise definition and even novel development of therapeutic strategies to overcome T-cell exhaustion. Therapeutic approaches to influence T-cell exhaustion have been developed to target proteins CTLA-4, PD-1, and PD-L1 and have proven to be effective in treating melanoma and non-small-cell lung cancer during ongoing trials. [9] TILs in TNBC patients also display immuno-suppressive phenotypes [10] and the number of TILs detected by TNBC patients is one of the highest of all breast cancer subgroups [11] which makes TNBC a valid target for further TILs research.

A valuable contribution to TILs research and any task involving visual analysis of histological images would be method automatization. Because while the manual examination continues to be widely applied in a clinical setting, it is subjective and not scalable to translational and clinical research studies involving large datasets of high-resolution whole slide tissue images (WSIs). Hence, there is a raised demand for reliable and efficient automated methods to complement the traditional manual examination of tissue samples.

With advancing technology and access to a large amount of data, deep learning methods have garnered an interest in computational pathology. There are multiple deep learning-based methods and pipelines that have been proposed for detection and segmentation tasks of WSIs. To stimulate the development of algorithms for automatic TILs evaluation, a special Tumor InfiltratinG lymphocytes in breast cancerER (TiGER) [12] challenge was formed. Within this competition, various algorithms were evaluated for the automated assessment of TILs in H&E stained histopathology WSIs that resulted in automatically acquired TILs scores. Those were later internally checked for significance as prognostic values and the concordance was reported. The clinical focus of the TiGER challenge is on Her2+ and TNBC. It is motivated by research and clinical data that show that Her2+ and TNBC have the worst prognosis making them an intense target of prognostic and predictive biomarker research aimed at improving patient management and prognosis.

This work is closely linked to the TiGER challenge. The goal is to develop a pipeline for HER2 positive and triple negative breast cancer H&E slides that segments tumor and stroma regions, detects TILs, and produces TILs scores as pictured in Figure 1.1 block 1-3.

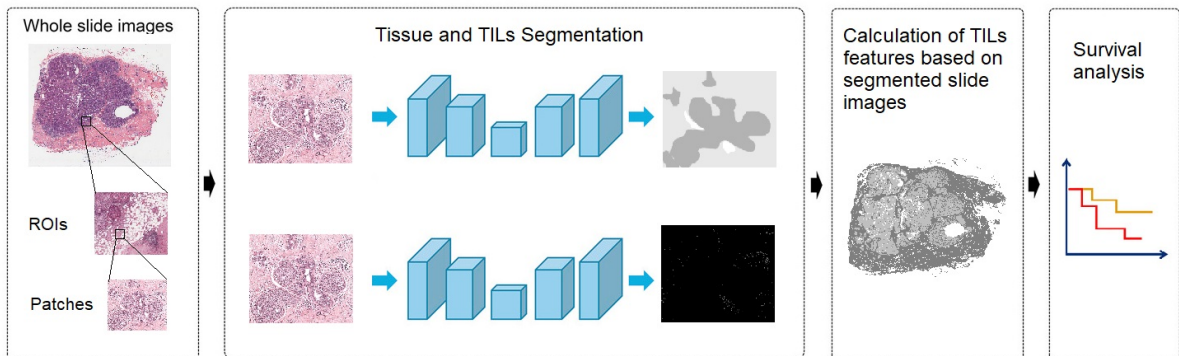


Figure 1.1: Abstract scheme to visually introduce the flow of this thesis work.

This work takes benefit of the annotated ROIs provided by the TiGER challenge for the development of patch-based automated tissue and TILs segmentation. As a step beyond the challenge, the scores based on the degree of lymphocytic infiltration are evaluated on the breast cancer TCGA-BRCA dataset generated by the TCGA Research Network and not on the hidden TiGER dataset which is not available after the end of the competition. The TCGA-BRCA clinical data enables independent broad survival analysis of different experimental TILs characteristics that can be calculated solely based on histological images (Figure 1.1 block 4). As a result, this work aims not only to develop a computational approach to compute TILs score on H&E images of Her2+ and TNBC but also experiment with different TILs scores and show detailed survival analysis based on publically available TCGA-BRCA dataset together with their predictive value for overall patient survival.

2 Related work

2.1 Deep learning-based semantic segmentation

The goal of semantic segmentation is to assign each image pixel to a category label corresponding to the underlying object. Due to the success of deep learning models in a wide range of vision applications, various deep learning-based algorithms have been developed and published in the literature [13]. One of the most prominent deep learning architectures used by the computer vision community include fully convolutional networks (FCNs) [14], encoder-decoders [15], generative adversarial networks (GANs) [16] and recurrent neural networks (RNNs) [17]. As Shephard, Adam et al. discuss [12] like tissue segmentation, TILs detection can be viewed as a semantic segmentation problem, since detection bounding boxes can be transformed into pseudo-segmentation masks. The focus of the following chapters is to superficially introduce the existing deep learning-based approaches for the histopathological tasks, that can be adapted or extended for tissue and TILs segmentation of breast cancer WSIs.

2.1.1 Fully convolutional networks (FCNs)

FCNs [14] are among the most widely used architectures for computer vision tasks and their general architecture consists of several learnable convolutions, pooling layers, and a final 1×1 convolution. Such models are used on segmentation problems in histology domain such as colon glands segmentation [18], as well as nuclei [19] and TILs [20] segmentation for breast cancer all performed on the Hematoxylin and Eosin (H&E) stained histopathology images. Moreover, the FCN method was applied for semantic segmentation of TCGA [21] breast data set [22], which is also used in this thesis. However, despite its popularity, the conventional FCN model has limitations such as loss of localization and the inability to process potentially useful global context information due to a series of down-sampling and a high sampling rate.

2.1.2 Encoder-decoder networks

A popular group of deep learning models for semantic image segmentation that aims to solve the aforementioned issues of FCNs is based on the convolutional encoder-decoder architecture [15]. Their model consists of two parts, an encoder consisting of convolutional layers and a deconvolution network that consists of deconvolution and unpooling layers that take the feature vector as input and generate a map of pixel-wise class probabilities. An example of such a convolutional encoder-decoder architecture for image segmentation is SegNet [23]. The SegNet's encoder network has 13 convolutional layers with corresponding

layers in the decoder. The final decoder output is fed to a multi-class soft-max classifier to produce class probabilities for each pixel independently. The main feature of SegNet is that the decoder uses pooling indices computed in the max-pooling step of the corresponding encoder to perform non-linear upsampling. This architecture also find a use in histopathology, e.g. colon cancer analysis [24]. There are several encoder-decoder models initially developed for biomedical image segmentation. Ronneberger et al. [25] proposed the U-Net model for segmenting biological microscopy images that can train with few annotated images effectively. U-Net has an FCN-like down-sampling part that extracts features with 3×3 convolutions and an up-sampling part. Feature maps from the encoder are copied to the corresponding decoder part of the network to avoid losing pattern information. Besides the segmentation of neuronal structures in electron microscopic recordings demonstrated in the original paper [25], U-Net was applied for numerous histopathology tasks such as nuclei segmentation [26, 27], individual colon glands segmentation [28], epidermal tissue segmentation of skin biopsies [29] and cell segmentation on triple-negative breast cancer patients dataset [30]. A further development of an encoder-decoder model for semantic segmentation of histopathology images is HookNet [31]. The architecture consists of two encoder-decoder branches to extract contextual and fine-grained detailed information and combine it (hook up) for the target segmentation. The model showed improvement compared with single-resolution models and was applied to segment breast cancer tissue sections [31].

Another widely used group of deep learning models for semantic segmentation are the atrous (or dilated) convolutional models that include the DeepLab family [32, 33]. The use of atrous convolutions addresses the decreasing resolution caused by max-pooling and striding and Atrous Spatial Pyramid Pooling analyzes an incoming convolutional feature layer with filters at multiple sampling rates allowing to capture objects and image contexts at multiple scales to robustly segment objects at multiple scales. DeepLabv3+ [34] uses encoder-decoder architecture including atrous separable convolution, composed of a depthwise convolution (spatial convolution for each channel of the input) and pointwise convolution (1×1 convolution with the depthwise convolution as input). Authors [34] demonstrated the effectiveness of DeepLabv3+ model on segmentation of H&E stained breast cancer [35]. Despite all the efforts, even this popular architecture has constraints in learning long-range dependency and spatial correlations due to the inductive bias of locality and weight sharing [36] that may result in the sub-optimal segmentation of complex structures.

2.1.3 Recurrent neural networks (RNNs)

RNNs [17] have proven to be useful in modeling the short/long-term dependencies among pixels to generate segmentation maps. Pixels can be linked together and processed sequentially to model global contexts and improve semantic segmentation. ReSeg [37] is an RNN-based model for semantic segmentation. Each layer is composed of four RNNs that go through the image horizontally and vertically in both directions to provide relevant global information, while convolutional layers extract local features that are then followed by up-sampling layers to recover the predictions at original image resolution. But despite all further developments that showcase the potential for histopathology image segmentation:

RACE-net [38] applied for segmentation of the cell nuclei in H&E stained breast cancer slides, Her2Net [39] segmenting cell membranes and nuclei from human epidermal growth factor receptor-2 (HER2)-stained breast cancer images, etc., an important limitation of RNNs is that, due to their sequential nature, they are comparably slower, since this sequential calculation cannot be easily parallelized.

2.1.4 Transformers

The Transformer in Natural Language Processing is an architecture that aims to solve sequence-to-sequence problems. These models rely on self-attention mechanisms and capture long-range dependencies among tokens (words) in a sentence without using RNNs or convolution. Transformers have also emerged in image semantic segmentation. Recent studies have shown that the Transformers can achieve superior performance than CNN-based approaches in various semantic segmentation applications [40]. The state-of-the-art Transformer-based semantic segmentation methods can be often applied either as convolution-free models or/and as CNN-Transformer hybrid models. Swin-Transformer [41] for instance is a pure hierarchical Transformer that can serve as a backbone for various computer vision tasks including semantic segmentation. To tokenize the image, it breaks the image into windows that further consist of patches. It constructs a hierarchical representation of an image by starting from small-sized patches and gradually merging neighboring patches into deeper Transformer layers. Swin-Transformer or its slightly modified successors found its application in the medical domain, often as a backbone, for example for colon cancer segmentation in H&E stained histopathology images [42] or gland segmentation [43]. A further popular fully transformer-based model for semantic segmentation is Segmenter [44]. The encoder consists of Multi-head Self Attention and Multi-Layer Perceptron (MLP) blocks, as well as two-layer norms and residual connections after each block and a linear decoder that bilinearly up-samples the sequence into a 2D segmentation mask. While performing well on scene segmentation [44], is not particularly used in the medical domain. In the field of medical image segmentation, TransUNet [45] was the first attempt to establish self-attention mechanisms by combining transformer with U-Net and proved that transformers can be used as powerful encoders for medical image segmentation. A novel positional-encoding-free Transformer SegFormer [46] set new state-of-the-art in terms of efficiency and accuracy in publicly available semantic segmentation datasets and applied for gland and nuclei segmentation [43]. This architecture remains promising also for semantic segmentation in medical applications due to the positional-encoding-free encoder and lightweight MLP decoder.

2.2 TILs as prognostic biomarker

The overall survival (OS) is the primary endpoint for prognostic analysis in this thesis, the survival methods are well established and include the Kaplan–Meier method [47] to estimate OS and Cox proportional hazard models [48] to quantify the hazard ratio (HR) for the effects of biomarker groups. The following chapter focuses on conducted research for the

development of TILs scores as a prognostic biomarker for survival analysis in breast cancer based solely in histological slides.

Amgad, M. et al. [49, 50]. assessed three variants of the TILs score:

1. Number of TILs / Stromal area
2. Number of TILs / Number of cells in stroma
3. Number of TILs / Total Number of cells

The results performed on the BCSS and NuCLS breast carcinoma datasets [22, 51] (the source datasets for TCGA part of TiGER dataset) showed the most prognostic TILs score to be the number of TILs divided by the total number of cells within the stromal region. A further breast cancer study [52] showed that the binarized tumor TILs infiltration fraction is predictive of survival, by analyzing the proportion of pixels in the image that were predicted as containing tumor as well as lymphocytes (number of pixels predicted as lymphocyte and tumor divided by the number of pixels predicted as tumor). Bai, et al. [53] also found associations of clinical outcomes in breast cancer with TILs scores based on the number of TILs divided by the number of TILs and tumor cells detected.

The stromal TILs (sTILs) have been shown to have prognostic value in HER2+ breast cancer and TNBC [49]. sTIL density was found significantly prognostic for OS not only while applied on H&E slides but IHC as well. [54] Applied on the TCGA-BRCA mixed with non publically available dataset, Thagaard, J. et al. [55] tried to mimic the approach of the pathologist and therefore defined tumor-associated stroma. Tumor-associated stroma includes a margin of 250 μ m from the border of the tumor into the surrounding stroma. The sTIL density was calculated as the number of TILs within the tumor-associated stroma per mm². The patient cohort was then stratified into two groups: high and low sTIL density by using maximally selected rank statistics for cutpoint selection. As a result sTIL density stratified the patients significantly into two distinct prognostic groups. For continuous variables, the sTIL density was divided by 300 and higher sTILs scores were associated with significantly prolonged overall survival. For the TCGA-BRCA dataset, a further TIL score was found significant as the overlapping area between lymphocyte-dense regions and stromal regions divided by the size of the stromal regions. [56] Whereas a study, that focused on TNBC cases of TCGA, did not observe any differences in OS neither while using a continuous variable of manually annotated TILs (scored by a pathologist and partitioned into eight different groups, e.g. < 1%, 10-20%, etc.) nor after applying the log-rank test [57]. On the other hand, Fassler, D. J., et al. [58] confirmed correlation of intratumoral TIL infiltration with increased OS in breast cancer in the TCGA-BRCA cohort. TIL infiltrate percentage was calculated as the number of predicted patches that were classified as positive for tumor and lymphocyte divided by total number of cancer patches. Another used definition of sTILs was the percentage of tumor stroma area containing a lymphocytic infiltrate without direct contact with tumor cells [59]. Furthermore, studies found a three-scale grading system for reporting TILs status to be applicable, instead of continuous or binary grouped TILs densities [60]. More advanced TILs-based features such as the Ball-Hall Index of spatially connected TILs regions (clusters) also showed association with survival, particularly within the BRCA dataset of TCGA [61].

Hence, there is no canonic method for the automatic determination of TILs score based on the H&E breast cancer tissue samples but number of TILs per mm^2 of stromal area is used most frequently.

List of Figures

1.1 Abstract scheme to visually introduce the flow of this thesis work. 2

List of Tables

Bibliography

- [1] B. S. Chhikara and K. Parang. "Global Cancer Statistics 2022: the trends projection analysis". In: (2022).
- [2] *Tiger - Grand Challenge*. URL: <https://tiger.grand-challenge.org/Home/>.
- [3] Q. D. Vu, S. Graham, T. Kurc, M. N. N. To, M. Shaban, T. Qaiser, N. A. Koohbanani, S. A. Khurram, J. Kalpathy-Cramer, T. Zhao, et al. "Methods for segmentation and classification of digital microscopy tissue images". In: *Frontiers in bioengineering and biotechnology* (2019), p. 53.
- [4] R. Salgado, C. Denkert, S. Demaria, N. Sirtaine, F. Klauschen, G. Pruneri, S. Wienert, G. Van den Eynden, F. L. Baehner, F. Pénault-Llorca, et al. "The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014". In: *Annals of oncology* 26.2 (2015), pp. 259–271.
- [5] C. Denkert, G. von Minckwitz, S. Darb-Esfahani, B. Lederer, B. I. Heppner, K. E. Weber, J. Budczies, J. Huober, F. Klauschen, J. Furlanetto, et al. "Tumour-infiltrating lymphocytes and prognosis in different subtypes of breast cancer: a pooled analysis of 3771 patients treated with neoadjuvant therapy". In: *The lancet oncology* 19.1 (2018), pp. 40–50.
- [6] A.-V. Laenkholm, G. Callagy, M. Balancin, J. Bartlett, C. Sotiriou, C. Marchio, M. Kok, C. H. Dos Anjos, and R. Salgado. "Incorporation of TILs in daily breast cancer care: how much evidence can we bear?" In: *Virchows Archiv* (2022), pp. 1–16.
- [7] M. V. Dieci, N. Radosevic-Robin, S. Fineberg, G. Van den Eynden, N. Ternes, F. Penault-Llorca, G. Pruneri, T. M. D'Alfonso, S. Demaria, C. Castaneda, et al. "Update on tumor-infiltrating lymphocytes (TILs) in breast cancer, including recommendations to assess TILs in residual disease after neoadjuvant therapy and in carcinoma in situ: a report of the International Immuno-Oncology Biomarker Working Group on Breast Cancer". In: *Seminars in cancer biology*. Vol. 52. Elsevier. 2018, pp. 16–25.
- [8] G. Gao, Z. Wang, X. Qu, and Z. Zhang. "Prognostic value of tumor-infiltrating lymphocytes in patients with triple-negative breast cancer: a systematic review and meta-analysis". In: *BMC cancer* 20.1 (2020), pp. 1–15.
- [9] M. A. Postow, M. K. Callahan, and J. D. Wolchok. "Immune checkpoint blockade in cancer therapy". In: *Journal of clinical oncology* 33.17 (2015), p. 1974.
- [10] W. Chung, H. H. Eum, H.-O. Lee, K.-M. Lee, H.-B. Lee, K.-T. Kim, H. S. Ryu, S. Kim, J. E. Lee, Y. H. Park, et al. "Single-cell RNA-seq enables comprehensive tumour and immune cell profiling in primary breast cancer". In: *Nature communications* 8.1 (2017), pp. 1–12.

- [11] A. Schneeweiss, C. Denkert, P. A. Fasching, C. Fremd, O. Gluz, C. Kolberg-Liedtke, S. Loibl, and H.-J. Lück. "Diagnosis and therapy of triple-negative breast cancer (TNBC)–recommendations for daily routine practice". In: *Geburtshilfe und Frauenheilkunde* 79.06 (2019), pp. 605–617.
- [12] A. Shephard, M. Jahanifar, R. Wang, M. Dawood, S. Graham, K. Sidlauskas, S. A. Khurram, N. Rajpoot, and S. E. A. Raza. "TIAger: Tumor-Infiltrating Lymphocyte Scoring in Breast Cancer for the TiGER Challenge". In: *arXiv preprint arXiv:2206.11943* (2022).
- [13] S. Minaee, Y. Y. Boykov, F. Porikli, A. J. Plaza, N. Kehtarnavaz, and D. Terzopoulos. "Image segmentation using deep learning: A survey". In: *IEEE transactions on pattern analysis and machine intelligence* (2021).
- [14] J. Long, E. Shelhamer, and T. Darrell. "Fully convolutional networks for semantic segmentation". In: *Proceedings of the IEEE conference on computer vision and pattern recognition*. 2015, pp. 3431–3440.
- [15] H. Noh, S. Hong, and B. Han. "Learning deconvolution network for semantic segmentation". In: *Proceedings of the IEEE international conference on computer vision*. 2015, pp. 1520–1528.
- [16] I. Goodfellow, J. Pouget-Abadie, M. Mirza, B. Xu, D. Warde-Farley, S. Ozair, A. Courville, and Y. Bengio. "Generative adversarial nets". In: *Advances in neural information processing systems* 27 (2014).
- [17] D. E. Rumelhart, G. E. Hinton, and R. J. Williams. "Learning representations by back-propagating errors". In: *nature* 323.6088 (1986), pp. 533–536.
- [18] A. BenTaieb and G. Hamarneh. "Topology aware fully convolutional networks for histology gland segmentation". In: *International conference on medical image computing and computer-assisted intervention*. Springer. 2016, pp. 460–468.
- [19] V. A. Natarajan, M. S. Kumar, R. Patan, S. Kallam, and M. Y. N. Mohamed. "Segmentation of nuclei in histopathology images using fully convolutional deep neural architecture". In: *2020 International Conference on computing and information technology (ICCIT-1441)*. IEEE. 2020, pp. 1–7.
- [20] M. Amgad, A. Sarkar, C. Srinivas, R. Redman, S. Ratra, C. J. Bechert, B. C. Calhoun, K. Mrazek, U. Kurkure, L. A. Cooper, et al. "Joint region and nucleus segmentation for characterization of tumor infiltrating lymphocytes in breast cancer". In: *Medical Imaging 2019: Digital Pathology*. Vol. 10956. SPIE. 2019, pp. 129–136.
- [21] D. A. Gutman, J. Cobb, D. Somanna, Y. Park, F. Wang, T. Kurc, J. H. Saltz, D. J. Brat, L. A. Cooper, and J. Kong. "Cancer Digital Slide Archive: an informatics resource to support integrated in silico analysis of TCGA pathology data". In: *Journal of the American Medical Informatics Association* 20.6 (2013), pp. 1091–1098.

- [22] M. Amgad, H. Elfandy, H. Hussein, L. A. Atteya, M. A. Elsebaie, L. S. Abo Elnasr, R. A. Sakr, H. S. Salem, A. F. Ismail, A. M. Saad, et al. "Structured crowdsourcing enables convolutional segmentation of histology images". In: *Bioinformatics* 35.18 (2019), pp. 3461–3467.
- [23] V. Badrinarayanan, A. Kendall, and R. Cipolla. "Segnet: A deep convolutional encoder-decoder architecture for image segmentation". In: *IEEE transactions on pattern analysis and machine intelligence* 39.12 (2017), pp. 2481–2495.
- [24] A. B. Hamida, M. Devanne, J. Weber, C. Truntzer, V. Derangère, F. Ghiringhelli, G. Forestier, and C. Wemmert. "Deep learning for colon cancer histopathological images analysis". In: *Computers in Biology and Medicine* 136 (2021), p. 104730.
- [25] O. Ronneberger, P. Fischer, and T. Brox. "U-Net: Convolutional Networks for Biomedical Image Segmentation". In: *Medical Image Computing and Computer-Assisted Intervention – MICCAI 2015*. Ed. by N. Navab, J. Hornegger, W. M. Wells, and A. F. Frangi. Cham: Springer International Publishing, 2015, pp. 234–241.
- [26] A. Lagree, M. Mohebpour, N. Meti, K. Saednia, F.-I. Lu, E. Slodkowska, S. Gandhi, E. Rakovitch, A. Shenfield, A. Sadeghi-Naini, et al. "A review and comparison of breast tumor cell nuclei segmentation performances using deep convolutional neural networks". In: *Scientific Reports* 11.1 (2021), pp. 1–11.
- [27] Z. Zeng, W. Xie, Y. Zhang, and Y. Lu. "RIC-Unet: An improved neural network based on Unet for nuclei segmentation in histology images". In: *Ieee Access* 7 (2019), pp. 21420–21428.
- [28] H. Pinckaers and G. Litjens. "Neural ordinary differential equations for semantic segmentation of individual colon glands". In: *arXiv preprint arXiv:1910.10470* (2019).
- [29] K. R. Oskal, M. Risdal, E. A. Janssen, E. S. Undersrud, and T. O. Gulsrud. "A U-net based approach to epidermal tissue segmentation in whole slide histopathological images". In: *SN Applied Sciences* 1.7 (2019), pp. 1–12.
- [30] M. E. Bagdigen and G. Bilgin. "Cell segmentation in triple-negative breast cancer histopathological images using U-Net architecture". In: *2020 28th Signal Processing and Communications Applications Conference (SIU)*. IEEE. 2020, pp. 1–4.
- [31] M. Van Rijthoven, M. Balkenhol, K. Siliņa, J. Van Der Laak, and F. Ciompi. "HookNet: Multi-resolution convolutional neural networks for semantic segmentation in histopathology whole-slide images". In: *Medical Image Analysis* 68 (2021), p. 101890.
- [32] L.-C. Chen, G. Papandreou, I. Kokkinos, K. Murphy, and A. L. Yuille. "Deeplab: Semantic image segmentation with deep convolutional nets, atrous convolution, and fully connected crfs". In: *IEEE transactions on pattern analysis and machine intelligence* 40.4 (2017), pp. 834–848.
- [33] L.-C. Chen, G. Papandreou, F. Schroff, and H. Adam. "Rethinking atrous convolution for semantic image segmentation". In: *arXiv preprint arXiv:1706.05587* (2017).

- [34] L.-C. Chen, Y. Zhu, G. Papandreou, F. Schroff, and H. Adam. "Encoder-decoder with atrous separable convolution for semantic image segmentation". In: *Proceedings of the European conference on computer vision (ECCV)*. 2018, pp. 801–818.
- [35] B. M. Priego-Torres, D. Sanchez-Morillo, M. A. Fernandez-Granero, and M. Garcia-Rojo. "Automatic segmentation of whole-slide H&E stained breast histopathology images using a deep convolutional neural network architecture". In: *Expert Systems With Applications* 151 (2020), p. 113387.
- [36] Y. Xie, J. Zhang, C. Shen, and Y. Xia. "Cotr: Efficiently bridging cnn and transformer for 3d medical image segmentation". In: *International conference on medical image computing and computer-assisted intervention*. Springer. 2021, pp. 171–180.
- [37] F. Visin, M. Ciccone, A. Romero, K. Kastner, K. Cho, Y. Bengio, M. Matteucci, and A. Courville. "Reseg: A recurrent neural network-based model for semantic segmentation". In: *Proceedings of the IEEE Conference on Computer Vision and Pattern Recognition Workshops*. 2016, pp. 41–48.
- [38] A. Chakravarty and J. Sivaswamy. "RACE-net: a recurrent neural network for biomedical image segmentation". In: *IEEE journal of biomedical and health informatics* 23.3 (2018), pp. 1151–1162.
- [39] M. Saha and C. Chakraborty. "Her2Net: A deep framework for semantic segmentation and classification of cell membranes and nuclei in breast cancer evaluation". In: *IEEE Transactions on Image Processing* 27.5 (2018), pp. 2189–2200.
- [40] C. Nguyen, Z. Asad, R. Deng, and Y. Huo. "Evaluating transformer-based semantic segmentation networks for pathological image segmentation". In: *Medical Imaging 2022: Image Processing*. Vol. 12032. SPIE. 2022, pp. 942–947.
- [41] Z. Liu, Y. Lin, Y. Cao, H. Hu, Y. Wei, Z. Zhang, S. Lin, and B. Guo. "Swin transformer: Hierarchical vision transformer using shifted windows". In: *Proceedings of the IEEE/CVF International Conference on Computer Vision*. 2021, pp. 10012–10022.
- [42] Z. Qian, K. Li, M. Lai, E. I. Chang, B. Wei, Y. Fan, Y. Xu, et al. "Transformer based multiple instance learning for weakly supervised histopathology image segmentation". In: *arXiv preprint arXiv:2205.08878* (2022).
- [43] A. Lin, B. Chen, J. Xu, Z. Zhang, G. Lu, and D. Zhang. "Ds-transunet: Dual swin transformer u-net for medical image segmentation". In: *IEEE Transactions on Instrumentation and Measurement* (2022).
- [44] R. Strudel, R. Garcia, I. Laptev, and C. Schmid. "Segmenter: Transformer for semantic segmentation". In: *Proceedings of the IEEE/CVF International Conference on Computer Vision*. 2021, pp. 7262–7272.
- [45] J. Chen, Y. Lu, Q. Yu, X. Luo, E. Adeli, Y. Wang, L. Lu, A. L. Yuille, and Y. Zhou. "Transunet: Transformers make strong encoders for medical image segmentation". In: *arXiv preprint arXiv:2102.04306* (2021).

- [46] E. Xie, W. Wang, Z. Yu, A. Anandkumar, J. M. Alvarez, and P. Luo. *SegFormer: Simple and Efficient Design for Semantic Segmentation with Transformers*. 2021. DOI: 10.48550/ARXIV.2105.15203. URL: <https://arxiv.org/abs/2105.15203>.
- [47] E. L. Kaplan and P. Meier. “Nonparametric estimation from incomplete observations”. In: *Journal of the American statistical association* 53.282 (1958), pp. 457–481.
- [48] D. R. Cox. “Regression Models and Life-Tables”. In: *Journal of the Royal Statistical Society: Series B (Methodological)* 34.2 (1972), pp. 187–202.
- [49] Z. Kos, E. Roblin, R. S. Kim, S. Michiels, B. D. Gallas, W. Chen, K. K. van de Vijver, S. Goel, S. Adams, S. Demaria, et al. “Pitfalls in assessing stromal tumor infiltrating lymphocytes (sTILs) in breast cancer”. In: *NPJ breast cancer* 6.1 (2020), pp. 1–16.
- [50] M. Amgad, R. Salgado, and L. A. Cooper. “MuTILs: Explainable, multiresolution computational scoring of Tumor-Infiltrating Lymphocytes in breast carcinomas using clinical guidelines”. In: *medRxiv* (2022).
- [51] M. Amgad, L. A. Atteya, H. Hussein, K. H. Mohammed, E. Hafiz, M. A. Elsebaie, A. M. Alhusseiny, M. A. AlMoslemany, A. M. Elmatboly, P. A. Pappalardo, et al. “Nucls: A scalable crowdsourcing, deep learning approach and dataset for nucleus classification, localization and segmentation”. In: *arXiv preprint arXiv:2102.09099* (2021).
- [52] H. Le, R. Gupta, L. Hou, S. Abousamra, D. Fassler, L. Torre-Healy, R. A. Moffitt, T. Kurc, D. Samaras, R. Batiste, et al. “Utilizing automated breast cancer detection to identify spatial distributions of tumor-infiltrating lymphocytes in invasive breast cancer”. In: *The American journal of pathology* 190.7 (2020), pp. 1491–1504.
- [53] Y. Bai, K. Cole, S. Martinez-Morilla, F. S. Ahmed, J. Zugazagoitia, J. Staaf, A. Bosch, A. Ehinger, E. Nimeus, J. Hartman, et al. “An Open-Source, Automated Tumor-Infiltrating Lymphocyte Algorithm for Prognosis in Triple-Negative Breast CancerMachine-Read TIL Variables in TNBC”. In: *Clinical Cancer Research* 27.20 (2021), pp. 5557–5565.
- [54] A. Kapil, A. Meier, A. Shumilov, S. Haneder, H. Angell, and G. Schmidt. “Breast cancer patient stratification using domain adaptation based lymphocyte detection in HER2 stained tissue sections”. In: (2021).
- [55] J. Thagaard, E. S. Stovgaard, L. G. Vognsen, S. Hauberg, A. Dahl, T. Ebstrup, J. Doré, R. E. Vincentz, R. K. Jepsen, A. Roslind, et al. “Automated quantification of stil density with h&e-based digital image analysis has prognostic potential in triple-negative breast cancers”. In: *Cancers* 13.12 (2021), p. 3050.
- [56] P. Sun, J. He, X. Chao, K. Chen, Y. Xu, Q. Huang, J. Yun, M. Li, R. Luo, J. Kuang, et al. “A computational tumor-infiltrating lymphocyte assessment method comparable with visual reporting guidelines for triple-negative breast cancer”. In: *EBioMedicine* 70 (2021), p. 103492.
- [57] K. E. Craven, Y. Gökmen-Polar, and S. S. Badve. “CIBERSORT analysis of TCGA and METABRIC identifies subgroups with better outcomes in triple negative breast cancer”. In: *Scientific reports* 11.1 (2021), pp. 1–19.

- [58] D. J. Fassler, L. A. Torre-Healy, R. Gupta, A. M. Hamilton, S. Kobayashi, S. C. Van Alsten, Y. Zhang, T. Kurc, R. A. Moffitt, M. A. Troester, et al. "Spatial Characterization of Tumor-Infiltrating Lymphocytes and Breast Cancer Progression". In: *Cancers* 14.9 (2022), p. 2148.
- [59] S. Meng, L. Li, M. Zhou, W. Jiang, H. Niu, and K. Yang. "Distribution and prognostic value of tumor-infiltrating T cells in breast cancer". In: *Molecular medicine reports* 18.5 (2018), pp. 4247–4258.
- [60] V. Kotoula, K. Chatzopoulos, S. Lakis, Z. Alexopoulou, E. Timotheadou, F. Zagouri, G. Pentheroudakis, H. Gogas, E. Galani, I. Efstratiou, et al. "Tumors with high-density tumor infiltrating lymphocytes constitute a favorable entity in breast cancer: a pooled analysis of four prospective adjuvant trials". In: *Oncotarget* 7.4 (2016), p. 5074.
- [61] J. Saltz, R. Gupta, L. Hou, T. Kurc, P. Singh, V. Nguyen, D. Samaras, K. R. Shroyer, T. Zhao, R. Batiste, et al. "Spatial organization and molecular correlation of tumor-infiltrating lymphocytes using deep learning on pathology images". In: *Cell reports* 23.1 (2018), pp. 181–193.