



**HAL**  
open science

## Deep Learning for Detecting BRCA Mutations in High-Grade Ovarian Cancer based on an Innovative Tumor Segmentation Method from Whole-Slide Images

Raphaël Bourgade, Noémie Rabilloud, Tanguy Perennec, Thierry Pécot, Celine Garrec, Alexis F. Guedon, Capucine Delnatte, Stéphane Bézieau, Alexandra Lespagnol, Marie de Tayrac, et al.

► **To cite this version:**

Raphaël Bourgade, Noémie Rabilloud, Tanguy Perennec, Thierry Pécot, Celine Garrec, et al.. Deep Learning for Detecting BRCA Mutations in High-Grade Ovarian Cancer based on an Innovative Tumor Segmentation Method from Whole-Slide Images. *Modern Pathology*, 2023, 36 (11), pp.100304. 10.1016/j.modpat.2023.100304 . hal-04196223

**HAL Id: hal-04196223**

**<https://hal.science/hal-04196223v1>**

Submitted on 26 Oct 2023

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

## **TITLE**

**Deep Learning for Detecting *BRCA* Mutations in High-Grade Ovarian Cancer based on an Innovative Tumor Segmentation Method from Whole-Slide Images.**

## **AUTHORSHIP**

Raphaël Bourgade<sup>1</sup>, Noémie Rabilloud<sup>2</sup>, Tanguy Perennec<sup>3</sup>, Thierry Pécot<sup>4</sup>, Céline Garrec<sup>5</sup>, Alexis F. Guédon<sup>6</sup>, Capucine Delnatte<sup>5</sup>, Stéphane Bézieau<sup>5</sup>, Alexandra Lespagnol<sup>7</sup>, Marie de Tayrac<sup>7</sup>, Sébastien Henno<sup>8</sup>, Christine Sagan<sup>1</sup>, Claire Toquet<sup>1</sup>, Jean-François Mosnier<sup>1</sup>, Solène-Florence Kammerer-Jacquet<sup>2,8</sup>, Delphine Loussouarn<sup>1</sup>

1: Department of Pathology, University Hospital of Nantes, 9 Quai Moncoussu, 44093 Nantes cedex 01, France.

2: LTSI—UMR 1099 Inserm, University of Rennes, 2 avenue du Professeur Léon Bernard, 35043 Rennes, France

3: Department of Radiation Oncology, Institut de Cancérologie de l'Ouest Nantes, boulevard Professeur Jacques Monod, 44800 Saint-Herblain, France

4: Biosit, UAR 3480 CNRS - US 18 Inserm, University of Rennes, 2 avenue du Professeur Léon Bernard, 35043 Rennes, France

5: Department of Medical Genetics, University Hospital of Nantes, 9 Quai Moncoussu, 44093 Nantes cedex 01, France.

6: Sorbonne University, National Institute of Health and Medical Research, Pierre Louis Institute of Epidemiology and Public Health, 184 rue du faubourg Saint-Antoine, 75012 Paris, France

7: Department of Molecular Genetics and Genomics, University Hospital of Rennes, 2 Rue Henri le Guilloux, 35000 Rennes, France

8: Department of Pathology, University Hospital of Rennes, 2 Rue Henri le Guilloux, 35000 Rennes, France

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

Corresponding author : Raphaël Bourgade

- tel : 0786252329
- mail : [raphael.bourgade@gmail.com](mailto:raphael.bourgade@gmail.com)
- postal address : 9 Quai Moncousu, 44093 Nantes cedex 01, France.

## **ABSTRACT**

*BRCA1/2* genes play a crucial role in repairing DNA double-strand breaks through homologous recombination. Their mutations represent a significant proportion of homologous recombination deficiency and are a reliable effective predictor of sensitivity of high-grade ovarian cancer (HGOC) to poly(ADP-ribose) polymerase inhibitors. However, their testing by next-generation sequencing is costly, time-consuming, and can be affected by various preanalytical factors. In this study, we present a deep learning classifier for *BRCA* mutational status prediction from HES-stained whole-slide images (WSI) of HGOC. We constituted the OvarIA cohort composed of 867 HGOC patients with known *BRCA* somatic mutational status coming from two different pathology departments. We first developed a tumor segmentation model according to dynamic sampling and then trained a visual representation encoder with momentum contrastive learning on the predicted tumor tiles. We finally trained a *BRCA* classifier on over a million tumor tiles in multiple-instance learning with an attention-based mechanism. The tumor segmentation model trained on 8 WSI obtained a dice score of 0.915 and an intersection-over-union of 0.847 on a test set of 50 WSI while the *BRCA* classifier achieved the state-of-the-art AUC of 0.739 in 5-fold cross-validation and 0.681 on the testing set. An additional multiscale approach indicates that the relevant information for predicting *BRCA* mutations is more located in the tumor context than in the cell morphology. Our results suggest that *BRCA* somatic mutations have a discernible phenotypic effect which could be detected by deep learning and could be used as a pre-screening tool in the future.

## **INTRODUCTION**

Epithelial ovarian cancer (EOC) is a highly lethal gynecologic malignancy and a significant cause of female cancer-related deaths<sup>1</sup>. The most common histological subtype of EOC is high-grade serous (HGSOC). Because of their silent course, high-grade ovarian cancers (HGOC) are frequently diagnosed at an advanced stage and are of poor prognosis. The standard treatment for patients with diagnosed advanced ovarian cancer consists of cytoreductive surgery and a combination of paclitaxel and platinum-based chemotherapy<sup>2</sup>. Despite standard therapy, 70% of the patients relapse within the subsequent 3 years<sup>2</sup>.

HGOC is characterized by defects in the DNA damage repair pathway known as homologous recombination. The *BRCA1* and *BRCA2* genes play a crucial role in repairing DNA double-strand breaks through homologous recombination<sup>3</sup>. Mutations in these genes are found in 17-25% of patients with HGSOC, the most common type of EOC<sup>4,5</sup>. In 2005, two studies demonstrated that in vitro, poly(ADP-ribose) polymerase inhibitors (PARPi) are effective at killing *BRCA*-deficient cancer cells, but have no effect on non-*BRCA*-deficient cancer cells. This phenomenon, known as synthetic lethality, first described by Hartwell<sup>6</sup>, occurs because PARPi inhibits the repair of DNA single-strand breaks through the base excision repair system. This mechanism leads to the accumulation of double-strand breaks that can be repaired by homologous recombination in *BRCA*-competent cells but not in *BRCA*-deficient cells, causing cell death.

Germline and somatic *BRCA1/2* mutations are known predictive markers of response to PARPi targeted therapy, both in relapse and first-line settings<sup>7-13</sup>. *BRCA1/2* somatic mutational status analysis is thus essential for selecting appropriate treatment.

Its characterization with next-generation sequencing (NGS) is widely available in developed countries, using DNA extracted from formalin-fixed paraffin-embedded (FFPE) tumor tissue from either archival or current tumor samples. However, NGS performed on FFPE tissue sections may be challenging, due to pre-analytical conditions or amount of cancer cells in the sample, which could affect molecular results<sup>14</sup>. Indeed, approximately 5% of FFPE NGS

1 analyses fail and must be repeated with no molecular results in 3% of cases<sup>15</sup>. Moreover, this  
2 analysis is quite expensive, time-consuming and not available or accessible to all patients.  
3  
4 Additionally, the growing demand for personalized medicine has led to an increasing use of  
5 cancer molecular genetics platforms, which can further strain resources and increase costs.  
6  
7 This potentially leads to delays in therapeutic management, as patients may have to wait for  
8 diagnostic results before they can receive the appropriate treatment.  
9

10 Digital pathology has enabled the acquisition of high-resolution images known as whole-slide  
11 images (WSI) for the application of computational techniques<sup>16</sup>. With the progress of computer  
12 vision and the development of convolutional neural networks (CNN), machine learning  
13 algorithms are able to extract information from hematoxylin-eosin-safran (HES)-stained WSI.  
14  
15 It has the significant potential to improve the standard clinical process in pathology.  
16  
17

18 In addition to computer-aided diagnosis, whose goal is to reproduce human tasks, such as the  
19 detection of lymph node metastases in breast cancer<sup>17,18</sup>, detection of mitosis<sup>19</sup>, or gastric  
20 cancer diagnosis<sup>20</sup>, artificial intelligence (AI) algorithms using WSI can predict patient survival  
21 and specific molecular features in the lung<sup>21</sup>, prostate<sup>22</sup>, brain<sup>23,24</sup>, colorectal<sup>25</sup>,  
22 gastrointestinal<sup>26</sup>, and breast cancer<sup>27,28</sup>.  
23  
24

25 In this study, we present CNN classifiers for *BRCA* 1/2 mutational status prediction from HES-  
26 stained HGOC WSI. As we assumed that the relevant information resides in the tumor, we  
27 developed a tumor segmentation model by using an innovative approach based on an efficient  
28 sampling strategy with a small annotated training dataset. Finally, we studied the impact of  
29 the resolution field on the final prediction through a multiscale approach.  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50

## 51 **MATERIAL AND METHODS**

### 60 OvarIA *BRCA* cohort

1 The OvarIA cohort consists of two independent cohorts of patients diagnosed with HGOC from  
2 biopsy or surgery and who underwent tumor *BRCA* mutational status testing by NGS in  
3 Department of Medical Genetics at the University Hospital of Nantes and Rennes. NGS library  
4 preparation was performed with *BRCA* MASTR Dx Kit (Multiplicom Agilent Technologies) for  
5 Nantes patients and with Advanta™ Solid Tumor NGS Library Prep Assay (Standard BioTools)  
6 for Rennes patients. The sequencing was performed with Illumina MiSeq for Nantes patients  
7 and Illumina NextSeq 500/550 for Rennes patients (Illumina, San Diego, California, US). The  
8 whole OvarIA cohort consists of 867 patients: 169 with *BRCA* mutation (*BRCA*-mut) and 698  
9 with no *BRCA* mutation (*BRCA*-wt). The first cohort included 551 patients (122 *BRCA*-mut and  
10 429 *BRCA*-wt) and the second cohort included 316 patients (47 *BRCA*-mut and 269 *BRCA*-  
11 wt), respectively from the Pathological Departments of the University Hospital of Nantes and  
12 Rennes. All variants of uncertain significance (VUS) and uninterpretable results have been  
13 discarded. One FFPE block per patient was collected and HES-stained in each Pathological  
14 Department. All HES-stained slides were finally reviewed and characterized by two  
15 gynecopathologists in the Pathological Department of the University Hospital of Nantes. This  
16 study only included high-grade serous and high-grade endometrioid subtypes and excluded  
17 mucinous subtypes. In decreasing order, the samples concern peritoneum, ovary, fallopian  
18 tube, and metastases site (omentum, node, colon, rectum, ileum, lung, liver, uteri). The slides  
19 were anonymized and then digitized on the same scanner at 20x magnification (0.46 μm/pixel)  
20 using a NanoZoomer S60 Digital slide scanner (Hamamatsu, Japan) at the Micropicell  
21 platform (IRS-UN unit, Nantes). The WSI were saved in ndpi format and converted to tiff format  
22 using openslide<sup>29</sup> and pyvips library.  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50

### 51 TCGA cohort

52 The diagnostic slides from the cancer genome atlas (TCGA) were downloaded from the  
53 genomic data commons portal (<https://portal.gdc.cancer.gov/>) in svsv format and constitute an  
54 external validation cohort. Any slide with tissue folds, blurred artifacts, or pen marks were  
55 excluded. A final dataset of 103 FFPE and H&E-stained samples of HGSOC with *BRCA* status  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1 available (21 *BRCA*-mut and 82 *BRCA*-wt) were retained, converted in tiff format, and  
2 downscaled to 20x magnification (0.5  $\mu\text{m}/\text{pixel}$ ).  
3  
4  
5  
6  
7

## 8 **TUMOR SEGMENTATION**

### 9 **Proposed pipeline**

10  
11  
12  
13 During the study design, we hypothesized that the relevant information for predicting *BRCA*  
14 somatic mutations could be mostly found in the tumor tissue which we have defined as the  
15 carcinomatous cells with the exclusion of stroma. We thus trained an ovarian cancer  
16 segmentation model to predict the tumor pixels. During training, 8 HES-stained slides were  
17 randomly selected from the first cohort (e.g. Nantes) and then pixel-annotated by a first  
18 gynecopathologist. To assess the robustness of our algorithm, 50 HES-stained validation  
19 slides were randomly selected from both cohorts (e.g. Nantes and Rennes) in equal  
20 proportions and were then pixel-annotated by a second gynecopathologist. Because of the  
21 different HES-staining protocols, we applied the Vahadane stain normalization algorithm  
22 during preprocessing to match the color spectrum distribution of the two medical centers<sup>30</sup>.  
23  
24 The validation was assessed using the dice coefficient (DC) and the intersection-over-union  
25 (IoU) score as evaluation metrics.  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43

### 44 **Data annotations**

45  
46 The 8 randomly selected training slides were annotated by a first gynecopathologist in less  
47 than 2 hours, using the opensource software QuPath (version 0.3.2)<sup>31</sup>. All the carcinomatous  
48 areas were delineated with the exclusion of necrosis. The polygonal annotations were then  
49 exported using the javascript object notation format, including X and Y coordinates  
50 corresponding to the annotated regions. These coordinates were then encoded into a binary  
51 tumor mask for strongly-supervised training.  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

## Training process

1  
2 Pre-processing techniques, such as tiling the region of interest into smaller patches, are often  
3 necessary when working with WSI due to their large size. One limitation of this approach is  
4 the necessity to predetermine certain hyperparameters, such as the size of the tile and the  
5 resolution level. This can significantly lengthen the pre-processing time as different  
6 combinations of these hyperparameters must be evaluated to determine the optimal  
7 combination. To address this issue, we used the *Deepflash2*, a python open-source library  
8 developed by Griebel et al.<sup>32</sup> in order to implement a more dynamic training process. Instead  
9 of tiling the WSI into fixed tiles by using a sliding window approach, the slides are saved to  
10 zarr files, a file storage format for chunked, compressed, and N-dimensional arrays. This  
11 format allows a memory-efficient loading of the slides which are then randomly sampled during  
12 training according to a probability density function. Each region of the WSI is weighted,  
13 determining the probability of being sampled at each epoch. One advantage of this dynamic  
14 approach is to use flexible tile shape and scaling factor at runtime. Although this sampling  
15 method is in itself a data augmentation process, we used additional operations such as  
16 flipping, rotating, mirroring, and color changes to increase diversity during training. Powered  
17 by the *Segmentation PyTorch models* package, *Deepflash2* allows the use of different state-  
18 of-the-art architecture (*U-Net*<sup>33</sup>, *UNet++*<sup>34</sup>, *LinkNet*<sup>35</sup>, *DeepLabv3+*<sup>36</sup>) and encoder (*ResNet*<sup>37</sup>,  
19 *Inception*<sup>38</sup>, *EfficientNet*<sup>39</sup>, *ResNeSt*<sup>40</sup>) combinations.  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43

## Hyperparameter optimization

44  
45 Hyperparameters have been set according to a grid search evaluated through 5-fold cross-  
46 validation. The best score was obtained using 512 x 512 pixels tile size, a downscaling factor  
47 of 2, and a sampling weight of 1 for the predicted tumor pixels, 0.7 for ovarian non-tumor tissue  
48 determined by the subtraction of the tumor mask and the whole tissue mask determined by  
49 the Otsu's method, and 0.1 for background regions (Figure 1). We used a U-Net architecture  
50 and an EfficientNet-B4 as an encoder to perform segmentation. We used a cross-entropy loss  
51 and a Ranger optimizer. We trained the model in mixed precision with a  $10^{-3}$  weight decay.  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65



1 Fifteen epochs with a batch size of 32 have been performed using the one-cycle policy with a  
2  $10^{-3}$  max learning rate.  
3  
4  
5

### 6 Post-processing

7  
8 In order to remove all the cutting artifacts localized in the tumor and over-predicted by the  
9 algorithm, we calculated the intersection of the predicted tumor mask with the tissue mask  
10 determined with Otsu's method.  
11  
12  
13  
14  
15  
16

### 17 Model assessment

18  
19 The performance of the models was assessed on a testing set of 50 WSI. As the task involved  
20 semantic segmentation and the tumor ratio within the images exhibited high variability, both  
21 the DC and the IoU were calculated. These metrics evaluate the similarity between the  
22 predicted binary mask (PM) and the ground truth (GT) delineated by the gynecopathologist.  
23  
24  
25  
26  
27  
28  
29  
30  
31

32 These metrics are defined as  
33  
34  
35

$$36 \quad \text{Dice} = \frac{2 \times TP}{(TP + FP) + (TP + FN)} \quad \text{IoU} = \frac{TP}{(TP + FP + FN)}$$

37  
38  
39  
40  
41 *Code Latex :*

42  
43  
44  $DC = \frac{2 \times TP}{(TP + FP) + (TP + FN)}$

45  
46  
47  $IoU = \frac{TP}{(TP + FP + FN)}$

48  
49  
50  
51 The true positives (TP) are the sum of all matching tumor pixels of the PM and the GT while  
52 the false positives (FP) and false negatives (FN) are the sum of tumor pixels that only appear  
53 in the PM or in the GT. We finally averaged the DC and the IoU over the 50 testing WSI.  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

## **BRCA CLASSIFICATION**

### **Proposed pipeline**

After segmentation, we trained a *BRCA* classifier by randomly splitting the OvarIA cohort into a development set (n=80%) and a testing set (n=20%). We trained five models using cross-validation on the development set and tested each of them on the testing set. To determine the most optimal scale for predicting *BRCA* mutation, we performed three different training schemes relying on the multiple instance learning (MIL) paradigm, and using momentum contrast (MoCo). MoCo is an unsupervised learning technique that aims to learn useful representations from unlabeled data. It involves training a model by maximizing agreement between two differently augmented views of the same input tile. Additionally, MoCo incorporates a momentum-based moving average of the model's parameters during training to stabilize and improve the learning of representations.

### **Data pre-processing**

The WSI were first tiled into non-overlapping patches of 512 x 512 pixels at 20x magnification (0.46  $\mu\text{m}/\text{pixel}$ ), according to the tumor mask predicted by our segmentation algorithm. To optimize computation, a maximum random subset of 5000 tiles with at least 50% of their surface covered by the tumor mask was selected from each slide. The first 2000 tiles randomly selected in this subset were retained for the classification, while the following 3000 were used for learning MoCo representations. If the tumor-predicted mask consisted of less than 2000 tiles, these tiles were all retained for classification and no tile was used to train MoCo representations. After tiling, 7 slides have been removed from the OvarIA dataset, because they contained no 512 x 512 pixels tiles with at least 50% of tumor area (Figure 2). This process generated 599 697 tumor tiles for self-supervised learning. However, because of their likely low impact during training, we decided to discard all slides with less than 200 predicted tumor tiles, (i.e. 85 slides) for a final total of 775 WSI and 1 040 149 tumor tiles used for

1 classification (details in Table 1). We used the MoCo repository available at  
2 (<https://github.com/facebookresearch/moco>) to train our model by randomly considering the  
3 following transformations: horizontal flip, grayscale, color jitter and gaussian blur. According  
4 to their popularity in feature extraction, we benchmarked different ResNet depths (18, 34, 50,  
5 and 101 layers). A first feature extraction has been conducted according to the imagenet  
6 parameters then a second after finetuning each model on their final layers. In both cases, the  
7 best performance was observed with the ResNet-50 which was thus chosen as an encoder  
8 and trained from scratch for 200 epochs on 4 GPU Nvidia RTX A4500 20 Go. We used the  
9 SGD optimizer with a momentum of 0.9, a weight decay of  $1e-4$  and a learning rate schedule  
10 with a cosine decay starting at  $3e-2$ .  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23

### 24 Training process

25 Due to the unbalanced dataset, we performed a stratified 5-fold cross-validation on the  
26 development set. As the impact of each tumor tile on prediction is unknown, we decided to  
27 use MIL technique with the attention-based model proposed by Ilse et al.<sup>41</sup>. After encoding,  
28 the feature vector of each tile is mapped to an attention score by a multi-layer perceptron. This  
29 attention score determines how much a given tile will contribute to the slide representation.  
30 The higher the score, the more the tile contributes to the final prediction. The slide  
31 representation is obtained by the sum of the vector of individual features for all tiles, weighted  
32 by the attention score. A second linear layer is then used to output the final prediction from  
33 the slide-level vector representation by minimizing a binary-weighted cross-entropy loss,  
34 according to the imbalanced classes.  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50

### 51 Evaluation criteria

52 Several classification metrics such as area under the ROC Curve (AUC) but also precision,  
53 recall, and F1-score have been used to evaluate the model performance. Precision is the  
54 proportion of TP predictions among all positive predictions while recall is the proportion of TP  
55 predictions out of all positive instances. Their combination determines the F1-score which  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

calculates their harmonic mean. It is particularly beneficial in our case since the classes are imbalanced. All of these metrics were calculated by their mean over the five validation folds of the development set. Each of these five models was then applied to the remaining testing set and their classification metrics were also averaged.

### External validation cohort

We performed an additional validation on an external dataset of EOC from TCGA. We selected 103 high-quality FFPE and H&E-stained slides. We then performed the same preprocessing steps as before, for a total of 111 727 tumor tiles. Their representations were provided by MoCo and each of our five previous models trained in cross-validation were evaluated on this external dataset. The final result is the AUC and the standard deviation averaged over the five models.

### Optimal observation field

To determine the most optimal observation field for predicting *BRCA* mutation, we conducted three additional approaches with different scaling. *Baseline approach* : a maximum of 2000 tiles of 512 x 512 pixels were randomly sampled in each WSI. This first “high-level” approach provides more contextual information. *Approach 2* : each of these tiles was divided into four tiles of 256 x 256 pixels (i.e maximum of 8000 tiles per WSI). Intuitively, this “low-level” approach brings more information in MIL, but less in the tumor spatial conformation. *Approach 3* : these four tiles were finally randomly reassembled into a new mixed tile of 512 x 512 pixels (i.e maximum of 2000 tiles per WSI) (Figure 3). This approach provides more information in MIL than the first approach, but with degraded information on the spatial configuration of the tumor.

### Computational configurations:

1 All analyses have been done under Python 3.7 using the Pytorch framework. The computation  
2 tasks were performed on an Intel Xeon Gold 6140 2.30 GHz processor with NVIDIA V100  
3  
4 Tensor Core GPU 32Go and 32 Go memory RAM. We used CUDA V10.2.89 Toolkit.  
5  
6  
7  
8  
9

## 10 **RESULTS**

### 11 **Tumor segmentation**

12  
13  
14  
15  
16  
17  
18  
19  
20 The first step of this study was to develop a deep-learning model for the automatic  
21 segmentation of HGOC. We used 58 HES-stained WSI randomly selected from the OvarIA  
22 cohort and splitted into a training set of 8 WSI from the first center (e.g. Nantes) and a  
23 validation set composed of 50 WSI from the two centers (e.g. Nantes and Rennes) randomly  
24 sampled in equal proportion. For a better assessment of the real performance of the model,  
25 the training set and the validation set were annotated by two different gynecopathologists. The  
26 similarity between the PM and the GT was computed by averaging the DC and the IoU over  
27 the 50 WSI and no significant difference was observed between the 2 cohorts. The  
28 segmentation model finally achieved an overall DC of 0.915 ( $\pm 0.05$ ) and an overall IoU of  
29 0.847 ( $\pm 0.079$ ).  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43

### 44 **BRCA mutation prediction**

45  
46  
47  
48 After segmenting the tumor over the 860 slides, we discarded 85 slides with less than 200  
49 predicted tumor tiles. We then trained several *BRCA* classification models on the 775  
50 remaining slides following three sampling approaches. In order to assess the robustness of  
51 our model, we next validated the *BRCA* classifier on an external independent test set. We  
52 used 103 FFPE and H&E-stained slides from the TCGA cohort with 21 slides *BRCA*-mut and  
53 82 slides *BRCA*-wt. All the results are presented in Table 2.  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1  
2 In order to increase their interpretability and according to their better results, the baseline  
3  
4 models trained in 5-fold cross-validation have been more precisely evaluated and achieved a  
5  
6 mean precision of 0,714 ( $\pm 0.02$ ), a mean recall of 0,731 ( $\pm 0.023$ ) and a mean F1-score of  
7  
8 0,722 ( $\pm 0.009$ ) on the internal testing set.  
9

## 10 11 12 13 14 15 **DISCUSSION** 16

17  
18  
19  
20 *BRCA1/2* mutations have been shown to be effective predictors of the sensitivity of HGOC to  
21  
22 PARPi. Their identification is crucial for the selection of appropriate therapeutic options.  
23  
24 Currently, somatic *BRCA1/2* mutation testing is conducted using NGS on FFPE tissue  
25  
26 samples, which can be challenging due to the poor quality of the DNA and its high level of  
27  
28 fragmentation. Additionally, tumor samples are often highly heterogeneous and can be  
29  
30 contaminated with DNA from normal tissue. Several studies have demonstrated the  
31  
32 effectiveness of CNN-based models for predicting specific molecular features in various types  
33  
34 of cancer, including lung<sup>21</sup>, prostate<sup>22</sup>, brain<sup>23,24</sup>, colorectal<sup>25</sup>, and gastrointestinal cancers<sup>26</sup>,  
35  
36 as well as breast cancer<sup>27,28</sup>. These studies have shown that CNN-based models can  
37  
38 accurately predict genomic alterations, such as mutations in specific genes, based on  
39  
40 morphological features extracted from WSI.  
41  
42

43  
44 In this paper, we present a deep learning-based model for predicting *BRCA1/2* mutation status  
45  
46 using an innovative tumor segmentation method. To our knowledge, our model has achieved  
47  
48 the best performance hitherto in predicting BRCA mutations in HGOC from WSI. Furthermore,  
49  
50 it is also the first model that has undergone evaluation on an external validation cohort.  
51  
52

53  
54  
55 Tumor segmentation is a classical task in computational pathology. It allows the identification  
56  
57 of regions of interest useful for complementary classification tasks<sup>42</sup>. However, pixel-level  
58  
59 segmentation requires precise annotations which are not always available. Indeed, annotation  
60  
61  
62  
63  
64  
65

1 is a costly and time-consuming task that requires a pathologist's expertise. Moreover, there  
2 are still few published annotation guidelines which leads to high inter-individual variability and  
3 bad interoperability of annotation protocols<sup>43</sup>. In their paper about ovarian cancer  
4 segmentation, Ho et al. have adopted a Deep Interactive Learning method to reduce the time  
5 of manual annotation<sup>44</sup>. They used a breast cancer segmentation model, pre-trained to  
6 segment high-grade invasive ductal carcinoma from triple-negative breast cancer images.  
7 They then finetuned it to segment HGSOc, thanks to the similarity of their morphological  
8 features. The training process is composed of multiple iterations of segmentation, assessment  
9 then correction of the prediction generated by the previous model. They thus initially predicted  
10 the HGSOc over 60 randomly selected WSI with their pre-trained breast cancer model and  
11 finally trained their own ovarian model after three iterations and the annotation of 25 WSI in  
12 3,5 hours. They then evaluated their model on 14 WSI and achieved an IoU of 0,74 for their  
13 best model. However, this approach ideally requires more than twenty WSI in training which  
14 may be difficult to obtain in rare diseases. Furthermore, this technique requires a pre-trained  
15 model on a tumor of more similar morphology.

16 A key improvement of our method is the use of a dynamic training process which considerably  
17 reduces annotation and pre-processing time. Thanks to the Deepflash2 library intersection  
18 and efficient sampling, we were able to train a segmentation model with less than 2 hours of  
19 manual annotations on 8 WSI. Our model was then evaluated on 50 WSI and obtained an  
20 overall DC of 0.941 ( $\pm 0.05$ ) and an IoU of 0.846 ( $\pm 0.079$ ). A particularity of our segmentation  
21 model is that, despite the few areas of stroma in the tumor mask labeled by the pathologist in  
22 the training set, we observed that, for the large majority of WSI, only the tumor pixels were  
23 predicted and not the stroma, as shown in Figure 2B. In some cases, this could be explained  
24 by a higher contrast of the tumor than the stroma with the rest of the non-tumor tissue. The  
25 difference between tumor mask delineated by the second gynecopathologist for the testing  
26 set and predicted tumor mask, explains some  $DC < 0.7$ . Indeed, it is sometimes difficult to  
27 exclude all the stromal tissue manually, especially for post-chemotherapy tumors or tumors  
28 with papillary or micropapillary architecture. This difference can however be reduced by post-

1 processing techniques, such as prediction smoothing. Nevertheless, as we seek to predict the  
2 somatic mutation status of *BRCA*, the objective of this segmentation step was to extract the  
3 tumor pixels while excluding as many non-tumor pixels as possible. Another reason for the  
4 decrease of the DC is the localized artifacts within the tumors. These artifacts were bypassed  
5 by the gynecopathologist while they are systematically removed during the post-processing  
6 with the intersection of the predicted mask and the tissue mask obtained by Otsu's method.  
7  
8  
9  
10  
11  
12  
13  
14

15 Several studies have focused on the use of deep learning for predicting gene mutations from  
16 routine FFPE and H&E-stained histology slides. Coudray et al. showed that six mutated genes  
17 in lung adenocarcinoma can be predicted with an AUC ranging from 0.733 to 0.856<sup>21</sup>. Other  
18 studies have demonstrated an AUC of 0.71 for the prediction of SPOP mutations in prostate  
19 cancer images<sup>22</sup>, and AUC ranging from 0.71 to 0.89 for the prediction of CTNNB1, FMN2,  
20 TP53, and ZFX4 mutations in hepatocellular carcinoma<sup>45</sup>.  
21  
22  
23  
24  
25  
26  
27  
28

29 To our knowledge, there are only two studies that have investigated the prediction of *BRCA*  
30 mutations in ovarian cancer<sup>44,46</sup>. Ho et al. recently conducted a study in which they aimed to  
31 predict *BRCA* mutations in HGSOc<sup>44</sup>. They included 609 HGSOc (119 *BRCA*-mut and 490  
32 *BRCA*-wt) and used a similar approach by first training a tumor segmentation model, and then  
33 training an additional classifier to predict *BRCA* mutations on the automatically extracted  
34 cancer patches. The authors performed an unbalanced sampling with a maximum of 1000  
35 tiles for non-mutated patients and 5000 tiles for mutated patients. They used a fixed tile size  
36 of 224 x 224 pixels and trained three *BRCA* classification models at various magnifications  
37 (5x, 10x, 20x). The slide-level score was then calculated by averaging all the patch scores of  
38 a WSI. They obtained an AUC ranging from 0.49 to 0.67 on the validation set and between  
39 0.40 and 0.43 on the testing set. Another study, conducted by Nero et al. also investigated the  
40 prediction of *BRCA* mutations in 664 ovarian cancer patients comprising 233 cases of somatic  
41 *BRCA1/2* mutations<sup>46</sup>. In contrast to Ho et al. they did not focus on the tumor and tiled the  
42 whole tissue slide. Each patch was then embedded into a low-dimensional feature  
43 representation before being weighted by an attention branch. They finally achieved an AUC  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65



1 of 0.7 on the training set and 0.55 on the testing set, improved at 0.59 by manually identifying  
2 areas of interest on a subset of 285 WSI.

3  
4 In our study, we combined both a tumor segmentation step and the use of an attention  
5 mechanism during classification. This allowed us to achieve the state-of-the-art AUC of 0.739  
6  
7  
8 ( $\pm 0.024$ ) in 5-fold cross-validation, an AUC of 0.681 ( $\pm 0.014$ ) over the internal testing set,  
9  
10 and an AUC of 0.631 ( $\pm 0.03$ ) over the TCGA dataset. Nevertheless, most of our patients were  
11  
12 recruited before 2019 and only underwent *BRCA1/2* mutation testing, which constitutes one  
13  
14 limitation of our study. Indeed, *BRCA1/2* mutations represent one of several causes of  
15  
16 homologous recombination deficiency (HRD), which is characterized by genomic instability  
17  
18 and also defines a target for PARPi. Tumors with similar morphological characteristics to those  
19  
20 of *BRCA*-mutated tumors may also harbor mutations in other genes in the HRD pathway. It  
21  
22 would be interesting to replicate this work on a cohort of tumors with HRD, as was done by  
23  
24 Lazard et al. in breast cancer, where they achieved an AUC of 0.86<sup>47</sup>. They also developed  
25  
26 an innovative visualization technique that allows for the automatic extraction of new  
27  
28 morphological features related to HRD.  
29  
30

31  
32 In their paper, Ho et al. have also studied the impact of resolution by training a deep multi-  
33  
34 magnification network (DMMN). This model fuses morphological features from both high and  
35  
36 low levels by the concatenation of patches at 20x, 10x, and 5x magnification. We decided to  
37  
38 confirm this assumption by conducting three different approaches at the same resolution (20x  
39  
40 magnification). The first approach consisted in tiling the tumor area in 512 x 512 pixels tiles  
41  
42 while the majority of similar studies tile in 256 or 224 pixels. This approach reduces the total  
43  
44 number of tiles but allows to get more contextual information about the tumor and its  
45  
46 architecture. These tiles were then divided into four tiles of 256 x 256 pixels. This step brings  
47  
48 four times more information during MIL, but reduces the contextual information, as the tiles  
49  
50 are encoded independently from each other in the CNN. This approach led to lower AUC of  
51  
52 0.721 ( $\pm 0.014$ ) for 5-fold cross-validation, AUC of 0.654 ( $\pm 0.011$ ) over the testing set and  
53  
54 AUC of 0.624 ( $\pm 0.024$ ) over TCGA dataset. Finally, we performed a last approach by randomly  
55  
56 reassembling the four previous 256 x 256 pixels tumor tiles into a unique 512 x 512 pixels  
57  
58  
59  
60  
61  
62  
63  
64  
65

1 mixed tumor tile. This approach brings as much information as the baseline, but reduces the  
2 tumor context by impairing its spatial architecture. This approach achieved the most  
3 disappointing results compared to the two others, with an AUC of 0.697 ( $\pm$  0.016), for 5-fold  
4 cross-validation, AUC of 0.635 ( $\pm$  0.015) over the testing set, and AUC of 0.619 ( $\pm$  0.024) over  
5 TCGA dataset. This multiscale approach suggests that the relevant information for predicting  
6  
7  
8  
9 *BRCA* mutations is preferentially located in the tumor context than in the cell morphology.

10 It could be interesting to compare this multiscale approach with the use of vision transformer<sup>48</sup>.

11 It is a model introduced in 2021 where the input image is split into fixed-size patches before  
12 being linearly embedded with their positional information and fed to a transformer encoder  
13 with a self-attention module. This module allows a global interpretation by capturing distant  
14 semantic relevances in an image while convolution is only focused on neighboring pixels.

15 However, the first vision transformer usually required large datasets to achieve similar or better  
16 performance than CNN on various tasks. This is the reason why Guo et al. in their paper, used  
17 a swin Transformer to predict microsatellite instability and key biomarkers in colorectal cancer  
18 from H&E stained images<sup>49</sup>. It is a hierarchical transformer whose representations are  
19 computed with a shifted windows. This architecture allows them to achieve a state-of-the-art  
20 AUC of 0.9 for MSI prediction using the MCO dataset composed of 1065 patients. This  
21 achievement will probably pave the way for the use of vision transformers on small datasets  
22 in computation pathology.

23 In conclusion, our results suggest that somatic mutations of *BRCA* have a phenotypic impact  
24 in HGOC. They also suggest that the relevant information for the prediction of *BRCA*  
25 mutational status seems to reside more in the tumor spatial conformation than in the cell  
26 morphology. It could also be interesting to investigate the phenotypic pattern of the tumor tiles  
27 with the highest probability of being *BRCA* mutated, according to the attention module in a  
28 future study. Moreover this work needs to be confirmed on an HGOC cohort with HRD. These  
29 AI-based models will probably be used more and more in the diagnostic routine to pre-screen  
30 tumors and optimize the preselection of patients for molecular determination. Even if this study

1 needs to be validated on a larger and multicenter cohort, it paves the way to clinical application  
2 with the future implementation of pre-screening tools for a more personalized medicine.  
3  
4  
5

### 6 **ETHICS APPROVAL**

7  
8  
9 The study was approved by two French ethics committees for the two cohorts: Sud-Ouest and  
10 Outre-mer CPP-registration N°21.00545.000010 for Nantes and Ouest II CPP-registration:  
11 N°22.02541.000097 for Rennes.  
12  
13  
14  
15

### 16 **AUTHOR CONTRIBUTIONS STATEMENT**

17  
18  
19 R.B and D.L. performed study concept and design. D.L., S.-F.K.J, C.S. and S.H. generated  
20 the patient cohort. D.L and C.S performed analysis and interpretation of data. C.G., S.B.,  
21 C.D., A.L and M.D.T. performed genomic analyses. J.-F.M. was the scientific supervisor of the  
22 tissue biobank. R.B. performed development of AI methodology and statistical analysis. R.B.  
23 and T.Per. developed the tumor segmentation model. N.R and T.Pec. pre-trained the feature  
24 extractor on their workstation. R.B. developed the *BRCA* classification model. R.B., A.G. and  
25 C.T. prepared the figures. R.B. and D.L. wrote the manuscript. All authors read and approved  
26 the final paper.  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39

### 40 **FUNDING STATEMENT**

41  
42 We are grateful to AstraZeneca for their financial support. We also thank the biological  
43 resource center for biobanking (CHU Nantes, Hôtel Dieu, Tumorotheque, Nantes, F-44093,  
44 France) for its contribution. T.Pec. is funded by a Chan Zuckerberg Initiative DAF grant (2019-  
45 198009).  
46  
47  
48  
49  
50  
51  
52

### 53 **DATA AVAILABILITY STATEMENT**

54  
55 All the TCGA dataset is available at <https://portal.gdc.cancer.gov/>.

56  
57  
58 The OvarIA dataset consists of confidential medical data from the Pathological Department of  
59 the University Hospital of Nantes and Rennes and is not open to the public. Any information  
60  
61  
62  
63  
64  
65

1  
2 and requests for resources should be directed to Delphine Loussouarn  
3 (delphine.loussouarn@chu-nantes.fr).  
4  
5  
6  
7  
8  
9

## 10 REFERENCE

- 11  
12  
13  
14  
15 1. Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates  
16 of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin.*  
17  
18 2021;71(3):209-249. doi:10.3322/caac.21660  
19  
20  
21
- 22 2. Ledermann JA, Raja FA, Fotopoulou C, et al. Newly diagnosed and relapsed epithelial  
23 ovarian carcinoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and  
24 follow-up. *Ann Oncol Off J Eur Soc Med Oncol.* 2013;24 Suppl 6:vi24-32.  
25  
26 doi:10.1093/annonc/mdt333  
27  
28
- 29 3. Caestecker KW, Van de Walle GR. The role of BRCA1 in DNA double-strand repair: past  
30 and present. *Exp Cell Res.* 2013;319(5):575-587. doi:10.1016/j.yexcr.2012.11.013  
31  
32  
33
- 34 4. Cancer Genome Atlas Research Network. Integrated genomic analyses of ovarian  
35 carcinoma. *Nature.* 2011;474(7353):609-615. doi:10.1038/nature10166  
36  
37  
38
- 39 5. Pennington KP, Walsh T, Harrell MI, et al. Germline and somatic mutations in  
40 homologous recombination genes predict platinum response and survival in ovarian,  
41 fallopian tube, and peritoneal carcinomas. *Clin Cancer Res Off J Am Assoc Cancer Res.*  
42  
43 2014;20(3):764-775. doi:10.1158/1078-0432.CCR-13-2287  
44  
45  
46
- 47 6. Hartwell LH, Szankasi P, Roberts CJ, Murray AW, Friend SH. Integrating genetic  
48 approaches into the discovery of anticancer drugs. *Science.* 1997;278(5340):1064-1068.  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65
7. Ledermann J, Harter P, Gourley C, et al. Olaparib maintenance therapy in patients with  
platinum-sensitive relapsed serous ovarian cancer: a preplanned retrospective analysis of  
outcomes by BRCA status in a randomised phase 2 trial. *Lancet Oncol.* 2014;15(8):852-

861. doi:10.1016/S1470-2045(14)70228-1

- 1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65
8. Pujade-Lauraine E, Ledermann JA, Selle F, et al. Olaparib tablets as maintenance therapy in patients with platinum-sensitive, relapsed ovarian cancer and a BRCA1/2 mutation (SOLO2/ENGOT-Ov21): a double-blind, randomised, placebo-controlled, phase 3 trial. *Lancet Oncol.* 2017;18(9):1274-1284. doi:10.1016/S1470-2045(17)30469-2
9. Coleman RL, Oza AM, Lorusso D, et al. Rucaparib maintenance treatment for recurrent ovarian carcinoma after response to platinum therapy (ARIEL3): a randomised, double-blind, placebo-controlled, phase 3 trial. *The Lancet.* 2017;390(10106):1949-1961. doi:10.1016/S0140-6736(17)32440-6
10. Moore K, Colombo N, Scambia G, et al. Maintenance Olaparib in Patients with Newly Diagnosed Advanced Ovarian Cancer. *N Engl J Med.* 2018;379(26):2495-2505. doi:10.1056/NEJMoa1810858
11. Ray-Coquard I, Pautier P, Pignata S, et al. Olaparib plus Bevacizumab as First-Line Maintenance in Ovarian Cancer. *N Engl J Med.* 2019;381(25):2416-2428. doi:10.1056/NEJMoa1911361
12. González-Martín A, Pothuri B, Vergote I, et al. Niraparib in Patients with Newly Diagnosed Advanced Ovarian Cancer. *N Engl J Med.* 2019;381(25):2391-2402. doi:10.1056/NEJMoa1910962
13. Coleman RL, Fleming GF, Brady MF, et al. Veliparib with First-Line Chemotherapy and as Maintenance Therapy in Ovarian Cancer. *N Engl J Med.* 2019;381(25):2403-2415. doi:10.1056/NEJMoa1909707
14. Kofanova O, Bellora C, Garcia Frascuilho S, et al. Standardization of the preanalytical phase of DNA extraction from fixed tissue for next-generation sequencing analyses. *New Biotechnol.* 2020;54:52-61. doi:10.1016/j.nbt.2019.07.005
15. Vos JR, Fakkert IE, de Hullu JA, et al. Universal Tumor DNA BRCA1/2 Testing of Ovarian Cancer: Prescreening PARPi Treatment and Genetic Predisposition. *JNCI J Natl Cancer Inst.* 2020;112(2):161-169. doi:10.1093/jnci/djz080
16. Griffin J, Treanor D. Digital pathology in clinical use: where are we now and what is

holding us back? *Histopathology*. 2017;70(1):134-145. doi:10.1111/his.12993

17. Ehteshami Bejnordi B, Mullooly M, Pfeiffer RM, et al. Using deep convolutional neural networks to identify and classify tumor-associated stroma in diagnostic breast biopsies. *Mod Pathol*. 2018;31(10):1502-1512. doi:10.1038/s41379-018-0073-z
18. Campanella G, Hanna MG, Geneslaw L, et al. Clinical-grade computational pathology using weakly supervised deep learning on whole slide images. *Nat Med*. 2019;25(8):1301-1309. doi:10.1038/s41591-019-0508-1
19. Veta M, van Diest PJ, Willems SM, et al. Assessment of algorithms for mitosis detection in breast cancer histopathology images. *Med Image Anal*. 2015;20(1):237-248. doi:10.1016/j.media.2014.11.010
20. Ba W, Wang S, Shang M, et al. Assessment of deep learning assistance for the pathological diagnosis of gastric cancer. *Mod Pathol Off J U S Can Acad Pathol Inc*. 2022;35(9):1262-1268. doi:10.1038/s41379-022-01073-z
21. Coudray N, Ocampo PS, Sakellaropoulos T, et al. Classification and mutation prediction from non-small cell lung cancer histopathology images using deep learning. *Nat Med*. 2018;24(10):1559-1567. doi:10.1038/s41591-018-0177-5
22. Schaumberg AJ, Rubin MA, Fuchs TJ. *H&E-Stained Whole Slide Image Deep Learning Predicts SPOP Mutation State in Prostate Cancer*. *Pathology*; 2016. doi:10.1101/064279
23. Chang P, Grinband J, Weinberg BD, et al. Deep-Learning Convolutional Neural Networks Accurately Classify Genetic Mutations in Gliomas. *AJNR Am J Neuroradiol*. 2018;39(7):1201-1207. doi:10.3174/ajnr.A5667
24. Mobadersany P, Yousefi S, Amgad M, et al. Predicting cancer outcomes from histology and genomics using convolutional networks. *Proc Natl Acad Sci U S A*. 2018;115(13):E2970-E2979. doi:10.1073/pnas.1717139115
25. Bilal M, Raza SEA, Azam A, et al. Development and validation of a weakly supervised deep learning framework to predict the status of molecular pathways and key mutations in colorectal cancer from routine histology images: a retrospective study.

*Lancet Digit Health*. 2021;3(12):e763-e772. doi:10.1016/S2589-7500(21)00180-1

26. Kather JN, Pearson AT, Halama N, et al. Deep learning can predict microsatellite instability directly from histology in gastrointestinal cancer. *Nat Med*. 2019;25(7):1054-1056. doi:10.1038/s41591-019-0462-y
27. Farahmand S, Fernandez AI, Ahmed FS, et al. Deep learning trained on hematoxylin and eosin tumor region of Interest predicts HER2 status and trastuzumab treatment response in HER2+ breast cancer. *Mod Pathol*. 2022;35(1):44-51. doi:10.1038/s41379-021-00911-w
28. Wang Y, Acs B, Robertson S, et al. Improved breast cancer histological grading using deep learning. *Ann Oncol Off J Eur Soc Med Oncol*. 2022;33(1):89-98. doi:10.1016/j.annonc.2021.09.007
29. Goode A, Gilbert B, Harkes J, Jukic D, Satyanarayanan M. OpenSlide: A vendor-neutral software foundation for digital pathology. *J Pathol Inform*. 2013;4(1):27. doi:10.4103/2153-3539.119005
30. Vahadane A, Peng T, Sethi A, et al. Structure-Preserving Color Normalization and Sparse Stain Separation for Histological Images. *IEEE Trans Med Imaging*. 2016;35(8):1962-1971. doi:10.1109/TMI.2016.2529665
31. Bankhead P, Loughrey MB, Fernández JA, et al. QuPath: Open source software for digital pathology image analysis. *Sci Rep*. 2017;7(1):16878. doi:10.1038/s41598-017-17204-5
32. Griebel M, Segebarth D, Stein N, et al. Deep-learning in the bioimaging wild: Handling ambiguous data with deepflash2. Published online November 12, 2021. doi:10.48550/arXiv.2111.06693
33. Ronneberger O, Fischer P, Brox T. U-Net: Convolutional Networks for Biomedical Image Segmentation. In: Navab N, Hornegger J, Wells WM, Frangi AF, eds. *Medical Image Computing and Computer-Assisted Intervention – MICCAI 2015*. Lecture Notes in Computer Science. Springer International Publishing; 2015:234-241. doi:10.1007/978-3-319-24574-4\_28

- 1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65
34. Zhou Z, Rahman Siddiquee MM, Tajbakhsh N, Liang J. UNet++: A Nested U-Net Architecture for Medical Image Segmentation. In: Stoyanov D, Taylor Z, Carneiro G, et al., eds. *Deep Learning in Medical Image Analysis and Multimodal Learning for Clinical Decision Support*. Lecture Notes in Computer Science. Springer International Publishing; 2018:3-11. doi:10.1007/978-3-030-00889-5\_1
  35. Chaurasia A, Culurciello E. LinkNet: Exploiting Encoder Representations for Efficient Semantic Segmentation. In: *2017 IEEE Visual Communications and Image Processing (VCIP)*. ; 2017:1-4. doi:10.1109/VCIP.2017.8305148
  36. Chen LC, Zhu Y, Papandreou G, Schroff F, Adam H. Encoder-Decoder with Atrous Separable Convolution for Semantic Image Segmentation. In: Ferrari V, Hebert M, Sminchisescu C, Weiss Y, eds. *Computer Vision – ECCV 2018*. Vol 11211. Lecture Notes in Computer Science. Springer International Publishing; 2018:833-851. doi:10.1007/978-3-030-01234-2\_49
  37. He K, Zhang X, Ren S, Sun J. Deep Residual Learning for Image Recognition. In: *2016 IEEE Conference on Computer Vision and Pattern Recognition (CVPR)*. IEEE; 2016:770-778. doi:10.1109/CVPR.2016.90
  38. Szegedy C, Vanhoucke V, Ioffe S, Shlens J, Wojna Z. Rethinking the Inception Architecture for Computer Vision. *IEEE Computer Society Conference on Computer Vision and Pattern Recognition*. Published online 2016:2818-2826.
  39. Tan M, Le QV. EfficientNet: Rethinking Model Scaling for Convolutional Neural Networks. *International Conference of Machine Learning ICML*. Published online 2019:6105-6114.
  40. Zhang H, Wu C, Zhang Z, et al. ResNeSt: Split-Attention Networks. *Conference on Computer Vision and Pattern Recognition (CVPR)*. Published online 2022:2736-2746.
  41. Ilse M, Tomczak JM, Welling M. Attention-based Deep Multiple Instance Learning. Published online June 28, 2018. doi:10.48550/arXiv.1802.04712
  42. Feng R, Liu X, Chen J, Chen DZ, Gao H, Wu J. A Deep Learning Approach for Colonoscopy Pathology WSI Analysis: Accurate Segmentation and Classification. *IEEE J*



*Biomed Health Inform.* 2021;25(10):3700-3708. doi:10.1109/JBHI.2020.3040269

- 1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52
43. Wahab N, Miligy IM, Dodd K, et al. Semantic annotation for computational pathology: multidisciplinary experience and best practice recommendations. *J Pathol Clin Res.* 2022;8(2):116-128. doi:10.1002/cjp2.256
44. Ho DJ, Chui MH, Vanderbilt CM, et al. Deep Interactive Learning-based ovarian cancer segmentation of H&E-stained whole slide images to study morphological patterns of BRCA mutation. *J Pathol Inform.* 2023;14:100160. doi:10.1016/j.jpi.2022.100160
45. Chen M, Zhang B, Topatana W, et al. Classification and mutation prediction based on histopathology H&E images in liver cancer using deep learning. *Npj Precis Oncol.* 2020;4(1):1-7. doi:10.1038/s41698-020-0120-3
46. Nero C, Boldrini L, Lenkowicz J, et al. Deep-Learning to Predict BRCA Mutation and Survival from Digital H&E Slides of Epithelial Ovarian Cancer. *Int J Mol Sci.* 2022;23(19):11326. doi:10.3390/ijms231911326
47. Lazard T, Bataillon G, Naylor P, et al. Deep learning identifies morphological patterns of homologous recombination deficiency in luminal breast cancers from whole slide images. *Cell Rep Med.* 2022;3(12):100872. doi:10.1016/j.xcrm.2022.100872
48. Dosovitskiy A, Beyer L, Kolesnikov A, et al. An Image is Worth 16x16 Words: Transformers for Image Recognition at Scale. Published online June 3, 2021. doi:10.48550/arXiv.2010.11929
49. Guo B, Li X, Yang M, Jonnagaddala J, Zhang H, Xu XS. Predicting microsatellite instability and key biomarkers in colorectal cancer from H&E-stained images: achieving state-of-the-art predictive performance with fewer data using Swin Transformer. *J Pathol Clin Res.* Published online 2023. doi:10.1002/cjp2.312

## **FIGURE LEGENDS**

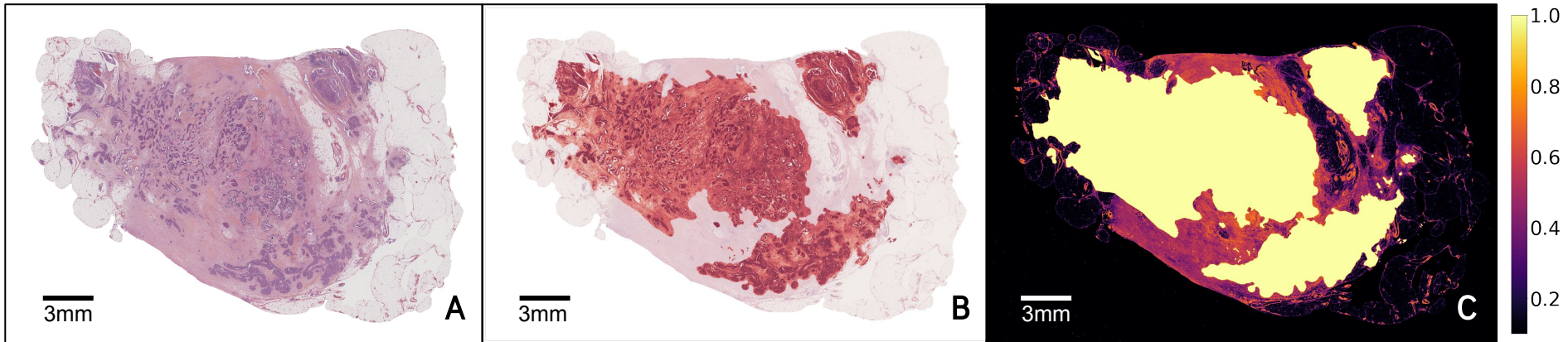
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

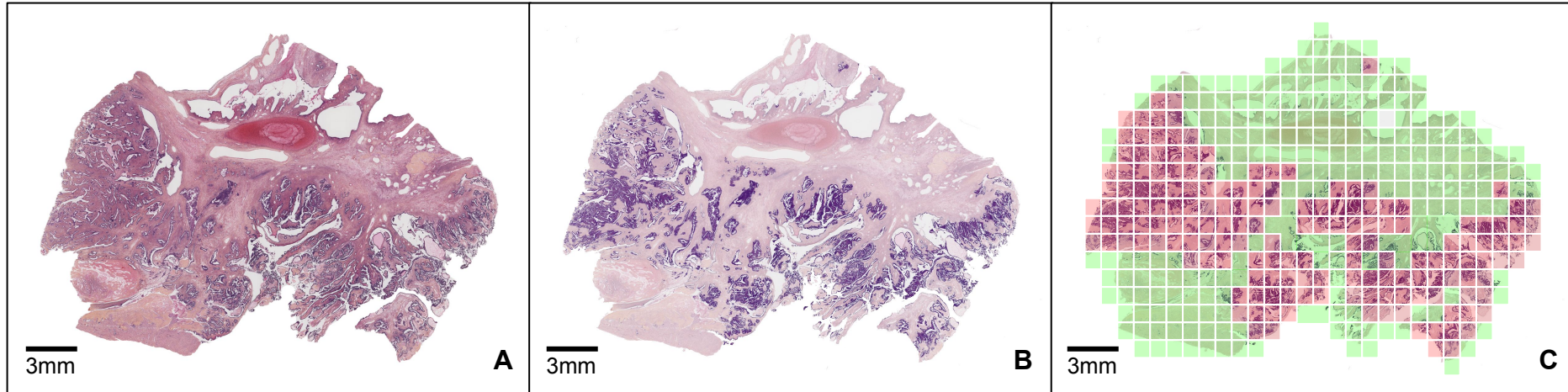
Figure 1: Original HES slide (A). Tumor mask annotated by a pathologist in red (B). Sampling weighted mask as a combination of the tumor mask and the tissue mask (C). The tumor mask

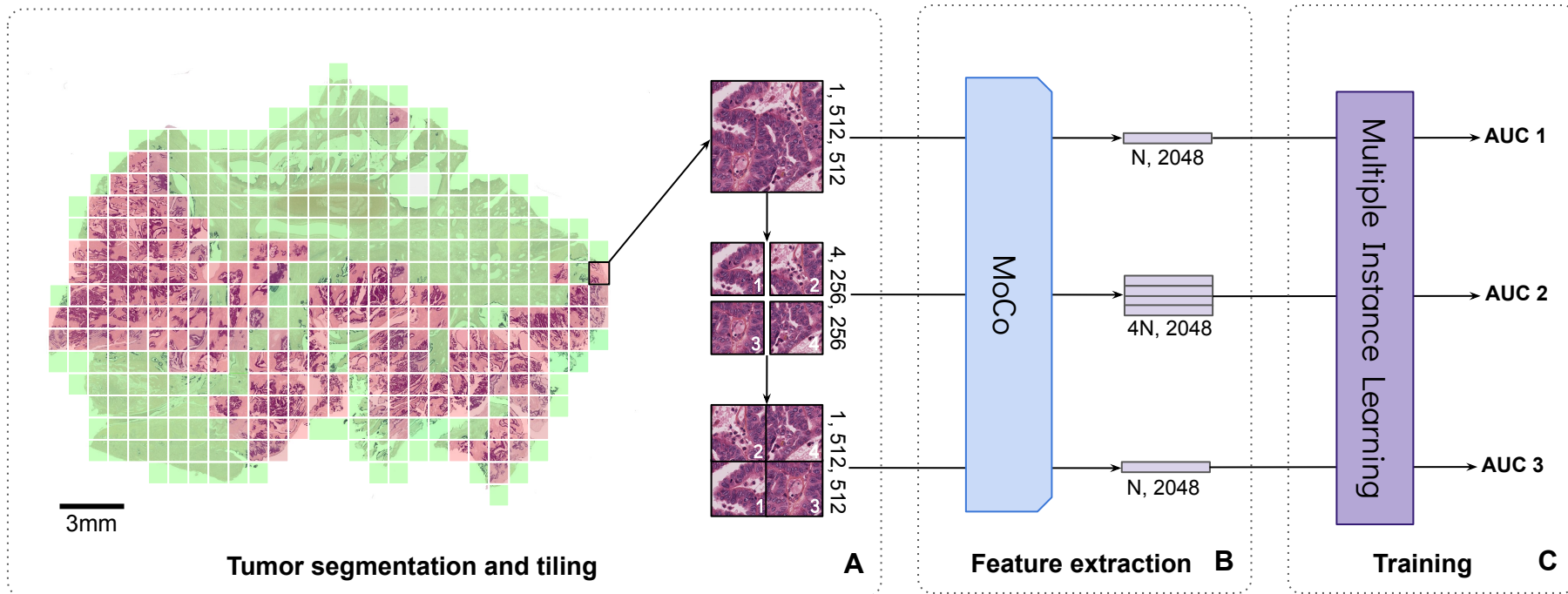
1  
2 in yellow has a sampling weight of 1 while the tissue mask in pink has a sampling weight of  
3 0.7 and 0.1 for the background.  
4  
5  
6  
7

8  
9 Figure 2: Original HES slide (A). Predicted tumor mask in purple (B). Tiling of the predicted  
10 mask: a maximum of 5000 tiles with more than 50% of their surface covered by the tumor  
11 mask are kept (in red). The green tiles include less than 50% tumor pixels and are therefore  
12 discarded (C).  
13  
14  
15  
16  
17  
18  
19  
20  
21

22 Figure 3: Overview of the multiscale approach. First, the tumor pixels are predicted by the  
23 ovarian cancer segmentation model. The predicted tumor masks are tiled into 512 x 512 pixels  
24 tiles. The resulting tiles are then separately divided into 4 tiles of 256 x 256 pixels and then  
25 randomly reassembled into a new mixed tile of 512 x 512 pixels (A). All of these datasets are  
26 then embedded into a low-dimensional space using a ResNet-50 as encoder, trained using  
27 the momentum contrast technique with N, the number of tiles per WSI (B). The embedded  
28 tiles are then scored through an attention mechanism and are finally fed to a decision module  
29 to predict BRCA mutation (C).  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65







	Dataset 1	Dataset 2	Dataset 3
Source	Nantes	Rennes	TCGA
Number of tumor tiles for self-supervised learning	331 998	267 699	none
Number of tumor tiles for classification :			
<i>BRCA</i> -mut	129 041	53 508	20972
<i>BRCA</i> -wt	460 832	396 768	90755

Table 1: The number of tumor tiles used to learn MoCo representations and perform the classification according to the MIL paradigm.

	5-fold cross-validation		Internal testing set		TCGA	
	AUC	Sd	AUC	Sd	AUC	Sd
Baseline	0,739	0.024	0,681	0,014	0,631	0,035
Approach 2	0,721	0.014	0,654	0,011	0.624	0.024
Approach 3	0,697	0.016	0,635	0,015	0.619	0.024

Table 2: Summary of performance metrics. Mean and standard deviation (SD) are computed over the five test sets of the cross-validation, the internal testing set, and the TCGA dataset. AUC, area under the (receiver-operating characteristics) curve. The baseline approach relies on 512 x 512 pixels tiles. Approach 2 relies on the division of these tiles into 4 tiles of 256 x 256 pixels. Approach 3 relies on the random reassembling of these 4 tiles into a new mixed tumor tile of 512 x 512 pixels.