

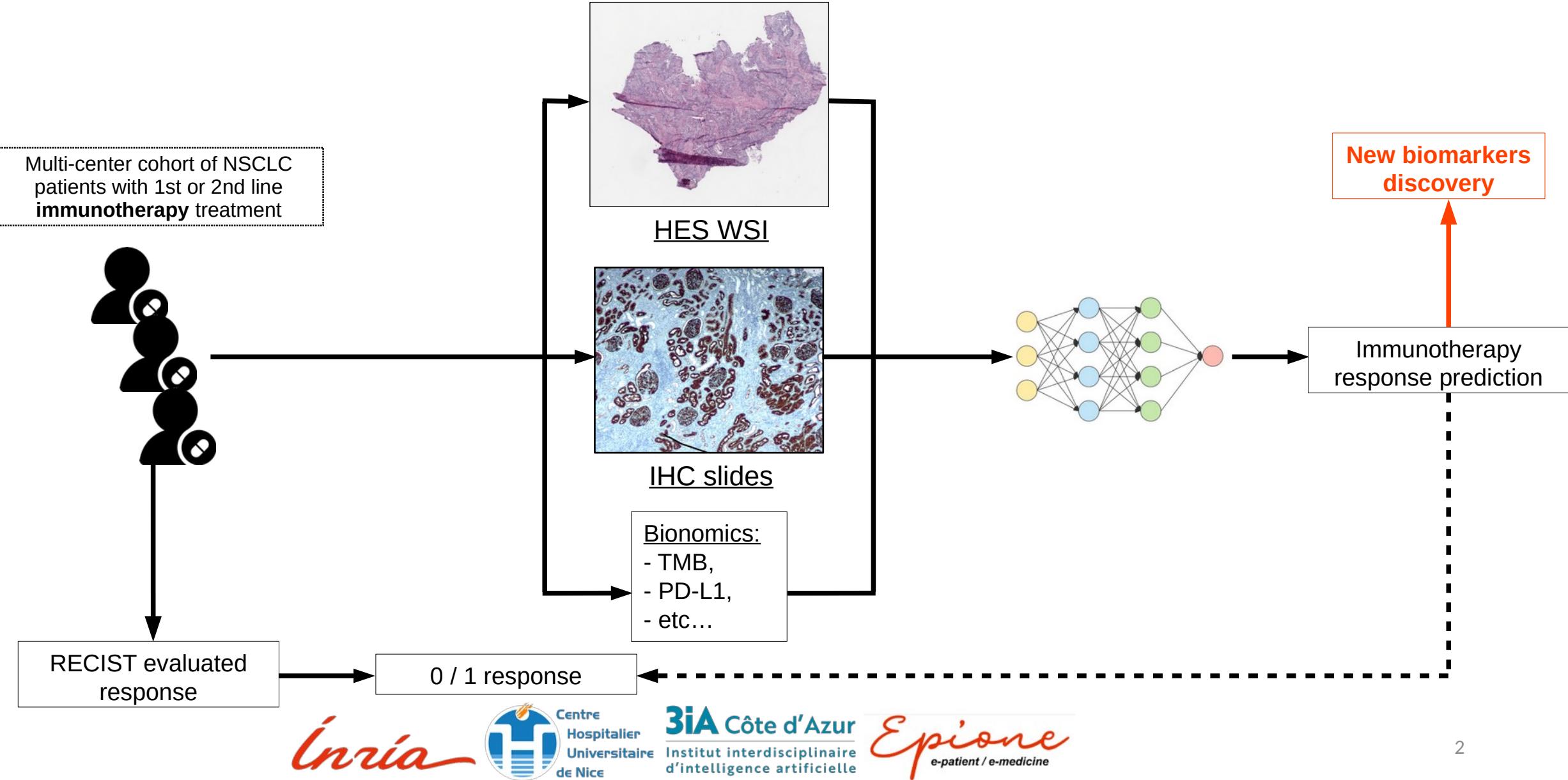
An introduction to machine learning for histopathology

Paul Tourniaire

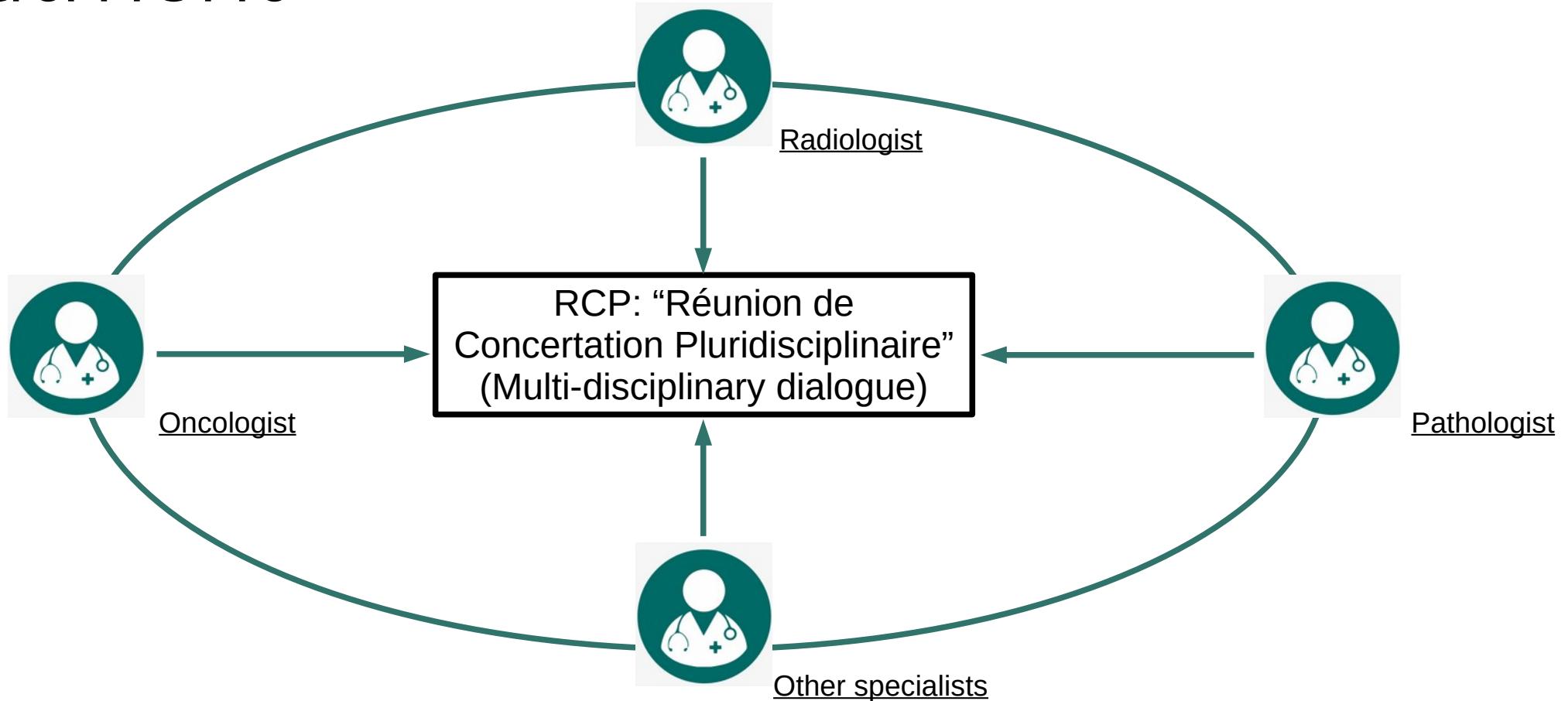


AI-based selection of imaging and biological markers predictive of therapy response in NSCLC

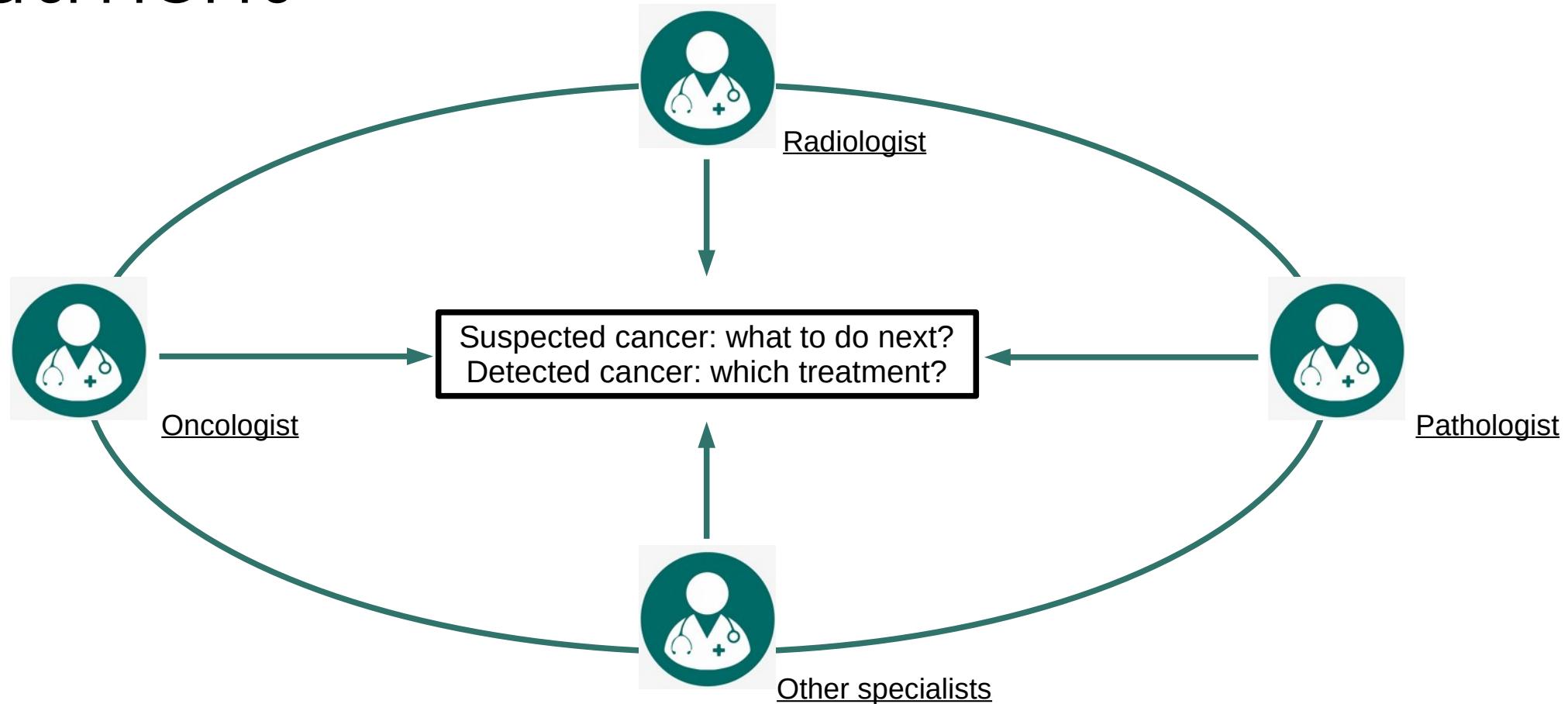
Paul Tourniaire, Hervé Delingette, Marius Ilié, Paul Hofman, Nicholas Ayache



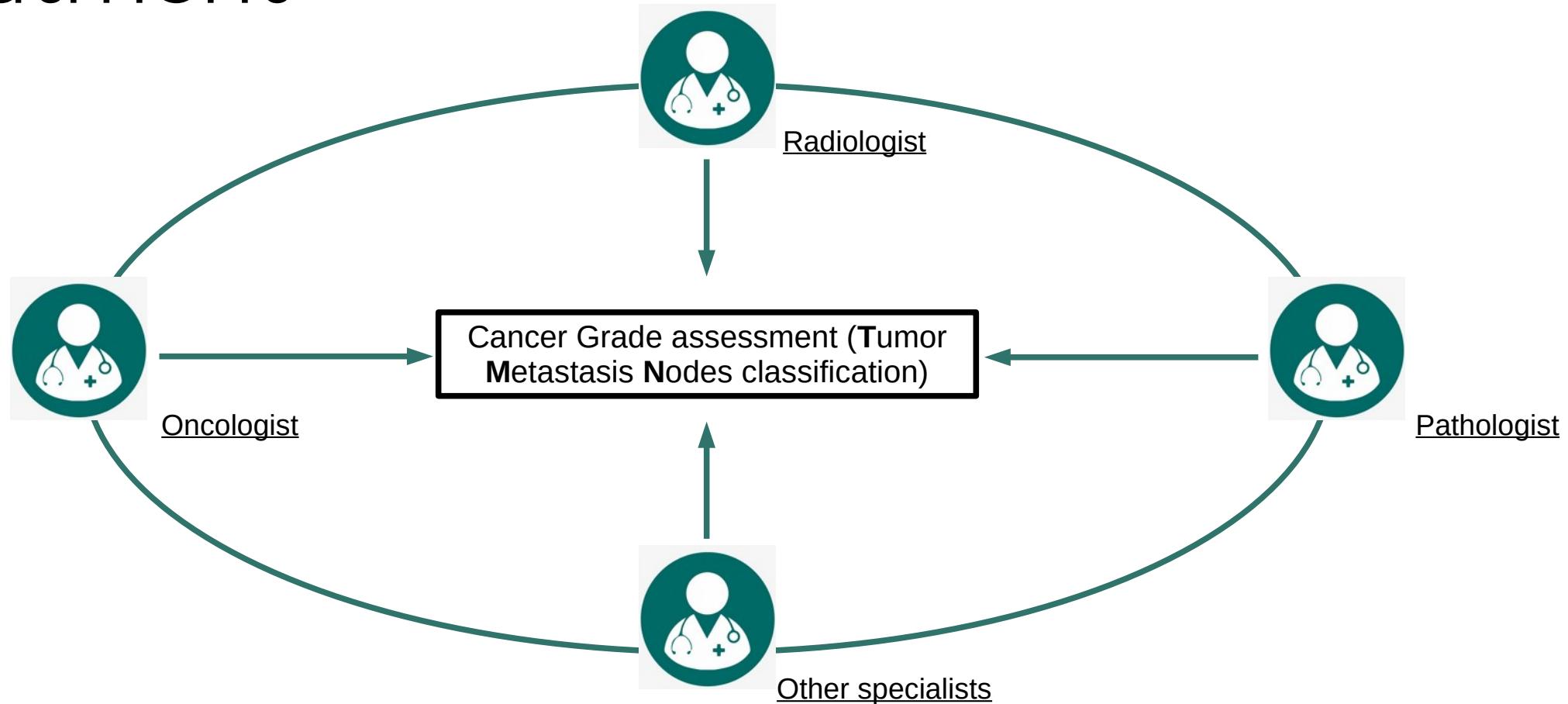
The role of the pathologist in cancer treatment



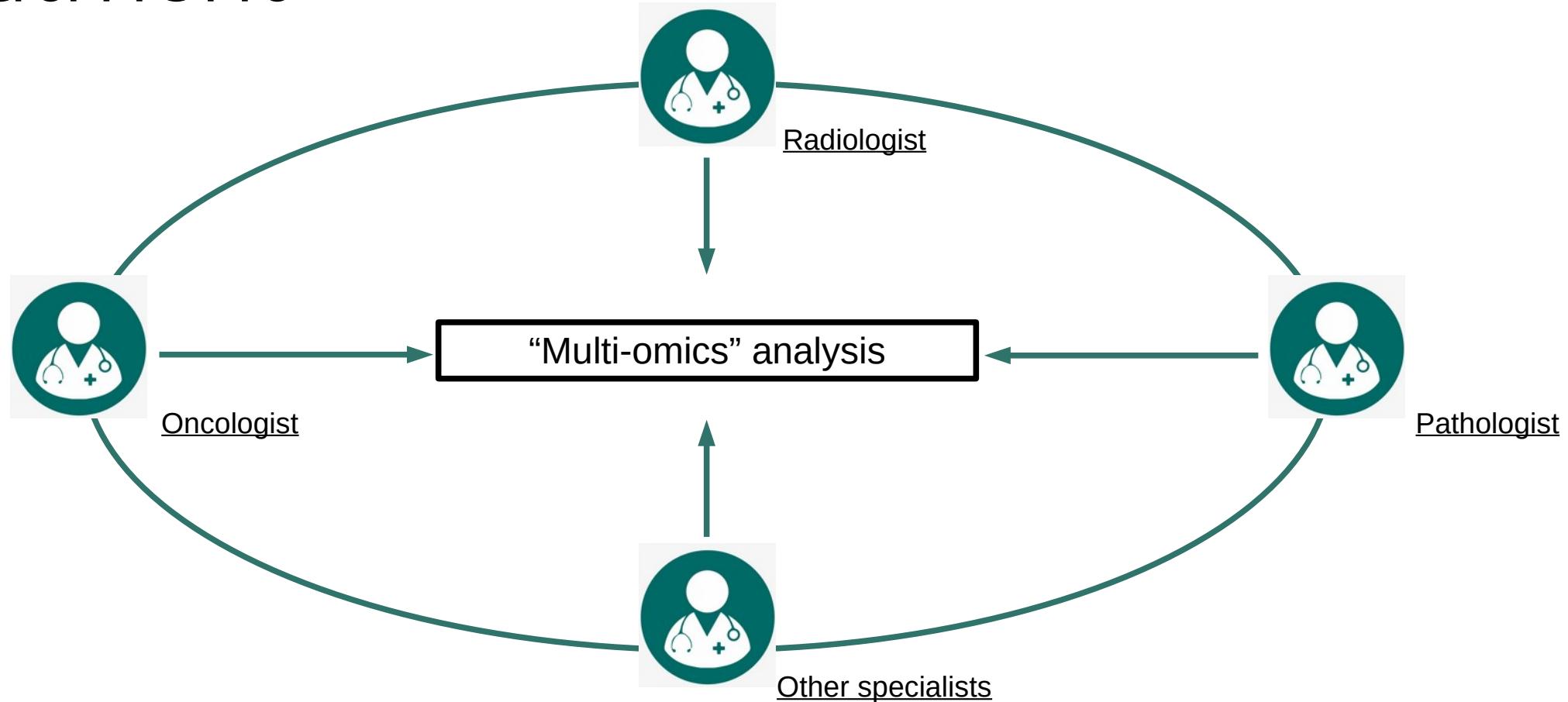
The role of the pathologist in cancer treatment



The role of the pathologist in cancer treatment



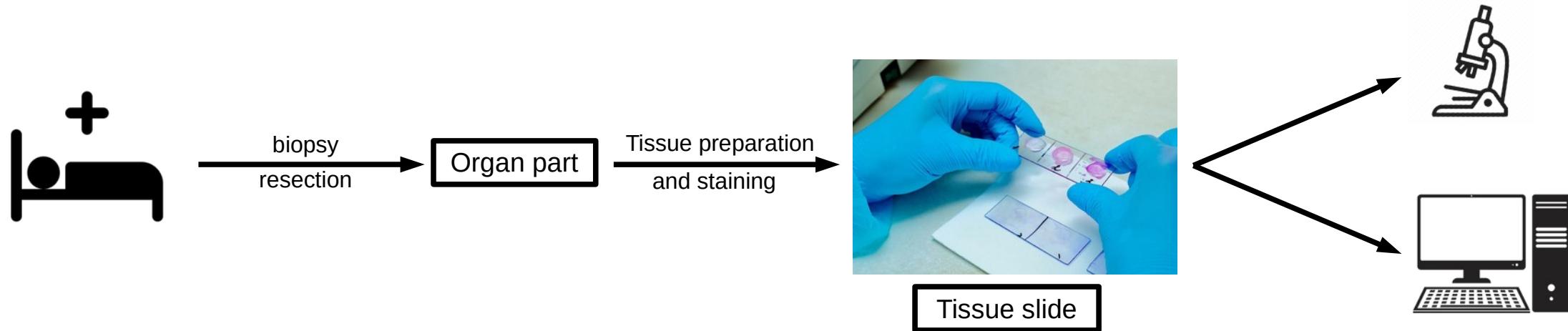
The role of the pathologist in cancer treatment



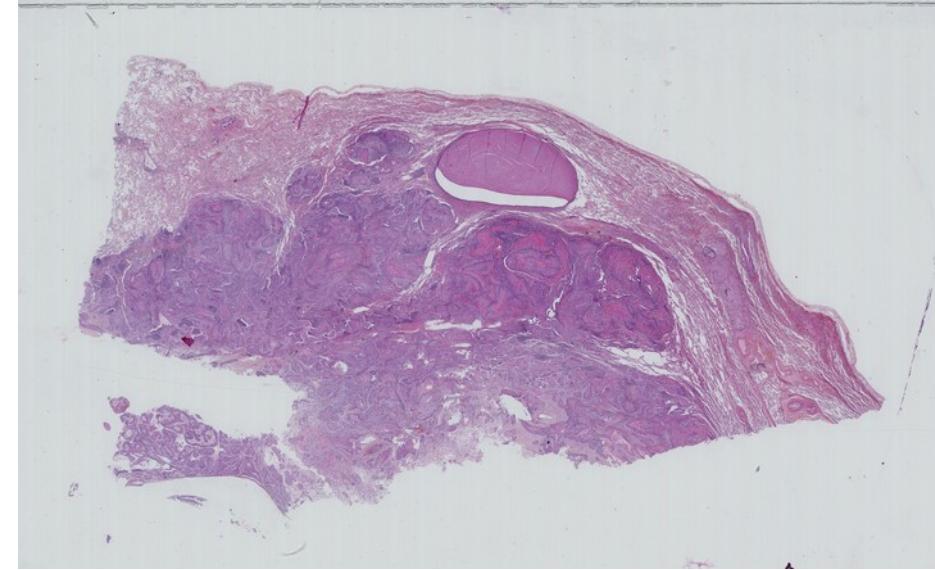
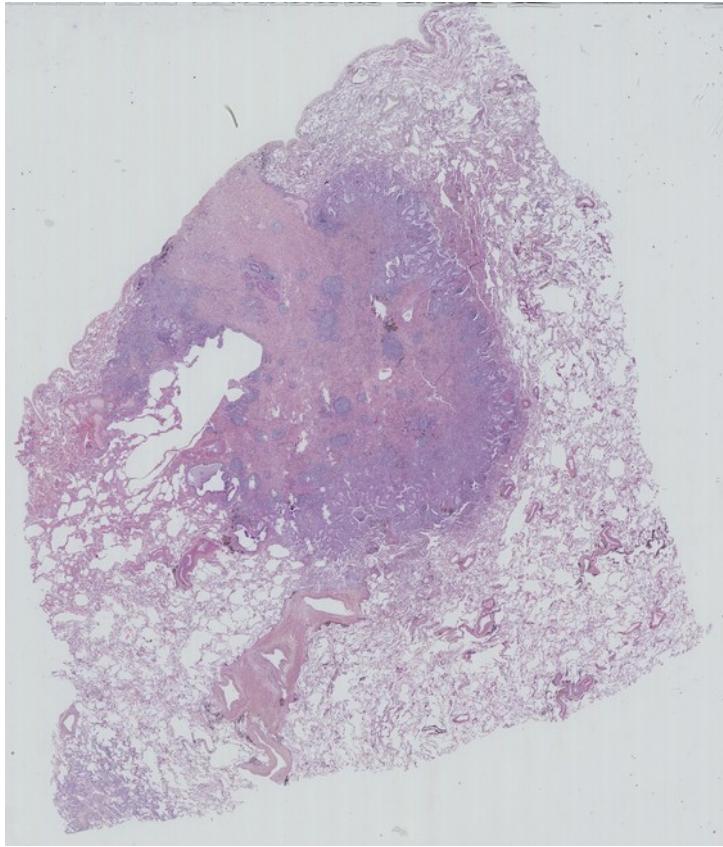
The role of the pathologist in cancer treatment

- Histopathology is the golden standard for cancer diagnosis.
- Ex: for lung cancer, histological types include adenocarcinomas, squamous cell carcinoma, sarcomatoid carcinoma, etc...
- Diagnosis is based on **tissue slides**.
- Tissue slides are thin tissue samples generated from the extraction of a small part of the affected organ.

From the patient to the image



Tissue slide examples



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Pyramidal files

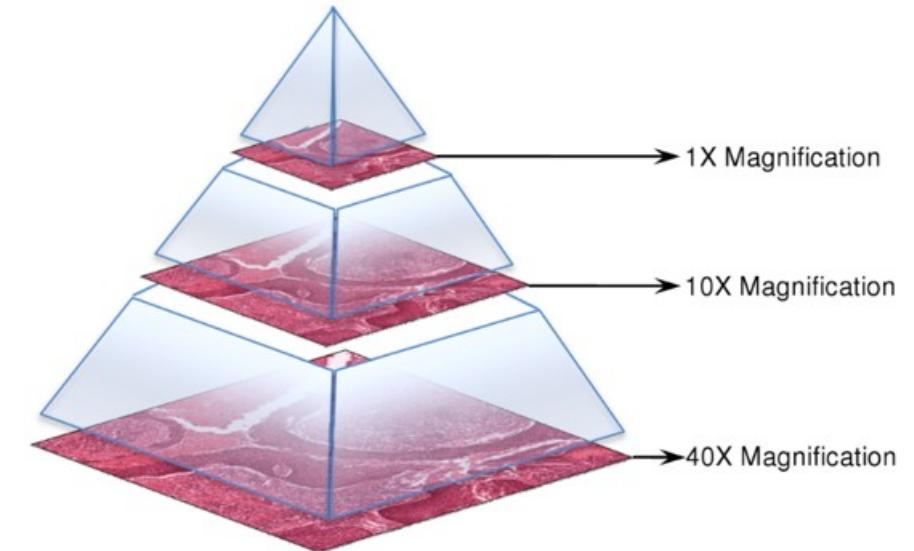
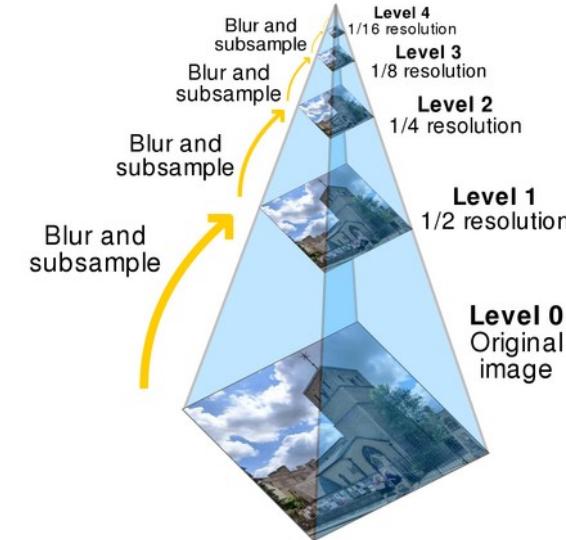
Image source:

Top

[https://en.wikipedia.org/wiki/Pyramid_\(image_processing\)#/media/File:Image_pyramid.svg](https://en.wikipedia.org/wiki/Pyramid_(image_processing)#/media/File:Image_pyramid.svg)

Bottom

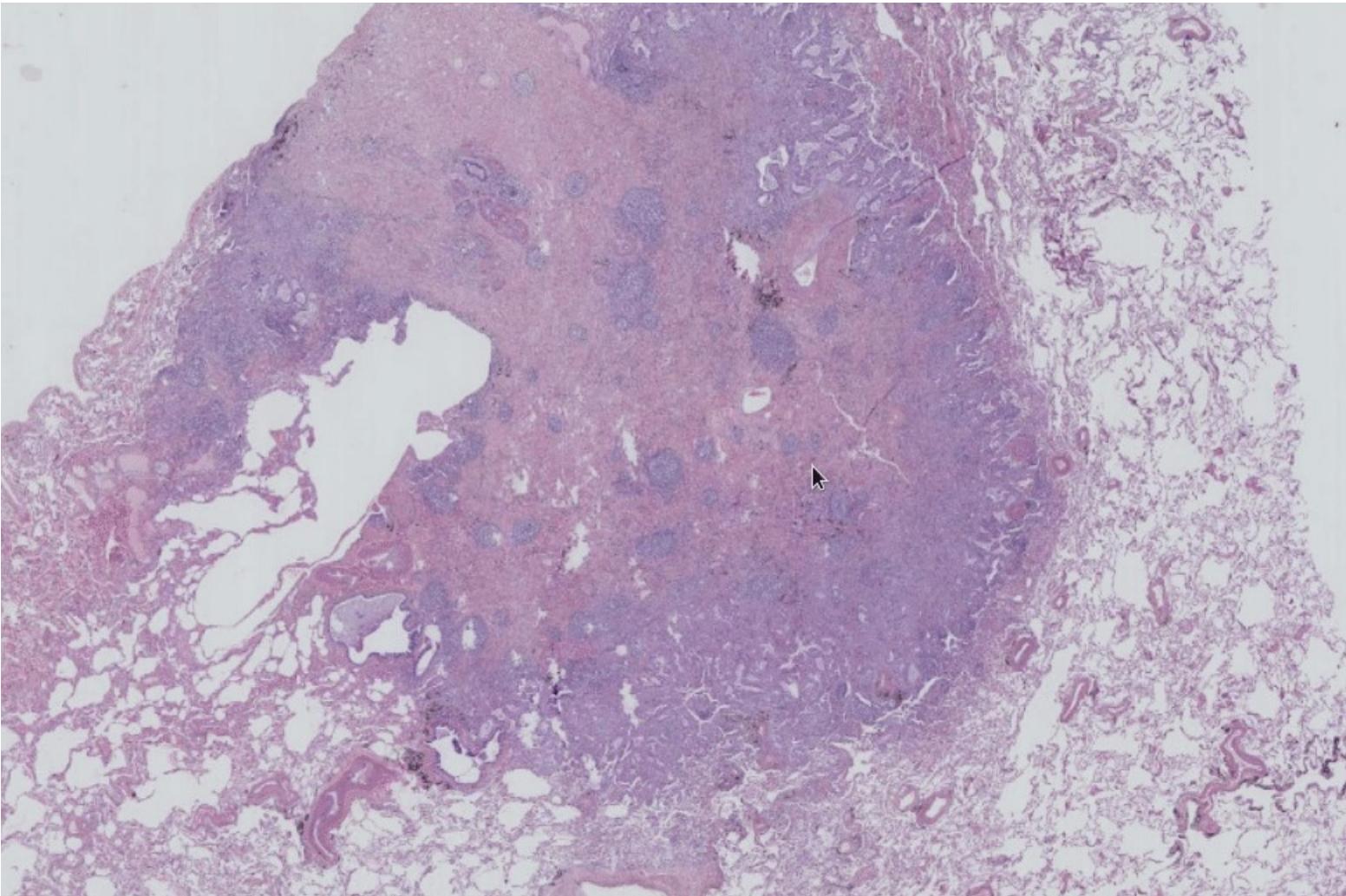
<http://tupac.tue-image.nl/node/95>



Computational tools for histology

- General python tools for computer vision:
 - opencv, numpy, scikit-image, scikit-learn, PIL, etc...
- Python tools dedicated to whole slide imaging and pyramidal image files:
 - openslide, pyvips, staintools, etc...
- Dedicated libraries offer easy-to-use programs and functions to open, display, pre-process, or analyse WSIs.
- Ex: deepzoom from openslide.

Deepzoom example



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A peek on the state of the art

- WSIs (Whole Slide Images) are mainly used for **tumor classification/detection**. Here are a few examples:
 - Lung cancer subtyping and mutation prediction (Coudray, N., Ocampo, P.S., Sakellaropoulos, T. et al. Classification and mutation prediction from non-small cell lung cancer histopathology images using deep learning. *Nat Med* 24, 1559–1567 (2018). <https://doi.org/10.1038/s41591-018-0177-5> and X. Wang et al., "Weakly Supervised Deep Learning for Whole Slide Lung Cancer Image Analysis," in *IEEE Transactions on Cybernetics*, vol. 50, no. 9, pp. 3950-3962, Sept. 2020, doi: 10.1109/TCYB.2019.2935141.)
 - Prostate Gleason Grading (Bulten, Wouter et al. "Automated Deep-Learning System for Gleason Grading of Prostate Cancer Using Biopsies: a Diagnostic Study." *The Lancet Oncology* 21.2 (2020): 233–241. Crossref. Web.)
 - Tumor proliferation assessment in sentinel lymph node (Veta, Mitko, et al. "Predicting breast tumor proliferation from whole-slide images: the TUPAC16 challenge." *Medical image analysis* 54 (2019): 111-121.)
- Other subjects include cancer **outcome predictions** :
 - Mobadersany, Pooya, et al. "Predicting cancer outcomes from histology and genomics using convolutional networks." *Proceedings of the National Academy of Sciences* 115.13 (2018): E2970-E2979.
 - Bug, Daniel, et al. "Semi-automated analysis of digital whole slides from humanized lung-cancer xenograft models for checkpoint inhibitor response prediction." *Oncotarget* 10.44 (2019): 4587.

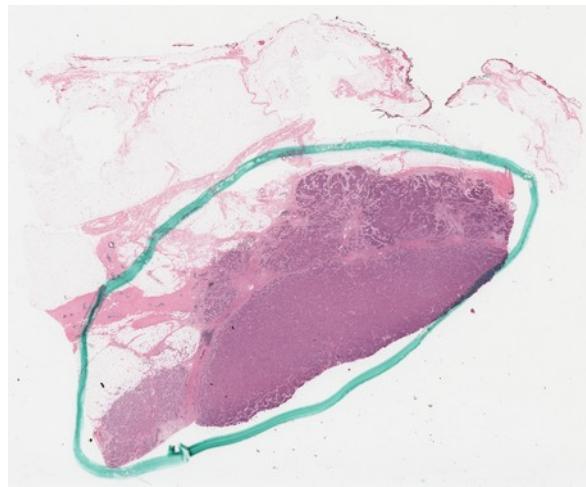
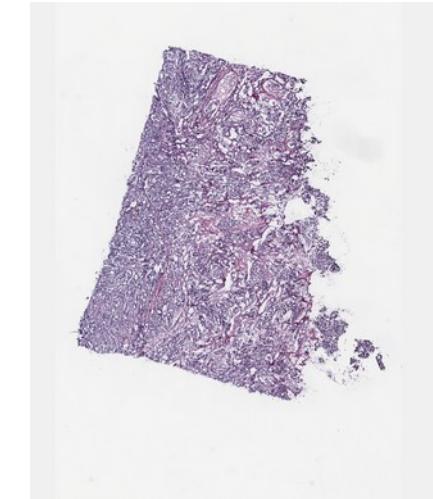
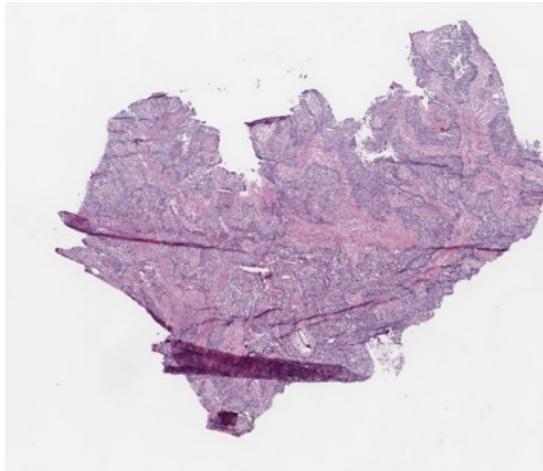
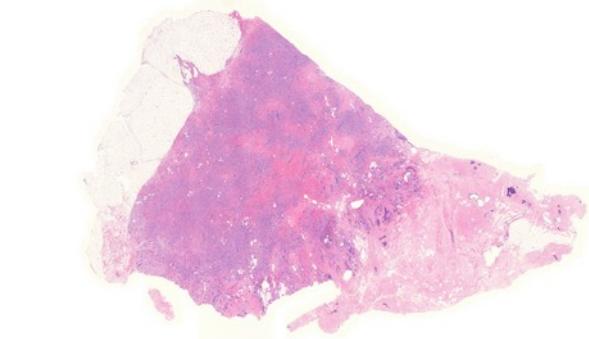
Issues and challenges

- Whole slide images are huge: the slide page 11 contains 63360 x 39168 pixels! And it's far from the largest files (up to 300,000 pixels in width or height).
- As a comparison: 8K images are 7680 x 4320 pixels, and images found in the ImageNet challenge have an average resolution of 469 x 387 pixels.
- Thus, they cannot be processed in one piece.
- A **tiling** approach is almost always considered: the image is split into smaller patches that are then processed.
- When tiling is done, the slide analysis becomes **weakly-supervised**: only the slide label is known. An aggregation method must then be devised to retrieve the slide-scale result. Therefore, the learning approach is part of **multiple instance learning (MIL)**

Issues and challenges (cont'd)

- WSIs are stained with hematoxylin and eosin (H&E stained images). The colour staining is not normalized, thus colour discrepancies between slides hinder learning tasks for histopathology.
- Many artefacts are observable on slides: air bubbles, tissue folds, pen markings, etc... → there needs to be a thorough pre-processing phase before stepping to machine learning models.
- Some articles dedicate a learning task to cleaning the slides, ex: Wang, Z., Hosseini, M. S., Miles, A., Plataniotis, K. N., & Wang, Z. (2020). FocusLiteNN: High Efficiency Focus Quality Assessment for Digital Pathology. 1–11. Retrieved from <http://arxiv.org/abs/2007.06565>

Staining gaps

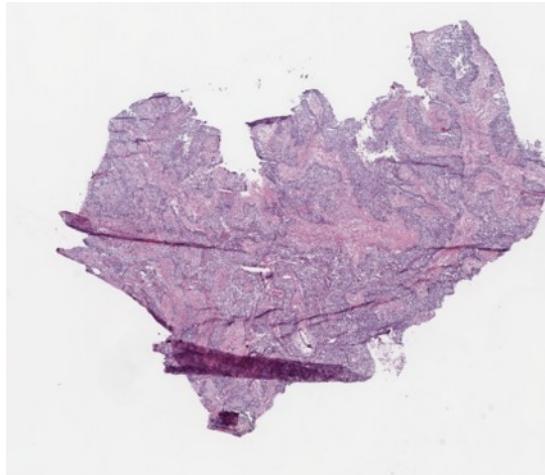


Original images downloaded from
<https://portal.gdc.cancer.gov/>

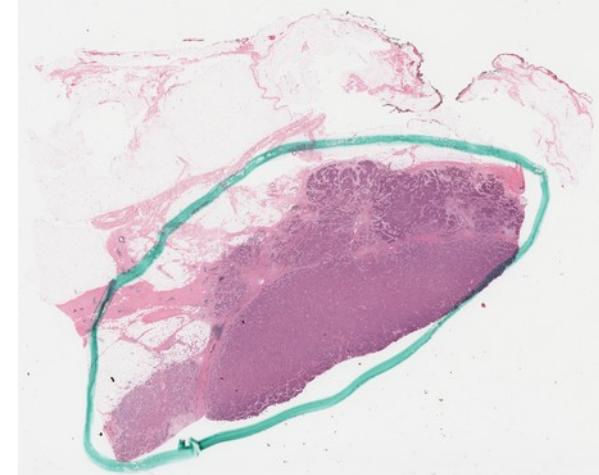
Artefacts



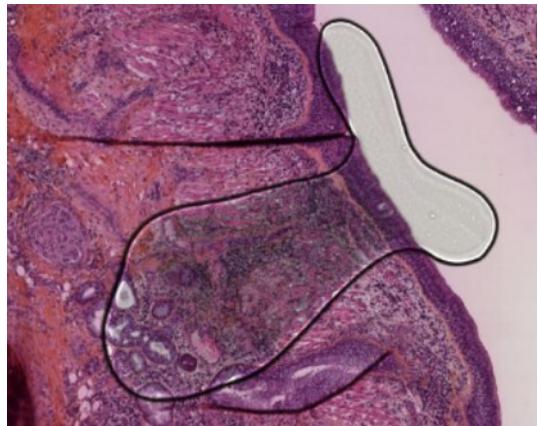
Tissue tears



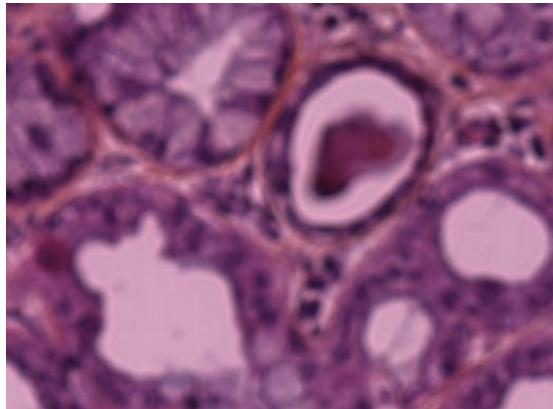
Tissue fold



Pen marking



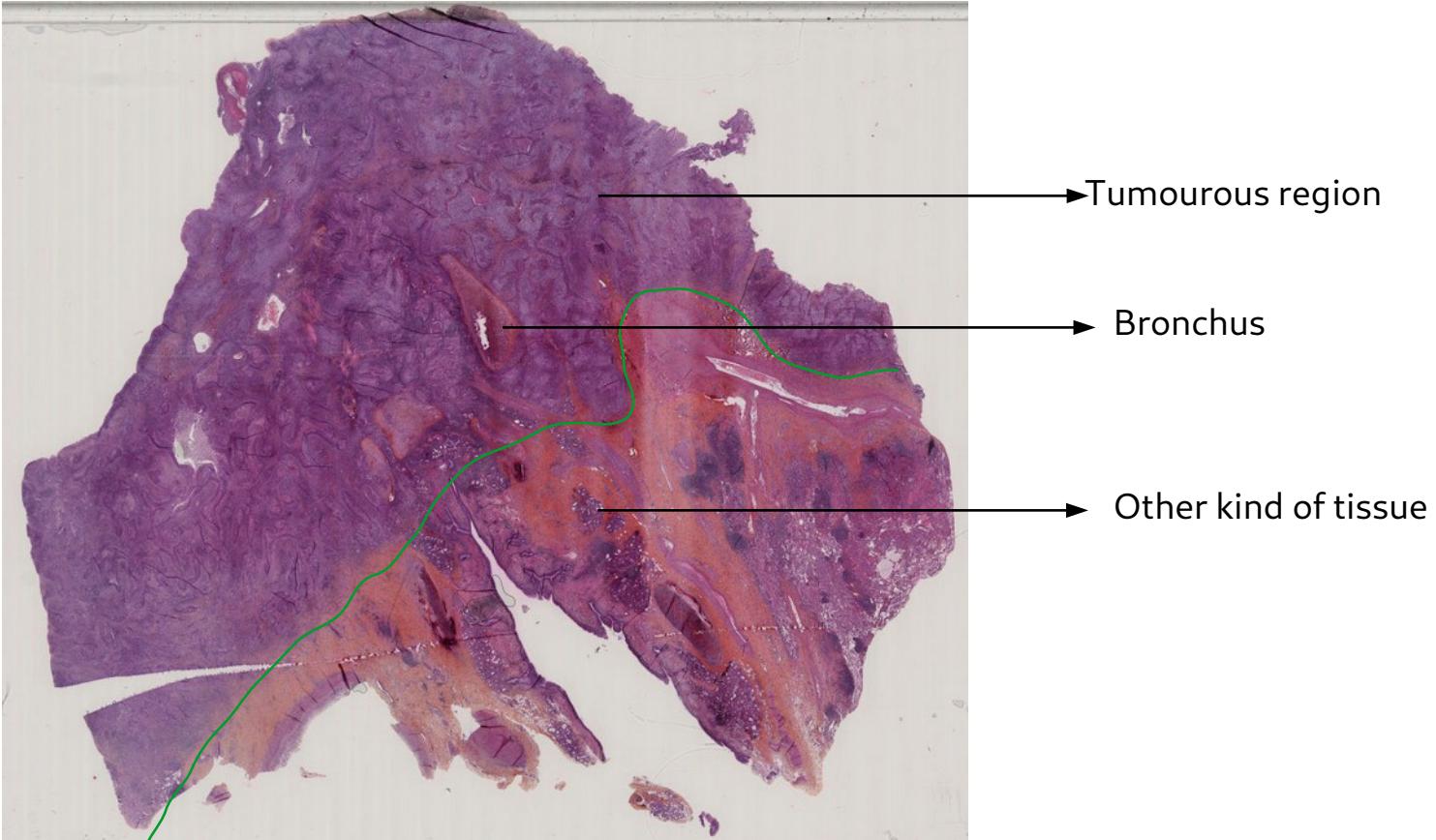
Air bubble



out-of-focus

Original images downloaded from
<https://portal.gdc.cancer.gov/>

Understanding a tissue slide



Lung tissue slide

Pre-processing a slide

- Multiple methods can be used successively to pre-process the slides
- The first task is to detect tissue regions → thresholding (binary, otsu, etc...)
- The second task is to filter out noisy elements (fat tissue, artefacts, ...) → morphological operations, laplacian and gaussian filters, etc...
- The third task is the tiling → use dedicated libraries
- The preprocessing methods are usually applied on a slide-level, using a down-sampled version of the image (1/8, 1/16, 1/32, etc...), available in the file.

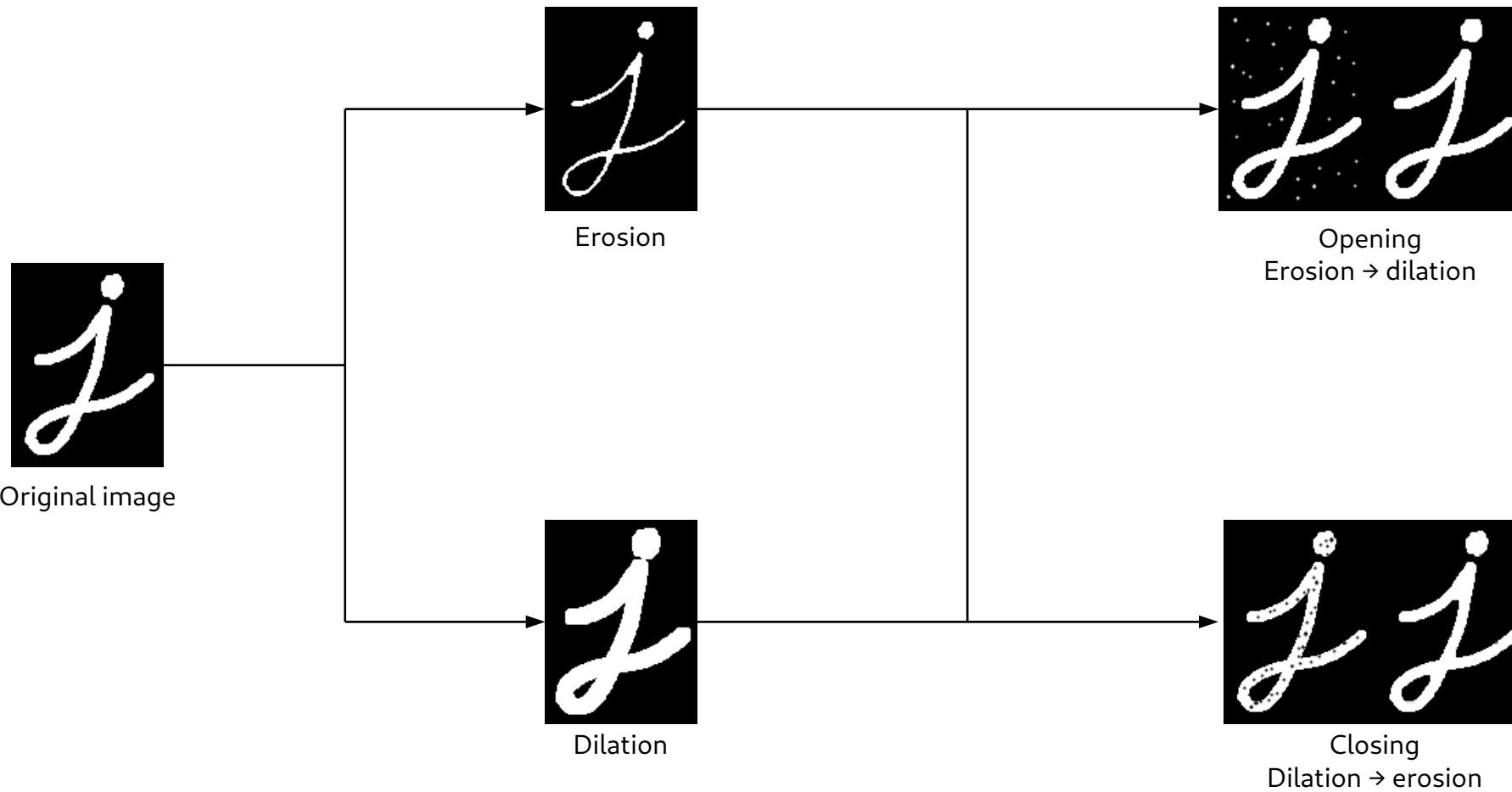
Image thresholding (Otsu*)



* N. Otsu, "A Threshold Selection Method from Gray-Level Histograms," in IEEE Transactions on Systems, Man, and Cybernetics, vol. 9, no. 1, pp. 62-66, Jan. 1979, doi: 10.1109/TSMC.1979.4310076.

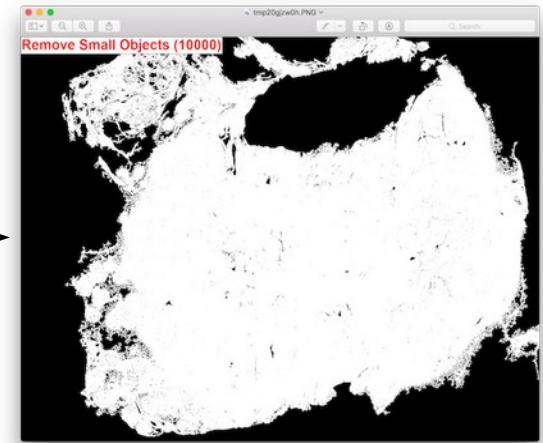
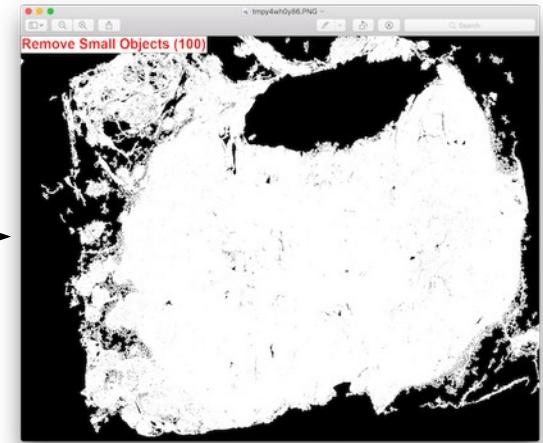
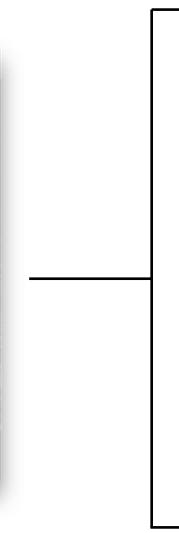
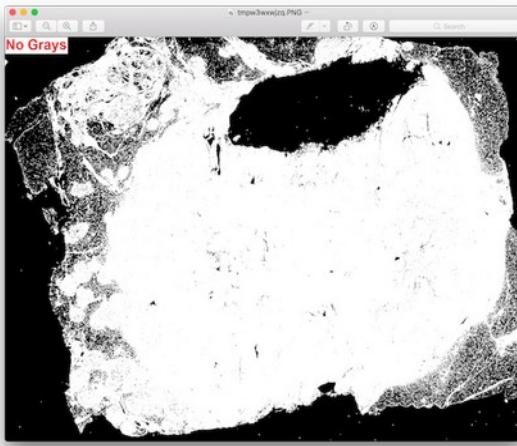
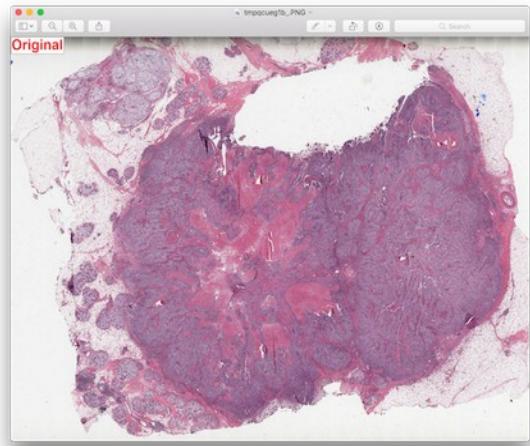
Snapshots from <https://github.com/deroneriksson/python-wsi-preprocessing/blob/master/docs/wsi-preprocessing-in-python/index.md>

Morphological operations



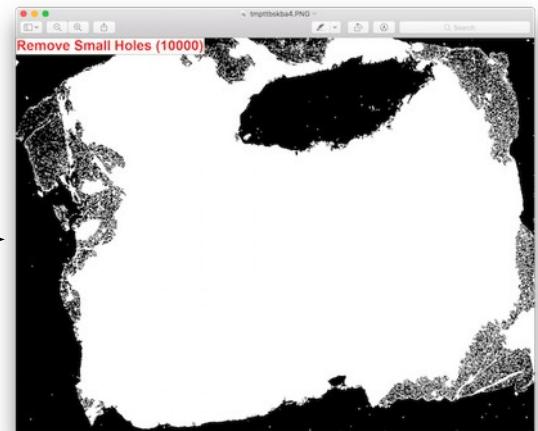
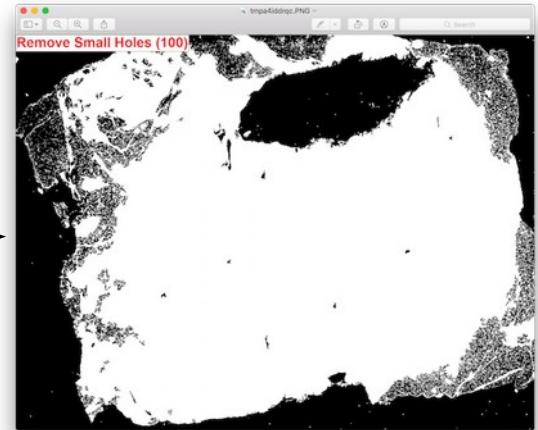
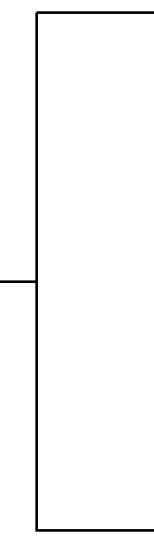
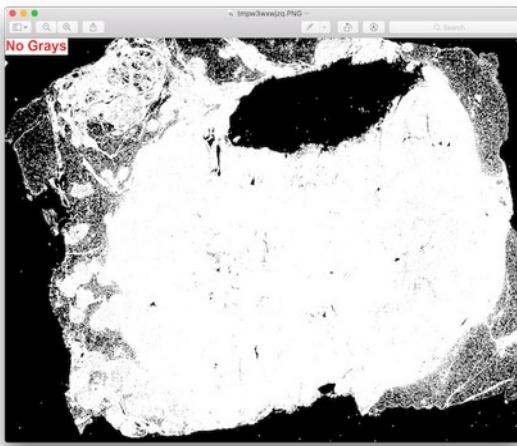
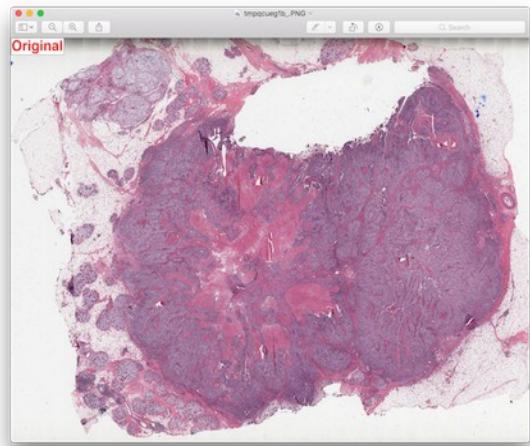
https://opencv-python-tutroals.readthedocs.io/en/latest/py_tutorials/py_imgproc/py_morphological_ops/py_morphological_ops.html

Remove small objects



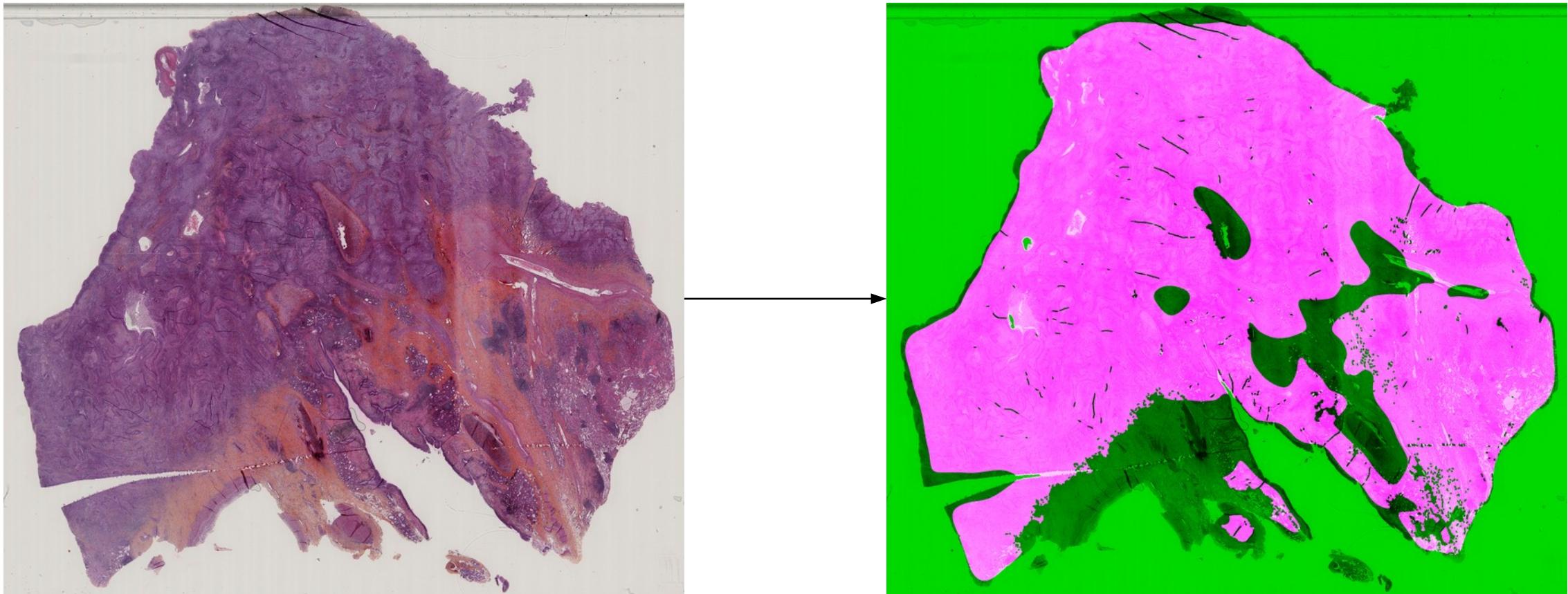
https://scikit-image.org/docs/dev/api/skimage.morphology.html#skimage.morphology.remove_small_objects

Remove small objects



https://scikit-image.org/docs/dev/api/skimage.morphology.html#skimage.morphology.remove_small_holes

A more elaborate filtering: HistoQC



Janowczyk, Andrew, et al. "HistoQC: an open-source quality control tool for digital pathology slides." JCO clinical cancer informatics 3 (2019): 1-7.
<https://github.com/choosehappy/HistoQC>

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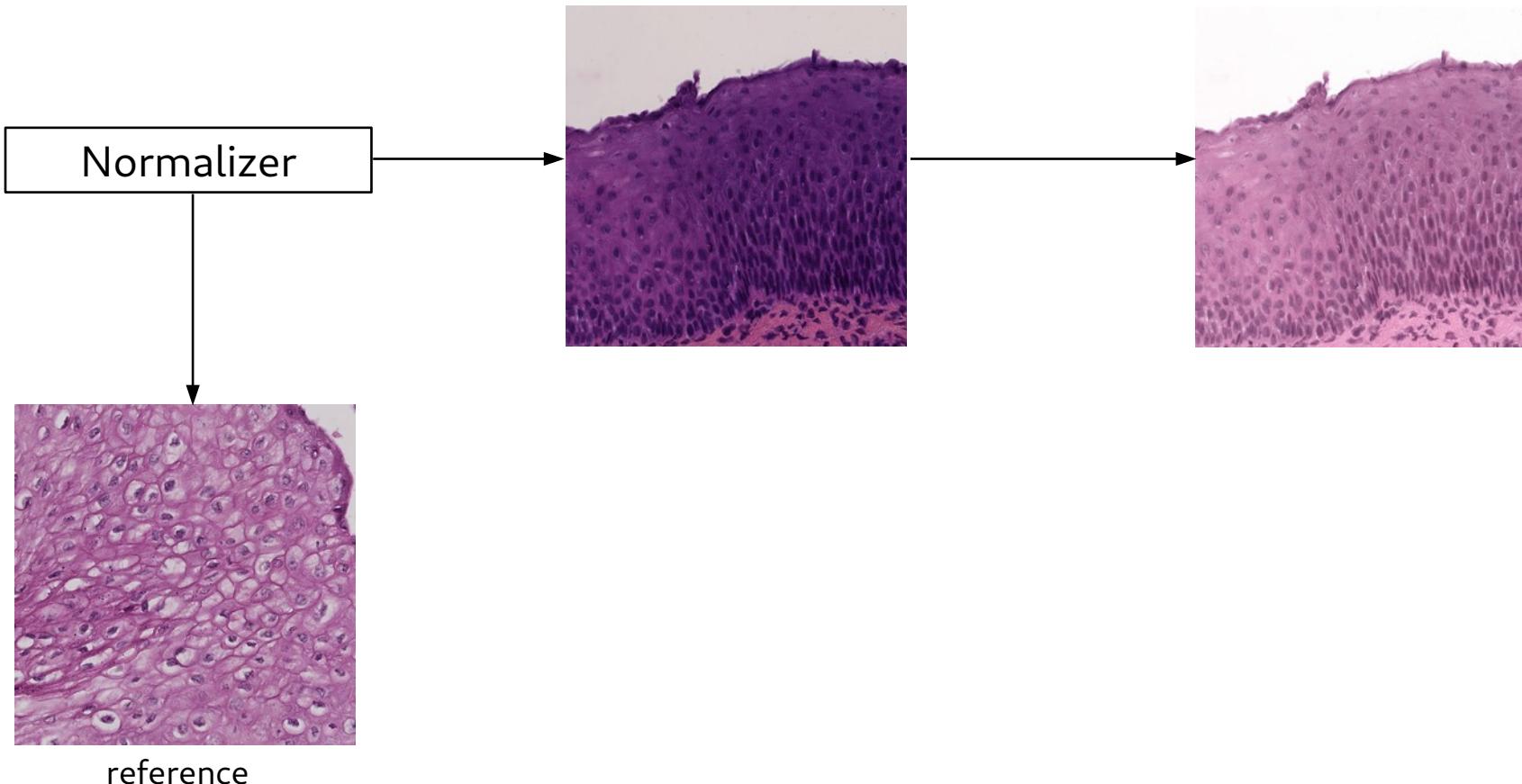
A more elaborate filtering: HistoQC

- Combination of image filters and classifiers to process the slides
- Random forest classifier based on image features (frangi, gabor, gaussian, median filters) to detect pen markings and coverslip edges.
- Classic filters e.g. Gaussian filter and morphological filters to detect blurry and smooth regions.

Janowczyk, Andrew, et al. "HistoQC: an open-source quality control tool for digital pathology slides." JCO clinical cancer informatics 3 (2019): 1-7.
<https://github.com/choosehappy/HistoQC>



Staining normalization: Macenko method*



Macenko, M., Niethammer, M., Marron, J. S., Borland, D., Woosley, J. T., Guan, X., ... & Thomas, N. E. (2009, June). A method for normalizing histology slides for quantitative analysis. In 2009 IEEE International Symposium on Biomedical Imaging: From Nano to Macro (pp. 1107-1110). IEEE.

<https://pypi.org/project/staintools/>



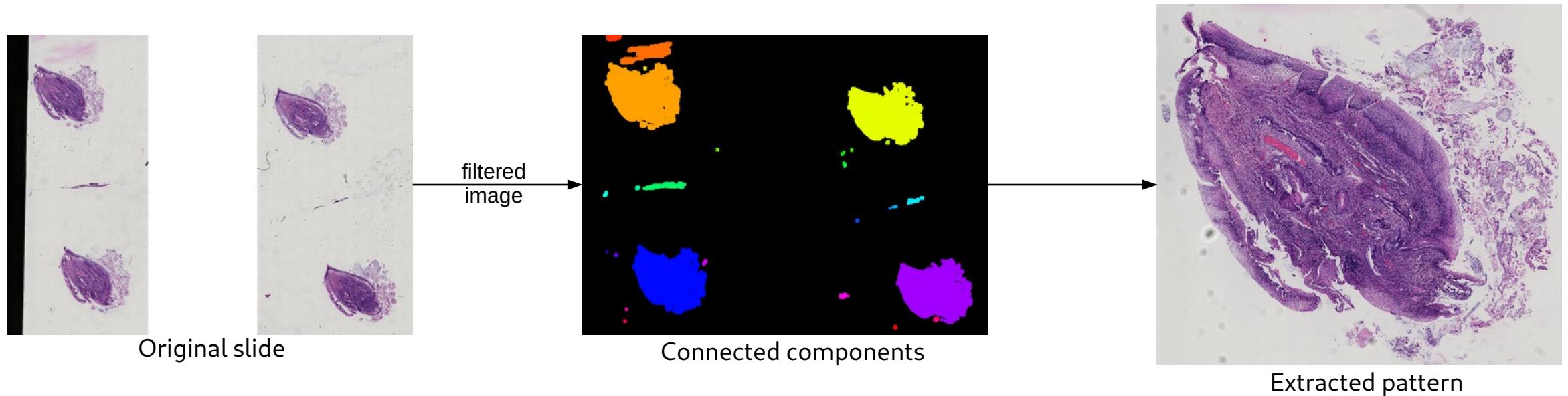
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Staining normalization

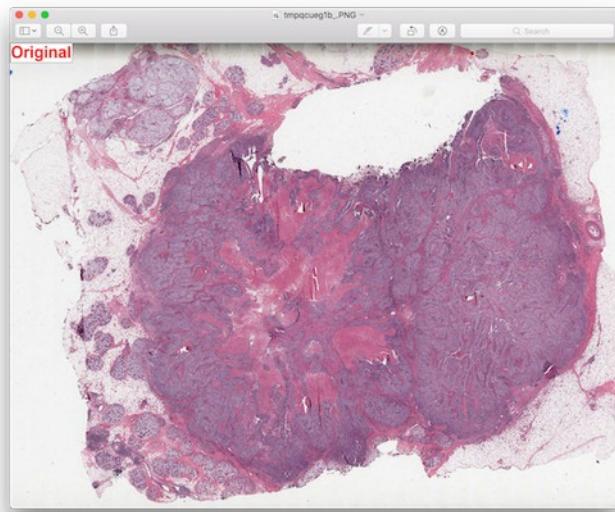
- Stain normalization is now, as well as slide cleaning, a research topic on its own.
See:
 - Nadeem, S., Hollmann, T., & Tannenbaum, A. (2020). Multimarginal Wasserstein Barycenter for Stain Normalization and Augmentation. Retrieved from <http://arxiv.org/abs/2006.14566>
 - Nishar, H., Chavanke, N., & Singhal, N. (n.d.). Histopathological Stain Transfer using Style Transfer Network with Adversarial Loss. (Dl), 1–10.
 - Self-supervised, H. I. U. (n.d.). Structure Preserving Stain Normalization of Histopathology Images Using Self-Supervised Semantic Guidance. 1–17.

Yet another issue: biopsy samples



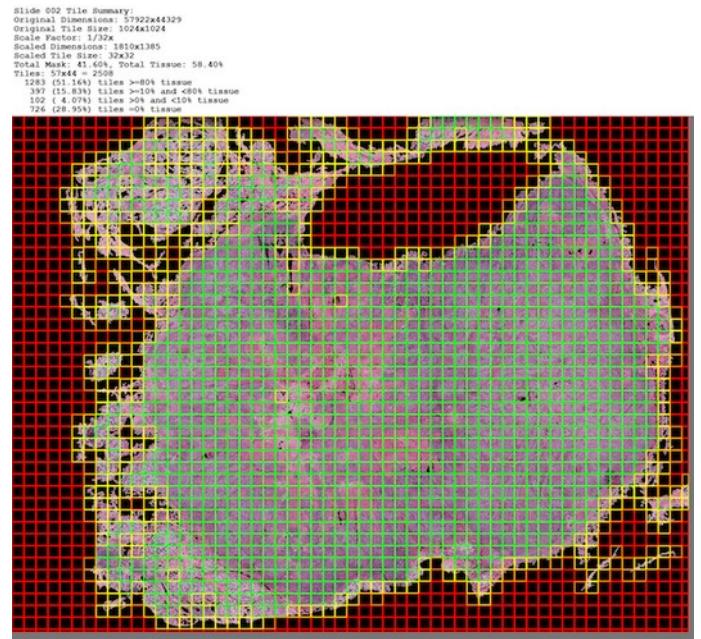
Slide taken from the TissueNet challenge (<https://www.drivendata.org/competitions/67/competition-cervical-biopsy/page/254/>)

Tiling

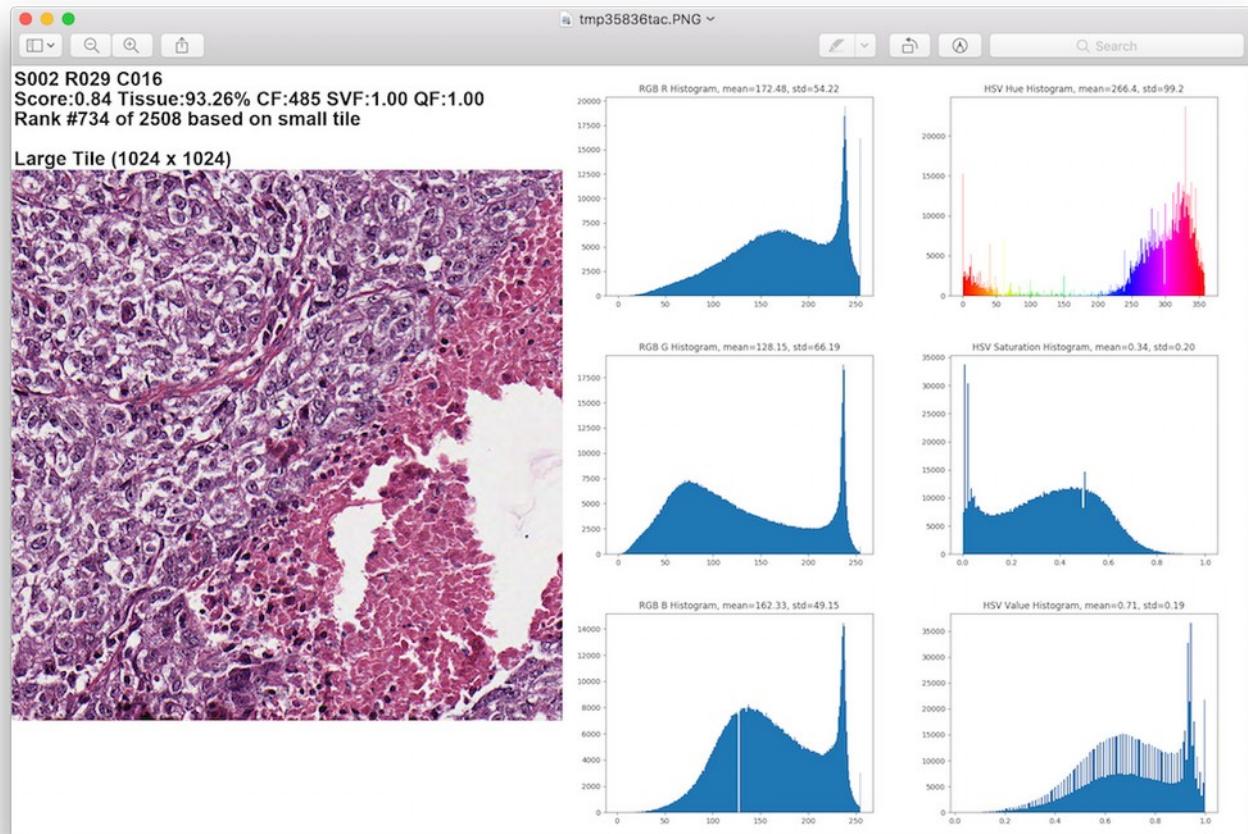


Slide 002 Tile Summary:
Original Dimensions: 57922x44329
Original Tile Size: 1024x1024
Scale Factor: 1/32x
Scaled Dimensions: 1810x1385
Scaled Tile Size: 32x32
Total Mask: 41.60%, Total Tissue: 58.40%
Tiles: 57x44 = 2508
1283 (51.16%) tiles >=80% tissue
397 (15.83%) tiles >=10% and <80% tissue
102 (4.07%) tiles >0% and <10% tissue
726 (28.95%) tiles =0% tissue

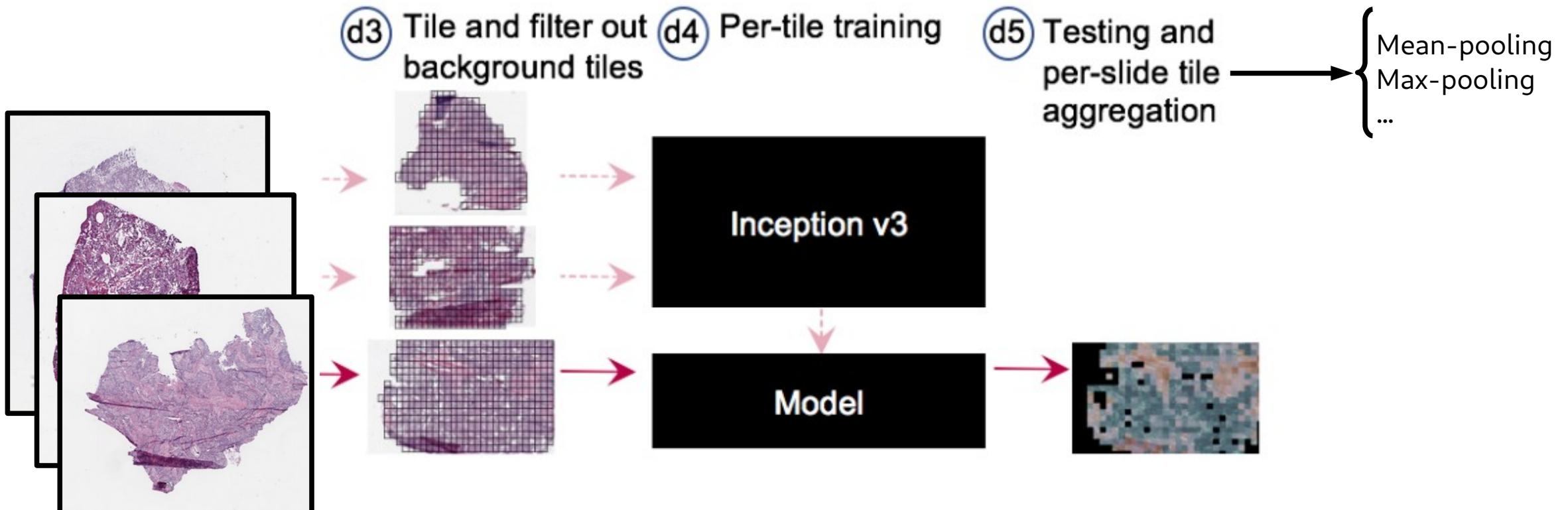
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726 (28.95%) tiles =0% tissue



A tile example



A pipeline example



Coudray, N., Ocampo, P.S., Sakellaropoulos, T. et al. Classification and mutation prediction from non–small cell lung cancer histopathology images using deep learning. Nat Med 24, 1559–1567 (2018). <https://doi.org/10.1038/s41591-018-0177-5>

Slide aggregation methods: an overview

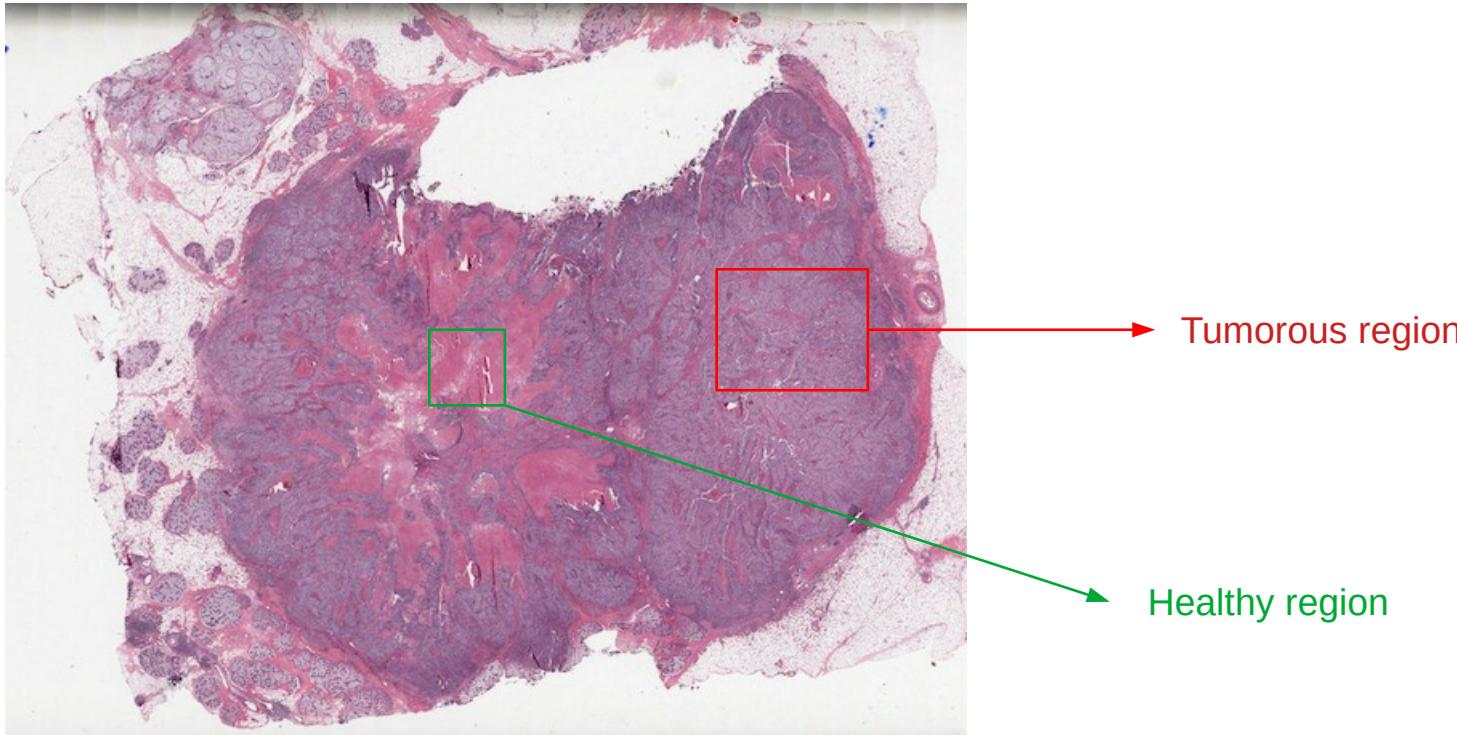
- For a m -class classification problem, there exist several methods:

- Mean-pooling: $c = \operatorname{argmax}_m \frac{1}{N} \sum_{k=1}^N p_{k,m}$ where N is the number of patches
- Max-pooling: $c = \operatorname{argmax}_m \max_k p_{k,m}$

are the most basic ones, but some articles have proposed different approaches, such as:

- Lu, M. Y., Williamson, D. F. K., Chen, T. Y., Chen, R. J., Barbieri, M., & Mahmood, F. (2020). Data Efficient and Weakly Supervised Computational Pathology on Whole Slide Images. 1–35. Retrieved from <http://arxiv.org/abs/2004.09666>
- Campanella, G., Hanna, M. G., Geneslaw, L., Miraflor, A., Werneck Krauss Silva, V., Busam, K. J., ... Fuchs, T. J. (2019). Clinical-grade computational pathology using weakly supervised deep learning on whole slide images. *Nature Medicine*, 25(8), 1301–1309. <https://doi.org/10.1038/s41591-019-0508-1>
- Courtiol, P., Tramel, E. W., Sanselme, M., & Wainrib, G. (2018). Classification and Disease Localization in Histopathology Using Only Global Labels: A Weakly-Supervised Approach. 1–13. Retrieved from <http://arxiv.org/abs/1802.02212>

The tiling issue



Cancer classification

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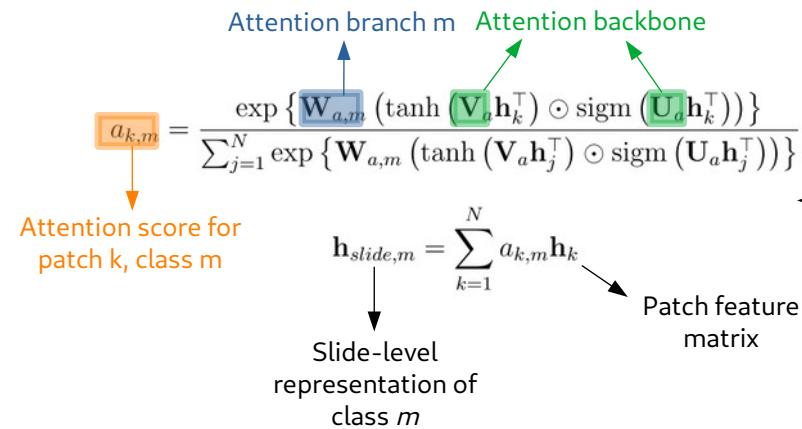
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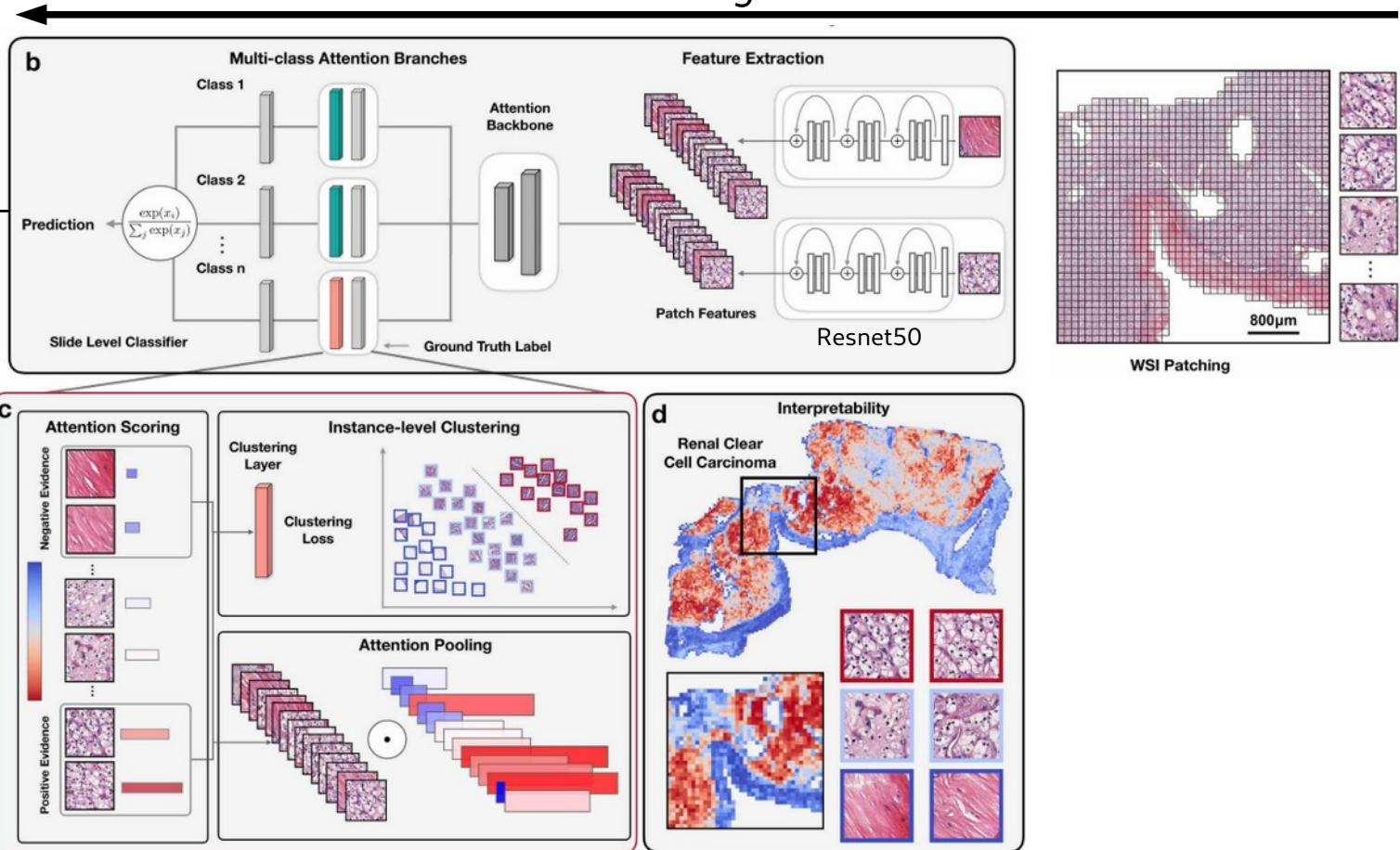
Method 1: CLAM

Reading direction for b



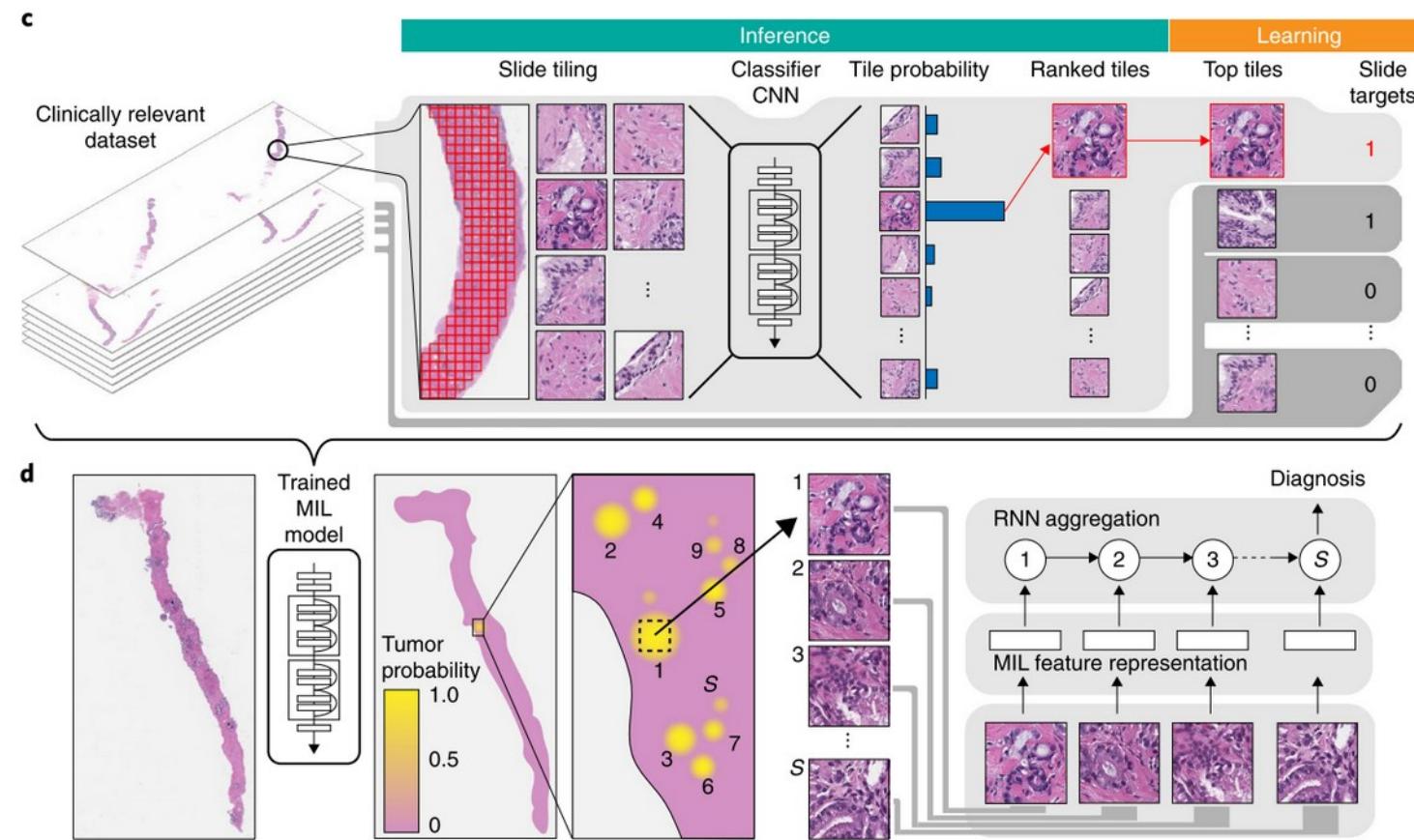
Clustering of patches with highest and lowest attention scores. The total loss is:

$$\mathcal{L}_{total} = c_1 \mathcal{L}_{slide} + c_2 \mathcal{L}_{patch}$$



*Lu, Ming Y., et al. "Data Efficient and Weakly Supervised Computational Pathology on Whole Slide Images." arXiv preprint arXiv:2004.09666 (2020).

Method 2: MIL + RNN



Campanella, G., Hanna, M. G., Geneslaw, L., Miraflor, A., Werneck Krauss Silva, V., Busam, K. J., ... Fuchs, T. J. (2019). Clinical-grade computational pathology using weakly supervised deep learning on whole slide images. *Nature Medicine*, 25(8), 1301–1309. <https://doi.org/10.1038/s41591-019-0508-1>

Method 3: CHOWDER

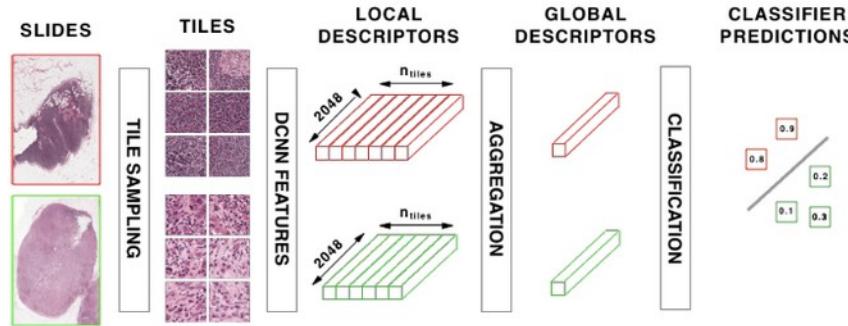


Figure 1: Description of the BASELINE approach for WSI classification via aggregation of tile-level features into global slide descriptors.

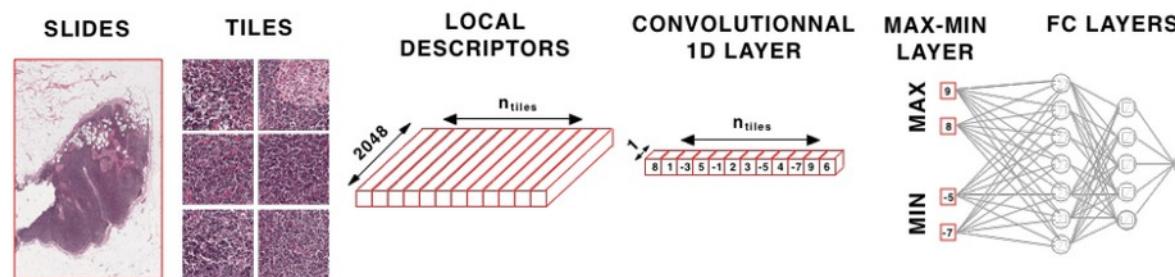


Figure 2: Description of the CHOWDER architecture (for $R = 2$) for WSI classification via MLP on operating on top positive and negative instances shown for a single sample mini-batch sample.

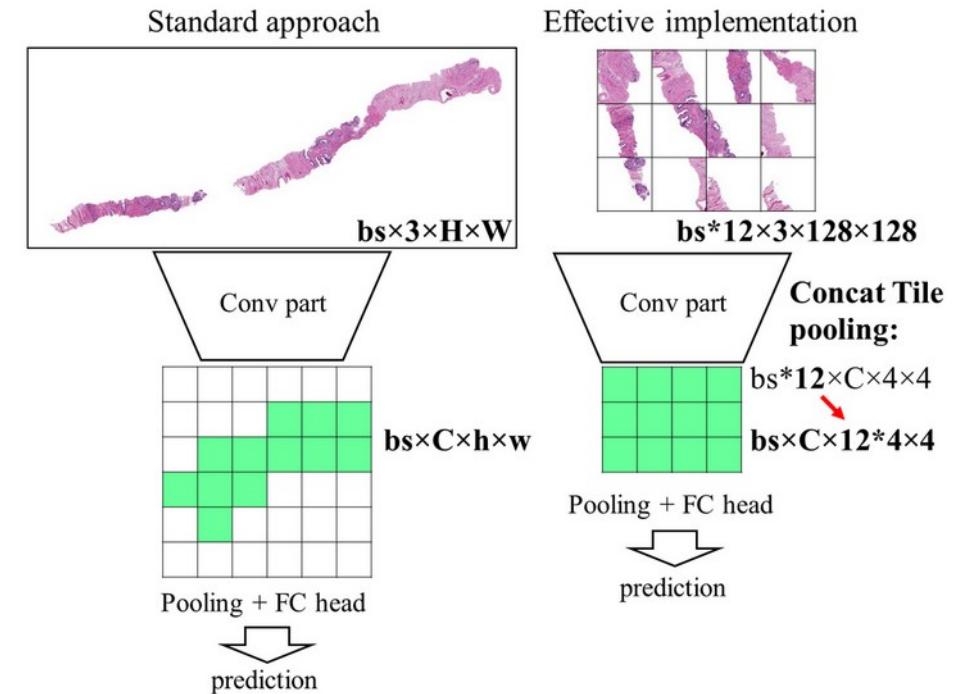
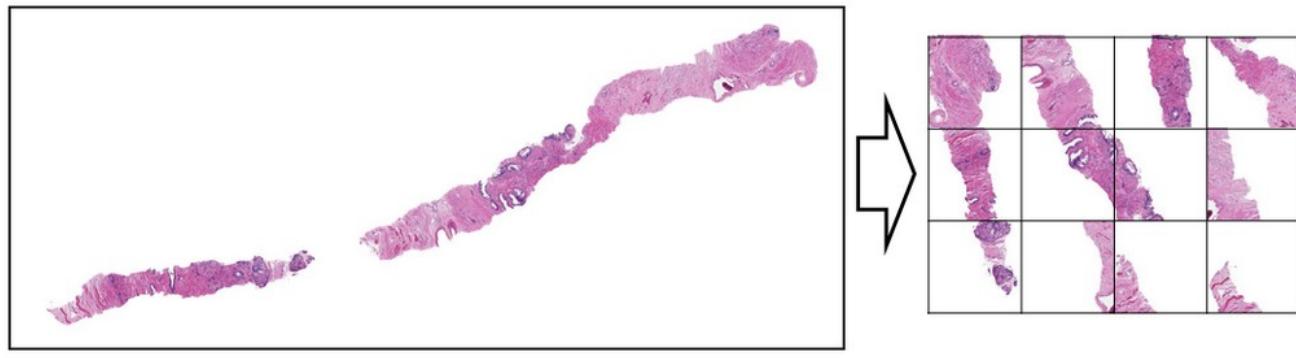
Courtiol, P., Tramel, E. W., Sanselme, M., & Wainrib, G. (2018). Classification and Disease Localization in Histopathology Using Only Global Labels: A Weakly-Supervised Approach. 1–13. Retrieved from <http://arxiv.org/abs/1802.02212>

Results comparison on lung

	Method 1: CLAM	Method 3: CHOWDER	Mean-pooling*
Dataset	TCGA	TCGA (+ external)	TCGA
Number of slides	1967	707 (+ 302)	1175
Task	NSCLC subtyping (adenocarcinoma vs. squamous cell carcinoma)	NSCLC subtyping (adenocarcinoma vs. squamous cell carcinoma)	NSCLC subtyping (adenocarcinoma vs. squamous cell carcinoma)
AUC	0.956	0.915	0.950

*Coudray, N., Ocampo, P.S., Sakellaropoulos, T. et al. Classification and mutation prediction from non–small cell lung cancer histopathology images using deep learning. Nat Med 24, 1559–1567 (2018). <https://doi.org/10.1038/s41591-018-0177-5>

Bonus Method: From PANDA challenge



<https://www.kaggle.com/iafoss/panda-16x128x128-tiles>

Survival prediction

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Method 1: Convolutional auto-encoder

- Survival prediction based on colorectal histopathology slides (660)
- ROI detection based on a deep-learning classifier pre-trained on 100,000 CRC patches
- Deep autoencoder training with latent space clustering for spatial and pattern proximity evaluation between tiles
- Patient descriptors based on patch clustering and patch probability of cluster transition
- Identification of tissue pattern interactions explaining survival

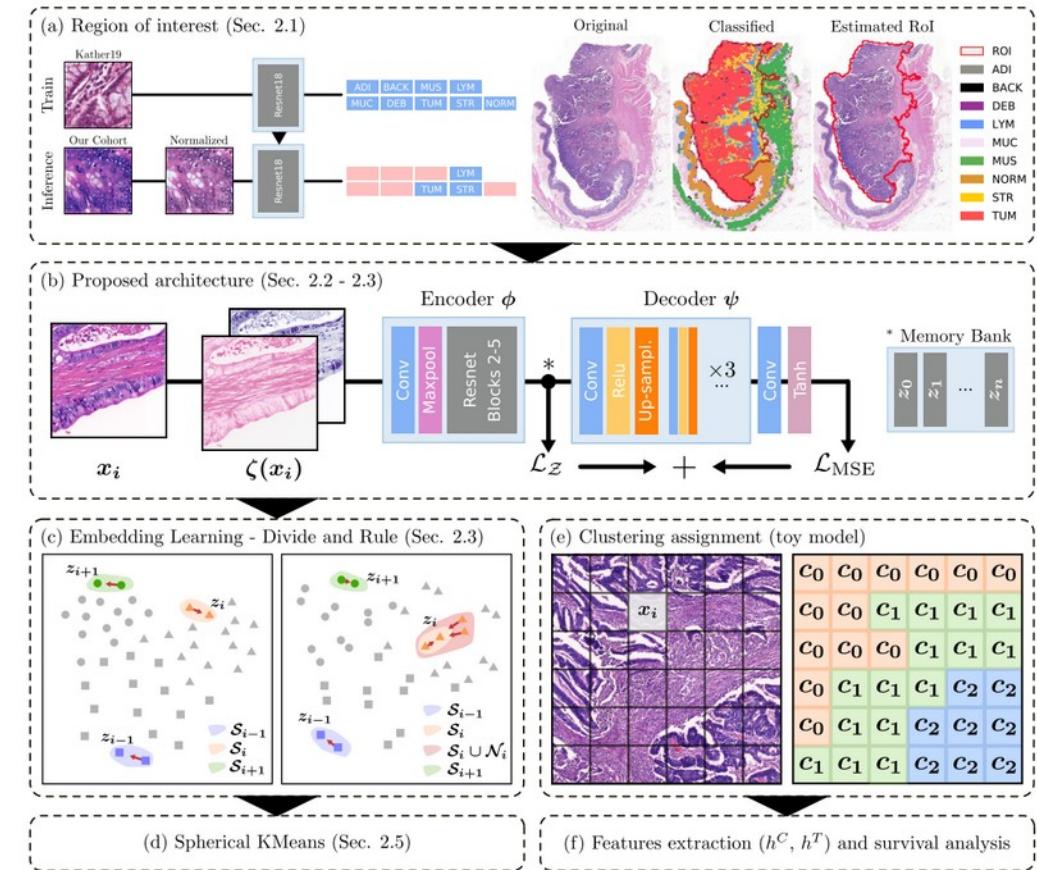
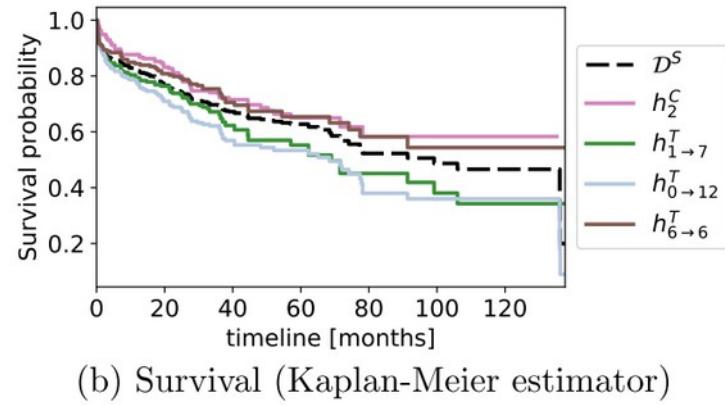


Fig. 1: The pipeline of the proposed approach. Estimation of the region of interest (a), learning of the embedding space (b-c), fitting of the cluster, assignment of all patient patches, and survival analysis (d-f).

Abbet, C., Zlobec, I., Bozorgtabar, B., & Thiran, J.-P. (2020). Divide-and-Rule: Self-Supervised Learning for Survival Analysis in Colorectal Cancer. Retrieved from <http://arxiv.org/abs/2007.03292>

Method 2: Multi-omics approach

- “Survival CNN” to predict patient outcome in the case of glioma
- Concordance found between molecular subtype and expected outcome
- Model further improved by the integration of genomics data to form a “GSCNN”, with superior results compared to basic integration of genomics

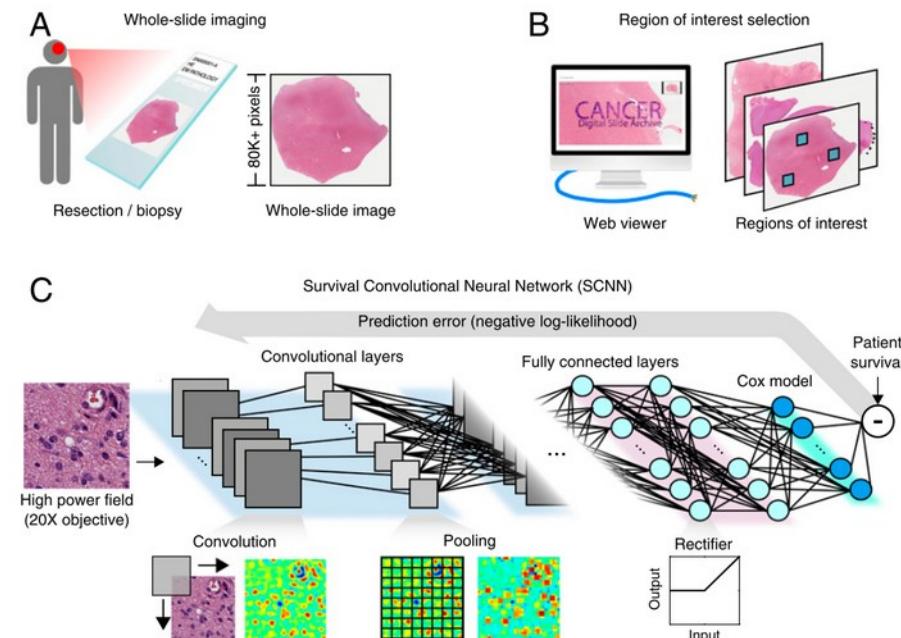
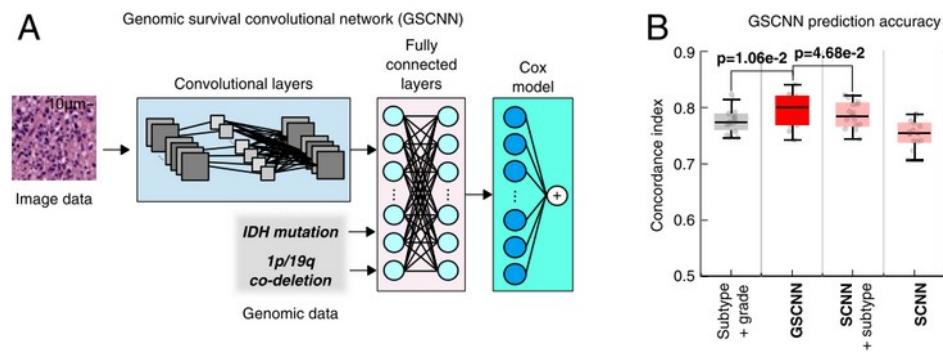


Fig. 1. The SCNN model. The SCNN combines deep learning CNNs with traditional survival models to learn survival-related patterns from histology images. (A) Large whole-slide images are generated by digitizing H&E-stained glass slides. (B) A web-based viewer is used to manually identify representative ROIs in the image. (C) HPFs are sampled from these regions and used to train a neural network to predict patient survival. The SCNN consists of (i) convolutional layers that learn visual patterns related to survival using convolution and pooling operations, (ii) fully connected layers that provide additional nonlinear transformations of extracted image features, and (iii) a Cox proportional hazards layer that models time-to-event data, like overall survival or time to progression. Predictions are compared with patient outcomes to adaptively train the network weights that interconnect the layers.

Mobadersany, P., Yousefi, S., Amgad, M., Gutman, D. A., Barnholtz-Sloan, J. S., Velázquez Vega, J. E., ... Cooper, L. A. D. (2018). Predicting cancer outcomes from histology and genomics using convolutional networks. *Proceedings of the National Academy of Sciences of the United States of America*, 115(13), E2970–E2979. <https://doi.org/10.1073/pnas.1717139115>

Conclusion

- Computational histopathology has now reached human-comparable results on cancer related tissues in many different tasks such as tumour classification, or survival prediction
- It also enables the discovery of new biomarkers for patient outcome, or a more accurate use of the existing ones (Ho, D. J., Agaram, N. P., Schueffler, P. J., Vanderbilt, C. M., Jean, M.-H., Hameed, M. R., & Fuchs, T. J. (2020). Deep Interactive Learning: An Efficient Labeling Approach for Deep Learning-Based Osteosarcoma Treatment Response Assessment. 1–10. Retrieved from <http://arxiv.org/abs/2007.01383>)
- By integrating multi-modal data to models, one could expect to reach better results than just exploiting histology images.
- However, there remain challenges that need to be taken on: inner-center and inter-center data heterogeneity, the lack of accurate annotations (because of time constraint), and efficient computational methods to handle such large datasets.
- Histopathology is now becoming a major field in medical imaging, spawning its own set of dedicated methods and frameworks.