

VIRTUAL H&E STAINING AND COMPUTER ASSISTED ASSESSMENT OF LYMPH NODE METASTASES IN COLORECTAL CARCINOMA AND NECK DISSECTIONS

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01 ABSTRACT

H&E-stain (Haematoxylin and Eosin) of formalin fixed and paraffin embedded tissues sections is the most often used staining in pathology for more than hundred years now. H&E staining is based on a sophisticated sequence of steps in a process based on the use of different chemicals, some of them known as toxic. Therefore, well educated technicians working and infrastructure are needed for performing these steps (1). We therefore attempted to find an alternative and developed an algorithm for virtually staining of tissue sections. This algorithm allows virtual staining of tissue sections avoiding the disadvantages mentioned above based on a high resolution scan of unstained sections. Virtual staining was supplemented by an application for the detection of lymph nodes metastases in these virtual stains. Proof of this concept was done by comparison of the results with standard histological evaluation on the same samples.

02 MATERIALS AND METHODS

632 lymph nodes were scanned using a commercially available scanner (Hamamatsu “NanoZoomer S360”) followed by the process of virtual staining using a research software optimized to the features of the scanner (admorai, Hannover). This software is characterized by a generator network trained on matched image data of unstained and stained tissue samples. Afterwards the same slides underwent a standard H&E staining. Blinded microscopic evaluations followed done by a group of five pathologists. The number of lymph nodes was assessed, added by the number of metastasis within these lymph nodes (Fig.1). Tissue sections were then H&E stained, scanned again and reevaluated. Results were compared to those obtained by virtual staining (Fig. 2). Moreover, the results were later on compared to a deep neural network (admorai, Hannover) trained to detect lymph node metastasis (Fig. 3).

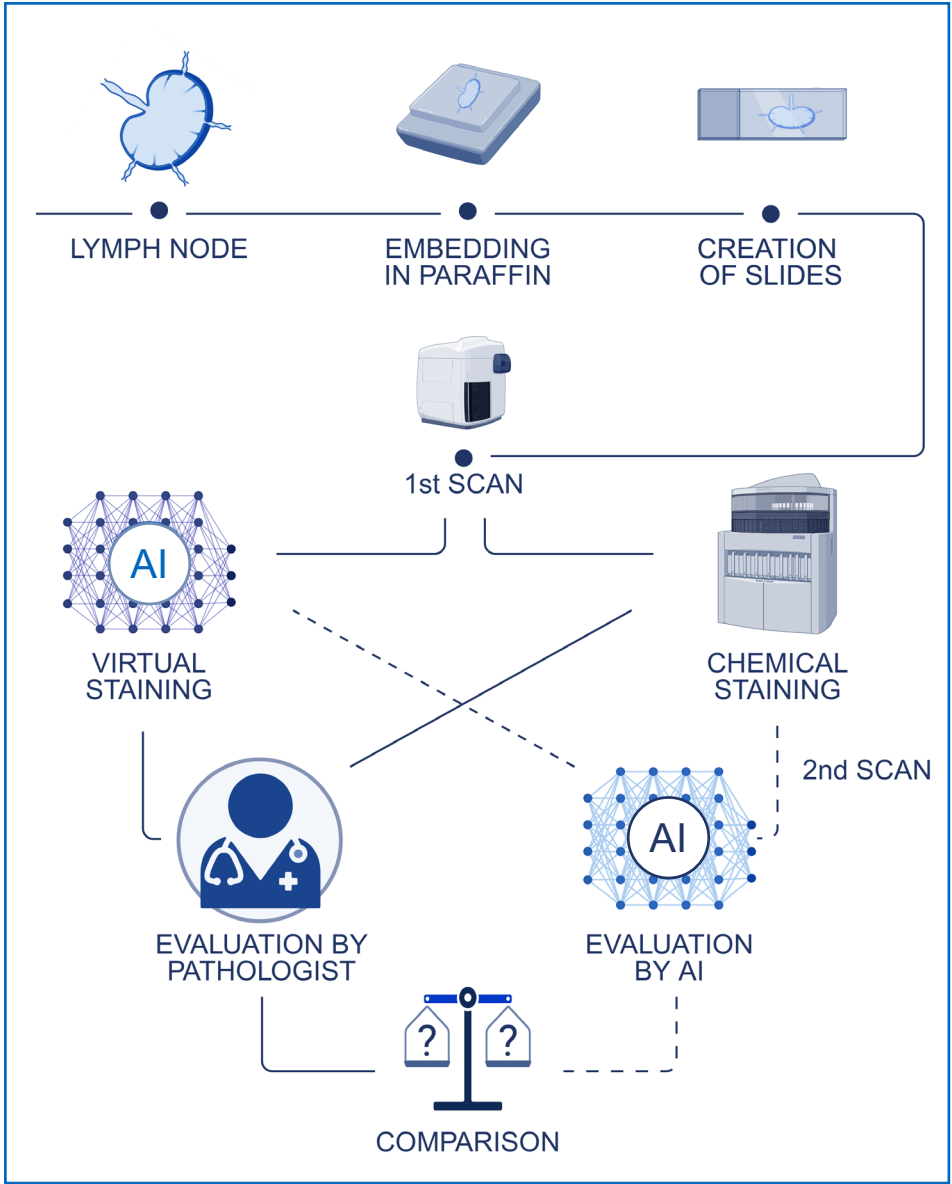


Fig. 1 The workflow to compare virtual and chemical staining

03 RESULTS

632 lymph nodes of colorectal carcinoma and neck dissections were virtually and later on chemical stained and evaluated. The comparison showed that while 632 lymph nodes were detected using a chemical staining only 604 lymph nodes were detectable with the virtual staining program. In addition 165 lymph nodes were found with metastasis using chemical staining but only 160 when staining virtually. In total the results of 39 in 230 slides differed between the two methods, suggesting a fallout rate of 16,9% (Fig. 4). However, the reasons for this deviation were too thick tissue samples or a not fitting level of focus during the scanning process. When just looking at the slides that are properly scanned the comparison between the methods showed an accordance of >95% (Fig. 5). Moreover, the deployed neural network was able to detect metastasis in lymph nodes of colorectal carcinoma with a sensitivity of >97% and a specificity of 97% in virtual and 89% in chemical stained lymph nodes.

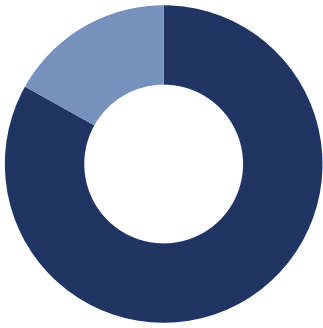


Fig. 4 Results of the comparison between virtual and chemical staining with concordance (dark blue) and deviation (light blue)

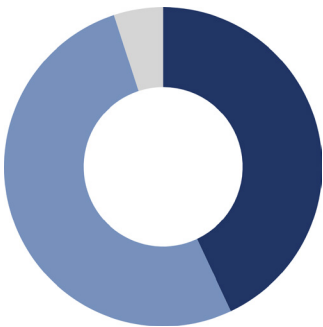


Fig. 5 Reasons for deviation in the results between virtual and chemical staining: too thick tissue samples (light blue), issue during the scanning process (dark blue), interobserver variability (grey)



Fig. 2 Comparison of virtual (left), unstained (middle) and chemical (right) stained lymph nodes

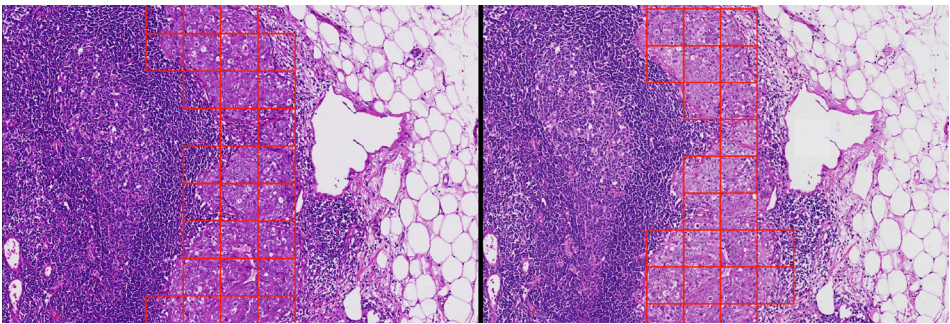


Fig. 3 Evaluation by detection network of virtual (left) and chemical (right) stained lymph nodes

04 OUTLOOK

These data demonstrate that it is possible to apply virtual staining in a sufficient and reliable way. The technique shortens handling time and the need of specialized technicians. It also avoids the use of toxic chemicals used in complex training procedures. Also, the technique saves tissue sections useable for additional stainings and/or molecular analysis. Even more, once scanned and processed the virtual stains can be used for new applications of automated evaluation. The current limitations of the system such as not useable tissue sections and incomplete scanning of sections hamper the use of the system. In fact, this seems to be a problem of preanalytical handling and needs to be solved in the next time (e.g. by optimized tissue sectioning).