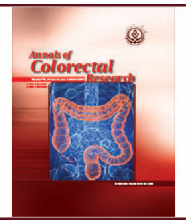
Brief Report

Ann Colorectal Res 2020;8(1):29-32.



Filling the Gap between Microscopic and Automated Analysis of the Tumor-Stroma Ratio

***Gabi van Pelt*[1*], *Rob Tollenaar*[1], *Wilma Mesker*[1]**

[1]*Department of Surgery, Leiden University Medical Center, Leiden, The Netherlands*

Corresponding authors:
Gabi van Pelt,
Department of Surgery, Leiden University Medical Center, Albinusdreef 2, 2333 ZA, Leiden, The Netherlands. **Tel:** +31 71 5262857; **Fax:** +31 71 5266750
Email: g.w.van_pelt@lumc.nl

Received: 16-10-2019
Revised: 06-03-2020
Accepted: 06-04-2020

Abstract

Determining the tumor-stroma ratio (TSR) using a conventional microscope is an easy to apply and highly reproducible method. Due to digitalization in the pathology workflow, the demand for automated analysis of the TSR method is rising. However, the process of automation is rather time consuming and needs validation before implementation in daily practice. In addition, international studies ask for exchange of digital images instead of the actual slides. This calls for an alternative digital scoring method.

This brief report describes the pitfalls of analyzing the TSR using digital images and proposes essential adaptations to create a standardized and reproducible scoring protocol. By using a circular annotation to mimic the microscopic method, these pitfalls can be avoided. Scoring the TSR digitally using a circular annotation does not take much additional effort compared to the microscopic method. When a fixed size of the annotation is saved, new cases can be scored in less than two minutes. With this brief report we propose an adjusted method for scoring the TSR on digital images to fill the gap between microscopic and automated scoring of the TSR. In addition, it opens the opportunity for application in daily diagnostics.

Keywords: Automation, Digital analysis, Microscopic analysis, Tumor-stroma ratio

Please cite this paper as:
Pelt GV, Tollenaar R, Mesker W. Filling the Gap between Microscopic and Automated Analysis of the Tumor-Stroma Ratio. *Ann Colorectal Res*. 2020;8(1):29-32. doi: .doi: 10.30476/ACRR.2020.46475.

Introduction

There is a continuous lookout for useful tumor-specific features in combination with digital pathology that may provide important independent predictors of tumor prognosis. Microscopic analysis of the tumor-stroma ratio (TSR) has proven its robustness and reproducibility as a prognostic clinical parameter for various types of epithelial cancers (1).

The field of clinical pathology is currently witnessing a transition from optical microscopy using glass slides to assessment of digitally scanned slides on a computer. Next to logistic advantages, this transition also facilitates large-scale use of computer algorithms to support pathology diagnostics. Analysis of entirely scanned whole-slide-images (WSI) is highly challenging due to the large data size (tens of gigabytes per WSI) and high complexity and variability of the images. Only in the last few years, fully automated computerized analysis of WSI has become feasible because of a

revolution in the field of digital pattern recognition: deep learning. Deep learning technology can be used to rapidly identify and classify nuclei in epithelial cancers, providing the basis for TSR automation (2). However, such a process is rather time consuming and needs validation before it can be implemented in daily practice. To fill the gap between microscopic and automated scoring of the TSR, an alternative is being investigated: determining the TSR on digital images. This will provide opportunities for (inter) national collaboration via exchange of digital images.

However, when implementing the standard TSR scoring protocol on digital images, multiple issues emerge, which make it difficult to score the TSR correctly. This brief report proposes a method to apply the TSR digitally in a standardized setting.

Current Procedure

A detailed protocol describing the microscopical determination of the TSR has recently been published (3). In brief, 4 µm thick haematoxylin and eosin (H&E) stained sections of the most invasive area of the tumor are selected. Areas appearing to have the highest amount of stroma are selected using a 2.5x or 5x objective. After switching to a 10x objective, image fields are scored in increments of 10%. Tumor cells are to be present at the four borders of the selected image field. Identifying one single image-field with high stroma content is decisive for a final stroma classification. A statistically determined cut-off value of 50% distinguishes between patients with a stroma-high (>50%) and stroma-low (≤50%) tumor (Figure 1) (4). The determination of the TSR takes only 1-2 minutes.

Pitfalls with Digital Analysis

Scoring the TSR on digital images requires adaptations to the current protocol for microscopic analysis, as there are some pitfalls using different software programs.

First, a slide scanned using one particular scanner can, when opened in different software programs with the same relative magnification, show different area sizes on the computer screen. Figure 2a shows an image viewed with three different software programs at a 2x relative magnification; all programs cover a different area size of the slide.

Secondly, differences in computer screen size will evidently show different areas of the slides; a larger screen will cover a larger area compared to a smaller screen (Figure 2b).

Third, the different coverage area of the image view on the computer screen might be a problem; some programs use full screen views (rectangular view, Figure 2c), while others use only three quarters of the screen (more square view, Figure 2d).

Most importantly, not using an annotation can cause a patient to end up in the wrong category. In full screen the case might appear to be stroma-low, while after placing an annotation a case should be categorized as stroma-high (Figure 3).

These issues cause variability in TSR determination directly from the screen. Different area sizes might lead to different scorings percentages, specifically for cases around the cut-off point. For these cases it is important to be able to meet all scoring criteria to obtain a reliable score.

New Approach

Due to the mentioned issues, we propose an adjusted approach for the estimation of the TSR when performed on digital images:

Select a slide viewer software program that allows for drawing circular annotations, preferably with the possibility of a fixed area. It is recommended to choose a software program in which it is possible to easily move the annotation around to find the most suitable area to score the TSR. Draw a circular annotation with an area size within the range of 2.54 mm^2 to 3.80 mm^2. This circle is comparable with the area of the field of vision of the most commonly used ocular lens diameters of 18 mm to 22 mm on a conventional microscope (an area of 3.46 mm^2 is commonly used). Using this annotation, the TSR can be scored optimally following the described protocol

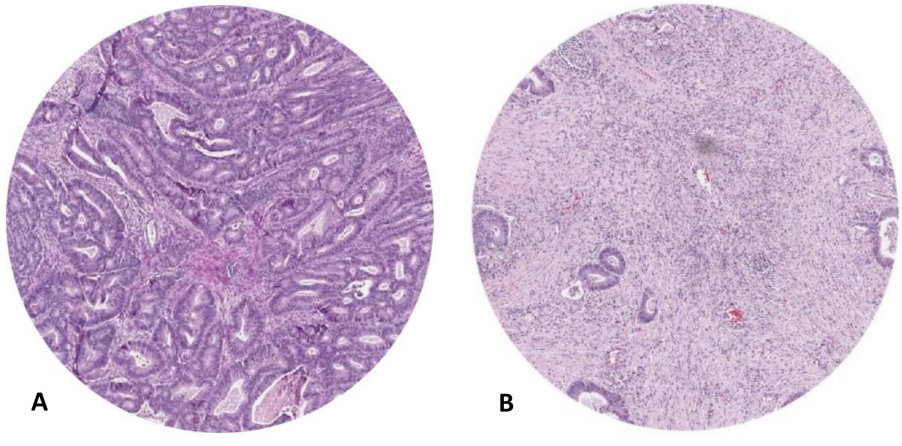


Figure 1: Examples of stroma-low (A) and stroma-high (B) haematoxylin and eosin (H&E) stained paraffin sections at the most invasive part of primary colon cancers.

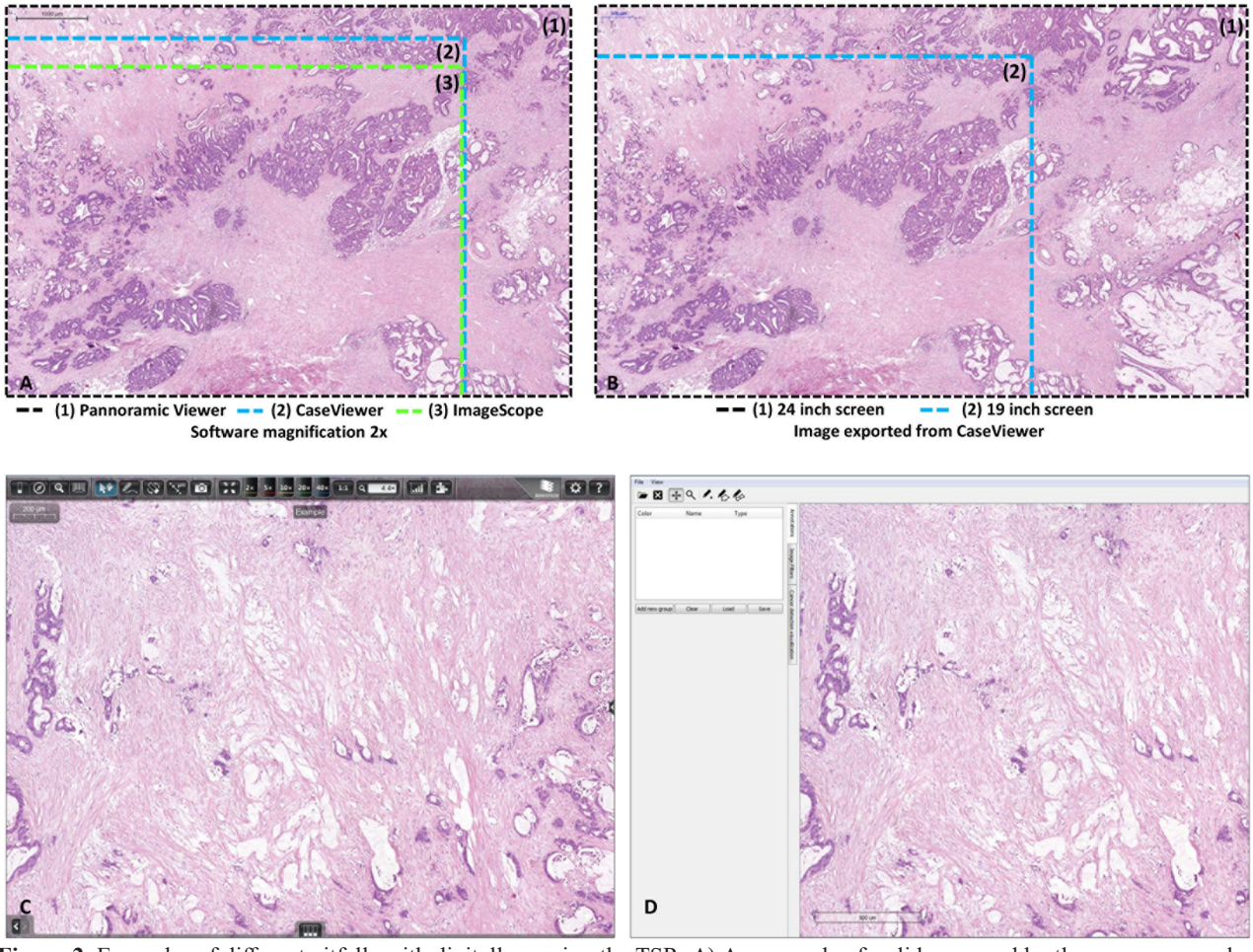


Figure 2: Examples of different pitfalls with digitally scoring the TSR. A) An example of a slide scanned by the same scanner but opened with three different image viewing software programs. All images are viewed with a 2x software magnification. The full image (1) is an image taken with Pannoramic Viewer (3D Histech), the blue lined image (2) is taken with CaseViewer (3D Histech) and the green lined image (3) is taken with ImageScope (Leica); B) An example of images taken from two different screen sizes, 24 inch (1) versus 19 inches (2); C) and D) Examples of two different software programs, which show different sizes of screen coverage; full screen (C) and three quarters of the screen (D).

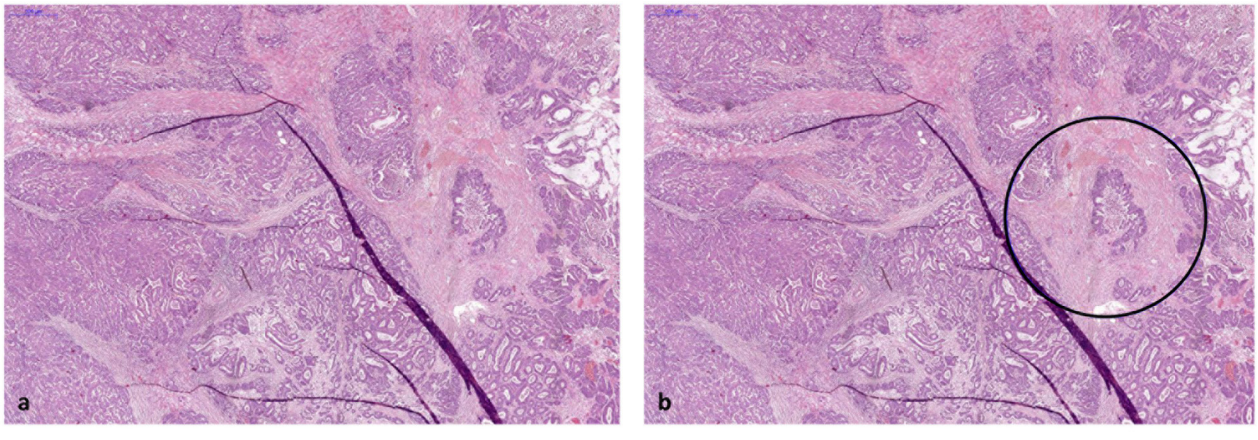


Figure 3: An example of a case that seems to be stroma-low, when scoring without annotation (A). However, when scoring with annotation, the case actually should be categorized as stroma-high (B).

for microscopic analysis.

The choice of software depends on the slide scanner used, as not all file extensions can be opened within each software program. In general, files created in .mrxs format are compatible with most software programs.

As not all diagnostic pathology laboratories have access to state of the art technologies or the sources to purchase a license for automated analysis software, this digital scoring method might also be used as a cheaper alternative. Many of the available slide viewer software programs can be downloaded free of charge from the internet; only a slide scanner is necessary.

Discussion

The transition from optical microscopy to automated analysis for determining the TSR is a time-consuming process and an alternative is needed to fill the gap. The implementation of the standard scoring protocol on digital images revealed multiple issues, which made it difficult to score the TSR correctly. With this brief report we propose an adjusted method for scoring the TSR on digital images. Scoring the TSR digitally using a circular annotation is easy to apply and does not take much additional effort compared with the microscopic method. As soon as the fixed size of the annotation is saved, every new case can be scored in less than two minutes.

This adapted method for scoring the TSR fills the gap between microscopic and automated scoring of the TSR, and opens the opportunity for application in daily diagnostics.

Author's contributions: GP wrote the manuscript. All authors have contributed to the intellectual content of this manuscript and revision of the article, and have given final approval to the manuscript.

Funding/Support: The authors did not receive any kind of funding or other financial support for this research.

Conflict of Interests: None declared.

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