# Mapping tumour tissue: quantitative maps of histological whole slide images

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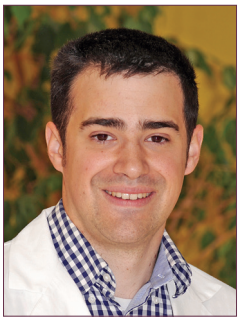

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## Histological imaging of tumour tissue

Immunohistochemistry (IHC) is the standard method to assess tumour tissue on a micro-scopic scale. IHC selectively highlights microscopic structures in the tissue and yields quantitative information that can be used to answer questions like: "How many immune cells are present in a given tumour?", "How many tumour cells are actively proliferating?", or "How many blood vessels are present in the tumour?". These questions are addressed by histopathologists who visually observe regions of immunostained slides of tumour tissue and count structures of interest, for instance, cells or blood vessels. In the clinic, this quantitative information can be then used to estimate the prognosis of a patient. For example, the number of blood vessels in tumour tissue is a prognostic factor for colorectal cancer patients [1].

Pathologists combine the excellent human vision and pattern recognition skills of the brain with an extensive training in tissue observation. Traditionally, pathologists use only a microscope to identify and assess structures of interest manually. However, the limitations of manual procedures are evident; besides the possibility of human error, the dimensions of tissue slides in high magnification are huge and it is not feasible to view the whole slide nor manually visualise or count any objects of interest. Therefore, microscopic structures such as blood vessels are only quantified in a small fraction of the entire tumour image [2]. However, tumour tissue is highly heterogeneous and adjacent tumour tissue areas may have very different properties [3]. This reflects a problem in traditional histopathology: if we only quantify objects in a small part of the whole image, we do not know how much the distribution of these objects varies in the rest of the image.

## Quantitative approaches in digital pathology

Digital pathology offers a solution to this problem. In principle, it is possible to use computer-based image analysis to automatically count objects in huge images. Microscopic tissue slides can be digitally scanned in high magnification to create digital whole slide images (WSI). Then, we can use automatic image processing procedures to extract all structures of interest from the original image.

The digitisation of histological slides and the data handling is a complex task that requires a cascade of several steps [4-6]. Several commercial tools are available that can be used to deal with and process WSI. Also, as part of the "open microscopy" project (www.openmicroscopy.org), various tools to handle WSI have been developed: OMERO, a software application capable of handling large WSI and BioFormats, a programming library that can be used by other applications such as ImageJ or Matlab to read and write WSI files.

Regardless of which tool is used to extract objects from WSI, the result is comparable: after an image processing procedure we know how many of these structures are present in a given image and where exactly these structures are located. The data on its own is not always useful, it needs to be evaluated by a trained human observer who can then extract clinically relevant information from this data. Consequently, an important question in digital pathology is the following: how can we efficiently present quantitative information from a WSI to a human observer?

## Example: how to count blood vessels in tumour tissue

In this work, we will give an example of how to analyse quantitative data derived from a WSI. As an example we will use the topic of blood vessel density in human tumours. Blood vessels play an important role in solid tumours as they supply the tumour cells with oxygen and nutrients. Blood vessels can be counted in immunostained images and their density is inversely correlated to patient survival in colorectal cancer and other cancer types [1]. Thus, the questions of how exactly blood vessels are distributed in tumour tissue might have relevant implications for the clinic.

We assume that our starting point is a tumour tissue stained by IHC so that blood vessels are of a specific colour. We can then scan this slide and feed the resulting WSI into a computer program that performs object recognition and records the exact position of each blood vessel within the tumour tissue [7,8]. Given the resolution and size of a WSI, it is relevant to ask how do we then make sense of this huge amount of data? The most straightforward approach would be to use only the total number of blood vessels and normalise them to the tissue area. The result is a single number, e.g. 100 blood vessels per $mm^2$. This simple approach is useful to characterise the overall degree of angiogenesis in tumours [9]. However, by breaking down all measurements to a single number, we lose all information about the spatial distribution of blood vessels.

## Quantitative maps of tumour tissue

Another approach to visualise the blood vessel distribution in tumour tissue is a map. In a map, each object of interest is displayed, for example, in a two-dimensional coordinate system. However, typically we are not interested in individual objects, but in distribution parameters on a higher scale, for instance, local density of objects. Thus, instead of displaying every single blood vessel in a tumour tissue section, we can instead derive a quantitative map of the local blood vessel density. In general, these maps represent the spatial description of a feature of interest in a given tumour. These type of quantitative maps enable us to evaluate objects in large images and show the result to a human observer. In Figure 1, the processing cascade from tumour tissue to data analysis is shown.

In our case, we are interested in a quantitative density map that displays the local density of blood vessels (as represented by a numeric value) at each location of the original WSI. Figure 2 shows an example of a WSI stained for blood vessels with CD34 and its corresponding blood vessel density as calculated automatically with the algorithm described in [7]. In the quantitative map of blood vessel density (figure 2D), each point in the image is assigned a colour that corresponds to the local blood vessel density at this particular location. Another interesting feature of quantitative maps of tumour tissue: The data represented in the map can be analysed by means of spatial statistics. For example, we can objectively identify statistically significant areas of high object density, i.e. hotspot areas (Figure 2D, Figure 1) [7].

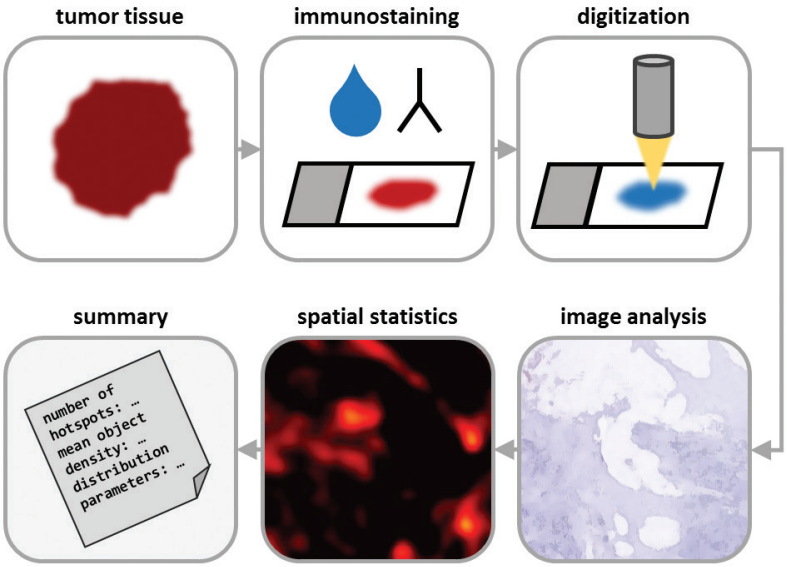


Figure 1: Processing pipeline to create and analyse a quantitative map of a histological whole slide image. A piece of tumour tissue is cut into slices, undergoes immunostaining and is digitized using a scanning microscope. Then, automatic image analysis produces a continu-ous map of features of interest, which can be analysed by means of spatial statistics.

## Established applications of quantitative maps

We have previously shown that quantitative maps of WSI are useful to assess blood vessel distribution [7]. Additionally, maps of tumour WSI have been successfully applied in a number of other studies. For example, they were shown to be useful to describe the density of immune cells in solid tumours [10]. In this case, the density of immune cells at the interface between tumour metastasis and healthy tissue was an excellent predictor of survival [11]. In breast cancer, specific spatial distribution patterns of immune cells were associated with different clinical parameters [12]. Another study showed that it is possible to assess many different antigens at once and, by image registration, compare the co-localisation of these antigens [13]. Alternatively, several antigens can be assessed simultaneously by using multiplex staining [14]. Even more information about spatial characteristics of histological slides can be acquired by a relatively novel technology called Matrix-Assisted Laser Desorption/Ionisation Imaging Mass spectrometry (MALDI IMS). This method has been used to measure the concentration of specific molecules in each location on a histological slide and produce a map of the distribution of each molecule of interest [15]. Ultimately, morphological analysis of WSI could be combined with MALDI IMS data and other "Omics" technologies [16].

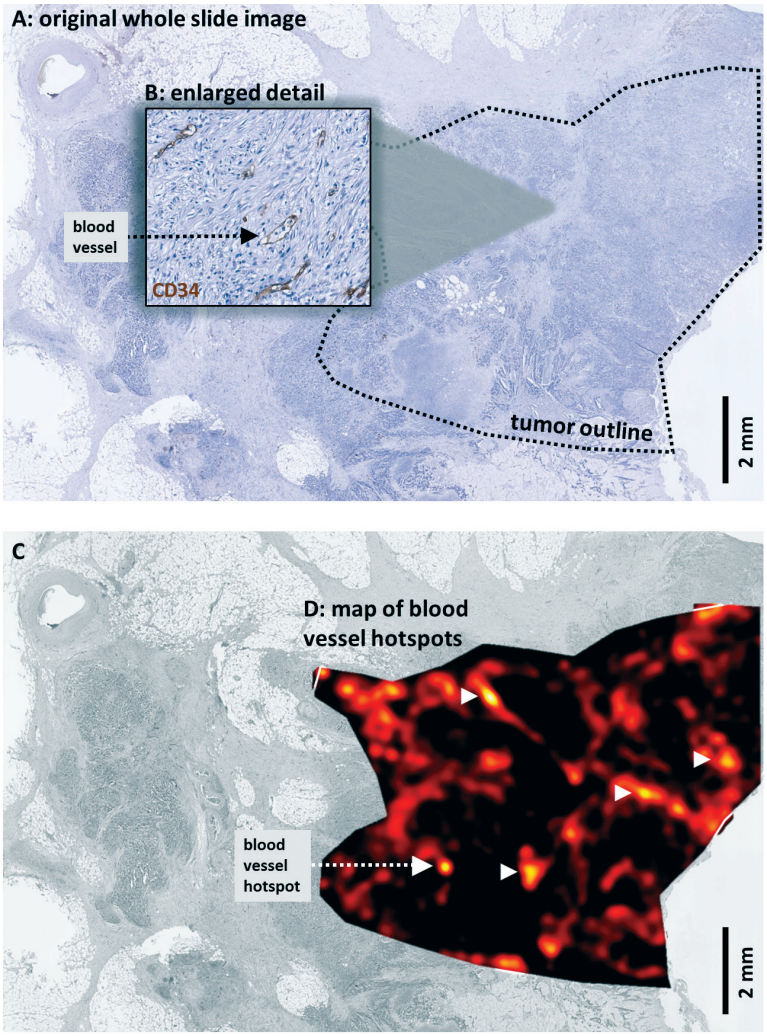


Figure 2: An example of a quantitative map: analysis of blood vessel hotspots in an image of a section through a colorectal carcinoma. A) Original whole slide image of a colorectal carcinoma, stained for the blood vessel marker CD34. B) Enlarged detail showing blood vessels in brown. C-D) Result of automatic analysis: the region of interest has been used to create a continuous map of blood vessel density. Black = low blood vessel density, yellow = high blood vessel density. Five statistically significant hotspots have been detected (white arrow-heads).

## Future directions for quantitative maps in histology

There are many interesting applications that could be addressed by quantitative maps in the future. For instance, it has been shown that proliferating tumour cells in hepatocellular carcinoma are predominantly located at the tumour margins as compared to the tumour centre [17]. However, to our knowledge, this finding has never been validated in WSI of different types of cancer. A quantitative map of proliferating tumour cells could be useful to model the distribution of proliferating tumour cells within tumours more accurately. Another example is the distribution of particular genetic alterations in a tumour. For example, breast cancer and gastric cancer often contain HER2 overexpressing cells. However, cells in adjacent tissue areas in these tumours sometimes show different degrees of HER2 overexpression. How exactly these cell populations are distributed in a tumour has never been investigated in whole slide images. This question could be addressed by using a quantitative map of HER2 positive cells within the tumour, which could potentially lead to clinically relevant conclusions.

## Summary

Continuous maps are a useful approach to visualise object distributions and calculate spatial statistics based on histological whole slide images. These maps are a very intuitive way of displaying spatial data and human observers can easily extract information from these maps. Thus, by using quantitative maps, the unsurpassed pattern recognition capacities of human observers are efficiently combined with the quantitative power of automatic computer based analysis.


References

1. Des Guetz G, et al. *"Microvessel density and VEGF expression are prognostic factors in colorectal cancer. Meta-analysis of the literature"*, Br J Cancer, 2006;94(12):1823–32.
2. Vermeulen PB, et al. *Second international consensus on the methodology and criteria of evaluation of angiogenesis quantification in solid human tumours.* Eur J Cancer 2002;38(12):1564–79.
3. Gerlinger M, et al. *Intratumor heterogeneity and branched evolution revealed by multiregion sequencing.* N Engl J Med 2012;366(10):883–92.
4. Ghaznavi F, et al. *Digital imaging in pathology: whole-slide imaging and beyond.* Annu Rev Pathol 2013;8:331-59.
5. Kothari S, et al. *Pathology imaging informatics for quantitative analysis of whole-slide images.* J Am Med Inform Assoc 2013;20(6):1099-108.
6. Pantanowitz L, et al. *Review of the current state of whole slide imaging in pathology.* J Pathol Inform 2011; 2, 36.
7. Kather JN, et al. *Continuous representation of tumor microvessel density and detection of angiogenic hotspots in histological whole-slide images.* Oncotarget 2015;6(22): 19163–76.
8. Reyes-Aldasoro CC, et al. *CAIMAN: an online algorithm repository for Cancer Image Analysis.* Comput Meth Prog Bio 2011;103(2): 97–103.
9. Eberhard A, et al. *Heterogeneity of Angiogenesis and Blood Vessel Maturation in Human Tumors: Implications for Antiangiogenic Tumor Therapies.* Cancer Res 2000;60(5),1388–93.
10. Halama N, et al. *Quantification of prognostic immune cell markers in colorectal cancer using whole slide imaging tumor maps.* Anal Quant Cytol Histol 2010; 32(6):333–40.
11. Halama N, et al. *Localization and density of immune cells in the invasive margin of human colorectal cancer liver metastases are prognostic for response to chemotherapy.* Cancer Res 2011;71(17):5670–7.
12. Nawaz S, et al. *Beyond immune density: critical role of spatial heterogeneity in estrogen receptor-negative breast cancer.* Mod Pathol, 2015;28,766-77.
13. Moles Lopez X, et al. *Registration of whole immunohistochemical slide images: An efficient way to characterize biomarker colocalization.* J Am Med Informatics Assoc 2015;22(1),86-99.
14. Isse K, et al. *Digital transplantation pathology: combining whole slide imaging, multiplex staining and automated image analysis.* Am J Transplant 2012;12(1):27-37.
15. Powers TW, et al. *MALDI imaging mass spectrometry profiling of N-glycans in formalin-fixed paraffin embedded clinical tissue blocks and tissue microarrays.* PLoS One 2014;9(9), e106255.
16. Heindl A, et al. *Mapping spatial heterogeneity in the tumor microenvironment: a new era for digital pathology.* Lab Invest 2015;95(4):377-84.
17. Waclaw B, et al. *A spatial model predicts that dispersal and cell turnover limit intratumour heterogeneity.* Nature 2015;525(7568):261-4.