Stain normalisation techniques for histopathology images

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Introduction

Computer vision is a field that has developed significantly over the last decade. Machine learning techniques in particular, mainly deep neural networks, have overcome many previously unsolved challenges. Given their success, it should be no surprise that researchers and developers are constantly looking for new areas where these methods could be of use.

One such area is that of medical image analysis. Reading an MRI, diagnosing a respiratory illness from an X-ray, or finding cancerous cells in a tissue sample are all tasks that normally require a highly trained professional to execute, but which can be translated to computer vision problems such as segmentation or classification. Precise computer vision techniques aiding in these tasks can be a huge help to healthcare workers worldwide.

I will be working on cancer segmentation in histopathology images in my projects. My work in this semester consisted of building the experiment pipeline and to study the literature on medical image recognition. A problem that very probably will arise is that the dataset I will be working with is relatively small. Since there is a lot more publicly available data, this in itself would not be an issue, but it creates another difficulty: histopathology images from different sources tend to have very high colour variaton. Below, I explain why this is a problem, and what can be done to circumvent it.

Review of stain normalisation methods

Stain normalisation and stain separation

Digital histopathology refers to the study of images taken by placing some tissue sample under microscope. Since these tissue samples naturally look transparent, staining is used for better visibility. Stains are chemicals added to the sample that colour specific parts with specific colours, while leaving other parts transparent. The most commonly used method is H&E staining, which uses haematoxylin – that stains the nucleus a darker purple – and eosin – that stains the extracellular matrix and cytoplasm pink. For various task, such as cancer detection, recognising these parts of the cell is paramount, so any computer vision model has to take into account the specifics of staining.

The problem comes from the huge differences that can occur between images taken at different labs. These can be due to different equipment or methods used for preparing the tissue, or different types of scanner used for capturing it. This obviously hinders the performance of any machine learning model that was trained on images coming from a single lab. Stain normalisation and stain separation techniques aim to minimise the effects of these sort of differences.

It is important to note the distinction between these two concepts. *Stain normalisation* refers to any technique that aims to, in some sense, normalise stained images – that is to say, apply a transformation that makes their colour histogram similar to that of a target image. *Stain separation*, on the other hand, aims to separate the different stains in the image. More formally, its goal is to write an image H as $H_1 + H_2 + \ldots + H_n$, where H_i represents a theoretical image taken of the same tissue sample, but using only the i^{th} stain. (Typically there are two stains, so $n = 2$ or 3, depending on whether we count the background or not.)

In the following sections, I will provide a brief review of different stain normalisation and separation techniques. I will mainly be relying on a study by Roy *et al.* [1] that reviews the most widely used and most influential methods.

Linear methods

The most simple methods use simple linear transformations to transform images. The two main examples are the Reinhard's method for stain normalisation [2] and Ruifork and Johnson's colour deconvolution for stain separation [3]. These two methods are important because the ideas present in them laid the foundation for later, more complex approaches. Mainly, their use of different colour spaces carries on to almost all future techniques.

Reinhard's method uses the $\ell\alpha\beta$ colour space, which has three axes corresponding to lightness, the green–red and the blue–yellow scales respectively. It prefers $\ell\alpha\beta$ to RGB because of the supposed smaller correlation between its axes.

Colour deconvolution, in contrast, uses the optical density or OD space. The OD space still has red, green and blue axes, which are calculated by taking the negative logarithm of the respective coordinate. The OD space works well for stained images due to Beer's law, that states that the concentration of a chemical solution is directly proportional to its absorption of light, where absorbance is defined as the logarithm of the ratio between incident and transmitted light. That means that if one can separate the different stains in OD space, then the coefficient of each stain vector will be directly proportional to the amount of stain present in that pixel. Ruifork and Johnston's method uses an S stain colour appearance matrix for the transformation, that is pre-calculated using laboratory data of the stains used.

Algorithmic non-linear methods

The fact that Ruifork and Johnston's colour deconvolution algorithm relies on a pre-calculated stain matrix is an obvious flaw that later methods have sought to correct. Both Macenko's [4] and Vahadane's [5] method have the same principle as basic colour deconvolution: in OD space, find a basis that represents individual stain vectors. They differ in the fact that they use no preliminary data, only information contained in the images themselves.

Macenko's method applies the presumption that each image contains a pixel that represents a single stain vector. Its main idea is to apply singular value decomposition to find the singular vector corresponding to the largest singular value. In theory, the two pixels that form the smallest and largest angle with the singular vector should be the stain vectors. In practice, due to noise, the minimum and maximum is computed with the top and bottom α percent disregarded, usually with $\alpha = 5$.

Vahadane's method uses continuous optimisation to find the W transformation map from the stain space to OD space, and the H representation in the stain space such that $V = WH$ where V is the image in OD space.

This is obviously equivalent to finding $\min \|V - WH\|$. Vahadane *et al.* add an additional sparsity constraint to H, assuming that most pixels are only stained with one stain. This is a realistic assumption, since different stains colour different parts of the tissue.

Machine learning methods

Naturally techniques using machine learning have been proposed to tackle the problem of stain normalisation. The earliest was that gained traction was proposed by Khan *et al.* [6] that used a relevance vector machine to learn to estimate the stain colour appearance matrix. Its obvious drawback is that relevance vector machines use what we would call today supervised learning – that is, they need data annotated beforehand to train on. Such data is hard to come by, which is why later techniques prefer unsupervised methods.

Conditional generative adversarial networks, or cGANs have been used by Zanjani *et al.* [7], and Saleh and Chalechale [8]. Zanjani *et al.* tried to learn the colour appearance matrix as an inner layer of a cGAN that had to reconstruct the $\ell\alpha\beta$ image from just its lightness channel.

Saleh and Chalechale's Stain-to-Stain Transformation (STST) also trains a cGAN to reconstruct the coloured image from a greyscale input, but not to directly learn the colour appearance matrix, but to learn a function that transforms any input so that it resembles the images it was trained on. Consequently, if it had been trained on samples stained and digitalised under similar conditions, it should learn to emulate those conditions regardless of samples.

Future work

In the future, I wish to test these methods in practice to measure their impact on model transferability between different datasets. In addition, I plan to experiment with different segmentation models and techniques to learn their capabilities.

References

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