The development and evaluation of a novel H&E stain quantification method

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Background

In pathology, tissue is stained with haematoxylin and eosin (H&E) stains for enhanced visualisation of tissue features. The staining method can be variable but has been tolerated due to the ability of the human visual system to adapt to variation. With the increasing adoption of digital pathology, and development of image analysis algorithms, stain variation can have a more profound impact. Despite this, methods of stain quality control are based on subjective interpretation of stained tissue controls that may vary in thickness and morphology. This poster presents a novel, physical technique for objective quantification of H&E stains in pathology.

Methodology

Stain assessment slides were prepared and a) characterised with H&E for a range of clinical stain times; b) compared with human liver tissue H&E stain response; and c) implemented in eight clinical laboratories, alongside tissue control slides, over a period of two weeks. See *Figure 1* for an example of the stain assessment slide implementation and measurement.

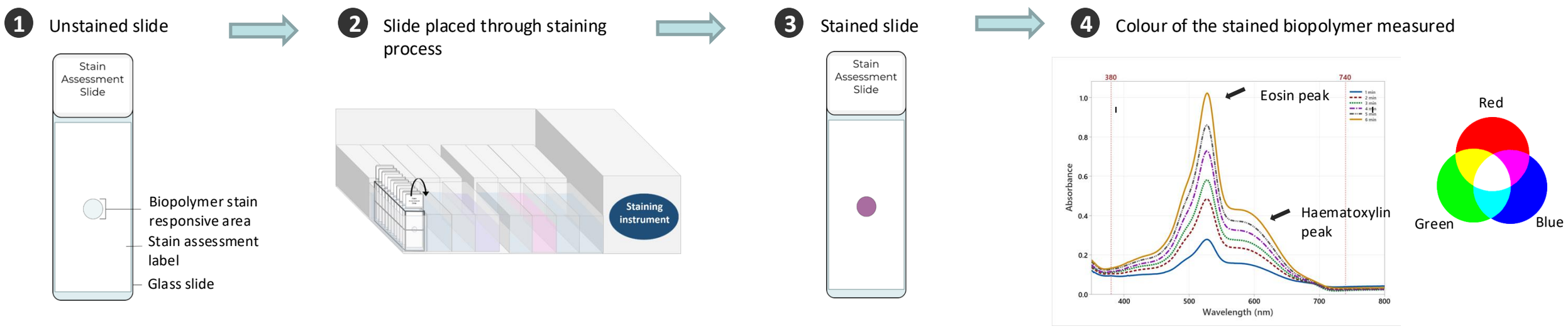

Figure 1. A diagram describing the process of using the biopolymer film on stain assessment slides to quantify H&E staining. The process involves (1) an unstained stain assessment slide comprising of a glass slide, a stain responsive biopolymer and a chemically resistant top label; (2) the stain assessment slides being placed into an automated staining instrument; (3) a stain assessment slide after H&E staining; and (4) colour measurement of the stained biopolymer film, illustrated here by examples of H&E stained biopolymer absorbance spectra (each coloured line depicting stain durations between 1 – 6 minutes), and a Red, Green and Blue (RGB) colour diagram.

Results

a) H&E characterisation

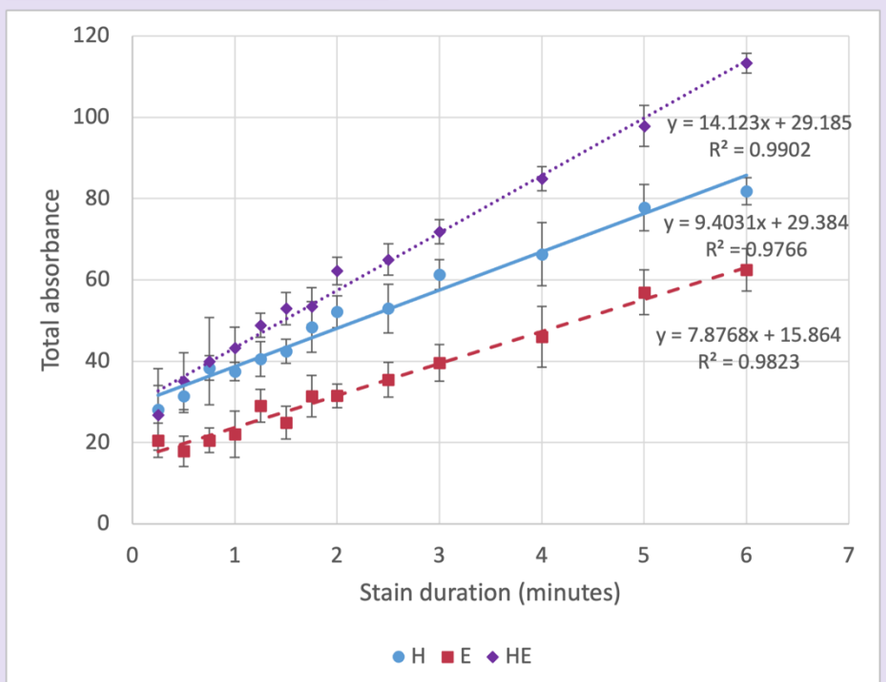

Figure 2 shows the total spectral absorbance (intensity) of H&E-stained biopolymer plotted against stain duration. The total absorbance increased linearly with stain duration for haematoxylin (r = 0.99), eosin (r = 0.99) and H&E combined (r = 0.99).

b) Tissue comparison

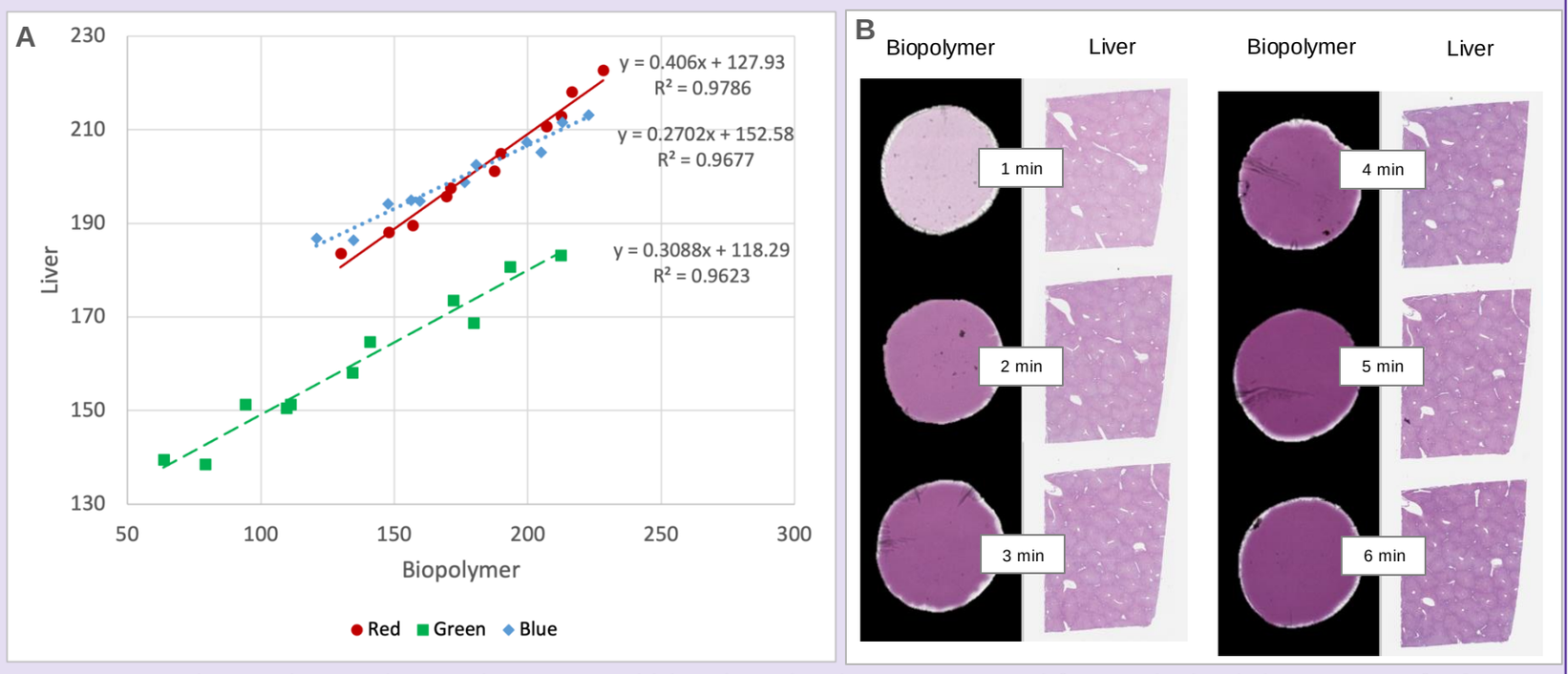

Figure 3. A shows the median red, green and blue (RGB) values, measured from whole slide images of H&E-stained biopolymer and liver tissue stained between 15 seconds and 6 minutes. A strong linear relationship was measured when comparing the biopolymer and liver tissue results for red (r = 0.99), green (r = 0.98) and blue (r = 0.99) values. For visualisation of the stained colour, **B** shows macro images of the biopolymer and liver tissue, stained with H&E between 1 and 6 minutes.

c) Implementation in clinical laboratories

Figure 4. Eight UK laboratories each stained 40 stain assessment slides and and 40 tissue control slides, using their standard H&E protocol, over a two-week period. The total absorbance was measured from the stain assessment slides and plotted in the boxplots shown in **A.** To analyse the potential impact of the stain variation measured, the QuPath automated cell counting algorithm (Bankhead *et al.,* 2017) was applied to 300 μm^2 regions of interest of liver tissue from the tissue control slides. ***B*** shows boxplots, with individual values plotted, displaying the spread of cell count results. The red dotted line indicates the average 'ground truth' cell count measured from the regions by three experienced pathologists. The laboratories results were significantly different for both total absorbance in **A** ($p < 0.00$) and automated cell count in **B** ($p < 0.00$).

Conclusion

The proposed novel technique reliably quantified stain uptake, providing an effective, quantitative method for laboratory quality control of stain variation. Results demonstrated a linear response to H&E staining, comparability to control tissue and demonstrable clinical utility in measuring stain. Inter-laboratory stain variation between eight laboratories was found to significantly affect stained colour and automated cell counts. If adopted into laboratory practice, this system could improve stain quality consistency in pathology which is important to underpin improving image analysis development and portability between institutions.

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Reference: Bankhead, P. et al. **QuPath: Open source software for digital pathology image analysis**. *Scientific Reports* (2017). https://doi.org/10.1038/s41598-017-17204-5