The use of a biopolymer film for quantitative H&E stain assessment and quality control in pathology

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Background

In pathology, tissue samples are stained with histochemical dyes for enhanced visualization of tissue features. The method of staining tissue can be variable but has been well tolerated due to the ability of the human visual system to adapt to variation. With the increasing adoption of digital pathology, and development of artificial intelligence algorithms, stain variation can have a more profound impact. Despite this, methods of quality control are based on subjective interpretation of stained tissue controls that may vary in thickness and morphology.

The proposed methodology is a novel technique for objective quantification of haematoxylin and eosin (H&E) staining, using a stain-responsive biopolymer film.

Methods

A stain assessment slide comprises a glass slide, a stain-responsive biopolymer film and a chemically resistant label overlaid. Stain assessment slides can be placed through the staining process and measured to quantify stain, see Figure 1.

H&E characterization

Stain assessment slides were stained between 15 seconds - 6 minutes with haematoxylin only, eosin only and H&E combined (equal time each stain). The stain response of the biopolymer film was measured in a spectrophotometer (Cary100 UV-Vis, Agilent technologies). Spectral absorbance was measured with respect to stain duration, and total absorbance calculated (see Figure 2).

Tissue comparison

Stain assessment slides and human liver tissue were stained simultaneously with H&E, between 15 seconds - 6 minutes (equal time each stain). All slides were digitized in a whole slide imaging scanner (Leica Aperio AT2). Stain response was measured digitally, comparing Red, Green and Blue (RGB) values between H&E stained biopolymer and liver (see Figure 3).

Implementation

A) Stain assessment slides were stained with H&E in three identical staining instruments, in one clinical pathology laboratory. 30 slides were stained in each instrument at one point in time, and 25 slides were stained in each staining instrument over a five day period. Spectral absorbance was measured, and total absorbance calculated (see Figure 4).

B) 40 stain assessment slides were stained in eight clinical laboratories, using their standard H&E protocol, over a ten day period. Spectral absorbance was measured and total absorbance calculated, with early results shown in Figure 5.

Methods – *Stain assessment technique*

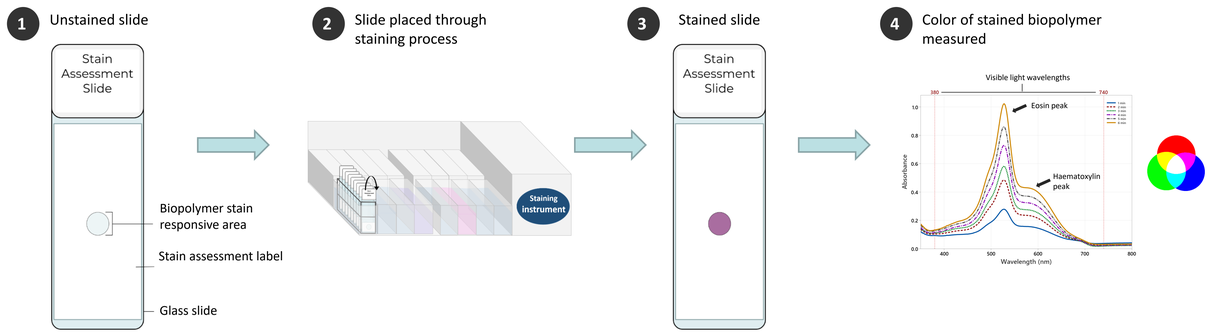

Figure 1. A diagram describing the process of using the biopolymer film on stain assessment slides to quantify H&E staining. The process is (1) an unstained stain assessment slide comprising of a glass slide, a stain responsive biopolymer and a chemically resistant top label; (2) stain assessment slides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

Results – *H&E characterization* – *Tissue comparison*

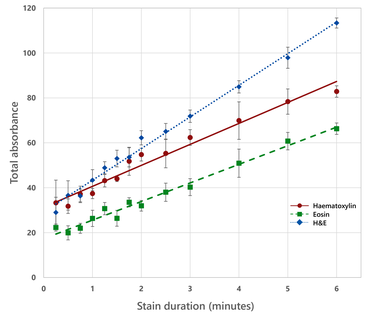

Figure 2. Total absorbance measured within visible wavelengths (380 – 740 nm) for biopolymer film stained with haematoxylin only, eosin only and H&E together, for staining durations of 1 – 6 minutes. Each point plotted is the average of 5 slides with error bars depicting one standard deviation from the mean.

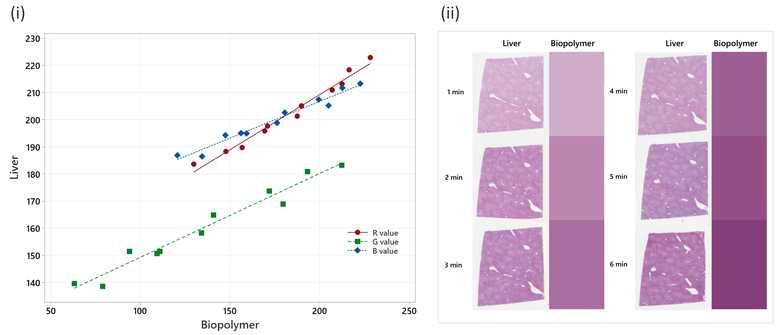

Figure 3. (i) Red, Green and Blue (RGB) color values of H&E stained human liver tissue and biopolymer film plotted against each other. Each point plotted represents the average value measured from 5 liver slides against 5 stain assessment slides measured for each stain duration between 15 seconds – 6 minutes. (ii) Shows macro images of the H&E stained human liver tissue and median color of biopolymer film, for visual representation of stained color between 1 – 6 minutes.

Results – *Implementation in one laboratory (5 days)* – *Implementation in eight laboratories (10 days)*

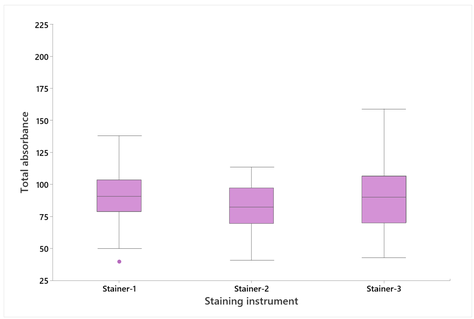

Figure 4. Boxplots showing the spread of total absorbance found within three staining instruments in one clinical laboratory, where 25 stain assessment slides were stained in Stainer-1, Stainer-2 and Stainer-3 over a five day period. The color of the boxes represents the median color values measured in each instrument.

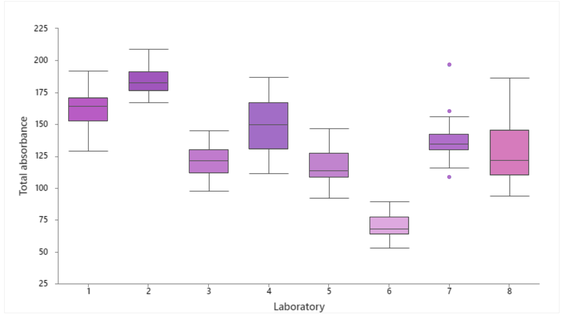

Figure 5. Boxplots showing initial results measured from stain assessment slides that were stained in eight laboratories. In each laboratory, forty stain assessment slides were placed through their default staining protocols over a ten day period. The color of the boxes represent the median color values measured, for visual representation of variation measured.

Results

H&E characterization

Total absorbance of H&E stained biopolymer increased linearly with stain duration for haematoxylin only (r = 0.99), eosin only (r = 0.99) and H&E combined (r = 0.99), see Figure 2. Mean coefficient of variation (C_V) across stain types and durations was 10% (±6).

Tissue comparison

Median RGB values for H&E stained biopolymer and liver tissue were plotted against each other. A linear relationship was found for R (r = 0.99), G (r = 0.98) and B (r = 0.99) values, see Figure 3. Mean C_V across stain durations for liver tissue was 2% (±1) and for biopolymer was 9% (±5).

Implementation

A) One clinical laboratory (five days)

The C_V at one point in time was found to be 6 – 9%. Across a five day period this rose to 23 – 30%. See Figure 4 for boxplots showing the spread across five days.

B) Eight clinical laboratories (ten days)

Initial results shown in Figure 6 show an average *intra*-laboratory C_V of 11% (±3) and an *inter*-laboratory C_V of 26% (±2). Large variation was observed between laboratories, for example the difference between the median total absorbance values for Laboratory 2 (median = 182) and Laboratory 6 (median = 68) was 91%.

Conclusion

The proposed novel technique reliably quantified stain uptake, providing an effective, quantitative method for laboratory quality control of stain variation. Results demonstrate linear response to H&E staining, comparability to control tissue and demonstrable clinical utility in measuring stain. If adopted into laboratory practice, this system could improve stain quality consistency in pathology. This is especially important to underpin the quality of digital pathology images, improving artificial intelligence development and portability.

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