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Effect of Formalin Fixing on Chromophore Saliency Maps Derived from Multi-Spectral Macropathology Skin Images

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INTRODUCTION

Macropathology refers to the assessment of surgically removed tissue under the naked eye and is the first stage of skin cancer diagnosis. Afterwards, skin specimens are preserved by formalin fixing. **Formalin fixing** is necessary for additional diagnosis, but distorts the optical properties (color, shape) of the tissue. However, in cases of strict tissue handling protocols (e.g. a pandemic) or tele-medicine, only fixed tissue images are available,

MultiSpectral Images (MSI) can assist in discerning concentrations of chromophore molecules in the skin[1] , which in turn facilitate skin cancer diagnosis.

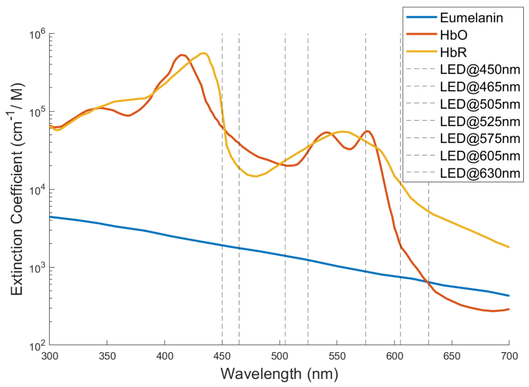

Fig. 1 Extinction spectra of skin chromophores and the wavelengths of our MSI system

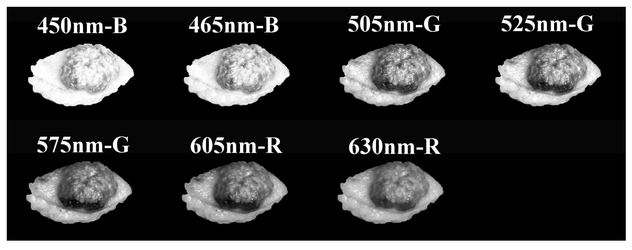

Fig. 2 Example of the MSI subimages captured at specific wavelengths and RGB camera filter

Hypothesis

After formalin fixing the values of total hemoglobin concentration map (HbT CrMap) are affected, while the values of melanin concentration map (Mel CrMap) remain the same.

Our **goals** are to:

- evaluate the influence of formalin fixing on MSI-based chromophore concentration maps
- investigate whether MSI-based CrMaps from fixed specimens can be of use during macropathology when untreated specimens are unavailable.

METHODS

Using multi spectral illumination and RGB camera we captured MSI pairs of **5 ex-vivo skin specimens before and after formalin fixing**. We generate MSI-based saliency maps (**CrMaps**) of **total hemoglobin and melanin concentrations**.

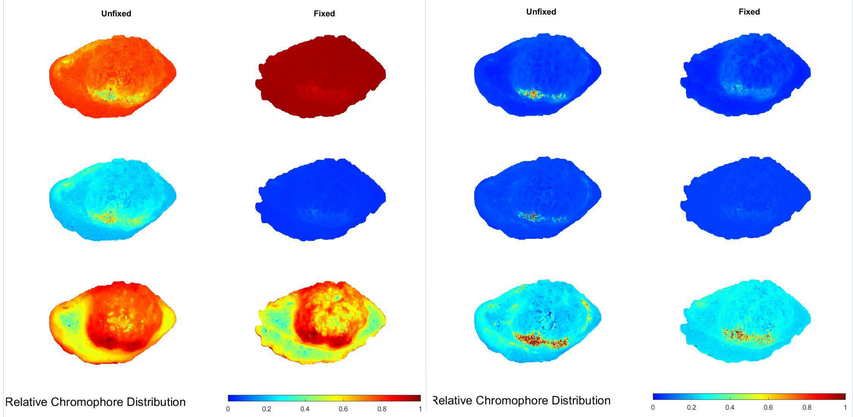

Fig 3 Example of relative concentration map for melanin (left) and total hemoglobin (right) for three different map generation methods.

Map generation methods were based on Absorbance Slope (AS)[2], Differential Absorbance (DA)[3] and Variation Index (VI)[4]. For evaluation purposes, **healthy tissue (Norm ROI), vessel area (HHbT ROI) and melanin-pigmented (HM ROI)** are identified in each MSI**.** Similarity between maps from the same specimen before and after fixing was quantified using Normalized Correlation Coefficient (NCC) and Histogram Integration (HI). High values indicate high similarity.

RESULTS

From visual evaluation and similarity measures, we observe that Mel CrMaps appear to not be affected by formalin fixing as much as HbT CrMaps. This is especially true for **CrMaps generated using the Variation Index method.** The decrease of HbT CrMap values after formalin fixing is consistent with reduced hemoglobin concentrations, because a portion of HbT is converted to hematin.

Relative concentration values from the total hemoglobin map is reduced after fixing, while this is not the case for melanin concentration.

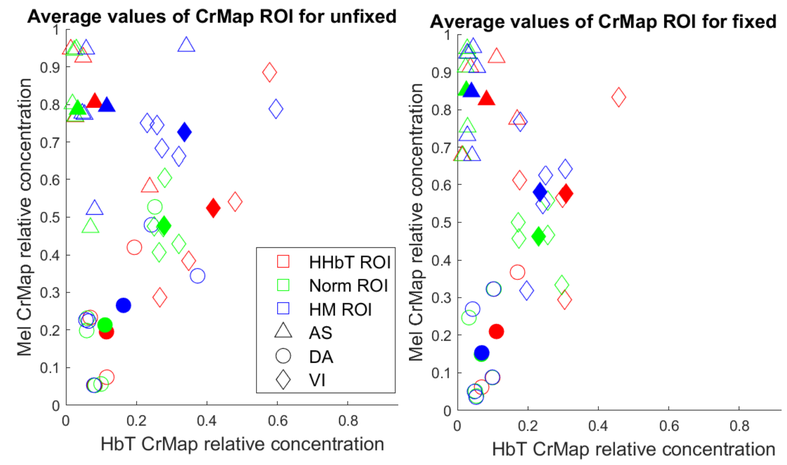

Fig. 4 Mean map values for unfixed tissue (left) and fixed tissue (right) color coded by tissue region of interest (ROI)

Areas that contain normal or melanin-pigmented tissue are affected less by formalin fixing, compared to areas with high blood concentrations.

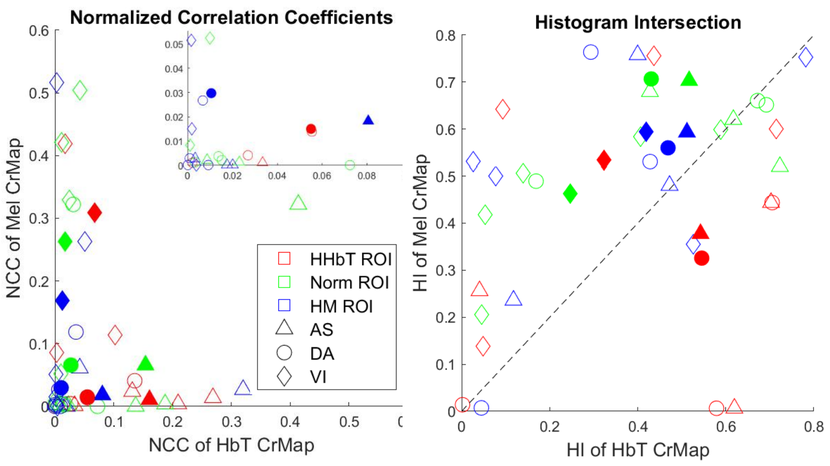

Fig. 5 Normalized correlation coefficient (left) and histogram integration (right) for CrMap pairs of unfixed and fixed tissue

CONCLUSIONS

MSI-bassed maps of melanin concentration are affected in a lesser extent by formalin fixing. Melanin maps generated from fixed tissue can be a useful tool in cases where unfixed tissue is unavailable.

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