

# Assignment E: Quantification of histochemical staining by color deconvolution

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## 1 Image data and tracked data inspection

**Question 1:** Explain the choice of stains and background region of interest (ROI) in each IHC image. i.e. Which histological stains are used and what are the RGB values, and why are they selected at the specific locations?

For the H\_E image, we know that purple/blue stain Fig.1a is the Hematoxylin while the pink stain is the Eosin. Hematoxylin is used to stain the nucleus parts, while Eosin stains the cytoplasm and extracellular regions, providing contrast to the nucleus part. On the other hand, IHC2 image is where H-Dab is used (Fig.1), which is oxidized by hydrogen peroxide in the presence of hemoglobin[1]. The oxidized DAB forms a brown precipitate, at the location of hemoglobin, allowing for identification and localization. The table bellow shows each mean RGB Hex transformed labels for each stain(Table.1).

Table 1: Stain Comparison, mean RGB to Hex Color values

Type	Stain 1	Stain 2	Background
H&E	f94a75	420d47	fcf3fc
IHC	7f3604	90add6	f9fafb

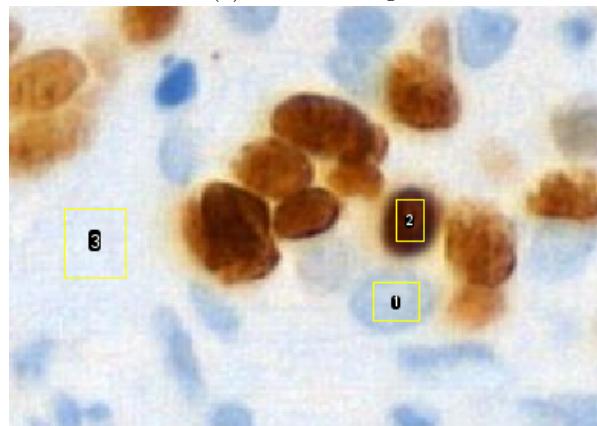
## 2 Methodology

**Question 2:** Present a color deconvolution workflow using a flowchart and description about the key components.

On Fig.2, the workflow of histological stain deconvolution is depicted. The main components of our method consist of calculating the Transmittance ( $T$ ) and converting it to Absorbance (OD- optical density matrix) using the Beer-Lamber Law[2]. With that formula, we can describe the relationship between the concentration of a substance and the amount of light absorbed or transmitted through it which will help us separate the different stain components in the histological image. Next, the deconvolution matrix is calculated with linear regression of our OD normalised matrix, to extract the individual coefficients for each IHC stain. Finally, we have to multiply the coefficients with the stain absorbance to get the image absorbance per stain and convert it to image transmittance for the representation of the staining intensity and the comparison of it across different regions of interest. For the deconvolution visualisation it is important to convert the values to intensity values (0-255).



(a) H&E staining



(b) H-DAB IHC

Fig. 1: IHC ROI

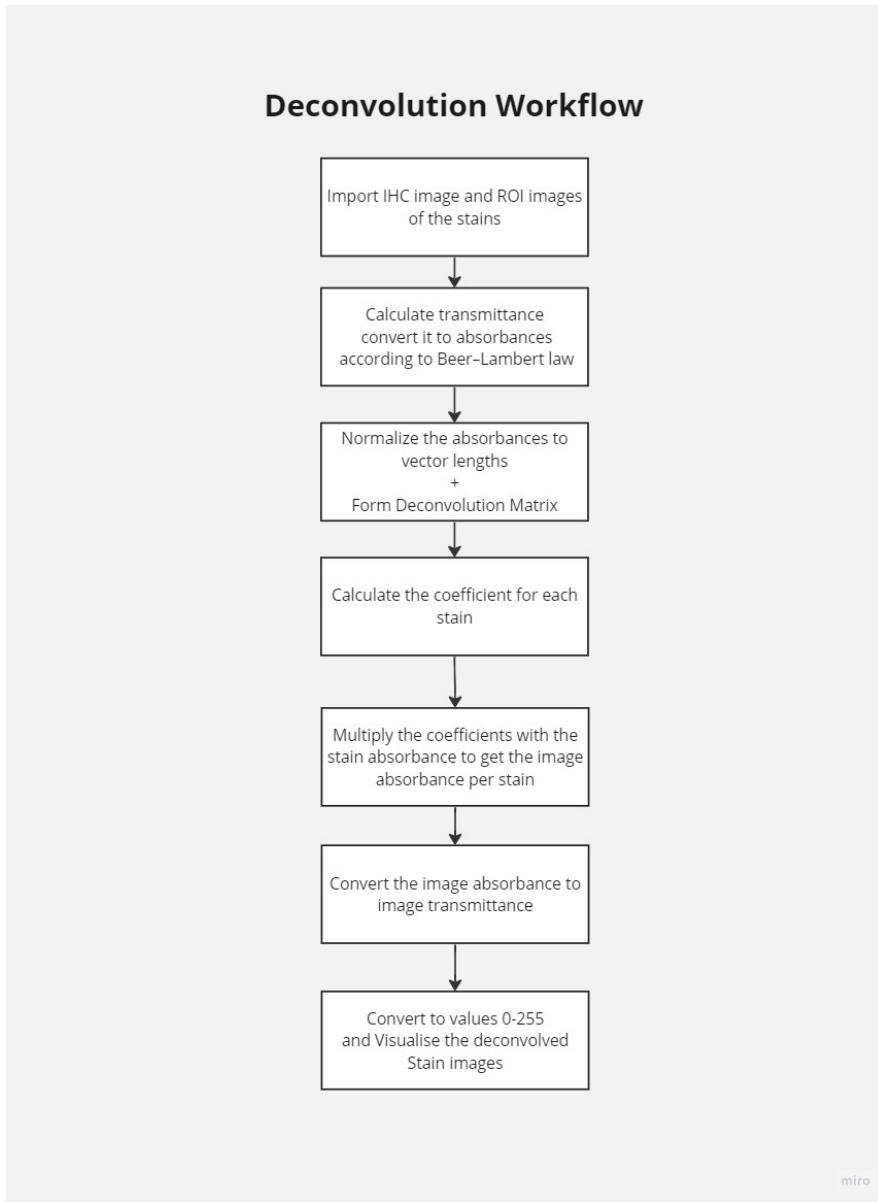


Fig. 2: Histological Stain Deconvolution workflow.

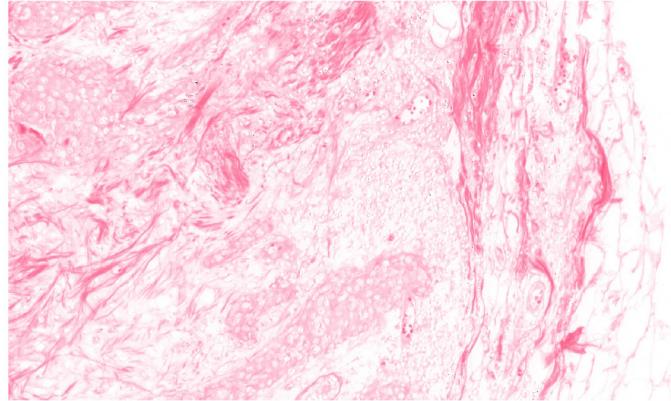
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### 3 Data analysis and discussion

**Question 4:** Use the final 2 figures in the Jupyter Notebook for each IHC image, explain the observations on the color deconvolved images. i.e. cellular or tissue structure stained by the selected histological stains

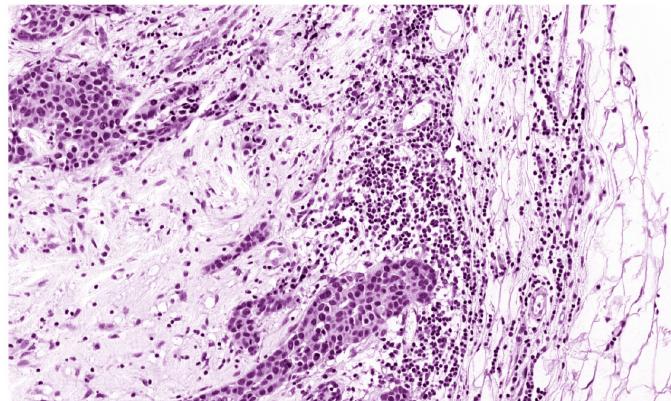
On Figure3, the pink Eosin stain seems to get properly assigned, as the extracellular and cytosol areas are coloured. Hematoxylin coloured purple seems to be more intense in the nucleus part accurately highlighting that areas. On the other hand on Figure4, DAB seems to stain the nucleus of interest but in various intensities. We cannot be sure if the oxidised areas are only the areas with only the cells of interest and not just random staining as it is discussed in Question 5. The blue hematoxylin regions mainly serve to provide contrast in the DAB staining and make it easier to visualize the normal cells(nuclei).

Deconvolved image for stain 1



(a) Deconvolution of EOSIN stain.

Deconvolved image for stain 2

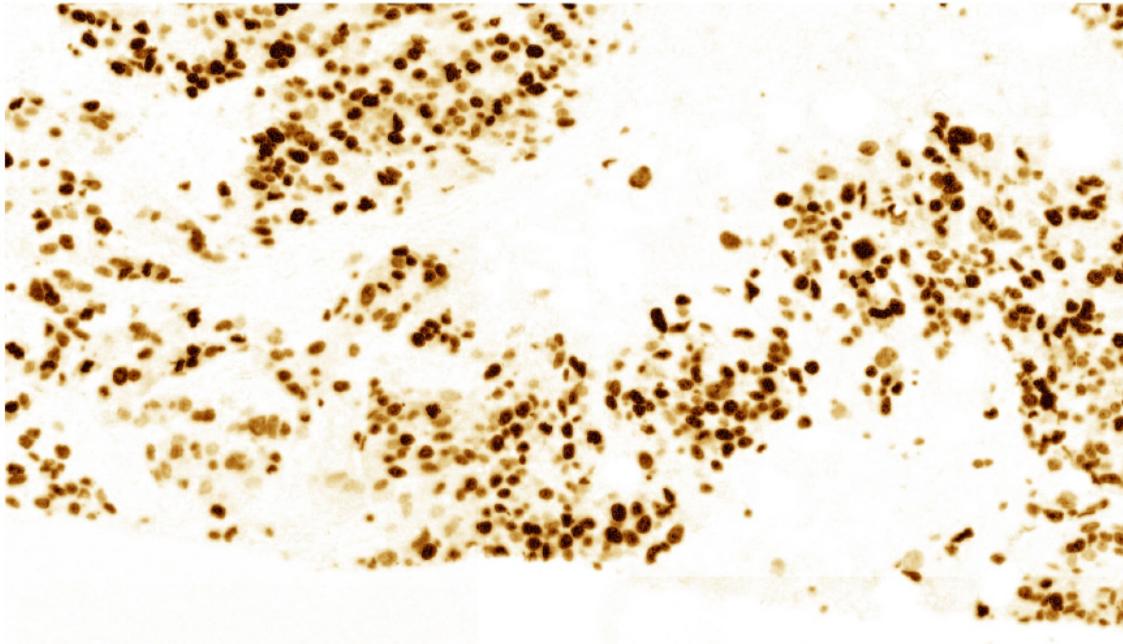


(b) Deconvolution of Hematoxylin stain.

Fig. 3: H&E, stain deconvolution

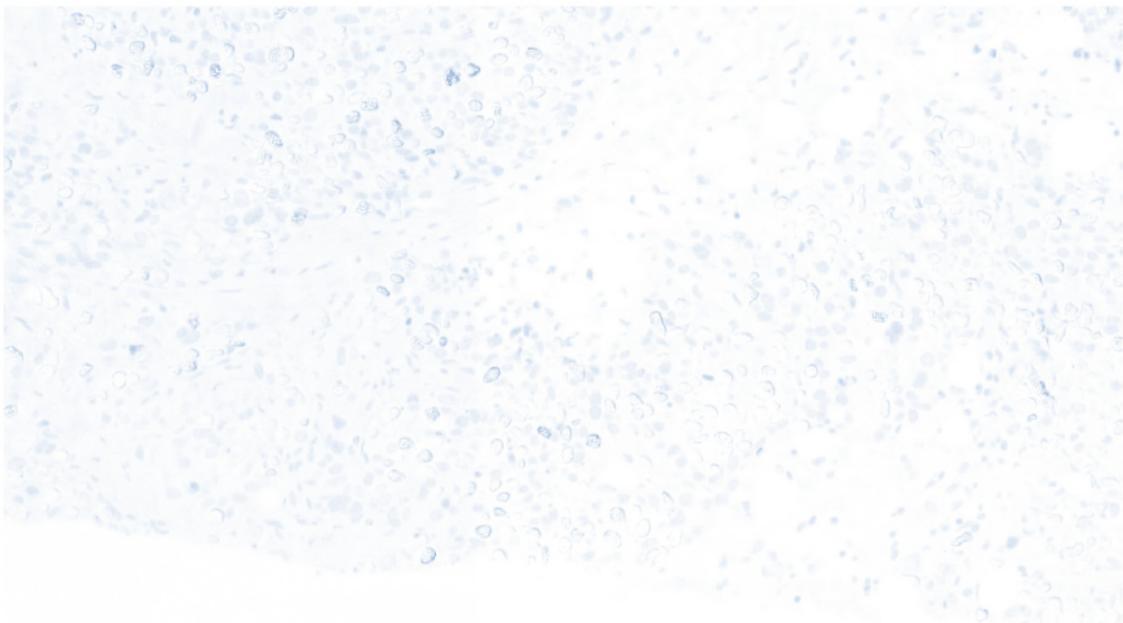
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Deconvolved image for stain 1



(a) Deconvolution of DAB stain.

Deconvolved image for stain 2



(b) Counter staining(hematoxylin).

Fig. 4: H-DAB, stain deconvolution

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**Question 5: Do you notice a difference in the output quality with IHC2.tiff compared to H\_E.tiff, where DAB was used? Do some internet search to understand why applying color deconvolution on DAB signal can be more challenging.**

Applying color separation techniques to DAB staining poses difficulties and can lead to a narrower range of color variations compared to HE staining. One drawback of DAB is its slow reaction with hydrogen peroxide, even in the absence of a peroxidase enzyme. Consequently, when DAB is combined with hydrogen peroxide, it should be used promptly to prevent the formation of a brown precipitate in the solution, which could cause undesired background staining. We end up with less contrast between the Dab and the background (even if we use hematoxylin for counter-staining) in comparison with the HE making the procedure more challenging.

## References

1. DAUDI, A., AND O'BRIEN, J. Detection of hydrogen peroxide by DAB staining in arabidopsis leaves. *BIO-PROTOCOL* 2, 18 (2012).
2. RUIFROK, A. C., AND JOHNSTON, D. A. Quantification of histochemical staining by color deconvolution. *Anal Quant Cytol Histol* 23, 4 (Aug 2001), 291–299.