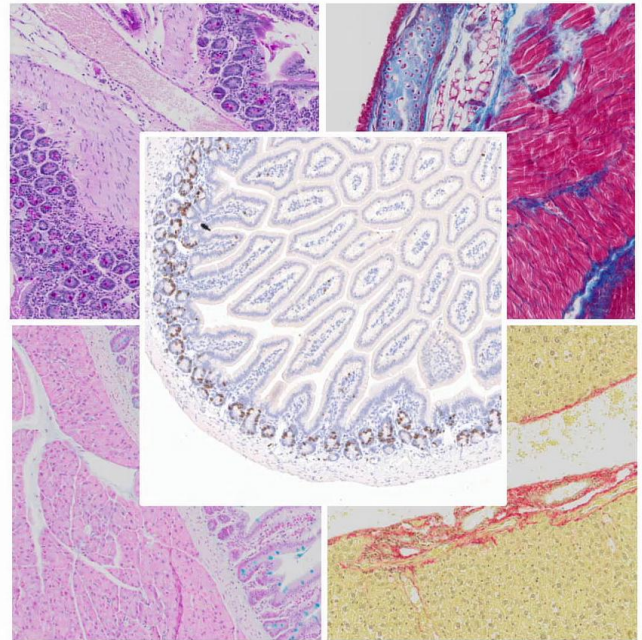


3,3'-Diaminobenzidine (DAB)



Histochemistry and immunohistochemistry stainings are very popular, and widely used to analyse and understand complex biological processes. DAB is one of these very popular staining. And in this video, we will discuss about some of its limitations.

Notes

Summary



0m 05s



- Absorption & Scattering
- Beer–Lambert–Bouguer law
- Quantifying DAB

To start we'll talk a bit about the principle of absorption and scattering of light. And the implication about the Beer-Lambert law. Finally, we'll see the implication for DAB quantification.

Notes

Summary

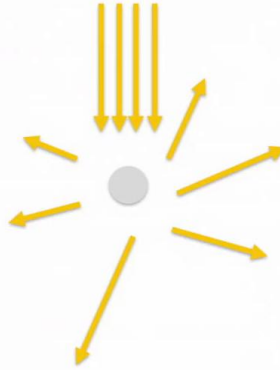


0m 23s

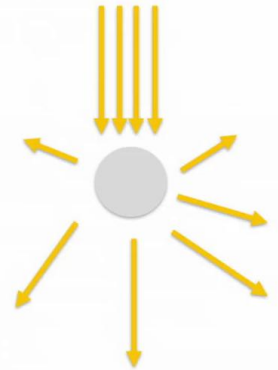
Scattering



Scattering



Scattering



Different Size = Different Effect

Let's consider some material with light going through it. A drop may have some molecules that absorb light. And, as a consequence the drop looks darker. With more molecules more light is absorbed. And the drop looks darker and darker. You might be familiar with the Beer-Lambert law that relates the attenuation of light to the properties of the material through which the light is traveling. The Beer-Lambert law correlates the absorbance of light to both the concentration of the attenuating species as well as the thickness of the material sample. Now what is scattering? It is a totally different process where the path of light is changed by the molecules. And another fact about scattering is that size matters and induces different effects.

Notes

Summary

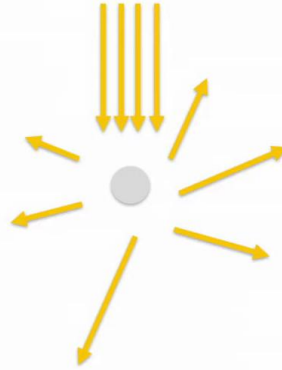


0m 38s

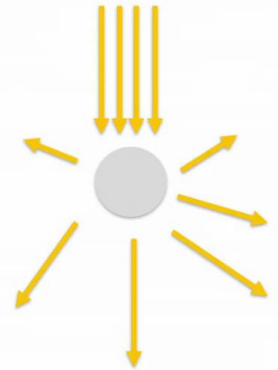
Scattering



Scattering



Scattering



Different Size = Different Effect

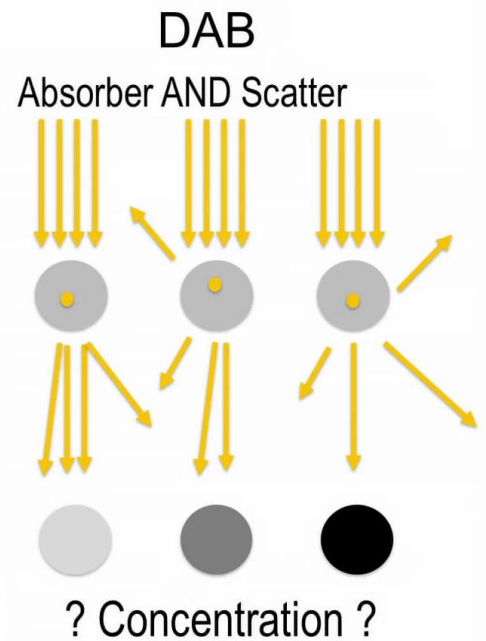
If you need to be convinced, think about waterdrops that induce very different visual effects in clouds and rainbows. In both cases we see waterdrops of different sizes scatter the light differently.

Notes

Summary



Consequence with DAB



As a consequence, in the case of DAB, which is an absorber and a scatterer, there is no relationship between the quantity of DAB and the darkness.

Notes

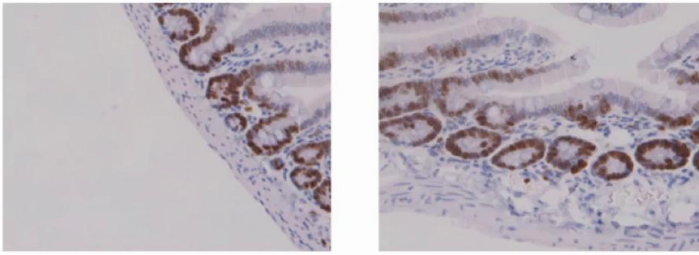
Summary



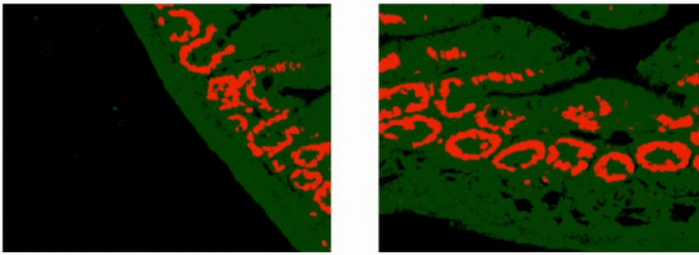
1m 48s

Quantifying DAB

Input



Output



So is it still possible to make some quantification from samples staining with DAB? The answer is yes! What we recommend you to do is to segment the DAB (Here the brown in the input image, as the red in the output image) and to segment the tissue area. Here the hematoxylin in blue (green in the output image).

Notes

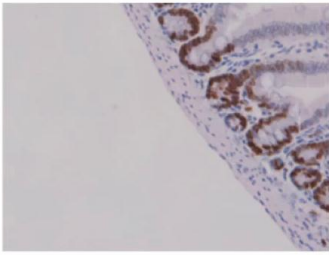
Summary



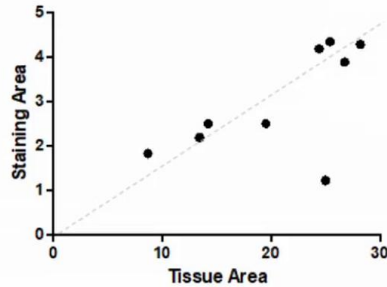
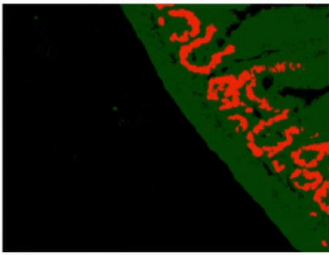
2m 01s

Quantifying DAB

Input

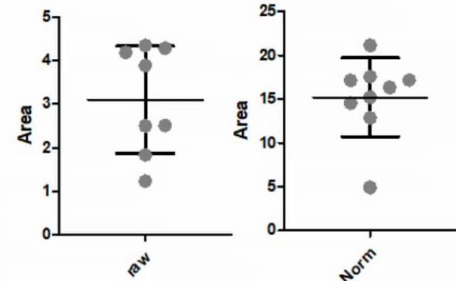


Output



The more Tissue Area analysed
The more DAB Area measured

$$\text{Norm Area} = \frac{\text{DAB Area}}{\text{Tissue Area}}$$



Normalisation decreases
the Deviation to the Mean

Why measure the tissue? Because there is a relationship between the DAB area and the tissue area. It makes sense, right? The more tissue there is the field of view, the more chances you have to get some DAB also. So if we measure both DAB and tissue areas, we can normalize the data. Which will help to reduce the deviation to the mean.

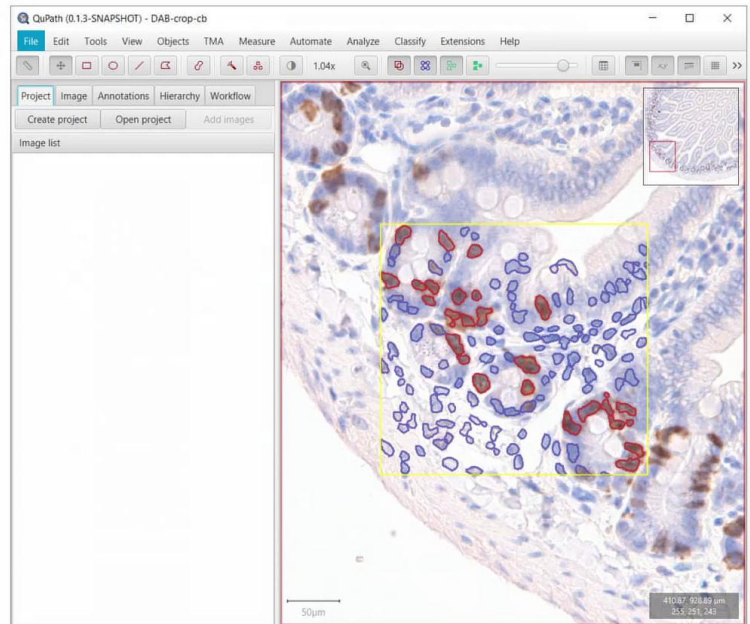
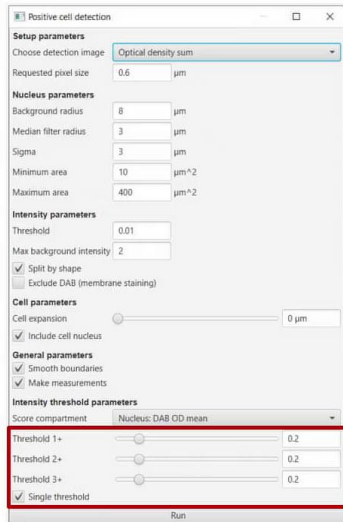
Notes

Summary



2m 27s

Quantifying DAB : Using QuPath



This is what we consider the safest way, meaning the least biased, to quantify your DAB. If you have to analyse whole slide images and you can't down scale your image, an option would be to use QuPath. It allows you to draw annotations (here's a yellow square), and then to detect the cell inside. And to define positive and negative cells you can use a threshold value. Regarding what we said earlier we recommend you to use only one threshold value to define either positive and negative cells. But please keep in mind that detecting individual cell is not an easy task. You may introduce bias if cells are clumped in different extents in different areas of your images. That's why we recommend you to prefer measuring area than positive cell numbers.

Notes

Summary



Conclusion



- Intensity of DAB is not so relevant
- Measuring DAB Area
(normalizing it using Tissue Area)

Ok! That's it for this video about quantifying DAB. Keep in mind: measuring area is safe. And normalizing it using tissue area is even better. Bye!

Notes

Summary



3m 49s