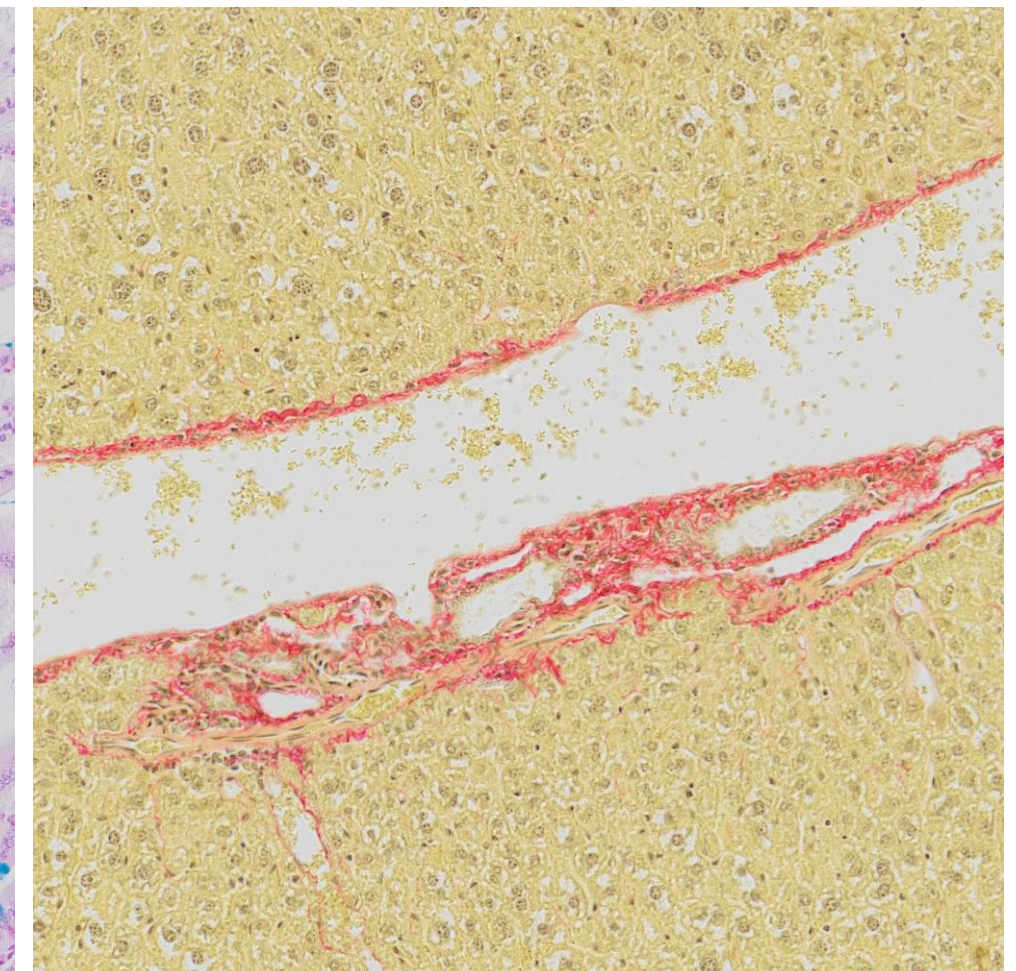
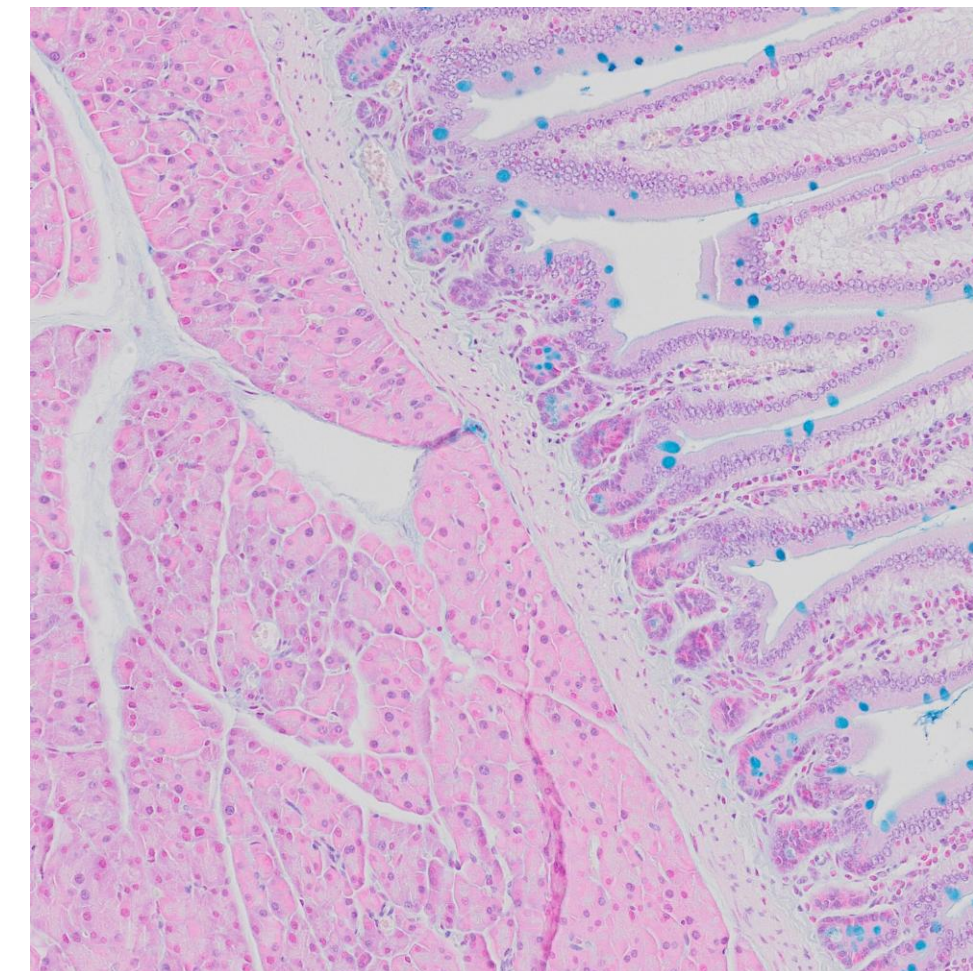
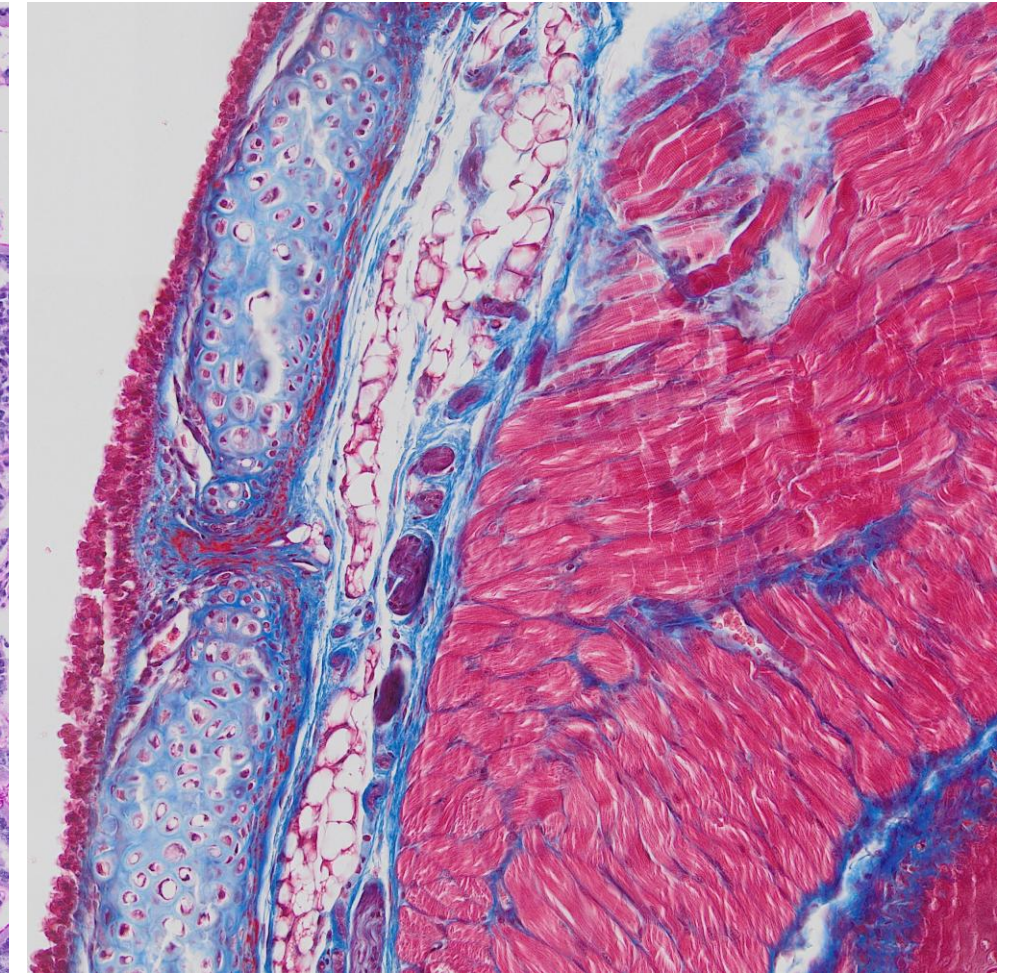
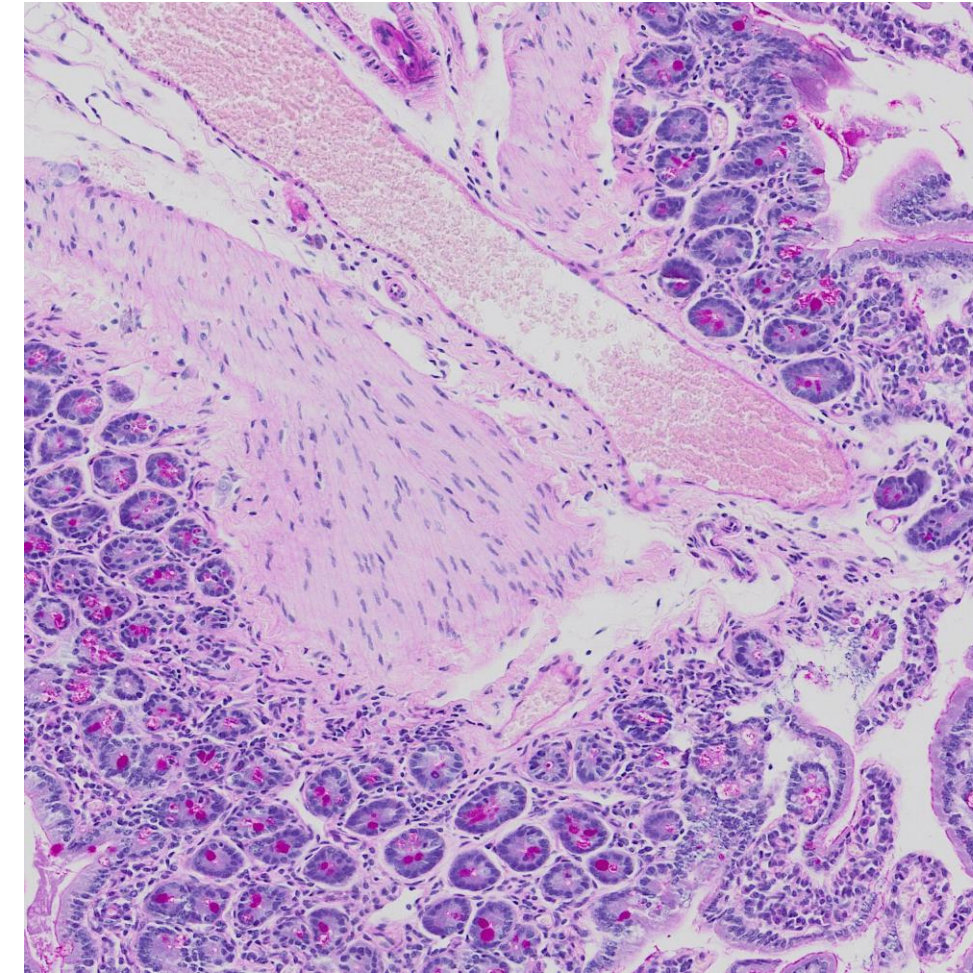


Quantifying DAB

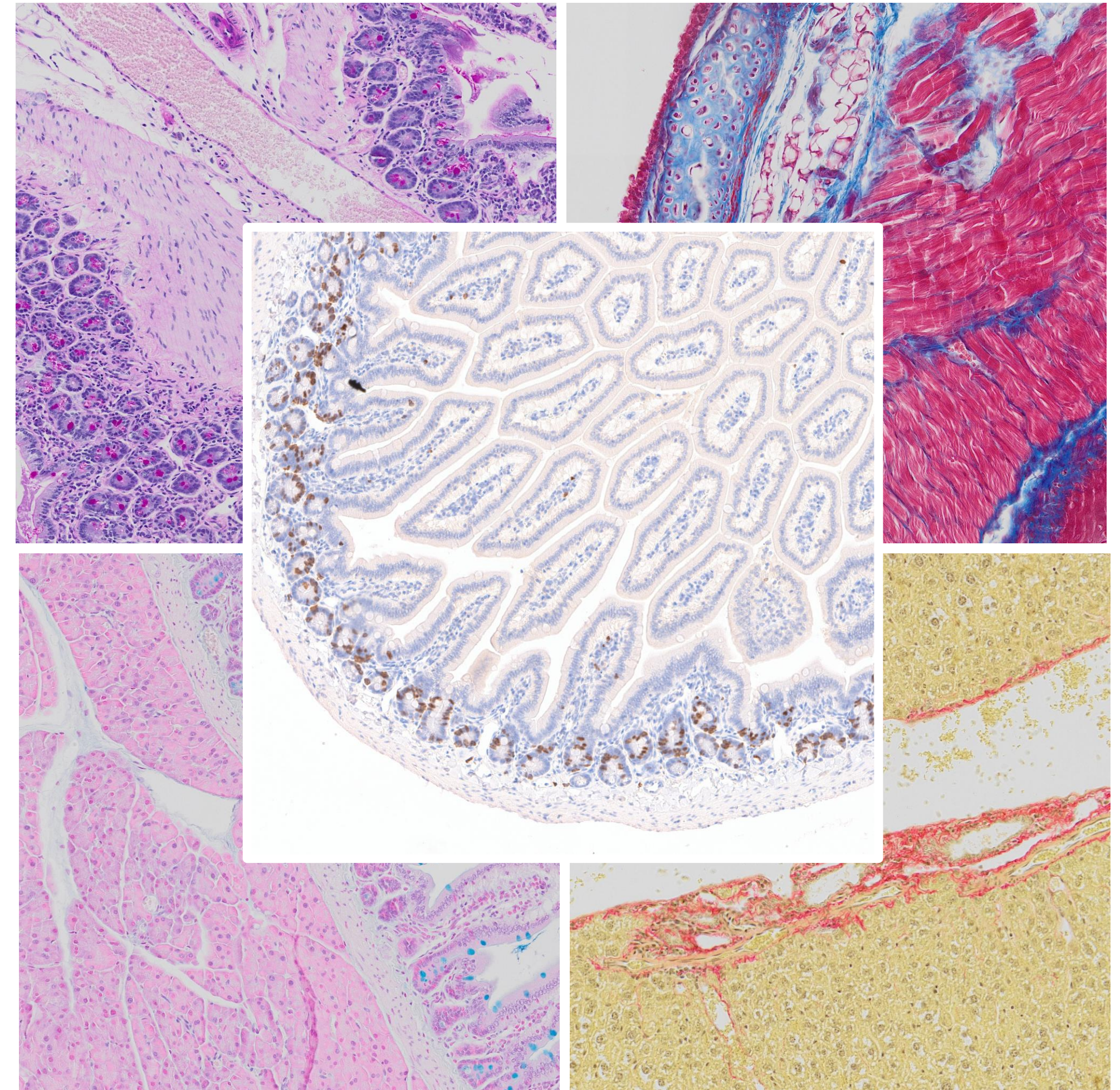
Image Processing & Analysis for Life Scientists

Olivier Burri, Romain Guiet & Arne Seitz

(Immuno)histochemistry Staining

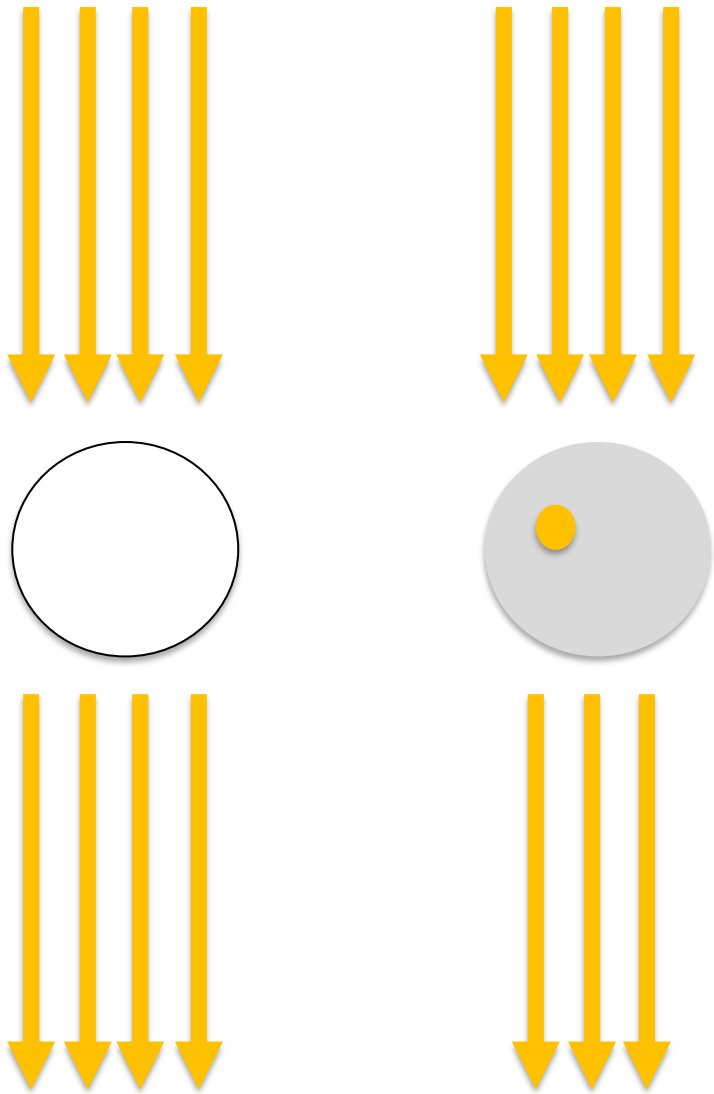


3,3'-Diaminobenzidine (DAB)



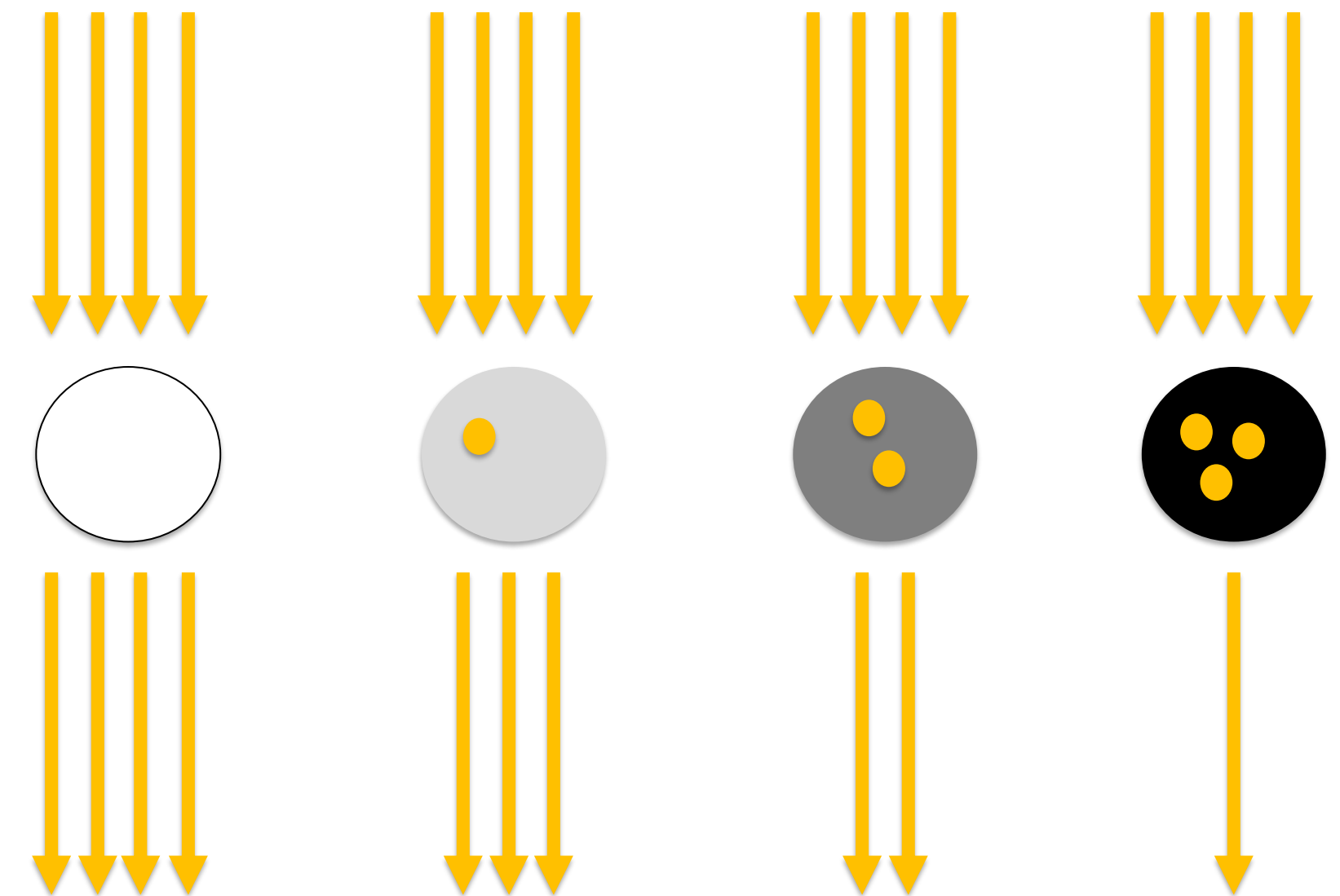
- Absorption & Scattering
- Beer–Lambert–Bouguer law
- Quantifying DAB

Absorption



Beer–Lambert–Bouguer law

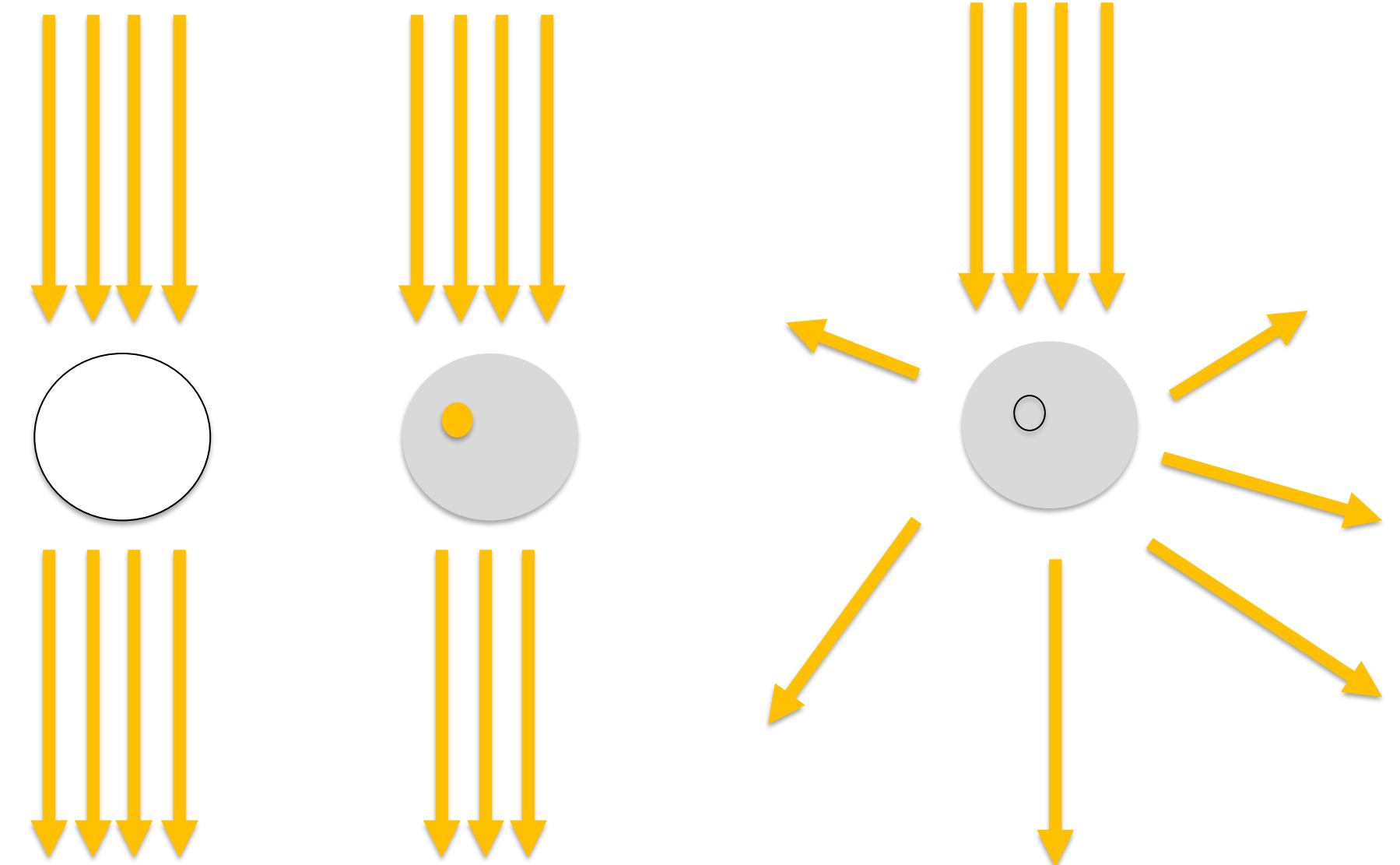
True Absorber



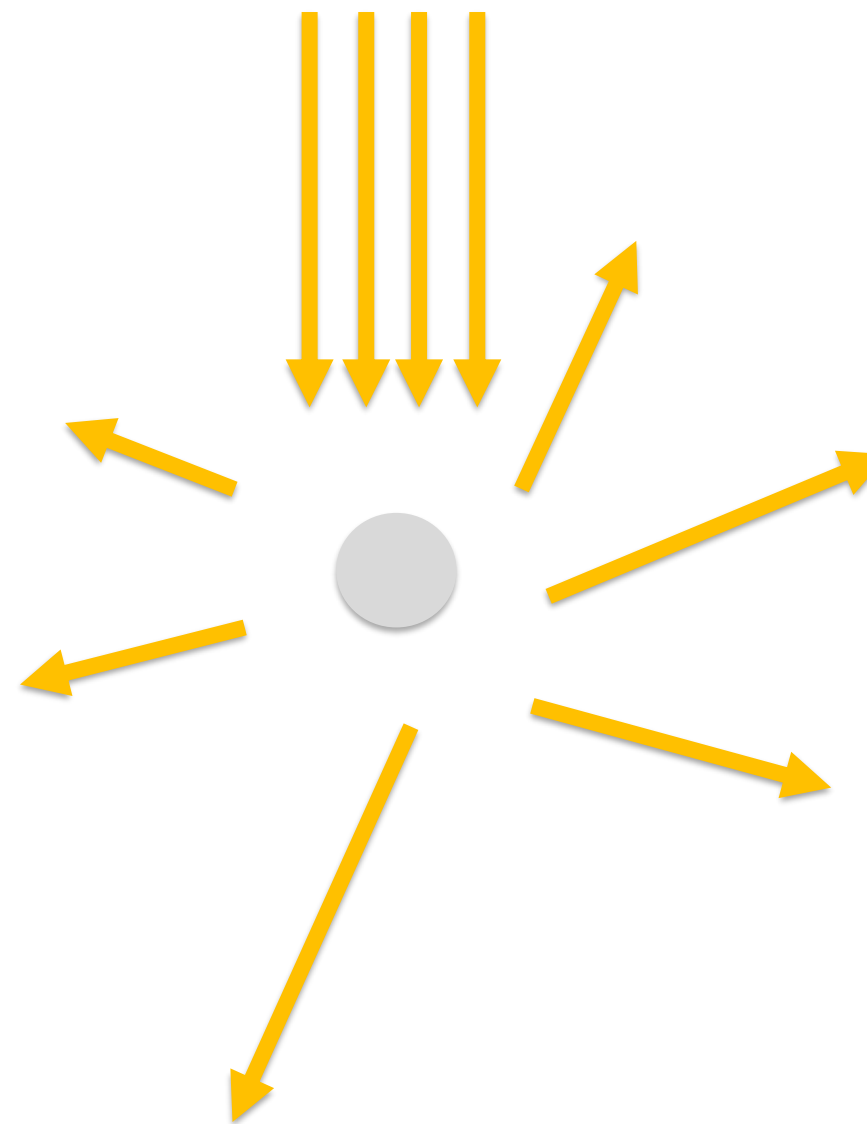
Concentration

Scattering

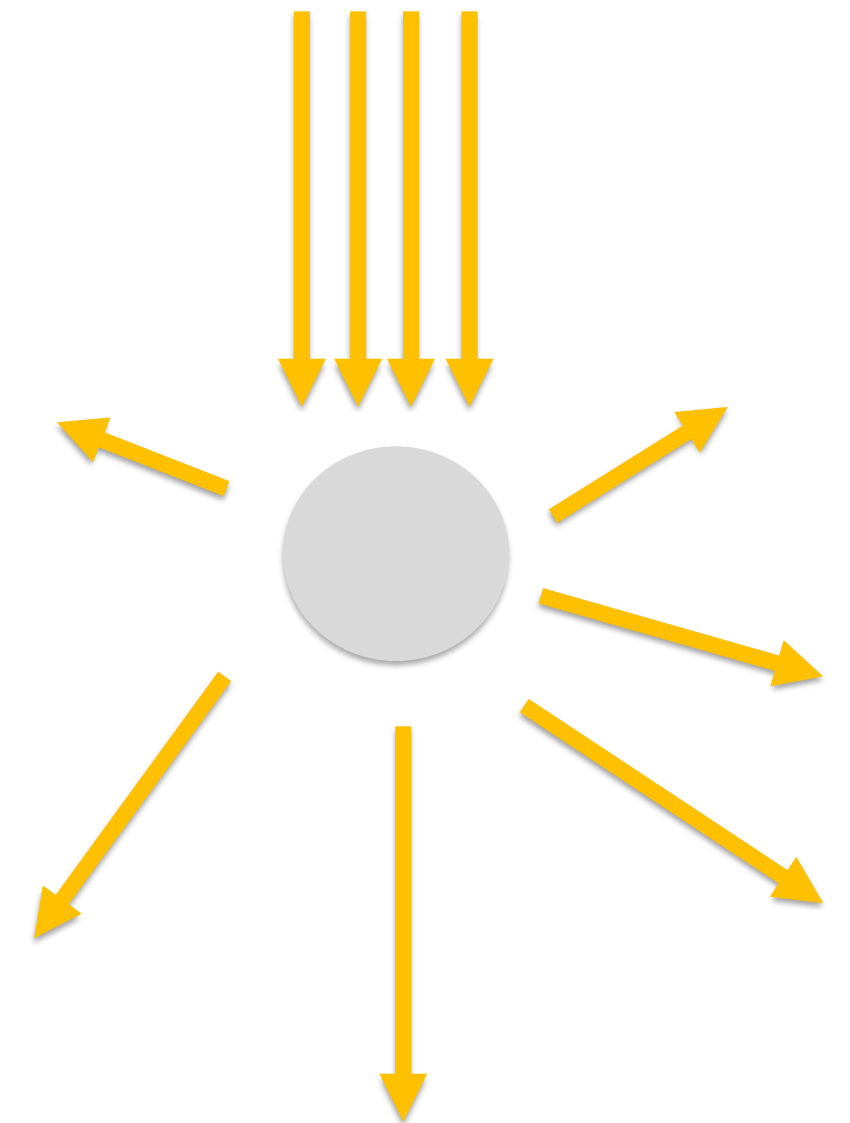
Absorption Scattering



Scattering

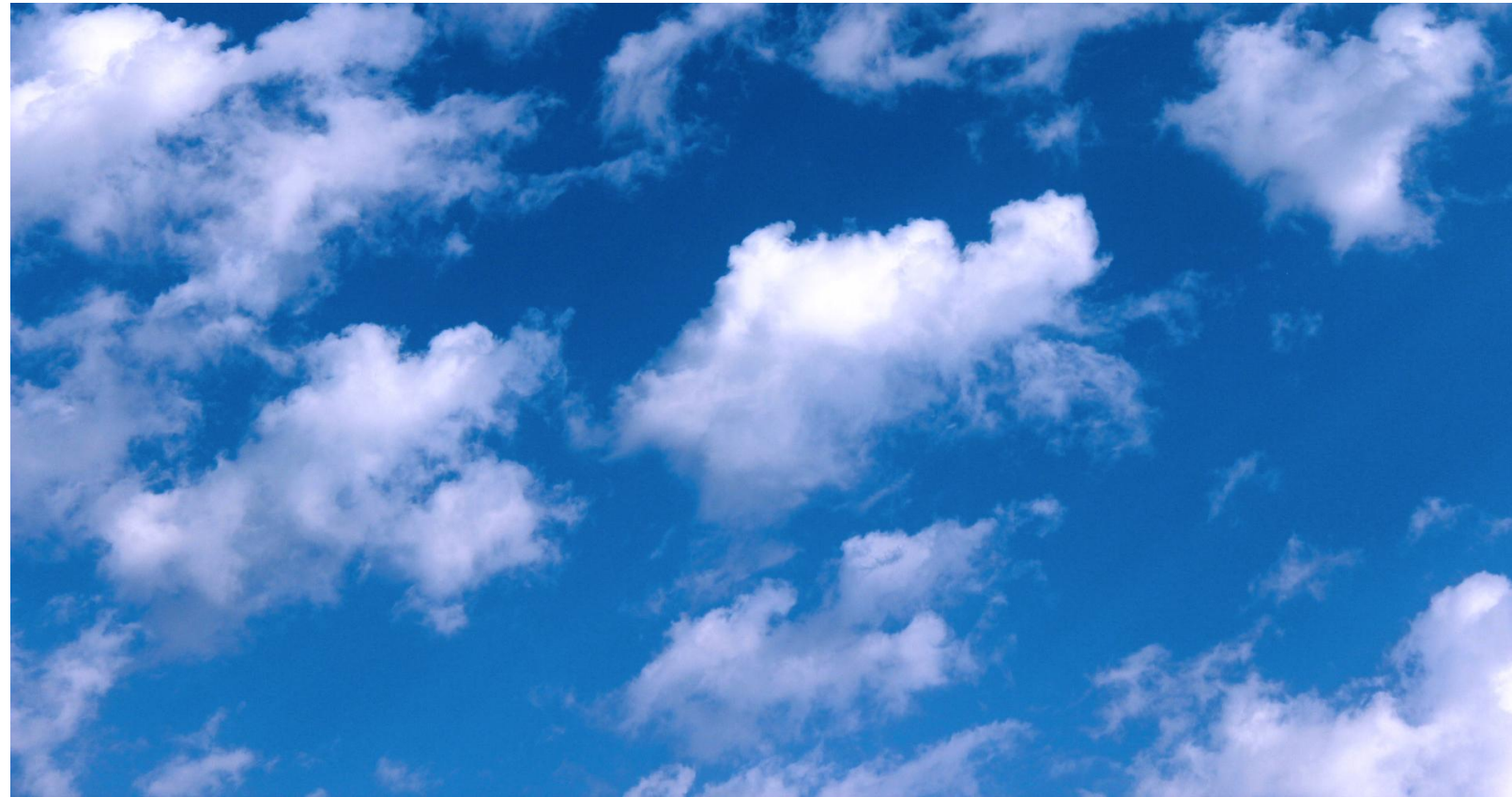


Scattering

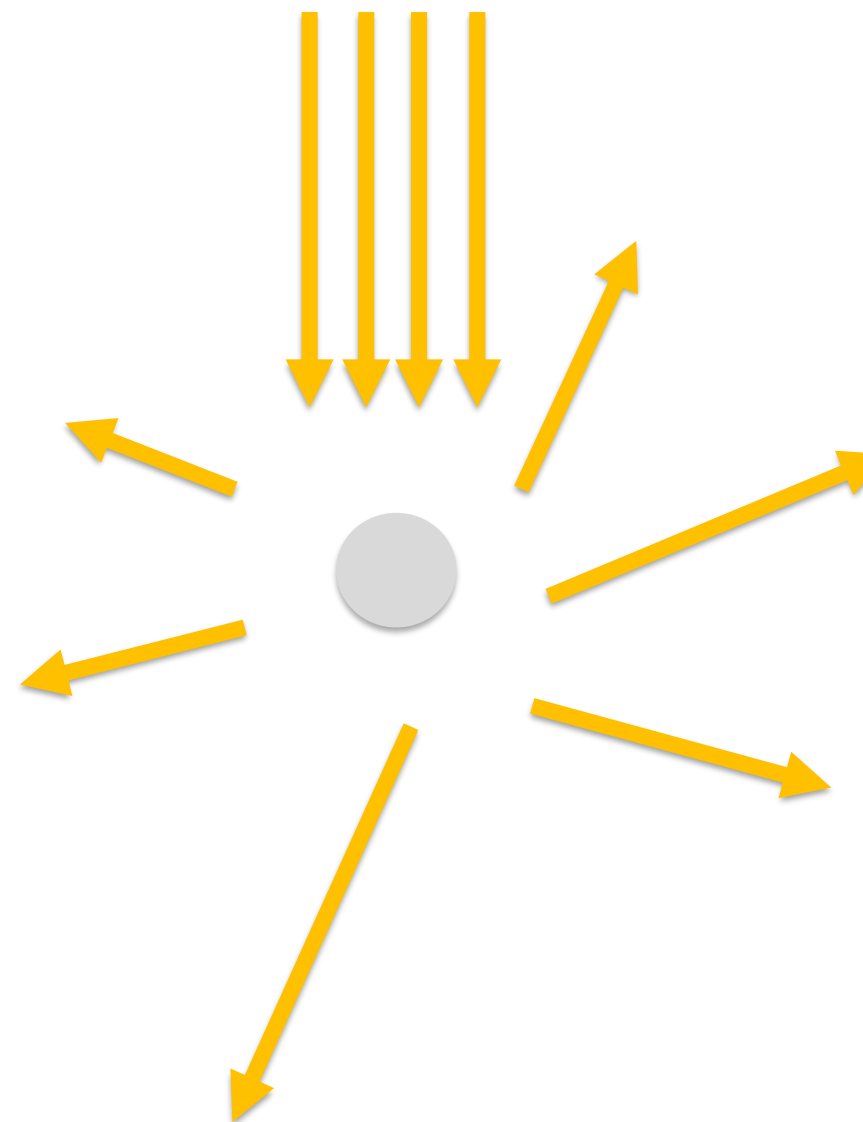


Different Size = Different Effect

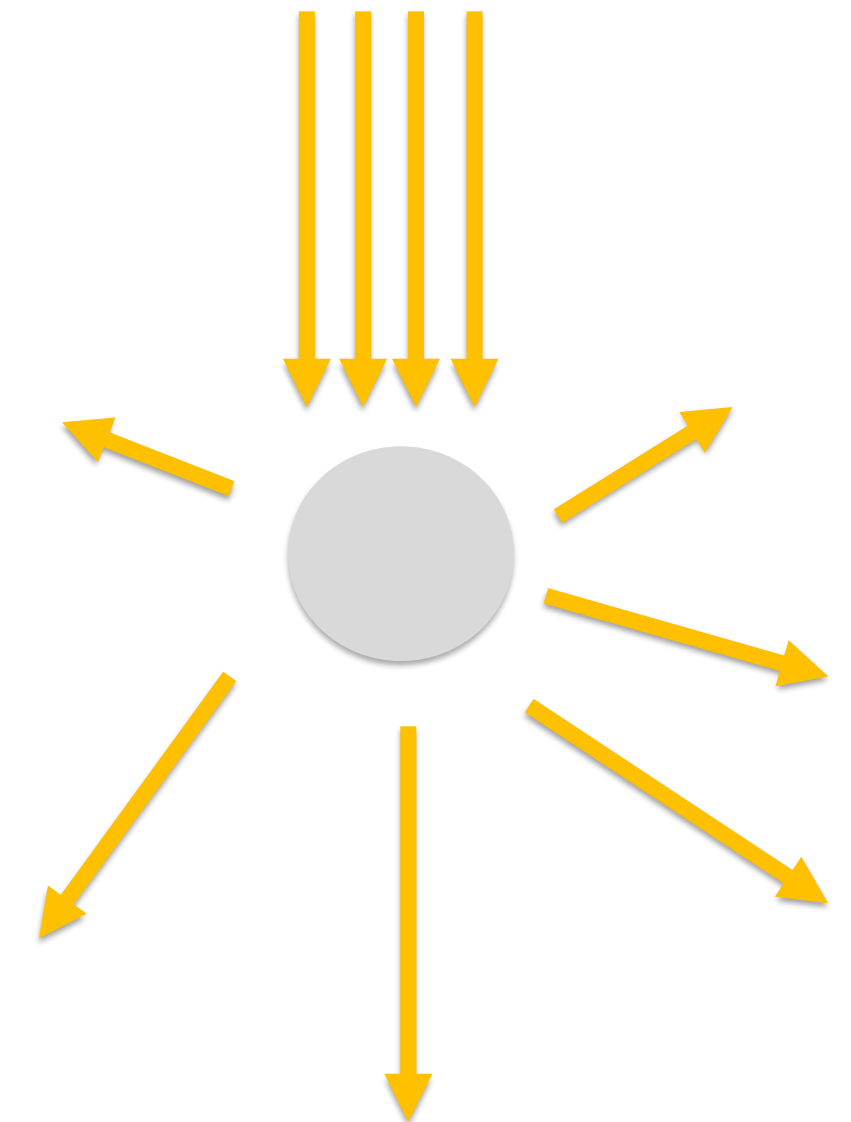
Scattering



Scattering



Scattering

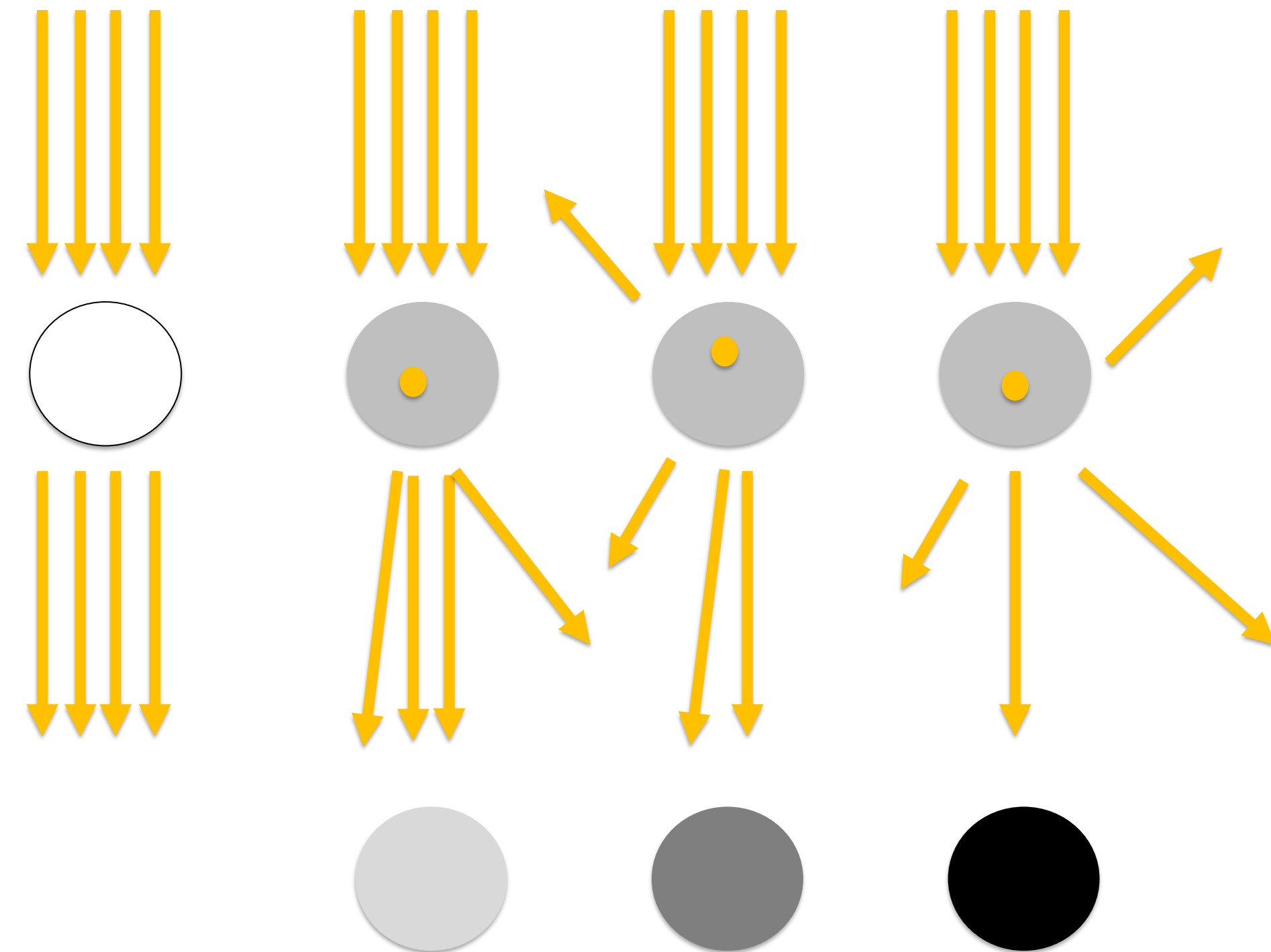


Different Size = Different Effect

Consequence with DAB

DAB

Absorber AND Scatter

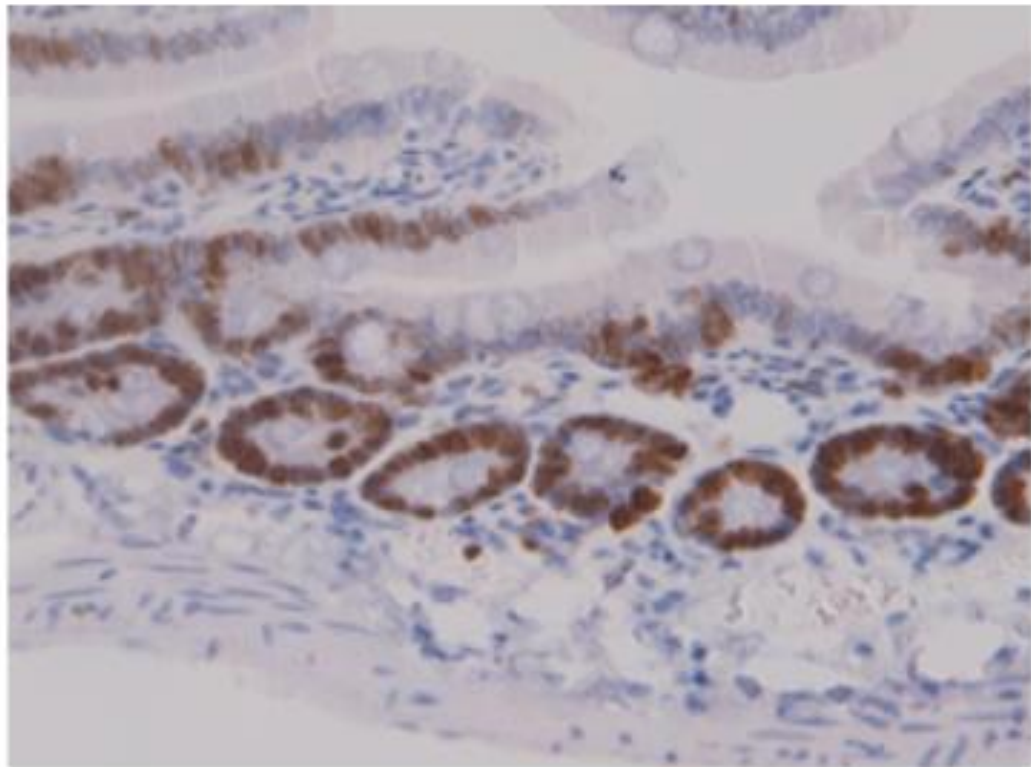
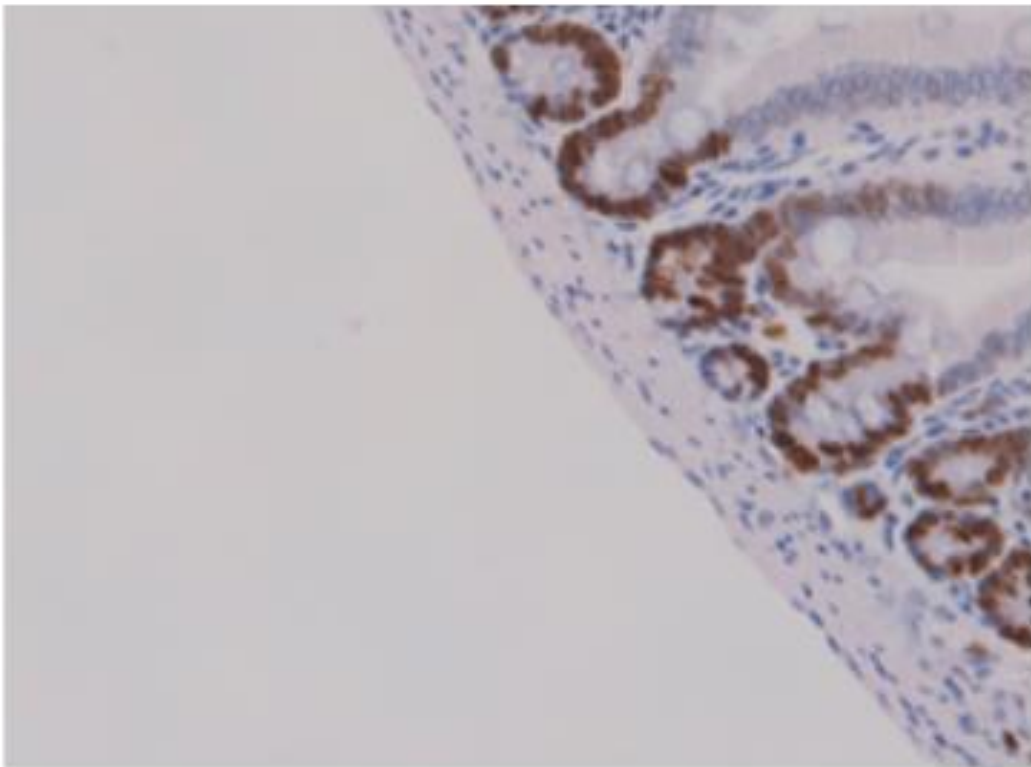


? Concentration ?

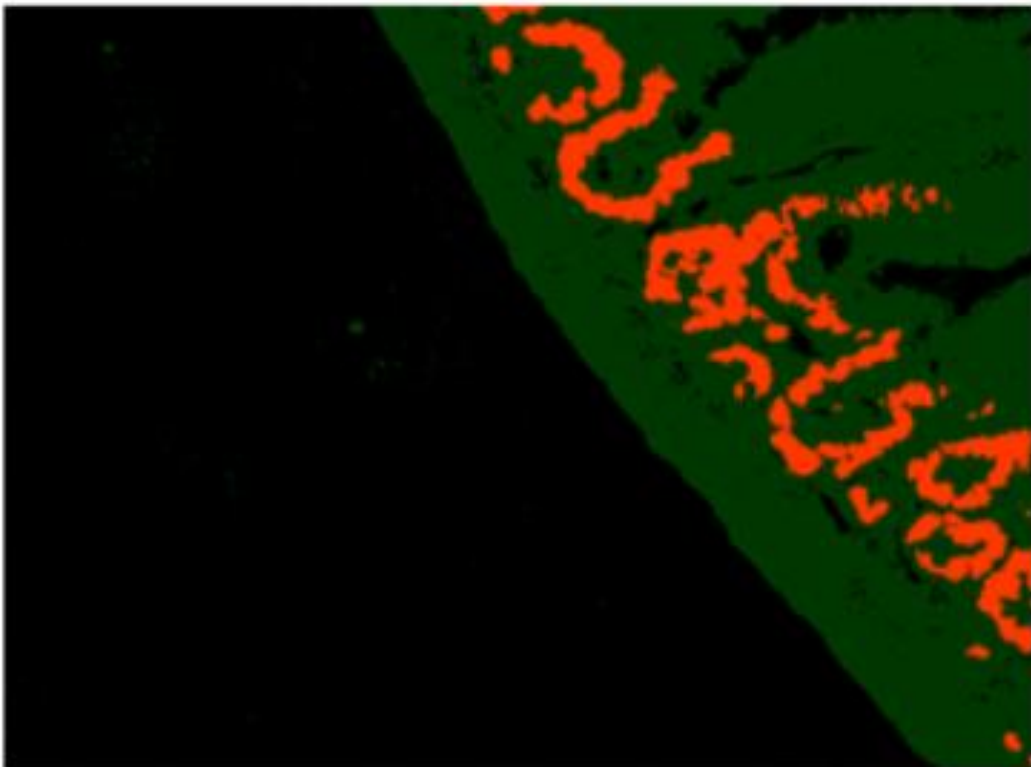
Quantifying DAB

Quantifying DAB

Input

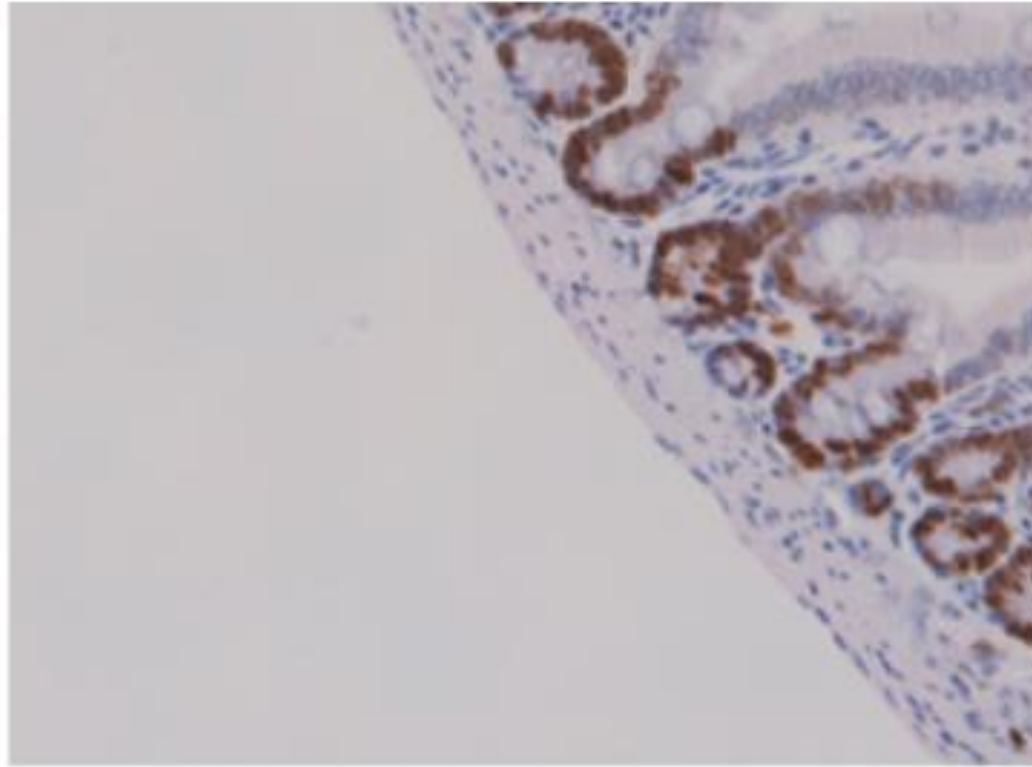


Output

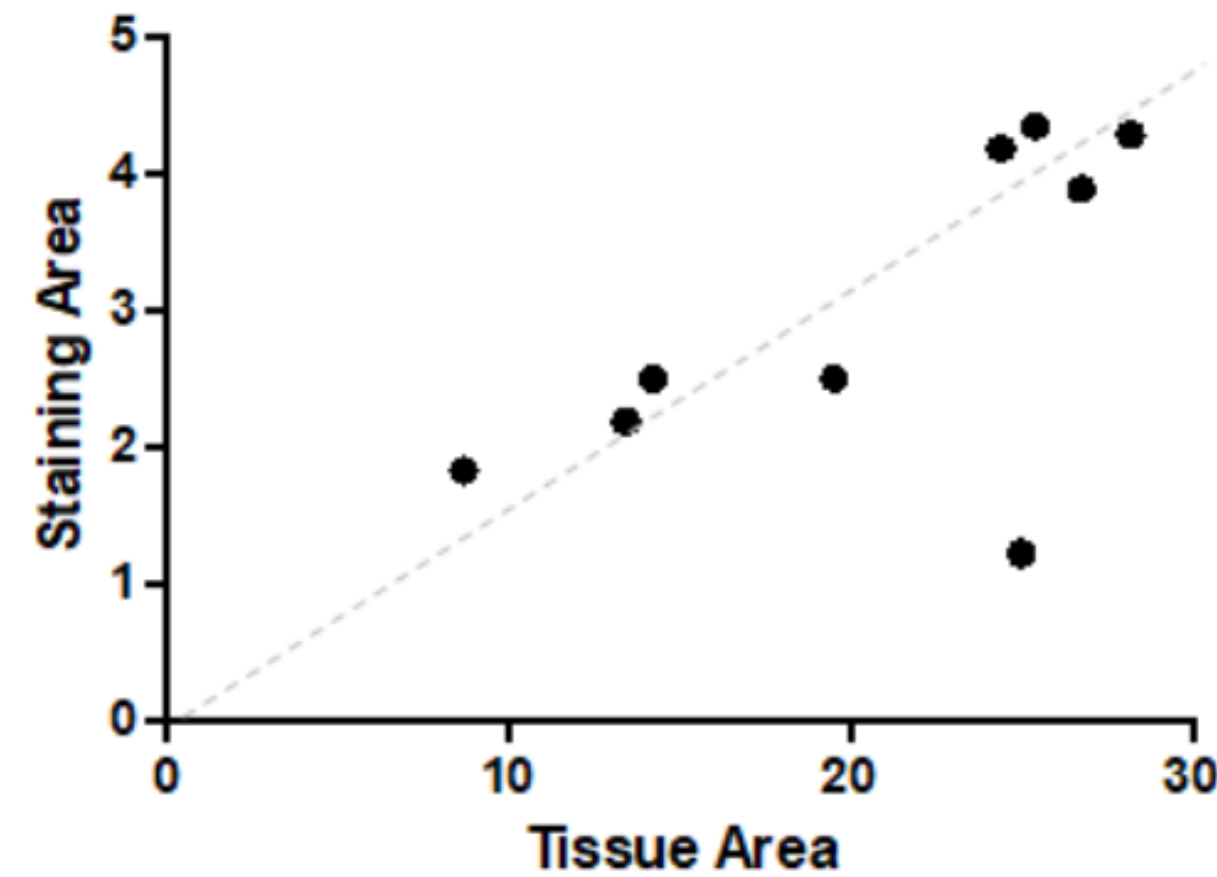
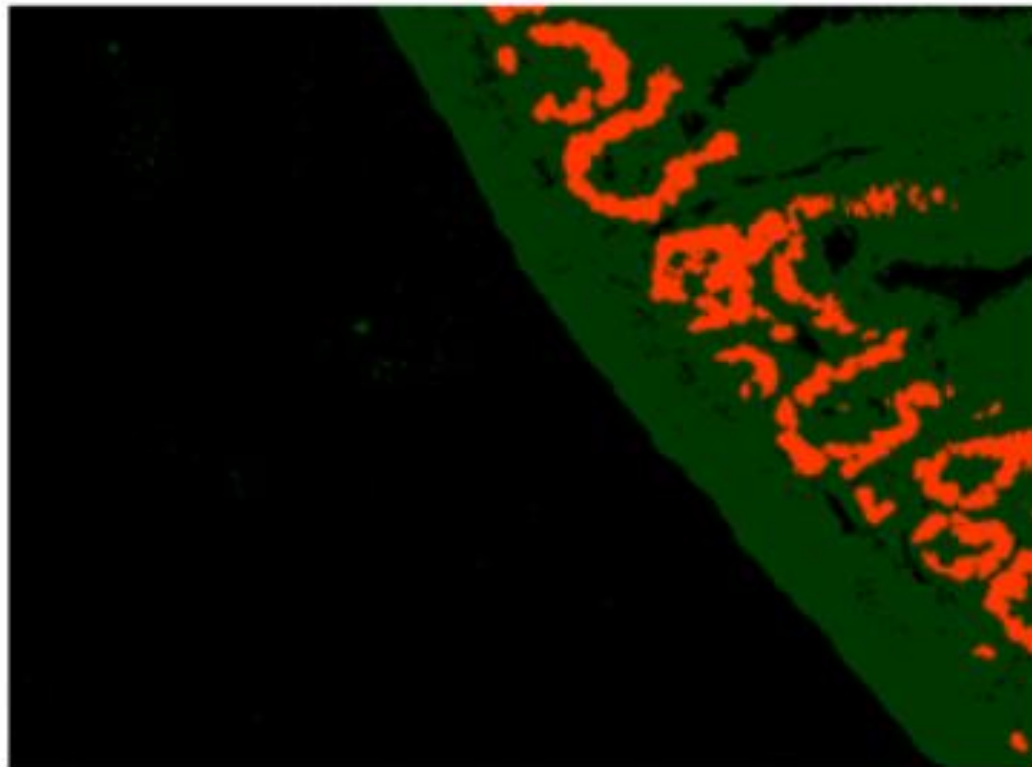


Quantifying DAB

Input



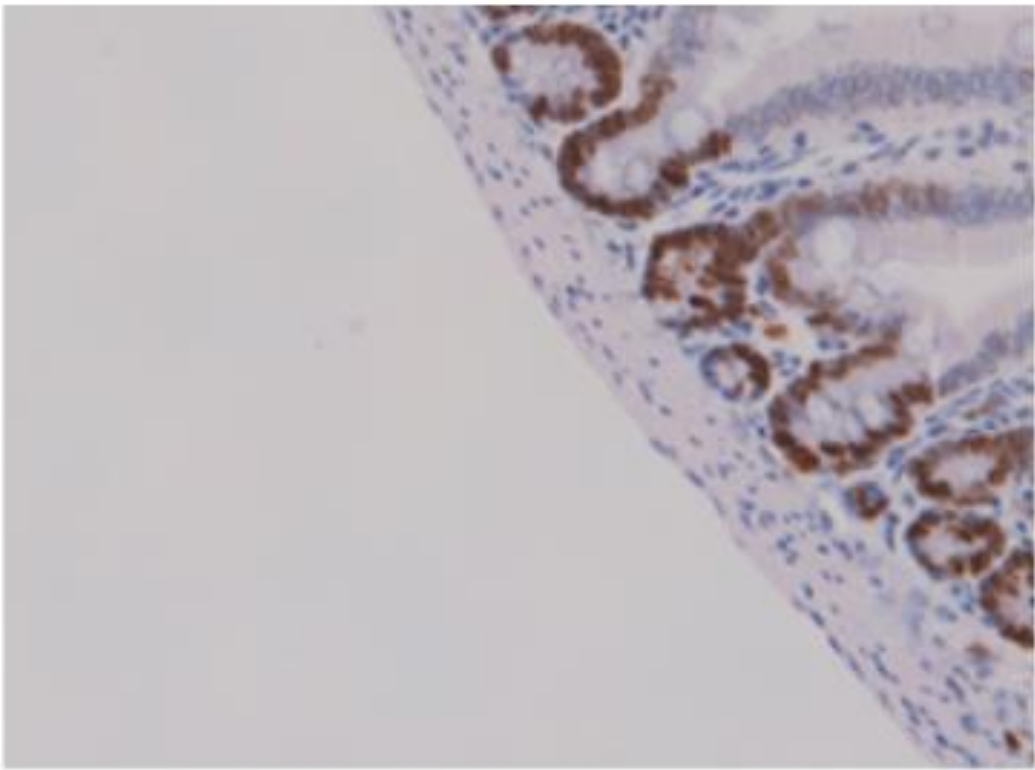
Output



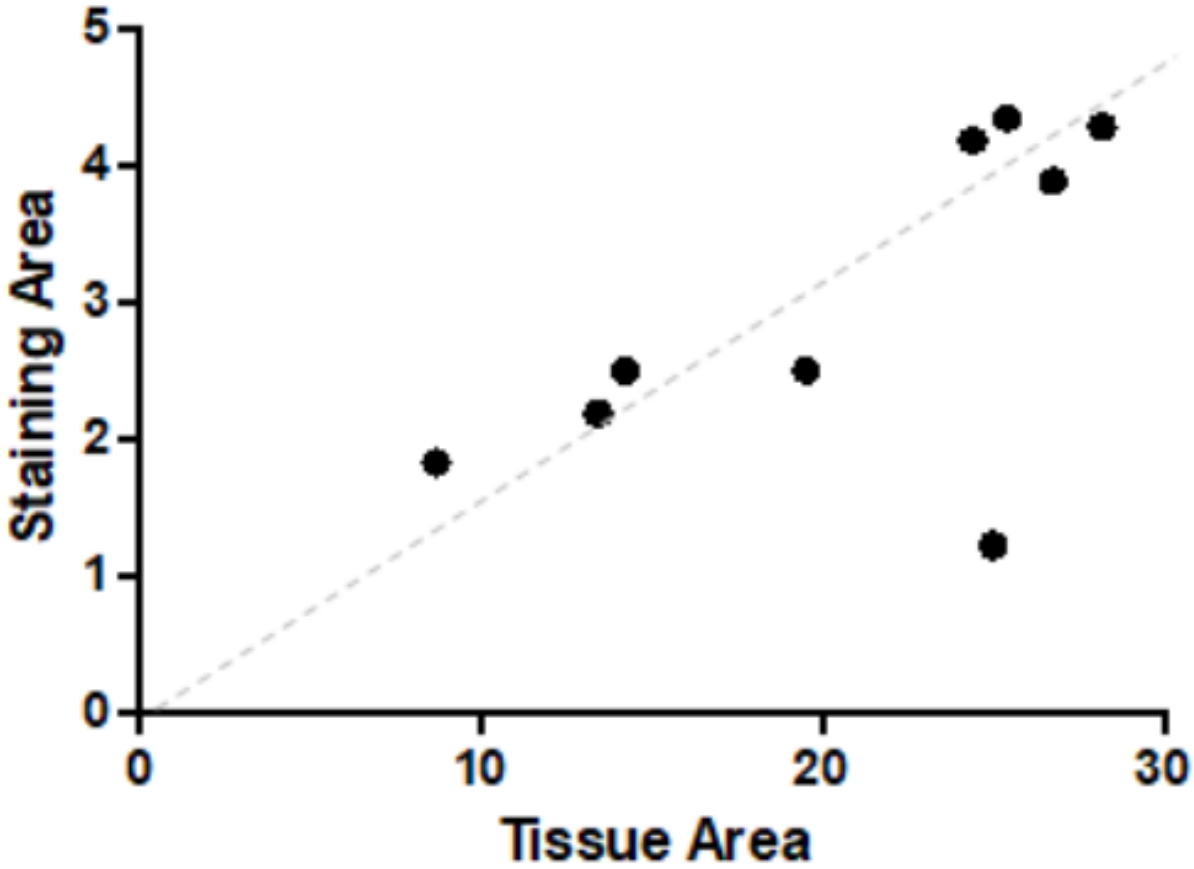
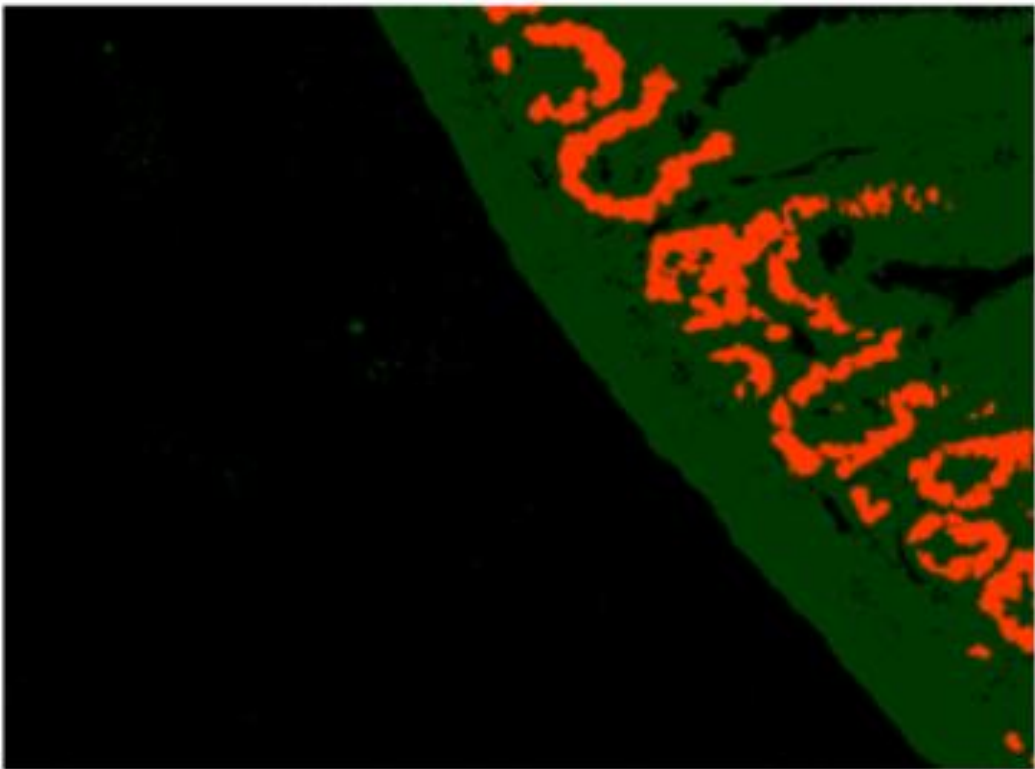
The more Tissue Area analysed
The more DAB Area measured

Quantifying DAB

Input

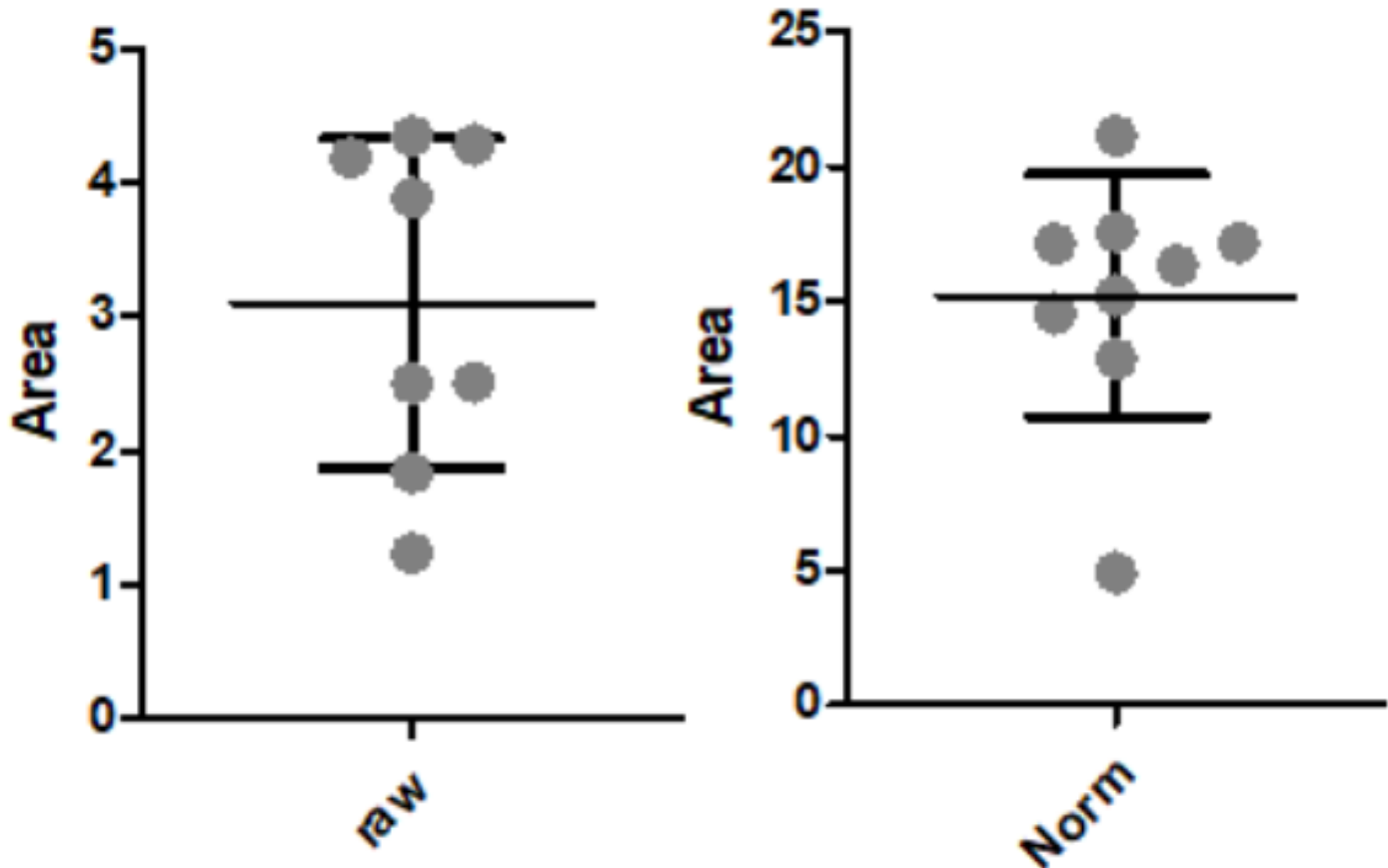


Output



The more Tissue Area analysed
The more DAB Area measured

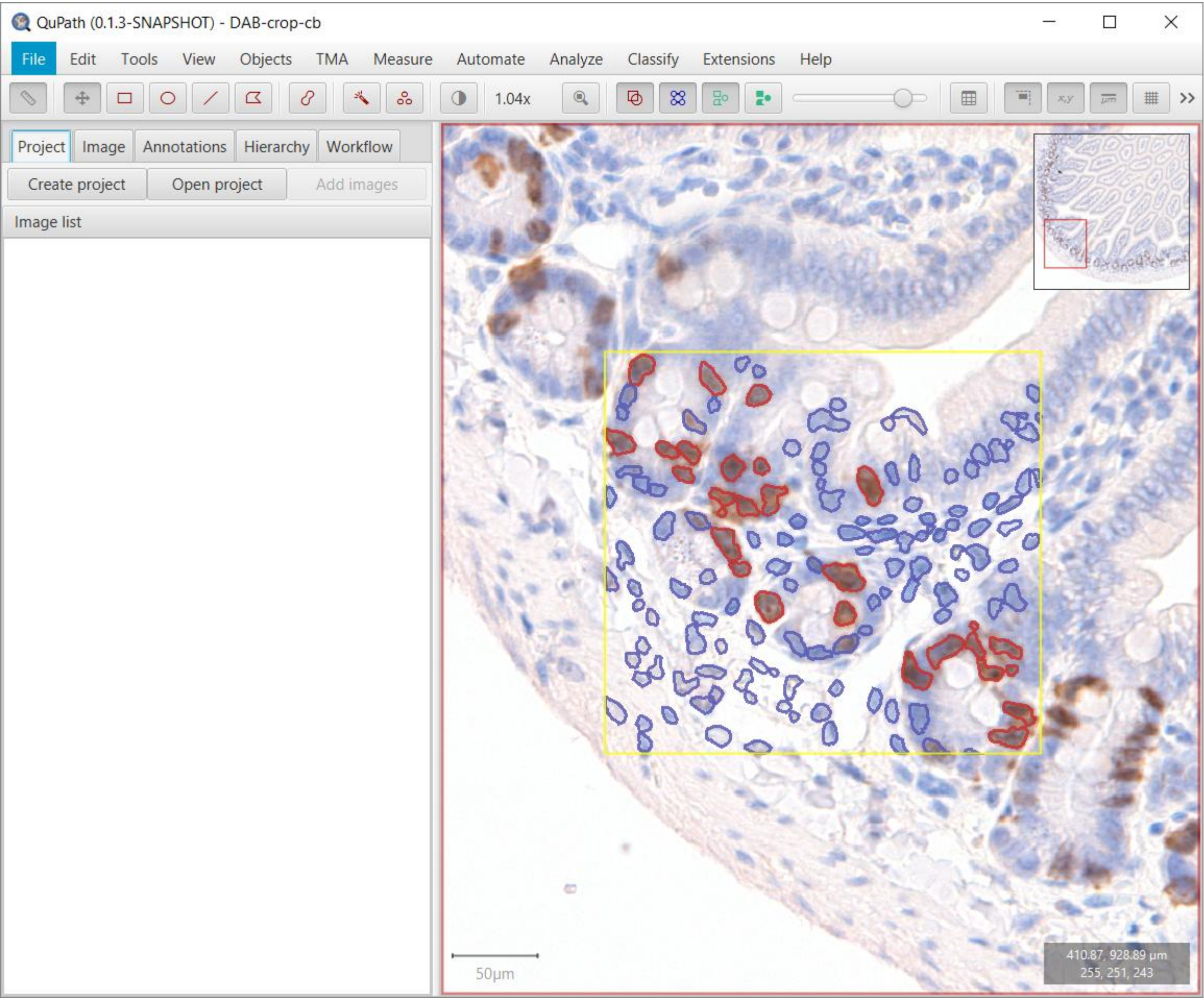
$$\text{Norm Area} = \frac{\text{DAB Area}}{\text{Tissue Area}}$$



Normalisation decreases
the Deviation to the Mean

Quantifying DAB

Quantifying DAB : Using QuPath



Quantifying DAB : Using QuPath

Positive cell detection

Setup parameters

Choose detection image: Optical density sum

Requested pixel size: 0.6 μm

Nucleus parameters

Background radius: 8 μm

Median filter radius: 3 μm

Sigma: 3 μm

Minimum area: 10 μm^2

Maximum area: 400 μm^2

Intensity parameters

Threshold: 0.01

Max background intensity: 2

☒ Split by shape

☐ Exclude DAB (membrane staining)

Cell parameters

Cell expansion: 0 μm

☒ Include cell nucleus

General parameters

☒ Smooth boundaries

☒ Make measurements

Intensity threshold parameters

Score compartment: Nucleus: DAB OD mean

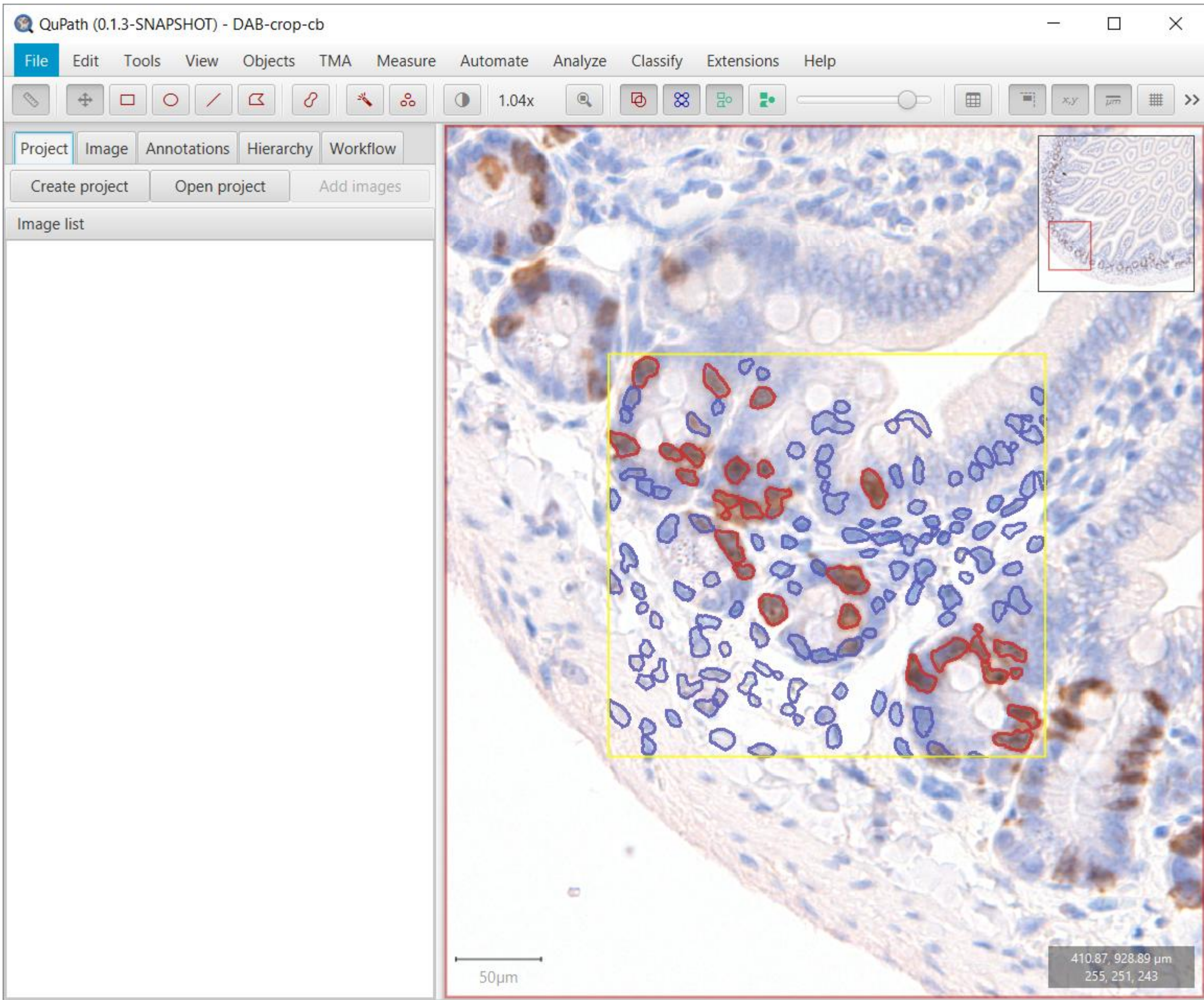
Threshold 1+: 0.2

Threshold 2+: 0.2

Threshold 3+: 0.2

☒ Single threshold

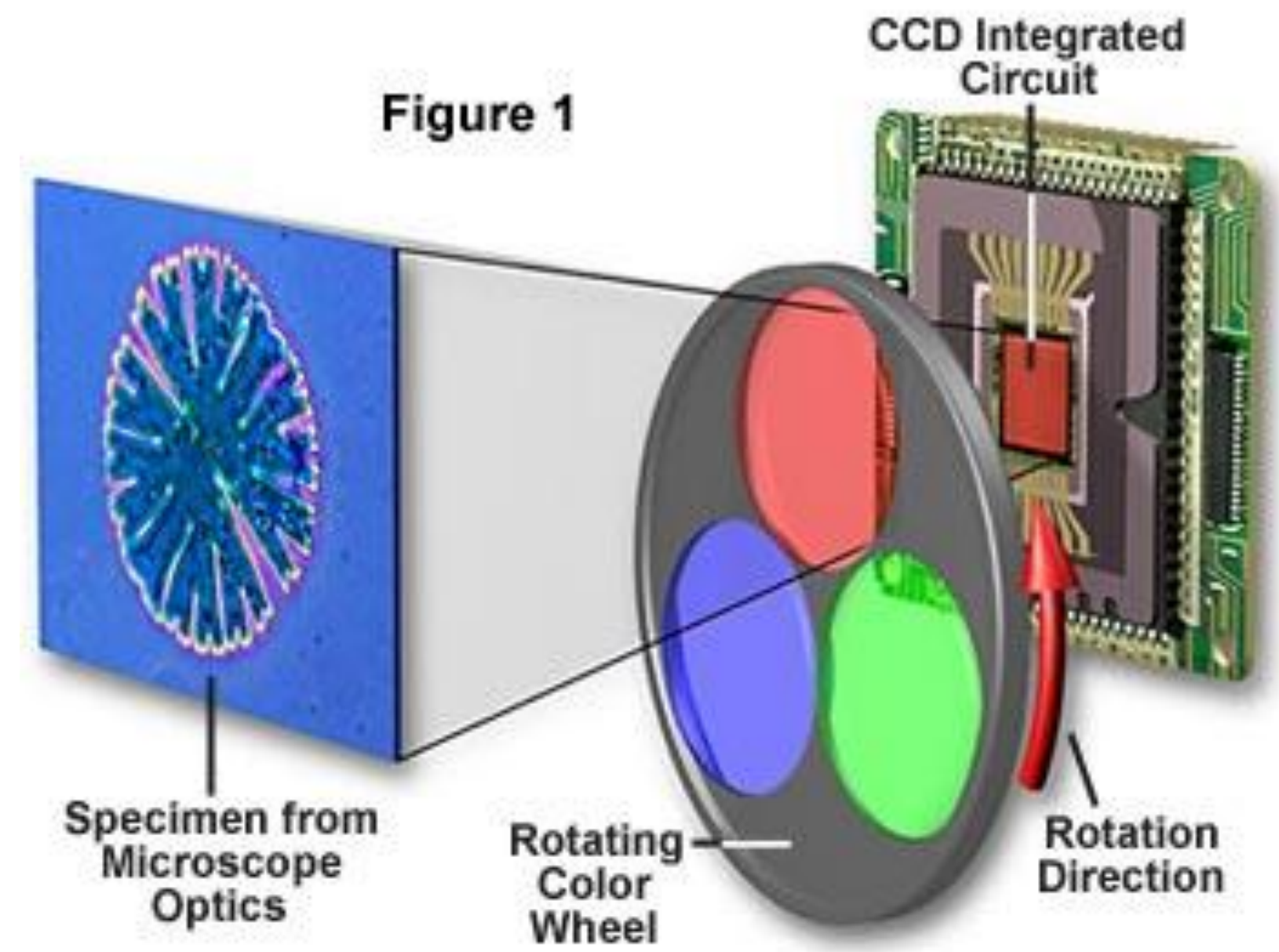
Run

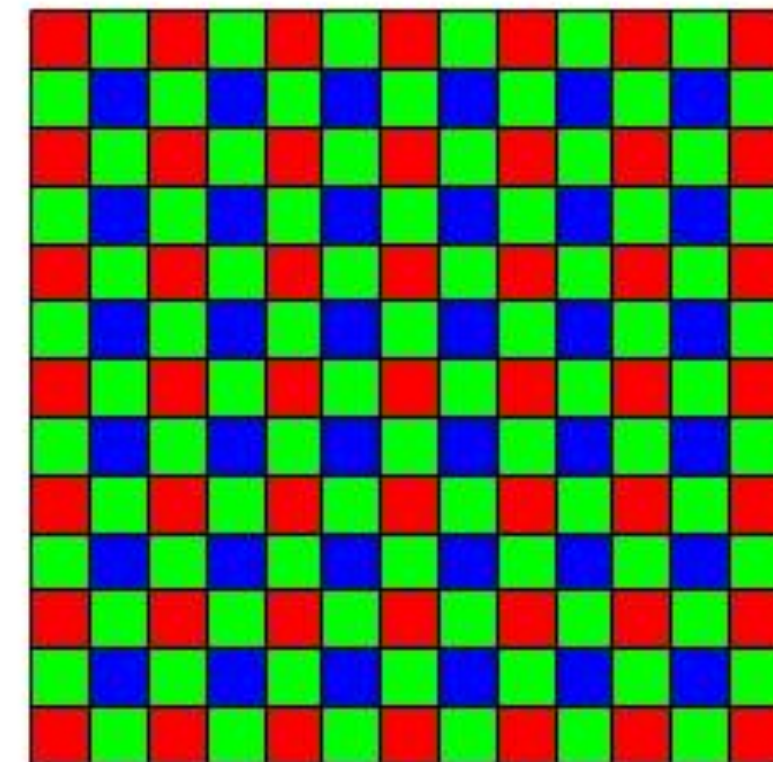


- Intensity of DAB is not so relevant
- Measuring DAB Area
(normalizing it using Tissue Area)

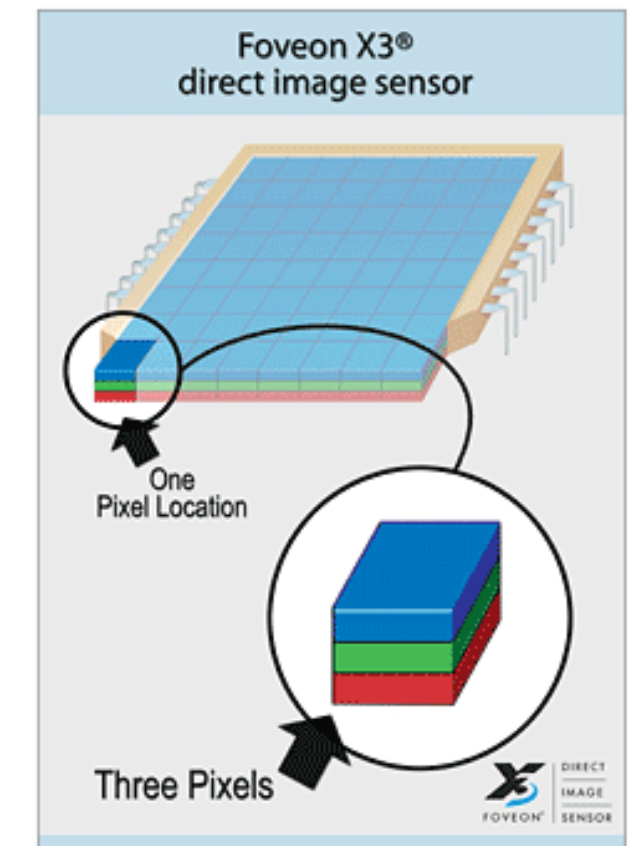
Sequential Color Three-Pass CCD Imaging System

Figure 1

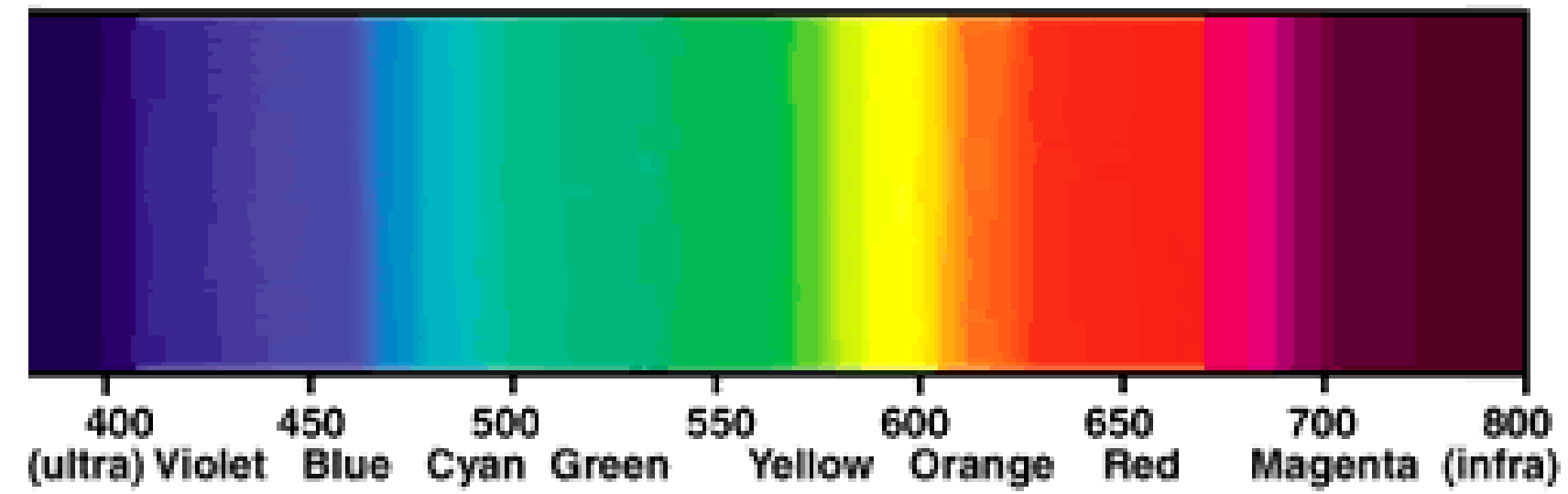




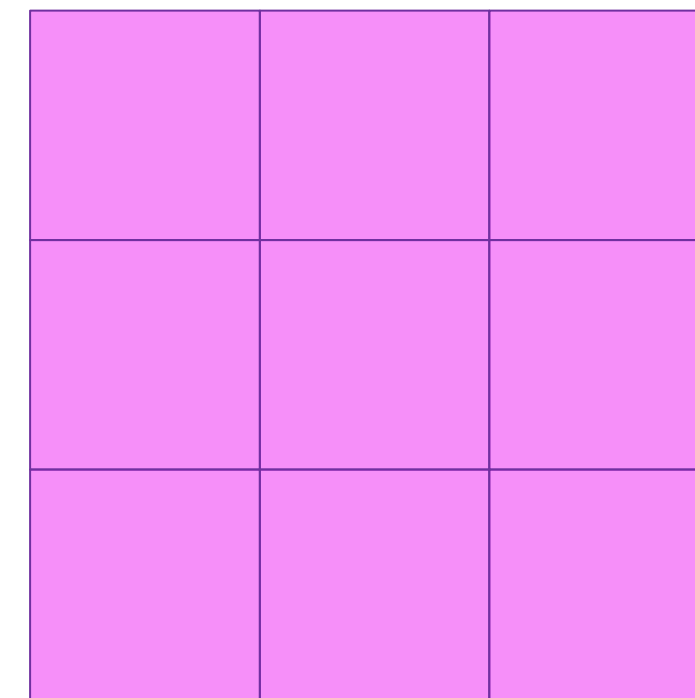
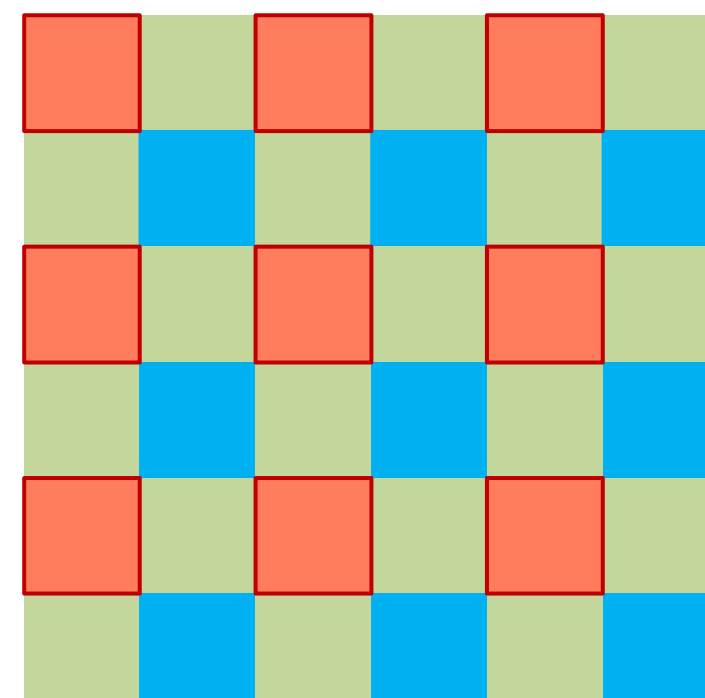
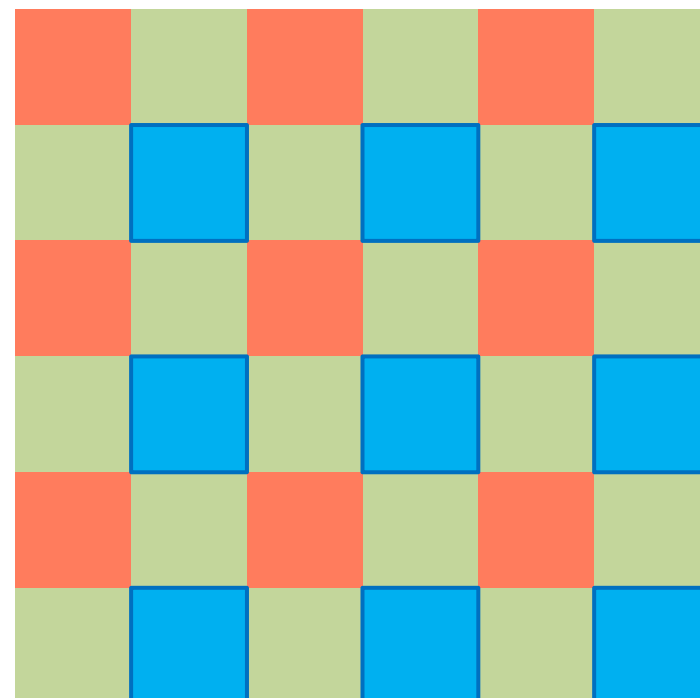
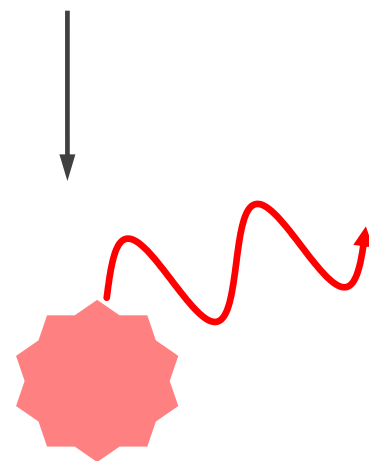
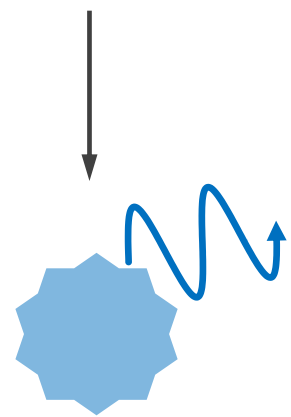
Bayer filter



THE VISIBLE SPECTRUM • Wavelength in Nanometers



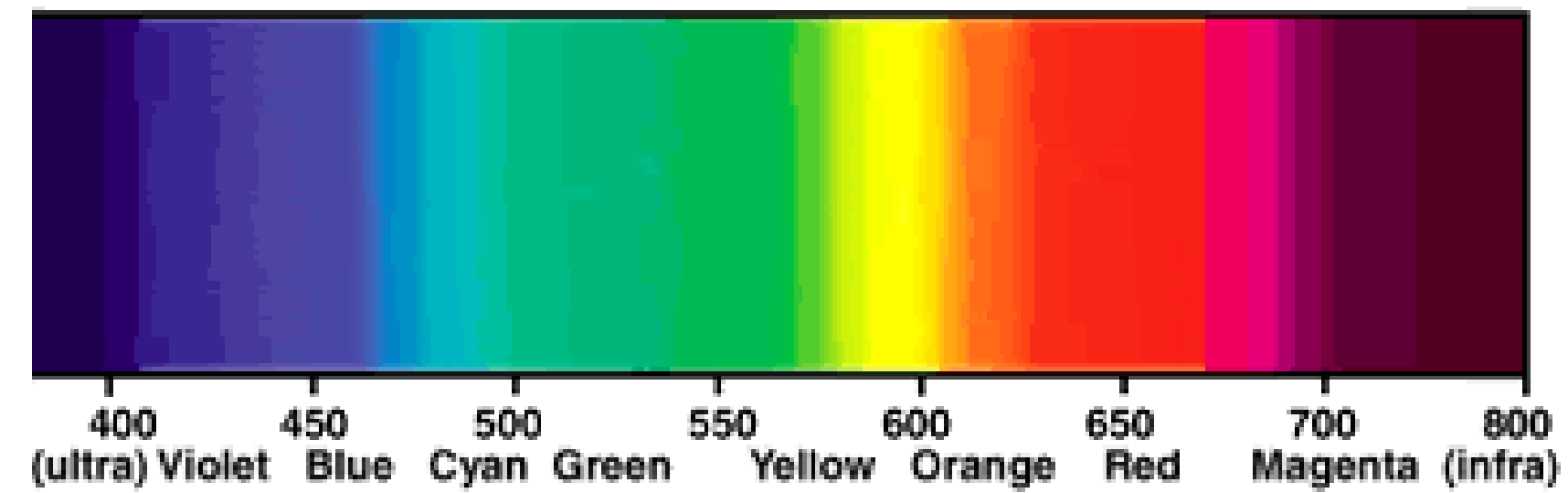
450nm + 650nm



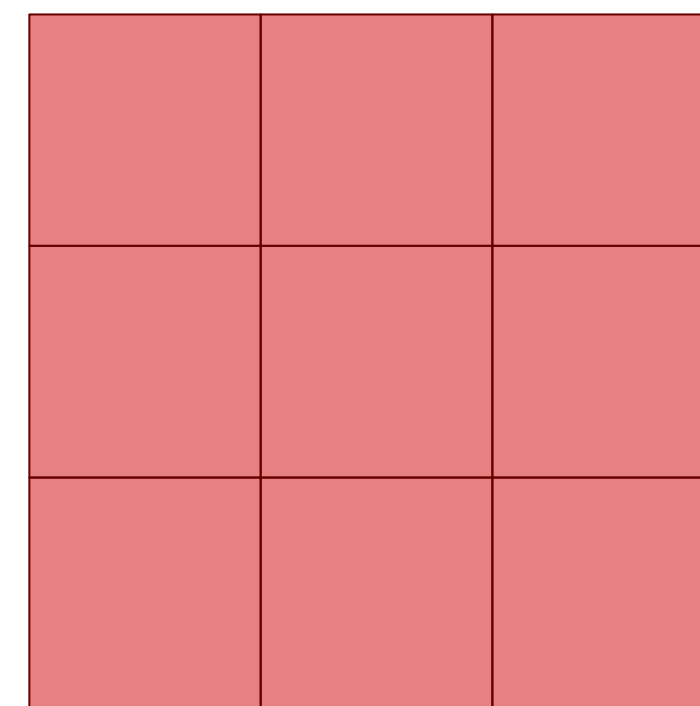
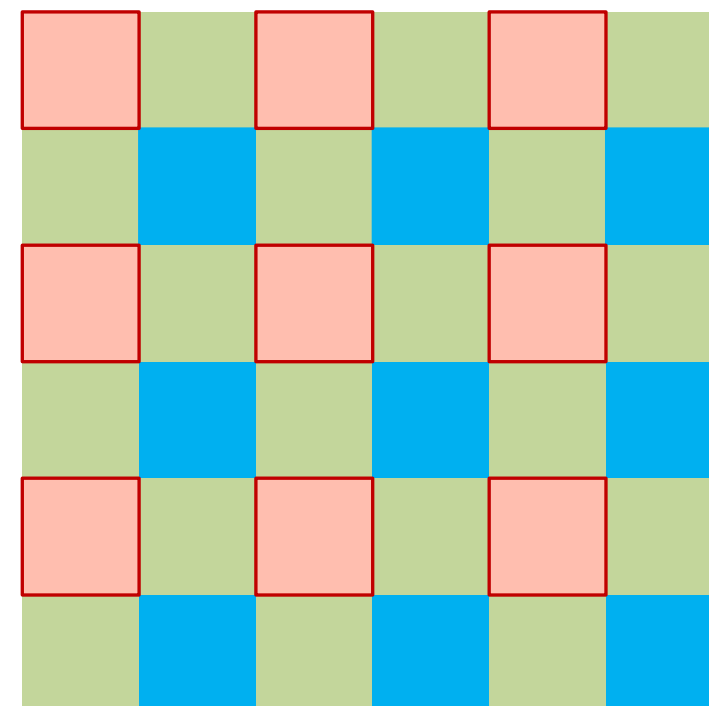
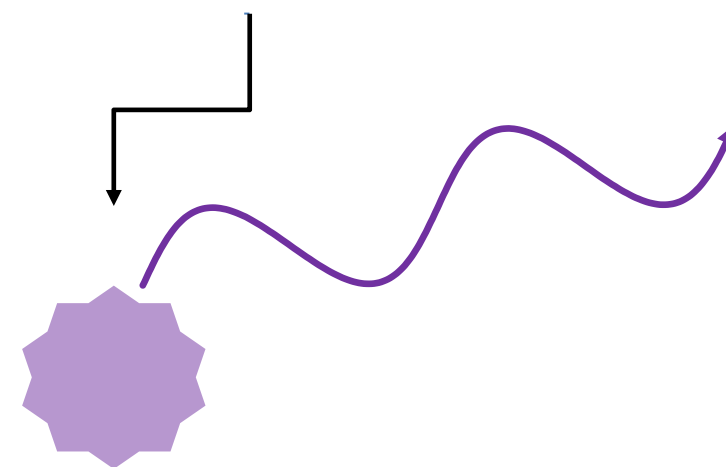
(R; G; B)

(255; 0; 255)

THE VISIBLE SPECTRUM • Wavelength in Nanometers

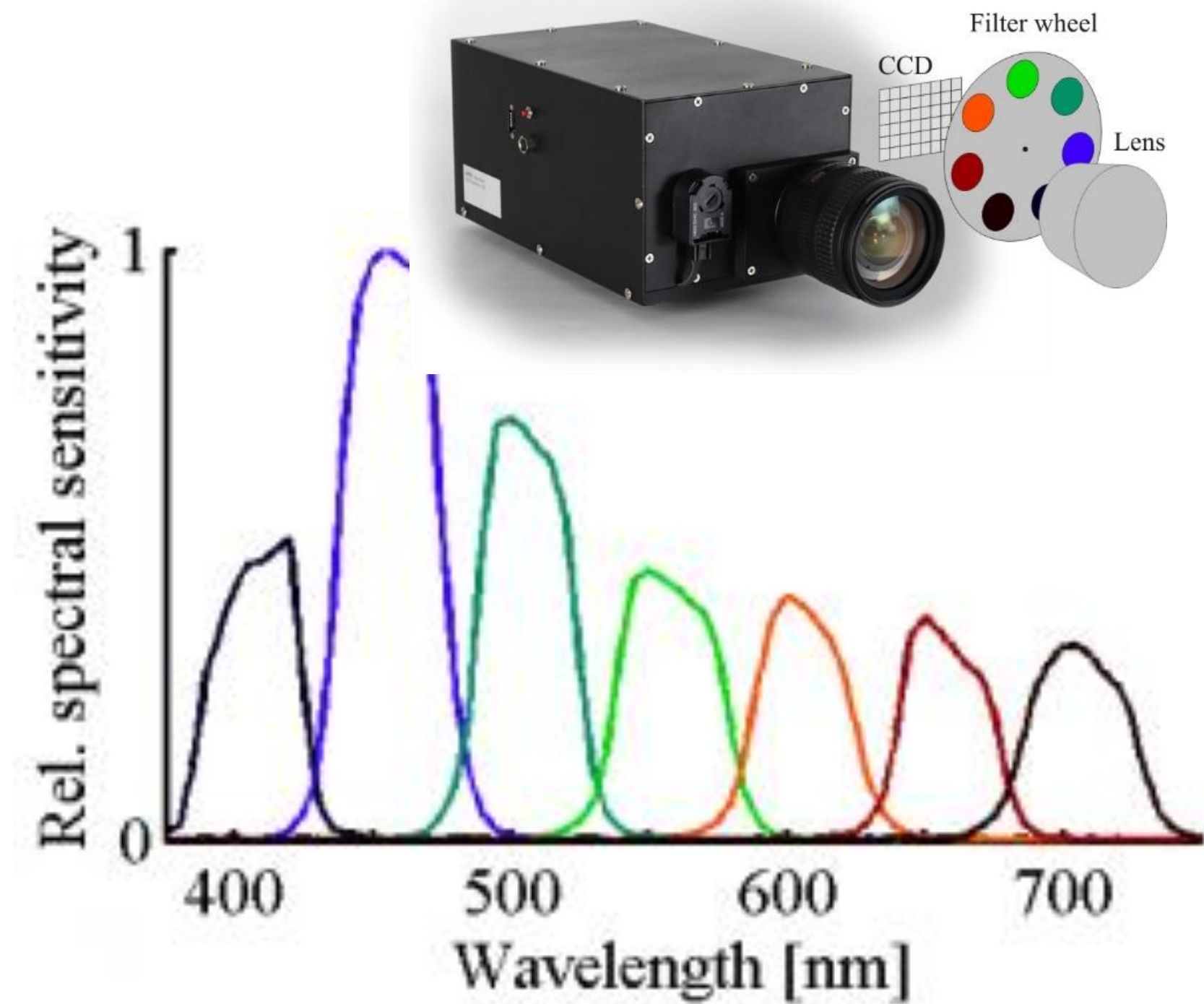
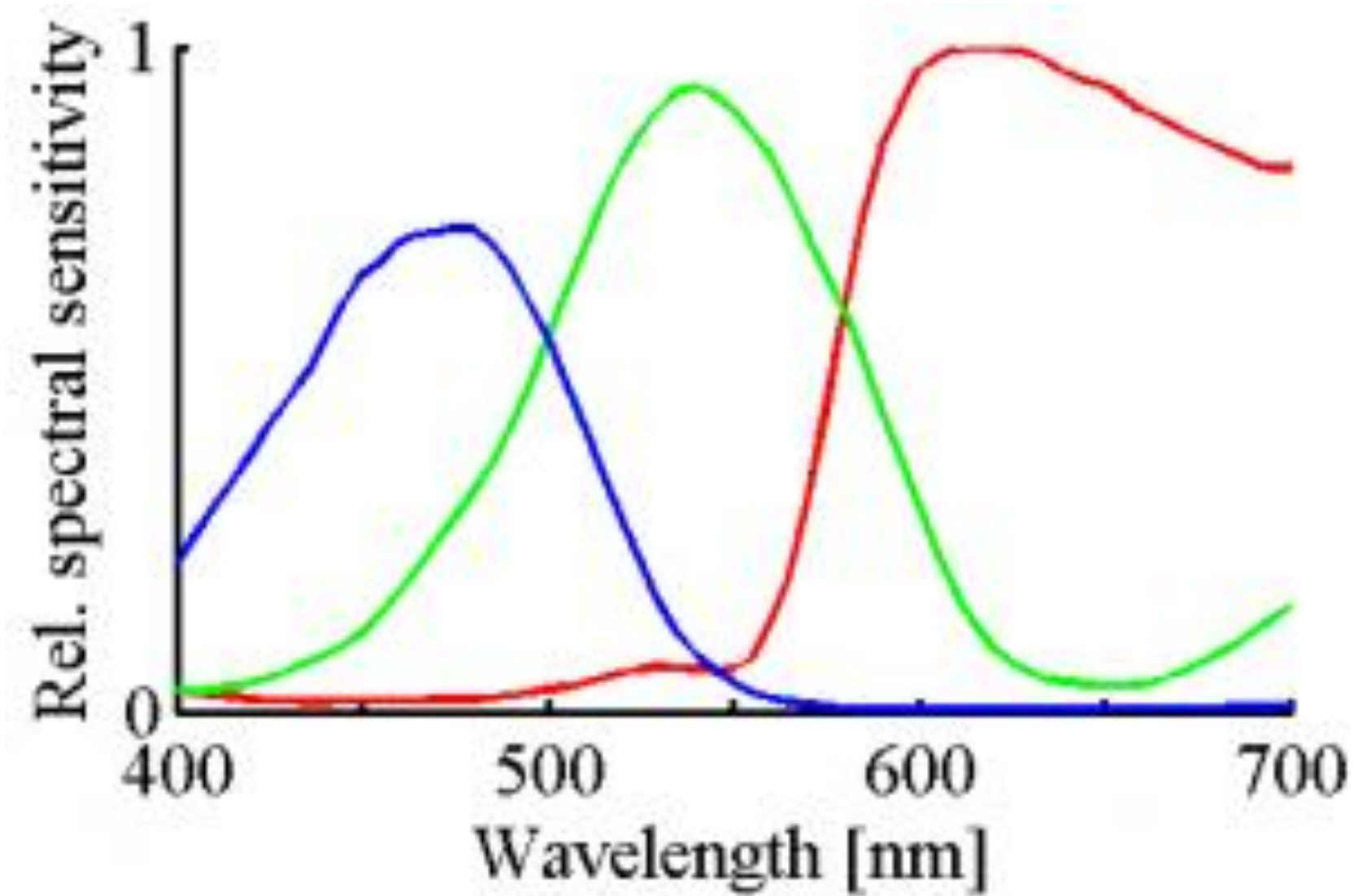


700nm



(R; G; B)

(120; 0; 0)



Source: <http://www.lfb.rwth-aachen.de/en/research/basic-research/multispectral/>