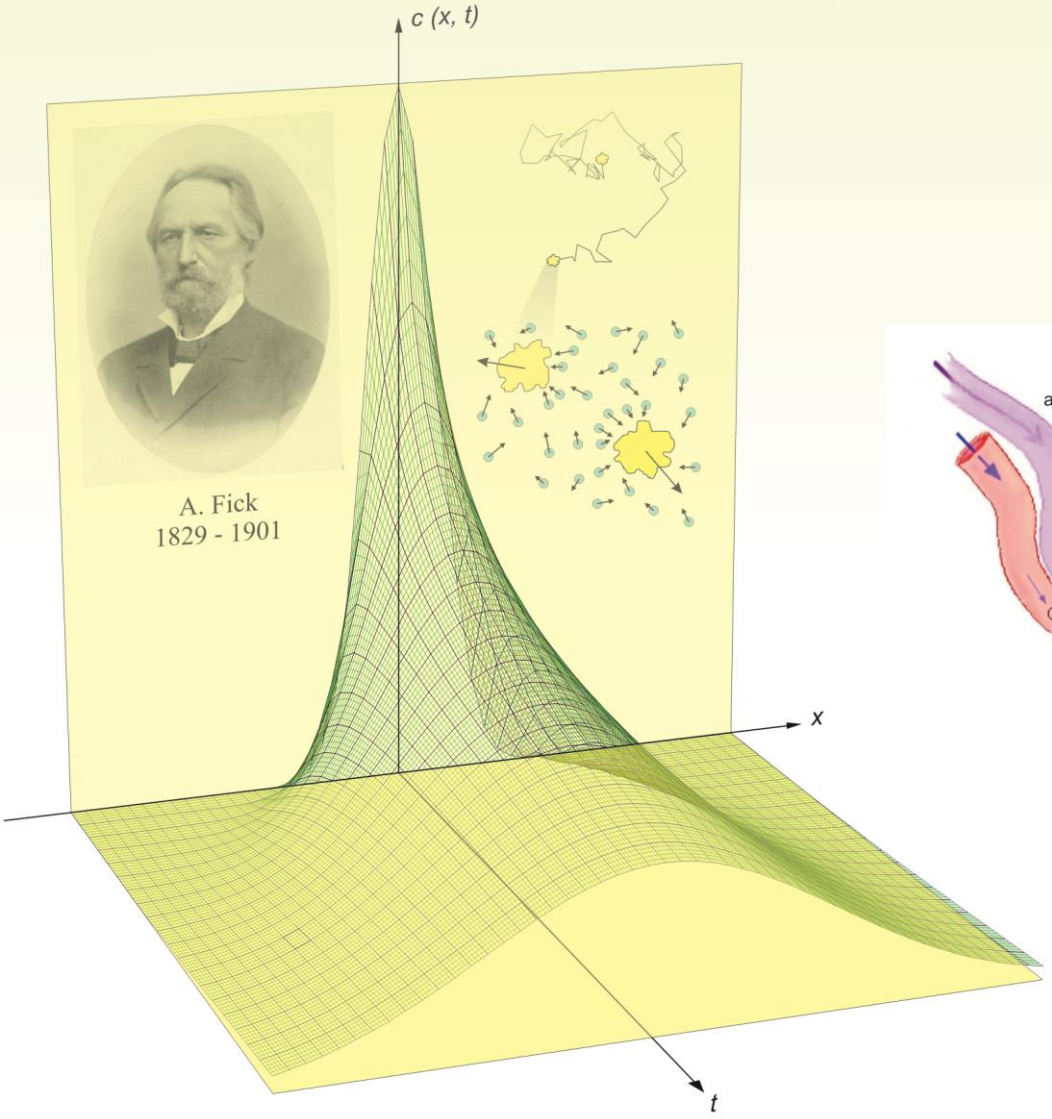
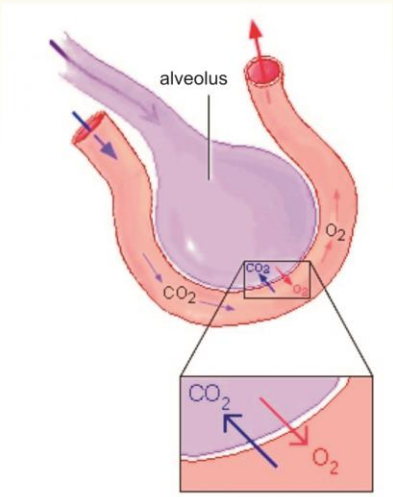
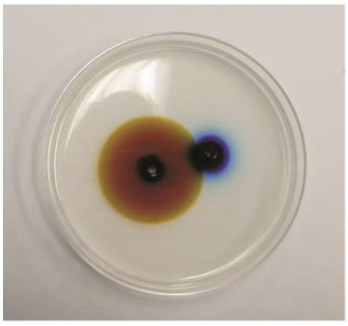
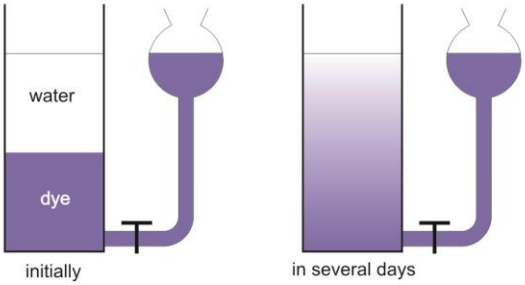
DIFFUSION

MATERIAL TRANSPORT, DETERMINATION OF THE DIFFUSION COEFFICIENT









SUMMARY:

DIFFUSION: spreading of particles of a substance due to thermal motion.

FICK'S FIRST LAW: the flow of particles per unit time across a unit area (flux) is proportional to the concentration drop, i.e. $J_\nu = -D \cdot \frac{\Delta c}{\Delta x}$, where coefficient D is the diffusion coefficient.

DIFFUSION COEFFICIENT (D): gives the amount of material diffused across a unit area in a unit time driven by a unit concentration drop. The unit of the diffusion coefficient is m^2/s. It depends on the size and shape of the molecule, on the interaction with the solvent and on the viscosity of the solvent.

FICK'S SECOND LAW: describes the spatial and temporal *changes* of the concentration as

$$D \cdot \frac{\Delta\left(\frac{\Delta c}{\Delta x}\right)}{\Delta x} = \frac{\Delta c}{\Delta t},$$

the spatial change of the concentration drop is linked to the temporal change of the concentration.

BROWNIAN MOTION: the random uncorrelated motion of particles due to collisions with the surrounding molecules.

MEAN SQUARE DISPLACEMENT (MSD): a measure of the differences of the positions of particles from a reference (starting, x_0) position:

$$MSD = \frac{1}{N} \cdot \sum_{i=1}^{N} (x_0 - x_i)^2$$

PIXEL: a point in the image. A digital image is a grid of points, each holding the digital value of the light intensity of the blue, green and red colors in a given spot.

GRAY SCALE: a brightness scale, where the brightness of each pixel is represented by a number between the maximum (the brightest spot) and the minimum (completely dark spot) value. An example would be 1024 for the maximum and 0 for the darkness.

Spreading of the particles due to the arbitrary thermal motion is known as diffusion. Sugar spreads in the coffee (even without stirring) or the rose scent spreads in the room, both by diffusion. This process goes on (it is noticeable) in case of thermal equilibrium until the distribution of the particles becomes more or less even in the entire volume. Diffusion is extremely important in the living organism. For example, the exchange of oxygen and carbon dioxide between the air in the alveoli of the lungs and the blood within the pulmonary capillaries, and later between the blood and the cells occurs by diffusion. Water, as a very small molecule passes the cell membrane by diffusion as well.
*In **this practice** we will learn the laws of diffusion and we will perform a measurement of a characteristic parameter of diffusion (diffusion coefficient) on a free diffusing system.*

Further readings:
Damjanovich-Fidy-Szöllősi:
III /2.1.

diffusion
Diffusion
diffúzió

THEORETICAL OVERVIEW

FICK'S LAWS

The main question according to the diffusion process is what parameters determine the "strength" of diffusion. To characterize this, we define the flow density of particles per second (also called in general: **flux**):

$$J_\nu = \frac{\Delta \nu}{\Delta t \cdot \Delta A}, \qquad (1)$$

that gives the amount of chemical material that passes through a unit area in unit time. Its unit is mol /(m^2·s).

diffusion coefficient
Diffusionskoeffizient
diffúziós együttható

The answer to the previous question is given by Fick's first law (for stationary diffusion), which sounds in its simplest form as:

$$J_\nu = -D \cdot \frac{\Delta c}{\Delta x}, \qquad (2)$$

where $\Delta c/\Delta x$ is the concentration change along a unit distance (along the x axis), or the **concentration drop (concentration gradient)**. Thus, the flow density of particles per second is proportional to the concentration drop (see Fig. 1). The coefficient of proportionality D is called the diffusion coefficient. D gives the amount of material that diffused through a unit area, in a unit time driven by a unit concentration drop. The SI unit of the diffusion coefficient is m^2/s. The diffusion coefficient depends on the size and shape of the diffusing particle and on the viscosity and temperature of the medium. For spherical particles the diffusion coefficient can be calculated from the Einstein-Stokes formula as:

$$D = \frac{kT}{6\pi\eta r}, \qquad (3)$$

where r is the radius of the particle, η is the viscosity and T is the temperature of the medium. The inverse proportionality of D to the size (r) (or molecular weight, to which the size is proportional) can be seen in the examples of Table 1.

diffusing particle (molecular weight)	medium	D (m^2/s)
H_2 (2)	air	$6.4 \cdot 10^{-5}$
O_2 (32)	air	$2 \cdot 10^{-5}$
CO_2 (44)	air	$1.8 \cdot 10^{-5}$
H_2O (18)	water	$2.2 \cdot 10^{-9}$
O_2 (32)	water	$1.9 \cdot 10^{-9}$
Glycine (75)	water	$0.9 \cdot 10^{-9}$
Serum albumine (69 000)	water	$6 \cdot 10^{-11}$
Tropomyosine (93 000)	water	$2.2 \cdot 10^{-11}$
Tobacco mosaic virus (40 000 000)	water	$4.6 \cdot 10^{-12}$

***Table 1.** Diffusion coefficients of some substances at 20 °C.*

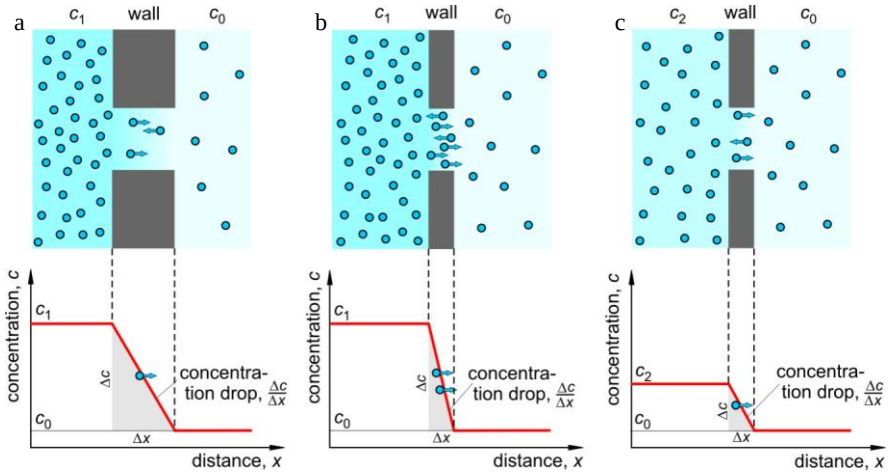


***Fig. 1.** Demonstration of Fick's first law. The concentration drop or **gradient** ($\Delta c/\Delta x$) determines the "strength" of the diffusion in a given system. In panels a and b, the **concentration differences are identical** but across different distances. In panels b and c, the concentration differences are different across the **same distance**. Thus, in the a and c panels the identical concentration gradients drive diffusion of the same "strength".*

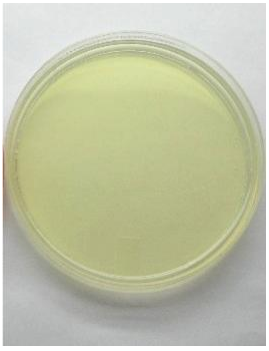


Fig. 2. *Agar-agar gel used as a two-dimensional diffusion surface.*

The second important question concerning diffusion is, how fast is the process of concentration equilibration. Fick's first law does not take into account the possibility of temporal changes in concentration. It is Fick's second law that describes the spatial and time changes of concentration (in one dimensional form):

$$D \cdot \frac{\Delta\left(\frac{\Delta c}{\Delta x}\right)}{\Delta x} = \frac{\Delta c}{\Delta t}. \qquad (4)$$

It can be seen that the change of the concentration over the next short time depends on the actual distribution of the concentration over space at the given time-point.

This is a rather difficult equation, which can not always be solved analytically to give a formula for c(x,t). In general cases numerical (computerized) methods are used, in which we take small Δt time-steps, and step-wise calculate the change of the concentration over time and space.

DETERMINATION OF THE DIFFUSION COEFFICIENT

We will apply Fick's second law to determine the diffusion coefficients of K^+ and colorful (lila) MnO_4^- ions (together with their hydration shells on a two-dimensional surface of a gel (Fig.2.). The prepared agarose gel (0.5% m/m%) provides a hydrated surface, on which the free diffusion of the ions will take place.
The solution of Eq. (4) in a special case when at the beginning of the experiment the material is concentrated into a very small (practically negligible sized) point yields a bell-shaped curve, which is broadening over time (See Fig. 3.). The concentration profile is rotationally symmetric, which means only the distance (r) from the center is the important spatial parameter:

$$c(r,t) = \frac{e^{\left(\frac{-r^2}{4Dt}\right)}}{4\pi Dt} \qquad (5)$$

This equation describes the average spreading of the molecules over time from their initial position. The broadening of the profile is rapid at the beginning of the diffusion experiment, and then gradually slows down.
The σ parameter describing the width of the bell-shaped curve is given by:

$$\sigma = \sqrt{2D \cdot t} \qquad (6)$$

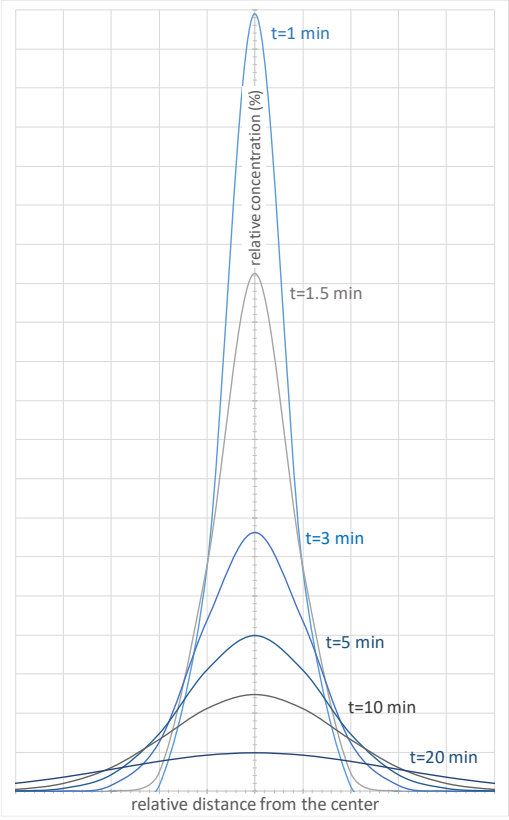


Fig. 3. *The concentration profile c(r,t) as a function of time and space.*

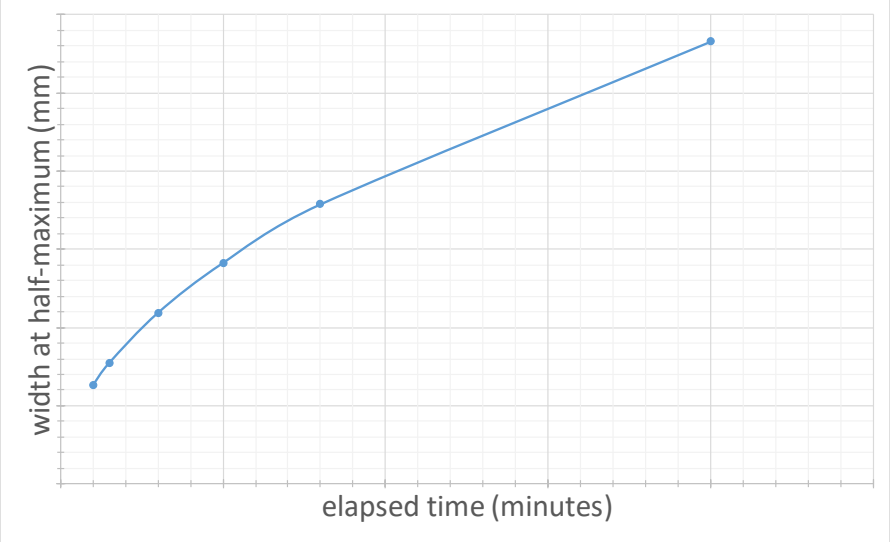


Fig. 4. *The width of the concentration profile over time follows a square-root function as described by Eq. (6).*

We can see from the graph that the width of the bell-shaped curved concentration profile generally follows a square-root function. From this function it is possible to determine the diffusion coefficient (D). To make the determination easier, we will measure the width of the curve as the width at half-maximum, FWHM, (w). This is the distance of two points where the concentration of the permanganate is half of the maximal concentration at the center.

$$w = 2\sqrt{\ln(4)\cdot 2\cdot D\cdot t}\,, \qquad (7)$$

It is convenient to linearize the equation, by taking the square-root of the time as the independent variable (x):

$$w = 2\sqrt{\ln(4)\cdot 2\cdot D} * x\,, \qquad x = \sqrt{t} \qquad (8)$$

This is the equation of a straight line, from the slope of the w-x graph the D diffusion coefficient can be determined. As can be seen, the unit of the diffusion coefficient is length squared over time (m^2/s). It can be seen that the FWHM is directly proportional to the σ parameter of Eq.(6).

The average distance covered (R) by diffusing particles over time can be calculated by using the D diffusion coefficient: (in three dimensions)

$$R_{average} = \sqrt{6\cdot D\cdot t} \qquad (9)$$

A sample series of images taken over the diffusion can be seen in Fig.5.

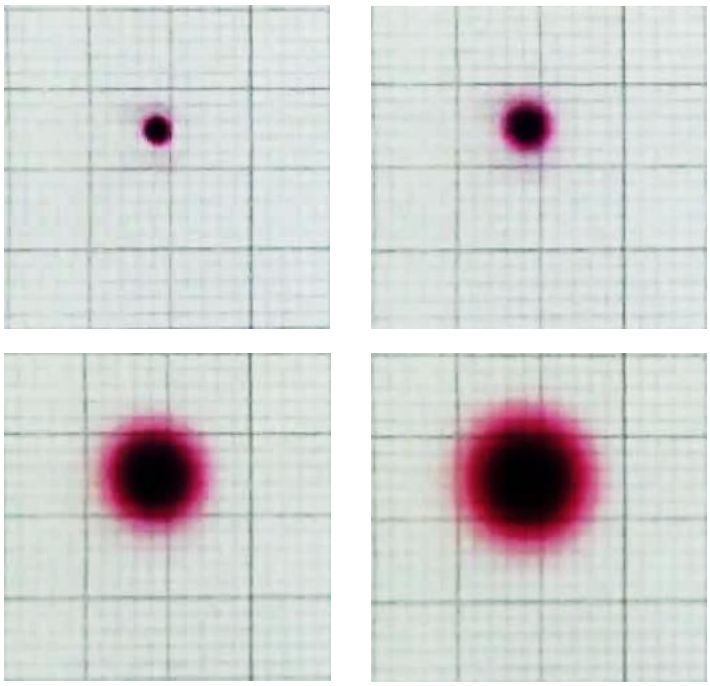
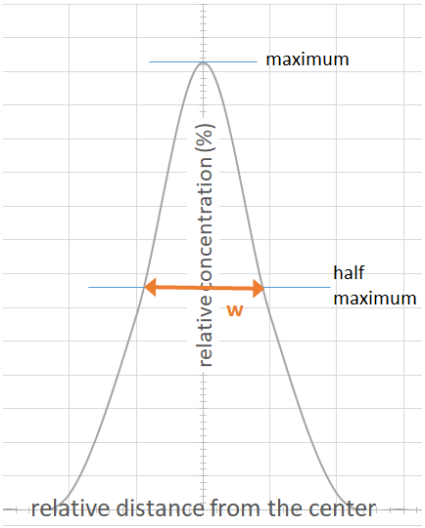


Fig. 5. *Sample images taken during the diffusion of potassium permanganate on an agarose surface. You will take similar images during the lab.*

The images taken by a simple usb camera connected to a computer can be used to determine the parameters of the concentration profile curve by image analysis.

During the lab we will use the ImageJ open-source scientific image processing software to analyze the images. Each image needs a separate **calibration**, which can be done by noting that the grids under the petri-dish have a 0.5 cm sized square.

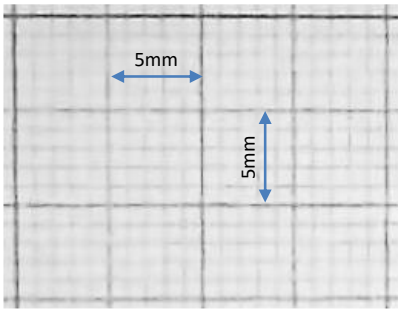


Before you start the experiment, make sure that the agar gel is fully visible on the camera image.

If necessary, ask your lab teacher to help in adjusting the position of the gel.

Ensure the workplace is evenly lit, and the image is free from strong light reflections.

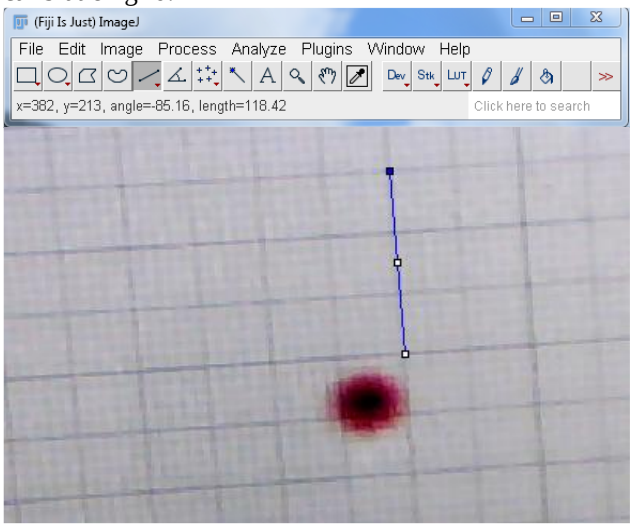
The calibration grid has 5mm markings in both directions:



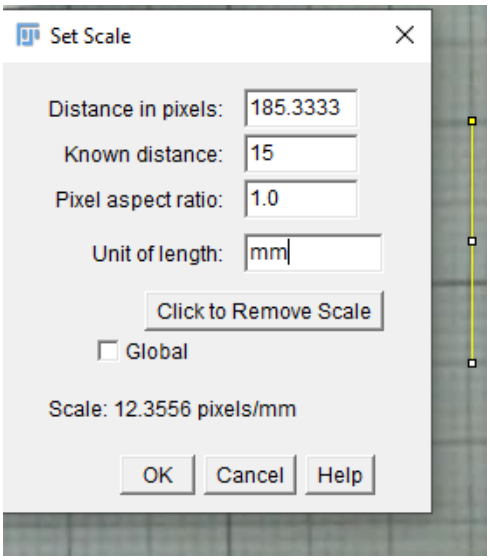
The steps are the following for the image analysis:

1. Open the image file, and draw a line along one of the lines of the calibration grid.

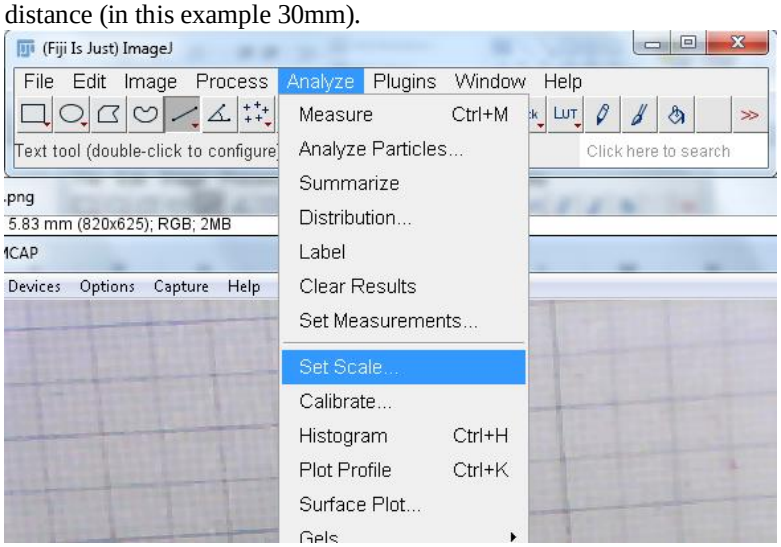
Draw an at least 15-25 mm long calibration line.



2. Use the "set scale" function to calibrate the camera image to the known distance (in this example 30mm).

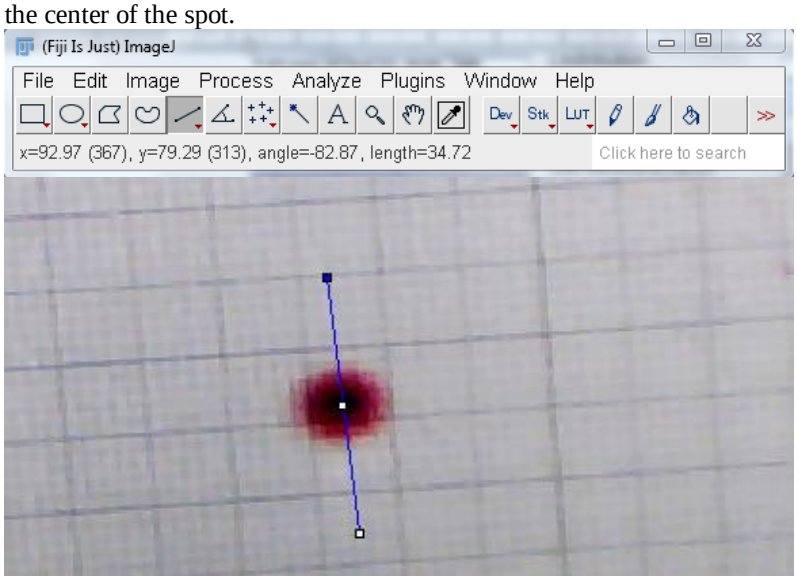


Set the correct scale and unit (here 15 mm) By ticking the "global" the same calibration can be used for all subsequent images.

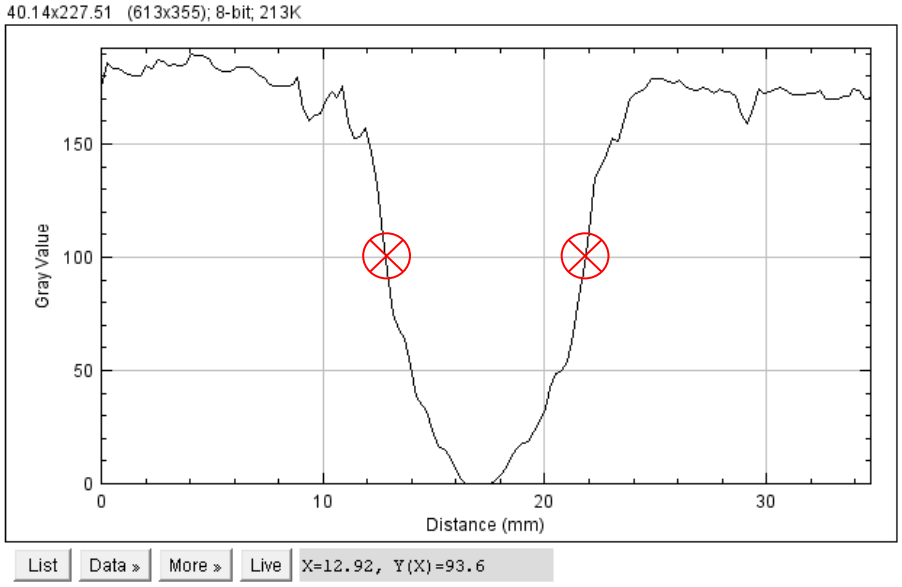


3. Draw a line through the visible viola spot. Make sure the line crosses at the center of the spot.

Since the image is now calibrated, it is not crucial that the line you draw is parallel to any grid line. The spot can be anywhere *along* the line, but it IS important to **cross the center** of the spot.



4. Select the „plot profile” menu to get an interactive image of the color intensity. Use that to read the „x” position of both half-maximum points, and insert them into the table.

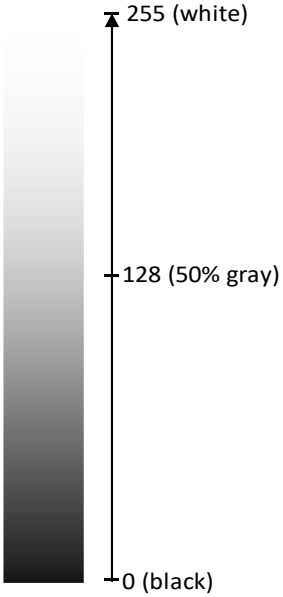


For consistent results you can pick a gray value corresponding to a grid-line and use that for every image. The two sample edge points are marked with a crosshair on the plot.

The „gray value” is the gray-scale value of a pixel (point of the image). The gray-scale value represents the brightness of the pixel on a numeric scale. These are recorded by the digital camera for every pixel. (In a colored image the red, green and blue intensities are separately recorded, the actual color is a mixture of these values)

5. Use the difference of the „x” data pairs obtained from each image as the width of the curve in the calculations.

A sample gray-scale (brightness scale) is shown here:
The brightest pixel has a value of 255, the completely dark has a value of 0. The number is proportional to the counted photons in each pixel.



8bit gray scale

PLAN OF THE EXPERIMENT

The diffusion will start as soon as the small $KMnO_4$ crystal will be placed on the surface of the agar-agar gel in the petri-dish.
You will take snapshot images of the gel surface, observing the spread of the viola color spot of the dissolved and diffusing permanganate ions.
Below is a table draft of the experiment:

Time after crystal is placed on the surface (min)	Actual time of the snapshot (min)	Left side half-maximum "x" position (mm)	Right side half-maximum "x" position (mm)	Width (mm)
0	-	-	-	0
1				
2				
3				
4				
5				
6				
…				
…				
…				

Table 1. *Experiment draft.*

TASKS

1. Start the image capture software.
2. Align the Petri-dish under the camera.
3. Make sure that the calibration grid is clearly visible on the image on the computer screen.
4. Take a test-snapshot image.
5. Prepare a timer on your mobile phone or use the stopwatch.
6. Open the Eppendorff tube, and slide the little viola KMnO4 crystal to the approximate center of the gel surface. Make sure the spot is visible on the camera image.
7. Start the timer as soon as the crystal is on the surface.
8. At approximately the prescribed time-points take a snapshot with the camera software and save it on the desktop of the computer.
9. After the last image is acquired, do the image analysis steps and extract the width data.
10. Make the appropriate graph, fit the theoretical function
11. calculate the diffusion coefficient of $KMnO_4$ on the gel surface.