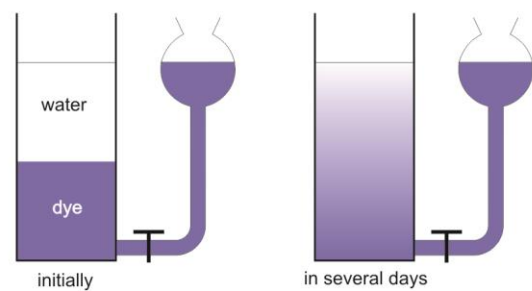
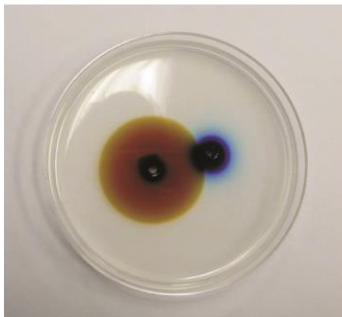
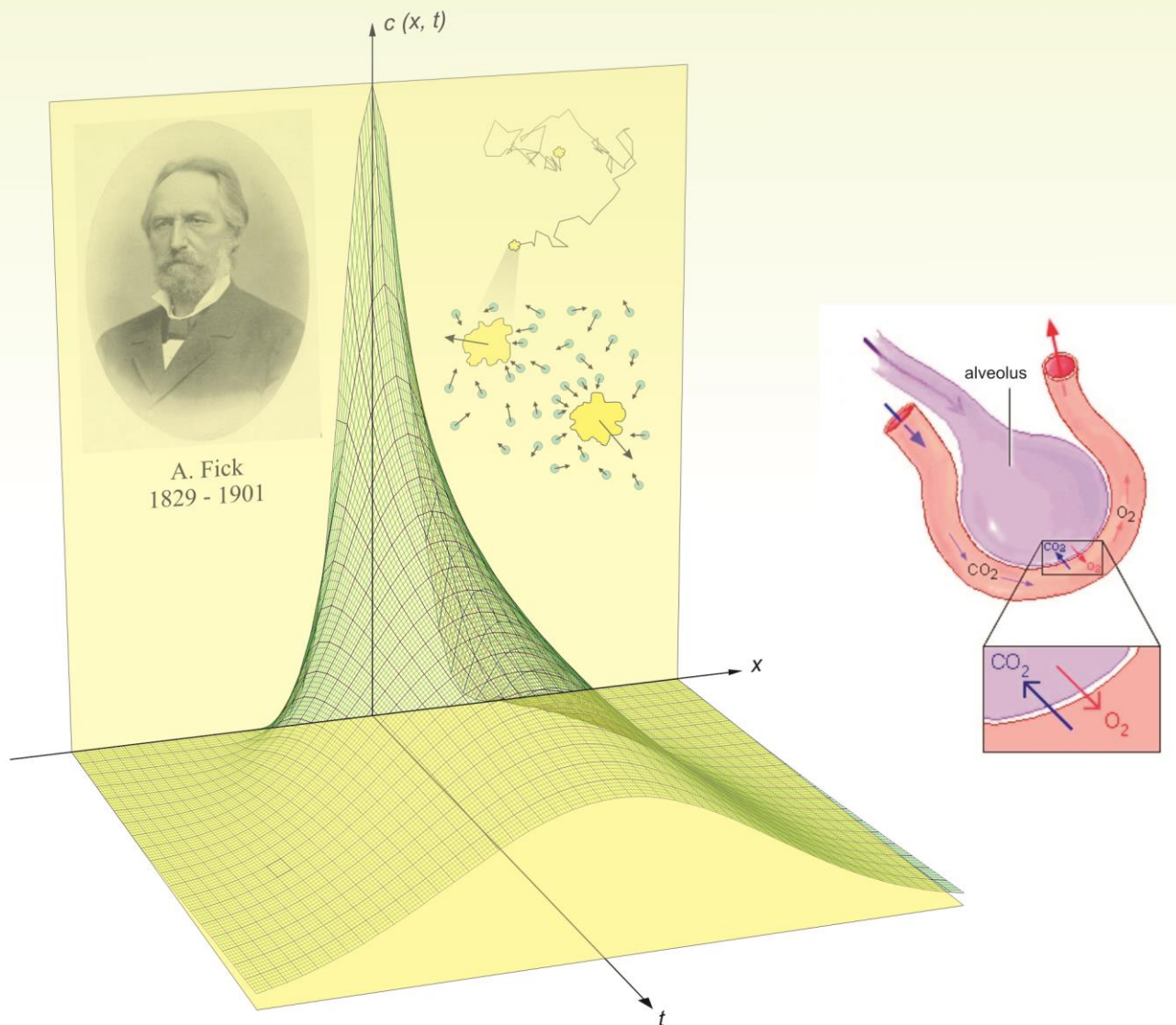


DIFFUSION

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. $J_v = -D \cdot \frac{\Delta c}{\Delta x}$, 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 in case of thermal equilibrium until the distribution of the particles becomes 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.



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**):

$$J_v = \frac{\Delta v}{\Delta t \cdot \Delta A}, \quad (1)$$

that gives the amount of chemical material that passes through a unit area in unit time. Its unit is mol/(m²·s).

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

$$J_v = -D \cdot \frac{\Delta c}{\Delta x}, \quad (2)$$

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 m²/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 in a free three-dimensional system the diffusion coefficient can be calculated from the Einstein-Stokes formula as:

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

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.

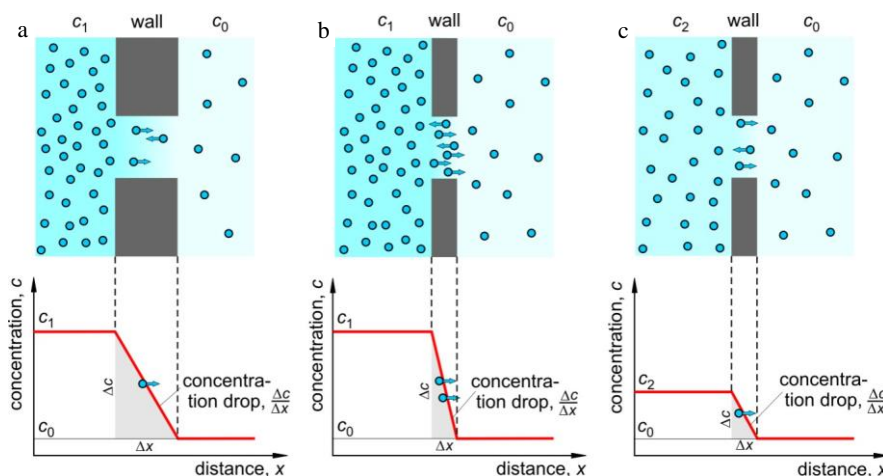
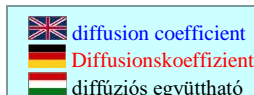


Fig. 1. Demonstration of Fick's first law. The concentration drop or **gradient** ($\Delta c/\Delta x$) determines the flux ("strength") of the diffusion in a given system. In panels a and b, the **concentration differences are identical** but across different distances. In panels b and c, the concentration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



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 ₂ O (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.

The II. Fick's law can also serve as a definition equation for the D diffusion coefficient. The I. Fick's law does NOT take into account any temporal change in the concentration, and as a result can serve as a source for the determination of the diffusion flux only at the moment in which the gradient is determined (or in the case of a stationary diffusion when the concentration gradient does not depend on the time). As soon as the diffusion starts, due to the flux the concentrations, and as a consequence the gradient(s) will be changed. This dependence is accounted for in the II. Fick's law.

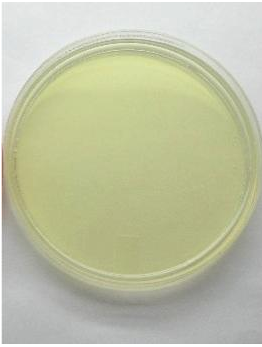


Fig. 2. Agar-agar gel used as a two-dimensional diffusion surface.

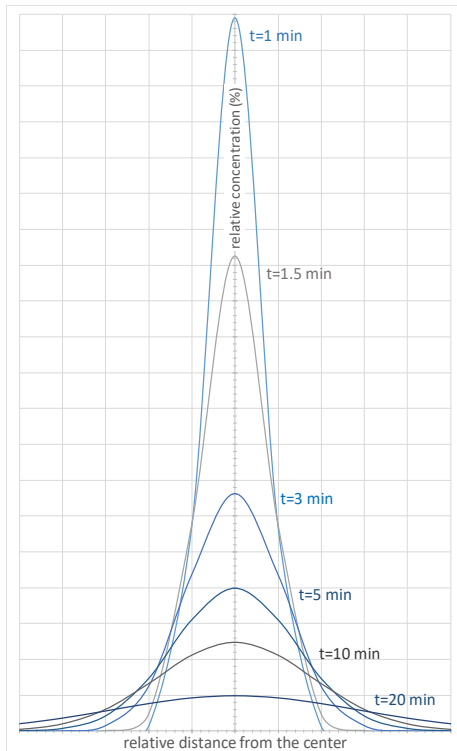
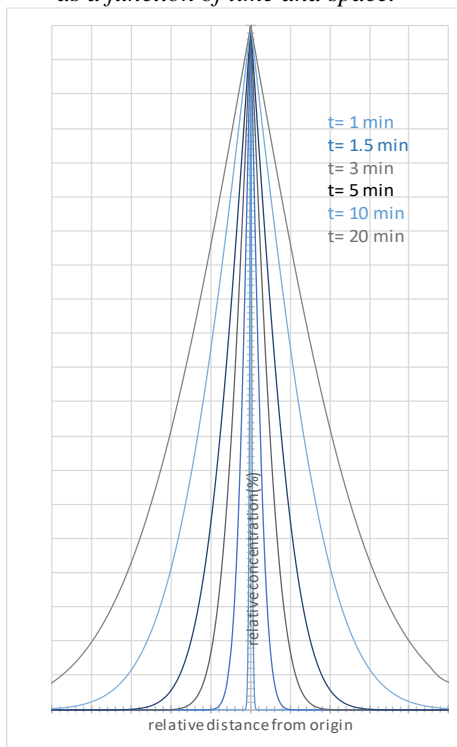


Fig. 3. The concentration profile $c(r,t)$ as a function of time and space.



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):

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

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, or the diffusion of protons (together with their hydration shells) in a two-dimensional surface or arrangement of an agarose gel (0.5% m/m%) (Fig.2.). A practically free diffusion will take place starting from a single point, and in this case the solution of Eq. (4) exists as a formula provided that at the beginning of the experiment the material is concentrated into a very small (practically negligible sized) point. The concentration profile is in this case rotationally symmetric, which means only the distance (r) from the center is the important spatial parameter. We consider two important special cases:

- A given amount of material diffuses from the origin (as is the case in the release of neurotransmitters).
- Diffusing particles are released from a large reservoir which keeps the concentration constant in the center during the diffusion process.

In the first case the solution of Eq.(4) is a Gaussian bell-shaped curve, the exact formula depends on the dimensionality. For two dimensions (planar diffusion) the concentration can be given as a function of the distance from the origin (r) and

time (t) as $c(r,t) = \frac{e^{\left(\frac{-r^2}{4Dt}\right)}}{4\pi Dt}$ which is shown in the upper part of Fig. 3. Since the total

amount of material corresponds to the area under the curve (which is constant), as the curve broadens it also has a decreasing maximum. In the second case the maximum is constant (bottom of Fig. 3.) and the area under the curves is increasing over time, made possible by the existence of the reservoir.

In both of the cases the width of the curves follow a square-root function of the time as seen in Fig. 4.

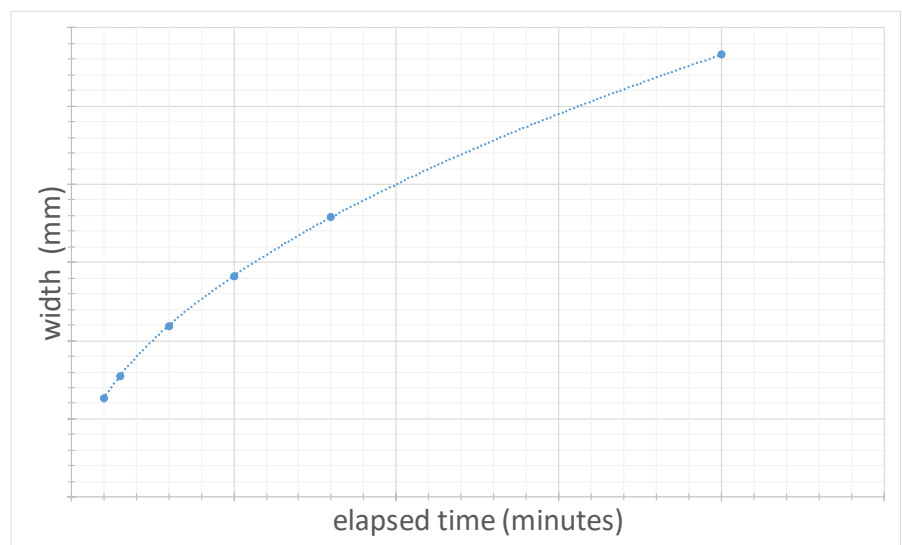


Fig. 4. The width of the concentration profile over time follows a square-root function.

The width of the curves can be described by the width of the colorful spots (e.g. the width at which the color intensity drops to 1%). This is connected to the D diffusion coefficient as:

$$w = 6 \cdot \sqrt{2 \cdot D \cdot t} \quad (\text{constant amount of material}) \quad (5)$$

$$w = \sqrt{8\pi \cdot D \cdot t} \quad (\text{constant center concentration}) \quad (6)$$

If we introduce a new parameter as $x = \sqrt{t}$ then the $w(x)$ function will have the shape of a straight line, and the D can be determined from the slope. Thus for the determination of the diffusion coefficient we have to take images from the process, determine the diameter of the colored spots and plot that as a function of the square-root of the elapsed time.

As apparent from the equations, the unit of the diffusion coefficient is length squared over time (m^2/s). The average distance covered (R) by diffusing particles over time can be calculated by using the D diffusion coefficient, and it does not depend on which of the two special cases one has, but it does depend on the dimensionality.

In three dimensions we get:

$$R_{\text{average}} = \sqrt{6 \cdot D \cdot t} \quad (7)$$

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

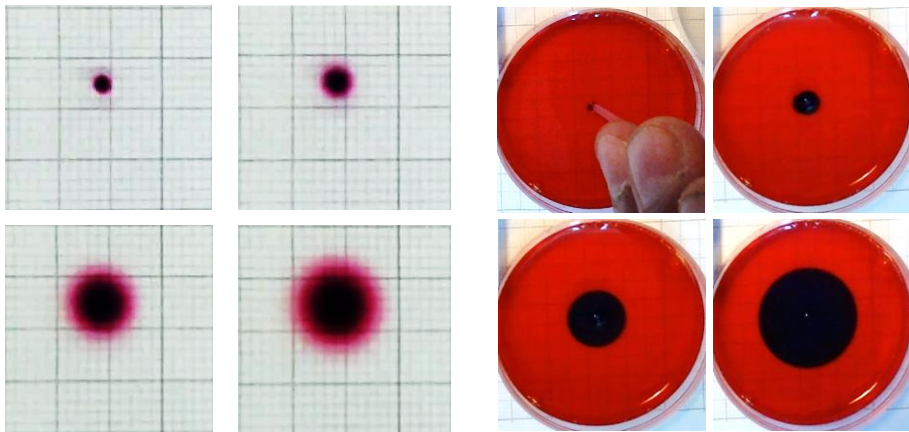
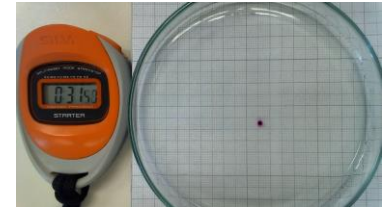


Fig. 5. Sample images taken during the diffusion of purple colored potassium permanganate (left) and protons (right) in native or congo-red stained agarose gel. You will take similar images during the lab.

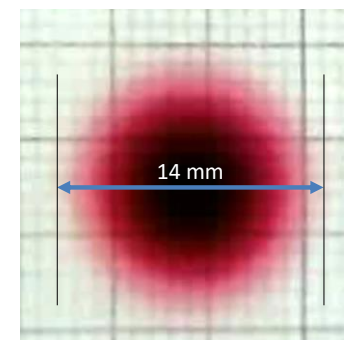
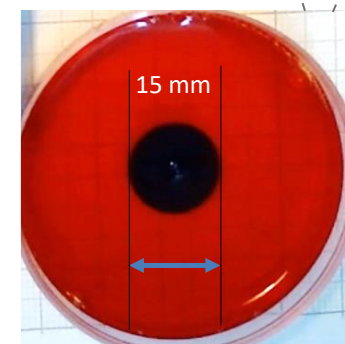
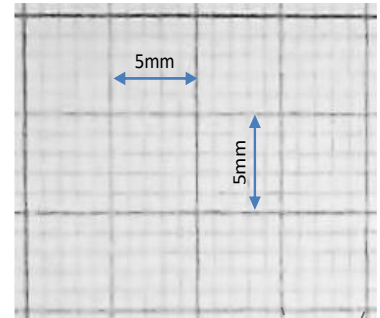
The images taken by a simple web camera connected to a computer can be used to determine the width of the concentration profile curves. It is even possible to determine the concentration profile if the camera is calibrated. During the lab we use a simple calibration mechanism: the distances can be determined by placing a mm-grid under the petri-dish containing the agarose gel. We will not determine the full concentration profile, instead only the w width parameter will be used for further calculations. **As a simplification w will be taken as the diameter of the colored spot.** In the case of the KMnO_4 diffusion the drop of the concentration by increasing distance from the center can also be seen, while in the case of the proton diffusion only the characteristic color change of the congo-red indicator dye is visible, which marks the position of a certain pH in the gel. The diffusion coefficient can be determined in both cases from Eq. 5.

Before beginning with the experiment it is important to control the camera setup. The images should be free from disturbing reflections, and the mm-grating under the gel must be also visible.



A sample image at the early stage of the diffusion process.

The calibrating grid has a thick line network in both directions at 5mm distances.



determination of the spot diameter

planned elapsed time from start of the experiment (min)	exact time elapsed (s) at which the image was taken	square-root of the elapsed time	spot diameter (mm)
0,5			
1			
1,5			
2			
2,5			
3			
4			
5			
7			
10			
15			
20			
25			
30			
35			
40			
45			

Table 1. a possible arrangement of the experimental data.

PLAN OF THE EXPERIMENT

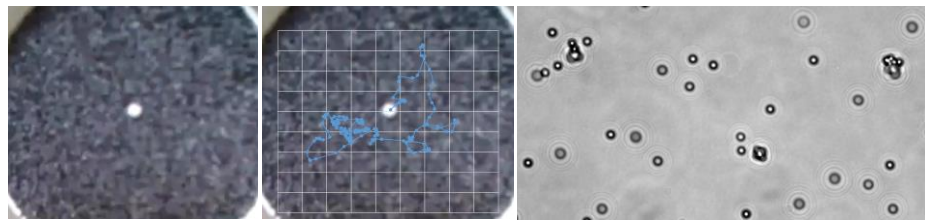
The diffusion will start as soon as the small KMnO_4 crystal will be placed on the surface of the agar-agar gel, or the concentrated acid solution is pipetted into the small hole in the center of the gel in the petri-dish.

You will take snapshot images of the gel surface and evaluate the diameter of the spots. A sample arrangement of the data table is shown in Table 1.

TASKS

1. Demonstration experiments:

We will observe the random movement of particles on the macroscopic and microscopic scale. The macroscopic experiment shows the random motion of a Styrofoam sphere on the surface of fluidized poppy-seeds. The poppy-seeds serving as “solvent molecules” move so fast that they are blurred on the images. Random collisions make the motion of the Styrofoam sphere follow the Brownian motion law. We will also observe the Brownian motion of plastic $1\text{ }\mu\text{m}$ diameter microspheres under the microscope with 100x objective and oil immersion. The Airy disks are also visible.



macroscopic and microscopic Brownian motion

2. Individual measurement task:

Verify before the start of the experiment that the camera can take acceptable images, free from disturbing reflections, and the mm-grid below the agarose gel is also clearly visible on the image. Take a sample image before you start the diffusion process. Take care to start the timer exactly when the diffusion starts. Take images at the prescribed times, and save them on the desktop.

3. Data evaluation:

Determine the diameter of the spots from the camera images. Plot the diameter as a function of the square-root of the elapsed time. Determine the slope of the linear trendline, and **use Eq.5. to determine the D diffusion coefficient.**

$$w = 6 \cdot \sqrt{2 \cdot D \cdot t} \rightarrow D = \frac{\text{slope}^2}{72}$$

Calculate the time needed to reach an average distance in the given sample cases.