Diffusion Processes in the extracellular space of the brain

Fredrik E Pettersen
f.e.pettersen@fys.uio.no

June 16, 2013

Contents

1 Introduction

This is a project in computational neuroscience based on some eight articles describing various aspects of diffusion in the extracellular space of the brain. The aim of the project is more or less to be an introduction to the models describing diffusion processes in the extracellular space of the brain, a space in which many important processes takes place. Among them are the delivery of nutrients and drugs to the brain-cells (specified later), as well as ion transport essential to the communication processes between brain-cells. The project starts with briefly introducing neuroscience in general and motivating the theme, before summing up the most important models and measurement techniques availible (in the early 2000's). We will also point out some other fields of modern physics that are quite similar to the problem at hand.

2 Background material

For all practical purposes, the brain of a rat is considered equal to the brain of a human in this project.

2.1 Basics of the brain

The human brain consists of two types of cells; the neurons and neuroglia. Neurons are tasked with signal processing and transport, while the glia are thought to have more janitoral tasks. The neurons are bathed in a salt solution that is mainly Na^+ and Cl^-. Inside the neurons, a highly regulated salt solution of mainly K^+ sets up a potential difference relative to the outside of the cell of approximately -65mV. The neurons are in constant communication with each other through action potentials, which are disturbances in the membrane potentials of neurons. These action potentials are generated in the body of the cell, called the soma, from where they propagate down the axon without loss of amplitude. This is achieved by constantly amplifying the signal using ion pumps (see the Hodgin-Huxley model of the action potential [7]). After propagating down the axon, the action potential reaches a synapse which is a gate to another neuron. If the action potential is of significant strength, vesicles carrying neurotransmitters merge with the synapse membrane, letting the neurotransmitters diffuse to the dendrite of the other neuron. If enough neurotransmitters reach the post-synaptic side, the signal continues propagating to the soma of this neuron, and the entire process starts over again.
The interest of this project lies, mainly, in the diffusion processes that take place in the space between these types of cells, the so-called extracellular space (ECS). This is a narrow space ($\sim 10 - 100$ nm [5]) with a highly complicated geometry (Figure 1). Surprisingly, the ECS adds up to 20% of the total brain volume. We can understand this by realizing that every part of a cell must be separated from another cell by the ECS. Since the cells consists of axons and dendrites which can be viewed as (somewhat) fractal, we see that this means separating a vast amount of surface area from other surface areas.

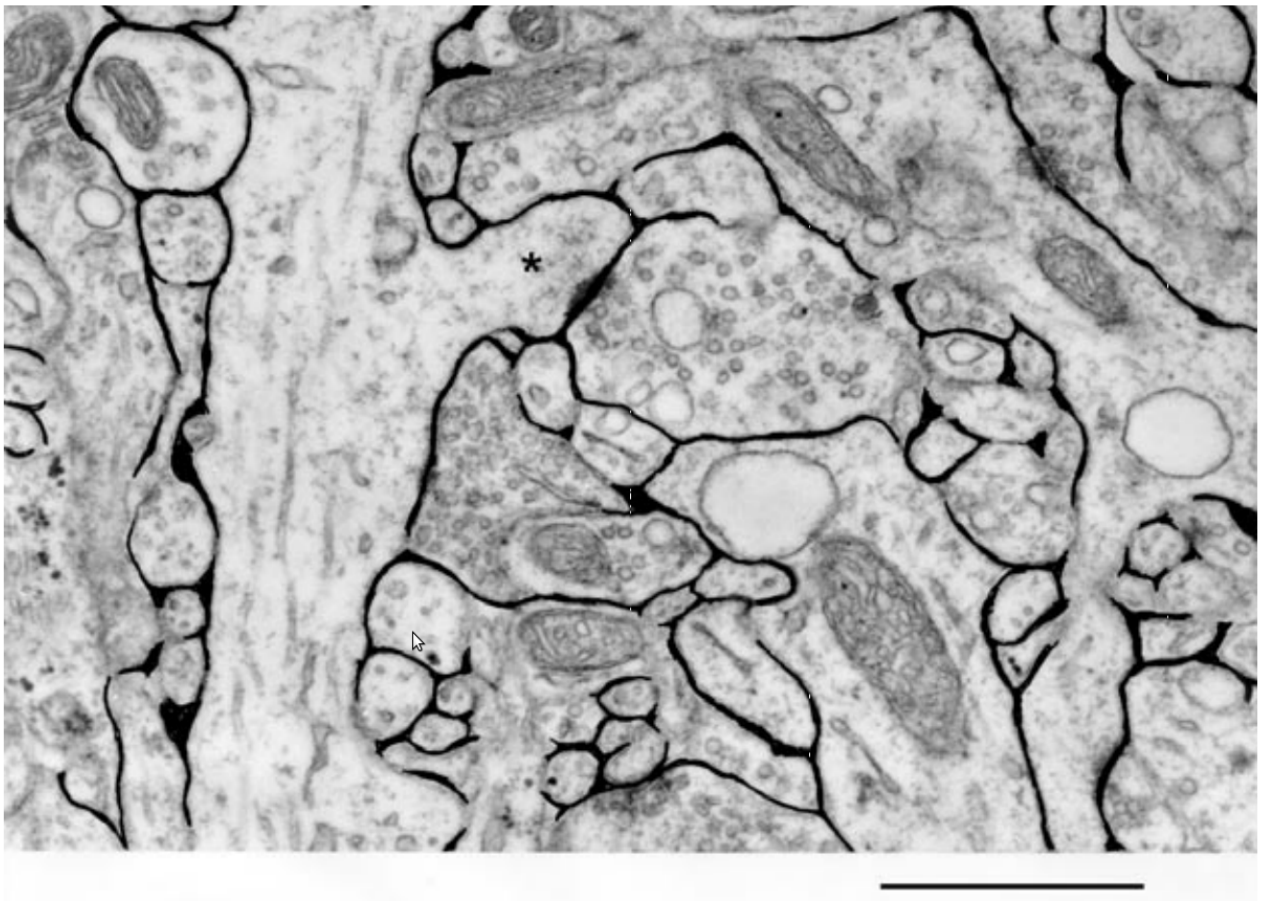


Figure 1: Electron micrograph of a small region of the cerebral cortex of a rat with a prominent synapse. The black areas of the picture indicate the ECS, which may be reduced in size as a consequence of the processing which prepare the sample for the micrograph. The asterix (*) indicates the post-synaptic side of a synapse on a dendrite. On the other side of the synaptic cleft one can make out the pre-synaptic terminal containing several small, round vesicles filled with neurotransmitter molecules. The scale bar under the figure indicates a distance of about 1μm. Figure taken from Nicholson [5].

The ECS is thought to support the diffusion of oxygen and nutrients to the neurons and glia, and diffusion of carbon dioxide and other waste from these cells through the blood - brain barrier and into the bloodflow.

2.2 Diffusion in general

Diffusion is a transport process which in its well known macroscopic form has been attributed to Adolf Fick. In 1855, building on the earlier experimental work of Graham, Fick formulated the macroscopic law later known as Fick's law

$$\text{flux} = -D \times \text{concentration gradient} \tag{1}$$

Where $[D] = \frac{m^2}{s}$ is the diffusion constant. Fick's law leads to the well known partial differential equation in the concentration, C.

$$\frac{\partial C}{\partial t} = D\nabla^2 C \tag{2}$$

Einstein later (1905) proposed the most useful relation between the diffusion constant and a fluid viscosity;

$$D = \frac{k_B T}{6\pi\eta r}, \tag{3}$$

where T is the temperature, k_B is Boltzmann's constant, η is the fluid viscosity with units $[\eta] = m^2 s^{-1}$. There are several relations on this form, relating the diffusion constant to various other easily measurable quantities. The most relevant relation for the study of diffusion in the ECS would be

$$\langle r^2 \rangle = 2dDt \tag{4}$$

in the limit of large t (that is in steady state). Equation 4 relates the root-mean-square displacement of a particle after a time t (where t is large) in a d dimensional space. There are two good reasons to consider the transport mechanisms in the ECS as diffusion processes. Firstly, diffusion goes seamlessly from micro to macro scale. In our case, this is perfect since the channels of the ECS are very narrow, but not nececarily narrow enough to consider all the microscopical effects. Second, the geometry of the problem is somewhat similar to diffusion in porous media.

2.3 Why diffusion in the extracellular space

Though there are several reasons to study diffusion processes in the ECS this project has a specific goal in mind. The Einstein relation, eq. (3), relates the diffusion constant to the viscosity of the medium in which the diffusion is taking place. From the definition of viscosity, μ, we have

$$v_d = \mu F \tag{5}$$

where $F = qE$ is the standard electrical force acting on a charged particle. We can also define the current from the drift velocity of the particles as

$$J = cqv_d = \sigma E \tag{6}$$

where $\sigma = c\mu q^2$ is the electrical conductance, in this case, of the ECS. Inserting this in the Einstein relation, eq. (3), lets us express the conductivity in terms of the diffusion constant as

$$\sigma = \frac{cq}{k_B T} D \tag{7}$$

Equation (7) is trivially generalizable to tensor notation, where the conductance and diffusion "constant" are both 2. order tensors.

We are interested in the extracellular conductance for measurement purposes. An important goal in the field of computational neuroscience is to model networks of neurons on such scale that the network can resemble an actual brain. This poses many challenges, the sheer number of neurons in the neocortex ($\sim$ 17 billion) being one of them. To date, the largest system simulated is said to resemble the brain of a cat, but the low complexity of the model neurons makes the real similarity to a cat brain questionable.

To verify the network models, they are (among other things) related to measurements of local field potentials (LFP) in the ECS. When signals pass through neurons, they will echo into the ECS and create measurable potentials which vary strongly in space. For a single neuron consisting of N compartments, the LFP is found by summing the contributions from each compartment, as we see from eq. (8). This formula relies on a set of approximations: The first is the use of the quasistatic approximation of Maxwell's equations, meaning that the electical an magnetic fields are effectively decoupled. This approximation is to a large degree fulfilled for the frequencies inherent in neural activity (less than $\sim 10^3$Hz). Further, the electrical conductivity in the ECS, σ, is assumed ohmic, frequency independent, homogenous and isotropic, meaning that it is the same in all directions. The last two assumptions can be dropped, making the

conductivity a 2. order tensor.

$$\phi(\mathbf{r},t) = \frac{1}{4\pi\sigma}\sum_{n=1}^{N}\frac{I_n(t)}{|\mathbf{r}-\mathbf{r_n}|} \tag{8}$$

When we include more than one neuron we will also need to introduce the current source density (CSD), $C(\mathbf{r},t)$, which is a coarse-grained measure of the net volume density of transmembrane current entering or leaving the ECS. Setting $C(\mathbf{r},t) = \sum I_n\delta^3(\mathbf{r}-\mathbf{r_n})$ now gives us a relation between the LFP and CSD if we insert the expression for CSD into eq. (8).

$$\phi(\mathbf{r},t) = \frac{1}{4\pi\sigma}\iiint_V \frac{C(\mathbf{r}',t)}{|\mathbf{r}-\mathbf{r}'|}d^3r' \tag{9}$$

Notice that $\delta^3(\mathbf{r})$ denotes the three-dimensional Dirac-delta function. Equation 9 still requires the conductivity to be scalar, but can be rewritten to provide the opposite relationship. The final equation is then

$$\nabla\cdot(\sigma(\mathbf{r})\nabla\phi(\mathbf{r},t)) = -C(\mathbf{r},t) \tag{10}$$

which is a nonlinear, second order, partial differential equation. It also supports the possibility of σ being a tensor.
Equation (10) lets us relate the LFP to the CSD through the conductivity in the ECS, which is a quantity we will need to estimate. Through eq. (7) we have related it to the diffusion constant (tensor), which is a quantity that is somewhat simpler to measure.

3 Mathematical models

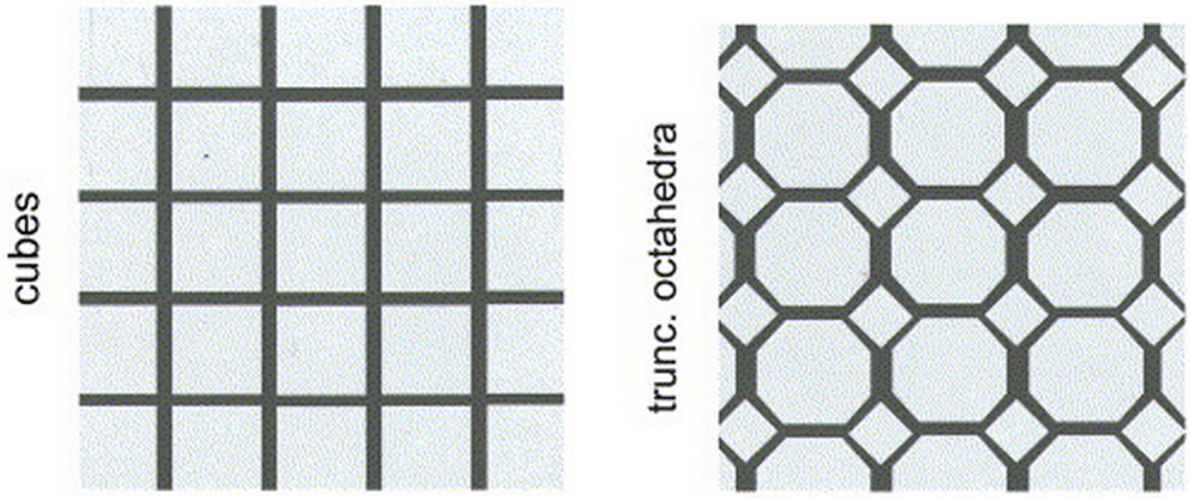
Diffusion in the ECS is, naturally, modeled by a diffusion equation, but rather a modified one than the basic diffusion equation 2. Since the geometry of the ECS is very narrow, molecules diffusing in this space will not be subject to free diffusion, as the normal diffusion equation assumes. The diffusing molecules will bounce off cell membranes (we are now considering macromolecules on such a scale that speaking off a cell membrane makes sense) and other molecules. There may even be molecules absorbed by cells, getting stuck onto membranes or going through similar processes. We therefore see it fit to introduce a modified version of the diffusion equation which is more similar to the one governing diffusion in porous media.

$$\frac{\partial C}{\partial t} = D^*\nabla^2 C + \frac{s}{\alpha} - k'C \tag{11}$$

Where we have introduced an effective diffusion constant D^* defined from the tortuosity, $\lambda = \sqrt{\frac{D}{D^*}}$, which is a parameter saying something about the reduction in the diffusion constant compared to free diffusion (usually measured in a low percentage agar solution in water). The tortuosity can also be interpreted as a measure of the "twistyness" of the media in which the diffusion is taking place. The relative volume fraction the ECS accounts for is defined as α, s is a source term, and the $k'C$ term models the uptake of the diffusing molecules by cell membranes and other processes which might remove diffusing molecules from the system. Note that eq. (11) is only one of several possible equations used to model this kind of diffusion, and that terms accounting for the (now assumed absent [5]) bulk flow in the ECS, and other possibly contributing terms, are not included. The model does, however, illustrate the general idea behind the modeling of diffusion in the ECS.

3.1 Numerical simulations of tortuosity

In 2003 Hrabětová and Nicholson conducted numerical simulations of diffusion in an artificial, porous 3D media to determine the tortuosity, λ, as a function of the volume fraction, α [3]. The ECS was modeled as the gaps between geometric figures, starting with cubes and advancing to more complicated shapes (see Figure 2). The models all have a regular spacing in common.
In their simulations the authors found that the tortuosity reached a maximum of $\lambda_g(\alpha = 0) = \sqrt{3/2} \approx 1.225$, which is substantially different from the normal value of $\lambda = 1.6$. These results suggest that other effects than geometric hindrance must be considered in the explanation of the effective diffusion constant. Personally I suspect that the observed maximum tortuosity comes from the regular geometry used in the simulations, and I have tested this hypothesis by the means of molecular dynamics (see section 5.2).
I first measured the self-diffusion constant of a liquid at some temperature and density, and later measured the self-diffusion constant of a liquid of the same density at the same temperature in a nanoporous matrix. The, using the definition of tortuosity $\lambda = \sqrt{\frac{D}{D^*}}$ I got $\lambda \simeq 1.41$. This is not 1.6 which it should be, but it is still larger than the estimated maximum value of $\lambda \leq 1.225$, and this is a rather rough estimate. The self-diffusion constant was measured using the Einstein relation, eq. (4), averaging the rms displacement of all movable atoms.



(a) Regular cubic lattice (b) octahedra

Figure 2: A cross section of the 3d lattices used in the simulations by Hrabětová and Nicholson [3]. The gray spaces between the geometric figures is meant to simulate the ECS. The authors used $20 \times 20 \times 20$ cubes in their simulations. Figure taken from Hrabětová and Nicholson.

4 Measurement techniques for brain diffusion characteristics

Measurement of the diffusion constant in the ECS can be done in 4 different ways, where one has the obvious advantage of not having to remove the brain from the skull. For in vitro measurements there main types of measurement are optical, and ion-sensitive micro-probes.

4.1 Optical measurements

In 1993 Nicholson and Tao developed a new method, the integrative optical imaging (IOI) method, to measure diffusion properties in the ECS [5, 6]. The basic approach is to eject a small volume ($\sim$ 1nL or less) of macromolecules labeled with a fluorescent tag into the tissue of interest (here the ECS), and record the spatial distribution of these molecules by a cooled charge-coupled device (CCD) camera every few seconds. This method also (naturally) requires the use of a microscope. The CCD camera transfers the pictures to a computer which calculates the effective diffusion constant. A comparison with unhindered diffusion is required, as is the case for the TMA measurements.
The IOI method has proved to work rather well, but it has one fundamental problem to overcome; the camera only takes pictures in 2d, and hence the slices used for these measurements must be optically thin. A further complication is that the diffusion should happen in 3d, but will be limited. On a brighter note, the uptake term in equation 11 is assumed to be zero ($k' = 0$) since the molecules are so large.
Today, there has been some research done on further developing the IOI method by replacing the traditional fluorescent dyes or molecules with quantum dots that label the tracer molecules [2]. The main advantages of using quantum dots as fluorescent tags are: Reduced photobleaching, which is the loss of fluorescence that occurs when dye molecules are exposed to light. Tunable emission spectra; the emitted frequency from a quantum dot is inversely proportional to its size meaning that one can chose the color of the probe. Finally, a quantum dot will also be brighter than normal fluorescent dyes.

4.2 TMA^+ measurements

All TMA measurements are usually done on brain slices kept under well controlled conditions. Though the method is invasive, it does not have to be done in vitro, but an in vivo measurement requires anesthetized animals and a lot of skill!
Tetramethylammonium (TMA^+) is the simplest quaternary ammonium cation consisting of four methyl groups attached to a central nitrogen atom, and is positively charged. Measurements using TMA^+ rely on the controlled release of very small amounts of TMA from a micro-pipette (using for example pressure ejection) into the tissue of interest, and measuring the corresponding concentration change some distance away (usually $\sim 100\mu$m). The change in concentration is measured by an appropriate ion-selective micro-electrode. Using ion-selective micro-electrodes we can measure the TMA concentration in time as well as space with the real time iontophoresis (RTI) method developed by Nicholson and Phillips in 1981 [] and get a resolution on the order of a minute.
Within the realm of TMA measurements there are a few variations. As suggested, we can measure both the spatial concentration distribution, making an analogous method to the radiotracer method, described below. One can also utilize the RTI methods to get a time resolution as well as a spatial one. A third possible method was developed by Chen and Nicholson in 2002 [1] using a sinusoidal source. Because the ions have to diffuse to the probe we will measure a phase lag in the oscillating steady state solution at the micro-electrode.

4.3 Radiotracer methods

The radiotracer methods are perhaps the most intuitive methods for measuring diffusion characteristics in the ECS, and were also the first methods applied quantitatively (1962 []). Although the method is not generally in use today because it requires interaction with radioactive substances (which one now tend to limit), the results of these early experiments are still used as verification because they offer an independent reference and the method is still sound. The

basic method was to perfuse the bilateral ventriculoscisternal cavities of an anesthetized animal, usually a dog or monkey, with a radiolabeled probe molecule for a longer period. Often several hours. After this period was over, the brain of the animal was quickly removed, frozen and sliced at $0.4 - 1$ mm thickness parallel to the perfusion. One could now measure the spatial distribution of radioactivity in each slice to determine the diffusion parameters D^* and α. As mentioned, modern health and safety measures limits the interaction with radioactive substances. It is also more and more frowned upon to use larger animals, like monkeys, in these kinds of experiments, and the TMA^+ measurements are just as good or better. The radiotracer methods are therefore not commonly used today.

4.4 Diffusion Tensor Imaging

This is a non-invasive measurement which has its obvious advantages in its ability to be used on living humans. Diffusion tensor magnetic resonance imaging (DTI) is, as the name suggests, a type of magnetic resonance imaging. In isotropic media, the diffusion process is fully described by a single scalar coefficient, the diffusion constant. With isotropy we mean that the medium in which the diffusion process is taking place is uniform in all directions which means that the diffusion will be equal in all directions. The ECS, however (and tissue in general) is anisotropic. In the presence of anisotropy, diffusion can no longer be characterized by a single scalar constant, but requires a tensor $\mathbf{D}$, which fully describes the mobility along each direction and the correlation between these directions [4]. The diffusion tensor is given in 12. We notice that it must be symmetric, meaning that we have $D_{ij} = D_{ji}$.

$$\mathbf{D} = \begin{pmatrix} D_{xx} & D_{xy} & D_{xz} \\ D_{yx} & D_{yy} & D_{yz} \\ D_{zx} & D_{zy} & D_{zz} \end{pmatrix} \tag{12}$$

DTI measures the self diffusion tensor of water molecules in the ECS using six or more gradients.

5 Other possible modeling approaches

In this part we will briefly look to other fields of study which might have similar problems to solve. The main issues of the established models for simulating diffusion in the ECS is the length scale on which it happens. The einstein relation is (to my knowledge) only derived for homogenous media, which means that the mean free path for a diffusing molecule must be much larger than the size of the molecule. It the ECS we are typically in a grey area with respect to the validity of the homogenous media assumption.
There are, however, some fields which operate with similar geometries within numerical statistical mechanics, and we will look closer at two possibly relevant fields here.

5.1 Random walks/flow on a percolating cluster

Percolation theory is the study of connectivity in random media. There are ways to study percolation, in this project we will only look briefly at the basics. The theory will be described in 2d, but it is trivially generalized to 3 or more dimensions.
We typically start with a matrix of random numbers drawn from the uniform distribution, and pick some porosity, $p \in [0, 1]$. For each site (entry) e_{ij} in the matrix we will now say that it is occupied if $e_{ij} \leq p$ and that two occupied sites are connected if they are neighbours (depending on what kind of neighbouring criteria we want). This gives us clusters of connected, occupied

sites with complex geometries, see Figure 3. If a cluster stretches from one side of the matrix to the other we say that we have percolation, and call that cluster the percolating cluster. Now, there are many interesting aspects of this kind of system in itself, but we would like to model diffusion in the ECS with this kind of system. To do this we can either simulate flow on the percolating cluster (which might not be a good model considering the absence of bulk flow in the ECS), or we can do Monte Carlo simulations of random walks on the percolating cluster. For a random walker we can easily measure the diffusion constant using equation (4) where $\langle r^2 \rangle$ denotes the rms displacement of the walker relative to its starting position.
Interestingly, percolation theory might offer an explanation to some of the observations done by Chen and Nicholson in 2000 (cited in section 5 of [3]) with respect to unexpected behaviour of λ under hyperosmotic stress. This stress increases α from 0.24 to 0.42, but λ remains more or less constant. Percolation theory tells us that as the porosity (analogous to α) increases, this will increase the mass (size) of the percolating cluster, but the contribution will be to the so-called dangling ends. These are the parts of the percolating cluster which, if removed, will not remove percolation.

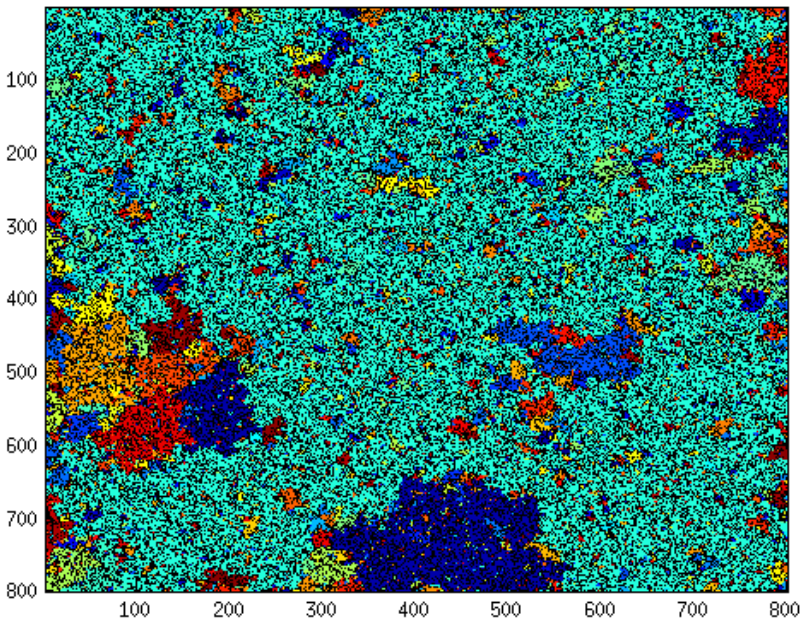


Figure 3: A percolating cluster (light blue) on an 800×800 matrix with porosity $p = 0.6$.

5.2 Molecular dynamics

Molecular dynamics is the simulation of the dynamics of atoms and molecules using classical, Newtonian mechanics in the sense that the molecules are affected by a potential, and that the sum of forces describes the dynamics. Their dynamics are then integrated forward in time, and used to describe for example flow in nanoporous media. This means that the system is fully described by the position and velocities of all the atoms. Again, there is a vast variety in the level of complexity here and we will only look at the simplest example, namely the Lennard-Jones potential, eq. (13). This potential consists of an r^{-12} term which denotes the Pauli repulsion at short ranges, and an r^{-6} long range, attractive Van Der Waals term. The relative distance between two atoms is denoted by r. The Lennard-Jones potential is derived

to simulate Argon in the Van Der Waals equation of state.

$$U = 4\epsilon \left[\left(\frac{\sigma}{r} \right)^{12} - \left(\frac{\sigma}{r} \right)^{6} \right] \tag{13}$$

It is possible to do simulations of flow in nanoporous materials using the Lennard-Jones potential, although it is a far from perfect model, by only allowing some of the atoms to move. We will then have a matrix of stationary atoms, simulating a wall (note that there will still be forces acting from these atoms), and a liquid inside this matrix.
The problem with applying molecular dynamics on our diffusion in ECS problem is the need for a potential that describes the system.

6 Outlook

As should now be apparent, diffusion in the ECS is a complex problem which is not fully understood. In my opinion the mathematical models are getting close to their explanatory limit while still being relatively intuitive. I base this assumption on the observed maximum value of the tortuosity of $\lambda \leq 1.225$, and assume that a sufficent model of λ will be rather complex in the existing framework. My suggestion is then to employ a combination of models of multiple length-scales, say the diffusion equation 11 for larger length scales, and some other model for the tortuosity based on smaller scale pysics. These models could be based on molecular dynamics, random walks, or something else. There are already techniques available for multi-scale simulations, but these are not necessarily very good and will probably need to be tailored to the task.

References

[1] Kevin C Chen and Charles Nicholson. "Measurement of diffusion parameters using a sinusoidal iontophoretic source in rat cortex". In: *Journal of Neuroscience Methods* 122.1 (2002), pp. 97–108.

[2] Maxime Dahan et al. "Diffusion dynamics of glycine receptors revealed by single-quantum dot tracking". In: *Science Signaling* 302.5644 (2003), p. 442.

[3] Sabina Hrabetová, Charles Nicholson, et al. "Contribution of dead-space microdomains to tortuosity of brain extracellular space." In: *Neurochemistry international* 45.4 (2004), p. 467.

[4] Denis Le Bihan et al. "Diffusion tensor imaging: concepts and applications". In: *Journal of magnetic resonance imaging* 13.4 (2001), pp. 534–546.

[5] Charles Nicholson. "Diffusion and related transport mechanisms in brain tissue". In: *Reports on progress in Physics* 64.7 (2001), p. 815.

[6] Charles Nicholson and Lian Tao. "Hindered diffusion of high molecular weight compounds in brain extracellular microenvironment measured with integrative optical imaging". In: *Biophysical journal* 65.6 (1993), pp. 2277–2290.

[7] David Sterratt et al. *Principles of computational modelling in neuroscience*. Cambridge University Press, 2011.