B1/B2t: Experimental and numerical models for the thermal selection of DNA

Thermal non-equilibrium systems likely played an essential role as a selection pressure for early life. We will recreate such a system in the laboratory and use temperature gradients to study the selective accumulation of DNA. The experiment will include a brief introduction to laboratory practice, construction of heat flow chambers with built-in microfluidics, accumulation of DNA in the constructed heat flow chambers, and subsequent analysis of the concentration distribution within the heat flow chamber. Comsol Multiphysics will be introduced as a numerical modeling tool so that the interns can replicate the experiment in a finite element simulation and freely explore the parameter space.

Inhalt

Introduction

In this experiment, you will get first contact with the effect of thermophoresis, i.e. the directed movement of solute molecules in a temperature gradient. This effect is already used in drug discovery to determine the in vitro efficacy of drugs. Still, in this experiment, we will use thermophoresis in combination with convection to concentrate dissolved DNA.

Before the experiment day, read through this guide and address the small **TASK**s (bold) in preparation for the initial discussion on the experiment day.

In the first part of the experiment, you will get a short laboratory introduction about the handling of pipettes and the setup of the experimental chambers. After assembling the chambers, they fill them with a DNA solution and apply a heat flux to the experimental chamber. The resulting convection of the liquid and thermophoresis of the dissolved DNA will cause it to accumulate at the bottom of the chamber. Since the accumulation process takes some time (about 3-4h), you will get an introduction to numerical modeling of this problem using Comsol software during this time. This will allow them to predict experimental results and explore the parameter space independently and quickly. Once the DNA has been accumulated, they freeze the heat flow chambers and slice the contents into uniform pieces, analyzing the concentration profile by Nanodrop spectroscopy for comparison with the numerical results.

References

Please read the following publications including supplementary information to familiarize yourself with the subject.

[1] Heat flows in rock cracks naturally optimize salt compositions for ribozymes
T. Matreux, K. Le Vay, A. Schmid, P. Aikkila, L. Belohlavek, A. Z. Çalışkanoğlu, E. Salibi, A. Kühnlein, C. Springsklee, B. Scheu, D. B. Dingwell, D. Braun, H. Mutschler, C. B. Mast, **Nature Chemistry** doi.org/10.1038/s41557-021-00772-5 (2021)

[2] Formation mechanism of thermally controlled pH gradients
Thomas Matreux, Bernhard Altaner, Johannes Raith, Dieter Braun, Christof Mast and Ulrich Gerland, **Communications Physics** doi.org/10.1038/s42005-023-01126-y (2023)

[3] Escalation of Polymerization in a Thermal Gradient
Christof B. Mast, Severin Schink, Ulrich Gerland and Dieter Braun
PNAS 110, 8030-8035 (2013), doi:10.1073/pnas.1303222110

[4] Zur Theorie des Clusiusschen Trennungsverfahrens
P. Debye, **Annalen der Physik**. 5, 36 (1939), doi:10.1002/andp.19394280310

[5] Why molecules move along a temperature gradient
Stefan Duhr and Dieter Braun**, PNAS** 103, 19678–19682 (2006), doi:10.1073/pnas.0603873103

Theory and model

Thermophoresis

A way to understand thermophoresis of charged molecules in fluids is given by Braun [5] with the Assumption of a local thermodynamic equilibrium with moderate thermal gradients ∇T. In this regime, we describe the movement v_T of dissolved particles linearly by defining the thermodiffusion coefficient $D_T = -v_T/\nabla T$. Applying a static temperature gradient in a solution causes solutes (e.g. DNA) to drift due to their specific thermophoresis. The resulting difference in concentration is counteracted by the diffusion of the solutes $j_D = -\nabla c \cdot D$, where ∇c denotes the concentration gradient and D the solute- and solvent-specific diffusion coefficient. Together, the flow sums up to:

(Eq. 1) $\boldsymbol{j}_{total} = -D \cdot \nabla c - c \cdot D_T \cdot \nabla T$

After some time, a steady state will be reached at which diffusion and thermophoresis cancel each other, yielding a static concentration profile:

(Eq. 2)   **TASK**: *derive an equation for the steady-state concentration profile. Discuss the meaning of the value $S_T = D_T/D$, also called the "Soret-coefficient ". Also, estimate typical values for the depletion/accumulation, given a Soret coefficient of $S_T = 0.01\frac{1}{K}$ and a temperature difference of $\Delta T = 10K$.*

A simple description for the size of the Soret coefficient is given in [5], assuming a moderate temperature gradient (where the temperature along a solute particle does not change significantly) and hence, a local equilibrium condition. Accordingly, the concentration ratio between two locations x_1 and x_2 at which a free energy difference ΔG persists, is given by a Boltzmann distribution:

(Eq. 3) $\frac{c(x_1)}{c(x_2)} = \exp(-\Delta G/kT)$

with k the Boltzmann constant and T the absolute temperature. To calculate ΔG, we for now, only consider the ionic contribution to thermophoresis, also called the capacitor effect. The thermophoretic motion of charged solutes (such as DNA) in salty solutions is triggered here by counterions arranging themselves around the solute (according to the Debye Hückel theory) and being more or less thermally disturbed depending on the temperature. At high temperatures/strong disturbances, they are less able to shield the solute's charge, creating an electric field. At low temperatures, good shielding occurs, resulting in a low field strength due to the solute charge. The energy of this field can be calculated from the energy density of the electric field for a spherical capacitor. With the temperature difference ΔT between the locations x_1 and x_2, where the Debye-Hückel length $\lambda_{DH}(T)$ is correspondingly different, the following derivation is thus obtained:

(Eq. 4) $\frac{\Delta G}{\Delta T} = \frac{\delta(Q_{eff}^2 \lambda_{DH}/2A\epsilon\epsilon_0)}{\delta T}$

With $\lambda_{DB} = \sqrt{\frac{\epsilon(T)\epsilon_0 kT}{2ec_s}}$, with ϵ the temperature-dependent permittivity of the solvent, e the charge of one electron, and c_s the salt concentration.

TASK: *Research on the temperature dependence of the relative permittivity of water. How does the Debye-Hückel length change when the temperature is raised/lowered?*

By comparing Eq. 3 and Eq. 2, we obtain an equation for the capacitor-effect-related Soret coefficient $S_{T,CE}$:

(Eq. 5) $S_{T,CE} = \frac{dG}{dT} \cdot \frac{1}{kT}$

By using Eq. 5 and Eq. 4, this leads to

(Eq. 6) $\qquad S_{T,CE} = \frac{A}{kT} \cdot \left(\frac{\beta \sigma_{eff}^2 \lambda_{DH}}{4\epsilon\epsilon_0 T} \right)$

Introducing the temperature dependence $\beta \equiv 1 - \frac{T}{\epsilon} \cdot \frac{\delta\epsilon}{\delta T}$ of the permittivity ϵ and the charge density $\sigma_{eff} = \frac{Q}{A}$. The second dominant contribution to thermophoresis is the temperature dependence of the hydration entropy $s_{hyd} = S_{hyd}/A$, with which we get a complete expression for S_T:

(Eq. 7) $\qquad S_T = \frac{A}{kT} \cdot \left(-s_{hyd} + \frac{\beta \sigma_{eff}^2 \lambda_{DB}}{4\epsilon\epsilon_0 T} \right)$

TASK: *Discuss what hydration entropy means and how it could affect temperature and the interaction with the solute particle.*

TASK: *Do you expect an increase or decrease S_T with a rising temperature T?*
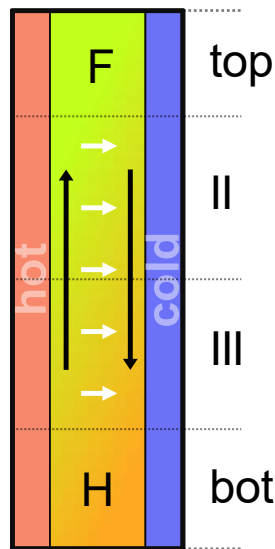

Figure 1. Schematic of thermogravitational accumulation: A liquid-filled gap between a heated (red) and a cooled (blue) plate leads to concurrent fluid convection (black arrows) and solute thermophoresis (white arrows). Both effects result in the effective accumulation of solute compounds with strong thermophoresis (compound H), while compounds with weak or no thermophoresis will remain homogeneously distribute inside the volume.

Thermogravitational accumulation

Thermophoresis is, however, only one component of thermogravitational accumulation. The concentration profile described by Equ. 2 only provides minor changes in the absolute concentration of typical solutes ($S_T \sim 0.1 - 0.001 \frac{1}{K}$), which requires sophisticated fluorescent labeling to provide a good signal/noise ratio in estimating the local concentrations. Interestingly, the effect gets amplified by the thermally induced fluid convection interplay. As the solutes move along the temperature gradient by thermophoresis (from hot to cold $S_T > 0$), they will be pushed into an unidirectional flow field, dragging them toward one edge of the chamber (bottom-cold edge for $S_T > 0$). This effect allows solutes to be concentrated (bottom) or depleted (top) over several orders of magnitude. To calculate the convection, we start with a Navier-Stokes model:

(Eq. 8) $\qquad \rho\dot{\boldsymbol{u}} = -\nabla p + \eta\Delta\boldsymbol{u} + (\eta + v)\nabla(\nabla \cdot \boldsymbol{u}) + \boldsymbol{f}$

With the fluid density $\rho(\boldsymbol{x})$, the dynamic viscosity η, the kinematic viscosity $v \equiv \eta/\rho$, and the volume force density $\boldsymbol{f} \equiv \boldsymbol{F}(\boldsymbol{x})/V$, and p the local pressure. The latter is simply defined by the temperature-

dependent density fluctuations inside the chamber, leading to $f_y = -\frac{\rho(x)}{\rho_0} \cdot g$, with g the gravitational acceleration, and ρ_0 an arbitrary reference density of the solvent.

The results show a laminar convection flow $\boldsymbol{u}$ inside the cell, which contributes to the solute movement as it drags it with it. Neglecting the turning points, the velocity of the fluid in the z-direction yields:

(Eq. 9) $\qquad u_z = -\frac{\Delta T \beta g \rho_0}{6\eta} \cdot a^2 \cdot \left(\frac{1}{2} \cdot \frac{x}{a} - \frac{\frac{3}{2}x^2}{a^2} + \frac{x^3}{a^3} \right),$

With x being the coordinate in the direction of the width a of the chamber, and $\beta = -\frac{1}{\rho_0} \cdot \frac{\delta\rho}{\delta T}$ the local temperature dependency of the fluid density ρ. This is achieved by solving the time-dependent drift-diffusion equation

(Eq. 10) $\qquad \frac{\delta c}{\delta t} = \nabla \cdot [D\nabla c - (\boldsymbol{u} - S_T \cdot D\nabla T)c]$

TASK: *Discuss the different contributions in Eq. 10. Assume a DNA strand with a length $n = 50$ nucleotides within a chamber of width $a = 170\mu m$, and a temperature difference of $\Delta T = 20K$ between the hot and the cold side. The diffusion and Soret coefficient can be approximated by $D(n) = 643 \cdot n^{-0.46}$ and $S_T = (5.3 + 5.7 \cdot n^{0.73}) \cdot 10^{-3} K^{-1}$. Calculate the maximal velocity $u_{z,max}$ inside the chamber, as well as the drift velocity v_T by thermophoresis.*

Using the previous equations, Debye [4] worked out a steady state solution to the thermogravitational accumulation problem, resulting in the concentration profile over the height y of the chamber:

(Eq. 11) $\qquad c(y, t \to \infty) = \exp\left(-\frac{\frac{q}{120}}{1+\frac{q^2}{10080}} \cdot S_T \cdot \Delta T \cdot \frac{y}{a} \right),$

With the quality factor $q \equiv \frac{\Delta T \beta g \rho_0}{6\eta} \cdot \frac{a^3}{D}$.

TASK: Determine the optimal (=extremal) value of the pre-factor $\alpha \equiv \frac{\frac{q}{120}}{1+\frac{q^2}{10080}}$ used in Eq. 11. What are the maximal possible accumulation ratios c_{max} within a $20mm$ high chamber ($y = 20mm$), given the settings from the previous task? Discuss the effective dependence of Eq. 1 to the temperature difference ΔT.

Introduction to COMSOL

Comsol is a software for the numerical solution of complex differential equation systems. These usually describe physical problems (diffusion, drift, fluid motion) but also chemical reactions or couplings of several such problems. As you can read in reference [4], the analytical description of thermophoretic accumulation is only approximate, limiting its predictive power to the. For this reason, in the following, we will generate a complete model that solves thermophoretic drift, diffusion, and fluid motion simultaneously and time-dependently.

User interface

Start-up procedure:

- Start Comsol
- Start the "Model Wizard"
- Choose "2D"
- Search for the physics modules and add them to the model:
    - Heat Transfer in Solids (ht)
    - Laminar flow (spf)
   - Transport of Diluted Species (tds)

    Click "Done".

- Choose "Stationary" study by double-clicking and clicking "done".

You now see the user interface of Comsol with a base model, including all physics modules necessary to solve heat conduction, fluid motion, and the transport of dilute species (DNA in our case).

The work sequence in comsol is as follows:

1. Definition of global parameters:
   Here, we will set important variables, such as temperature difference, Soret coefficients etc.
2. Definition of geometry
   A geometrical shape of our reaction vessel will be created.
3. Definition of Component couplings
   We will define base operations on different parts of our geometry (e.g. calculating the average of a certain sub-volume of our reaction vessel).
4. Selection of an assignment of materials
   We will select the material (here: water) to be modeled.
5. Refinement of physics modules
   We will set all necessary parameters and boundary conditions to the **ht**, **spf** and **tds** modules.
6. Optimization of the mesh
   The mesh is a subdivision of our previous geometry into a finite number of sub-volume elements. All differential equations will be solved numerically for each of these elements.
7. Definition of study steps:
   We will set how the respective physics modules are to be solved (steady state, time-dependent).
8. Definition of result nodes:
   In the last step, we will define plots and tables to present and export the modeled data.
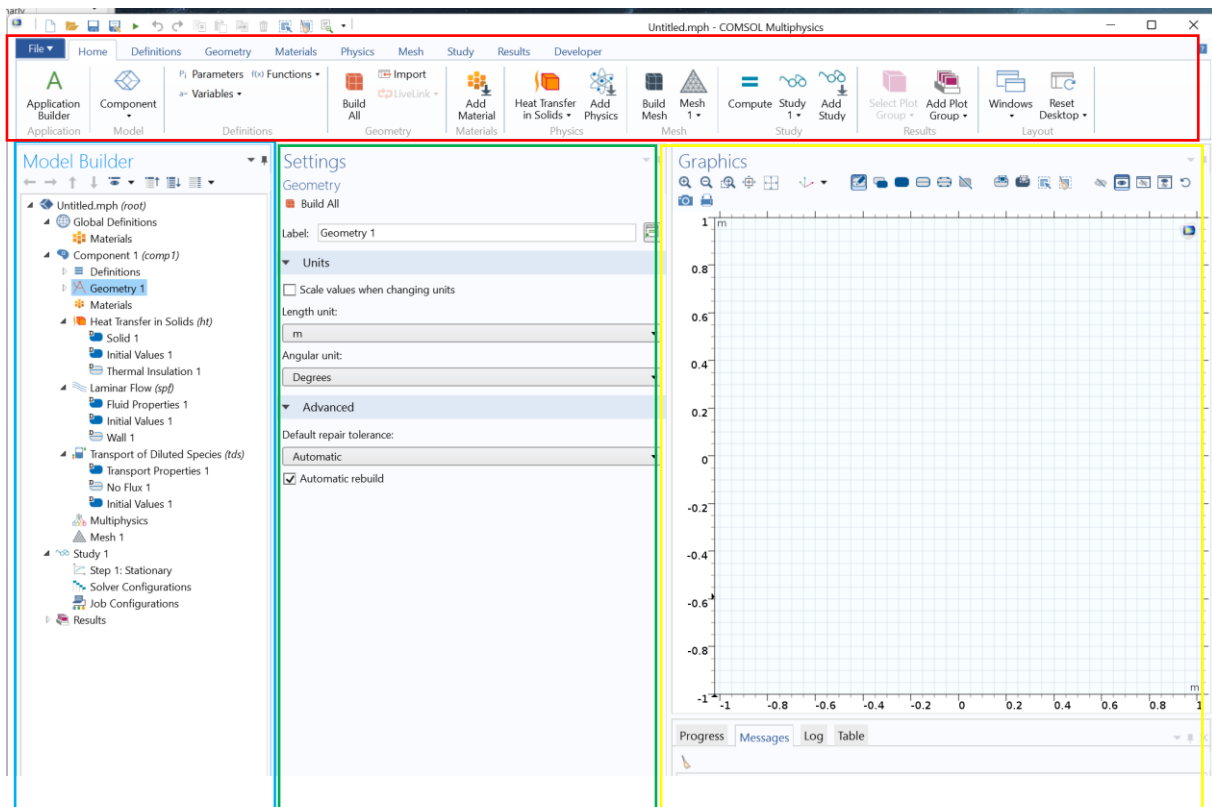
The GUI is separated into at least 4 parts:

- Red: Quick access to commands. Here, the most used tools are presented via icons. All commands are also accessible via context menus within the model builder (blue).
- Blue/model builder: steps 1-8 of the above-described sequence are represented by a tree structure. Here, the complete model is defined.
- Green/settings and properties: Upon selecting a node within the model builder, the settings/properties section shows all parameters that can be defined for the selected node.
- Yellow/graphics: Display of geometry features and results (plots/maps).

Step by step guide for a coupled Multiphysics simulation

1. Definition of global parameters
   - Open the "Global Definitions" node and add a "Parameter" node using the context-menu (right-click on "Global Definitions").
   - Add parameters:

Name	Expression
STn	(5.3+5.7*n^0.73)*1e-3[1/K]
Dn	643*n^(-0.46)[um^2/s]
DTn	STn*Dn
n	100
Tcold	26[degC]
Thot	Tcold+deltaT
Width	0.17[mm]
ChannelLe...	15[mm]
deltaT	90[K]
c_init	1[uM]
Height	35[mm]
Partitions	4

Note, that all introduced new parameters can be used within the "Expressions" field as variables (e.g. "n", the length of our DNA).

- o Change the parameters to match the settings used in your experiment.

2. Definition of geometry
   - o Open the Nodes "Component 1" -> "Geometry 1" and add a rectangle using the context menu.
   - o Set the width to "Width" and the height to "Height" within the "Settings" section. Both variables, "Width and "Height" were defined within step 1. Press "build all objects" to update the graphics representation.
   - o Add 4 more rectangles that devide the rectangle into equal parts by parametrizations (e.g. not using actual numbers, but parameters that you add in "Global Definition" -> "Parameters"). This has the advantage of quickly changing the model without fiddling deep inside the model-builder tree.

3. Definition of component couplings
   - o Open the nodes "Component 1" -> "Definitions" and add 4x "Average" nodes using the context menu ("Component Couplings"->"Average").
   - o For each of the just created Average nodes, select one of the 4 above created rectangles. We will later use these couplings to calculate the average concentration of each rectangle.
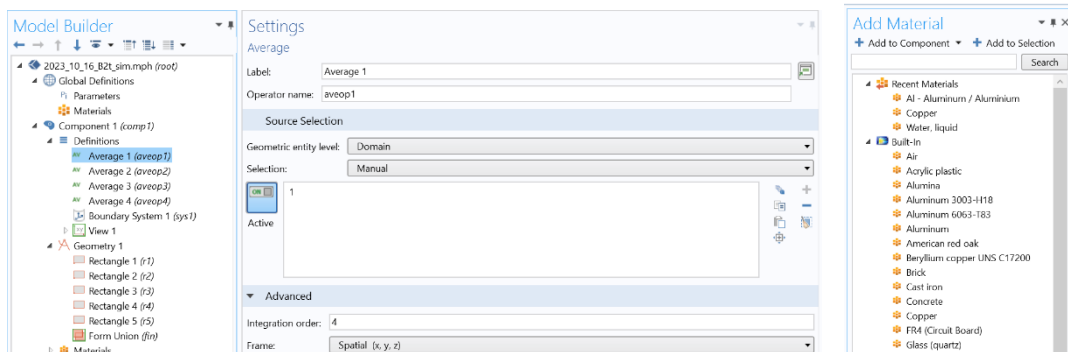

4. Selection of an assignment of materials
   - o Open the nodes "Component 1" -> "Materials" and select "Add material from library" by using the context menu.
   - o In the just appeared "Add Material" section, open the node "build-in" and select "Water".

5. Refinement of physics modules
   - o Set boundary conditions for the heat transfer module:
     - ▪ Add 2x Temperature boundary condition by using the context menu on the "Heat Transfer in Solids" node.
     - ▪ Set the temperature of the "Temperature 1" node to "Tcold" and the temperature of the "Temperature 2" node to "Thot".
       Hint: Most physics-nodes provide an "Equation" field within the Settings section that provides more insight on what this node actually does.
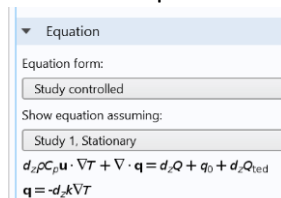
- Select the complete left and right side for the both Temperature boundary conditions, respectively, to implement a horizontal temperature gradient.

o Set boundary conditions of the laminar flow module:
- Add the subnodes "Volume Force" and "Pressure point constraint" (from the context menu on "Laminar Flow" -> "Points")
- Select the just-created subnode "Volume Force 1" and select "All domains". Add the expression "-g_const*mat1.def.rho" to the y-component of the volume force.

▼ Volume Force
Volume force:
F   0
-g_const*mat1.def.rho
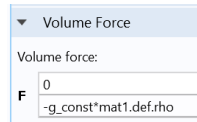
Explanation: "g_const" is a comsol-native value of the gravitational acceleration. "mat1.def.rho" accesses the property "rho" (density) of "mat1" which is the name of our only material, water, which we just added above. Try to find the "rho" sub-node within the material "mat1" by diving down the model-builder tree (node: "Materials").

- Select the node "Pressure Point Constraint 1" and select an arbitrary point in the graphics section. The selected point will change color from black to blue.
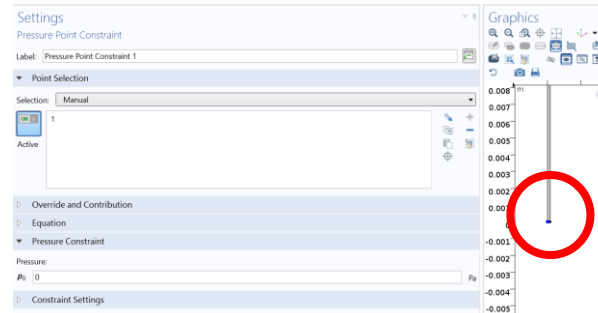
- Select the "Laminar Flow" node and make sure that "Compressible flow" is selected in the Settings section under "physical model"
TASK: Why needs water (mostly assumed incompressible) set as compressible fluid here? **HINT**: check how the equation changes in the settings section -> "Equation".

o Set boundary conditions of the Transport of diluted species module:
- Click on the "eye"-icon at the top of the model builder and activate the Advanced Options for Physics, Study and Results.
- Select the "Transport of Diluted species (tds)" - node and make sure that "Nonconservative form" is selected in the settings section under "Advanced Settings". Check how the equations change upon changing this parameter.
- Select the tds-subnode "Transport Properties" and make sure "Temperature (ht) ist selected in the settings section under "Model Input"->"Temperature"
- Under "Model Input", also change the velocity field under "Convection".

▼ Convection
Velocity field:
u   User defined
u-DTn*Tx
v-DTn*Ty
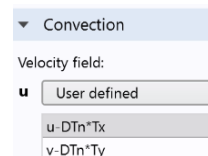
This will set the drift part of the drift-diffusion-equation (also see Eq. 10 and the equations shown in comsol under "Equation"). u and v are the x and y component of the fluid velocity vector **u**. We add the product $-D_T \cdot \nabla T$ which represents the thermophoretic drift velocity. Tx and Ty are (hardcoded

by COMSOL) the x and y component of the temperature gradient ∇T, respectively.

- Under Model Input, also make sure to select "Water, liquid (mat1)" in the Diffusion section and replace the diffusion coefficient value with Dn.

- Right click on the tds-node to add a "Flux" boundary conditions through the context-menu and select it.
- In the settings section, select "All boundaries" under Boundary selection and "General inward flux" under "Inward Flux". Make also sure to check "Species c".
- Add the expression "-c*(-tds.u*tds.nxmesh-tds.v*tds.nymesh-tds.w*tds.nzmesh)" in the field "$J_{0,c}$". This is required as the default "No Flux 1" boundary condition only set the diffusive fluxes to zero at the boundaries (compare with a look at the equation view), but not the drift part of Eq. 10. Above expression is the scalar product of the drift part of Eq. 10 and the unit-vector perpendicular to the boundaries of the reaction vessel (tds.nx(y)mesh).
- Select the node "Transport of Diluted Species (tds)" -> "Initial Values 1" and set the initial concentration to "c_init" (as defined in the parameters).
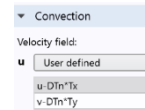
6. Optimization of the mesh
   o Select the "Mesh" node and select the Element size "Fine" (you can change this also later on).

7. Definition of study steps:
   o Open the "Study" node and add one more Stationary Solver using the context menu -> "Study Steps" -> "Stationary" -> "Stationary" and one Time dependent Solver.
   o Select "Step 1: Stationary" and make sure only the "Heat Transfer in Fluids (ht)" is checked within the settings section under "Physics and Variables Selection".
   o Make sure that in the "Values of Dependent Variables" section, "User controlled" (Settings), "Initial expression" (Method) and "Zero solution" (Study) is selected for both "Initial values of variables solved for" and "Values of variables not solved for".
   o Select "Step 2: Stationary" and make sure only the "Laminar flow (spf)" is checked. Make sure that in the "Values of Dependent Variables" section, "User controlled" (Settings), "Initial expression" (Method) and "Zero solution" (Study) is selected for "Initial values of variables solved for". In the "Values of variables **not** solved for", set the fields as shown here:

   o Select "Step 3: Time Dependent" and make sure only "Transport of Diluted Species (tds)" is checked. Set the solution-time via clicking on the range-icon, and use the mode "Number of values", starting from 0, stopping at the experimental time (lets start with "3[h]" here) and set the number of values to 50.
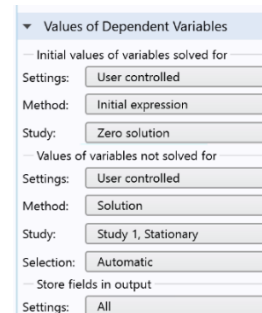
Now, save the file and solve the model by clicking "Compute" in the settings section of the "Study 1" node. The solution should take a few minutes.

8. Definition of result nodes

Default result nodes are automatically created. Select them ("Temperature", "Velocity", "Concentration"..) and examine e.g. the maximal concentration. Also play around with changing color scales etc.

- o To extract the average concentration of the previously defined sub-volumes, create a "Point Evaluation" node using the context menu on the "Derived Values" node.
- o Select one arbitrary point on the geometry and add the expressions:

Expression	Unit
aveop1(c)	uM
aveop2(c)	uM
aveop3(c)	uM
aveop4(c)	uM

The "aveop1/2/3/4" functions were defined by the component couplings above. The argument of these functions are any value calculated by COMSOL (here, the concentration c) and return the average of this value for the volume set for the respective component coupling.

- o To get the results for the average values, click on "Evaluate" in the Settings section of the "Point Evaluation 1" node. The results are displayed in a table on the right side of the program:

Time (s)	Average 1 (uM), Point: 1	Average 2 (uM), Point: 1	Average 3 (uM)
0	1.0011897641170533	1.0011779054059544	1.001177922059
220.40816326530611	-8.293055000405792	4.3711983791814495	1.788790611988
440.81632653061223	-65.66273218418513	3.4404696879265417	0.673693119512
661.2244897959183	36.293566988070076	1.6939090676888977	0.174721228065
881.6326530612245	34.13200291974526	1.083330106207627	0.074056337747
1102.0408163265306	34.13200291974526	0.6515630724935636	0.036087919829
1322.4489795918366	-2.9689710519018644	0.2197960387795005	-0.00188049808
1542.857142857143	-5.914516112118282	0.07564704375499884	-0.01000657847
1763.265306122449	-3.029643310019899	0.03895973351560854	-0.00698277451
1983.673469387755	-0.14477050792152435	0.0022724232762180636	-0.00395897054

- o You can either copy this data to a text file or set an table output by selecting "Tables" -> "Table 1" and set "On file" in the settings section under "Storage" -> "Store table". Define a filename and click "Update".
- o Now, you are ready to perform the tasks defined below.

Tasks

- Calculate the thermophoretic accumulation profile using the just assembled COMSOL model.
- Vary parameters of DNA length n, Temperature gradient ∇T, chamber width a and experiment time and calculate the accumulation profiles for each parameter sweep.
- If there is enough time:
  - o Explore further possibilities of COMSOL: Add a reaction node, disable the thermophoretic movement and calculate a chemical reaction of your choice (e.g. A+A <-> B).
  - o Vary the mesh size and quantify the change to the result.
- Plot & export the data and discuss it in the experimental protocol

Experiment and setup

Schedule

- Before the experiment day:
  - Read papers [1] - [5] (for overview, we won't discuss these in extreme detail)
  - Solve and discuss the tasks in the theory section above.
  - Read over the COMSOL-introduction.
- During the experiment day:
  - 1st colloquium: discussion of the theory-tasks & referenced papers
  - Introduction + starting the experiment (**with guidance**):
    - Pipetting-refresher + preparation of 50mer DNA
    - Assembling the heat-flow thamber & filling of sample
    - Starting the experiment
  - During experiment time (**alone**):
    - Live introduction to COMSOL
    - Solve the tasks within the COMSOL section above
    - Export the results from COMSOL for pos-analysis
  - After the experiment has finished (**with guidance**):
    - Freeze-extract DNA from heat flow chambers.
    - Measure concentration profile using the Nanodrop spectrometer.
- After the experiment day:
  - Prepare a detailed protocol that summarizes:
    - Thermophoretic accumulation – principles, results from theory-TASKS
    - Experimental protocol (steps taken, results measured)
    - Comparison with results from theory and COMSOL
    - Conclusion.
  - Submit protocol to examiner & 2nd colloquium (to discuss your written protocol).