QUICK GUIDE: SIMUL 5 (FIRST RELEASE, January 2007)

Vlastimil Hruska (vlastimilhruska@gmail.com), Bohuslav Gas (gas@natur.cuni.cz)

This is the first draft of the Simul 5 manual and it did not undergo larger text corrections so we apologize for some text errors or inaccurate formulations. The program Simul 5 and its manual will be updated - check our website www.natur.cuni.cz/gas.

The creation of Simul 5 manual was supported by the University Development Foundation (FRVS, Czech Republic), Grant number 733/2006. The support is gratefully acknowledged.

Program Simul 5 contains several integrated tools that help with various kinds of electrophoretic simulations. This tools and windows can be divided into several categories:

- Definition of **composition** and initial distribution of concentrations and mobilities.
- Setup of **running parameters**.
- Modifications of electrophoretic model (Ionic strength correction, in-build electroosmotic flow,...).
- **Exporting** (CSV file) and data evaluation tools.
- Rich **file management**: saving and opening of project files (SNA), composition files (CMP), simulation progress files (PSQ), detector files (DET).

Note that for new users all these integrated tools might make Simul environment relatively difficult to understand but usually most of these tools and variables can be kept as they are.

Used terms and units

Editable inputs 

User editable boxes. Changes are confirmed by <ENTER> button.

Dimmed (inactive) Buttons 

The button's availability is dependent on actual state of application (running simulation, playing PSQ file).

Right-click popup menu and set-up dialogs 
Some objects have popup menu (constituent in Composition list, list of graph profiles) or set-up dialog (Diffusion conditioning) accessible by right-click on the object. Objects are visualized by a change of the mouse cursor to a pointing-hand.

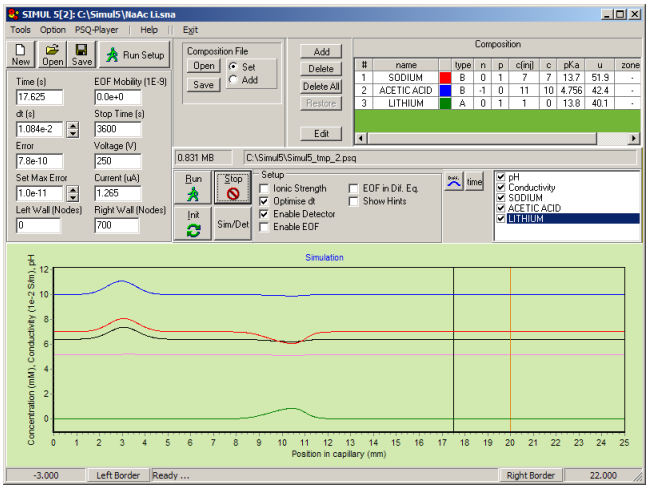
Units
Mobility unit = 10^{-9} $m^2.V^{-1}.s^{-1}$
uA = 10^{-6} A (micro-ampere)
mM = $mol.m^{-3}$
Node = spatial discrete unit (discrete division of capillary).
Step = one computation cycle where time is increased by one dt increment
1 MB = 1048576 Bytes (file size)

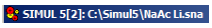
Note: the word "*check*" or "*uncheck*" means "to mark" or "to unmark" (usually in the context with checkboxes 

or radio-buttons 

).

Main Window



Title of the Main window 

Contains a name and a version of the program and a location of opened/saved project file. If more than one application is opened its identification number is showed in brackets [].

Main window menu

Menu item "Tools" contains a tool for a data exporting ("Export All to CSV"), defining composition ("Ampholines Wizard"), reduction of detector data size ("Detector Tools") and for processing simulated data ("Moments"). Item "Option" shows option window, which has mostly environment settings. "PSQ Player" is tool for playing recorded simulation PSQ-files. "Help" item contains "**Introduction**" with brief information about Simul concept, "**Simul 5 Guide**", which opens this manual and "**About**" dialog.

Status Bar 

Status bar shows in order: a position of the left corner with respect to "Zero position" defined in Option window, a status of application (running, ready,...) and a percentual progress of a running simulation, a duration of one simulation step, real time estimation, spent real time and a position of the right corner with respect to "Zero position".

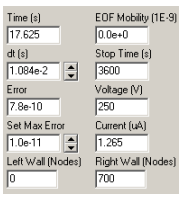
Project file management buttons (SNA file extension) 

The button "**New**" clears an actual simulation including all constituents. "**Open**" shows open dialog and opens project file (SNA) and also composition file (CMP) containing information about constituents, detector file (DET) if available and copy PSQ file. If checkbox "Ask before transfer PSQ" in Option is checked user is asked question before transfer of PSQ, which gives chance to refuse copying PSQ file when its size is too extensive. If PSQ file is copied and user continues in simulation new data are appended to the end of PSQ file otherwise a new PSQ file is created. The button "**Save**" shows save dialog and saves all project files (SNA, CMP, PSQ, DET).

Composition file (CMP file extension)

CMP file is automatically opened and saved with project SNA file. This tool enables handling with CMP file separately. Button "**Open**" shows open dialog and opens CMP file using "**Set**" or "**Add**" mode. "**Set**" mode deletes all actual constituents and transfers all constituent from the CMP file. "**Add**" mode keeps all actual constituent in project and transfers only constituents, which have a different name than actual constituents. Button "**Save**" saves actual composition to a CMP file. A typical use of this tool is for user-defined constituents, which are not in in-build database or for constituents, which are defined by "Advanced Setup".

Main window controls



"**Time (s)**" displays actual simulation time in seconds. A simulation time step dt is given by "**dt (s)**" edit. It can be managed by program automatically if checkbox "**Optimise dt**" located in "Setup" panel of the Main window is checked. This procedure is connected with "**Error**" display, which shows an error of the simulation and "**Set Max Error**" edit defining desired interval of error (0.1 to 10 multiple around the value of Set Max Error) - for more information see "Automatic dt optimization". "**Left Wall (Nodes)**" and "**Right Wall (Nodes)**" define a position of borders of the simulated space, which is currently calculated - for more information see "Walls". "**EOF Mobility (1E-9)**" contains mobility value of electroosmotic flow in 10^{-9} $m^2.V^{-1}.s^{-1}$ units - see "Simulation of EOF". "**Stop Time (s)**" is a final time of the simulation. The percentual progress is shown in the Status bar of the Main window. "**Voltage (V)**" and "**Current (uA)**" display actual voltage and current, respectively. The "Power Mode" in Run Setup window

defines which one can be edited and which one is dependent quantity. To display *Time* and *dt* value in not-rounded format click on "time" botton 

.

Automatic dt optimisation

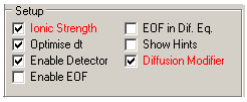
After checking checkbox "**Optimise dt**" in the Main window Setup program automatically try to determine suitable "**dt (s)**" value. It uses error guess of Predictor-Corrector procedure calculating numerical progress of simulated electrophoretic system. Error displayed in "**Error**" is kept in the range of 0.1 to 10 multiple of value in the "**Set Max Error**" control. If error is higher or lower than limits the actual dt is multiplied or divided by "1 + *dt_increment*", where *dt_increment* value is given in Run Setup window by "**dt Optimisation Increment**" control (by default set to 0.25). The change of dt value temporally increase "Error" therefore period of change should be chosen carefully. The period is given by "**Optimisation Interval (Steps)**" located in "Run Setup" window (by default every 150th step).

Simulation of EOF (electroosmotic flow) 

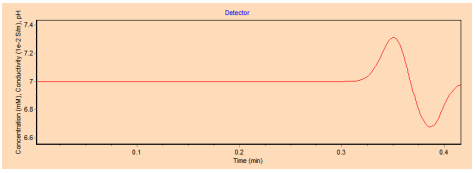
Mobility of EOF is given by "**EOF Mobility (1E-9)**" in 10^{-9} $m^2.V^{-1}.s^{-1}$ units. EOF velocity is calculated by multiplying EOF mobility by value of actual voltage divided by total capillary length. Total capillary length is a sum of "**Capillary Length**" and "**Additional Length**" values both defined in the Run Setup window. EOF mobility is used in two different ways how to simulate EOF. First one simulates EOF by moving detector position, which affects only detector data but not simulation itself. It is activated by non-zero EOF mobility and "**Enable Detector**" and "**Enable EOF**" checkboxes located in "Setup" panel of the Main window. The procedure is available only in the mode of constant voltage - see Power Mode. The second way includes EOF directly to the numerical model and causes that the simulated part of capillary is shifted. It is activated by "EOF in Dif. Eq." checkbox located also in "Setup" panel of the Main window.

Simulation Buttons


"**Run**" button starts or continues simulation and "**Stop**" button stops simulation. "**Init**" button initialise simulation, shows initial distribution of concentration, pH and conductivity, calculates current or voltage, sets walls to the left and right corners if "**Initiate walls position**" in Run Setup window is checked and process some other actions. "**Sim/Det**" button switches graph between Simulation and Detector profiles.

Setup (Main window)


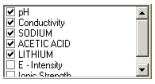
"**Ionic Strength**" performs ionic strength correction of thermodynamic dissociation constants and limit ionic mobilities (the McInnes approximation of the Debye-Hückel theory, the Onsager and Fuoss model). It becomes active after the initiation of simulation ("**Init**" button) and its text turns red. "**Optimise dt**" enables Automatic dt optimisation. "**Enable Detector**" shows detector line in the graph and acquires data in its position to the DET file. Detector position is given by "**Detector Position (mm)**" in Run Setup window. Period of saving is defined by "**Detector Input (Steps)**" in the Run Setup. "**Enable EOF**" and "**EOF in Dif. Eq.**" simulate EOF - see Simulation of EOF. Some components or buttons of the application have a floating help text (hint). This function is enabled and disabled by "**Show Hints**" checkbox.

Simulation and Detector Graph


Displays Simulation and Detector profiles - switched by "**Sim/Det**" button. Concentration profiles are in mM (mol/m^3) units, pH profile is in standard pH scale, conductivity profile is in 10^{-2} S/m, voltage profile (detector only) is in kV, current profile is in μA, intensity of electric field is in kV/m and ionic strength is in mM. Spatial axis of simulation graph (x-scale) is in metres (m) or millimetres (mm) - given by choice of "**X-axis unit**" in the Option window. The time axis is in minutes (min). Thickness (pen size) of profile lines in the graph can be changed by "**Profile Line Width (Points)**" in the Option window. To export profiles as CSV file click on "Tools"->"Export All to CSV" in the main menu. There are several ways how to handle with profiles by clicking on graph:

- Profiles can be zoomed by **left-click and dragging** to the right-down corner. Dragging to the opposite direction cancels zoom.
- **Right-click and dragging** allows to move with a graph content.

- **Left-click** on a profile shows Graph-Click Information Table. The tolerance of the click on the profile can be set by "**Profile Click Tolerance (Points)**" in the Option window.
- **Double right-click** on the graph shows all profiles in the full scales.



To hide or unhide specific profile check or uncheck a profile name in the list of the graph profiles. **Double left-click** on a profile name focuses chosen profile in the graph. **Right-click** on the list of graph profiles shows popup menu with choices: to "**Check all**" and "**Uncheck all**" names in the list. Other possibility how to zoom a specific constituent is a tool, which enables zoom a specific profile around its baseline. It is accessible by **right-click on constituent** in the composition list, choose "**Focus**" and then set a concentration radius around its baseline.

Graph-Click on Profile Information Table

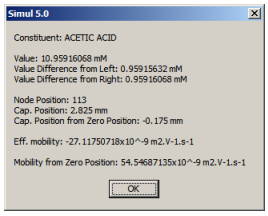
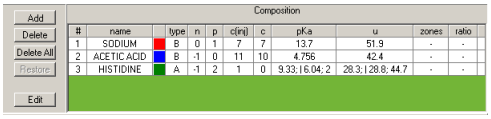


Table is shown by left-click on the graph profile. It contains following information: profile **name**, **value** and its **difference** from the left and right corner (if at least one corner value is equal to an undisturbed baseline (in CZE) then the difference is a response at the clicked point), **node position** (indexed from 0 to N) and **position** in the capillary. **Capillary position from zero position** is position relative to the user-defined "**Zero position**" (by default at the Injection site position), which can be defined in the Option window. **Mobility from Zero Position** is mobility calculated from simulation time, distance of the clicked point from the Zero position, actual voltage and the total capillary length (capillary length + additional length). Typical use of this is for the CZE mode where the Zero position is set to the initial position of the centre of a sample plug and then a click on a peak gives simulated mobility. If the clicked profile belongs to the constituent then the Table also contains **effective mobility** of the constituent in the given point (given by local pH).

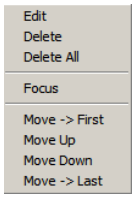
Diffusion Conditioning Tool

Left-click on "**Diffusion Conditioning**"  button starts diffusion mode by applying zero electric field for time, which can be set by right-click on the button. The diffusion mode is signalised by a red text "**Diffusion mode**" localized below "Composition File" tool DIFFUSION MODE . After clicking on the button the actual values of "**dt**" and "**dt optimisation increment**" are stored. After the start of the simulation ("**Run**" button) the diffusion time is counted down to zero in "**Time (s)**" display (renamed to "**Diff. time (s)**"). After reaching zero time the stored values are restored and standard voltage (or current) is applied. Checking "**Automatic Initial Diffusion**" in Run Setup causes that after clicking on "**Init**" button diffusion mode is automatically applied.

Composition (List of Constituents)

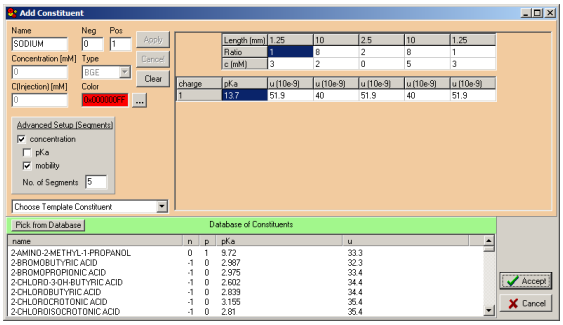


"**Composition**" contains the list of constituents created by "**Add**" button or by opening of CMP file or SNA file. Button "**Delete**" deletes specific constituent and button "**Delete All**" deletes all constituents in the list. If there are some changes in constituents after simulation is initialised then button "**Restore**" is enabled and a click on it restores all changes in constituent properties. **Double left-click** on specific constituent in the list or click on "**Edit**" button enables to edit chosen constituent in the "Add Constituent" window. All editable constituent properties are displayed in the list. In a case of *pKa* and *u* values - vertical line "**|**" divides values belonging to negative and positive charge numbers and individual values are separated by semicolon "**;**". Also, if values vary in different segments then the parentheses "**{}**" separates particular segments. The colour in the third column coincides with the colour of line of the concentration profile.



Right-click on row of the specific constituent shows a popup menu containing commands "Edit", "Delete" and "Deletes All" with the same meaning as stated above. Command "**Focus**" enables zoom of the specific profile in the graph around its baseline. The baseline is defined as an initial concentration in the right corner. The last four commands in the popup menu move with constituent position in the list.

Add Constituent



This window enables to define constituent properties (pKs ,mobilities,...) and initial concentration profiles. Add Constituent window contains all information about a new or edited constituent (name, charge numbers, initial distribution of concentration, ...).

"**Name**" is a unique identifier of constituent. If a new constituent has the same name as an already existing constituent then the new constituent replaces the old one. "**Neg**" and "**Pos**" are negative and positive limits of charge numbers (e.g. acetic acid neg = -1 and pos = 0 or histidine neg = -1 and pos = 2). The change of these values has to be confirmed by an "**Apply**" button.

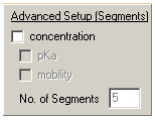
The "**Apply**" button reallocates grid fields located at the top-right area of the window. There are shown editable mobilities "**u**" and "**pKa**" data of all ionic forms and if segmentation is applied (in the Advanced Setup of this window) there is also grid field divided into user-defined number of segments. A button "**Cancel**" cancels changes in the charge numbers ("neg", "pos") and in the Advanced Setup. A button "**Clear**" removes all information about constituent and sets them to default values. It sets charge numbers to zeros so a text "Neutral Constituent" is displayed instead of mobility and pKa values.

"**Type**" is a drop-down list of four basic types of constituents (Analyte, BGE, Leading, Terminating) primarily designed for CZE and ITP modes simulation. It is a basic way how to set up an initial distribution of concentrations and it is disabled (for given constituent) when Advanced Setup is used. A construction of the initial distribution is simple - it divides distribution into three segments (Left, Injection and Right) and each segment is filled by certain concentration using information from chosen "**Type**" and entered values in the "**Concentration (mM)**" and "**C(injection) (mM)**" edits. It uses a following table of concentrations:

	Left Segment	Injection Segment	Right Segment
Analyte	0	C(injection)	0
BGE	Concentration	C(injection)	Concentration
Leading	0	C(injection)	Concentration
Terminating	Concentration	C(injection)	0

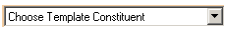
A position of a centre of Injection segment is given by "**Injection Site (mm)**" in the Run Setup window. The value can be set out of simulation space so user can for example create a distribution based only on two segments if the third segment is outside. Types Analyte and BGE are set automatically.

"**Advanced Setup (Segments)**" enables more sophisticated distributions of concentration, mobilities and pKa constants. When "**concentration**" checkbox is checked then a user can choose segmentation in "**No. of Segments**" and also whether pKa constants and mobilities are also segmented (checked "**pKa**" and "**mobility**"). If segmentation is applied then there are three additional rows in the upper right area:



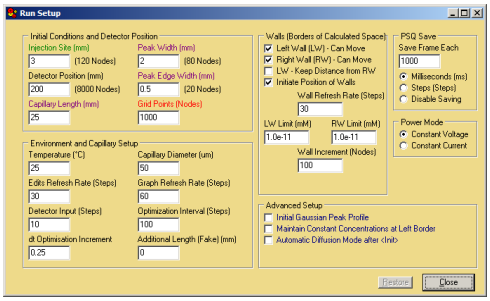
- "**c (mM)**" sets a concentration of the constituent for each segment.
- "**Ratio**" defines relative widths of segments.
- "**Length (mm)**" is a width of the segment in mm and it uses information from "ratio" and recalculates it to the lengths using "Capillary Length (mm)" value in the Run Setup window.

All segment are smoothly connected with a measure of smoothness given by "Peak Edge Width (mm)" in the Run Setup window. "**Choose Template Constituent**" drop-list bar enables to use segmentation in already created constituent.



Note: Each constituent setup is independent on the others but be careful when basic and advanced modes are mixed. Especially, when "Gaussian Initial Peak Profile" in the Run Setup is applied because it can mix gaussian shapes of the basic setup with rectangular shapes of the advanced setup.

Run Setup 



Run Setup window contains some important parameters, which affect concentration distribution, number of nodes, wall management (simulation borders), PSQ saving and others. It is divided into several panels.

"**Initial Conditions and Detector Position**" panel contains the most important parameters. "**Injection Site (mm)**" is a position of the centre of the Injection segment when constituent "Type" property (in Add Constituent) is active for one constituent at least. The Injection Site position is also used by "Zero Position" in the Option window. "**Detector Position (mm)**" is position of detector (light brown vertical line in the graph) when "Enable Detector" in the Main window Setup is checked. If "Enable EOF" in the Main window is checked then program can imitate presence of EOF by continuous shifting of detector with mobility given by "EOF Mobility (1E-9)" value (Main window). For saving of simulation nodes detector can be placed out of the simulation space. If so the detector starts collecting data after it reaches the simulation space. Detector data can be viewed by clicking on "Sim/Det" button (Main window). "**Capillary Length (mm)**" is a simulated length of the capillary. For purposes of the calculation of the actual current it can be combined with Additional Length (Run Setup), which will be described below. The capillary length is also used when segmentation is applied (Advanced Setup in Add Constituent). "**Peak Width (mm)**" is a width of the Injection Segment when constituent "Type" property (Add Constituent) is active for one constituent at least. "**Peak Edge Width (mm)**" is measure of the initial smooth connection of all segments, which create the initial distribution of concentration, mobility and pKa profiles. "**Mesh Density (Nodes)**" is discrete division of the simulated space (note: the number of used nodes is Mesh Density value + 1). The higher this value is the less numerical oscillations are created on steep boundaries but the simulation is slower. The smoothness of steep boundaries can be alternatively improved by decreasing of Voltage or Current value (Main window), which slows down electromigration and allows more diffusion on problematic boundaries.

"**Environment and Capillary Setup**" panel mostly contains refresh rates and dt optimisation parameters. "**Temperature °C**" is used for the calculation of diffusion coefficients from mobilities. "**Capillary Diameter (um)**" is in μm unit and serves for the calculation of the current from the current density, which is not displayed but it is primarily calculated during simulation. "**Edits Refresh Rate (Steps)**" is a frequency how often are values of edits (time, error, ...) in the Main window updated. "**Graph Refresh Rate (Steps)**" is a frequency how often is graph updated. "**Detector Input (Steps)**" is a frequency how often are data collected by detector. If collected data are too large simulation and some other operations (Open, Save,...) might be slowed. The size of collected data in the actual project can be halved by "Detector Data Tool" available in "Tools/Detector Tools" in the main menu. "**Optimization Interval (Steps)**" is a frequency of dt optimization if "Optimise dt" in the Main window is checked. It should not be too high because there is a certain time period needed for a stabilization of Error value (Main window) when a dt value is changed. "**dt Optimisation Increment**" defines factors, which are applied when dt is increased or decreased (decreasing factor is little bit higher). For more details see Automatic dt Optimisation. "**Additional Length (Fake) (mm)**" is non-simulated length of the capillary, which is only used for calculation of the intensity of electric field and consequently for the voltage and the current recalculation. This function can prevent from artificial changing of the electric field (and current) caused by local changes in conductivity. It remembers initial conductivity of the node in the right corner and its value is used during simulation as the conductivity of the non-simulated part.

"**Walls (Borders of Calculated Space)**" panel manages behaviour of moving walls (borders of simulated space). Left and right walls are drown by black vertical line in the graph. "**Left Wall (LW) - Can Move**" and "**Right Wall (RW) - Can Move**" defines if program can automatically change their position when "**LW Limit (mM)**" and "**RW Limit (mM)**" are exceeded by approaching boundary in one or more concentration profiles. "**LW - Keep Distance from RW**" causes that the left wall keeps constant (actual) distance from the right wall (note: it should be handled carefully because suitable distance between walls can increase after certain time and it must be done manually). "**Initiate Position of Walls**" determines if wall positions

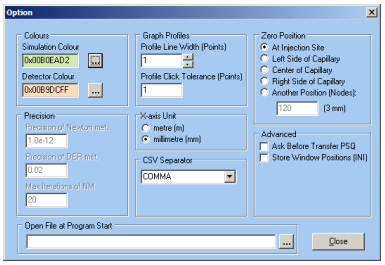
are initially set to the left and right corner or left as they are. "**Wall Refresh Rate (Steps)**" is a frequency between two automatic recalculations of the wall positions.

"**PSQ Save**" panel manages continuous saving of simulation frames after defined period. "**Save Frame each**" value defines frequency between saving of a new simulation frame. "**Milliseconds (ms)**" and "**Steps (Steps)**" choices define if Save Frame value is in ms of simulation time or in steps. "**Disable Saving**" disable or finish saving of frames to the temporary PSQ file. PSQ file is automatically saved with a project and the file can be played in "PSQ Player" tool in the main menu.

"**Power Mode**" panel defines power mode - "**Constant Voltage**" or "**Constant Current**".

"**Initial Gaussian Peak Profile**" checkbox in the "**Advanced Setup**" panel causes that for initial profiles, handled by certain constituent "Type" choice (Add Constituent property), is used Gauss function instead of default diffused rectangular function. Gaussian variance parameter is calculated using both Peak Width and Peak Edge Width values. "**Maintain Constant Concentrations at Left Border**" will maintain constant concentration of all constituent at the left corner. "**Automatic Diffusion Mode after <Init>**" automatically enable diffusion mode after each clicking on INIT button (Main window).

Option (Main Menu Item)

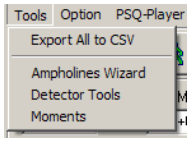


All option values and setups are stored in the Simul INI file. If user wants to restore the default setup then "SIMUL 5.ini" file have to be deleted manually. In the panel "**Colours**" the "**Simulation Colour**" and the "**Detector Colour**" of graph background can be set.

Panel "**Precision**" contains a precision and an iteration stop conditions. "**Precision of Newton Met.**" value is a precision (stop) parameter for pH calculation from electroneutrality equation. "**Precision of DET met.**" value is stop parameter in Predictor/Corrector calculation. "**Max Iterations in NM**" value defines maximum number of iteration in the Newton method (pH calc.) - important when simulated system is composed strictly from strong electrolytes. All these values are by default disabled to be edited. But if a user has a urgent need to change them and is sure how to do it then shortcut Ctrl+Alt+P will enable or disable their editing.

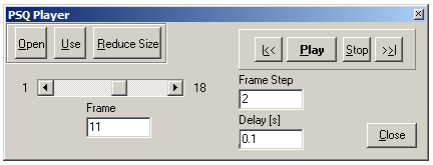
"**Open File at Program Start**" contains file name (SNA, CMP, PSQ), which will be automatically opened after the each start of the program Simul. It is a tool designed for changing e.g. default values. "**Profile Line Width (Point)**" value in the "**Graph Profiles**" panel defines pen size of lines in the graph. "**Profile Click Tolerance (Points)**" is the maximum distance of the mouse click from a profile line, which call Profile Information. "**X-Axis Unit**" defines unit of simulation graph x-axis. "**CSV Separator**" is actual separator used in profiles export to the CSV file (Tools/Export All to CSV). "**Zero Position**" is a value used in the calculation of the average mobility in the Profile Information. There are several predefined possibilities or it can be placed to user defined position. "**Ask Before Transfer PSQ**" checkbox in the "**Advanced**" panel will send a question - if saved PSQ file is about to be transferred to temporary PSQ file - every time during opening of a saved project. Transfer is necessary if user wants to continue in the saved simulation. If this checkbox is not checked then PSQ file is automatically copied. This feature was created for a case where a user handles with large PSQ files and do not want to continue in a simulation. It is to avoid of copying large PSQ files that consumes a lot of time. "**Store Window Positions (INI)**" save actual positions of most of windows to the INI file.

Tools (Main Menu Item)



"**Export All to CSV**" will export all simulation and graph profiles to CSV file (comma separated value), which can be imported to various data evaluating programs (Excel, Origin). "**Ampholines Wizard**" helps to create a large number of ampholytes (typically for IEF simulations). "**Detector Tools**" shows actual size of detector data points and can halve them if their size is too big. "**Moments**" is a tool, which calculates area and some other statistic moments of a certain part of a chosen profile.

PSQ-Player



"**Open**" button opens PSQ file, which is automatically played. The number of frames is displayed on the right side of the scroll bar, which indicates a position of the actual frame. The frame number is in the "**Frame**" edit. Setting a "**Frame Step**" value to higher value than 1 skips certain number of frames (1 - play all, 2 - play every second frame and so on). "**Delay (s)**" slows down frame playing. Buttons in the right top panel manage playing. "**|<<**" button rewinds the file to the beginning, "**Play**" starts playing, "**Stop**" stops playing, "**>>|**" rewinds the file to the last frame. Button "**Use**" transfers data of the displayed frame to the simulation (initialise simulation using the frame composition) and PSQ file is transferred to the temporary PSQ file. After that user can continue in the simulation and new frames will be appended to the PSQ file. "**Reduce File**" button allows reducing size of the opened file.