



PhyNexus

**Operating Instructions for
PhyTip[®] ME 200 and ME 1000 Purification Systems**

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Operating Instructions for PhyTip[®] ME 200 and ME 1000 Purification Systems

Introduction

In order to realize the benefits of PhyTip columns for protein purification and enrichment, PhyNexus has developed the PhyTip ME 200 and ME 1000 Purification Systems as the platform of choice for routine use. When used with the PhyNexus Operating Software and the PhyNexus Instrument Stand, this system offers a range of flow rates that maximizes the purification and enrichment efficiency of the various affinity resins available for PhyTip columns.

These operating instructions are divided into the following sections:

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1. *Unpacking*

Each system contains the following:

1. Multi Channel System Controller
2. Power Supply
3. Instrument Stand
4. Software CD, includes manual/instructions for use
5. 5 ea 200 μ L Sample Trays

If these five separate items are not in the original outer packing, please call PhyNexus at 408-267-7214.

Remove all contents from their individual packs and prepare to install the system.

2. *Setting up the system*

This section is divided into:

1. Initial location and set up of system parts
2. Installing the software
3. Powering up the system

Initial location and set up of system parts

Once all contents have been removed from their individual packs, locate the instrument stand and place it close to the computer to be used for operation. Remove the multi channel system and attach to the stand using the hook locator on the multi channel system to rest on the holder at the front of the stand. Locate the Software CD and install in the computer.

Install the software in the PC

The PhyNexus Operating Software is designed to operate with PCs running Windows XP, 2000, NT, 98. With the computer turned on, insert the software disc into the CD or DVD drive of the system, double click on the setup icon and follow the install instructions on the screen. At the end of install, connect the communications cable for the multi channel system into the serial port. The computer should now be ready to communicate with the multi channel system.

Powering up the system

Connect the power supply cable to the communications cable attached to the multi channel system and plug in the power supply (note: plug the power supply into the connector encased in the cable, not into the multi channel device directly). With the communications cable attached to the serial port on the computer, the power supply connected and on, and the software installed in the computer, the system is now ready to use.

3. Using the PhyTip ME 200 and ME 1000 Purification Systems

This portion of the manual is divided into the following sections:

1. Instrument Stand
2. Easy To Use Quick Guide Software Instructions
3. Easy To Use Quick Guide Operating Instructions
4. Detailed Software Information and Instructions

Instrument Stand

The PhyNexus stand has been designed to aid the process of Capture – Purify – Enrich™ using PhyTip columns. The stand holds the multi channel system in place to allow reproducible positioning of the PhyTip columns over the sample, the wash buffers and finally the elution buffer used for enrichment. By maintaining the optimum positioning, the system is able to accurately pipette the desired volumes required to capture the specific proteins, purify the proteins and finally enrich them in a small volume of elution buffer, e.g. 10 µL.

Description

The PhyNexus Stand has three components: Sample Plate Holder, Adjustable System Holder and Stand.

The Adjustable System Holder can be moved vertically from a lowered position over the sample to an upper resting position at the top of the Stand – at an angle of 30° to the vertical. This allows for freedom of movement of the multi channel system away from a sample plate at the end of the purification and enrichment process.

The Sample Plate Holder is used to hold a sample plate beneath the PhyTip columns. It can be adjusted by turning the four adjustment screws at the corners; this allows for fine adjustment of the height of the sample plate and keeps the plate level with respect to the end of the PhyTip columns.

Directions for alignment of the table with the Multi Channel System.

1. Place the multi channel on the Adjustable System Holder using the hook on the pipette to attach it to the holder. The communications cable to the computer should be placed behind the stand. Raise the Adjustable System Holder to the top resting position.
2. Place an empty sample plate on the Sample Plate Holder. Remove two PhyTip columns from their shipping box and place at the two ends of the multi channel (positions 1 and 8 for an 8 channel system, positions 1 and 12 for a 12 channel system). Lower the Adjustable System Holder gently down until the PhyTip columns enter into the wells of the empty sample plate. Adjust the height of the PhyTip column above the bottom of the well to approximately 1 mm. Use the knurled knob on the Stand to set a rough base point for the Adjustable System Holder and then use the adjustment screws on the Sample Plate Holder for fine adjustment of the height and level of the plate.
3. Reset the multi channel system to its resting position and replace the PhyTip column in their shipping box. Execute the method to be run on the computer using the PhyNexus Operating Software and follow the instructions indicated. The method will begin with a “conditioning step” followed by instructions to add the required number of PhyTip columns. After this, place the sample plate with sample, wash buffer and elution buffer on the Sample Plate Holder. Lower the Adjustable System Holder gently to its lower stop position with the PhyTip columns inserted into the wells containing the sample to be purified and enriched. Continue with the automated method.

Easy to use Quick Guide Software Instructions

The PhyNexus Operating Software has been designed for simple to use semi-automated methods that have been preprogrammed into the system. Methods manage the entire Capture, Purify and Enrich process, protocols manage the individual steps. A method is a series of protocols with pauses and prompts built in between steps. To load the software onto a PC, simply insert the CD into the CD or DVD drive of the PC and follow the installation instructions on the screen.

For simplest use of the system, run the preprogrammed methods for any of the given affinity resins. See below to load and execute a method file.

Easy to Use Quick Guide Operating Instructions

With the software installed, multi channel system connected to the computer and powered on, attached to the Adjustable System Stand:

1. Prepare Samples ready for Capture – Purify – Enrich.
2. Prepare Wash Buffer(s) ready for Purification process
3. Prepare Elution Buffer ready for Enrichment process
4. Check Sample Plate Holder level
5. Load appropriate Method File
6. Follow instructions as described in the software
 - a) Condition System
 - b) Capture
 - c) Purify 1
 - d) Purify 2
 - e) Enrich
7. Store/analyze samples

1. Preparing samples ready for Capture – Purify - Enrich

It is recommended that prior to the process of purification and enrichment by PhyTip columns, all samples be checked for particulates and clarity. If the final solution to be purified appears cloudy or contains particulates, it is recommended to spin the samples down one more time at 3,000 g for 5 minutes.

Samples should now be dispensed into appropriate holders ready for the capture step. For low to medium throughput operations, e.g. 2-8 samples per run. It is recommended to use either a deep well 96-well plate (for higher volume, 1000+ PhyTip columns) or a v-bottom 96-well polypropylene plate (for lower volume, 200+ PhyTip columns), placing samples down column 1, in well positions A1, B1, C1, D1 etc.

2. Preparing wash buffer(s) ready for Purification process

In column 2 (well positions A2, B2, C2, D2 etc), it is recommended to aliquot the correct volume of Wash Buffer 1, aligned to the specific sample in column 1. In column 3 (well positions A3, B3, C3, D3 etc), aliquot the second Wash Buffer, if necessary. Refer to the Product Information Sheet for the appropriate volumes of buffer.

3. Prepare Elution Buffer ready for Enrichment process

In the final column, add the required volume of Elution Buffer. If using 1000+ PhyTip columns, the initial Capture and Purify steps may have been performed in a deep well plate. For the final Enrichment step, a v-bottom 96 well plate should be used to contain the 15 µL of Elution Buffer. For 200+ PhyTip columns, the Capture and Purification steps will have been performed in a v-bottomed 96-well plate, so just add the 10 µL of Elution Buffer to column 4 of the plate (well positions A4, B4, C4, D4 etc).

4. Check Sample Plate Holder Level

Place an empty 96-well sample plate on the Sample Plate Holder. Remove two PhyTip columns from their shipping box and place at the two ends of the multi channel system (positions 1 and 8 for the 8 channel system, positions 1 and 12 for the 12 channel system). Lower the Adjustable System Holder gently down until the PhyTip columns enter into the wells of the empty sample plate. Adjust the height of the PhyTip columns above the bottom of the well to approximately 1 mm. Use the knurled knob on the Stand to set a rough base point for the Adjustable System Holder and then use the adjustment screws on the Sample Plate Holder for fine adjustment of the height and level of the plate.

Reset the pipette to its resting position and replace the PhyTip columns in their shipping box.

5. Load the appropriate Method File

To load the appropriate Method File, go to File on the user screen. Enter Open and the choice of Protocol or Method will be shown. Enter Method and hit OK. This will take you to folders containing the preprogrammed method files available to run. Select the required file and press open. The Method will now be available for the system to run. To verify the method, click on Method – Edit, this displays all of the linked protocols that constitute the given method.

Execute the method to be run by clicking on Method – Execute and follow the instructions indicated. Do not attach PhyTip columns until instructed to do so after starting the method. The PC is in constant communication with the multi channel controller; please do not run other programs while running the PhyNexus Software.

6. Follow the instructions as described in the software

The method will begin with a “preparation step” which prepares the multi channel system for use. This will be followed by instructions to add the required number of PhyTip columns in the appropriate positions.

After this, place the sample plate with sample, wash buffer and elution buffer on the Sample Plate Holder. Lower the Adjustable System Holder gently to its lower stop position with the PhyTip columns inserted into the wells containing the sample to be purified and enriched. Continue with the automated method by pressing OK.

7. Store/analyze samples

Once the final enrichment step has been completed, samples are now ready for neutralization (if required) and then storage/analysis. Reminder: if using Protein A or Protein G affinity resins, the final elution step uses a low pH buffer and it is recommended to add approximately 5 μ L neutralization buffer to bring the solution to a physiological pH.

To change or modify the initial Protocols or the Methods, check in the Detailed Software Information and Instructions section.

Detailed Software Information and Instructions

Methods on the multi channel system perform the Capture, Purify and Enrich process for all of the defined samples. **Protocols** manage the individual steps of the Capture, Purify and Enrich process. A method is a series of protocol instructions that fully automate the entire process of purifying a column with sample. To load the software onto a PC, simply insert the CD into the CD or DVD drive of the PC and follow the installation instructions on the screen.

For simplest use of the system, run the preprogrammed methods for any of the given affinity resins. To do this, go to **File**, then **Open**. This will bring down a screen that shows either Method or Protocol, click on **Method** to show all of the stored Methods. Open the standard method IMAC; this will bring down the operational screen. Now go to **Method** and click on **Edit**; this will show all of the individual steps of the method.

Click on any one of the individual steps of the methods to show the options screen. This section deals with the operation of the multi channel system (controller). Actions that are available are:

Top Section:

<i>Execute:</i>	executes a stored protocol, e.g. Capture
<i>Audible Alert:</i>	introduces an audible alert at the end of an action
<i>Home (controller):</i>	homes the multi channel system to a zero volume
<i>Pause for Operator:</i>	builds in an automatic pause in the method and allows operator to build in an automatic comment or prompt between actions
<i>Comment:</i>	allows operator to manage writing methods by addition of comments during script
<i>Blowout:</i>	causes multi channel system to blow out any air from the syringe

Protocols

Protocols define the flow rates, volume and pauses associated with the process of Capture-Purify-Enrich. There are a series of standard protocols that have been stored in the software, e.g.:

200+ ProA Condition
200+ ProA Capture
200+ ProA Wash 1
200+ ProA Wash 2
200+ ProA Elute

These protocols represent a series of steps that would be used for the 200+ PhyTip columns containing Protein A affinity resin; there are others for 200+ Protein G, IMAC and Glutathione plus 1000+ Protein A, Protein G, IMAC and Glutathione.

The *standard methods* have been written to incorporate a series of the *standard protocols* so that the process of Capture-Purify-Enrich can simply be carried out without the need for further programming.

200 + Protocol

As an example of these protocols when using the standard 200+ Protein A Method, there are several “Execute” steps that reference the following steps:

200+ Pro A Condition

Volume (mL)	Rate (mL/min)	Times to do	Delay (sec)
0.01	1	1	0

Condition: This conditions the multi channel system to prepare for the complete process of moving liquids over the resin bed in the PhyTip columns. The final process of elution will often require only 10 μ L of elution buffer to be moved over the resin bed and then expelled. In order to be sure that all of this liquid can be blown out, a sufficient reservoir of air must be added to the multi channel system before the PhyTip columns are attached. In this case, 10 μ L of air is moved into the multi channel system at a rate of 1 mL/minute.

200+ Pro A Capture

Volume (mL)	Rate (mL/min)	Times to do	Delay (sec)
0.17	0.25	1	10
-0.17	0.25	1	10
0.17	0.25	1	10
-0.17	0.25	1	10
0.17	0.25	1	10
-0.17	0.25	1	10
0.17	0.25	1	10
-0.172	0.25	1	10

Capture: Passes the sample over the resin bed enabling the Protein A affinity resin to bind the protein of interest. In this case 170 μL of sample is moved into the PhyTip (Intake) and 170 μL is moved out (Expelled). Intake can be seen as the positive number and expel the negative number, also note that all expel steps are colored red. Note that there are four expel steps. Therefore, four cycles of Capture and that at the end of the fourth cycle 172 μL is expelled from the PhyTip column. This extra volume is part of the original 10 μL Condition step and helps to remove as much sample solution as possible prior to the Wash steps.

The Flow Rate is 250 $\mu\text{L}/\text{min}$ (expressed as 0.25 mL/min) with a delay of 10 seconds at the end of each step. This delay step of 10 seconds is built into the system to allow for the full equilibration of all PhyTip columns in all positions.

To modify any of the parameters, click on the parameter line and an “Edit Protocol Step” screen will display. This has several boxes that can be modified (see Section B, Detailed software Information): *Volume* (sets the desired volume), *Volume Units*: (references the volume, with mL or μL available), *Direction* (Expel or Intake), *Volume Prior to this Step* (indicates last volume input), *How Many Times* (number of times to repeat sequentially this specific step), *Rate* (the flow rate), *Rate units* (shows mL/hr , mL/min , $\mu\text{L}/\text{hr}$, $\mu\text{L}/\text{min}$) and *Delay* (in seconds).

200+ Pro A Wash 1

Volume (mL)	Rate (mL/min)	Times to do	Delay (sec)
0.17	0.5	1	10
-0.17	0.5	1	10
0.17	0.5	1	10
-0.17	0.5	1	10

Wash 1: This is the first of two washing (Purification) steps, here two cycles of moving 170 μL of Wash Buffer 1 is accomplished at a flow rate of 500 $\mu\text{L}/\text{min}$ (expressed as 0.5 mL/min) with a delay of 10 seconds at the end of each step. Note that on the final expel step, the volume is the same as all the intake steps. At this first step, there is no need to expel any further volume since the next step is another wash step.

200+ Pro A Wash 2

Volume (mL)	Rate (mL/min)	Times to do	Delay (sec)
0.18	0.5	1	10
-0.18	0.5	1	10
0.18	0.5	1	10
-0.182	0.5	1	10

Wash 2: This is the second wash step, again two cycles at the same flow rate as Wash Step 1. Now however, there is a larger volume of wash buffer (180 μL) and at the final expel step there is an extra 2 μL of volume expelled from the PhyTip column (-182 μL).

200+ Pro A Elute

Volume (mL)	Rate (mL/min)	Times to do	Delay (sec)
0.05	0.5	1	10
-0.05	0.5	1	10
0.05	0.5	1	10
-0.05	0.5	1	10
0.05	0.5	1	10
-0.05	0.5	1	10
0.05	0.5	1	10
-0.05	0.5	1	10
0.05	0.5	1	10
-0.056	0.5	1	10

Elute: The final step in the purification process uses 5 cycles of rapid flow elution over the resin bed. In this case, although only 10 µL is being used as the Elution volume, the protocol is stored as 50 µL. This difference between actual physical volume and programmed volume allows for all of the Elution buffer to pass over the resin bed, thus maximizing the total yield of purified protein that can be obtained from the column. It is essential to “overdrive” this step (50 µL to pick up 10 µL of liquid) to overcome the surface tensions and pressures that are caused by the initial insertion of the tip, and bed resistance in the tip. The required balance is to pull up enough to get the fluid to move, but not too much such that the volume of fluid pulls an air bubble.

As a programming guideline, if a higher volume for elution is needed, add 40 to the final volume number to obtain the programmed elution volume, e.g. if 30 µL is the final elution volume required, program $30 + 40 = 70$ µL for the final elution volume. **This guideline is valid for elution volumes less than 75 µL. For elution volumes greater or equal to 75 µL, simply program that same volume.**

Again note that the final step expels 56 µL to maximize the volume of Elution Buffer returned (Note: Initial Conditioning step has Intake volume of 10 µL. Capture has excess expel of 2 µL; Wash 2 has excess expel of 2 µL; and the final Elution step has an excess expel of 6 µL, thus balancing the Intake and Expel for the entire process).

1000+ Protocol

Below is an example of the protocols for a 1000+ PhyTip column system using IMAC as the affinity resin:

1000+ IMAC Condition

Volume (mL)	Rate (mL/min)	Times to do	Delay (sec)
0.09	3	1	0

1000+ IMAC Capture

Volume (mL)	Rate (mL/min)	Times to do	Delay (sec)
0.45	0.25	1	20
-0.45	0.25	1	20
0.45	0.25	1	20
-0.45	0.25	1	20
0.45	0.25	1	20
-0.45	0.25	1	20
0.45	0.25	1	20
-0.47	0.25	1	20

1000+ IMAC Wash 1

Volume (mL)	Rate (mL/min)	Times to do	Delay (sec)
0.9	0.5	1	20
-0.9	0.5	1	20
0.9	0.5	1	20
-0.9	0.5	1	20

1000+ IMAC Wash 2

Volume (mL)	Rate (mL/min)	Times to do	Delay (sec)
0.5	0.5	1	20
-0.5	0.5	1	20
0.5	0.5	1	20
-0.52	0.5	1	20

1000+ IMAC Elute

Volume (mL)	Rate (mL/min)	Times to do	Delay (sec)
0.25	0.5	1	20
-0.25	0.5	1	20
0.25	0.5	1	20
-0.25	0.5	1	20
0.25	0.5	1	20
-0.25	0.5	1	20
0.25	0.5	1	20
-0.25	0.5	1	20
0.25	0.5	1	20
-0.3	0.5	1	20

Note: The initial conditioning step of 90 µL and the subsequent combination of excess expels total 90 µL. Also the final elution volume of 20 µL requires an intake/expel of 250 µL.

As a programming guideline, if a higher volume for elution is needed, add 230 to the final volume number to obtain the programmed elution volume, e.g. if 30 µL is the final elution volume required, program $30 + 230 = 260$ µL for the final elution volume. **This guideline is valid for elution volumes less than 250 µl. For elution volumes greater or equal to 250 µl, simply program that same volume.**

Manual methods programming

The ME software not only includes standard Methods and Protocols that can be simply modified, but also a section that allows the user to change COM ports etc. The standard windows screen shows five basic headings:

File – Protocol – Method – Options – Help.

File is the standard New/Open/Save/Save as found in most Windows applications.

Protocol contains Execute/Edit. Execute begins the loaded Protocol and Edit allows the user to Edit the loaded Protocol.

Method contains Execute/Verify/Edit. Execute begins the loaded Method, Verify checks that the Method can be operated and Edit allows the user to Edit the loaded Method.

Options contain Communications/Syringe. Communications sets the COM port for the system and Syringe verifies the multi channel system type (200+ or 1000+).

Help shows the About information regarding the system.

1. Introduction

PhyNexus controller software is a graphical user interface application which is intended to allow the user to perform the following tasks:

1. Open, verify, and execute protocol files which perform the individual steps of Capture, Purify and Enrich.
2. Edit and save protocol files.
3. Open, verify, and execute method files which execute protocol files and other commands.
4. Edit and save method files.
5. Perform manual intake, expel, and blowout / home operations.

The controller software is intended for use on 32-bit Microsoft Windows platforms (Windows '95, '98, Me, NT, 2000, and XP) and communicates with the multi channel purification system.

1.1 Conventions

The multi channel purification system will be referred to as the controller.

The controller software will afterwards be referred to as the ***app***.

Menu and submenu options will be referred to using the -> notation. For example, the Open option under the File menu is referred to as File->Open.

1.2 Definitions

A protocol is a series of steps which command the controller to intake or expel liquids through the PhyTip columns. Each step of a protocol specifies a volume (positive to intake, negative to expel), a rate, and a number of times to repeat. An optional post-step delay may also be specified.

A method is a series of steps which group protocol(s) together and also provide some user-interface functionality. The following actions can be steps of a method:

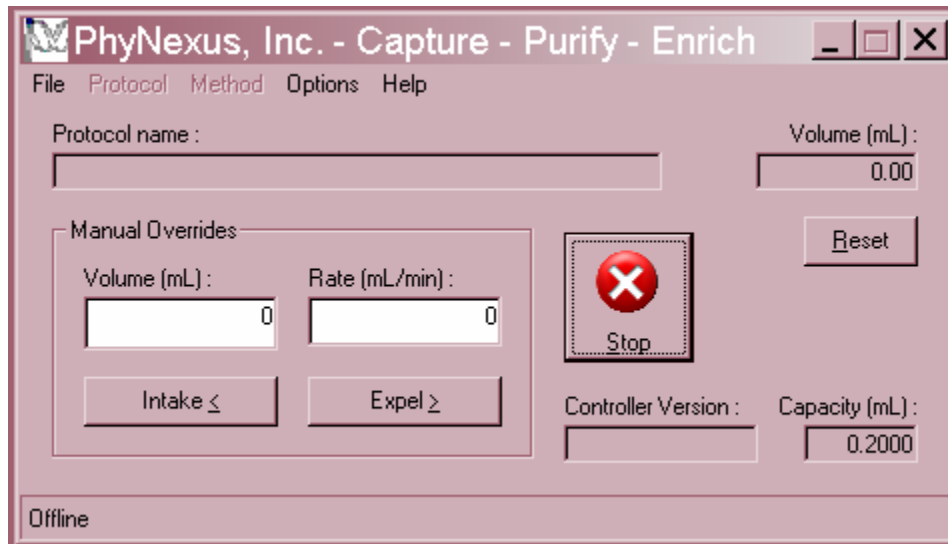
1. Execute protocol (specified by filename).
2. Audible alert (beep).
3. Pause (display message, and wait for user input to either continue method or abort).
4. Blow out contents.
5. Reset to home position.
6. Comment (solely for documentation of the method file – not seen by the operator).

1.3 Compatibility

Version 2.0 (or greater) of the app is compatible only with PhyTip ME 200 and ME 1000 Purification Systems supplied by PhyNexus.

2. How to open and execute a protocol

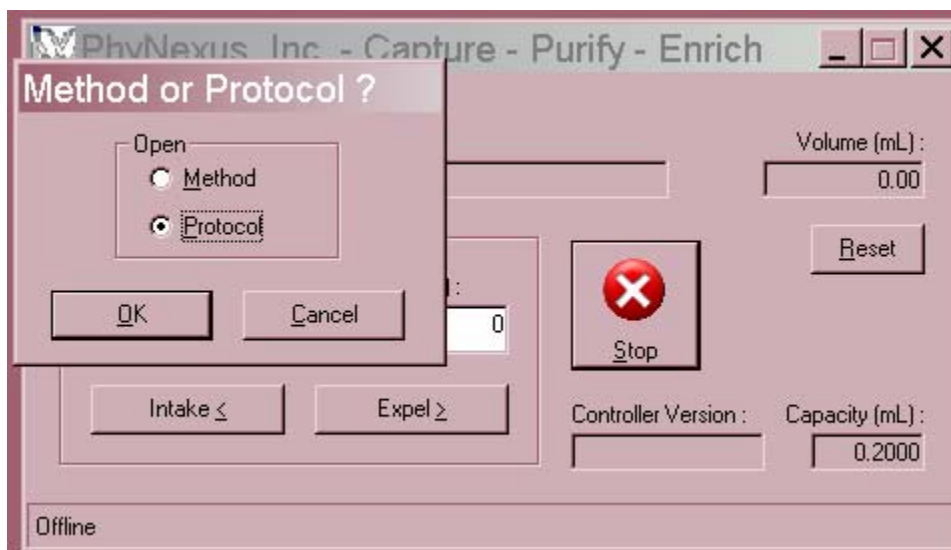
The following window appears when the app first runs:



The **status bar** (lower portion of the screen) shows the app status at any time. Initially the app is not communicating with the controller, so the status bar shows the message “Offline” when it starts.

2.1 Open protocol file

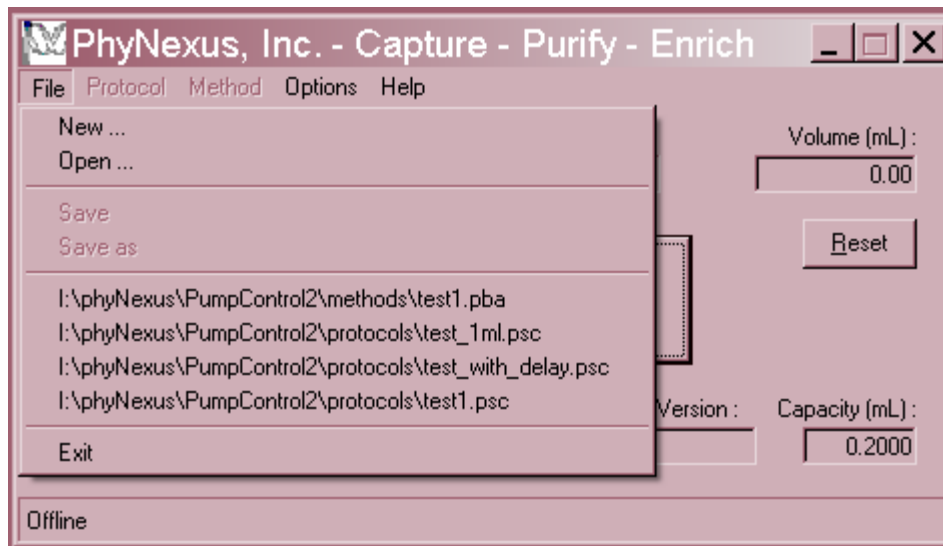
Use the File->Open menu option and select Protocol to browse for a protocol file, as shown below:



Protocol files are assumed to have a .psc file extension. If the protocol file was opened successfully, then it may be verified, executed and/or edited.

If the app displays an error message when you try to open the protocol file, then it may have been corrupted, e.g. via disk error or hand-editing, or the file selected is simply not a protocol.

The last four successfully-opened files are added to an **MRU** (most-recently-used) list in the file menu, as shown below:



When the filename is selected from the MRU, the app attempts to open the file. If the file cannot be opened or no longer exists, then it is removed from the MRU.

2.2 Execute protocol

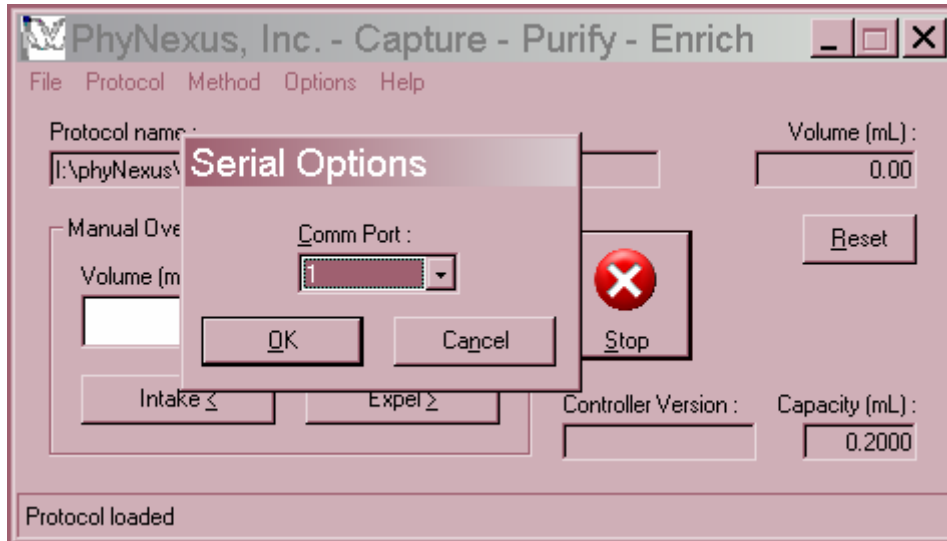
Before executing the protocol, make sure of the following:

1. Controller is correctly mounted on the Adjustable System Holder.
2. App's serial parameters are set to match those of the controller.
3. App's capacity matches the controller capacity.

After these items have been checked, the protocol can be executed.

2.2.1 Check serial port

The app's serial port setting may be adjusted by selecting the Options->Serial menu, which pops up a dialog as follows:



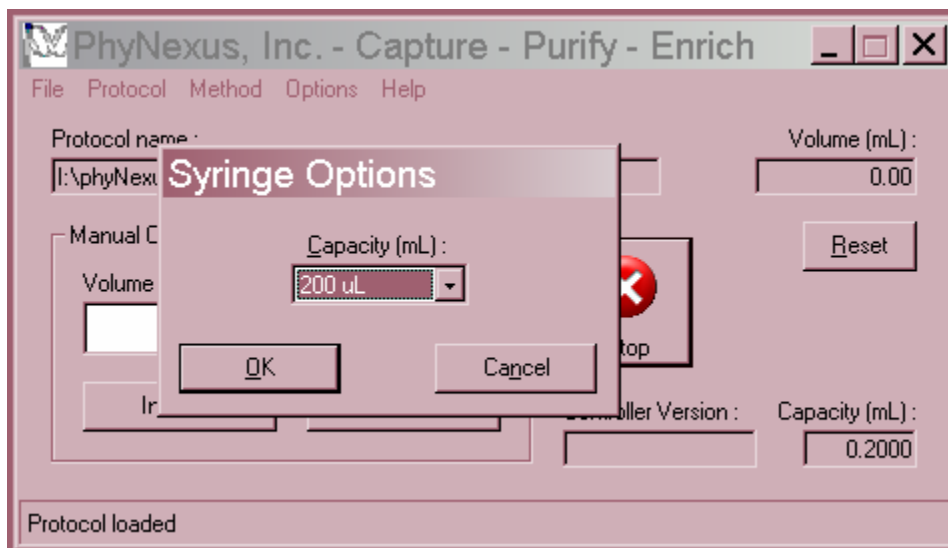
Make sure that the port setting corresponds to the one on the PC that is connected to the controller. Initially, the app uses port 1, but remembers the last setting used (See *registry settings* below).

Notes:

- Settings for the comm port can only be changed when the app is not communicating with the controller (not executing a protocol, method or manual override command). Pressing 'Stop' terminates communication, and allows these settings to be changed (see below).

2.2.2 Check capacity setting

The capacity setting may be adjusted via the Options->Syringe menu, which pops up the following dialog:



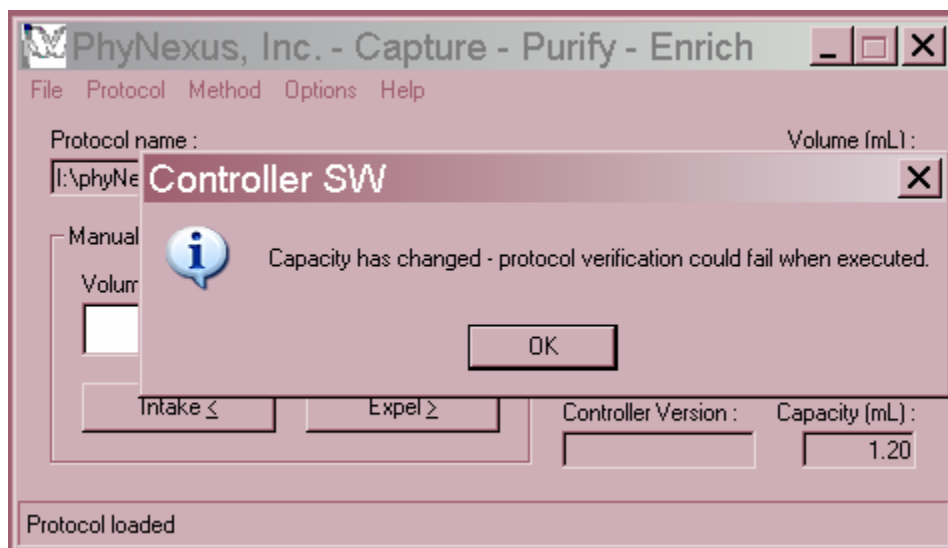
The setting shown above is for the 200 microliter capacity. The app is initially set up for 200 microliters, but remembers the last settings used (See *Registry Settings* below).

Also note that the current capacity is displayed on the main window of the app.

The capacity setting can only be changed when the app is not communicating with the controller (not executing a protocol, method or manual override command). Pressing 'Stop' also stops communication so that these settings can be changed.

2.2.2.1 Capacity and verification

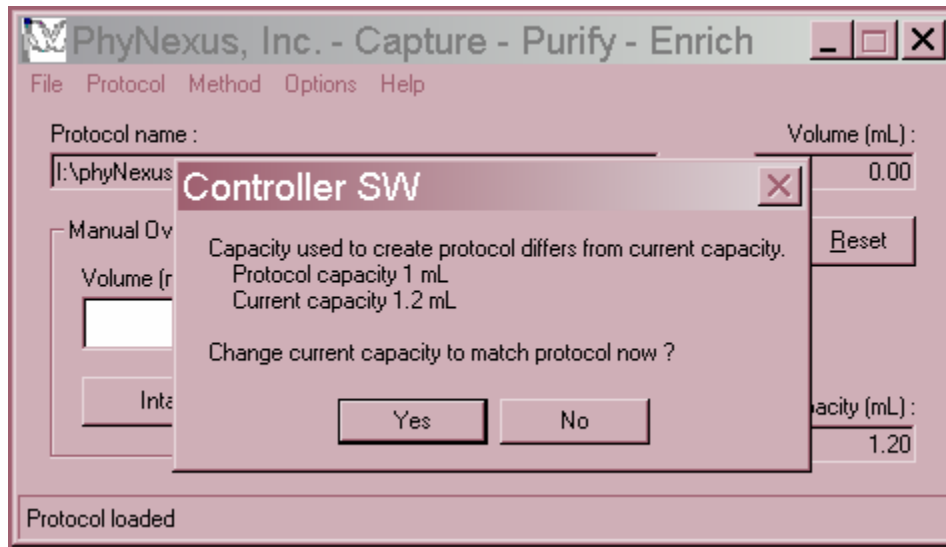
If the capacity setting is changed while a protocol is open, the user is prompted as follows:



This message indicates that the protocol may not execute properly with the new capacity setting.

2.2.2.2 Capacity and protocols

If a protocol specifies a different capacity than the current app settings, the user is prompted as follows when the protocol file is opened:



Answer “yes” to set the app’s capacity setting to match the protocol, or “no” to do so later.

Note: Answering “no” may prevent the protocol from executing properly.

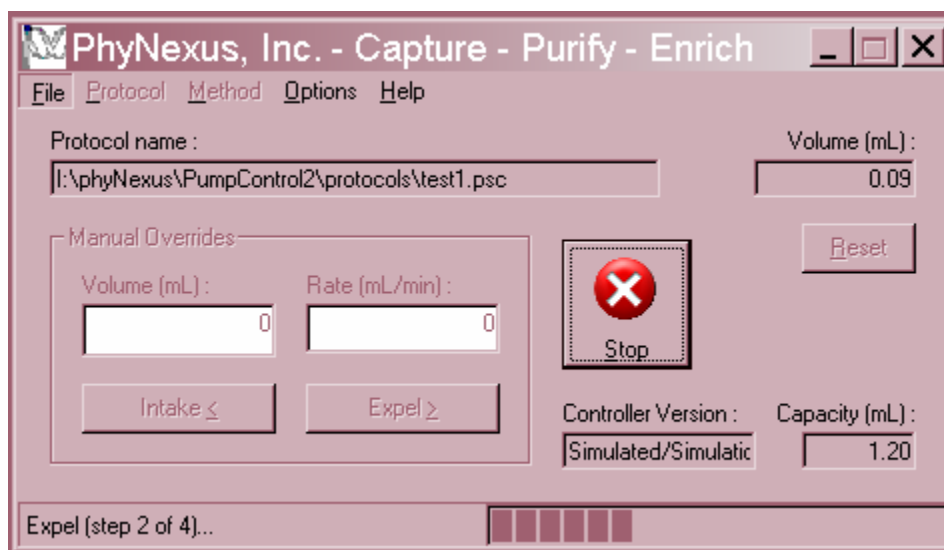
2.2.3 Start protocol execution

Once the protocol has been opened, and the controller has been prepared for protocol execution, select the Protocol->Execute option. The app will establish communication with the controller (go online), get the controller’s software version and capacity, disable the controller’s keyboard / speaker / LCD display, and get the current volume. The protocol will then be automatically *verified*, as noted in section 2.3 below, using the controller’s current volume. Then the app will instruct the controller to execute the intake or expel steps contained in the protocol.

The app displays the following during protocol execution:

1. Status bar displays the protocol step and step type (intake/expel).
2. **Progress bar** (to the right of the status bar text) shows how far along the protocol is in total execution time, as calculated by the volume and rate of each step.
3. **Volume** displays the current volume in the syringe, as reported by the controller after the most-recently-executed step.
4. **Controller Version** shows the firmware version of the controller. In this case it's simulated, but with an actual controller this would display a version number such as 1.4. See section 1 above for compatibility information.

An example is as follows:



When the protocol is completed, the status bar will display “*Protocol execution complete*” and the Volume indicator will show how much volume is in the controller at the end of the protocol.

The protocol is automatically terminated if an error occurs during execution, and the error is shown in the status bar. See “Possible errors and corrective action” below (section 2.2.3.2).

2.2.3.1 Stopping protocol execution

If the protocol needs to be stopped before it's complete, then press the Stop button. Pressing stop terminates the app's execution of the protocol, but the controller will continue executing the last command issued by the controller until it has been completed.

2.2.3.2 Possible errors and corrective action

If a ***Port in use*** error results when the protocol is executed, then some other application is using the serial port selected (See section 2.2.1 above). Possibly a modem is plugged in and is being used or the HyperTerminal app (which is found on most versions of Windows). To fix this, select a different serial port from the Serial Options dialog and try again, or find the application which is using the serial port, and shut it down.

The error ***No response from controller (offline)*** indicates that the serial port selected is not actually connected to the controller. Select a different serial port and try again.

Controller error (command not echoed, bad checksum, invalid parameter or command) indicates a problem communicating with the controller over the serial cable, or an incompatible version of controller firmware. Check that the serial cable is in good condition, and that the controller firmware version is supported by the app (see section 1 above).

2.3 Verify protocol

Verification is performed automatically by the app prior to executing a protocol, but may also be invoked manually. In this process, the app checks that the following rules are followed during the protocol:

1. Volume never falls below zero.
2. Volume never rises above the capacity (see also section 2.2.2.1).
3. Flow rates are within limits for the capacity (see Appendix D).

When verification is performed automatically just prior to protocol execution, the initial volume is assumed to be that reported by the controller.

A protocol may be manually verified using the Protocol->Verify menu option. In this case, whatever current volume is presently reported by the controller is assumed to be the initial volume for verification purposes. If the app has not yet gone online, then the initial volume is assumed to be zero.

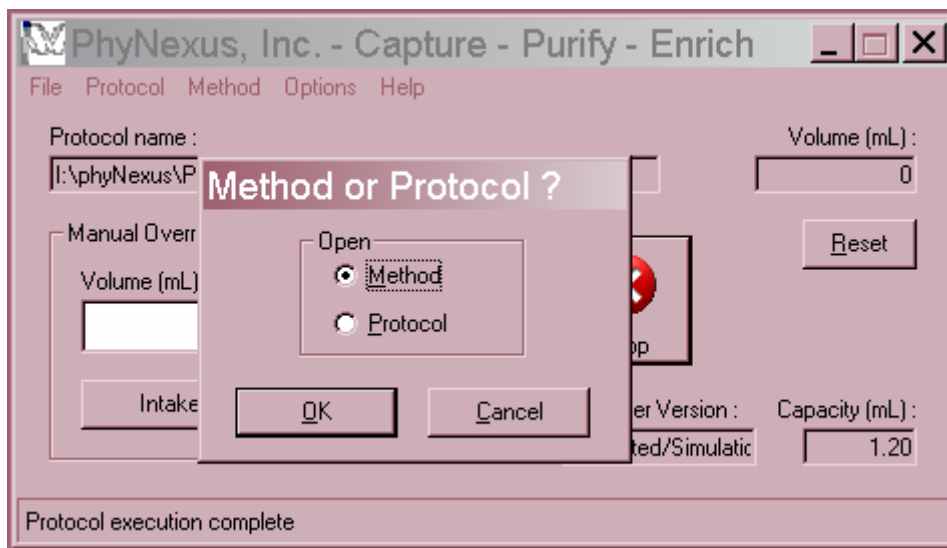
If protocol verification fails, then an error message appears showing the line in the protocol at which the error occurred.

3. How to open and execute a method

Handling of methods is similar to that of protocols.

3.1 Open Method

Use the File->Open menu option and select Method to browse for a method file, as shown below:

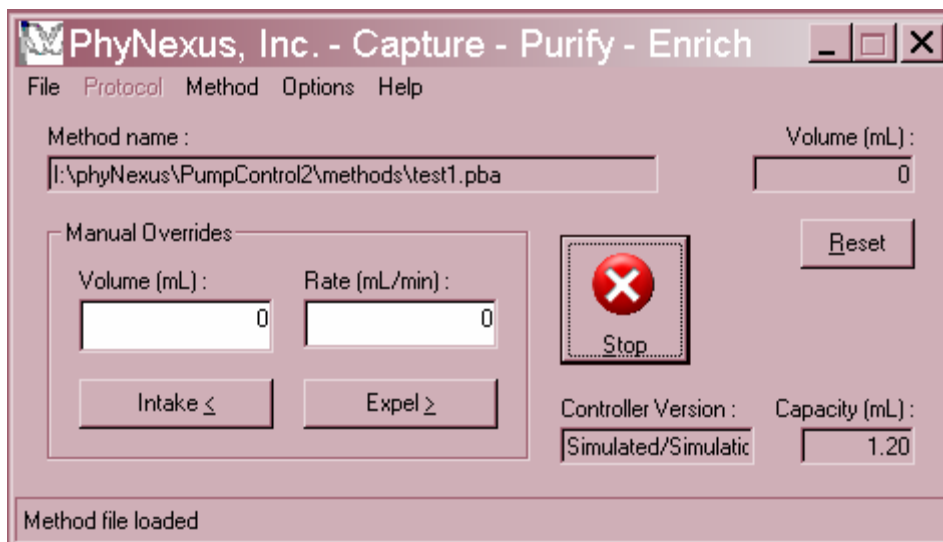


Method files are assumed to have a .pba file extension. If the method file was opened successfully, then it may be executed and/or edited.

If the app displays an error message when you try to open the method file, then it may have been corrupted, e.g. via disk error or hand-editing, or the file selected is simply not a method.

The **MRU** (most-recently-used) list in the file menu also handles method files.

After opening the method file, the display will indicate the currently-open method, as shown below:



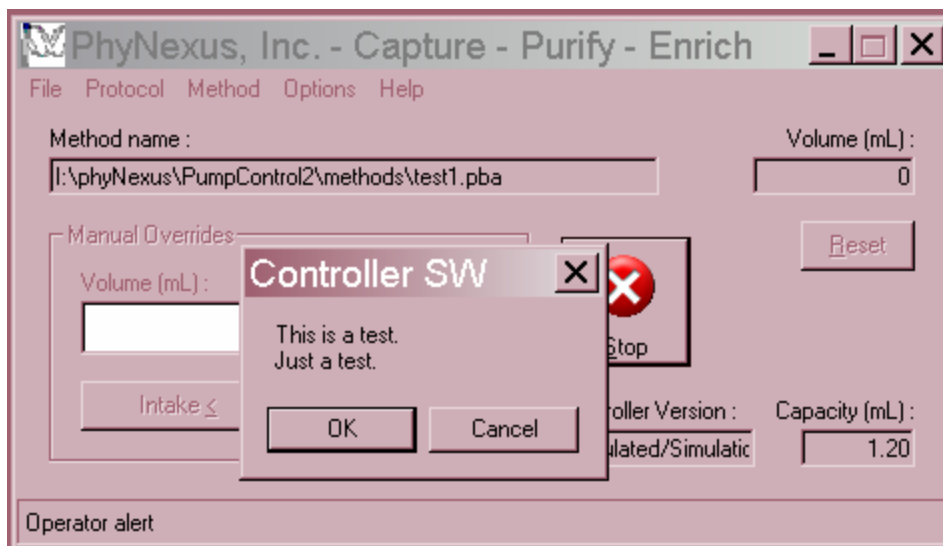
3.2 Execute method

Before executing the method, make sure of the following (see section 2.2 above):

1. Controller is correctly mounted on the Adjustable System Holder.
2. App's serial parameters are set to match those of the controller.
3. App's capacity setting matches the controller's capacity.

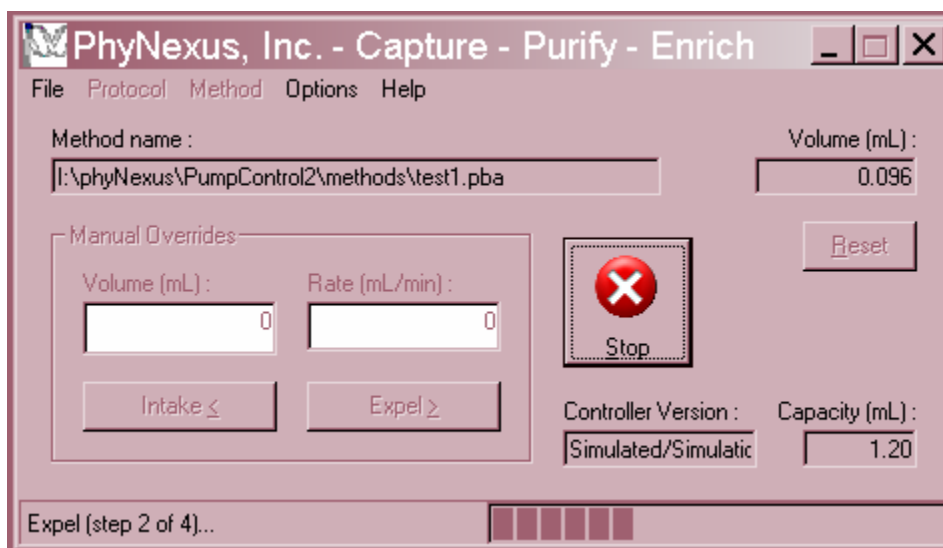
To execute the currently-open method, select the Method->Execute menu option. The app will then go online with the controller, much as it would while executing a protocol.

When a Pause method command is executed, the app displays the message specified in the command, and stops to wait for operator intervention, as shown:



The operator can press **OK** to proceed with method execution, or **Cancel** to abort the method.

The appearance of the app while executing a protocol within a method is very similar to that when simply executing a protocol standalone – the only difference is that the method name is shown instead of the protocol name, as shown here:



Similarly, the Blowout and Home commands cause the status bar to be updated, and Volume to be zeroed.

See section 2.2.3 above for information on stopping execution, and possible errors that may occur, since this information applies equally to methods as well as protocols.

4. Manual Commands

The controller can be instructed to perform individual intake and expel steps when the app isn't running a protocol. The following procedure should be used:

1. Verify that the controller is mounted in the Adjustable System Holder correctly and the appropriate PhyTip columns are attached.
2. Verify that the app's serial parameters match those of the controller.
3. Verify that the app's capacity setting matches that of the controller.
4. Enter the volume and rate in the text boxes under the *Manual Overrides* frame, and then press the *Intake* or *Expel* button.

This will cause the app to go online with the controller and execute the intake or expel step. Status will be shown in the status bar, and the Volume indicator will be updated when the operation is completed, if successful.

See section 2.2.3.2 above for troubleshooting.

4.1 Reset volume

The Reset button may be used to manually blow out the controller contents, and reset its volume to the "home" position (zero).

4.2 Stopping manual commands

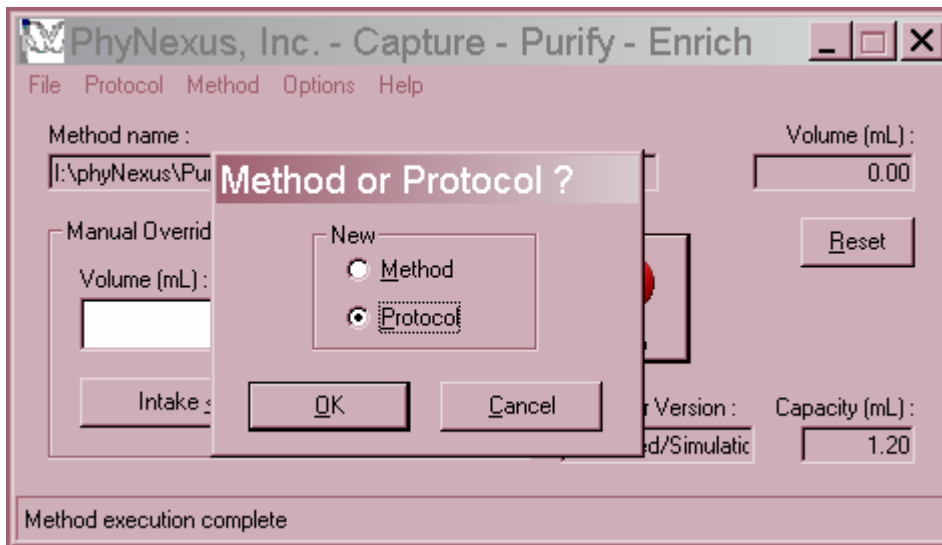
Manual intake/expel operations may be aborted using the Stop button. As with aborting protocol or method execution, pressing stop will only prevent the app from issuing further commands to the controller, but the controller will finish executing the last command it was given. When this last command is complete, the app will display the current volume as last reported by the controller.

Note: Communications and controller settings can only be changed when the app is not communicating with the controller (not executing a protocol, method or manual override command). Pressing 'Stop' terminates communication, and allows these settings to be changed.

5. Protocol editing

5.1 How to create a new protocol

To create a new protocol, select File->New and select Protocol, as shown below:



Then press OK, which will pop up the editor dialog:



Press the **Add** button to insert new steps. New steps may be edited after they have been added (see below).

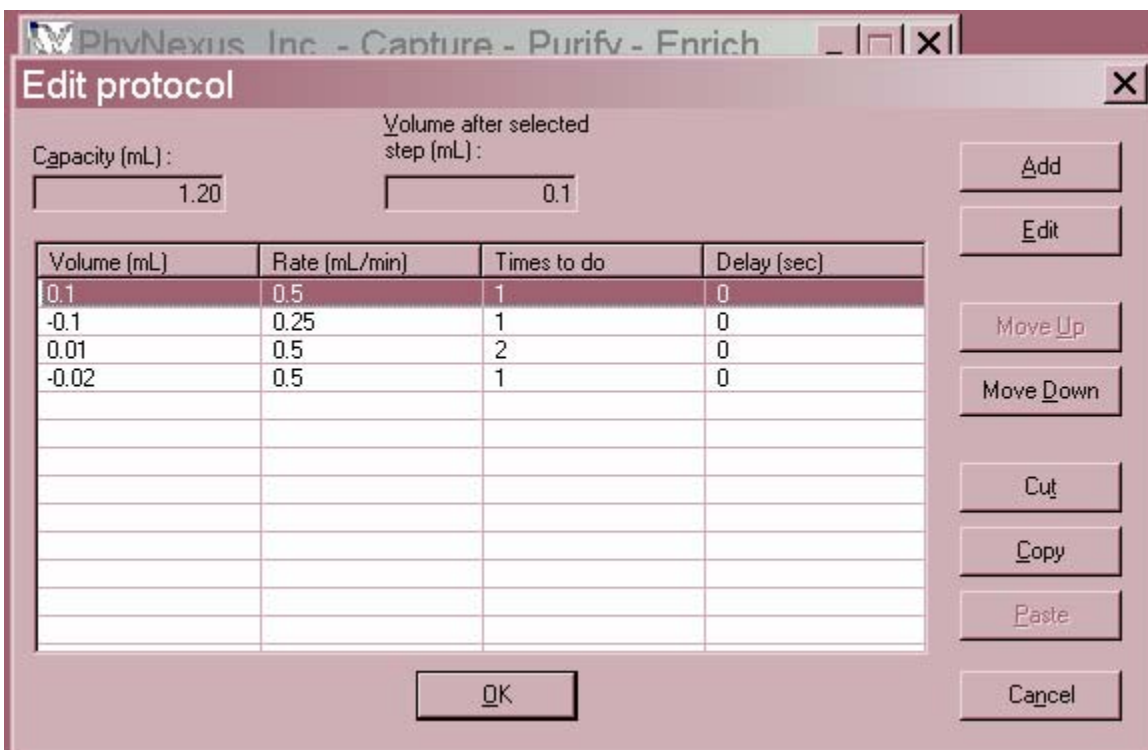
Steps may be re-ordered, either individually or in groups, by selecting the steps to be re-ordered, and using the **Move Up** and **Move Down** buttons.

A single step or a group of steps may be cut, copied, or re-inserted, using the **Cut**, **Copy**, and **Paste** buttons – these work as most Windows users would expect, and the actions of the buttons may also be invoked by pressing <Ctrl-X>, <Ctrl-C>, and <Ctrl-V>, respectively.

When a single step is selected (highlighted using the mouse or keyboard), the **Volume after selected step** indicator shows the volume in the syringe after the current step has been executed. The volume at the beginning of the protocol is assumed to be the value in the **Initial volume** edit box. The user may change the Initial volume, as desired.

5.2 How to edit an existing protocol

To edit a protocol, select Protocol->Edit to pop up the editor dialog, as described above. An example is shown below:



See next pages for a description of creating and editing steps.

5.3 Create protocol step

To create a new protocol step, press the Add button – this pops up the following dialog:

Delay (sec)
0
0
0
0

Note: If no protocol step was selected in the editor dialog, then the new step is placed at the end of the protocol. If any protocol steps were selected, then the new step is placed at the end of the selected steps. The *Move Up* or *Move Down* button may be used to move the new step, if desired.

Make sure to enter the correct values and units for the volume and rate, select the direction (intake/expel), the number of times to execute the step, and the post-step delay, if any. Delay times are rounded to the nearest millisecond. If a step is to be repeated, then the post-step delay is also repeated when executed.

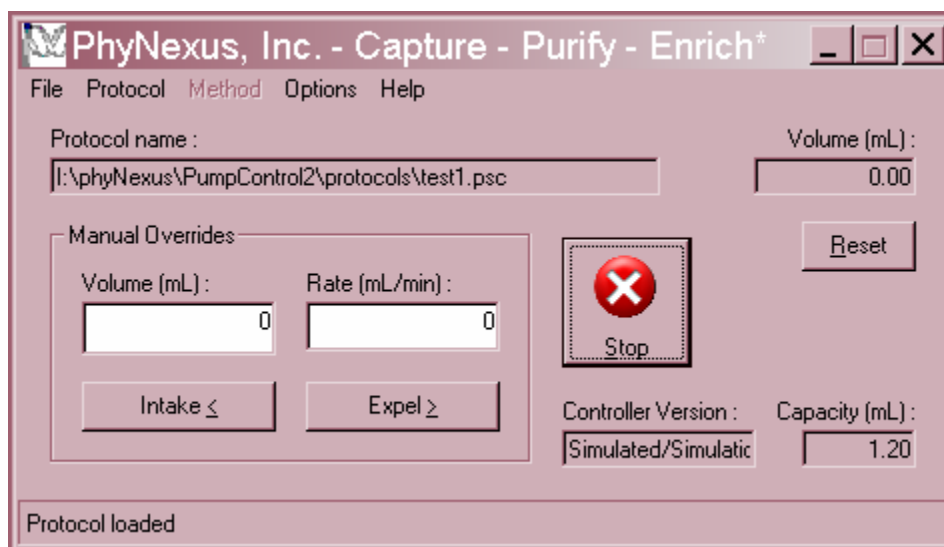
The ***Volume prior to this step*** indicator shows the calculated volume prior to the step being added. This dialog will not allow the volume to exceed the capacity, and also checks the rate against the capacity (see Appendix C).

5.4 Edit protocol step

To edit an existing protocol step, select the step in the editor window, and either double-click on the step, or press the **Edit** button. The protocol step dialog described above is then shown.

5.5 Save protocol to file

After a user presses **OK** in the editor window, the protocol is assumed to have been changed, and the app's caption indicates this via an asterisk next to the title, as shown below :



The asterisk means that there are changes to the protocol that have not been saved. Select File->Save to save the changes back to the previous filename, or File->Save As to save changes to a new filename.

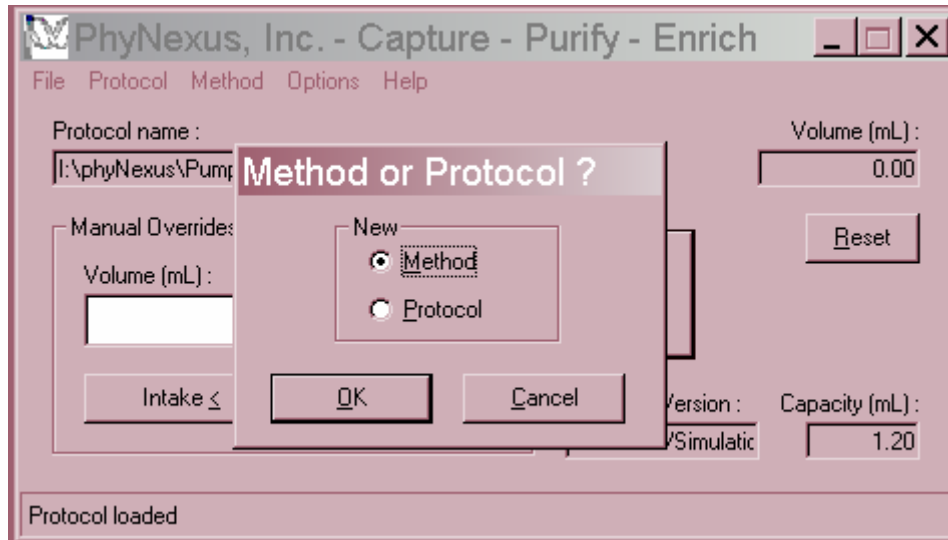
Note that the app's current capacity setting is also saved to the protocol file as a comment (see Appendix B).

6. Method editing

The steps involved in creating and editing a method are similar to that for a protocol.

6.1 How to create a new method

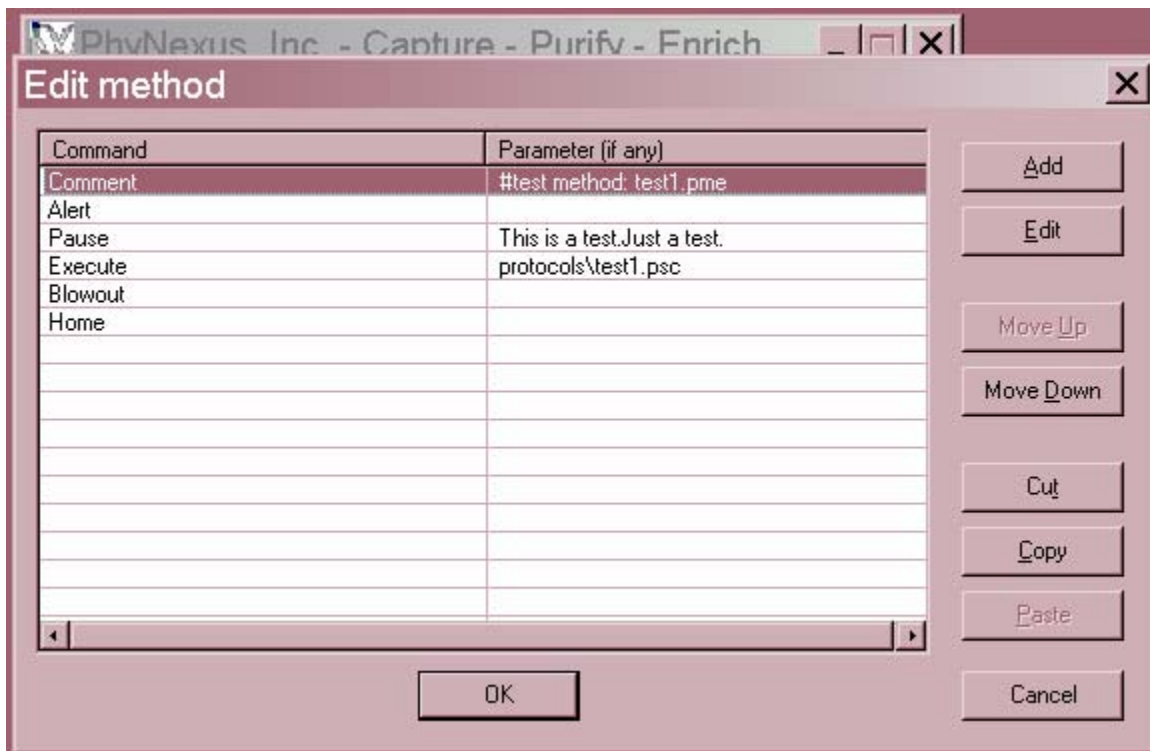
To create a new method, select File->New and select Method, as shown below :



Then press OK, which will pop up the editor dialog:

6.2 How to edit an existing method

Select Method->Edit to pop up the editor dialog. An example is shown below:

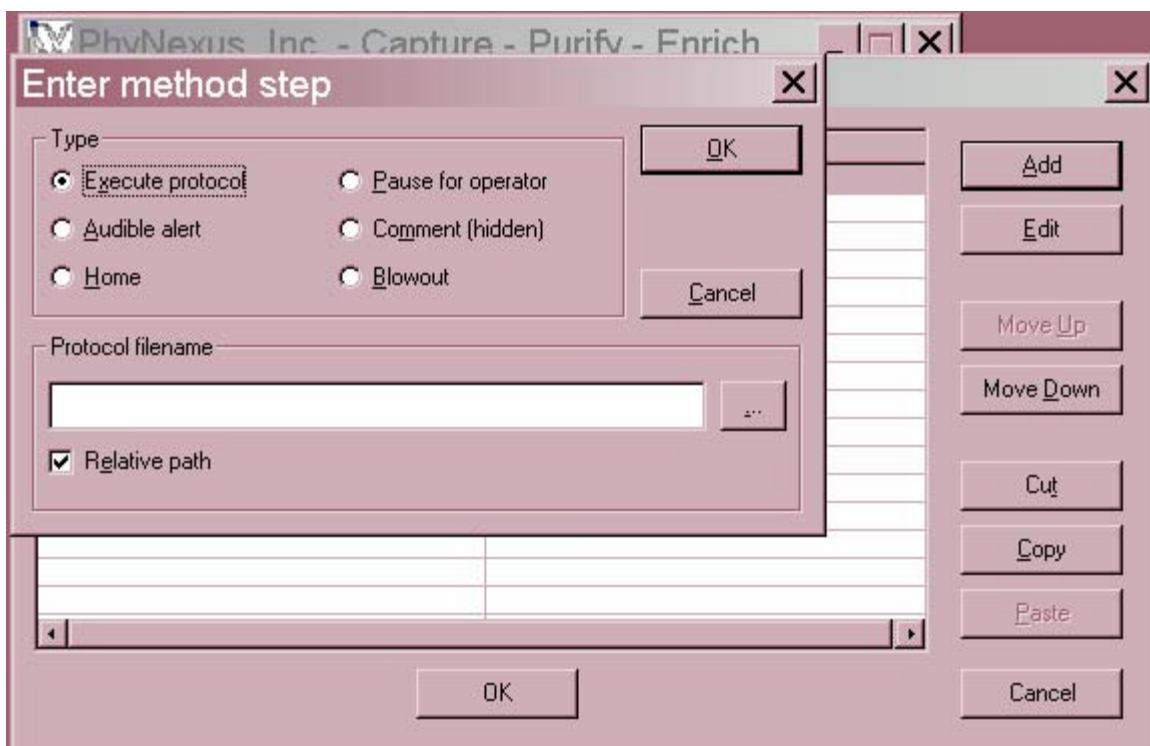


Note that the method shown above contains one of each of the six kinds of steps which may compose a method.

See below for a description of creating and editing method steps.

6.3 Create method step

To create a new method step, press the Add button – this pops up the following dialog :



Note: If no method step was selected in the editor window, then the new step is placed at the end of the method. If any steps were selected, then the new step is placed at the end of the selected steps. The *Move Up* or *Move Down* button may be used to move the new step, if desired.

Select the type of method step desired – this will cause the entry field(s) at the bottom of the dialog to change appearance as needed. Enter the parameter needed, as follows:

1. Audible Alert: no parameter needed.
2. Home: no parameter needed.
3. Pause for operator: enter message to display for operator. Note that this message may contain multiple lines, as needed.
4. Comment: enter any information needed to document this method.
5. Blowout: no parameter needed.
6. Execute protocol: enter path to protocol file. The browse button may also be used.

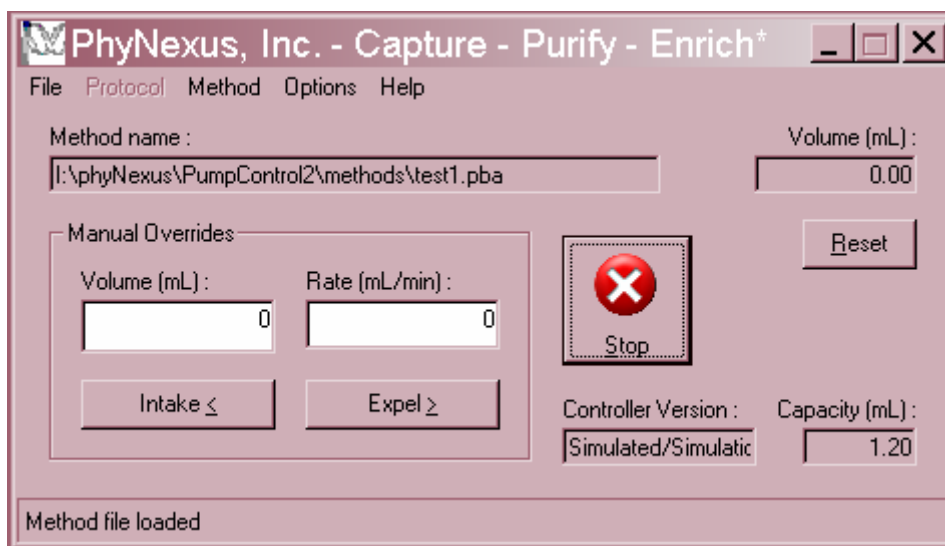
If **Relative Path** is clicked, then the path will be shortened to be relative to the app's executable file, if the path can be found somewhere under the app's installation directory (such as the protocols and batch folders).

6.4 Edit method step

To edit an existing method step, select the step in the editor window, and either double-click on the step, or press the **Edit** button. The method step dialog described above is then shown.

6.5 Save method to file

After a user presses **OK** in the editor window, the method is assumed to have been changed, and the app's caption indicates this via an asterisk next to the title, as shown below:



The asterisk means that there are changes to the method that have not been saved. Select File->Save to save the changes back to the previous filename, or File->Save As to save changes to a new filename.