Microlab STAR User Manual

for User Software 3.0

including Options and Accessories

HAMILTON Bonaduz AG

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1 General Information

Hamilton's Microlab STAR is the next generation pipetting workstation. This User Manual is designed to help you get the most out of your Microlab STAR.

You should read through the entire manual before beginning to operate your instrument. This first chapter should be read with particular attention. It contains important information about the use of the Microlab STAR and this manual.

1.1 About this Manual

This manual is to help users operate the Microlab STAR correctly and safely.

To achieve that aim, Chapter 3 of the manual will describe the different components of the Microlab STAR and how they work. Then, in succeeding chapters, we will describe what can be done with each – the basic operations of aspirating and dispensing liquids. The manual describes both the hardware and software of the Microlab STAR to the extent that a user needs to know them in order to operate the instrument.

After introducing you to the various parts of the Microlab STAR, we show you step by step how to perform typical operations using those components. Sample methods for typical applications guide you through the programming. When you have worked through this manual, you should be quite well able to operate the Microlab STAR.

Warnings and *notes* are included in this manual to emphasize important and critical instructions. They are printed in italics in the left margin of the page, begin with the word 'Attention' accompanied by the '!' symbol, or the word 'Note', as appropriate.

This manual refers to User Software release 3.0 for the Microlab STAR.

1.2 Additional Microlab STAR Manuals

A detailed software reference for the Microlab STAR is to be found in the online help of the User Software. This online help will answer any question you may have about details of the Microlab STAR User Software.

The manner in which the Microlab STAR and its components are to be serviced is described in the *Microlab STAR Service Manual*. This manual will be made available to Hamilton-authorized service technicians.

Whenever a manual amendment is issued, detailed instructions on amending the existing manual will be provided.

1.3 Intended Use of the Microlab STAR

The Microlab STAR is a robotic pipetting workstation, in other words, a sampler used for pipetting liquid samples in an automated process suitable for medium to high throughput with a high degree of flexibility in pharmaceutical, veterinary and genetics applications.

A user will typically wish to carry out low, medium, or high volume contamination-free pipetting with disposable tips or with steel needles.

At the present time, the Microlab STAR is classified as a general laboratory instrument and is not specifically validated as an *in vitro* diagnostic device.

1.4 Operation

The operator of the Microlab STAR must have attended an appropriate training course. The procedures contained within this manual have been tested by the manufacturer and are deemed to be fully functional. Any departure from the procedures given here could lead to erroneous results or malfunction.

1.5 Safety

The following section describes the main safety considerations, electrical and biological, in operating this product, and the main hazards involved.

1.5.1 General Precautions

When using Microlab STAR, Good Laboratory Practices (GLP) should be observed. Suitable protective clothing, safety glasses and protective gloves should be worn.

During Microlab STAR operation, do not place hands in the way of moving parts or on the working deck. Keep your head and hands away from the work surface of the Microlab STAR when it is in operation – the pipetting arm and channels move fast and it is possible to sustain an injury. In general, never lean over the Microlab STAR when working with it.

When working with samples, do not switch tubes around after they have been identified by the barcode reader. This could result in incorrect test data.

When working with samples which will be used in particularly sensitive tests, take into account evaporation and condensation that may occur while the method is running.

Perform test runs i) with deionized water and ii) with the final liquids, prior to routine use. Test for all the liquid classes you are going to use.

Liquid level detection needs to be explicitly tested when working with foaming liquids.

If sampling aggressive liquids, use filter tips.

During operation, the Microlab STAR should be shielded from direct sunlight and intense artificial light.

NOTE

Discard used tips. Do not reuse them.

Use filter tips for tasks which are sensitive to cross-contamination (aerosols).

Note that foam may affect the accuracy of liquid level detection.

As a general safety principle, the inner diameter of sample tubes, reagent vessels, etc. must be greater than the channel diameter of 9 mm.

If the system is paused, do not wait too long before resuming the run. Loss of liquid from a full tip may result in invalid data.

Do not overfill reagent containers, tubes, or other liquid containers.

Switch off the power of the Microlab STAR during maintenance.

Set up the Microlab STAR with its back to a wall to preclude any rear access to the work area.

The breakdown of the power supply during a run may cause the loss of data. If the loss of data is unacceptable, use an independent power supply.

Opening the front window during a run will lead to a system abort and may cause the loss of data.

To avoid computer breakdowns, ensure that there is always enough storage capacity on your hard drive.

Never disable any security measure.

Do not leave tips or needles picked up on the pipetting channels for long periods (such as overnight). This may cause damage to the O-rings.

For repair or shipment, all mechanical parts must be put in their rest positions. A Microlab STAR sent away for repair must also be decontaminated if it was in a laboratory environment with infected or hazardous materials. The Microlab STAR must be repacked in the original shipping crate and only by an authorized service technician. There should be no containers or tips on the Microlab STAR during transportation.

Only original HAMILTON Microlab STAR-specific parts and tools may be used with the Microlab STAR, e.g. carriers, racks, tips, steel needles, and waste containers. Commercially available liquid containers, such as microtiter plates and tubes, may of course be used.

If working with contaminated samples, the user need not touch them. The Microlab STAR will drop its used tips into a waste container that should be emptied as soon as it is full.

The Microlab STAR products conform to European norms as regards interference immunity. However, if the Microlab STAR is subjected to electromagnetic RF fields, or if static electricity is discharged directly onto the Microlab STAR, its Liquid Level Detection ability (see below) may be negatively affected. It is therefore recommended that the Microlab STAR be kept away from other equipment that emits electromagnetic RF fields in the laboratory, and that static electricity be minimized in its immediate environment.

1.5.2 Electrical Safety Precautions

Before removing a mechanical or electrical component, the Microlab STAR must first be switched off and disconnected from the main electricity supply and PC.

1.5.3 Biohazard Precautions

If the Microlab STAR becomes contaminated with biohazardous material, it should be cleaned in accordance with the maintenance procedures given in the section “Maintenance” (3.7). Observe and carry out the maintenance procedures given. Failure to do so may impair the reliability and correct functioning of the Microlab STAR.

If working with biohazardous samples, observe and carry out the maintenance procedures paying particular regard to cleaning and decontamination. Wear gloves when handling the pipetting arm and channels, the carriers, racks, and containers, and the tips and steel needles. Avoid touching the discarded tips in the waste container. Any surfaces on which liquid is spilled must be decontaminated.

1.5.4 Computer Precautions

Guard against software viruses. Use only manufacturer’s original installation CD-ROM sets for the operating system, and the original HAMILTON software.

Only the HAMILTON User Software and the firmware protocol (CoCo/KuSt, cf. *Microlab STAR Service Manual*) may be used to control the Microlab STAR.

1.6 Warranty Statement

HAMILTON Bonaduz AG, CH-7402 Bonaduz / Switzerland is the manufacturer of the Microlab STAR.

The Microlab STAR is sold in accordance with the general conditions of sale of HAMILTON Bonaduz AG.

HAMILTON warrants this product to be free of defects in material and workmanship for a period of 12 months from the date of delivery, ex works Bonaduz.

HAMILTON or the authorised HAMILTON representative will repair or replace, at its option and free of charge, any product that under proper and normal use proves to be defective during the warranty period.

HAMILTON shall in no event be liable or responsible for any incidental or consequential damage, either direct or contingent.

HAMILTON accessories and consumable products, e.g. carriers, racks, tips, steel needles, and waste containers, are warranted to be free of defects in material and workmanship at the time of delivery only.

The above warranty shall not apply if:

- the product has not been operated in accordance with the user manual;
- the product is not regularly and correctly maintained;
- the product is not maintained, repaired or modified by a HAMILTON-authorized representative or user;
- parts other than original HAMILTON parts are used, except for liquid containers such as microtiter plates and tubes;
- the product or parts thereof have been altered without written authorization from HAMILTON Bonaduz AG;
- the product is not returned properly packed in the original HAMILTON packaging.

HAMILTON reserves the right to refuse to accept any product that has been used with radioactive or microbiological substances, or any other material that may be deemed hazardous to employees of HAMILTON. Such a product has to be properly decontaminated and marked.

HAMILTON endeavours to provide prompt and satisfactory service.

1.7 Customer Service

Customer service will be provided in the first instance by the network of HAMILTON representatives. In the event of any problem experienced with your Microlab STAR, the first recourse is your local HAMILTON representative. For further problems requiring hardware or software expertise, the Technical Support department at HAMILTON Bonaduz AG will be available by phone, fax or e-mail to deal with your queries. Here is their address, phone, fax and e-mail:

Europe, Africa, and Asia:

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HAMILTON Company
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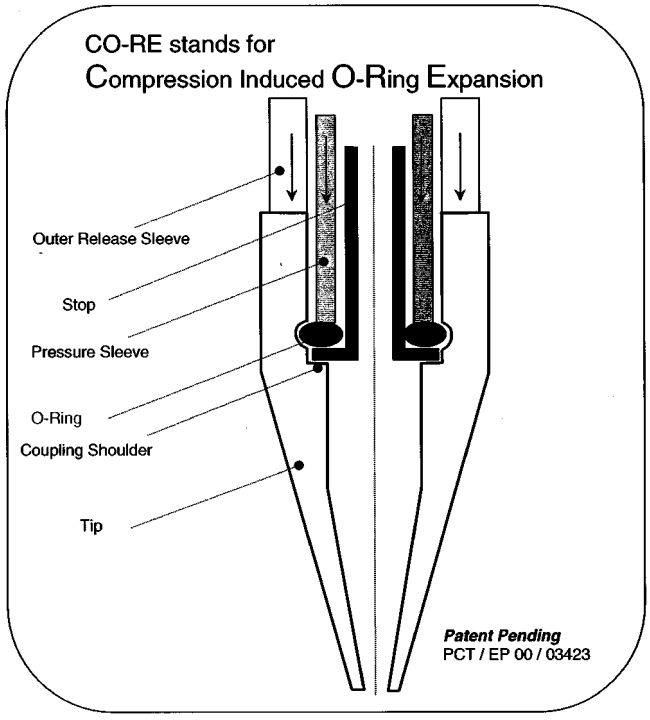
2 The Art of Pipetting

In this chapter the process of pipetting with the Microlab STAR is described. Pipetting means transfer of small quantities of liquid from one container to another. A pipetting operation is achieved by aspirating (drawing) liquid from a source container, then transferring and dispensing (dropping) it into a target container.

2.1 The Air Displacement Pipetting Principle

The Microlab STAR is based on the **air displacement pipetting** principle, comparable to the functioning of hand pipettes. Air displacement means that the liquid is aspirated into and dispensed from a disposable tip or needle by the movement of a plunger. Between the plunger and the liquid surface is air. No system liquid of any kind is involved in the Microlab STAR.



The Air Displacement Pipetting Principle

The air displacement principle has the following advantages:

- Independent and modular design of pipetting heads for most flexible assay programming.
- CO-RE (compression-induced O-ring expansion) technology for the flexible coupling of tips or needles to the pipetting channel.
- Tips or reusable needles of three sizes on the same head in the same run.

- The construction principle enables pressure-based liquid level detection and aspiration monitoring.
- No contamination or dilution by system liquids.
- No drops due to moving tubes.
- No problems with corroded tubing, pumps, etc.
- Same commonly accepted pipetting principle as for hand pipettes.

2.2 From Aspiration to Dispensing

In this section we describe in detail the processes involved in a simple pipetting step.

2.2.1 Tip Pick-up

The first task for the Microlab STAR is to pick up a disposable tip or a reusable steel needle. For tips, special carriers typically holding 5 tip racks of 96 tips are placed on the instrument deck. Steel needles may be picked up directly from the wash station, or from a separate needle rack, which typically is a tip rack on a tip rack carrier with needles instead of tips.

2.2.2 Aspiration

The first step within an aspiration and dispense cycle is to aspirate a variable amount of blowout air, which is used at the end of the (last) dispense, to blow the liquid out of the tip. This is done with the tips still in the air.

To start the aspiration of liquid, the tip must make contact with the liquid. This may be done by moving the tip to a **fixed height**. This height must be chosen to be permanently below the liquid level, to prevent the aspiration of air. On aspiration, the tip follows the falling liquid level (if so specified) according to the volume aspirated. The distance to follow is computed from the known geometry of the (first segment of the) liquid container.

More elegantly, and with greater safety, the liquid level of the vessel to be aspirated from can be detected. This can be provided by STAR's **Liquid Level Detection** (LLD) feature, based on either capacitive (cLLD) or pressure (pLLD) signal detection. For conductive liquids, capacitive LLD should normally be used. The sensitivity of the capacitive LLD that is to be used depends on the vessel size and the conductivity (or polarity) of the liquid that is to be detected. For a solution of 0.1% NaCl in distilled water, the sensitivities are:

cLLD Setting	Sensitivity Level	Vessel
1	Very High	384-well plates
2	High	96-well round-bottom plates
3	Medium	96-well flat-bottom plates
4	Low	Tubes

NOTE

Using an ionic buffer in your assay in place of distilled water may help to overcome liquid level detection problems.

Use only original Hamilton labware carriers. For a proper capacitive liquid level detection, a sufficient conductive coupling of carrier and labware (tubes or microplates) is crucial.

For non-conductive liquids, or in case of an insufficient electrical coupling between container bottom and carrier, pressure LLD should be used. Pressure LLD only works with new and empty tips for the aspiration of liquids. The suitable settings depend on the tip size and on the type of liquid.

300 µl channel:

pLLD Setting	Sensitivity Level	Tip	Liquid
1	Very High	Standard	Low boiling point, low viscosity
2	High	Low	Low boiling point, low viscosity
3	Medium	Standard	Water or higher viscosity
4	Low	Low	Water or higher viscosity

1000 µl channel:

pLLD Setting	Sensitivity Level	Tip	Liquid
1	Very High	Standard	Low boiling point, low viscosity
2	High	High	Low boiling point, low viscosity
3	Medium	Standard	Water or higher viscosity
4	Low	High	Water or higher viscosity

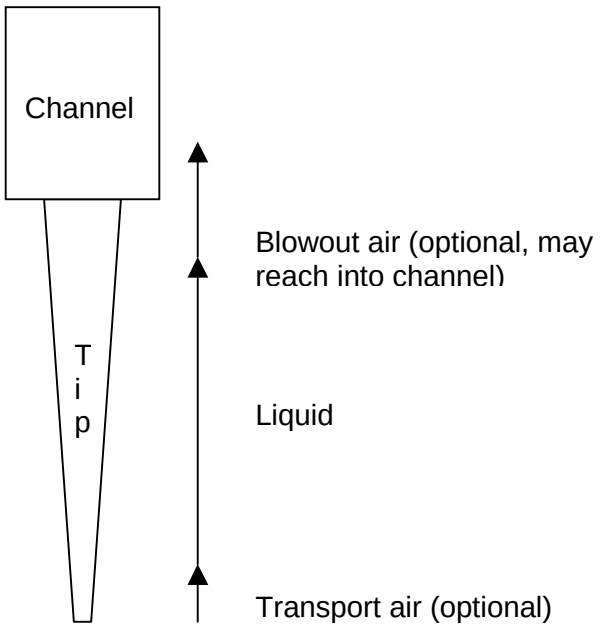
In the case of **aspiration from foaming liquids**, capacitive liquid level detection in particular may not detect the surface properly. As an alternative, try pressure LLD, or a combination of both. If you are using a combination of both LLD types, the maximum height difference between the two independent LLDs can be used as a parameter.

The following table gives dead volumes for both pressure- and capacitance-based liquid level detection in various containers.

Labware	V_{min}/µl	Carrier
Tubes, 16 mm x 100 mm	200	SMP-CAR-24
Tubes, 12 mm x 75 mm	150	SMP-CAR-32
Eppendorf tubes 1.5 ml	50	SMP-CAR-EPIL
Eppendorf tubes 0.5 ml	50	SMP-CAR-EPIS
96-well PCR plate (200µl/well)	50	PLT-CAR-L5PCR
96-well flat-bottom microplate	75	PLT-CAR-L5MD
384-well flat-bottom microplate	50	PLT-CAR-L5MD
96-deepwell microplate (archive)	150	PLT-CAR-L5AC

Once the liquid surface is detected, an additional immersion depth (typically 2mm) is reached to prevent the aspiration of air, and **aspiration** starts. The tip follows the falling liquid level (if so specified) according to the volume aspirated. Then, the tip leaves the liquid slowly and heads for the vessel to dispense into.

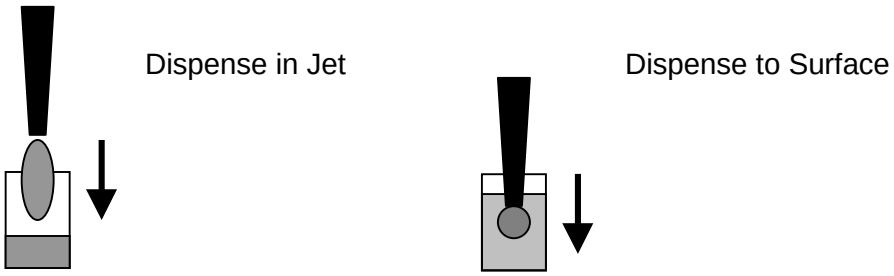
Finally, to prevent droplet formation, a variable amount of transport air is aspirated. After an aspiration step, the situation within the tip looks like this.



The situation within the tip and the channel after aspiration.

2.2.3 Dispense

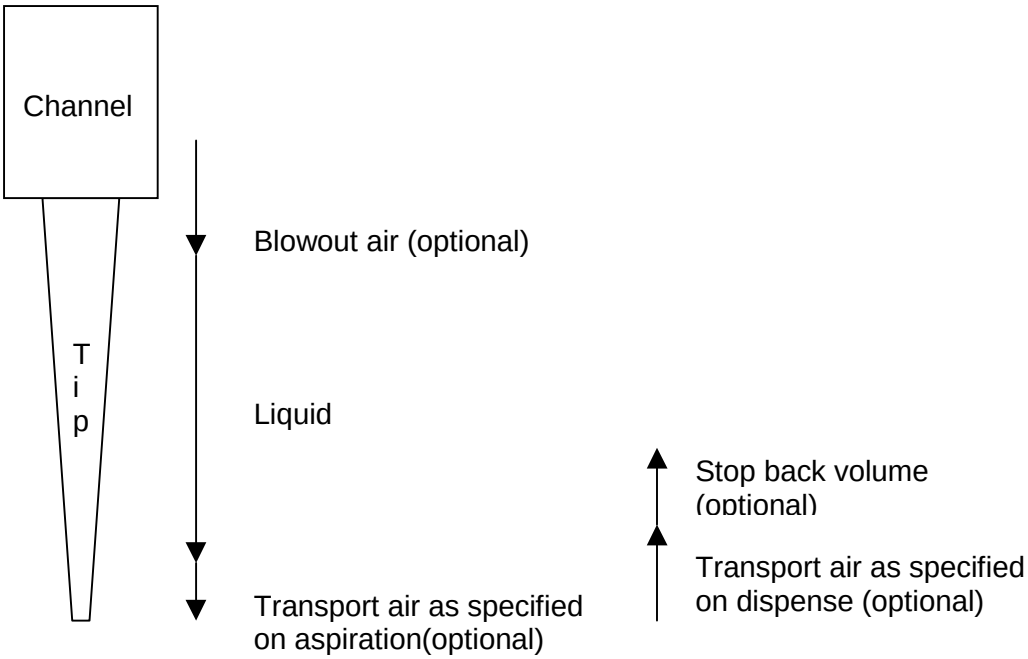
The transport air that was aspirated at the end of the aspiration step is first dispensed with the tip still in the air. Dispensing of the liquid may now occur in two different modes: to the liquid surface or in a jet.



To ensure that the specified accuracy is achieved, volumes below 20 µl should always be **dispensed to a liquid surface**. For dispensing to a liquid surface use cLLD to detect the position of the surface and then dispense by following the rising liquid level. For volumes larger than 20 µl the liquid can be **dispensed in a jet** without touching the surface. To dispense in a jet specify a position a couple of millimeters above the surface and dispense by following the rising liquid level. For dispensing in a jet, a varying amount of blowout air is used to make sure that all liquid is dispensed from the tip.

However, dispensing to the surface of an empty vessel (touch off) is also possible. For dispensing into an empty container, position the tip less than 1 mm (typically 0.4 mm) above the bottom of the container and dispense, following the rising liquid level.

After the dispense, the situation within the tip looks like this:



Situation on dispense: Firstly, transport, liquid, and blowout volume are dispensed, followed by an aspiration of stop back volume (in the case of partial volume dispenses) and transport air.

2.2.4 Tip Drop-Off

The final step is to eject the used tip into the waste container of the Microlab STAR. A needle will be placed back into the wash station, where the wash process may be started directly.

2.3 Avoiding Contamination

If cross-contamination is a concern, consider the following approaches:

- Use only HAMILTON tips on the Microlab STAR.
- Use new tips for every pipetting step to avoid carry-over between different wells or containers.
- Use filter tips to avoid contamination of the pipetting channel by jets, aerosols, etc.
- Always dispense to a surface. Dispensing in a jet may produce aerosols and thus cause cross-contamination.
- Always dispense using a residual volume, i.e., do not completely empty the tip on dispense. This can be achieved e.g. by aspirating 10 µl and dispensing only 9 µl.

2.4 Liquid Classes, Pipetting Modes, and More

In general, pipetting on the principle of air displacement (as with hand pipettes) is sensitive to

- the manner of pipetting (e.g. surface or jet),
- tip or needle type
- environmental effects (temperature, pressure, humidity), and
- liquid type.

The instrument's behavior is determined by specifying the pipetting mode (e.g., surface or jet mode) and the liquid class. Pipetting mode and liquid class represent two independent sets of information which both have to be specified. For aspiration, three modes are possible:

- Simple "Aspiration", for all standard cases,
- "Consecutive Aspiration" for aspiration with a tip that has already aspirated liquid, and
- "Aspirate All" for aspiration of all the liquid within a cup (specify a volume larger than what is expected within the cup). In this case, aspiration monitoring is deactivated and the tip will follow the falling liquid level (if specified) to the bottom of the container, staying there for the rest of the aspiration.

For dispensing, four modes are possible:

- "Surface Dispense Part Volume" for dispensing only a part of the liquid in the tip to a surface, leaving a residual volume in the tip,
- "Surface Dispense Empty Tip" for dispensing all the liquid in the tip to a surface,
- "Jet Dispense Part Volume" for dispensing only a part of the liquid in the tip in a jet, i.e. without touching a surface, leaving a residual volume in the tip,
- "Jet Dispense Empty Tip" for dispensing all the liquid in the tip in a jet.

The **liquid class** stores all relevant background parameters, such as flow rates and volume corrections, for one pipetting cycle, i.e. for one aspiration and the subsequent dispense(s). Depending on the pipetting mode chosen, only a subset of the parameters of the liquid class is active. According to the different dependencies listed above, liquid classes have attributes related to their intended use: tip type, liquid name, and dispense mode.

Different liquid classes are provided with the User Software and optimized for different liquids, tip types, and important pipetting processes, such as aspiration followed by dispensing either to a surface or in a jet. HAMILTON has optimized the standard liquid classes with great care to assure the best pipetting accuracy. To change HAMILTON standard liquid classes, store the class under a different name first. For special applications, the user can define his or her own liquid class to achieve the highest accuracy with the compounds and volumes of interest. For this purpose a Liquid Editor comes with the Microlab STAR User Software. It is described in Chapter 11 below.

NOTE

Always use the same liquid class for one aspiration and dispense cycle. Otherwise uncontrolled residual volumes may be left within the tip or other errors relating to the sum of volumes may occur.

2.4.1 Liquid Handling Examples

Here are some examples of frequently-used combinations of liquid classes and pipetting modes:

1. Aspirate ≥20µl of a water-like liquid, dispense 5 µl into an empty 96-well plate; use standard tips; change tips every cycle:

Liquid Class:	StandardVolume_Water_DispenseJet
Aspiration Mode:	Aspiration
Dispense Mode:	Jet Dispense Empty Tip
Detection:	Aspiration: LLD = pressure or capacitance or both, submerge to a depth of 2mm, following liquid level
	Dispense: Fixed height of 5mm, not following liquid level

2. Aspirate a water-like liquid, single dispense into a pre-filled 96-well plate; use low volume tips; change tips every cycle:

Liquid Class:	LowVolume_Water_DispenseSurface
Aspiration Mode:	Aspiration
Dispense Mode:	Surface Dispense Empty Tip (in the liquid class selected here, the blowout volume is 0)
Detection:	Aspiration: LLD = Pressure or Capacitance or both, Submerge Depth 2mm, following liquid level
	Dispense: Capacitance LLD on, following liquid level

3. Aspirate ≥20µl of a water-like liquid, dispense the same amount into an empty 96-well plate; keep tips:

Liquid Class:	StandardVolume_Water_DispenseJet
Aspiration Mode:	Aspiration
Dispense Mode:	Jet Dispense Empty Tip (empty tip only)
Detection:	Aspiration: Capacitance LLD, submerge depth 2mm, following liquid level
	Dispense: Fixed height of 5mm, not following liquid level
Comment:	On first aspiration, pre-wetting of the tip by 1-3 mixing cycles is necessary to equalize conditions for initial and subsequent dispenses.

4. Aliquoting of liquid means aspirating a given volume all at once and dispensing several partial volumes (aliquots) in a jet to different containers. In this frequently-used pipetting procedure, measurements have revealed that the accuracy of the first and the last aliquot are often not within the specified range. Therefore, to dispense e.g. 10 aliquots of 20 µl of a **water-like liquid** with the ML-STAR, aspirate 240 µl and dispense 20 µl directly back into the container. This is followed by dispensing 10 of the 20 µl aliquots. The last aliquot of 20 µl is discarded into another container or ejected with the tip. In addition, after the dispense of every aliquot, a given amount of air is aspirated and dispensed with the next aliquot.

Liquid Class:	StandardVolumeWaterAliquotJet
Aspiration Mode:	Aspiration
Dispense Mode:	Jet Dispense part volume
Detection:	Aspiration: Capacitance LLD, Submerge Depth 2mm, following liquid level
	Dispense: Fixed height of 5mm, not following liquid level

The following table gives sample values and results for pre- and post-aliquot volumes (please note that these are not technical specifications):

Chan-nel Type	Tip Type	Liquid	Pre-wet	V [µl] main aliq.	No. of Aliq.	V [µl] pre-aliq.	V [µl] post-aliq.	CV%	R%	Class
300	Std	Water	Yes	20	12	20	20	1.5	-2.6	A
300	Std	Water	Yes	50	4	50	20	2.0	-1.1	A
300	Std	Serum	Yes	20	12	40	40	2.0		B
300	Std	Serum	Yes	50	4	70	50	4.5		B
1000	Std	Water	Yes	10	12	20	>10	3.9	-3.8	A
1000	Std	Water	Yes	20	12	20	20	2.5	-3.2	A
1000	Std	Water	Yes	50	4	50	20	2.0	-1.5	A
1000	High	Water	No	20	12	20	20	5	-1.6	C
1000	High	Water	No	50	12	50	50	2.5	-1.2	C
1000	High	Water	No	100	8	50	100	1.5	-0.9	C
1000	High	Water	No	200	4	50	100	1.5	-1.5	C
1000	High	Serum	Yes	100	8	100	100	1.0	-1.1	D

Table of Aliquots. *Tip types are: Std.=Standard Volume Tip (300µl), High=High Volume Tip (1000µl). Pre-wet: If "Yes", pre-wetting by 3-fold mixing on aspiration with aspiration volume necessary. V(main aliq.): Volume of main aliquot, V(pre-aliq.): Volume of pre aliquot, V(post-aliq.): Volume of post aliquot. CV: Precision (coefficient of variation, for definition see specs), R: Trueness (for definition see specs). The R and CV values mentioned here are typical results for measurements. Class: Liquid class to be used. A:"StandardVolume_Water_AliquotJet", B: "StandardVolume_Serum_AliquotJet", C: "HighVolume_Water_AliquotJet", D: "HighVolume_Serum_AliquotJet". The dispense mode for all cases is "Jet Dispense, Empty Tip".*

2.5 Process Control

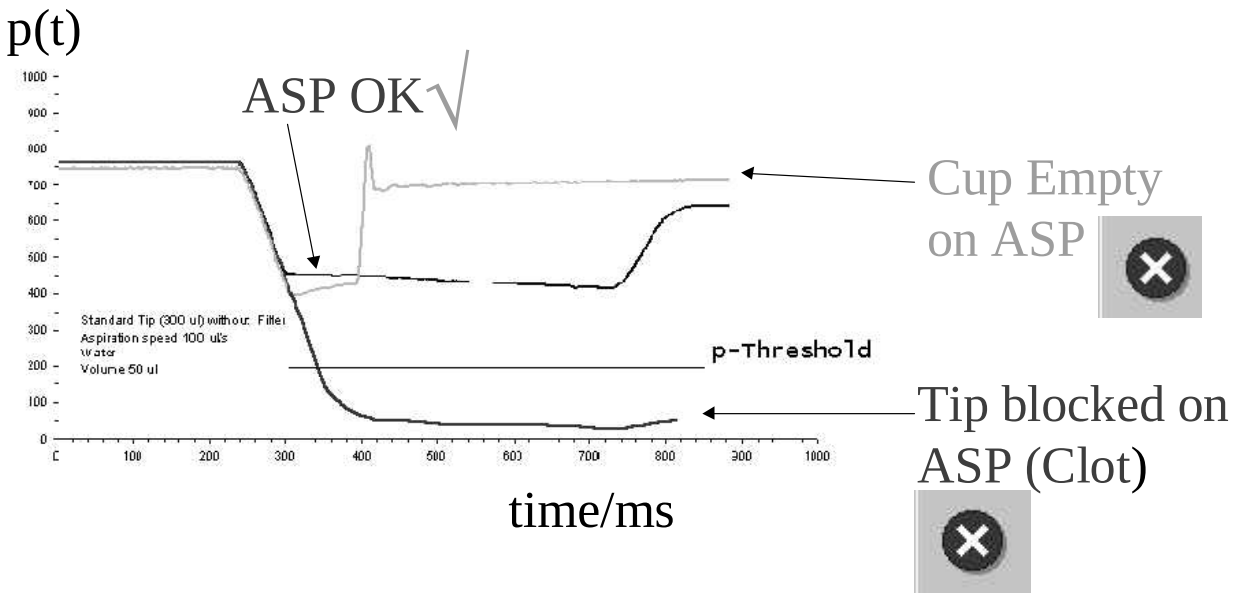
2.5.1 Monitored Air Displacement

The Microlab STAR is equipped with an aspiration monitoring feature. During the aspiration process, the pressure within the pipetting channel is measured in real time. Analyzing the shape of the p(t) curve, the system can distinguish the following situations:

- A correct aspiration takes place.
- Air is aspirated into the tip (because, for example, the cup has not been filled properly).
- A clot blocks the tip.

The aspiration monitoring can be switched on and off for each individual aspiration step of a method using the appropriate commands (see section 2.4). For pressure-based clot detection, a threshold can be given in arbitrary A/D (analog/digital) values (typically 100 A/D

values). The range of A/D values of the pressure sensor is from around 800 (at ambient pressure) to <10 A/D values for 18 mbar below ambient pressure. For comparison, the hydrostatic pressure of 100 µl of water in our standard tip is around 2 mbar.



Aspiration monitoring based on pressure.

NOTE

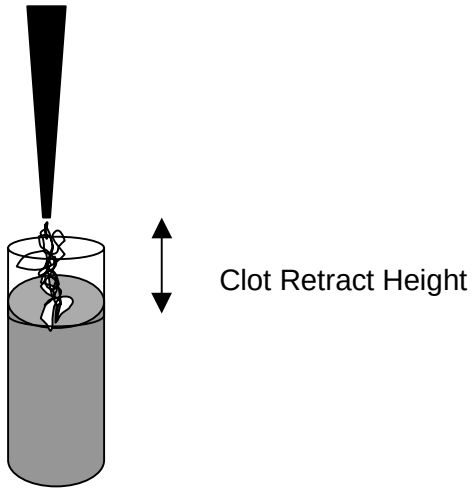
Pressure-based monitoring works only with unused disposable tips (not with needles).

The volume range for the monitoring depends on the specific assay. The lower limit is in many cases an aspiration volume of 50µl.

Pressure-based monitoring has been optimized for aqueous solutions only.

2.5.2 Capacitance-Based Clot Detection

In addition to pressure-based clot detection, the ML STAR is equipped with capacitance-based clot detection, too. This detection approach works for an aspiration with capacitance liquid level detection switched on. The system measures the conductive signal when the tip leaves the liquid after aspiration. Due to the air gap between tip and liquid, the capacitance signal will vanish once a given height is reached (the “clot retract height”, which is specified within the liquid class). If a clot is present, it bridges the distance and the signal will remain, resulting in an error message. A typical clot retract height is 2-5 mm. This is illustrated below.



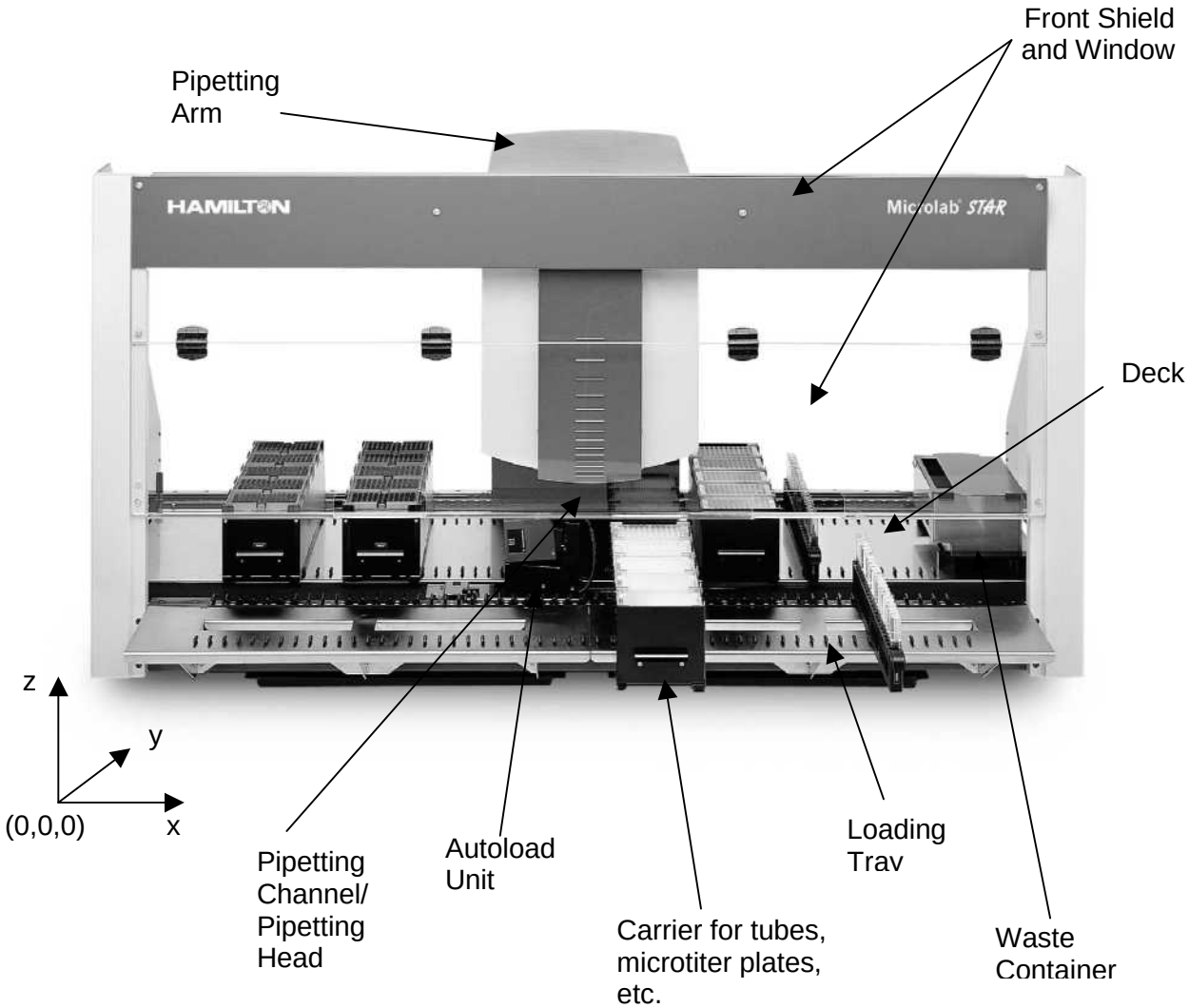
This clot detection is independent of pressure-based monitoring.

3 Description of the Microlab STAR

3.1 The Standard Microlab STAR

STAR stands for Sequential Transfer and Aliquoting Robot. The Microlab STAR performs pipetting operations on liquids in containers placed on its work surface.

The basic model Microlab STAR contains a work surface, called a deck, for placing movable carriers. These carriers hold reagent containers, such as tubes, microtiter plates, or other kinds of labware.



The Microlab STAR

The Microlab STAR's deck is divided into 54 equal tracks (T) for loading carriers with predetermined positions. This obviates the need for precise measurement of positions. The deck has partitions for a maximum of 54 specialised 1-T carriers for sample tubes, or a maximum of 9 6-T carriers for microtiter plates and CO-RE tips. An additional partition space is provided for the tip waste container.

The instrument's internal coordinate system is shown in the figure, located at its origin.

The Microlab STAR is equipped with a pipetting arm typically containing 8 pipetting channels which work independently. The pipetting arm can move in an X direction, whereas each pipetting channel can move relatively independently both in a Y and a Z direction. The Microlab STAR supports pipetting with disposable tips or with needles.

The pipetting channels have a set "travelling height" of 245 mm above the origin, or 145 mm between the tip of the disposable tip and the deck of the instrument. That means that when a channel is to move from one location on the deck to another, it automatically does so at that particular height. This is a safety precaution, so that channels will not collide with any items that may be on the deck.

The instrument is equipped with a front shield and a hinged transparent window made of plexiglass. This window is equipped with a magnetic switch and it is locked during a run.

ATTENTION

An aborted run (stopped by opening the front window) cannot be restarted. If you need to open the window during a run, click "Pause" in the Run Screen, wait until the instrument stops and then open the window.

3.2 Options

Options are defined as components or configurations that are part of the instrument initially provided to the customer as specified by that customer. Options cannot be added later, whereas accessories can (see Section 3.3 "Accessories" below). The instrument's configuration is set within the configuration editor of the User Software.

3.2.1 4, 8, 12 or 16 Pipetting Channels

The Microlab STAR comes with 4, 8, 12, or 16 pipetting channels working in parallel for simultaneous transfer of liquids.

Instruments with 4,8, or 16 channels are best operated with carriers, holding microplates and tip racks in landscape orientation (e.g. PLT-CAR-L5MD), whereas carriers with portrait orientation for microplates and tips (e.g. PLT-CAR-P3MD) are best suited for the 12-channel Microlab STAR. In addition, increasing the number of channels to 12 or 16 reduces the "random access space" of the Microlab STAR, i.e., the space that can be reached with all pipetting channels. This is shown in the following table:

No. of Channels	**Y_{min} /mm**	**Y_{max} /mm**
	Rel. to Instr. Coordinate System	
4 or 8	77.5	77.5 + 465
12	113.5	113.5 + 393
16	149.5	149.5 + 321

The random access range of the different numbers of channels is indicated by brackets at the left side of the deck layout display in the User Software (see chapter 6 for an account of deck layouts).

To guarantee random access to sample carriers, only the inner tube positions should be used. The number of blank positions to be used for the different instrument configurations is listed in the following table:

No. of Channels	SMP-CAR-32		SMP-CAR-24		SMP-CAR-12	
	Front Blanks	Rear Blanks	Front Blanks	Rear Blanks	Front Blanks	Rear Blanks
4 or 8	0	0	0	0	0	0
12	3	3	2	2	1	1
16	5	5	4	4	2	2

The 16-channel Microlab STAR is intended as a batch-type processor, i.e., random access to all positions is not possible. By contrast with an 8-channel Microlab STAR, the methods for a 16-channel instrument should be set up in such a way that all positions are processed in batches of at least 8 (or better 16, to optimize the pipetting speed) with 8 (or 16) simultaneous aspirations or dispenses at identical x-coordinates (see section 8.9 for more).

NOTE

If pipetting positions outside the random access range of the instrument are used, the system may crash. However, a strictly batch-type process can eliminate these problems.

3.2.2 High and Low Volume Channels

Different pipetting heads are available for the different volume ranges:

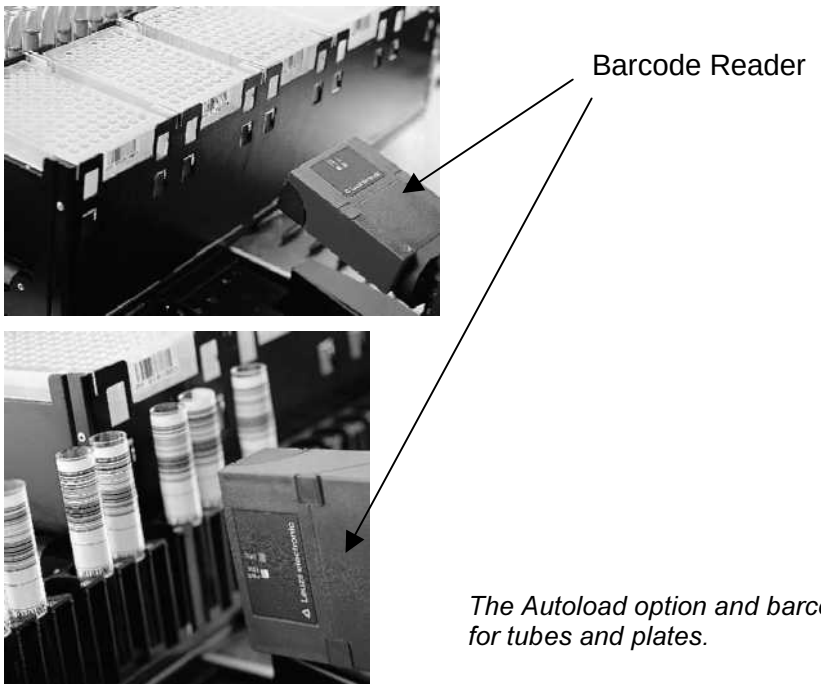
a) from less than 1 µl to 300 µl (for the use of low volume tips = 10 µl, and standard tips = 300 µl), or

b) from 5 µl to 1000 µl (for the use of standard tips = 300 µl, and high volume tips = 1000 µl).

Currently, a mixing of high and low volume channels on one and the same instrument is not feasible.

3.2.3 Autoload Option

The Autoload option, like the options mentioned above, belongs to the Microlab STAR configuration initially specified by the customer and cannot be added later.



The Autoload option and barcode orientations for tubes and plates.

The autoload unit is a device enabling automatic loading of the Microlab STAR. It contains a loading head that

- moves in an X direction,
- shunts carriers onto and off the Microlab STAR, and
- reads the barcodes of carriers, tubes, and microtiter plates.

There is a presence sensor that identifies the tubes present on a carrier.

Barcode reading is only possible in conjunction with the Autoload option.

Equipped with autoload, the ML STAR can read the following barcode types:

- ISBT Standard
- Code 128 (Subset B and C)
- Code 39
- Codabar
- Code 2 of 5 interleaved
- UPC A/E
- JAN/EAN8

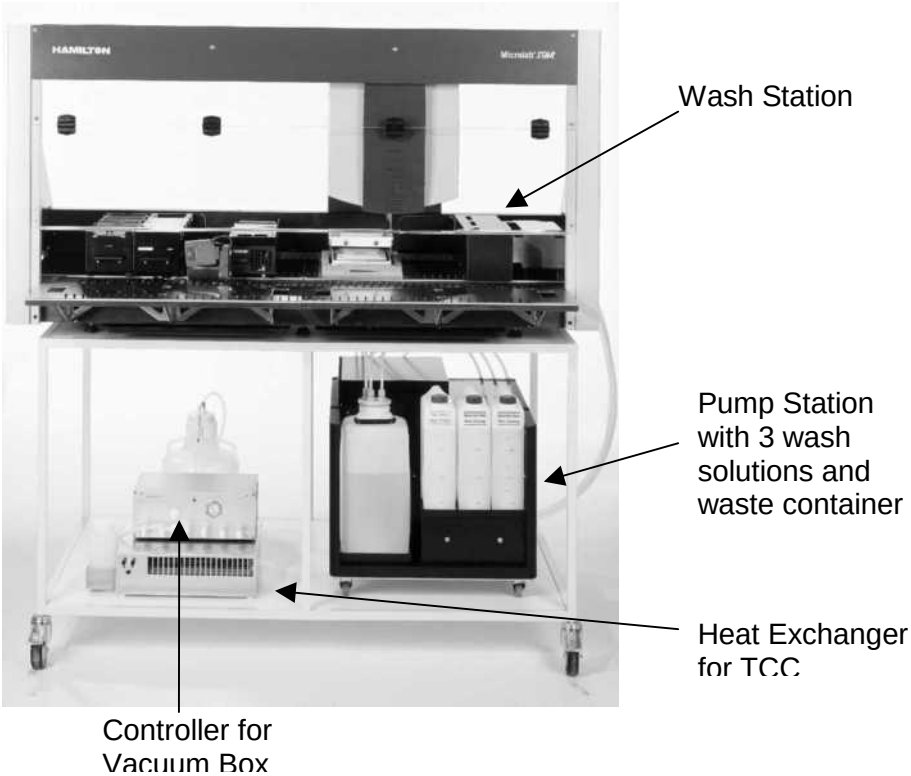
NOTE

In addition, barcodes must have a minimum readability (i.e., good contrast, size, correct orientation and distance between bars) to be fully functional.

Ensure the correct barcode orientation for tubes and plates (see picture on previous page).

3.3 Accessories

Accessories are defined as additional components that can be ordered later by the customer and installed either by HAMILTON personnel, or by the customer. They include carriers, a wash station, a temperature-controlled carrier and an automatic vacuum system, which are described in this section of the manual. Note that the availability of accessories is subject to change. Please ask for a current list.



The Microlab STAR with Carriers, Wash Station, Temperature-controlled Carrier, and Vacuum System.

3.3.1 Carriers

The labware is placed on special carriers which are loaded onto the Microlab STAR. HAMILTON provides a wide range of standard carriers for microtiter plates, tubes, tips etc. All standard carriers can be added to the deck by the user.

The naming of carriers follows a systematic nomenclature: **X-Y-Z-Ann**.

X: stands for the type of labware, placed on the carriers, e.g., TIP (= tips),

PLT (plates), SMP (=samples)

Y=CAR for carrier

Z: describes the labware details, e.g.,

L: landscape orientation

P: portrait orientation

Number: number of items placed on the carrier (plates or tips)

MD: medium density (96- or 384-well microplates)

HD: high density microplates (1536)

AC: 96-well archive plates

Ann identifies the part number revision (e.g. A00)

Example: PLT-CAR-L5MD-A00 is a carrier for 5 medium density (96- or 384-well) microplates in landscape orientation.

A carrier must always be identified (e.g in deck layouts and methods) by the unique descriptor with which it is tagged.

3.3.2 Disposables

CO-RE tips come in 3 sizes: low-volume, standard, and high-volume (approximately 10, 300, and 1000 µl respectively). One blister pack contains 5 racks of 96 tips, giving a total of 480 tips. CO-RE tips are available in boxes of 4000-6000, depending on the tip size. CO-RE tips are produced under sterile conditions, i.e. the tips are RNAse- and DNAse-free.

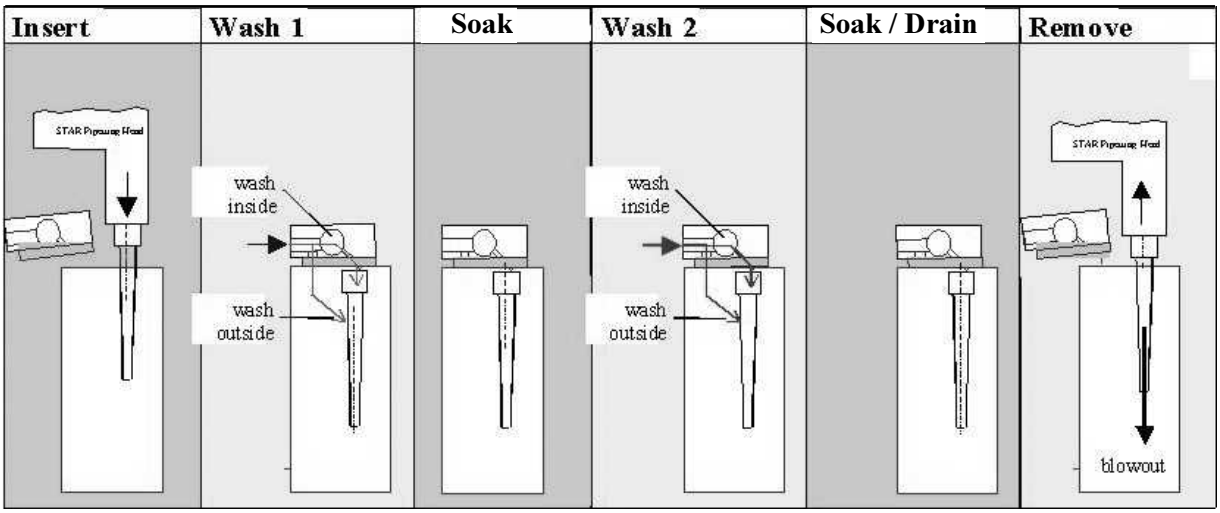
Reusable needles with 50, 300, and 1000 µl volumes may be used instead of tips. Only HAMILTON needles and disposable tips should be used for coupling to the pipetting channel of the Microlab STAR.

HAMILTON steel needles and disposable tips for the ML-STAR

3.3.3 Needle Wash Station

The needle wash station is a device for the washing and drying of steel needles parallel to the pipetting process. The wash station has the width of a normal microplate carrier (6T) and is mounted on the deck of the Microlab STAR. All tubing and electrical connections are routed through the docking station at the rear of the instrument. Along with each wash station comes a pump station with three reservoir containers for wash solutions and a waste container. This pump station is placed below the bench on which the Microlab STAR rests.

The principle of the wash station is illustrated in the following figure, where a typical procedure is shown.



Schematic drawing of needle wash process: Needles are placed into the wash module, washed from inside and along the outside with 1) wash and 2) rinse solution. The pipetting channels blow air through the needles to expel any residual liquid from them.

One wash station consists of three individual 8-fold wash modules **for the same needle type**: High Volume (1000 µl), Standard Volume (300 µl), or Low Volume (50 µl).

To enable parallel pipetting with an 8-channel Microlab STAR, the following cycle of steps takes place:

1. fresh needles are picked up from the first module for pipetting, used, and then placed back into the same module. Washing starts.
2. fresh needles are picked up from the second module for pipetting, used, and then placed back into the same module. Washing starts.
3. fresh needles are picked up from the third module for pipetting, used, and then placed back into the same module. Washing starts.
4. The next set of needles is picked up from the first module again, which in the meantime has washed and dried all 8 needles. The process is repeated again.

The needle wash station needs to be installed by a trained Hamilton engineer.

NOTE

Do not use disposable tips with the wash station.

To use two different needle types, two wash stations of different type are needed.

For a 16-channel instrument, two independent wash stations are necessary to enable uninterrupted pipetting with one needle type.

A maximum of 2 wash stations (each with 3 wash modules and its own pump station) can be installed on one Microlab STAR.

The carry-over of the wash station depends on the wash program. Typical values are 10^{-5} to 10^{-6}.

The wash parameters can be set within the User Software, prior to each wash step.

For each of the two wash solutions (or wash and rinse solution), a set of parameters can be specified:

- The **"rinse time"** is the length of time liquid flows through the wash chamber.
- The **"soak time"** is the length of time wash liquid is held within the wash chamber.
- The **"flow rate"** is given in millilitres per second for both wash liquids.

For the drying of needles an additional parameter is used:

- The **"draining time"** gives the length of time allotted for draining the liquid from the needles.

A set of default parameters is given for the wash process within the relevant dialog boxes of the User Software.

To empty the waste liquid container or to refill the wash solutions, lift the container by the handle. The valve at the bottom automatically closes, sealing the container. To reattach the container, simply slot it back into its original position.

The liquid containers of the wash station. The waste container is placed to the left of the blue container and has a volume of 12 L.

Customers can order one of two complete variants of the wash station: "regular", suitable for most uses, and "chemical-resistant", specially designed to meet the needs of those working with aggressive chemicals. Each variant is complete with 3 wash modules and pump station. For each variant there are high-, standard- and low-volume options. The part numbers for the various options are given in the table below:

	Regular	Chemical-Resistant
Low Vol.	182575	182722
Std. Vol.	182574	182721
High Vol.	182573	182720

NOTE

A table of chemical compatibilities with the wash station can be found in appendix F. The information listed is based on laboratory tests with raw materials and should be interpreted as a guideline only.

Chemical resistance is not the only criterion for the selection of wash liquids. Also consider local regulations for handling and storage of wash liquids as regards toxicity, contamination, fire protection, etc.

ATTENTION

The needle wash station for Microlab STAR is not explosion-proof. When working with flammable or explosive fluids or vapors, the necessary precautions must be taken.

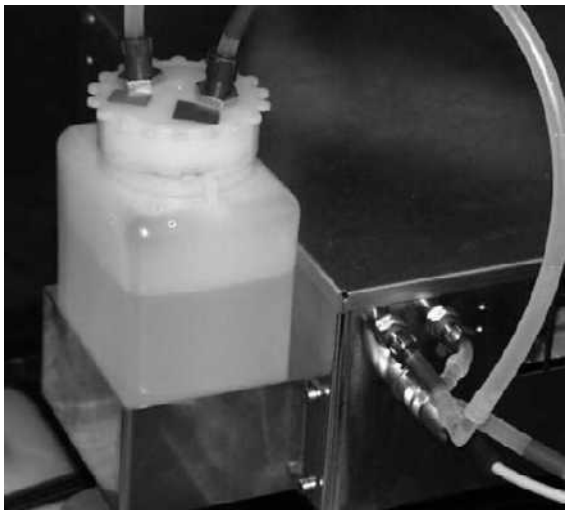
3.3.4 Temperature-Controlled Carrier (TCC)

The temperature-controlled carrier is a device to heat and cool microplates. The TCC has four positions for microplates, which are all of the same temperature. The TCC may heat microplates up to 60°C, or cool them down to 22°C below ambient temperature. The TCC is able to read barcodes on the microplates (with the autoload option). It consist of a plate carrier with peltier elements on the instrument deck and a service station with a heat exchanger below the bench. For the heat exchanger, a water-based heat exchange liquid is used in a closed cycle; it is stored in a reservoir.

Typical times (at 40% rel. humidity) to heat and cool the TCC are ($T_{ambient}$ = 20°C):

$T_{ambient}$ to 60°C	20 mins
60°C to $T_{ambient}$	20 mins
$T_{ambient}$ to 4°C	15 mins
4°C to 60°C	25 mins

The temperature-controlled carrier (TCC).



The heat exchanger solution reservoir.

NOTE

Ensure there is always enough liquid (1 L) within the reservoir.

Allow air exchange between the exchanger and ambient air.

The default position of the TCC is on the instrument deck. Never leave the TCC unloaded on the autoload tray.

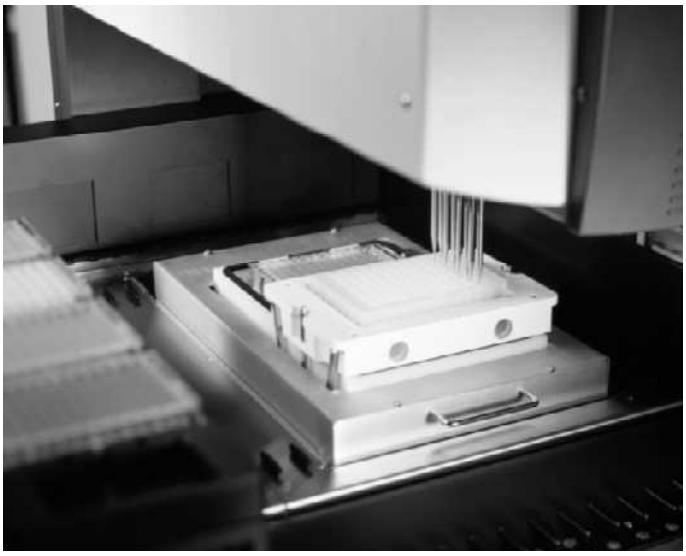
Replace the heat exchanger solution twice yearly.

Do not use SMART Steps to load a TCC with the autoload function.

Always ensure that the TCC is on the deck at the beginning of the loading process.

The TCC will be installed by a trained Hamilton service engineer. A maximum of 2 TCCs can be placed on one Microlab STAR.

3.3.5 The Automated Vacuum System (AVS)

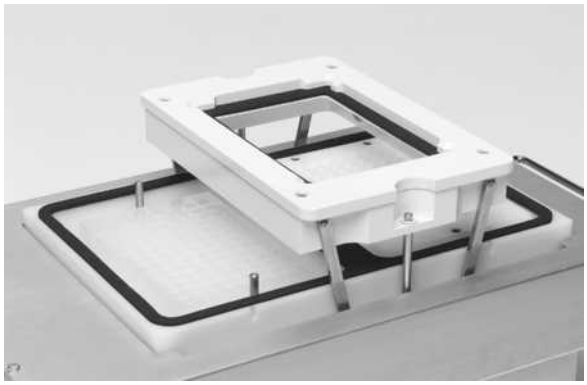


The Automated Vacuum Box System consists of a vacuum box, a vacuum controller and the controlling software. The Vacuum Box is fitted to the deck of the Microlab STAR and is controlled by the workstation‘s computer. For this integration, a special deck has to be mounted on the Microlab STAR. The vacuum box accommodates a wide variety of 96-well filter and collection plates and is automated, so that the filter plate can be transported from a waste/conditioning chamber to an elution chamber. The vacuum box is compact and a maximum of two can be used in parallel on the Microlab STAR deck with the same controller. Filter plates can be placed onto or removed from the system by hand or with the help of a robotic arm (see the section on iSWAP below).

The vacuum controller allows for user-defined vacuum settings. The software integrates seamlessly with MICROLAB® Vector. Loading is handled with a step in the method.

We now turn to a description of the components of the automated vacuum box system.

Vacuum Box

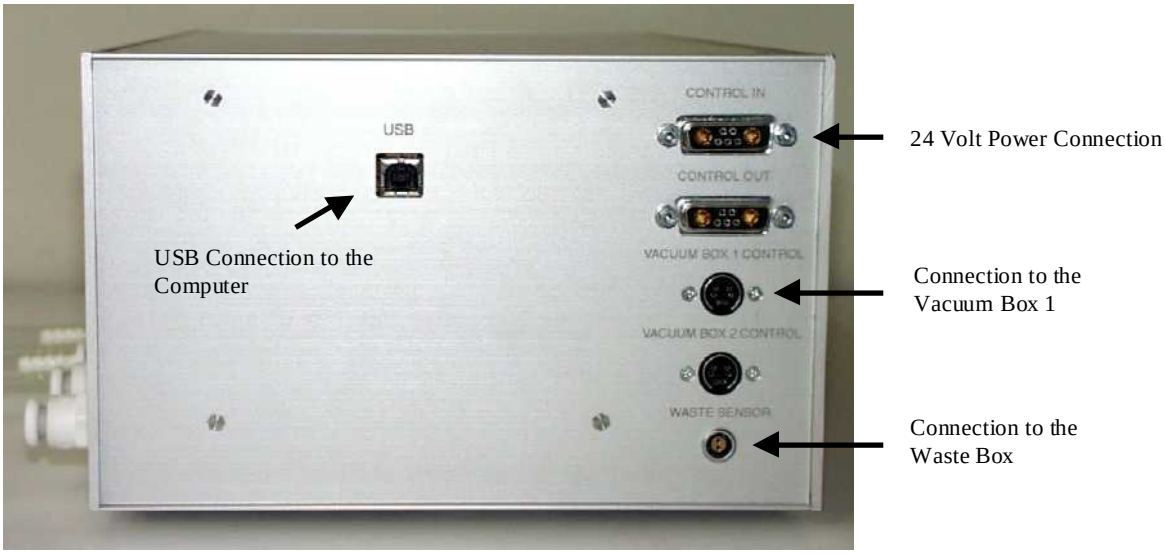


The picture on the left provides a bird’s-eye view of the vacuum box, showing the conditioning chamber on the right and the elution chamber on the left, which is covered in this picture by the carriage. A lid gasket in the carriage provides the sealing surface for the filter plate and a chamber gasket provides the sealing surface for the carriage. In the picture on the right, the carriage is on the way to the elution chamber.

The picture above shows the ports on the vacuum box. There are three ports into which is fed the tubing that goes to the vacuum box controller. There is also a push-button switch that allows the user to manually operate the carriage. An electronic control port is used for power and communication.

Vacuum Box Controller

One Controller can control up to two vacuum boxes. The front of the controller has eight ports. Six of these are needed for conditioning, elution and waste removal. There is also a vacuum exhaust port for venting vapors and a “waste out“ port for draining liquid from the conditioning chamber.



Pictured above is the right side of the controller with the different connections. The communication with the computer is via a USB cable. As the controller requires 24 volts, a power supply with 24 volts is delivered together with the controller.

Waste Container

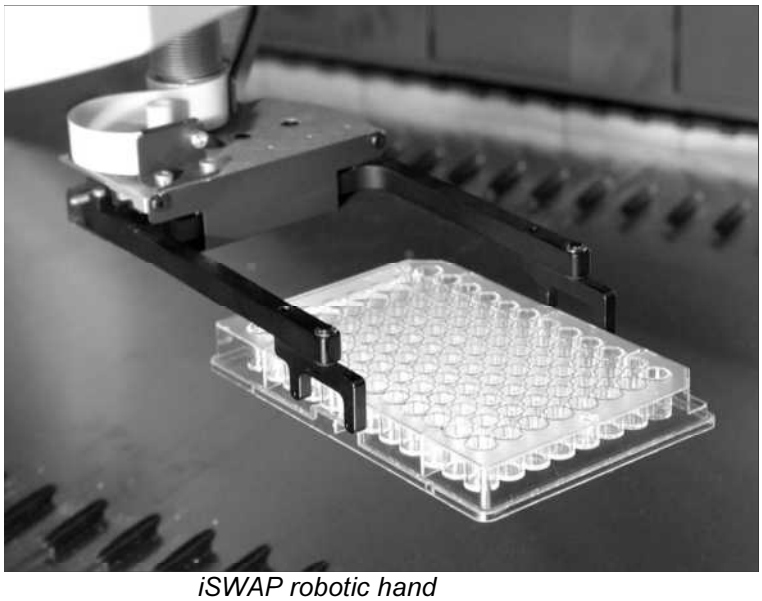
The waste is collected in the closed-system waste container with liquid level monitoring to prevent overflows.
The waste container with a volume of 10 litres should preferably be placed below the level of the instrument. The waste level sensor has to be connected with the controller.

The AVS and its components are to be installed by a Hamilton service engineer.

3.3.6 iSWAP

The iSWAP (internal Swivel Arm Plate Handler) transports microplates, covers of microplates, archive plates or filter plates used for the vacuum box to and from positions on the deck of the Microlab STAR IVD. The plates can be placed in landscape or portrait orientation and rotated 180 degrees. In addition, the iSWAP is able to load and unload plates to and from a plate stacker on the left side of the instrument outside the working area (with some restrictions, this can also be done on the right side). Like Microlab STAR's pipetting channels, iSWAP has a "travelling height" of 145 mm above the deck (245 mm above the origin).

The iSWAP is mounted on the pipetting arm and parked below the cover of the pipetting arm. In this position it does not affect the movement of the pipetting channels.



iSWAP robotic hand

iSWAP can be chosen by the customer as an ordering option, but can also be installed later by a service technician at the customer's request.

3.4 Computer Requirements

The Microlab STAR is controlled by a dedicated user software program which controls all functions for daily work routine, method programming, running methods, and other services.

The Microlab STAR and the computer controlling it may be linked in two different ways:

- By a serial interface (RS232), or
- By a Unified Serial Bus interface (USB).

The Microlab STAR automatically recognizes the type of communication in use.

The Microlab STAR requires a recent model of PC including a mouse, CD-ROM drive, high resolution monitor and graphics (SVGA or better), 1-2 serial ports for a USB, about 1 GB HDD and ≥128 MB RAM, and Windows 2000 (service pack 1 or better) as the operating system.

To avoid any loss of data, we recommend a UPS (uninterruptable power supply) for the PC.

NOTE

Microlab STAR's functioning has been verified using Windows 2000 exclusively. Running the Microlab STAR under any other operating system than Windows 2000 may lead to severe problems and malfunction.

3.4.1 User Software

The Microlab® STAR User Software is the controlling software for the Microlab® STAR.

It is a Windows™-based, menu-driven interface allowing the user to define deck layouts and methods, and then to run the Microlab® STAR.

The Microlab® STAR User Software allows the user to program and run different methods for aspirating and dispensing liquids.

NOTE
Each programmed method has to be validated by the user.

3.4.2 Firmware

Firmware is the set of instructions that are downloaded to the Microlab STAR by the manufacturer to enable the instrument to carry out its functions. Normally, the User Software is used to control the instrument. However, the Microlab STAR can be controlled directly with the CoCo firmware protocol (CoCo means 'Communication and Control'). This is done by controlling actions of the Microlab STAR with single firmware commands. Firmware commands are listed in the *Service Manual* for the use of Hamilton engineers servicing the instrument.

3.5 Installation and Set-Up

The Microlab STAR will be unpacked and installed and initial set-up performed by a trained HAMILTON technician. The customer need only ensure that a suitable control PC is available for installation of the ML STAR User Software.

Make sure that the Microlab STAR is connected to a 115 to 230 V AC (50 or 60 Hz) socket. The Microlab STAR automatically recognizes any voltage within that range, without user intervention. Select the appropriate voltage at the pump station of the needle washer. The needle washer does not recognize the voltage automatically.

3.6 Power / Voltage

3.6.1 Basic Microlab STAR

We recommend using a Uninterruptible Power Supply (UPS) for the Microlab STAR.

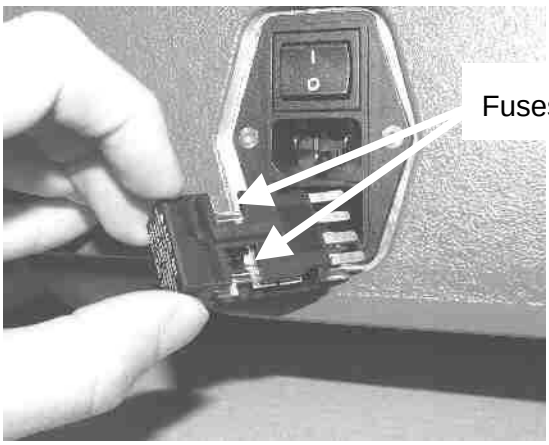
Ensure that the instrument is correctly earthed when connected to the power supply.

The mains plug is on the left hand side of the instrument towards the rear (see picture 1 below).

The fuses for the instrument are situated in the mains power switch (see picture 2 below).

Plug the mains cables for the computer and the instrument into the same electrical outlet. Connect them only to an earthed outlet.

Picture 1



Picture 2

ATTENTION

Always keep the instrument voltage selector at 220-240 V~

Place the appropriate fuse in the mains power switch before switching on the instrument.

The technical specifications regarding electrical power are listed in the following table.

Maximum power consumption:	600W
Voltage:	115 / 230 V~ -15 % + 15 %
Frequency:	50 / 60 Hz ± 5 %
Delayed action fuse:	
115 V~:	6.3 A
230 V~:	3.15 A
Overvoltage category	II
Degree of pollution	2

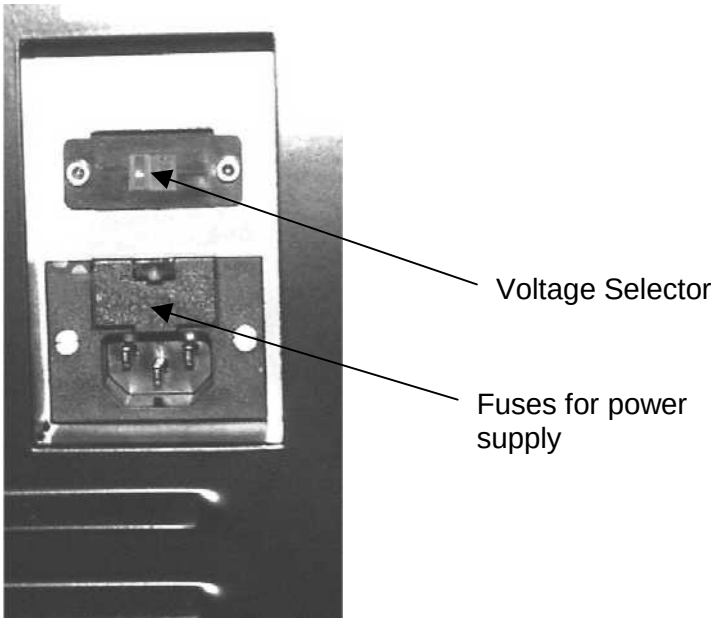
3.6.2 Needle Wash Station

The needle wash station has its own power supply. The mains plug is on the rear of the pump station (see picture below).

Ensure that the the needle wash station is correctly earthed when connected to the power supply. Connect the wash station only to an earthed outlet.

Ensure that the voltage selector is correctly set before operating the needle wash station.

The fuses for the instrument are situated next to the mains power switch (see picture below). The pump station has two fuses for the power supply which can be accessed by opening the cover above the mains plug.



Fuses for power supply of wash station.

The technical specifications regarding electrical power for the wash station are listed in the following table:

Maximum power consumption:	140 W
Voltage:	115 / 230 V~ -15 % + 15 %
Frequency:	50 / 60 Hz ± 5 %
Delayed action fuse:	
115 V~:	3.15 A (T3.15L250)
230 V~:	1.6 A (T1.6L250)
Overvoltage category	II
Degree of pollution	2

3.7 Maintenance

A short preventive maintenance including volume verification should be carried out twice yearly. Typically, the service and volume verification are carried out by a trained HAMILTON service engineer.

To clean the instrument deck use the procedures required by GLP. To preserve plastic materials from damage, do not use organic solvents.

If a spray is used, do not point the spray directly at the autoload unit or at electrical boards or connectors.

3.8 Verification

The Microlab STAR will be verified by a trained HAMILTON technician upon initial set-up, and thereafter at regular intervals for a period of time specified by service agreements. HAMILTON recommends that this verification take place four times yearly.

For volume verification in the field, HAMILTON will supply a verification tool, based on gravimetrical measurements (the “gold standard” of volume verification). The detailed specifications are listed in the specification table at the end of this chapter.

Conditions are valid only for the HAMILTON verification kit.

3.9 Disposal

After the life cycle of the instrument has terminated, the Microlab STAR may be shipped to the original manufacturer or retailer. Otherwise local disposal regulations are to be observed.

3.10 Training

Training in operation of the Microlab® STAR and the User Software will be provided by HAMILTON personnel upon initial set-up.

3.11 Technical Specifications

3.11.1 Accuracy (Trueness and Precision) Specifications

The pipetting specifications for the Microlab STAR are given in the following table. The differences in the specifications are due to the accuracy of the gravimetrical measurement, which to a great extent depends on the quality of the balance, as well as on the stability of the environmental conditions (pressure, humidity, temperature).

300 µl (Low Volume) Pipetting Channel							
		Design Specs		**Final Testing Specs**		**Field Verification Specs**	
V_{Pip}/µl	**V_{Tip}/µl**	**|R|/%**	**CV/%**	**|R|/%**	**CV/%**	**|R|/%**	**CV/%**
200	300	1	0.75	1.5	1	2	1.5
50	300	2	0.75	2.5	1	-	-
10	300	5	2	6	2.5	-	-
5	10	2.5	1.5	3	2	6	5
2	10	5	3.5	6	4	-	-
1	10	5.0	6	-	-	-	-
1000 µl (High Volume) Pipetting Channel							
		Design Specs		**Final Testing Specs**		**Field Verification Specs**	
V_{Pip}/µl	**V_{Tip}/µl**	**|R|/%**	**CV/%**	**|R|/%**	**CV/%**	**|R|/%**	**CV/%**
1000	1000	1	0.75	1.5	1	2.0	1.5
500	1000	1.5	0.75	2	1	-	-
200	300	1	0.75	1.5	1	-	-
100	300	1.5	0.75	2	1	-	-
50	300	2	0.75	2.5	1	-	-
10	300	5	2	6	2.5	9	6

V_{Pip} and V_{Tip} are the volumes of liquid and tip, respectively. Design specs are the design specifications which have been verified in the design phase of the Microlab STAR. Final testing specifications and field verification specifications are given, too. The trueness and precision specification is valid for 8 channels. The basis for the calculations is given in Appendix E, "Principles of Calibration".

The design specifications mentioned above are valid under the following conditions, obtained for measurements at HAMILTON Bonaduz:

- Test method: Gravimetric testing at Hamilton. The scatter of the test method must be less than 1/6 of the specified precision (for one channel).
- Trueness/Precision The values given refer to use of 8 pipetting channels.
- Test size: ≥ 12 single pipettings per channel with disposable CO-RE tips (pick-up and dispense, tip used only once) per channel and specified volume
- Test mode: Volumes ≥ 20µl as jet dispense, < 20µl as (liquid) surface dispense
- Acceptance criteria: Measured values are within specifications if less than the values appearing in the table on p. 40.
- Test temperature: 20 ± 2°C
- Test fluid Deionized water with 0.1 % NaCl, 0.01% Tween

NOTE

No warranty can be given that the above specifications for trueness and precision are met with any other liquid or environment than the ones specified.

Optical test methods such as fluorescence or absorbance plate reading tend to have internal scatters in the range of about 5%.

The operating temperature range for the Microlab STAR is from 15 to 35° C, with a relative humidity of 30 - 85% with no condensation.

4 Design of the User Software

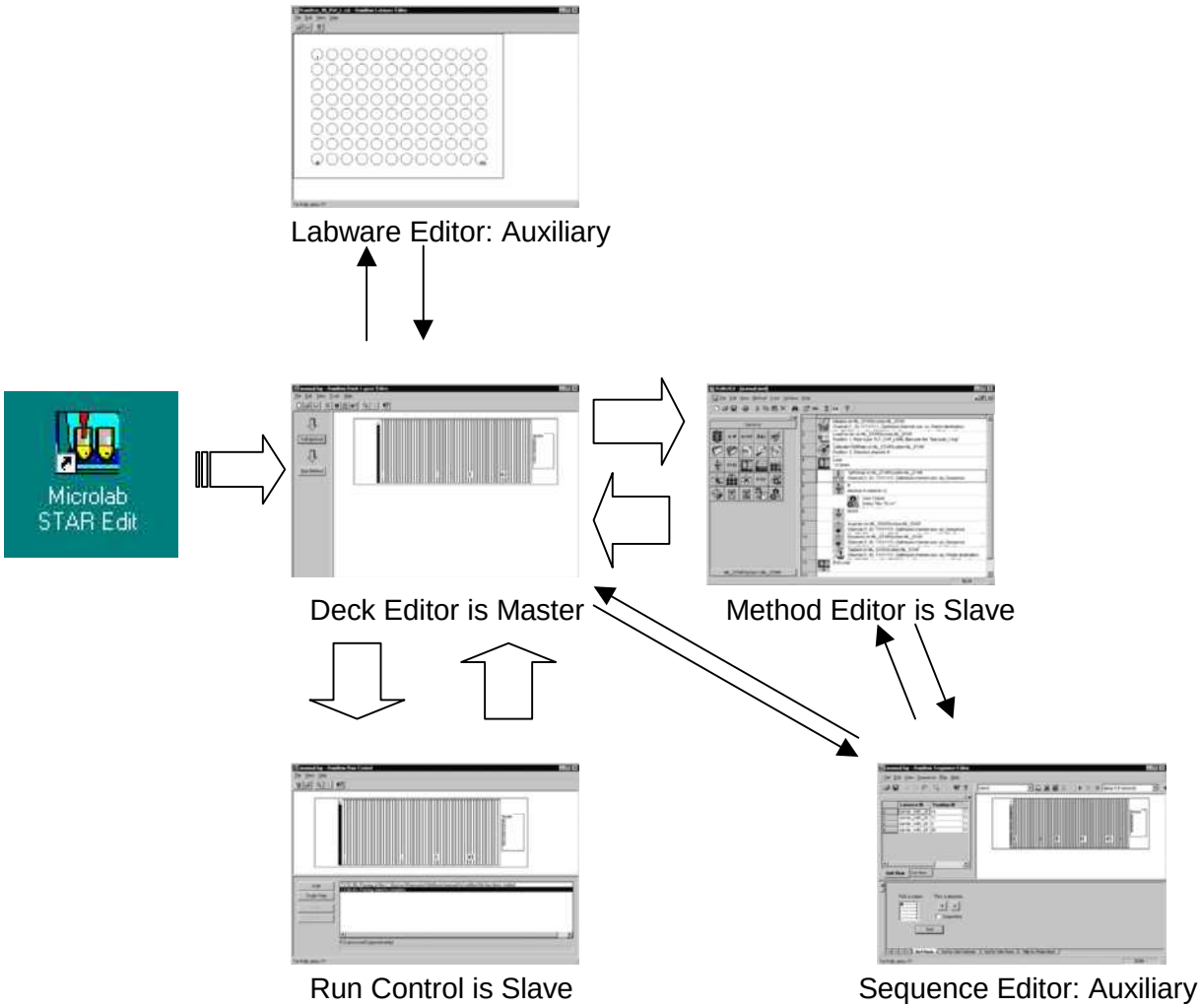
4.1 Overview

The Microlab® STAR User Software is the controlling software for the Microlab® STAR.

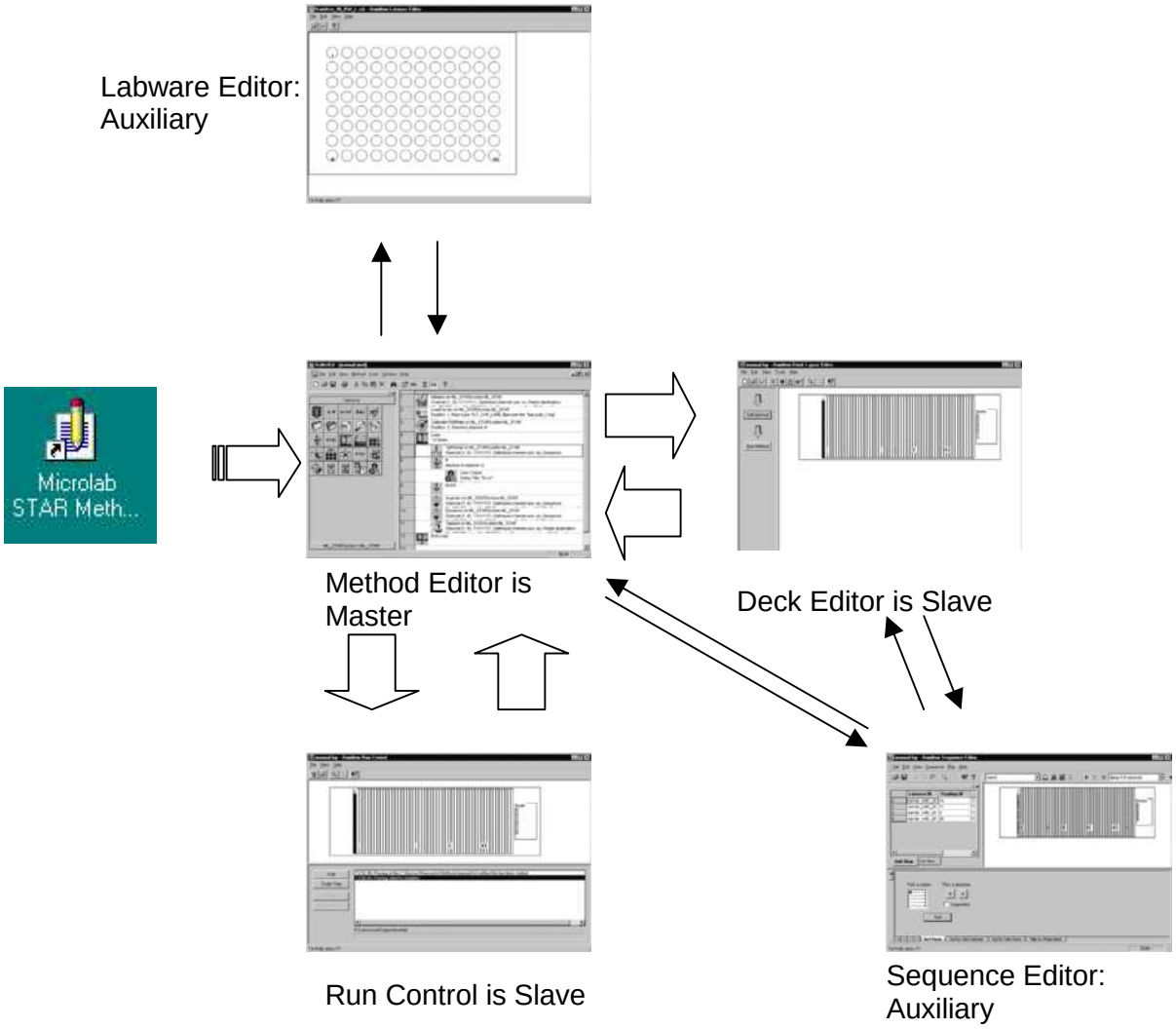
It is a Windows™-based, menu-driven interface allowing the user to define deck layouts and methods, and then to run the Microlab® STAR.

The Microlab® STAR User Software allows the user to program and run different methods for aspirating and dispensing liquids. Note that each programmed method has to be validated by the user. The deck layout is defined within the Deck Layout Editor, whereas the aspiration and dispense steps are defined using the method editor. An independent run control is used to run the methods programmed.

For ease of use, there are two different options for programming. In both cases, a “master” or central editor is selected, which can either be the method or the deck layout editor. Whatever editor is started first is the master editor. All other (“slave”) editors and the run control are then called from the master editor, using easy access shortcut buttons. If you make changes within one of the slave editors, the master editor is automatically updated once you quit the slave editor. This is illustrated below with the deck editor as master:



Alternatively, the user may start with the method editor as the master editor (if there is no shortcut to the method editor, create it manually. The relevant executable file is ...\hamilton\bin\HxMethEd.exe):



The auxiliary modules and tools (small arrows in the above figures) are called from the tool menu by menu items or tool icons. Finally, the method is executed using the independent run control. In some cases, one may wish to define special labware items or sequences (pipetting patterns - see below) using the labware editor and the sequence editor.

4.2 Methods and Deck Layouts

The Microlab STAR User Software allows an *n:n* relation between deck layouts and methods. This means that one deck layout may be used for different methods or one method uses different deck layouts, e.g., to link two Microlab STARs, or a Microlab STAR and a Microlab SWAP.

The run control allows you to start either deck layouts (with the corresponding method if there is only one), or, in general, methods. These methods are then either executed or simulated.

4.2.1 "Save As", or Saving Methods and Deck Layouts under Different Names

Due to the *n:n* relation between deck layouts and methods, the "Save as" option needs to be used in the following way:

To save a method under a different name, still referring to the old deck layout:

- Select "Save As" in the method editor and save the old method A under a new name B. The new method B is still linked to the old deck layout A.

To save a method and the corresponding deck layout under a new name:

- Start the Method Editor as the (first opened) master editor.
- Select "Save As" to save method A under the new name method B.
- Start the Deck Editor to save deck layout A under the new name deck layout B. Close the Deck Layout Editor.
- Link method B to deck layout B (Methods->Instruments). Save method B.

4.3 Structure of the User Software

The User Software consists of a set of modules and tools (programs) which work together. These modules and tools are grouped into a general (instrument-independent) and a specific (instrument-dependent) group. Given that structure, a system with different HAMILTON instruments (even non-HAMILTON instruments, suitably adapted) can be controlled and operated in the same manner, in the same environment and at the same time. The general modules get all the specific information they need about instruments (such as pipetting steps, or information about the probe head) from the instrument-dependent modules.

The general modules with a common "look and feel" for all HAMILTON instruments include:

- Deck layout editor (for mapping the position of labware on the deck)
- Method editor (for programming methods)
- Sequence editor (for defining pipetting sequences)
- Labware editor (for defining custom labware to be used in deck layouts)
- HSL Method editor (for programming methods using text entry mode)
- Run execution (for running methods on the instrument).

The general tools include:

- Version info.

The Microlab STAR-specific editors include:

- Liquid Editor (for defining liquid classes)
- Configuration Editor (for configuring the User Software to a particular instrument)

The Graphical Method Editor lets the user write methods in a graphical, syntax-free environment. Technically, this editor is a code generator producing a low-level code which is then interpreted by the run control. This low level code is called HSL (Hamilton Standard Language), because it provides a common programming approach for all Hamilton instruments. HSL can be accessed directly using the HSL Method Editor. HSL is a syntax-dependent language that offers even more flexibility than the Graphical Method Editor. The instrument-specific steps (e.g. aspiration, dispense - see below) are the same for the

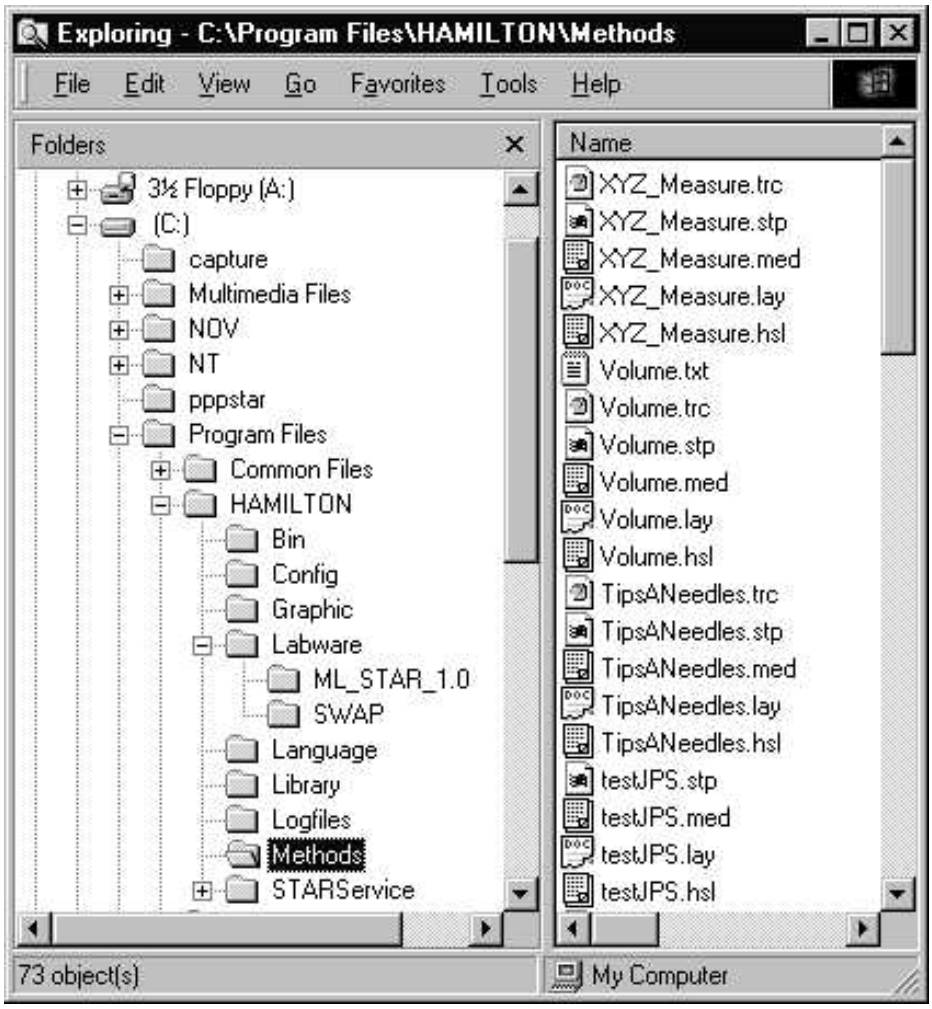
graphical and the HSL method editor. The general syntax used in the HSL is the C/C++ notation and associated language constructs.

4.4 Access Rights

The User Software does not define any user access restrictions. However, specific access rights may be restricted to trained users within the operating system, or by applying write-protection to specific files.

4.5 File Structure

The installation generates the following default directory structure, if not requested otherwise.



Note in particular the file contents of the following directories:

Bin: Executables, DLLs

Config: Configuration Files

Labware: Labware Definitions

Logfiles: Com Traces (Port Trace), Tip Counter File (TipCount.xls)

Methods: Methods (*.med, *.hsl), Deck Layouts (*.lay), Method Traces (*.trc)

Library: HSL libraries of commonly-used additional functions

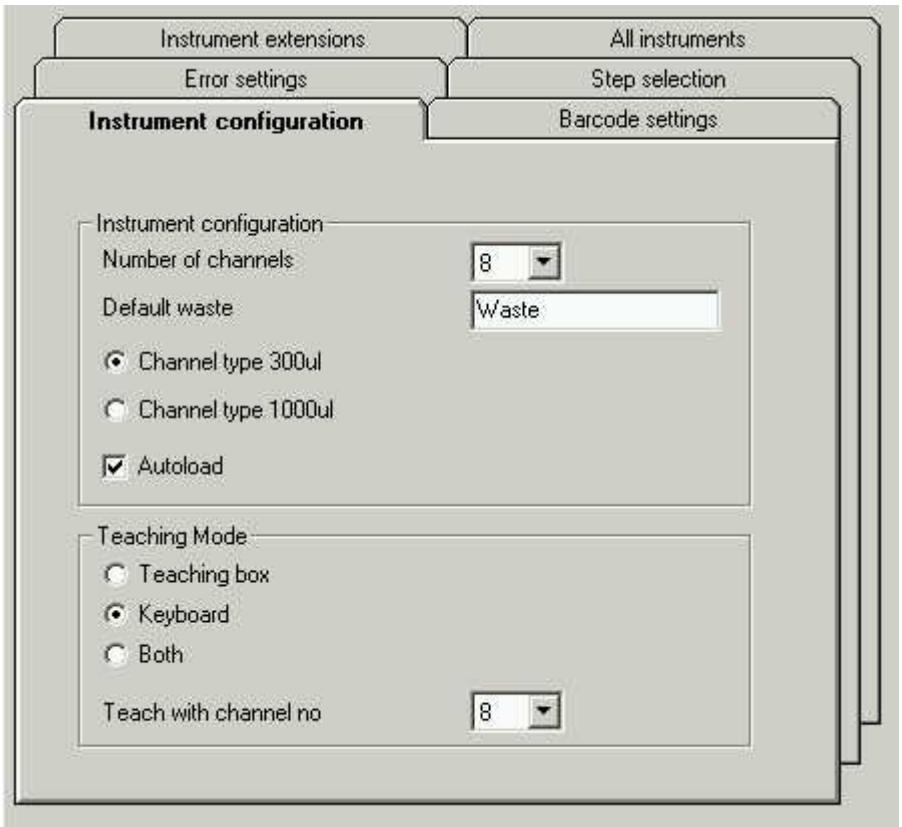
c:\barcodes In addition, this directory is used by the sample tracker to store Microlab AT-like barcode and register files.

5 Instrument Configuration

As described in Chapter 3, the Microlab STAR is available in several variants. To configure the User Software to a specific instrument configuration, the Configuration Editor is available within the Deck Layout Editor under Tools->Configuration:

The first decision to be made - prior to a run - is whether to run the method with the instrument or to simulate the run without using the instrument. Run simulation is a tool to test whether a method has been programmed correctly. The run simulator creates a method trace file with pipetting pattern and other useful information.

Click the Advanced button to access the instrument configuration screen. Different tabs are visible:

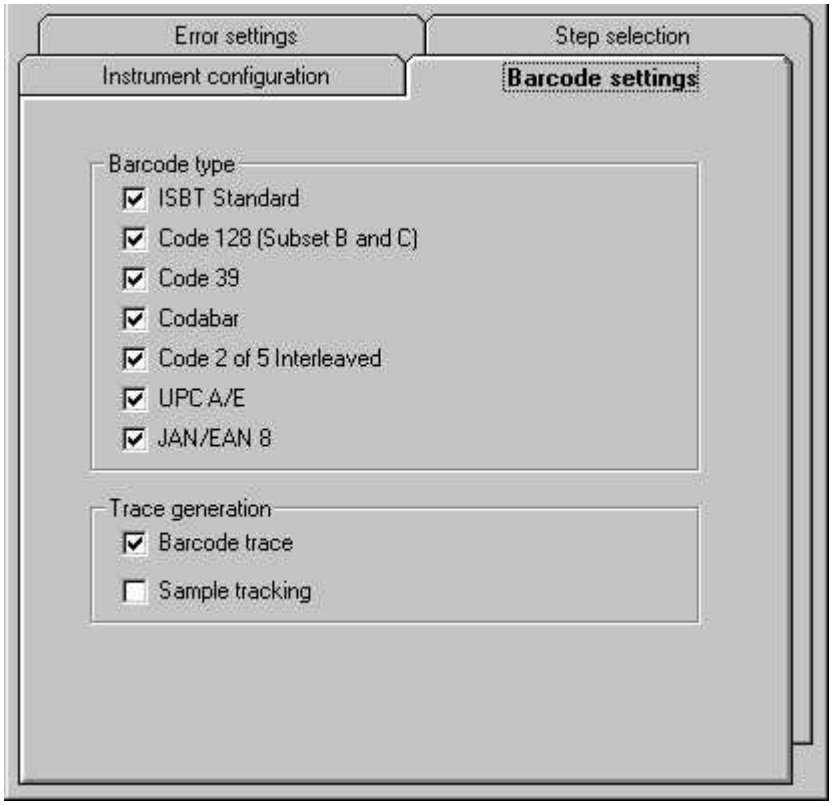


The tab Instrument configuration

Select your instrument configuration. Waste16 (as in picture above) is the correct sequence of the waste container for disposable tips. Teaching mode refers to the possibility of “teaching” positions on the instrument deck, which can be done moving the arm by the

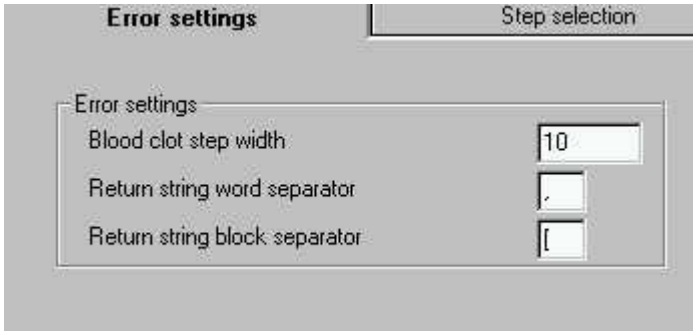
keyboard or by a teaching box (accessory). The channel to be used for “teaching” is to be specified, too.

The next tab refers to barcode handling:



The different barcodes which are allowed on the instrument can be selected here. In addition, the generation of a barcode trace file (barcodes_N.txt) can be switched on and off, storing rack barcode and track position on deck as well as tube (or plate) barcodes and positions within the rack. The sample tracker, which generates a database of liquid transfers in the background can be switched on here, too. This sample tracker is used to generate a Microlab AT-like barcode file using a special filter tool.

The error settings tab allows you to specify the step width in mm for blood clot error recovery (the channels move up on request by the step width). The return string separators are used to define the syntax of single step return values.



The step selection finally allows you to exclude steps such as the move commands from the method editor.

Only if “instrument” is selected within the configuration editor is the additional tab “communication settings” visible.

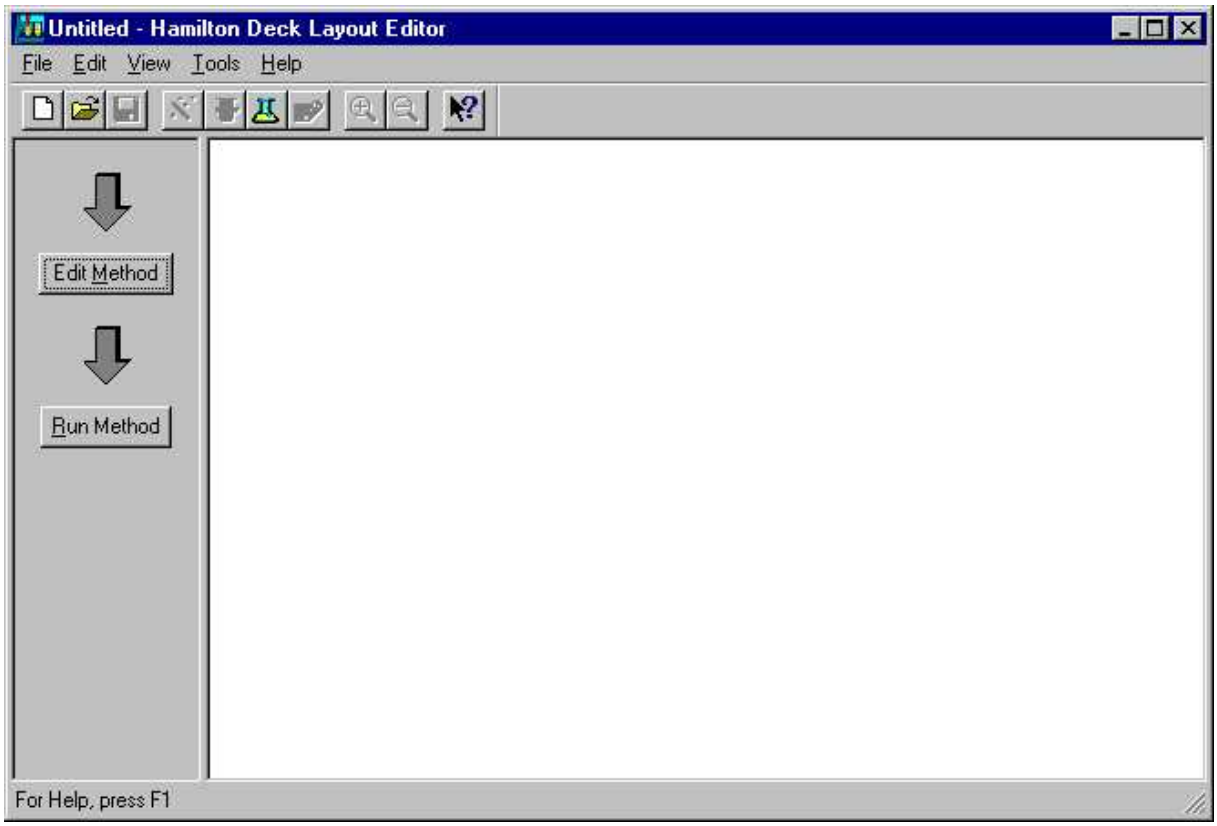
Select the type of communication and the com port for communication via RS232 interface.

Click “save” to activate the new settings or “abort” to leave the settings unchanged.

6 Defining the Deck Layout

In the Hamilton Deck Layout Editor, the user is prompted to tell the system which labware (carriers, racks, or containers) is to be used in the procedure, and where these items are placed on the deck. This is called “defining a deck layout”.

The 'Hamilton Deck Layout Editor is started by double-clicking the shortcut icon displayed on the desktop of your PC.



Deck layout editor

The method editor and the run screen can be started from the deck layout editor by clicking the appropriate buttons located in the leftmost frame.

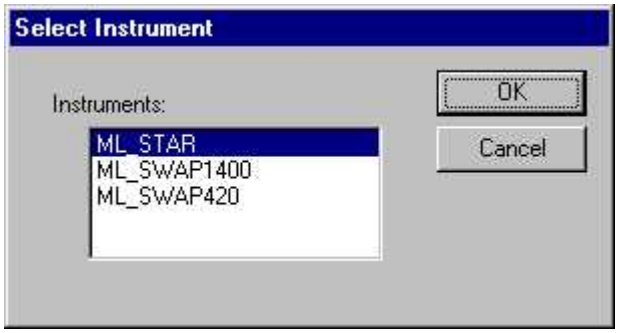
For access to the main modules, a toolbar is provided. In that toolbar the following choices are available (some of which are deactivated):

- New deck layout
- Open deck layout
- Save deck layout
- Method editor
- Run execution
- Labware editor
- Sequence editor
- Zoom in deck layout
- Zoom out deck layout

6.1 New Deck Layout

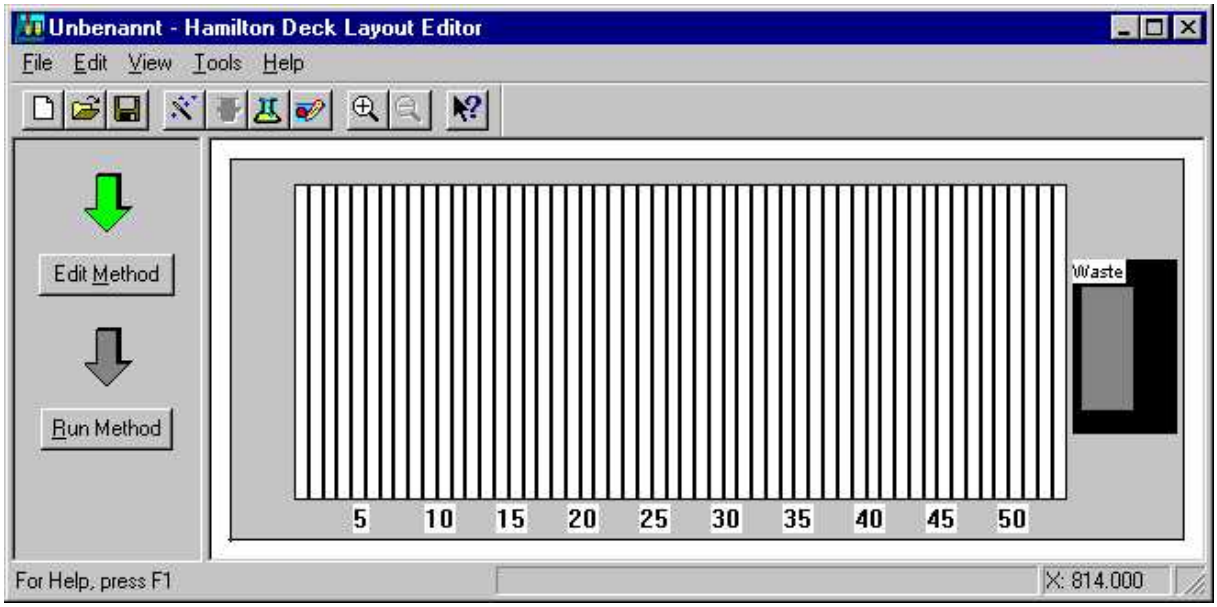
Under the File menu or in the toolbar, select New to start the definition of a new deck layout.

If multiple instruments are installed on your PC, a dialog window will appear. Select an instrument (e.g. 'ML_STAR' for Microlab STAR) and click OK. If there is only one instrument installed, the template (base definition of a deck layout) of that instrument is shown directly.



Select Instrument window

The deck layout displayed below shows the tracks of the predefined grids from the appropriate deck template.



Example: The Tracks of a Microlab STAR deck

Use the (+) zoom button to enlarge the deck view.

A deck template is an instrument-specific definition of dimensions, main grid and hidden grids, with preloaded standard labware such as a waste container. Deck templates can be customized.

6.1.1 Save Deck Layout

The complete loading of the deck may be stored under a chosen file name (extension: ".lay"), e.g. "MyDemo.lay". This is done using the Save, Save As... commands in the File menu.

After a deck layout has been defined and saved, you are ready to start programming your method.

6.1.2 Open Existing Deck Layouts

It is possible to load previously defined deck layouts (extension ".lay") by selecting "Open" under the File menu.

6.2 Adding Labware to the Deck Layout

Basically, the deck is freely configurable. The term labware refers here to carriers (available from Hamilton) as well as microplates (from various manufacturers) and tip racks. The general procedure is first to add a carrier for a special kind of plate to the deck and then add the plate to the carrier. There is a restriction, however, that carriers *for one type of microplate only* are available as standard parts. Therefore, do not place a deep-well microplate and a flat microplate (for example) on the same set of vertical positions in the deck layout.

ATTENTION

If a tube carrier is placed directly adjacent to a plate or tip carrier, using low volume tips may lead to a collision when the channel moves down to aspirate from the tube bottom.

6.2.1 Adding Labware to Track Positions on the Deck

The software comes with a set of ready-made labware definitions for the standard labware items that are most commonly used in laboratories. The carrier name and definition selected must always be identical to the name with which the physical carrier is labelled.

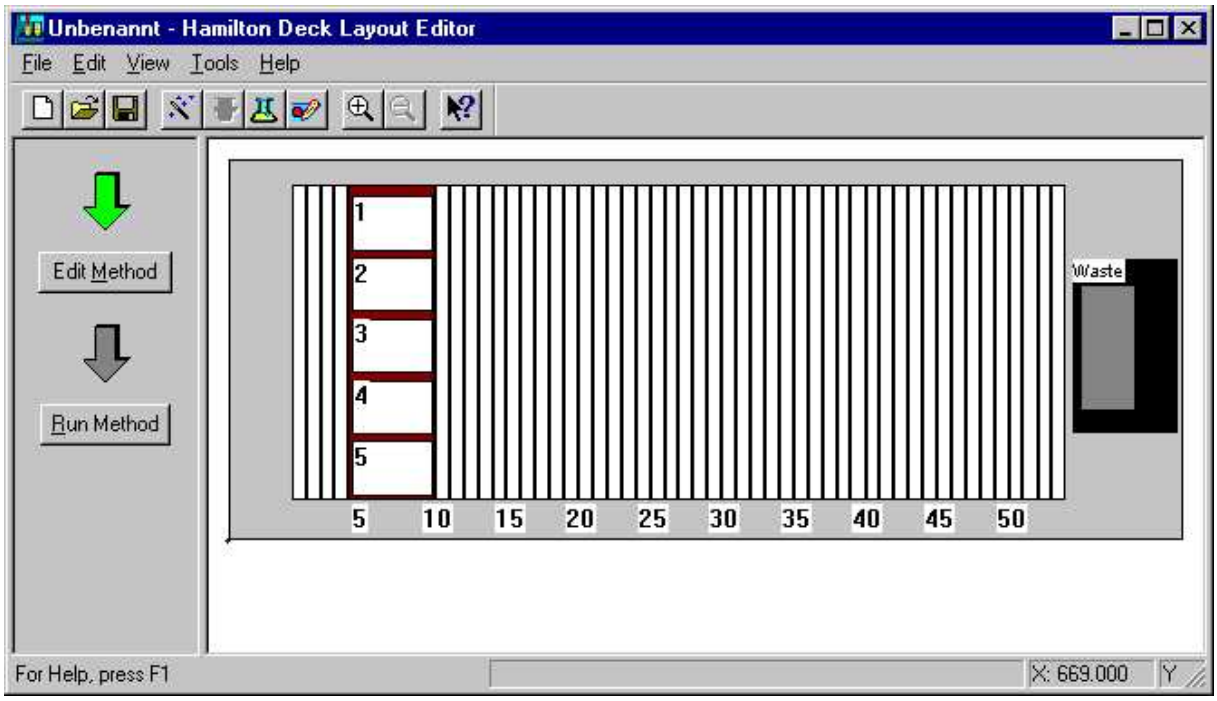
Here are some frequently used Hamilton standard carriers:

- Plate carriers for 5 flat 96- or 384-well microplates PLT-CAR-L5MD
- Plate carriers for 4 1536-well microplates PLT-CAR-L4HD
- Plate carriers for 5 96-deepwell (or archive) microplates PLT-CAR-L5AC
- Tip carriers for 5 tip racks of 96 tips each TIP-CAR-480
- Preloaded carriers for 32 or 24 tubes with defined diameter and length CAR24_Cup15x75
- Plate carriers for 5 96-well PCR microplates PLT-CAR-L5PCR
- Plate carriers for 3 96- or 384-well flat microplates in portrait orientation PLT-CAR-L3MD
- Plate carriers for 3 96-deepwell microplates in portrait orientation PLT-CAR-L3AC
- Carrier for 12 reagent containers of 100 ml each REA-CAR-L3AT
- Temperature-controlled carrier No 1 and No 2 CAR_TCC_1, CAR_TCC_2
- Wash Station No 1 and No 2 for needles: CAR_Wash_1_LowNeedle, etc.

Carrier definitions are stored under ...\hamilton\labware\ML_star. Files defining carrier templates have the extension *.tml. A carrier features a varying number of locations for the placement of labware, such as tip racks, microplates, etc.

To add a carrier to the deck layout, simply double-click on the appropriate track position. The “add labware” dialog box opens up. Type in a name for the carrier and click browse to select the PLT_CAR_L5MD from the \labware\ML_star directory. Click OK in the “add labware” dialog.

The carrier is now added to the deck layout.

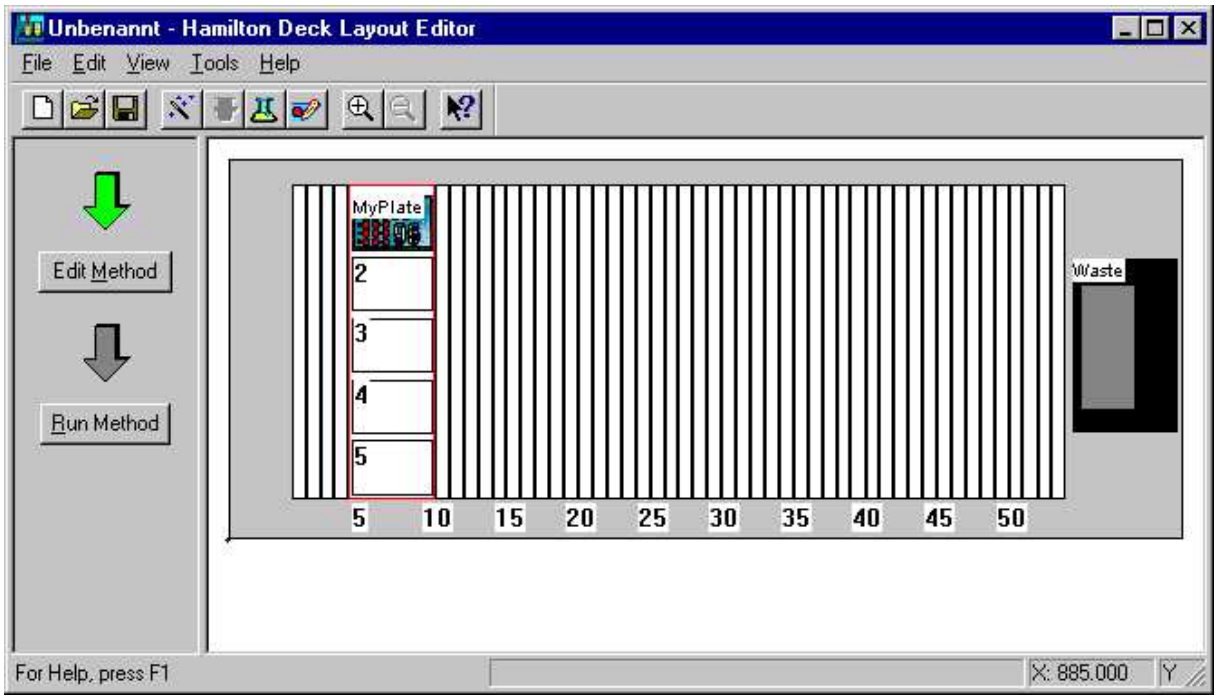


The next step is to add a plate to this carrier. The corresponding labware (plates, tip racks, etc.) is sorted by manufacturer and stored within the Labware directory. Standard plates from almost every major manufacturer are available:

- Nunc
- Falcon
- Greiner, etc.

Microplates, tip racks, etc. are labware items and generally called “racks”. The defining files have the extension “.rck”

To add a plate to the carrier, double-click on the desired carrier position. Again, the “add labware” dialog box opens. Select the Nun_96_Fl_L.rck (96-well flat-bottom Nunc plate in landscape orientation) from the labware\nunc directory. Give a name to the plate, e.g., “MyPlate”.



The plate is added to the site. Plates (or labware) may be moved between sites on one carrier by simple drag-and-drop.

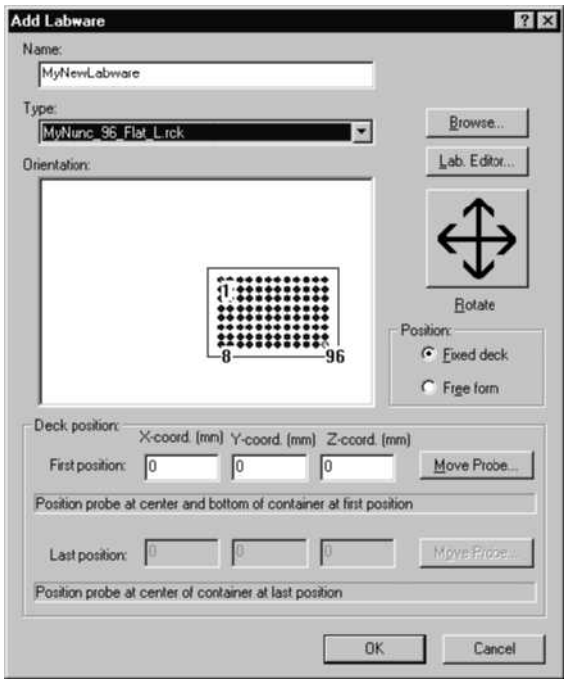
Besides plate and tip carriers, tube carriers can also be placed on the deck. These carriers are preloaded with tubes of a defined size (diameter and length). In fact, these carriers are racks (*.rck) in contrast to the other carriers, which are carrier templates (*.tml). The tube carriers are an example of racks which fit directly into the track grid of the Microlab STAR.

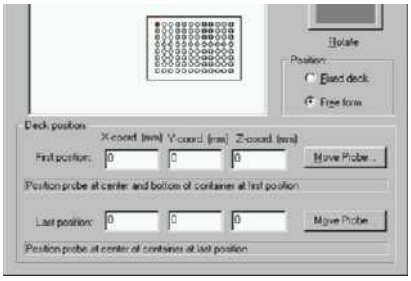
6.2.2 Adding Labware Directly to the Deck

To place labware (e.g. microplates) directly onto the deck (without using a carrier), select “Add Labware” from the Edit Menu. A dialog window opens up: Select the type of labware from the library and enter the x,y,z coordinates by hand.

You may enter a name by which the rack will be known on the deck.

In the type field, select the already listed labware item or press Browse to load another labware item to the list. Click OK. If the labware position falls outside the instrument deck, a message window opens to inform you.



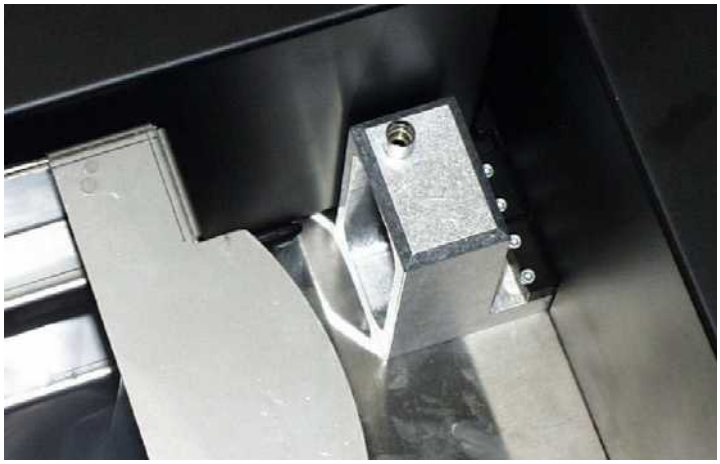


Use the 'Rotate' button to rotate the labware item in increments of 90 degrees and type the position in X,Y, and Z coordinates into the input boxes under Deck Position. Click OK.

If the labware item is to be placed at an angle other than 90, 180, 270 or 360 degrees, the 'Free form' mode will have to be used. Here, two sets of coordinates are required.

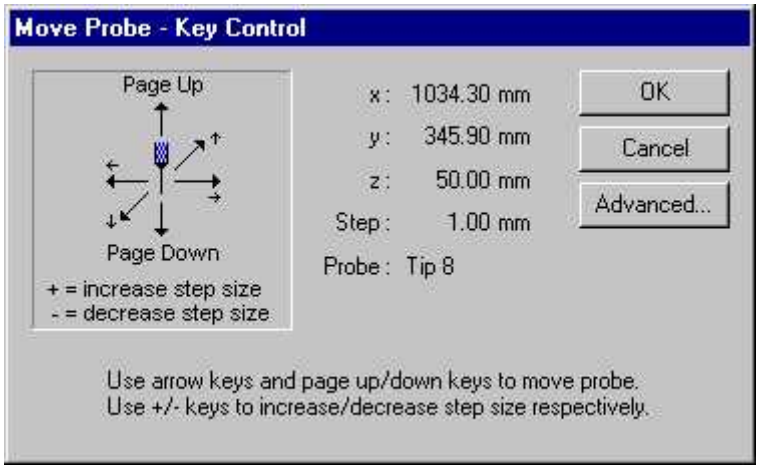
6.2.3 Teaching Labware

"Teaching" means guiding a channel or other probe to a particular location on the deck and assigning a name to that location. The User Software enables you to record the x-y-z coordinates of that point and to associate them with the name you have given the location. Afterwards, you need only specify the name of the location and ML-STAR "remembers" exactly where it is on the deck. The precise position of labware items can be "taught" in this way, using the needle from the teaching station. This is an accessory. The teaching station is placed on track 55, next to the waste container.

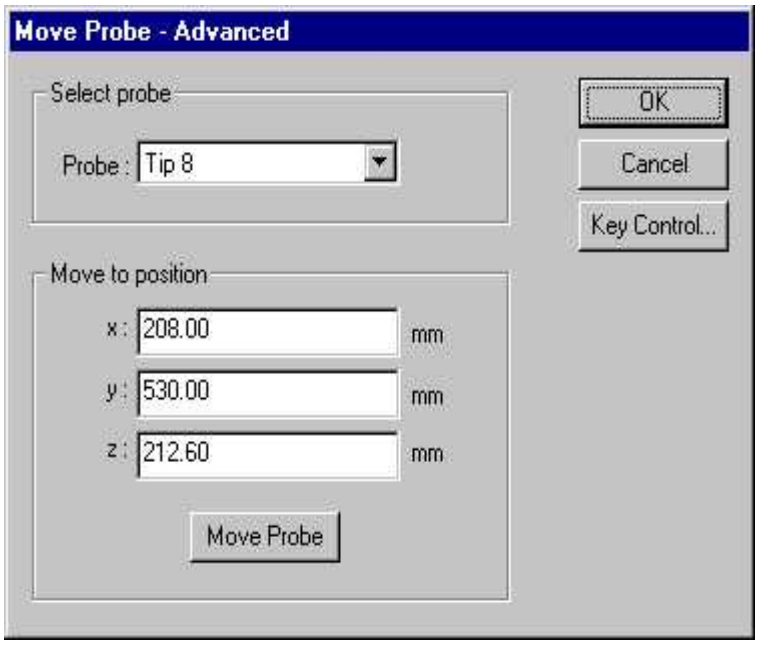


The teaching station with needle.

Right-click on the labware to be "taught" within the deck layout. Select "Move Labware" from the menu. Click on the Move Probe button. A needle is picked up from the teaching station. Note that the teaching station does not have to be added to the deck layout (software).



The dialog gives the current x,y,z coordinates and allows you to move the needle to any position on the deck. The Advanced section allows you to move directly to a position by clicking on Move Probe.



Clicking on Key Control brings you back to the previous dialog. Clicking OK ends the teaching process and writes the current position into the x,y,z coordinate fields of the move labware dialog.

Alternatively, select “Move Probe” from the tools menu.

NOTE

The reference points for “teaching” are the tip of the needle and the reference well of the rack, which is usually marked red (upper- and left-most well = A1).

6.3 Making Changes to the Deck Layout

It is possible to add additional labware to the deck any time by the procedures described so far.

To delete labware items from the deck, right-click the item to be deleted and select “Delete“ from the menu. In the message box that appears, click OK. The item is deleted from the deck.

To rename a given labware item, right-click the item to be renamed and select “Rename“ from the menu. In the message box that appears, change the name and click OK. The item on the deck is renamed.

To get the properties of a labware item, right-click the item of interest and select “Properties“ from the menu. The message box that appears will display the type and the name of the item.

For the definition of custom labware objects, refer to chapter 15.

NOTE

If you change a name or delete a labware item on the deck which is already used in a method, you must also make the appropriate changes to your method.

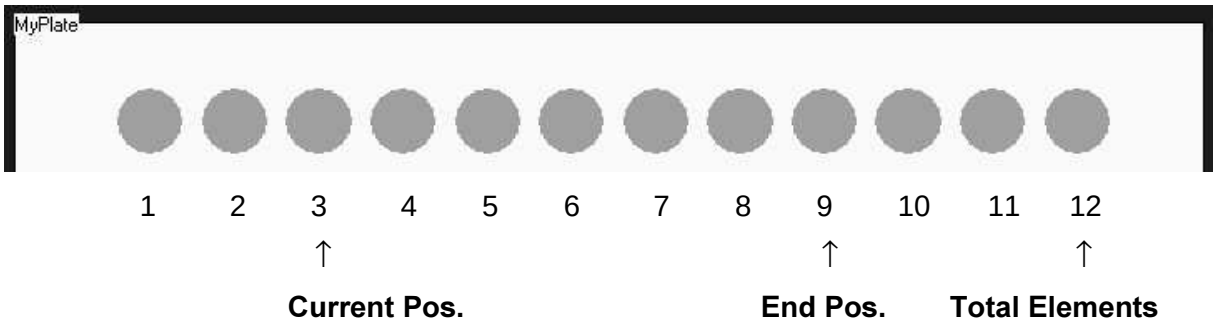
7 Sequences

A sequence is a defined series of pipetting events involving the transfer of liquid from one container to another. A sequence defines the order of containers in a rack. For example, it links the single wells of a microtiter plate together so as to treat them as one plate. When the probe is pipetting, it will follow this order (channel No 1->Sequence Position No 1, channel No 2 ->Sequence Position No 2, etc.) unless instructed to do otherwise.

A sequence such as the one shown below has three pointers (or important positions) which may change during a run:

- The current position (which is the first unused well in the current situation),
- the end position (the last position to be used for pipetting), and
- the total number of elements (the overall length of the sequence).

Consider this example:



Within the method, all three positions may be altered or requested by the appropriate functions.

A standard sequence is created automatically when the rack is placed on the deck. Custom sequences can be defined graphically by selecting the appropriate wells in the Sequence Editor, or “on the fly“ within the programming of the method.

In methods defining the pipetting steps to be performed, only the sequences and not the actual labware items are referred to. Pipetting always occurs according to a sequence.

7.1 Sequence Editing

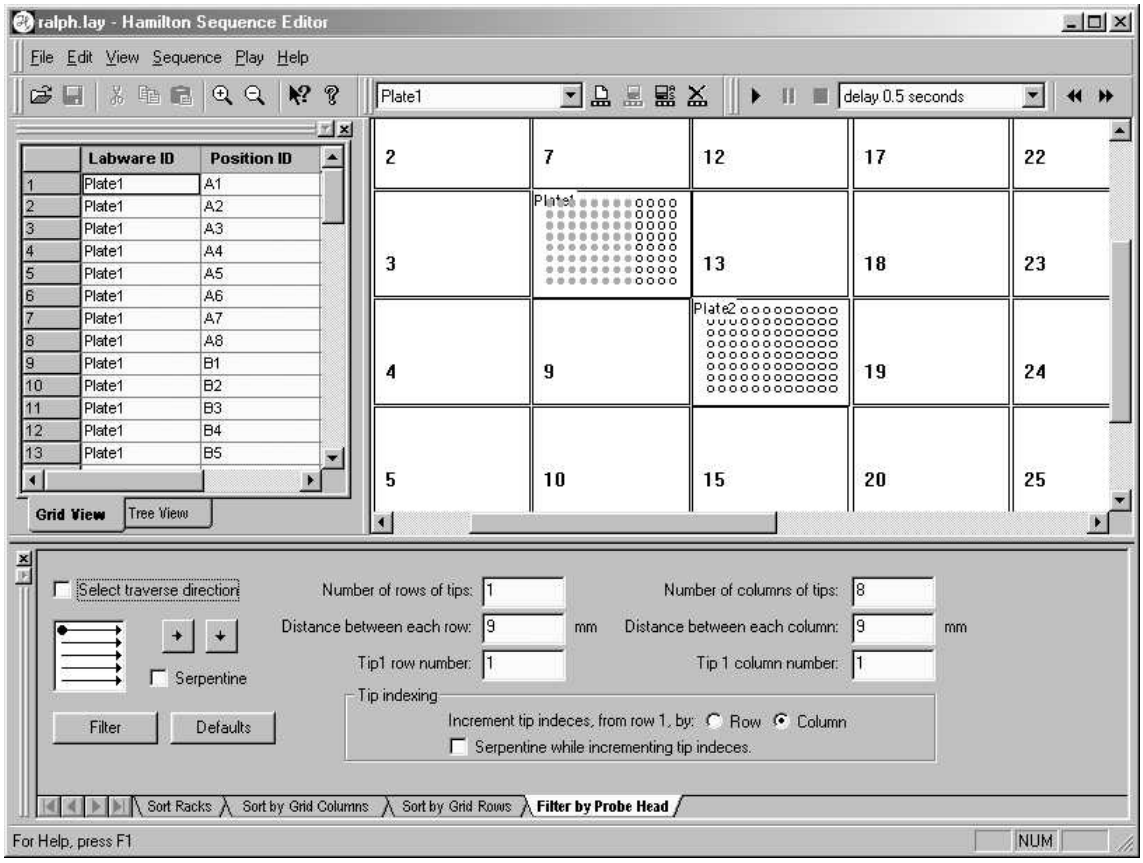
To edit or change sequences, start the sequence editor by selecting Tools->Sequence Editor in the Deck Layout Editor. The Sequence Editor is illustrated on the next page. When the sequence editor is started from the Deck Layout Editor, a new untitled sequence is created automatically. Add labware positions to this sequence by selecting (clicking on) positions (wells) in the deck layout view. The standard left-click, shift-left-click, control-left-click, and rubber-banding rectangle are available for selecting positions in the deck layout view.

As positions are added to the sequence, they are displayed in the grid in the left pane of the window. The grid contains one row for each labware position in the sequence. The order of the rows in the grid can be changed by standard cut, copy, paste, drag, drop, and delete operations, thereby changing the order of the positions in the sequence. The grid has a fixed width. X, Y, and Z columns may not be initially visible. Moving the vertical splitter or the horizontal scroll bar will allow these columns to be seen. Sequence information cannot be typed directly into the grid.

Additionally, the functions of the sort/filter panes can be applied to the selected rows of the grid (if selected) or all grid contents. Once the labware positions in the sequence are in the desired order, save the sequence. Saving fixes the order of each row in the grid as the order of the labware positions in the sequence. On saving, you are requested to give a name to the sequence.

Any existing sequence in the deck layout can be activated for viewing and/or editing by selecting the sequence name in the drop-down menu. Additional labware positions can be inserted in the active sequence by selecting them in the deck layout view. Existing positions can be removed. Positions can be reordered as described above. The sequence can be resaved to fix its positions according to the new order.

The tree view in the left pane displays the sequence from a tree-oriented perspective, where the tree's leaves are the positions in the sequence.



7.1.1 Adding Positions to a Sequence

Use the mouse to select labware positions to insert in the sequence as follows.

1. Left-clicking on labware position adds the position to the sequence. That means it either (a) inserts it in sequence ahead of the (first) selected row in the grid, or (b) appends the position after the last row in the grid if no rows are selected.
2. Rubber-banding a rectangle of wells adds a block of positions to the sequence. The block of positions is added in a manner similar to that employed when adding a single labware position. The block of positions is ordered according to default sequence for all labware object(s) involved.
3. Control-left-click on labware position toggles the position's status in the sequence. That means it inserts the position in the sequence if it's not already there (same as left-click on

labware position), otherwise it deletes the position with the largest sequence number from the sequence.

4. Shift-left-click on labware position adds a block of positions to the sequence. That means it adds all positions in the rectangle whose first corner has the coordinates of the most recently added position and whose opposite corner has the coordinates of the newly selected labware position. The block of positions replaces the most recently added position and is ordered according to default sequence for all labware object(s) involved.

NOTE

To select a labware position, click on it only once. If you click a second time on a labware position, it will be added to the sequence again.

7.1.2 Play

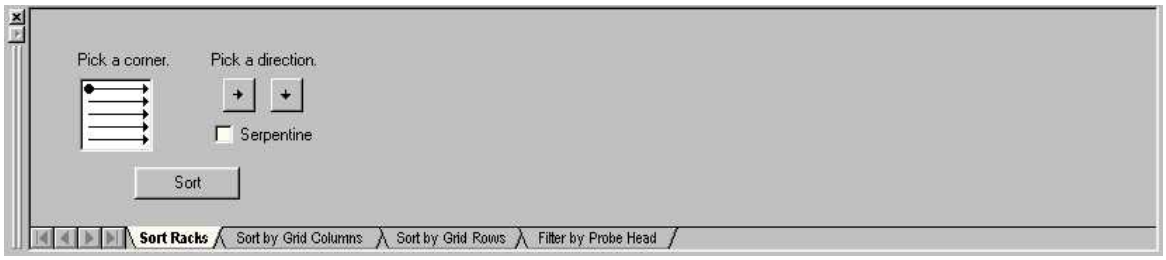
Play first deselects all positions in the sequence, then plays the sequence. As each position is selected, it is painted in the deck-layout view and highlighted in the grid and tree views.

Play can be paused at any time and resumed by clicking on play again.

7.1.3 Sorting Racks

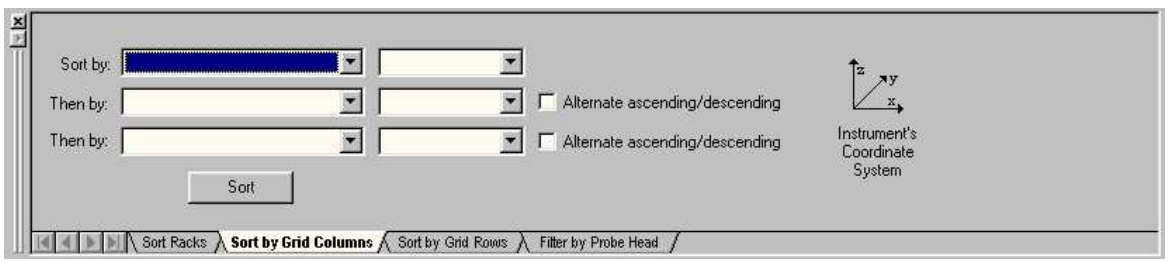
The "Sort Racks" dialog allows you to apply an order to labware positions in the active sequence. The order is applied to the currently selected labware positions, or to the entire sequence if no positions are selected.

Use this filter to create processing sequences across multiple racks in the direction required.



7.1.4 Sorting by Grid Columns

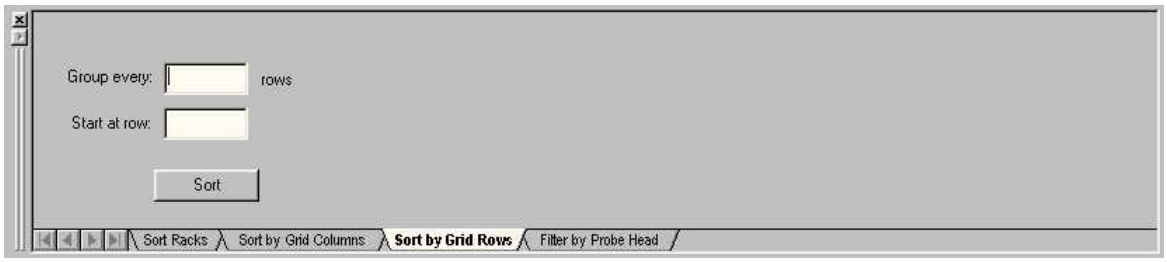
The "Sort by Grid Columns" dialog allows you to sort the contents of the grid based on specified criteria. Left-most combo boxes contain the grid's column headers: #, Labware, Position, X, Y, Z, and "(none)". Right-most combo boxes contain "ascending" and "descending". The sort is applied to the currently selected labware positions, or to the entire sequence if no positions are selected.



7.1.5 Sorting by Grid Rows

The “Sort by Grid Rows” dialog allows you to sequentially order every *i*-th row in a grid beginning with the *j*-th row. The sort is applied to the currently selected labware positions, or to the entire sequence if no positions are selected.

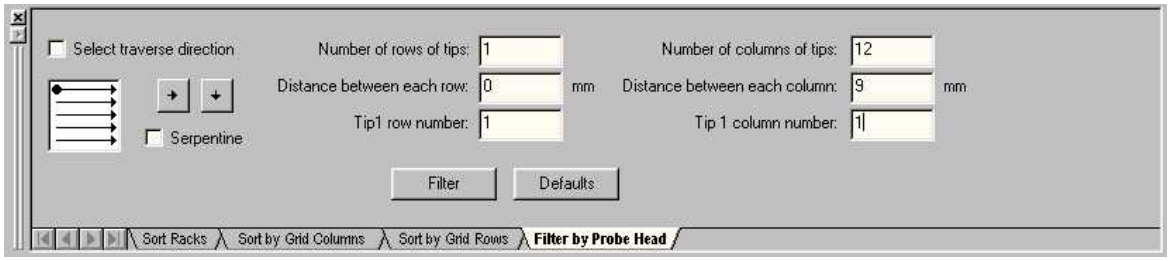
The sequence will be re-ordered by selecting the *i*-th grid row and moving that row to the “next” position.



7.1.6 Filter by Probe Head

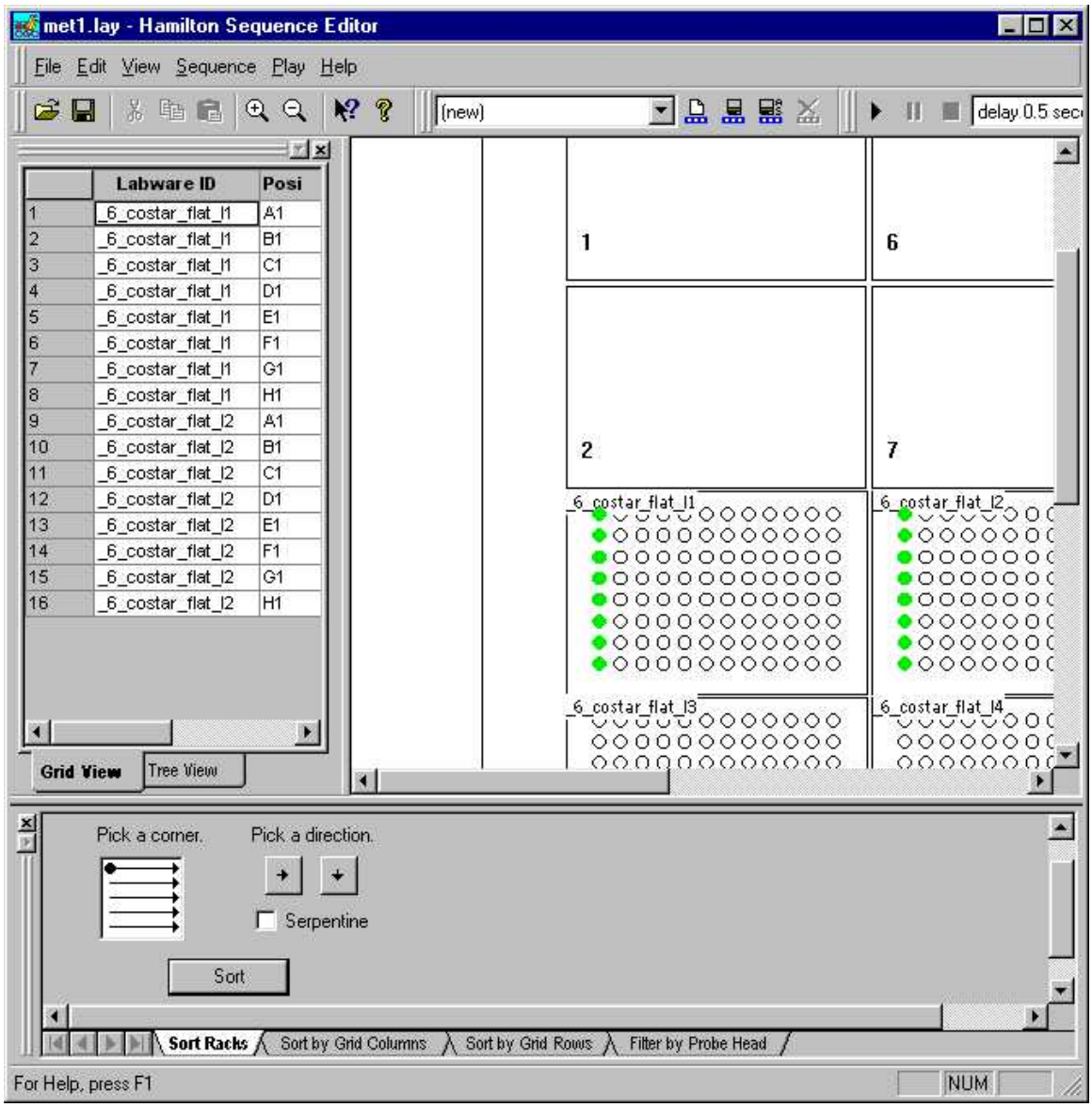
The “Filter by Probe Head” dialog allows you to (a) apply an order to labware positions in the active sequence, then (b) remove positions where the probe won’t fit. These functions are applied to the currently selected labware positions, or to the entire sequence if no positions are selected. The first function, (a), is optional.

Initial values for this tab are taken from the instrument’s configuration file. These initial values can be recalled using the Defaults button.

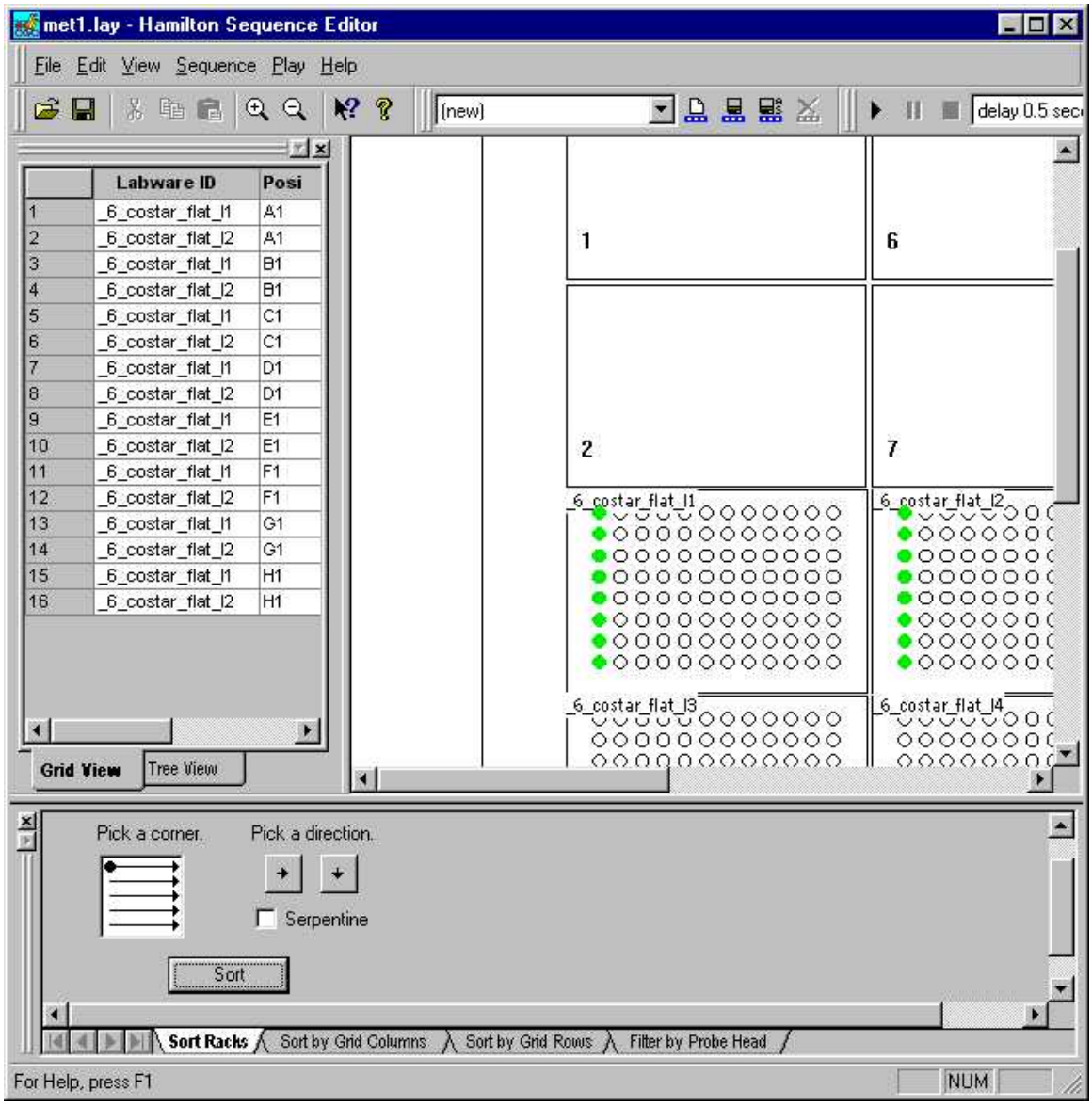


7.1.7 Sorting Racks Example

Here is an example of how rack sorting works. Given the following sequence across 2 racks, apply the Sort Racks filter, defining a direction across the racks.

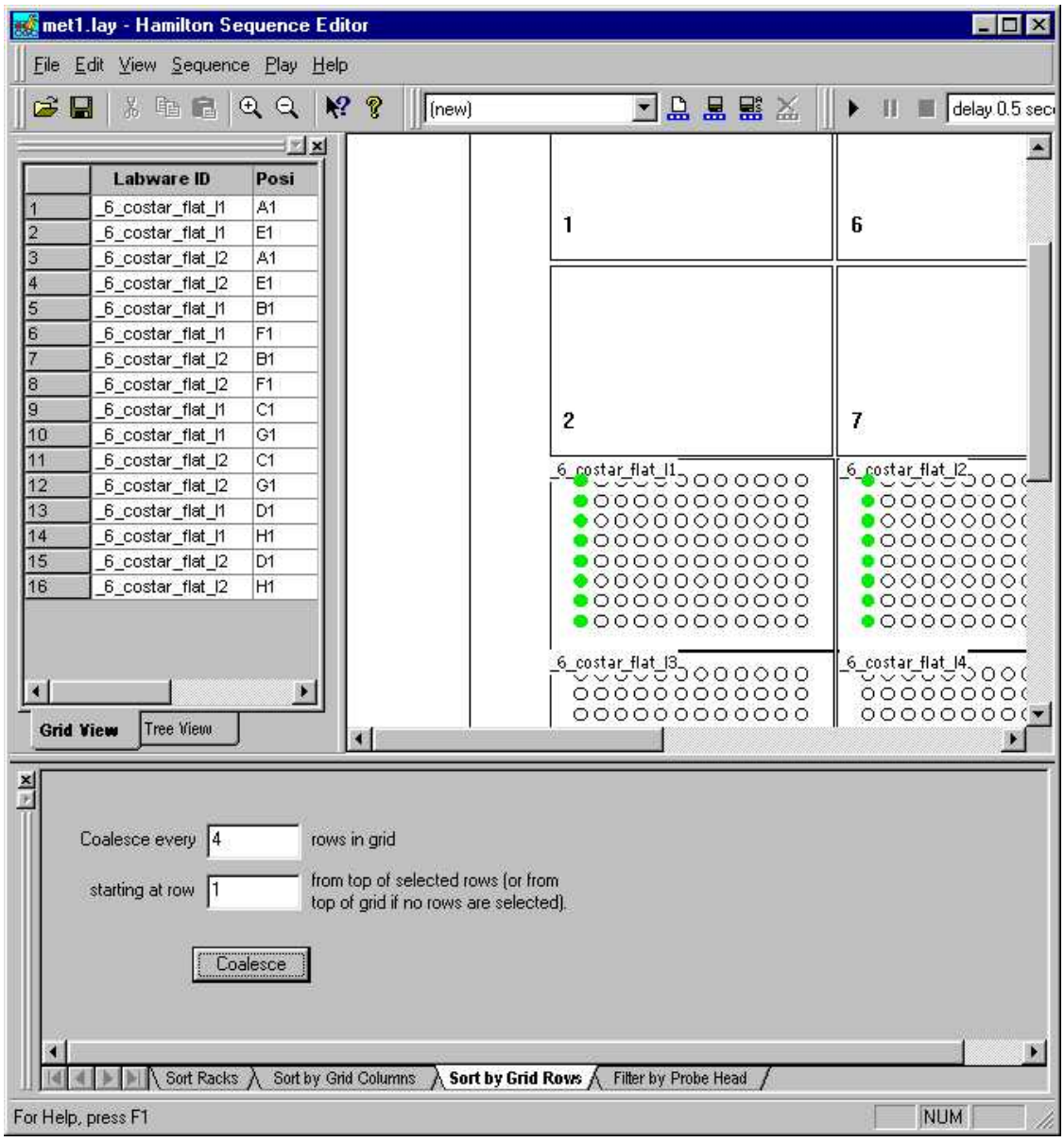


The sequence is re-ordered across the racks as follows:



7.1.8 Sorting Grid Rows Example

The same initial sequence if sorted using Sort by Grid Rows will result in the following:



In this case the initial sequence was:

Rack1 A1, B1, C1, D1, E1, F1, G1, H1

Rack2 A1, B1, C1, D1, E1, F1, G1, H1

Taking the 4th row after the first row gives

Rack1 A1, E1,

Rack2 A1, E1

Rack1 A1 is gone, so Rack1 B1, F1

Rack2 B1, F1

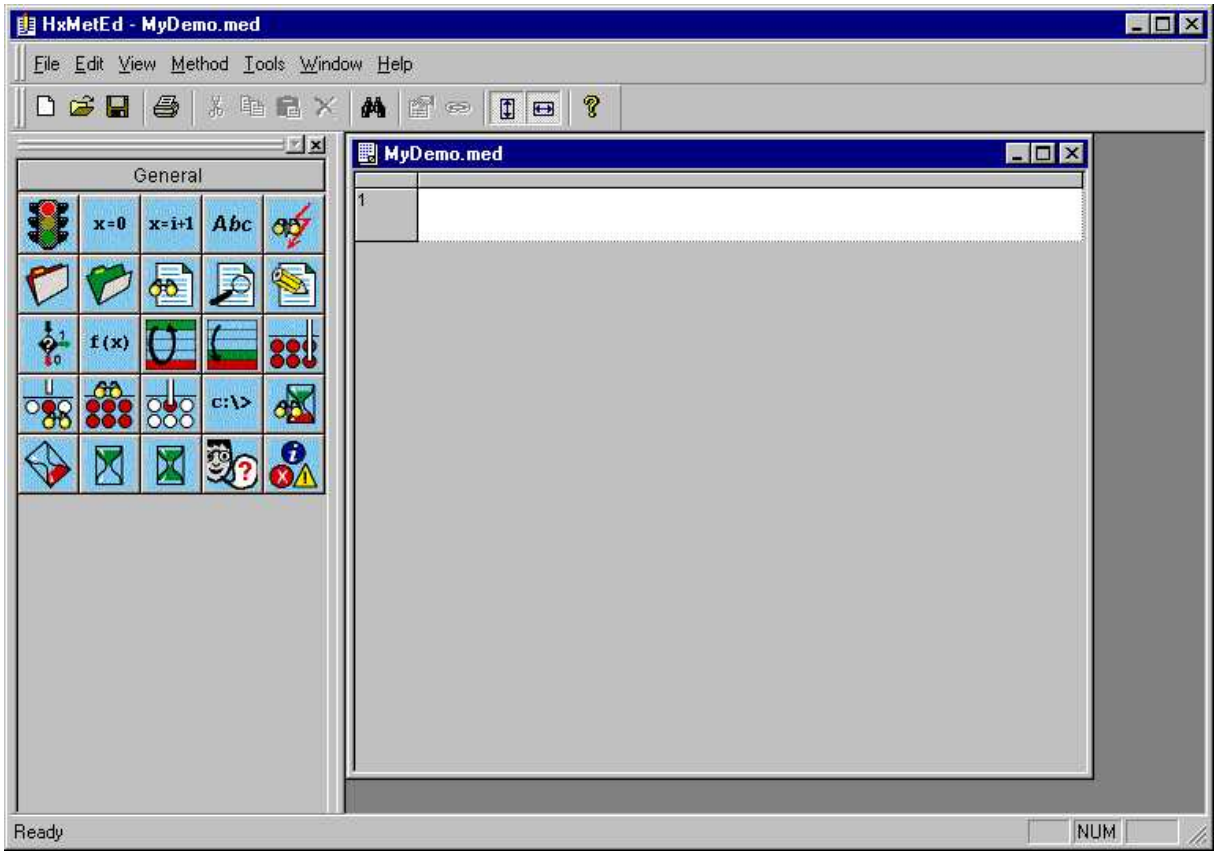
Rack1 B1 is gone, so Rack1 C1, G1... And so on.

8 Graphical Method Editor

Methods are short programs that string a number of specified commands (such as aspirate or dispense) together to instruct the instrument to perform a function. They can be as complex or as simple as the user desires. Access to a method editor is necessary to define or edit methods.

The Graphical Method Editor is a syntax-free editor that presents a batch-like graphical editing environment. Common constructs such as loops, conditionals and built-in functions are supported by this editor. It also provides a variable management system. This system simplifies variable usage by allowing implicit declarations and by supplying complete variable context to edit dialogs. The output of the Method Editor is a linked set of files that may be executed by the *Run Screen* or further customized using the *HSL Method Editor* (see chapter *9 HSL* Method Editor, page 96).

In most cases (but not necessarily), a method references one single deck layout. The *Method Editor* is accessed by clicking on the *Edit Method* button in the *Deck Layout Editor*, after the deck layout has been defined and saved, e.g. as “MyDemo” (.lay). Alternatively, the method editor may be started directly from the desktop.



Method Editor

The Method Editor is divided into 2 sections:

The ***Method View*** on the right is a grid containing the method.

The ***Toolbox Window*** on the left offers various elements that can be used to build the method, grouped by bars.

At the beginning of a new method, there is only one bar named *General*, containing the common language elements such as *if-else*, *loop*, *function call*, etc. No instrument-specific steps (e.g., aspirate) are visible so far.

All **steps can be disabled/enabled on request** by right-clicking a step or a block of steps and selecting disable/enable from the menu.

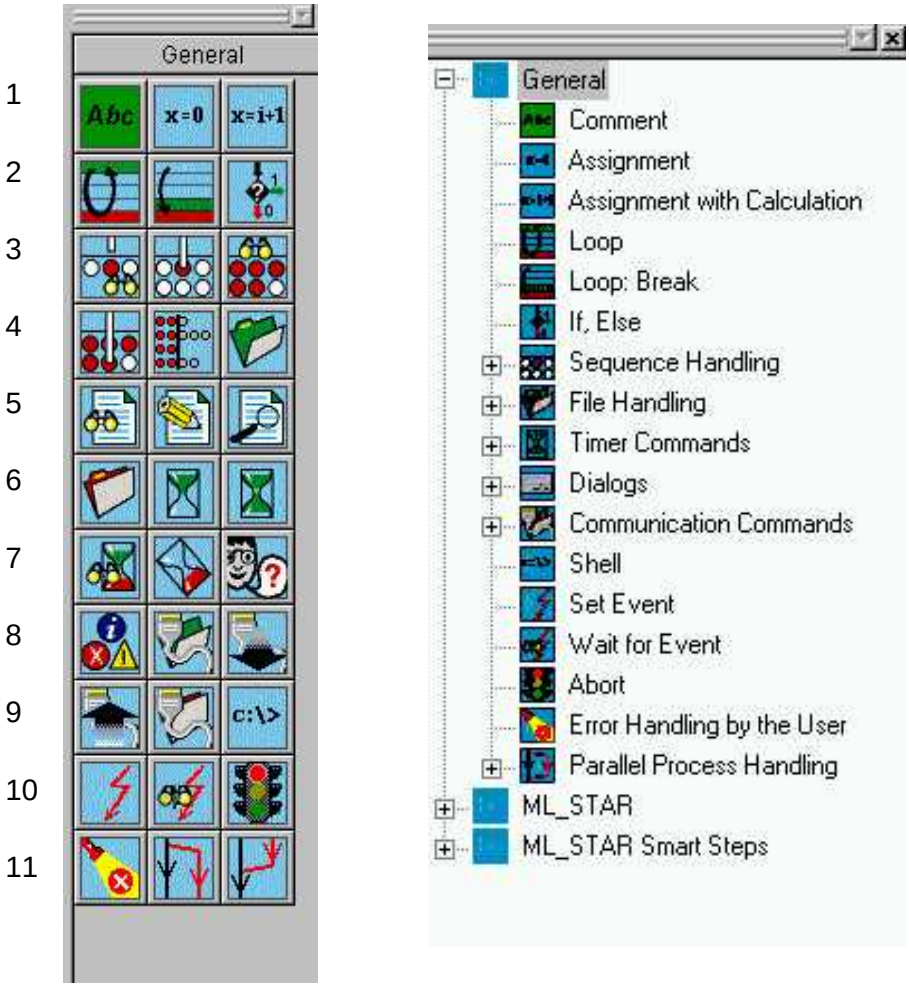
It is possible to load different methods into the editor and **copy steps between the methods**.

8.1 General Method Commands

A detailed description of all commands in the general method editor is directly available from the software. Invoke the Help menu for more information.

A number of standard commands are available, as you can see from the figure below. For an explanation of the command buttons on each line (numbered for convenience), see the listing following the figure.

Whatever input is necessary for the individual commands, all input fields labelled with text in brackets [*description*] contain optional information or the default may safely be accepted.



General commands listing in icon- and tree view. Right-click in the tool menu to switch between the different views.

Commands on line 1

- **Comment**: For any user-defined comments in a method
- **Assignment**: assign a variable
- **Assignment with calculation**: perform calculations

Commands on line 2

- **Loop**: Loop commands between start and end of loop
- **Loop break**: Terminates a loop
- **If-then, else**: Condition command

Commands on line 3

- **Sequence**: Get current position
- **Sequence**: Set current position
- **Sequence**: Get end position

Commands on line 4

- **Sequence**: Set end position
- **Sequence**: Adjust sequences
- **Open file**: To open a file before file operation

Commands on line 5

- **Read from file**: Read data out of a file
- **Write to file**: Write data into a file
- **Set position**: Set the file-pointer to a specific position

Commands on line 6

- **Close file**: To close a file after file operation
- **Timer**: start a timer
- **Timer**: wait for a timer

Commands on line 7

- **Timer**: read elapsed time from a started timer
- **Timer**: restart a timer
- **User Input**: display an input box at a particular point in the program

Commands on line 8

- User output: display an output box at a particular point in the program.

- **Communication Port Open**: Opens a com port
- **Communication Read**: Reads data from a com port

Commands on line 9

- **Communication Write**: Writes data to a com port
- **Communication Port Close**: Closes a com port
- **Shell:** call external program

Commands on line 10

- **Wait for a event:** waits for an event

- **Set event:** defines an event

- Abort: Abort Method

Commands on line 11

- Error handling by user: Defines a section with user-defined error handling

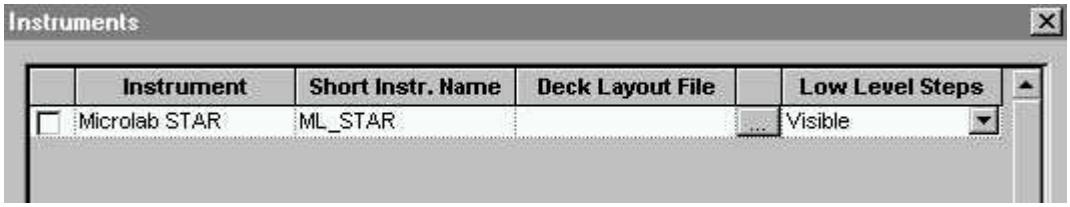
- Begin Parallel: Starts a bifurcation for programming parallel processes

- End Parallel: Ends a bifurcation for programming parallel processes

For a detailed description of the input dialogs that correspond to these icons, refer to the on-line help.

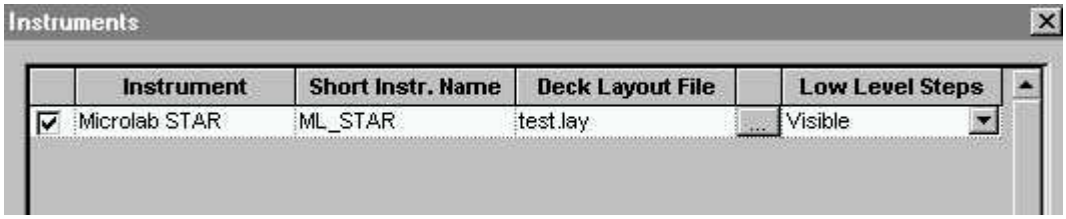
8.2 Using Microlab STAR-Specific Steps

To use instrument-specific steps (e.g. aspirate, dispense, etc.), the method has to be linked to an existing deck layout first. Use *Instruments...* in menu *Method* to open the following dialog:



The instruments dialog.

Click on [...] to browse for the deck layout you wish to write a method for. Select the layout from the file list and click Open.



If the selected deck layout corresponds to a Microlab STAR, the check box is enabled. Click OK to link method and deck layout. Keep the “Smart Steps” check box selected. Note that multiple deck layouts may be linked to one method to enable programming of different instruments within one method.

Once you have written a method using instrument specific steps, the “[...]“ button vanishes. Now the deck layout can no longer be separated from the method until all instrument-specific steps are deleted again.

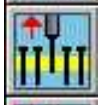
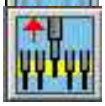
NOTE

Only after the method has been linked to a deck layout are the instrument-specific commands loaded into the method editor.

Linking the deck of another instrument to an ML STAR will result in an error message.

8.2.1 SMART Steps

SMART steps are powerfull commands for programming the Microlab STAR. Whereas single steps (see next chapter) are restricted to single actions like picking up tips, aspiration, dispense, etc., SMART steps combine many single steps for specific tasks like filling a microplate starting with tubes, aliquoting of reagent to a complete plate, loading the deck, etc. The available SMART steps are:

Icon	Command	Action performed	Parameters to be specified
	Load Deck	Load carrier on deck	Sequence, carrier calibration
	Pipette With Tips	Pipette using disposable tips	Pipette mode, volumes asp/disp control seq, liquid class, tip seq, tip handing/replacement, tip counter
	Unload Deck	Remove carrier from deck	Sequence
	Define Needle Wash	Specify parameters for needle wash	Wash sequence, start with liquid, settings
	Pick up Needles	Pick up Needles from Wash Station (or Racks)	Needle sequence
	Release Needles	Release Needles in racks or wash station (and start wash)	Needle sequence
	Pick up tips	Pick up disposable tips from tip rack	Tip sequence, tip counter
	Eject tips	Eject disposable tips into tip waste	none

The method editor allows you to freely combine SMART steps and single steps.

For all SMART steps, the instrument name is to be selected. This is set by default to the Microlab STAR's short name and cannot be changed within the SMART steps. Only in the case of two or more instruments linked together is a selection necessary.

8.2.1.1 SMART Step Pipette with Tips

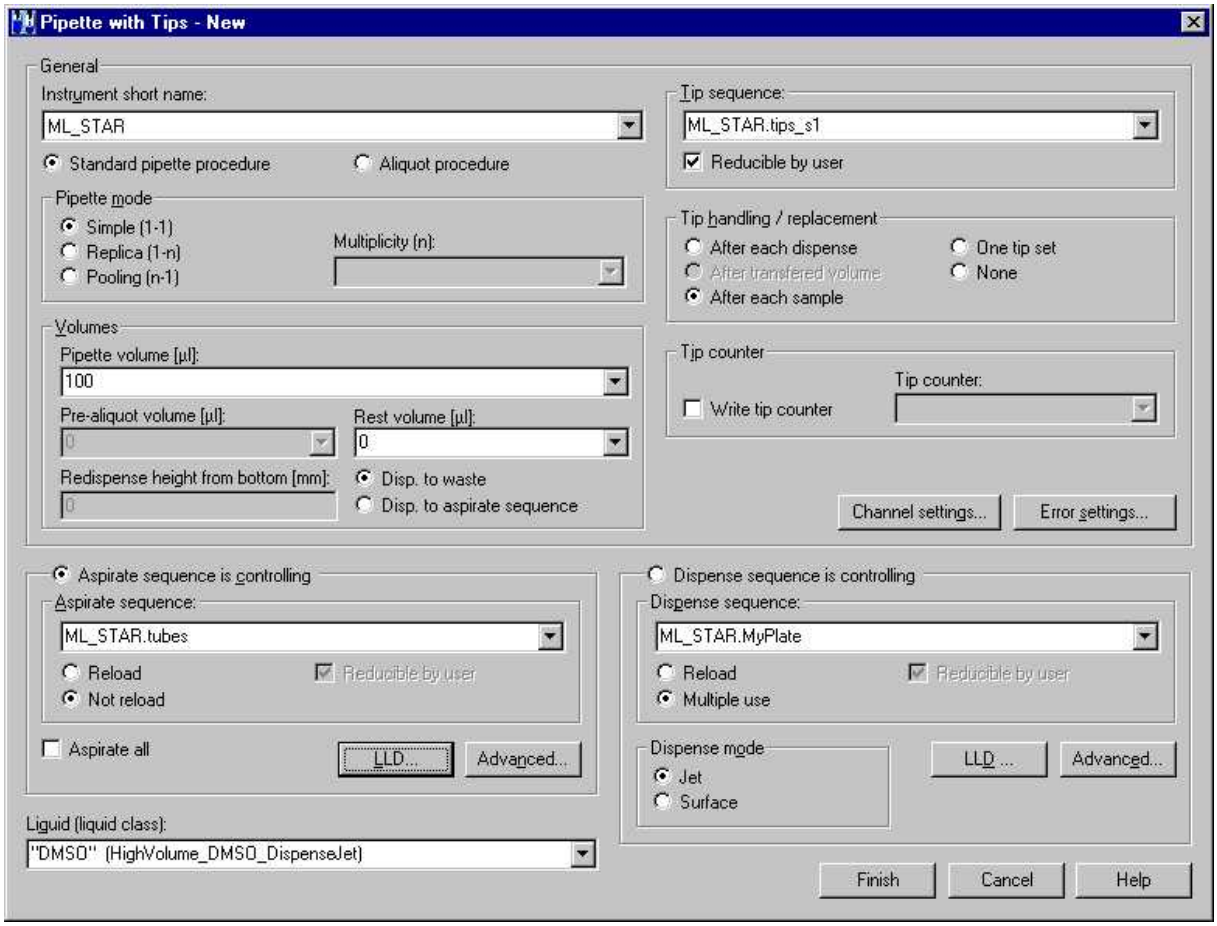
The SMART step pipette is a multifunctional command for various kinds of pipetting tasks. The Pipette Step allows the user, for example

- to copy one plate into another, or
- to transfer from tubes to a plate with variable numbers of samples, or
- to aliquot reagent into a plate,

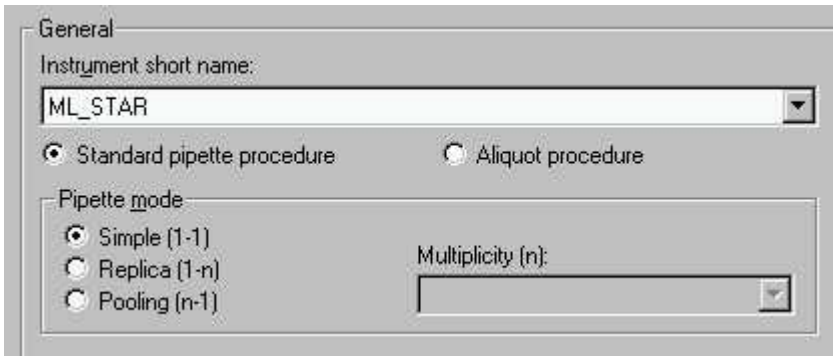
all within one step without taking explicit care about the instrument's initialization, single steps for aspiration and dispense, sequence handling, or loops. As is the case for all Microlab STAR user software components, SMART Steps too make use of sequences. Therefore, the pipetting task should be reflected within the sequences defined for this step. For the explanation of sequences we refer to chapter 7.

In this chapter, the general features of the SMART Step are explained; examples can be found in the chapter „Methods for Microlab STAR".

Dragging a SMART step into the method tree opens the SMART Step input dialog:



Let us now walk through the different sections of the SMART Step.

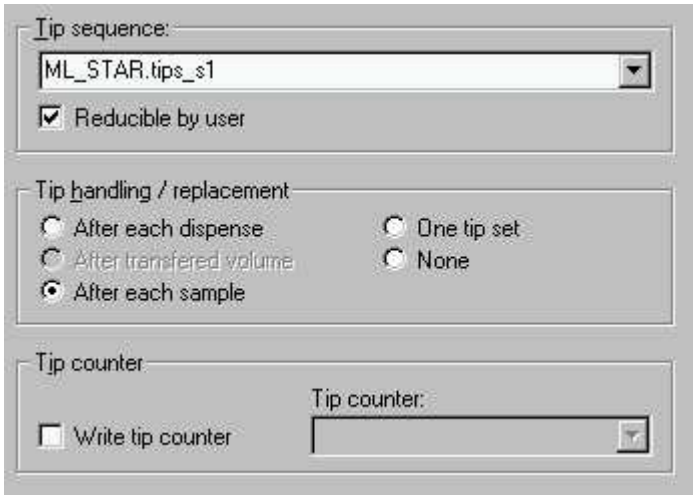


Standard or aliquoting mode must be selected. For **standard pipetting** mode, three different procedures are possible:

- "Simple" pipetting for simple aspiration/dispense cycles,
- "Replica" for cycles of aspirations/dispenses where the liquid from one source is dispensed into multiple target containers (no aliquoting), e.g., for a "double test" (tube 1 -> plate position A1, A2, tube 2 -> plate position A3,A4, etc.). The desired multiple can be chosen (e.g. 2 for a double test).
- "Pooling" for cycles of multiple aspirations/dispenses where liquid from multiple source containers is dispensed into one target container (e.g., tube 1, tube 2 -> plate position A1, tube 3, tube 4 -> plate position A2, etc). Here too, the desired multiple has to be chosen (e.g., 12 for pools of 12).

For the aliquoting procedure, the choices under pipetting mode are deactivated. Aliquoting means that one aspiration is followed by multiple dispenses (e.g., tube 1-> plate positions A1 to A12).

The next step is to select the appropriate tip handling:



First, select the tip sequence - where the tips are to be picked up from. The tip type is automatically retrieved from the sequence. If the tip sequence is used up, it will be automatically reloaded. If "Reducible by user" is checked, the tip sequence can be reduced on reloading.

Tip handling can be chosen to

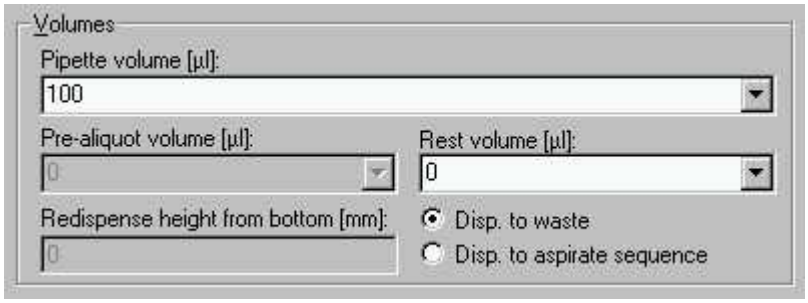
- replace tips "after each dispense" (if multiple dispenses are needed to transfer e.g. 900 µl with a standard tip of 300 µl),

- "after each sample" (multiple dispenses of the same sample are done with the same tip),
- with "one tip set", or
- without tip handling, "none". In this case, STAR will have to pick up tips prior to the pipette step and eject them after pipetting, using single steps.
- Only for replicas (and pooling), the option "after transfered volume" becomes active. This allows you to use fresh tips even for multiple aspiration and dispense cycles being performed with the same sample.

Finally, a **tip counter** can be specified, which enables the user to start with a set of tips, partly used in previous runs, at the correct position. To read a tip counter, it can be specified within the SMART Step load (see that section). A tip counter is specified by a name, e.g., „MyFirstStandardTipCounter". Note that the name has to be placed between quotation marks.

Within the SMART Step pipette, the current position of the tip sequence will continuously be stored under the name of the tip counter, if the check box "write tip counter" is checked. Note that a tip counter has to be written if it is to be read by the SMART Step load within the next run.

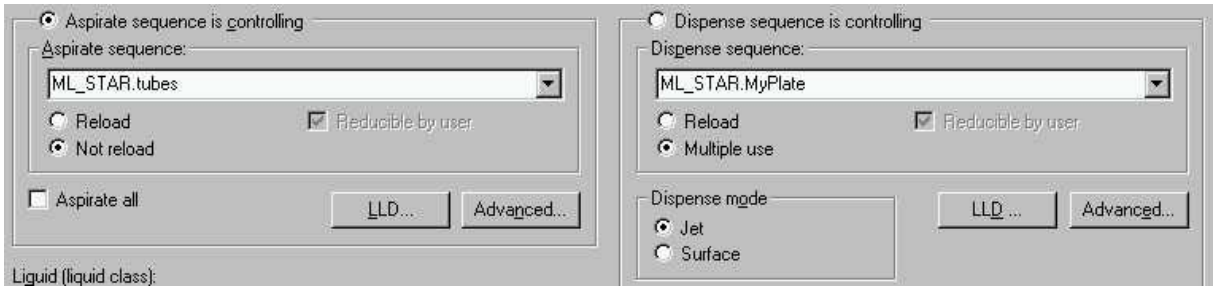
The next step is to define the **volume(s)** for the pipetting process. For the SMART Step pipette, one volume for all channels has to be defined.



If a volume for "simple transfers" is specified that exceeds the volume of the tip, multiple transfers with equally divided volumes will be performed automatically. A residual volume may be used too. Then the choice is to discard the residual volume back into the aspiration sequence, or to dispense it into the waste container.

In the case of aliquoting, a pre-aliquot has to be specified, too. This pre-aliquot is dispensed back into the aspiration sequence. A redispense height counted from the bottom of the container has to be given.

Now, the **aspiration and dispense** locations used within this step have to be defined.



The sequence of the aspiration and dispense is to be selected from the dropdown fields. In addition, the user can opt to reload a sequence: if during pipetting a sequence is used up (no more tubes are present to aspirate from), the system then asks for a new carrier of tubes to be loaded. If in addition the checkbox "reducible by user" is checked, then the user has the option of reducing the newly loaded sequence at runtime. This is especially helpful if the exact amount of sample tubes varies from run to run (or from tube carrier to tube carrier).

An important issue is to select the **controlling sequence**. This is either the aspiration, or the dispense sequence. As long as both sequences are of the same length, this selection does not influence the pipetting (if no pipetting error occurs).

Sequence handling in Pipetting Mode "Simple"

But think of the situation, where the aspiration sequence comprises e.g. 8 tubes, and the dispense sequence is the one of a 96-well plate. What to do now with the 88 remaining positions within the plate? If the aspiration sequence is controlling, the dispense sequence is reduced to the length of the aspiration sequence and the pipetting stops after dispensing into the first 8 positions of the plate sequence. If the dispense sequence is controlling, the aspiration sequence will be repeated until it reaches the length of the dispense sequence. This results in multiple (12 for an 8-channel instrument) transfers from the same 8 tubes to fill the complete plate. Then the (controlling) dispense sequence is finished and pipetting stops.

NOTE

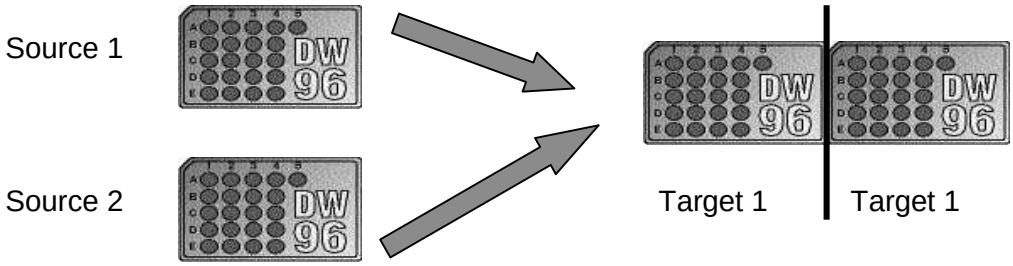
The SMART Step pipette always equalizes the length of aspiration and dispense sequences.

The following table gives an overview of the different situations and examples. L is the length of the sequence.

	$L(S_{asp}) > L(S_{disp})$	$L(S_{asp}) < L(S_{disp})$
Aspiration Controlled	**Case 1: Seq(Disp) is repeated to length of Seq(Asp)** Use: -Copy 2 plates into 1 (after another) -With reloading of Seq(Disp): Transfer available tubes to as many plates as needed.	**Case 3: Seq(Disp) is reduced to length of Seq(Asp)** Use: -Transfer a variable number (<96) of tubes into a 96-well plate (left partly filled).
Dispense Controlled	**Case 2: Seq(Asp) is reduced to length of Seq(Disp)** Use: -Transfers from many tubes to a plate and stops when plate is filled. -With reloading plate sequences: Automatically fill as many plates as needed with liquid from tubes.	**Case 4: Seq(Asp) is repeated to length of Seq(Disp)** Use: -Transfer reagent from a container (or, e.g., 8 tubes) to a complete plate. -Copy tubes repeatedly into plate. -With reload of aspiration sequence: Automatically reload tubes until plate is filled.

The 4 cases are illustrated in the following diagrams:

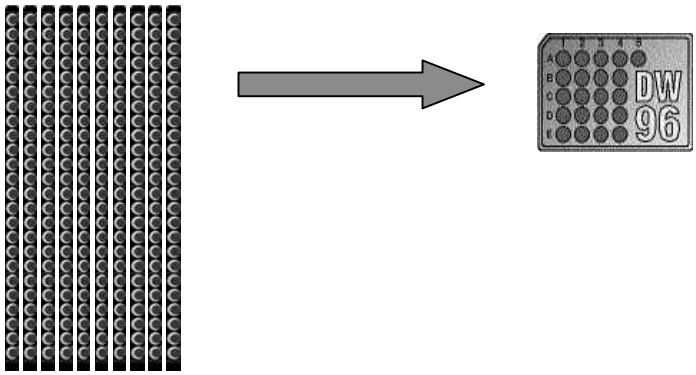
Case 1: L(S_{asp}) > L(S_{disp}), Control: ASP



The target sequence is repeated to match the length of the source sequence.

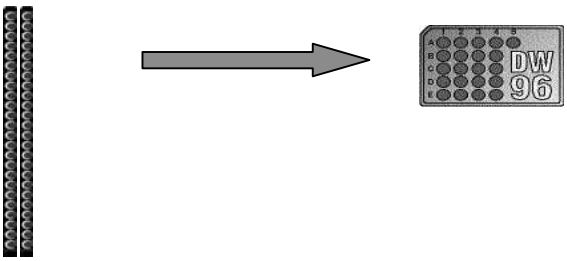
Another example for this case: All Tubes to Plate(s). Reloadable plate sequence to load all tubes to as many plates as needed.

Case 2: L(S_{asp})> L(S_{disp}), Control: DISP



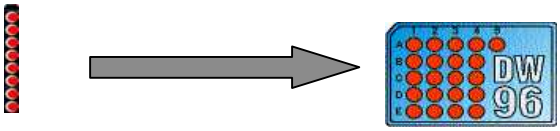
Source sequence is cut to match length of target sequence; stops when plate is full (prepare plate for assay)

Case 3: L(S_{disp}) > L(S_{asp}), Control: ASP



Target sequence is cut to match length of source sequence; stops when tubes are processed (how many samples today?). If L(S_{asp})>96, plate is filled again!

Case 4: L(S_{disp}) > L(S_{asp}), Control: DISP



Source sequence is repeated to match length of target sequence; stops, when plate is full (Distribute Buffer, Reagents...). Also used for aliquoting.

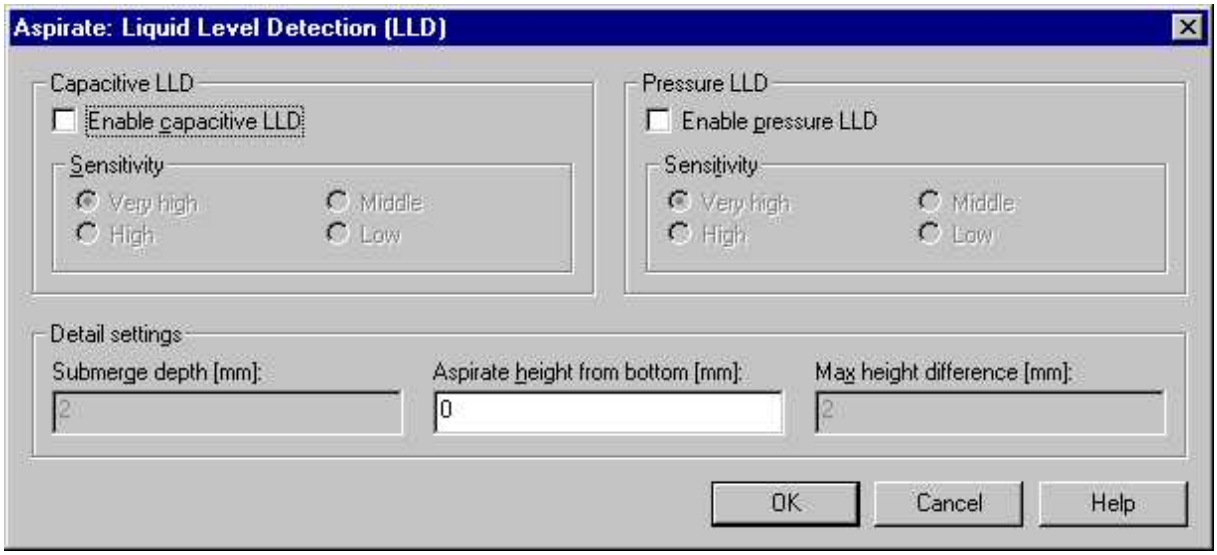
Sequence handling in Pipetting Mode Pooling and Replica

In these two cases, the aspiration sequence should be selected as controlling.

Sequence handling for Aliquot Procedure

In the case of aliquoting, the (longer) dispense sequence is to be selected as controlling.

For the aspiration, the check box “aspirate all“ enables aspiration of the residual liquid from a container (specify a volume larger than the expected residual volume within the container). On dispense, the dispense mode “surface“ or “jet“ has to be selected. For aspiration and dispense, the LLD (liquid level detection) settings have to be specified by clicking on the **LLD** button:

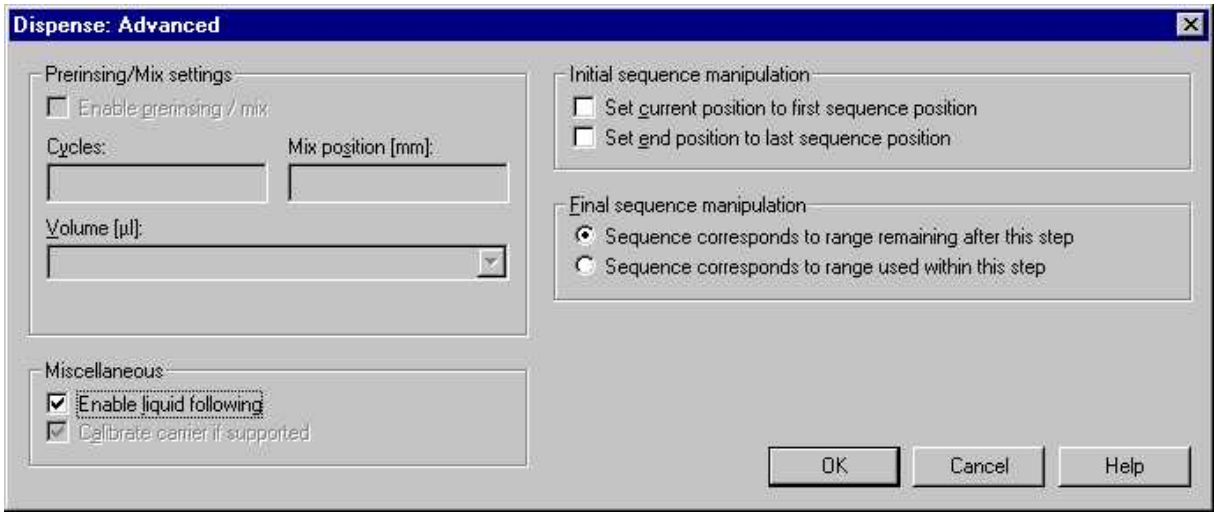


Within the LLD dialog, capacitance, pressure, or both LLD types may be switched on. A sensitivity setting is necessary (see table in chapter 2). If LLD is used, a submerge depth has to be specified, if a fixed height is prefered, the distance counted from container bottom is to be specified. For the parallel use of both LLD types, a maximum height difference has to be given, within which both LLDs have to respond.

The next step is to select the appropriate liquid from the dropdown field.

The selection by attributes (tip type, dispense mode) is automatically made, so that only the liquid name has to be selected.

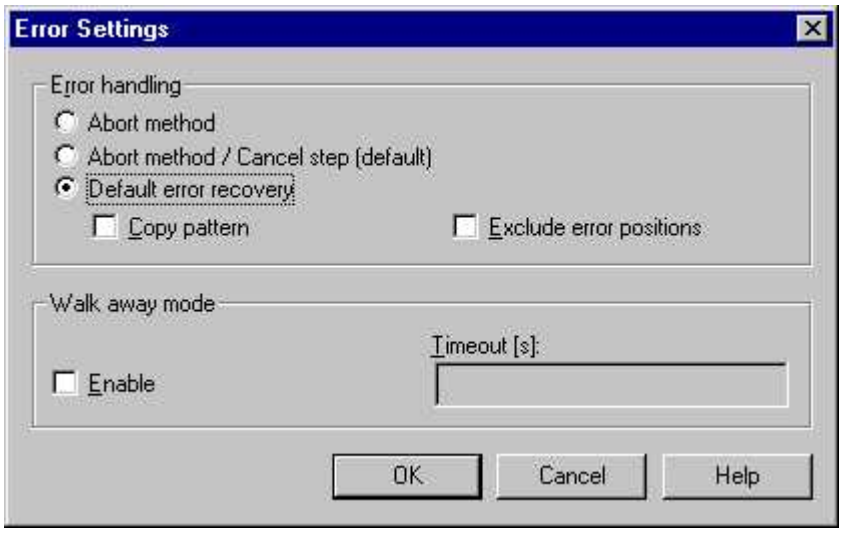
Under “**advanced**“, pre-rinsing and liquid following can be specified.



Furthermore, sequence manipulations can be made (separately for aspiration and dispense sequences). If the sequences which this SMART Step is going to work with have been used within this method in preceding steps, the sequence counters can be reset using the two check boxes for initial sequence manipulation. If other steps are following this SMART Step, the status of the sequence which is passed back can be defined by the radion buttons under “final sequence manipulation“.

Under **channel settings**, the user may specify a channel pattern manually, as well as the number of the channel which will be used to calibrate (1536-well) carriers.

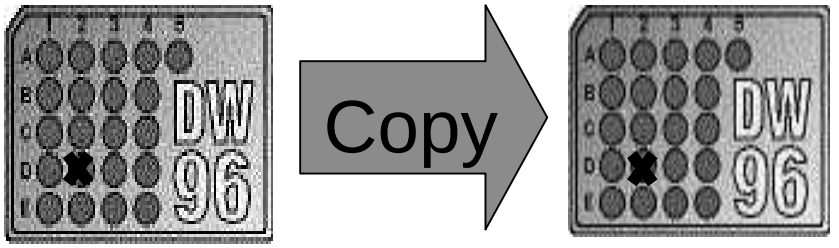
The **error settings** dialog allows you to specify an error handling approach:



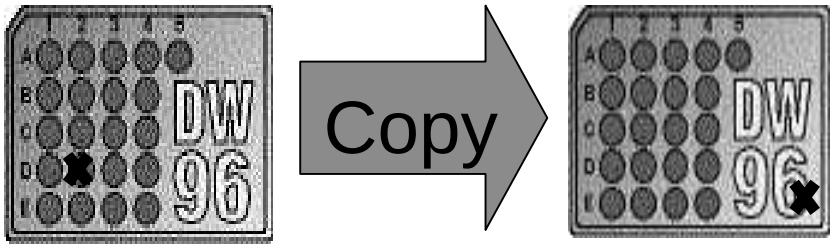
The choices in case of an error are: abort the method (only abort button on runtime error dialog); give the choice (in runtime) to abort or to cancel (continue with user-defined error handling, if programmed, otherwise abort); or use the fixed default error recovery (recommended) pre-programmed for the SMART Step. In this case two choices can be made:

- "Copy pattern" means that in case of an error on aspiration, the corresponding well of the dispense sequence will be left out (the pattern is kept). If "copy pattern" is not checked, the dispense sequence positions will all be pipetted, leaving an unpipetted well at the end of the sequence.

Copy Pattern Checked



Copy Pattern NOT Checked



- "Exclude error positions" means exclude the erroneous positions from aspiration and dispense sequences. If this option is enabled and an error occurs during an aspirate or dispense step, the erroneous position will be excluded from the sequence by removing the corresponding element from the aspirate or dispense sequence. The next time these sequences are used, the erroneous well will not be pipetted.

In addition, **walk-away** mode can be enabled. If this checkbox is checked, a timeout has to be specified, after which the error dialog on runtime will automatically close down again to continue with the selected error handling: Abort, Cancel, or default error recovery.

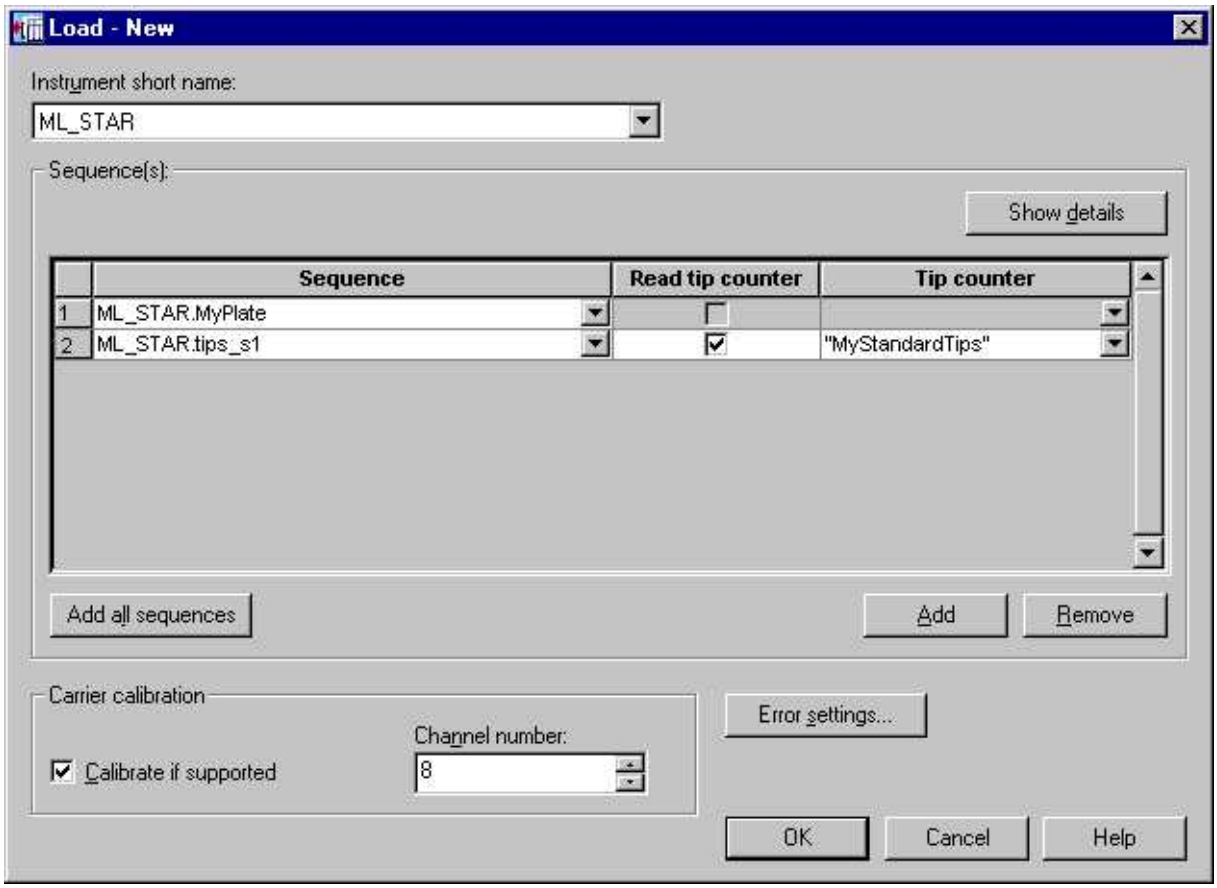
NOTE

Exception: If the option "default error recovery" is chosen and an error occurs for which the default is set to abort (e.g. a hardware error), the walk-away mode is left on and the dialog waits for user interaction. The other default settings correspond to those of the single steps (see chapter 15.4 for further explanations of walk-away error handling).

8.2.1.2 SMART Step Load/Unload

This SMART Step is used to automatically load the instrument deck with all labware necessary for the run. These commands work with autoload and manual load instruments. For a manual load instrument, the user is requested to load/unload the carriers by hand.

Dragging the **load** command to the method tree opens up a dialog box:



The procedure for loading the instrument follows logically from our concept of sequences. Whatever carriers have to be loaded to ensure that the sequence which is used within the method is available *will* be loaded. If, for example, a sequence of samples spans 3 tube carriers, all 3 carriers will be loaded automatically to their locations (tracks) as defined in the deck layout.

The dialog box has three main buttons. Their functions are:

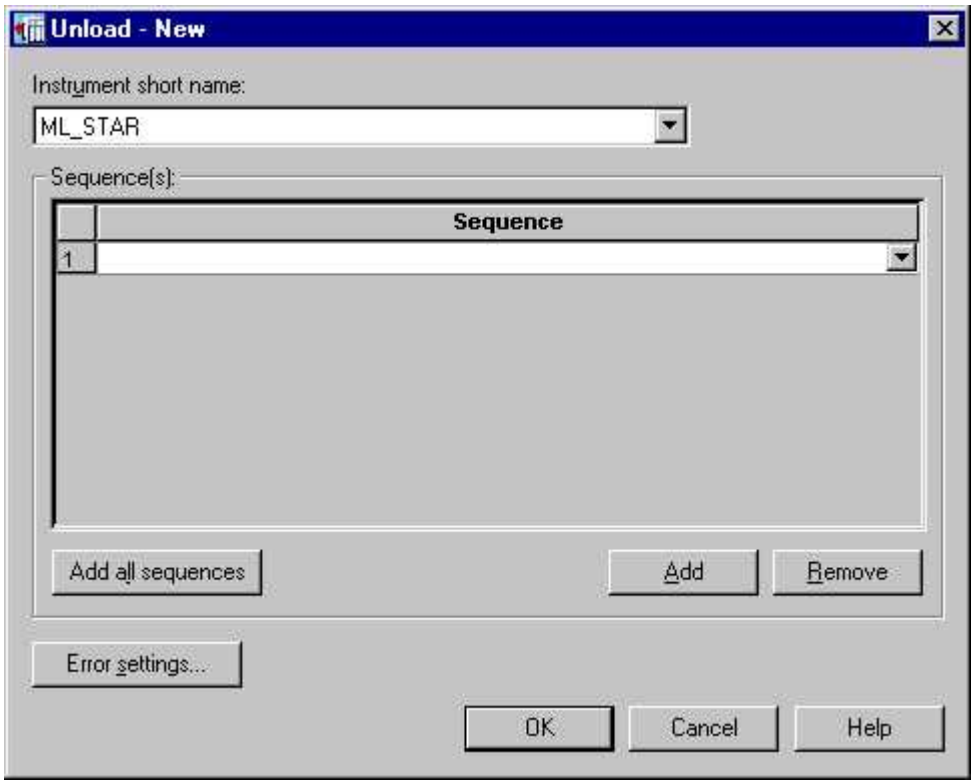
- to load all sequences to the deck,
- to add a specific sequence which can be selected from the dropdown field, and
- to remove a selected sequence.

In the case of a tip sequence, the SMART Step gives you the option to read the current position of a tip counter, which has to be specified. In this example, the tip counter “MyStandardTips” is read and the current position of the sequence is set to the value of the tip counter.

In addition, sequences may be reduced on loading (click on “show details”). If a carrier for 1536-well microplates is being loaded, it may be calibrated using the channel selected in the dropdown field.

Click on “Error Settings” to define the error handling for this step. This is similar to the error handling of the SMART Step Pipette.

To **unload** the deck, use the SMART Step Unload. It is very similar to the loading step.



Here, all carriers involved in the specified sequences are unloaded from the instrument deck.

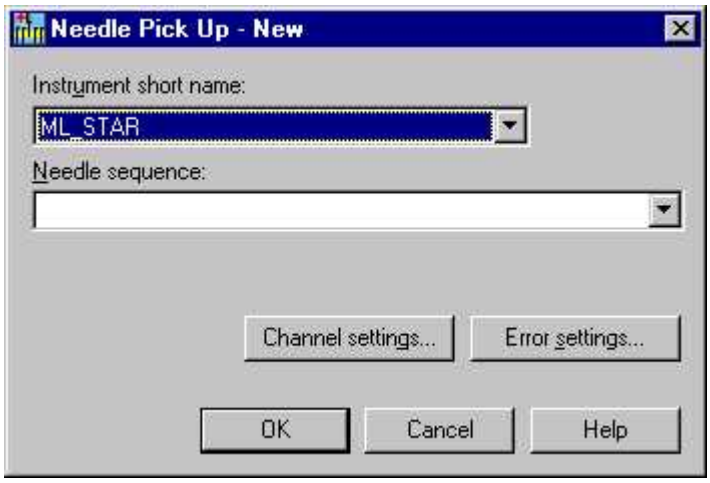
8.2.1.3 SMART Steps for Washing Needles

For the washing of needles, three SMART Steps are available. The idea is that needles can be used like disposable tips without bothering about the organization of the three wash modules of a wash station.

To do this, the **needle pick up** command

- waits until a wash module is ready,
- picks up needles from a wash station (or from a separate needle rack, which is in fact an empty tip rack filled with needles: e.g., the lowneedle_l.rck).

Dragging the command to a method tree opens the following dialog:



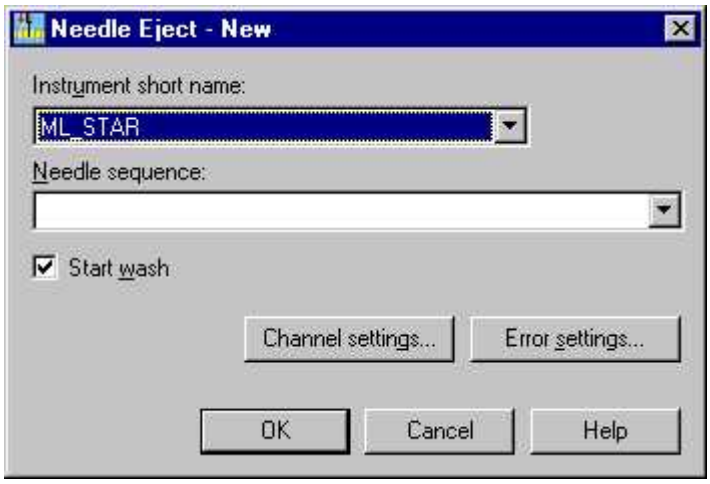
The sequence requested here is the sequence running over all (three) modules of a wash station, thus having 24 positions (8 from each module). This sequence has to be generated using the sequence editor. The SMART Steps automatically take care of handling the individual wash modules by incrementing the appropriate sequences in the background.

NOTE

For 4- and 12-channel instruments, an appropriate sequence has to be created with the sequence editor. Thus for a 4 channel instrument, the sequence should hold positions A1,A2,A3,A4,B1,B2,B3,B4,C1,C2,C3,C4, where A,B,C are the different wash modules.

The needle eject command

- places the needles into the next available wash module, and
- starts the wash process (on request).



For both commands channel patterns can be set manually. The error settings are again similar to those of the SMART Step pipette.

An additional SMART Step is available to set the wash parameters; see the section on the wash station.

8.2.1.4 SMART Steps for Picking up and Releasing Tips

Two further SMART steps allow you to pick up tips from a rack and then dispose of them in the tip waste after use.

Here is the dialog for the “pick up tips” step:

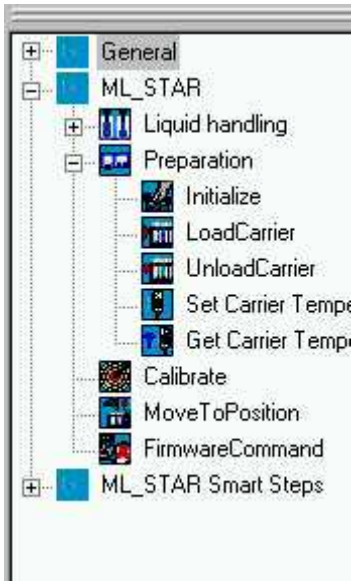
The dialog asks you to specify a tip sequence. You also have the option of specifying a tip counter.

Here is the dialog for the “tip eject” step:

For “channel settings” and “error settings”, see the description of previous SMART steps.

8.2.2 Single Steps

Now look at the *Toolbox Window*. You will find that, for each deck layout or instrument you selected, there is now an additional bar containing the instrument-specific steps.

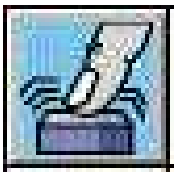
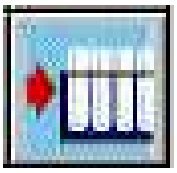
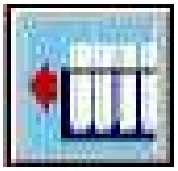
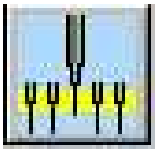
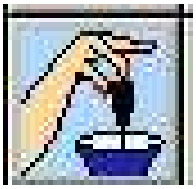
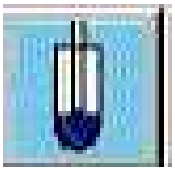


Method Editor with instrument-specific steps in two different views.

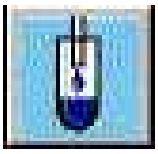
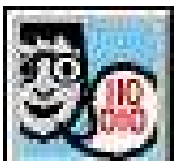
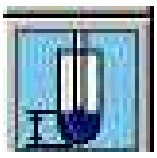
NOTE

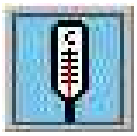
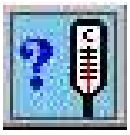
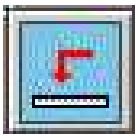
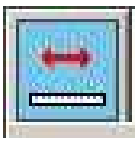
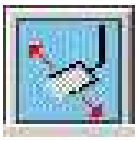
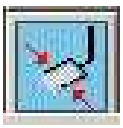
To change between the different views, right click within the tool window and select "tree view".

The table on the next two pages gives a brief outline of the available ML-STAR-specific commands. These instrument-specific single commands are used in both the Graphical and the HSL Method Editors.

Command	Icon	Action performed	Parameters to be specified
Initialize		Initializes the instrument	Waste destination, channel
Load Carrier		Loads a carrier on the deck	Carrier position[1], rack type
Unload Carrier		Removes a carrier from the deck	Carrier position
Tip Pickup		Picks up a CO-RE tip or needle	Sequence, tip type, channel
Tip Eject		Discards the tip into the tip waste or the needle into the wash station or rack	Waste destination, channel
Aspirate		Draws liquid from a container	Sequence, volume, liquid name, channel, mix settings, LLD

[1] The carrier position gives the track number on the Microlab® STAR deck where the carrier will be inserted. For microtiter plate carriers which are 6 tracks wide, the carrier position refers to the leftmost edge of the carrier.

Command	Icon	Action performed	Parameters to be specified
Dispense		Drops liquid into a container	Sequence, volume, liquid name, channel, mix settings, LLD
Calibrate 1536		Measures precise position of a high-density microtiter plate before aspirating or dispensing	Carrier position, channel
MoveTo Position		Moves the pipetting head to an absolute position, or to one relative to the current position	Mode, direction, value
Firmware Command		Sends a low-level firmware command to the instrument	Firmware command, parameter
Get last liquid level		Gets the z position of the last liquid level detection	
Start Needle Wash		Starts a needle wash module	Name of wash module
Wait for Wash		Waits for the needle wash module to be ready	Name of wash module

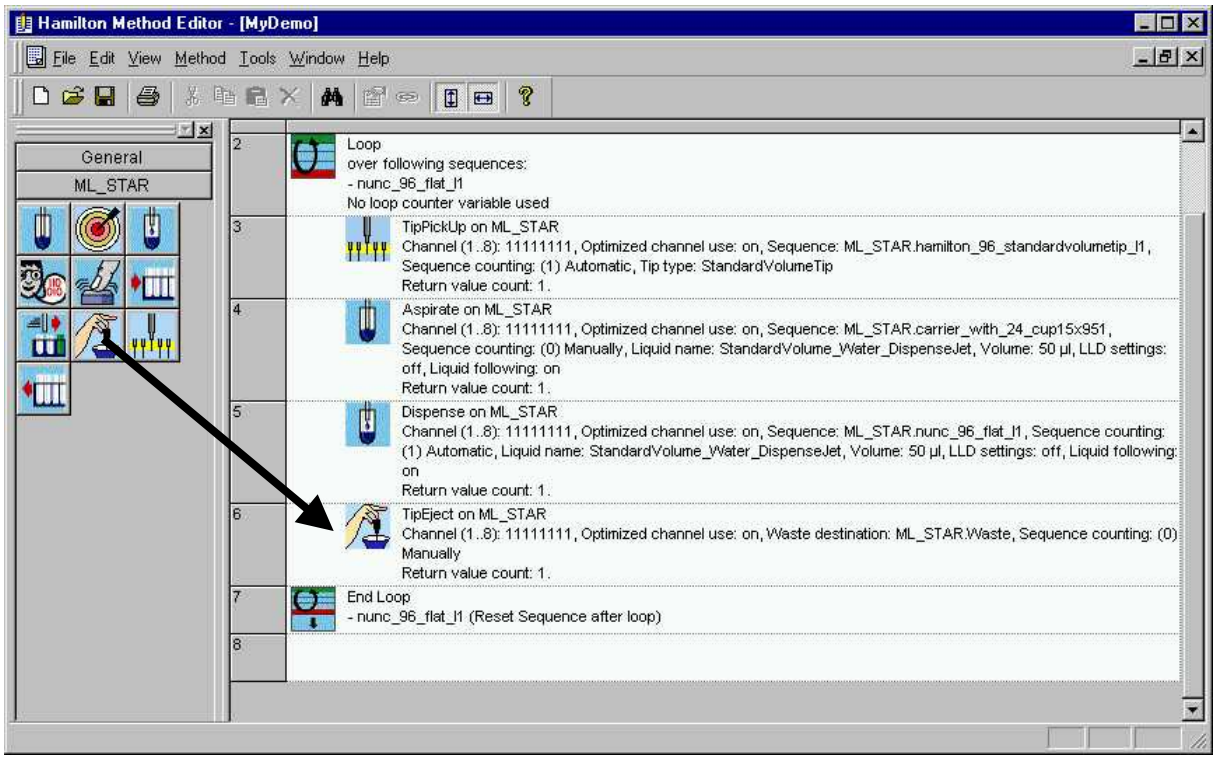
Command	Icon	Action performed	Parameters to be specified
Set Temperature		Sets the temperature of a TCC	Carrier name, temperature settings
Get Temperature		Retrieves the temperature of a TCC	Carrier name, bind return value to get temperature in °C (see chapter about binding return values)
Get Plate		Picks up a plate from the defined position	Position of plate to be located and picked up by iSWAP
Place Plate		Sets a plate down in a defined position	Movement type, transport mode, plate sequence, sequence counting, lid sequence, complex movement parameters
Move Plate		Transfers a plate to another sequence	Move to sequence
Open Gripper		Spreads the fingers of iSWAP's robotic hand	Transport mode, plate sequence, sequence counting, lid sequence
Close Gripper		Closes the fingers of iSWAP's robotic hand	Transport mode, plate sequence, sequence counting, lid sequence

NOTE

Initialization must be the first instrument-specific step in each method.

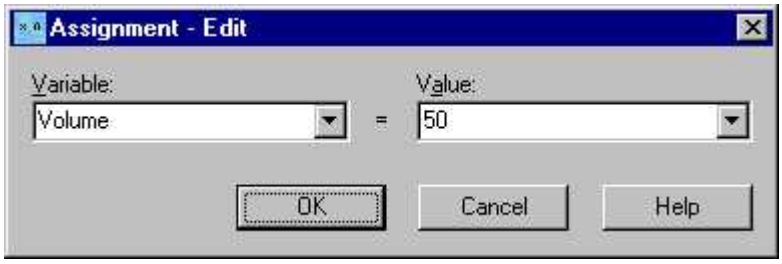
8.3 Writing a Method

You can now easily write a method by dragging icons from the toolbox on the left and dropping them into the method window on the right.



A simple method

Inserting a new command or double-clicking on an existing command opens the appropriate dialog to edit the command-specific parameters, for example the *Assignment* command, used to define and assign variables:



Assignment parameter dialog

8.4 Editing a Method

A method can be edited by the usual Windows copy-and-paste techniques. Mark the step or the block of adjacent steps within the method editor and right-click the method window to access the cut/copy/paste menu. To insert a step from the buffer, click on the line above which the step should be inserted, and then paste.

8.5 Variables

Within a method, the user may typically wish to define and process variables. The Graphical Method Editor recognizes three types of variables: integers (numbers), floats (floating point numbers), and strings (text variables).

Variables can be newly defined within the dialogs, if they occur

- on the left-hand side of an equation (e.g. x=0, or x=t+1), or
- within edit fields that hold only one variable.

If not explicitly requested (as is the case in the “Open file” dialog), the variable types are identified automatically. For example, f=5 will generate a variable f of type integer.

NOTE

To define a string (text) variable by assignment, the text must be placed within quotation marks, e.g. s=“Test“. s is then of type string*.*

Variables once defined show up in the drop-down fields of all input fields of a method.

12 predefined array variables of dynamic length are available in the array library.

For variable names, do not

- *place numbers at the beginning,*
- *use blanks or signs other than the underscore (“_”).*

Variable names are case sensitive.

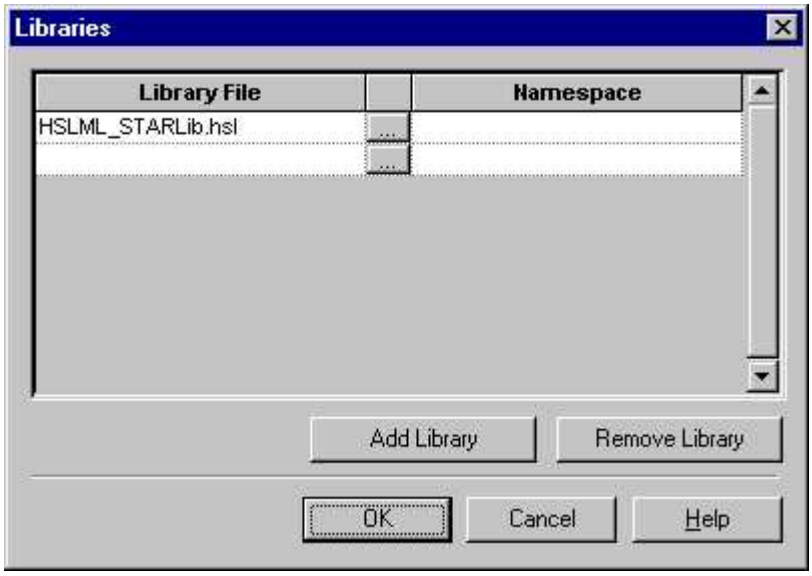
To define a path within a string variable, use the double backslash (“\\“) rather than the single backslash (“\“) - e.g. “c:\\program files\\hamilton\\methods\\“.

For calculations with variables, note that a=4, b=3 are integers. c=a/b is therefore 1 (also an integer). To get the floating point result, define a or b explicitly as a float by assigning a=4.0 an/or b=3.0.

8.6 Using Library Functions: the ML STAR Library

In addition to the toolbox elements, several low-level standard library functions are available, such as the mathematical function library. To use such functions, you have to import the appropriate library.

Open the *Libraries* dialog (menu *Methods/Libraries...*):



Libraries dialog

Click the browse button ("...") to select a library. Use this dialog to import all the libraries you need, and to remove libraries you no longer need.

This example links to the HSLML_STARLib.hsl library. This library contains some interesting functions for the Microlab STAR.

Note that if your method uses a library function, the library in question is indicated in the list of referenced libraries and can't be removed.

You can also write your own library function using the *HSL Method Editor* (see chapter 9 HSL Method Editor, page 96) and use it like any of the standard libraries.

Once you close this dialog with OK, the mathematical library functions (for example) are available under their own bar and can be invoked like standard commands. In this example, the following functions are linked and now made available within the method:

Pressure based aspiration monitoring off

Pressure based aspiration monitoring on

Capacitance based clot detection off

Capacitance based clot detection on (the clot heigth must be specified in the liquid class)

Measure container volume with n channels (auto-increment 1=yes, 0=no), if pattern is reduced (e.g. 11000111), all sequence positions are used

Get container volume of i^{th} channel in ml, as measured in previous „Measure Container Volume“ Step.

Set threshold for pressure based clot detection.

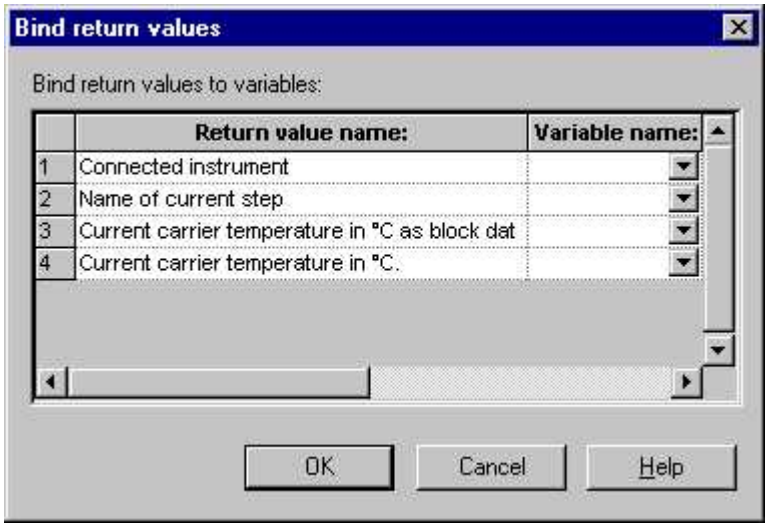
Additional functions available include the generation of a Microlab AT-like barcode file, the execution of a Gemini worklist, the formatting of an ML-STAR barcode file, and the deletion of an ML-STAR barcode file. Refer to the online help for more information on these functions.

The libraries available include:

Library	*This library function handles*
HSLArrLib	array variables
HSLDevLib	devices
HSLDlgLib	dialogs
HSLErrLib	errors
HSLFilLib	files
HSLMthLib	mathematical functions
HSLSeqLib	sequences
HSLStrLib	string variables
HSLTimLib	timer
HSLTrcLib	trace files

8.7 Binding Instrument Step Return Values

If an instrument step returns information, it can be bound to a variable by opening the *Bind return values* dialog (select step of interest and select menu *Edit/Bind return values...*). For example, the get temperature step of the temperature-controlled carrier returns the temperature and other information:



Bind Return Values dialog

These pieces of §information can be stored in a variable for further processing or output. The dialog generally lists the return values, each with an editable field to define the variable the return value is to be bound to.

8.8 Working with Worklists

8.8.1 File Formats

Frequently, you will want to import and export worklists. The “open file” command of the Method Editor is designed to enable such file handling. The data source type (format) is identified from the extension of the worklist’s file name. The editor supports:

Microsoft Excel	.xls
Microsoft Access Database	.mdb
Text Files	.txt, .csv, .tab, .asc

No other extensions are supported.

8.8.2 Worklist Handling with Microsoft Excel

You can specify a subset of the available data when you first open a Microsoft Excel workbook. In a workbook file, you can open a single worksheet, a named range anywhere in the workbook, or an unnamed range in a single worksheet.

The following table lists the conventions for these settings.

To open this object to read...	...use this syntax
Entire worksheet in a workbook file	Specify the sheet as *sheetname***$**, where *sheetname* is the name of the worksheet. **Important:** You must end the worksheet name with a dollar sign ($).
Named range of cells in a worksheet or workbook file	Specify the named range as *NamedRange*, where *NamedRange* is the name you assigned to the range in Microsoft Excel. **Important:** You must name the range in Microsoft Excel before attempting to open it.
Unnamed range of cells in a single worksheet in a workbook file	Specify the sheet you want to open as *sheetname***$** and the range as *FirstCellInRange***:***LastCellInRange*. For example, to access cells A1 through Z256 in a worksheet called Sales, you would specify `Sales$A1:Z256`.
To open this object to write	**...use this syntax**
Entire worksheet in a workbook file	Specify the sheet as *sheetname*, where *sheetname* is the name of the worksheet. **Important:** You must **not** end the worksheet name with a dollar sign ($).
To append to an object	**...use this syntax**
Append to an **existing** worksheet in a workbook file	Specify the sheet as *sheetname***$**, where *sheetname* is the name of the worksheet. **Important:** You must follow the worksheet name with a dollar sign ($).
Append to a **not yet existing** worksheet in a workbook file	Specify the sheet as *sheetname*, where *sheetname* is the name of the worksheet. **Important:** You must **not** follow the worksheet name with a dollar sign ($).

NOTE

You cannot specify a value in a range that exceeds the maximum number of rows, columns, or sheets for the worksheet or workbook. For more information on these values, see your Microsoft Excel documentation.

The format of all entries within one column of the excel file must be identical: text, number, etc.

8.9 Writing Batch-type Methods for a 16-Channel Microlab STAR

As discussed in chapter 3.2.1, the 16-channel Microlab STAR is intended to be used as a batch-like processor. This means all 16 channels should aspirate and dispense simultaneously, to allow maximum parallelization and highest pipetting speed. In this case, the reduced random access space of a 16-channel Microlab STAR does not cause any problems.

To achieve a batch-like process, all sequences involved must consist of blocks of at least 8, or better 16 positions. Sequence positions within a block must have

- the same x-positions, and
- decreasing y-positions from channel 1 (rear-most) to 16 (front-most).

To program a method using single steps

- Select the option “keep pattern” within the “Channel Settings” dialog in all single steps (tip pick-up, tip eject, aspirate, dispense). Do **not** use the option “all sequence positions”.
- Even if an error occurs and “Exclude Channel” is selected as an error recovery, the pattern will be kept.

To program a method using SMART Steps

- Select “Copy Pattern”
- Do **not** select “Exclude Erroneous Positions”.
- If sequences are reloadable (in runtime during the pipette command), **deselect** the option “reducible by user” to maintain the original block-wise structure of the sequences.

9 HSL Method Editor

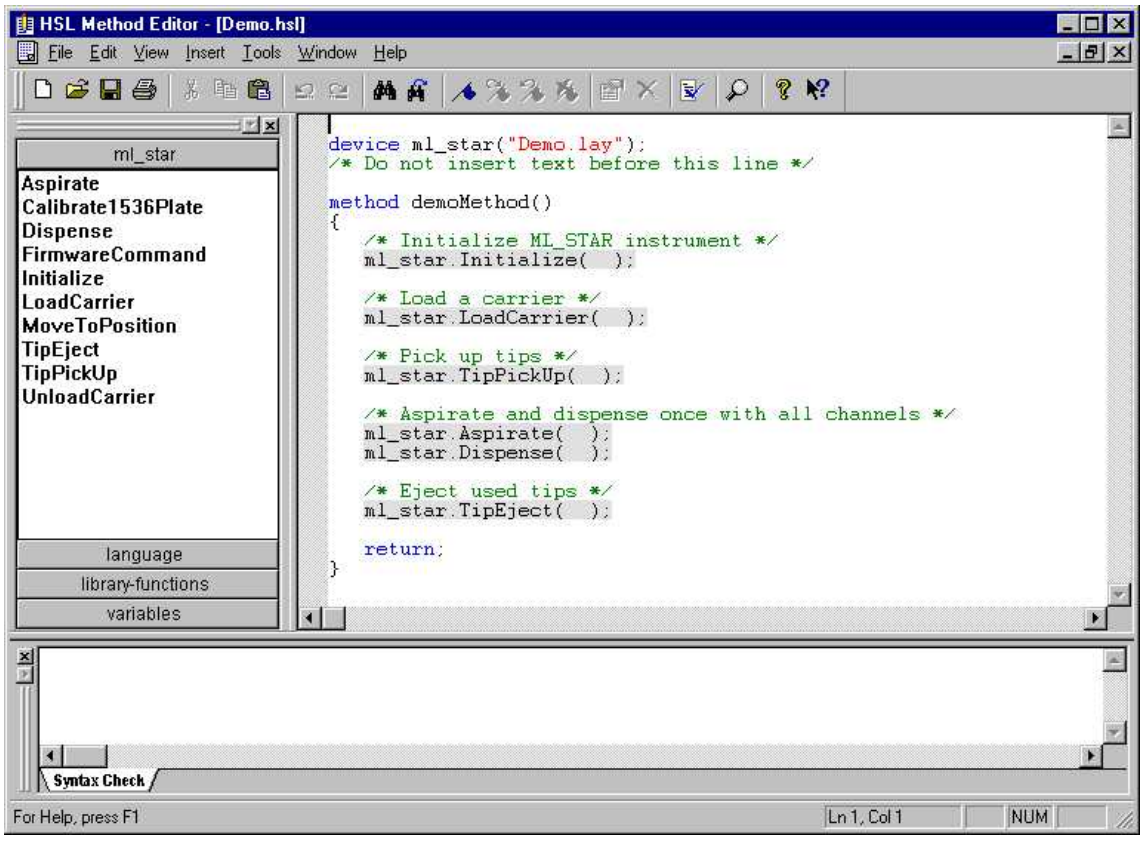
The HSL method editor gives the user access to the underlying HSL code, offering higher flexibility for programming methods. Usually this method editor is used only to customize methods written with the graphical method editor. Of course, a complete method can also be written using the HSL method editor.

The *HSL Method Editor* is a general-purpose text-like editor that can be used to create methods based on *HSL* (*HAMILTON Standard Language*) for any instrument system. It is a sub-component of the User Software. Parameters for instrument steps are entered using dialog boxes, and may include variables. The editor makes use of the same instrument-specific steps as the graphical method editor. It allows methods to be created, saved, modified and printed.

To use the *HSL Method Editor*

- Open Windows Explorer
- Go to folder \Program files\Hamilton\Bin
- Create a shortcut to the executable file named HxHSLMetEd.exe on the desktop.

Use the *HSL Method Editor* shortcut you have placed on the desktop to start it. Then use the *New* menu to create a new method based on an existing deck layout, or open a previously written method by means of the File->Open menu.



HSL Method Editor

The *HSL Method Editor* window is divided into 3 sections:

The ***Method View*** on the right is a general-purpose text-like editor window containing the method written in *HSL*. The general syntax used in HSL is the C/C++ notation.

Instrument steps are shown with a gray background. For safety reasons it is not possible to change or type in these commands directly. Double-clicking an instrument step displays a step dialog where you can change specific parameters. To see all step parameters in the *Method View*, use *View All Stepdata* in the *View* menu.

The ***Toolbox Window*** on the left offers various elements that can be used to build the method, grouped by the following bars:

Bar name	Contents
ML_STAR *(as example of a device)*	Each device defined in the method shows its own instrument bar containing all the steps the instrument can execute.
language	Contains the common *HSL* language elements such as *if-else*, *loop*, *function call*, etc.
library function	A set of standard library functions such as mathematical functions, trace functions, string manipulation functions etc.
variables	Different variable types.

All these elements can be used by dragging them from the appropriate bar and dropping them into the *Method View*, or by using the *Insert* menu. Alternatively, language elements, library functions and variables (but not instrument steps) can also be typed directly into the *Method View*.

When an instrument step is inserted, the appropriate step dialog is displayed so you can define the specific parameters needed.

After writing a method, invoke Syntax Check from the *Tools* menu to check your method.

The ***Syntax Check Window*** at the bottom shows the results of the syntax check, if performed. If any error is listed in this view, double-clicking on it will set the cursor to the incorrect statement in the *Method View*.

If the HSL method editor is used to edit a method that was written with the graphical method editor, insert the HSL code only between two adjacent comment lines. Leave the comment lines and all code in between unchanged. Save your changes and close the HSL method editor. If this method is reopened with the graphical method editor, your changes will be visible as a greyed-out, unchangeable HSL code block.

10 Run Control

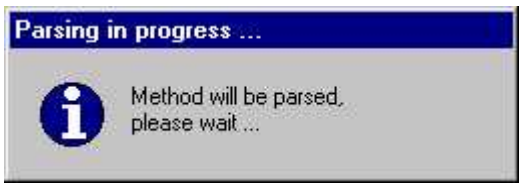
Run Control enables you to execute your methods.

During execution of a method, the instrument steps are automatically logged and displayed in the log window as well as written in a trace file with the same name as the relevant layout file. The Run Screen shows a static view of the deck layout. Because there is an open n:n relation between methods and deck layouts (see chapter 4), the Run Control may start either deck layouts (*.lay), or methods (*.hsl). A further alternative is to start linked methods (*.cmt).

The run control may be accessed by the shortcut buttons of deck layout or method editor.

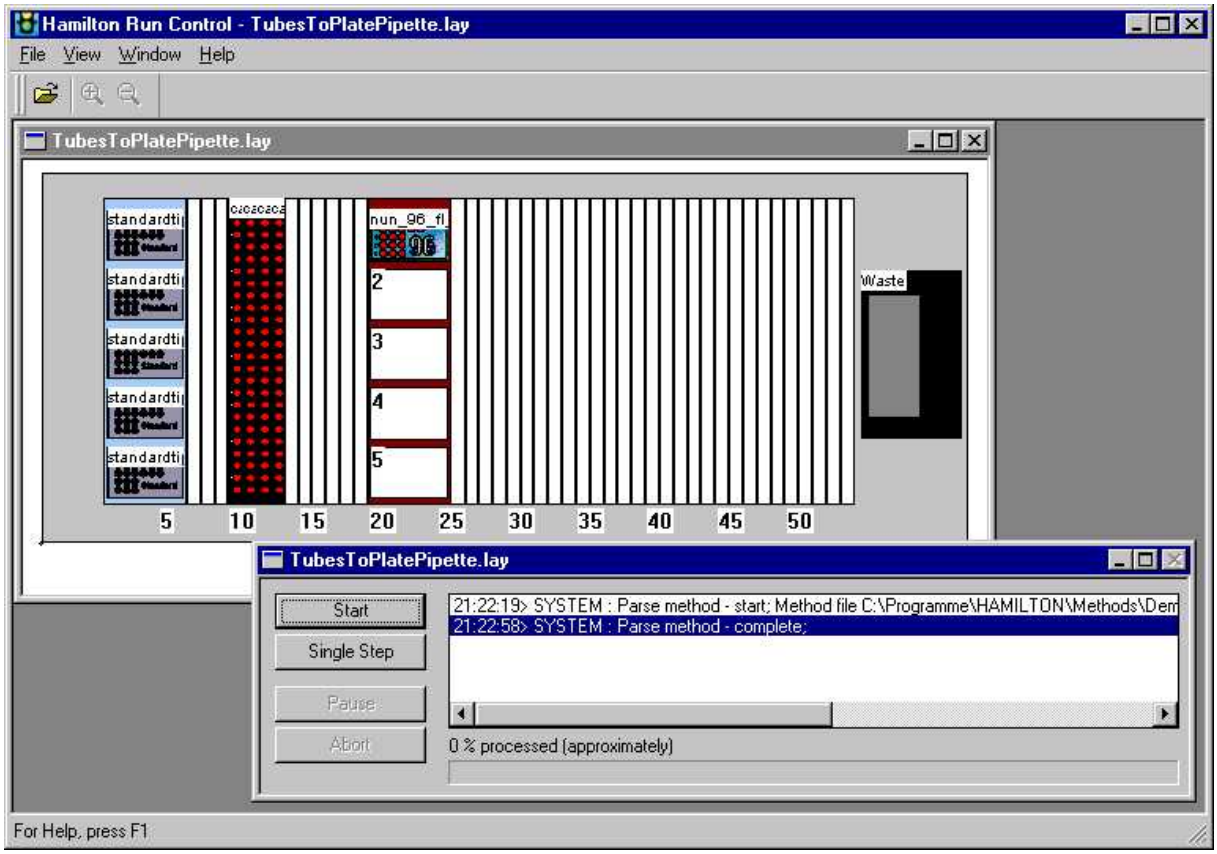
Then, the method of interest is automatically loaded into the Run Control. Alternatively, the Run Control may be started by double-clicking the “Microlab STAR Run ” shortcut on the desktop. Then, the method (or the deck layout) of interest must be opened from the run control by selecting File->Open from the menu.

On opening the method, a dialog box appears for a period of time, informing the user that the method is being parsed, i.e., analyzed. This procedure may take up to a minute, depending on the size of the method.



Parsing a method

After loading and parsing a method you can use the *Start* button to execute it.



A method ready to run

Each executed instrument step is logged in the log window.

The choices within Run Control are

- To start a method
- To abort a method
- To use single steps (system pausing after each single step)
- To pause a method (to be continued later)

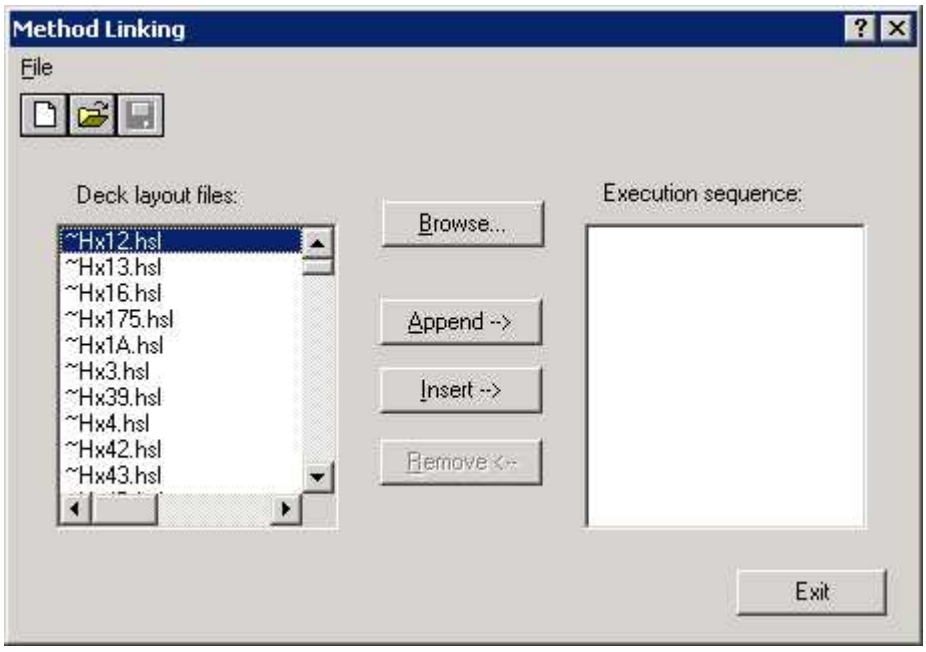
Clicking on the deck layout view of the Microlab STAR within Run Control adds the Tools menu to the menu list. Here you will find shortcuts to access the configuration and liquid editor. The configuration editor is used to switch between simulation and instrument mode.

NOTE

To simulate a run, always set the instrument configuration to autoload (even for a manual load instrument). Don't forget to set the configuration back to manual load when performing a run with a manual load instrument.

10.1 Method Linking

The Microlab STAR user software allows you to link different methods together prior to starting the merged or linked run. To do so, open the Deck Layout Editor and select “File->Method Linking”. A dialog appears:



Select the first method of interest and click on the buttons to add the method to the list on the right-hand side. It is recommended to select HSL files (.hsl) rather than layout files (.lay) or method files (.med).

Select File->Save to generate a chained method file (.cmt). Close the dialog by clicking on exit. Close the deck layout editor. Open the Run Control by double-clicking the shortcut on the desktop. Open the newly created .cmt file and execute it.

11 The Microlab® STAR Liquid Editor

11.1 Concept of Liquid Classes

As mentioned in chapter 2 The Art of Pipetting, page 12, the background parameters for pipetting are managed using liquid classes. A liquid class is a set of parameters specifying the aspiration and dispense behavior appropriate for a given liquid (e.g. water, DMSO,....). In all aspirating and dispensing steps, a valid liquid class must be selected.

Standard liquid classes will be supplied along with the User Software (Water, DMSO, Glycerine, Precinorm). The standard liquid classes cover a wide range of applications, and you will probably not need to make any changes to the parameter settings.

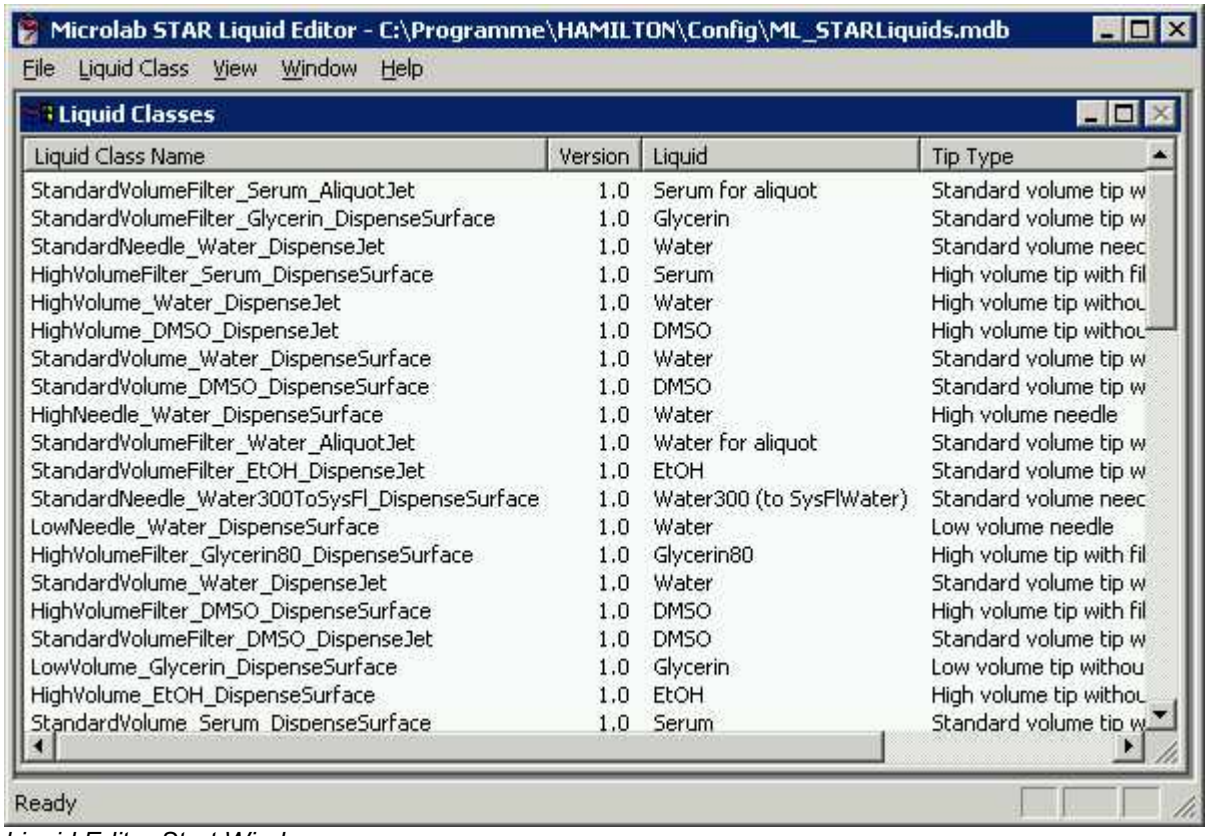
For special applications, you can define your own liquid class. This custom liquid class can be used just like the pre-defined classes.

Defining liquid classes independently serves to simplify the steps in the method and allows the complete set of parameters to be defined once for all pipetting tasks.

To define a custom liquid class, and to display the parameters of the liquid classes defined, the Liquid Editor should be used.

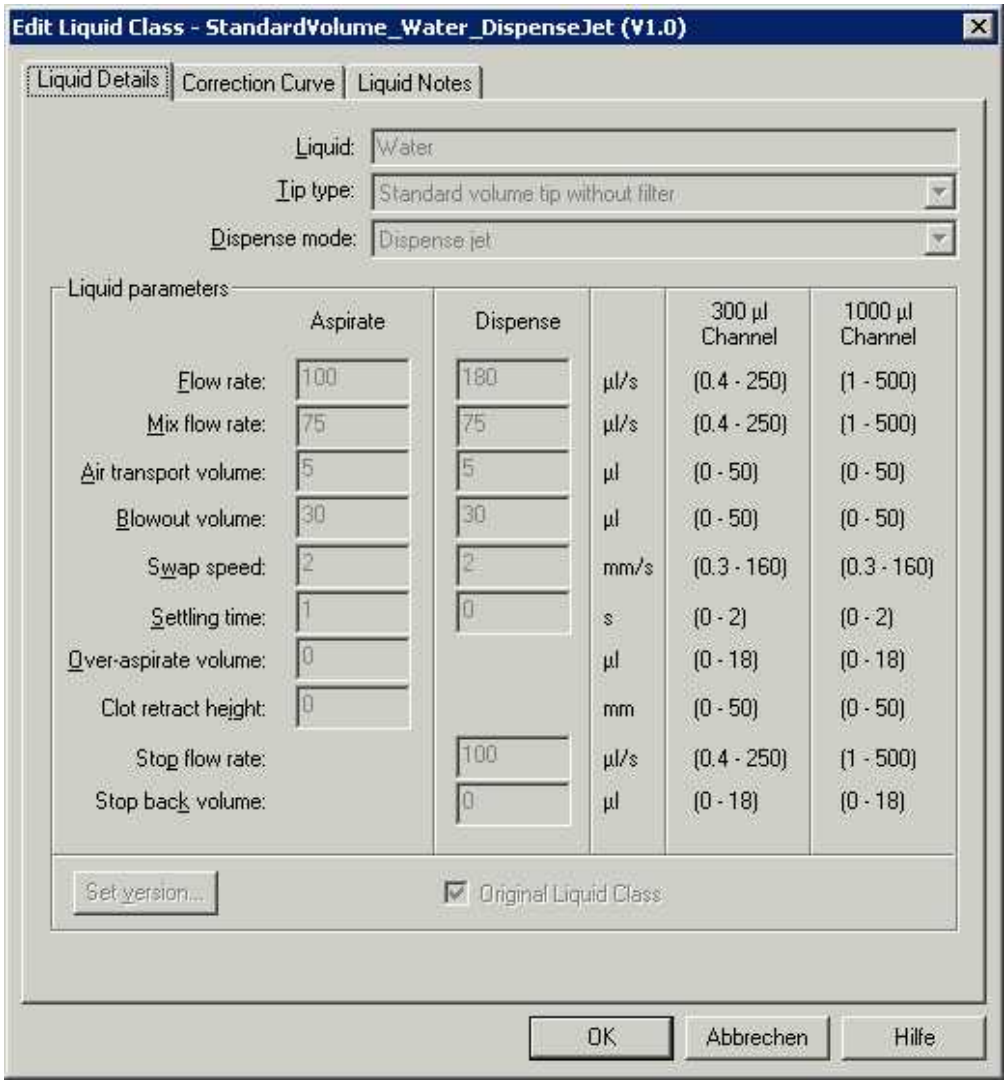
11.2 Editing Liquid Details

To start the Liquid Editor, select "ML_STAR Liquid Editor" from the Tools menu of the Deck Layout Editor. The Liquid Editor start window is displayed:



Liquid Editor Start Window

Select for example “StandardVolume_Water_DispenseJet”. Click Edit to open the Liquid Details window and go to the Liquid Details tab:



Liquid Details Window

The Liquid Details tab has two sections. At the top, the attributes of the liquid class are shown: Liquid Name, tip type, dispense mode.

NOTE
A liquid class is valid only for the defined set of attributes.

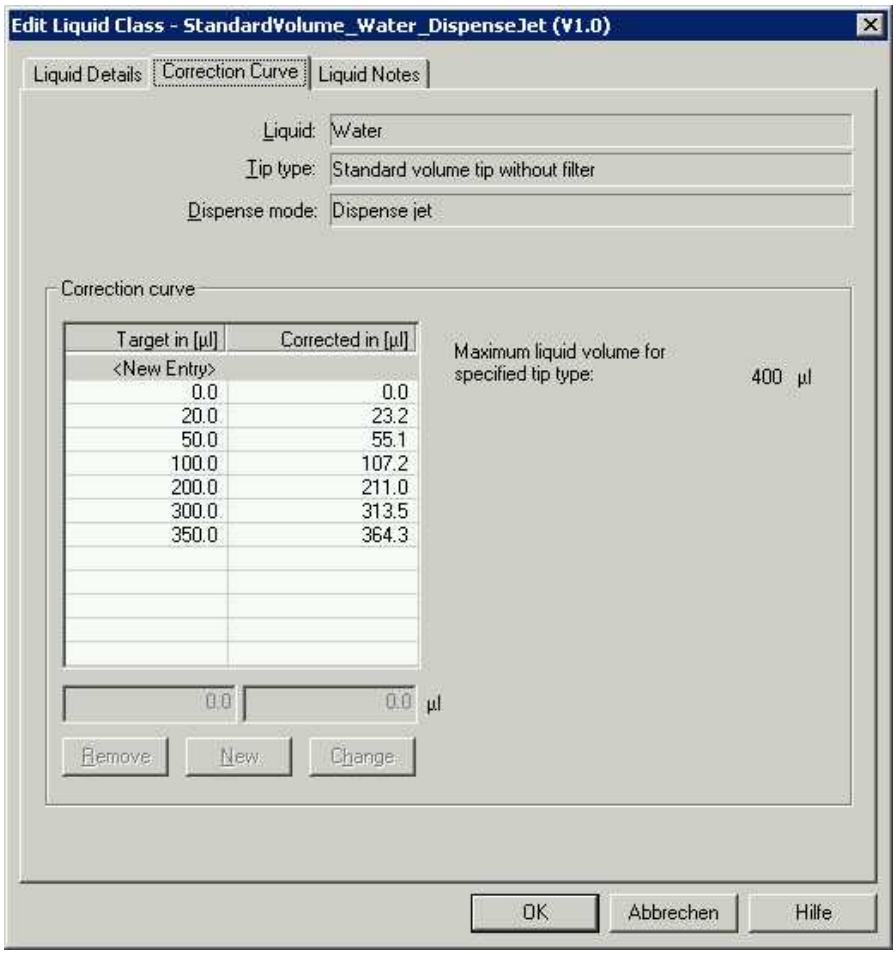
The liquid parameters section on the left specifies the appropriate instrument parameters for aspirating and dispensing.

Here is what the various parameters mean:

- **“Flow rates”** and **“Mix flow rates”** are volume flows of liquid in µl/s; they correspond to plunger speeds for aspirating, dispensing and mixing.
- **“Air transport volume”:** air for transport is aspirated at the end of the aspirate step and automatically dispensed again as an extra volume in the first part of the dispense step.
- **“Blowout volume”:** blow-out air is taken up first during aspiration, if dispensing will later be done using the “empty tip” dispense mode. In the dispense step, the entire volume including blow-out air is dispensed.

- **"Swap speed"** is the speed at which the dispensing head is drawn up out of the liquid.
- **"Settling time"** is the time the dispensing head has to wait in the liquid after aspiration/dispensing until it begins to withdraw.
- **"Over-aspirate volume"** is a kind of pre-wetting volume: on aspirating e.g. 20µl of liquid, first more than 20µl is aspirated (20µl+Over Asp. Vol.), so as to pre-wet the tip. Then this volume is immediately dispensed again (still in the aspirate step).
- **"Clot retract height":** a parameter for recognizing blood clots, which determines how high the dispensing head is allowed to travel up out of the liquid if there is a residual cLLD signal after aspiration. It is measured in mm from the height of the liquid surface upwards. If this distance is exceeded, an error message is generated.
- **"Stop flow rate":** dispensing speed of the plunger (expressed as a stream of liquid volume in µl/s), at which the dispensing step terminates abruptly. If "dispense flow rate" is equal to "stop flow rate", the dispense breaks off abruptly after dispensing the volume without slowing down beforehand. If "Stop flow rate" is equal to zero, the plunger movement becomes gradually slower during the dispense until it stops.
- **"Stop back volume":** volume which is aspirated again immediately after the dispense (as part of the dispense step). This volume is aspirated automatically as quickly as possible.

The next tab defines the correction curve:



A correction curve has a target volume and a corrected volume. The “target volume“ is the volume to be dispensed. The “corrected volume“ is the volume (plunger travelling distance x diameter) that actually needs to be aspirated for this purpose. In aspirate or dispense steps, the “target volume“ which will actually be dispensed into the vessel must be entered. The corrected volumes are usually determined gravimetrically. Accordingly, a corrected volume of 105 µl does not mean that 105 µl of liquid will be dispensed. When the tip is emptied, 100 µl are dispensed. The correction is mainly due to the properties of the air column above the liquid.

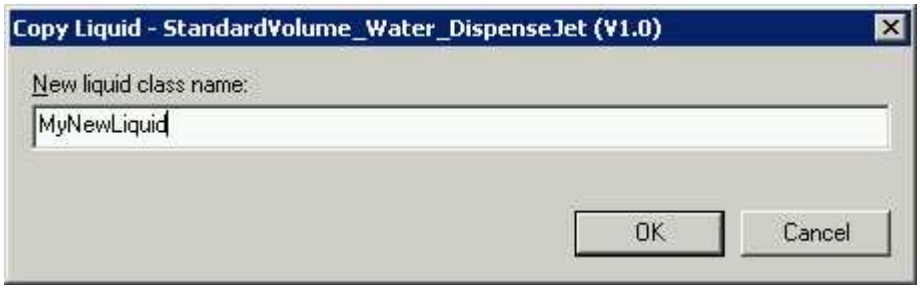
The definition of liquid classes allows you to pipette any given liquid with high accuracy. Liquid classes are also available from HAMILTON’s Application Engineering Group for custom purposes, if you need them.

NOTE

The standard liquid classes supplied with the instrument have underscores (_) in their file names. These liquid classes cannot be changed by the user. They can be copied, saved under a different name and then edited.

11.3 Defining a Custom Liquid Class

To define a custom liquid class, select a pre-defined liquid in the Liquid Editor start window and click Create. The Copy Liquid window pops up:



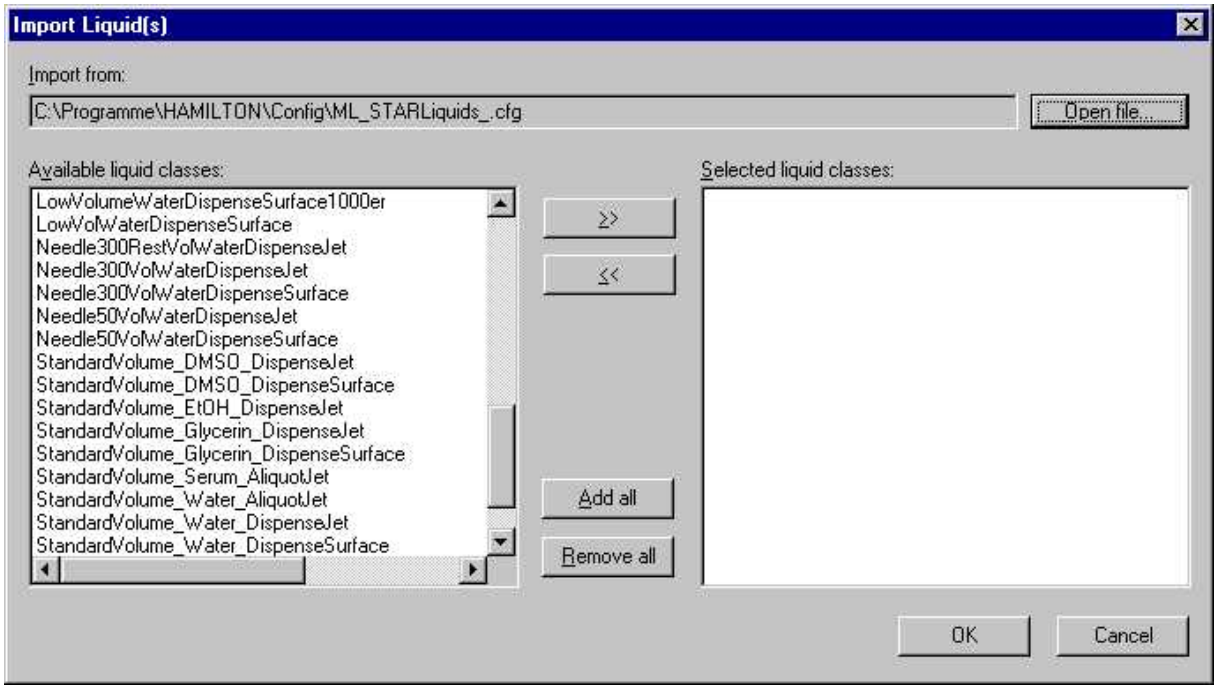
Copy Liquid window

Type in the name of the new custom liquid class and click OK.

Back in the Liquid Editor start window, select your new liquid class and click Edit. Now all the parameter input fields in the Liquid Details window are active, so you can make whatever changes you require.

11.4 Importing and Exporting Liquids

A liquid is stored in a configuration file (.cfg). Opening the Liquid Editor loads liquids defined in the standard liquid configuration file (...\Hamilton\Config\ML_STARLiquids.cfg). If other liquids are needed from another liquid configuration file, they can be imported. Click Import liquid(s)... in the Liquid Editor start window to display the Import Liquid(s) window:



Import Liquid(s) Window

Open the desired liquid configuration file using the Open file... button. Select the required liquids. Close the dialog with OK to import all the liquids selected.

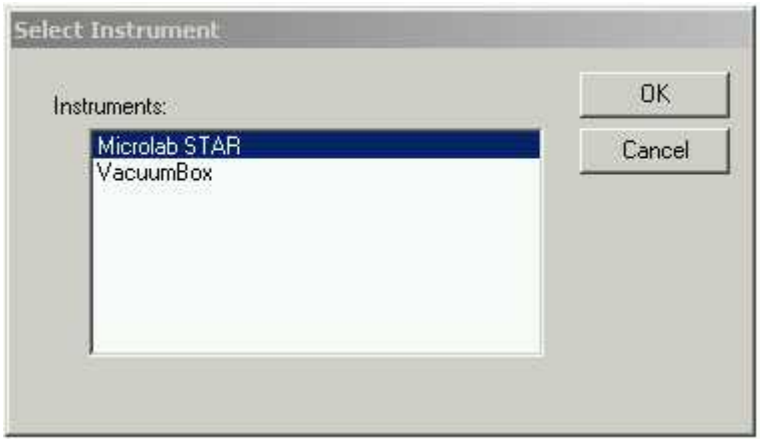
In addition, a similar dialog is available for exporting liquid classes to configuration files. This dialog is accessed by clicking on Export.

12 Microlab AVS – Automated Vacuum System

12.1 Integration of the AVS

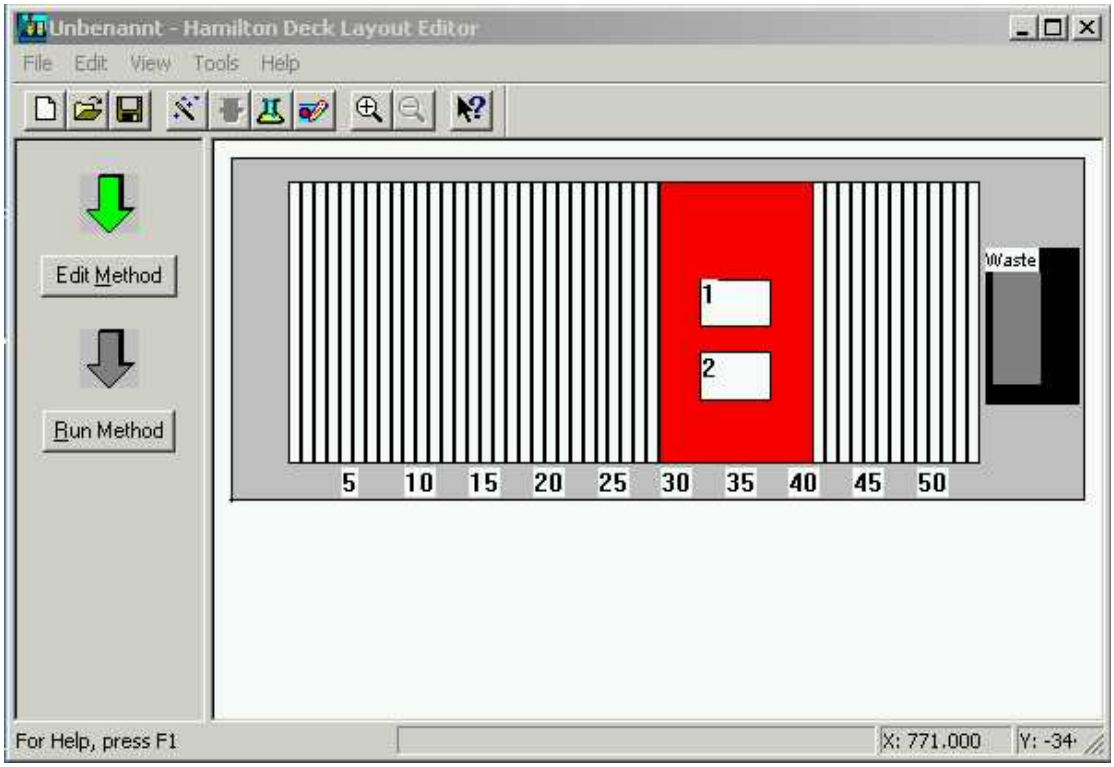
The Microlab AVS is a separate software component that comes with the vacuum box. It has to be installed separately. For convenience, you may wish to add the vacuum box permanently to the deck layout.

The following example describes the programming of the Automated Vacuum System. As a first step to writing the method, a deck layout with an integrated vacuum box has to be created. Therefore, open the Hamilton Deck Layout Editor window and select “New“ from the “File“ menu. This window appears:



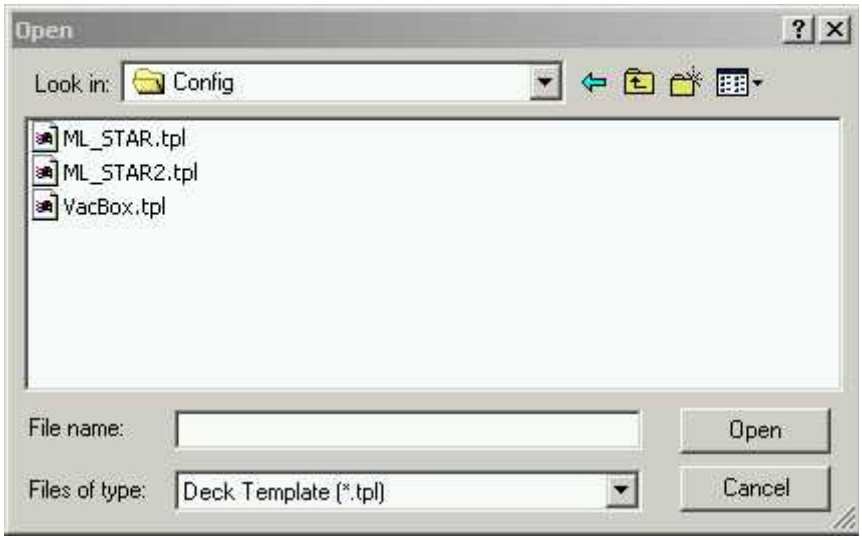
Select the Microlab STAR instrument and click OK. An “empty“ STAR deck layout will appear.

You have now to select the vacuum box under “Add labware..“ from the labware folder “VacBox“ under “MLStar“ and put it in the right place on the deck (track 31 for first vacuum box).



To add a filter plate on the conditioning site of the vacuum box, double-click on position 2 of the vacuum box and select the right filter plate. The same filter plate can be selected for the elution site of the vacuum box on position 1. On the same STAR deck layout, all required carriers can be added with the “Add Labware..“ command under the “Edit“ menu. Finally, select “Save“ from the “File“ menu to save your deck layout with the integrated vacuum box.

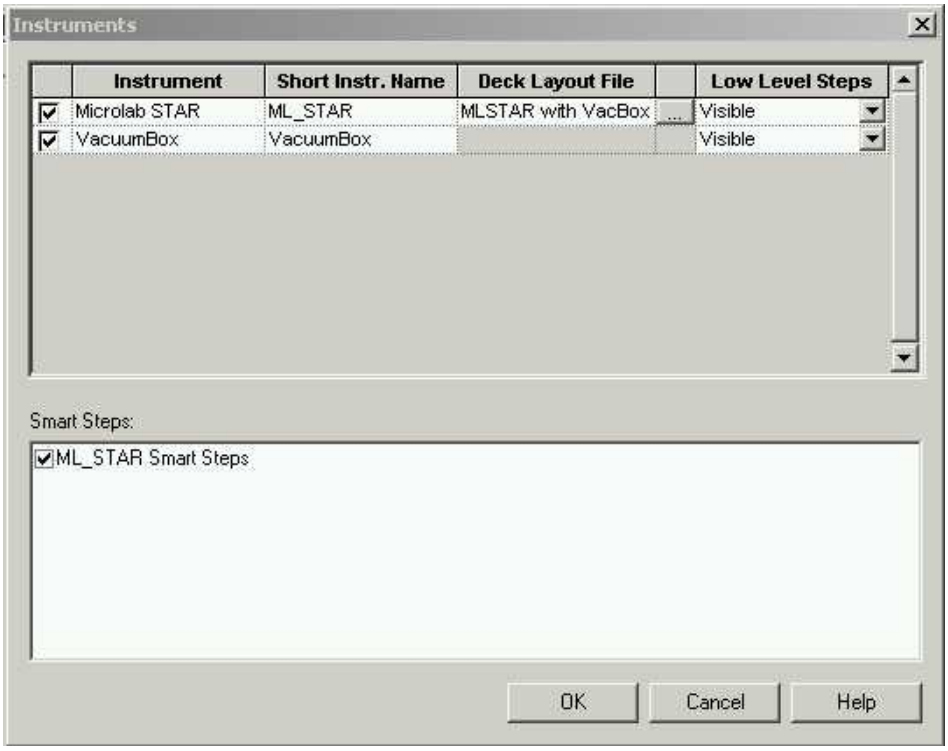
There is also a possibility to permanently change the default STAR deck layout, which might be helpful sometimes. This can be done by modifying the configuration. To integrate the vacuum box in the configuration of the ML STAR, you have to do the following: open the Hamilton Deck Layout Editor window and select “Open“ from the “File“ menu. Then open the “Config“ folder and change the “Files of type:“ to “Deck Template“.



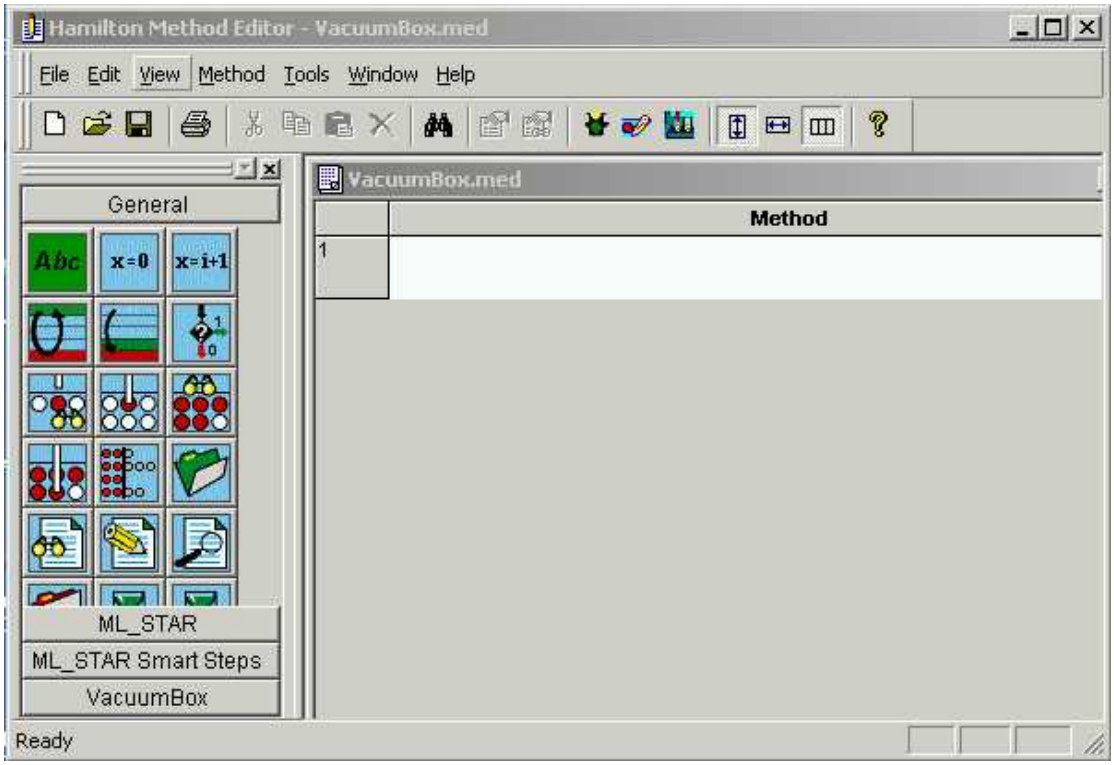
From the three different available deck layouts select the „ML_STAR2.tpl“ and open it. Select the vacuum box under „Add labware…“ and put it on the right place on the deck. Select “File“ and click “Save“. Your default Microlab STAR deck layout is now changed. Whenever you open a new STAR deck layout, the vacuum box will already be placed on the deck.

Close the Hamilton Deck Layout Editor and open the Hamilton Method Editor. Select “New“ under the “File“ menu and save your method with a new name. Click on “Instruments…“ under the “Method“ menu.

Click on the grey Deck Layout File button associated with your Microlab STAR instrument. A list of deck layouts will appear. Select the Microlab STAR deck layout you wish to associate with this method. The Layout window reappears with the selected layout file showing. Afterwards select the „VacuumBox“ instrument in order to get all the specific vacuum box commands.



After clicking „OK“, three new sets of commands are now accessible via command bars in the lower left of the Hamilton Method Editor window, namely the Microlab STAR-specific commands „ML_STAR“ and „ML_STAR Smart Steps“ and the vacuum-box-specific command „VacuumBox“.

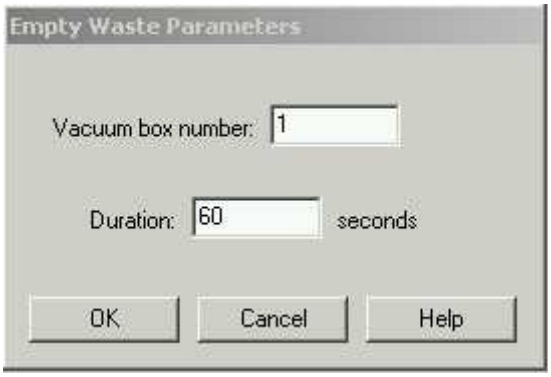


12.2 Commands for the AVS

There are three commands associated with running the vacuum box. These are in alphabetical order and are described below.

Empty waste command:

Empty waste



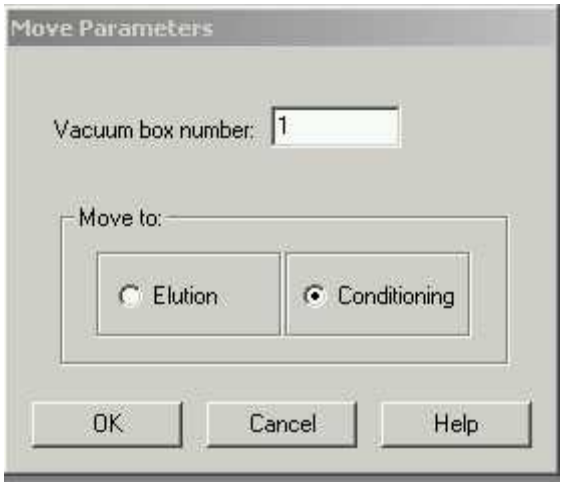
This command turns on the waste pump inside the vacuum box controller so that the waste tray in the conditioning chamber can be evacuated. Select the vacuum box to evacuate. Vacuum box number 1 is the default. The pump duration will need to be determined by trial and error. Generally, 25 seconds are adequate for emptying a full waste tray (200 ml). If the waste bottle is full when this command is running, an error dialog box will appear. The user then can empty the waste bottle and continue the program. Variables can be used for the vacuum box number and duration of pumping action.

NOTE

The capacity of the waste tray is 200ml. Program your methods to activate the vacuum box waste pump before the capacity is reached, so that this tray does not overflow.

Move command:

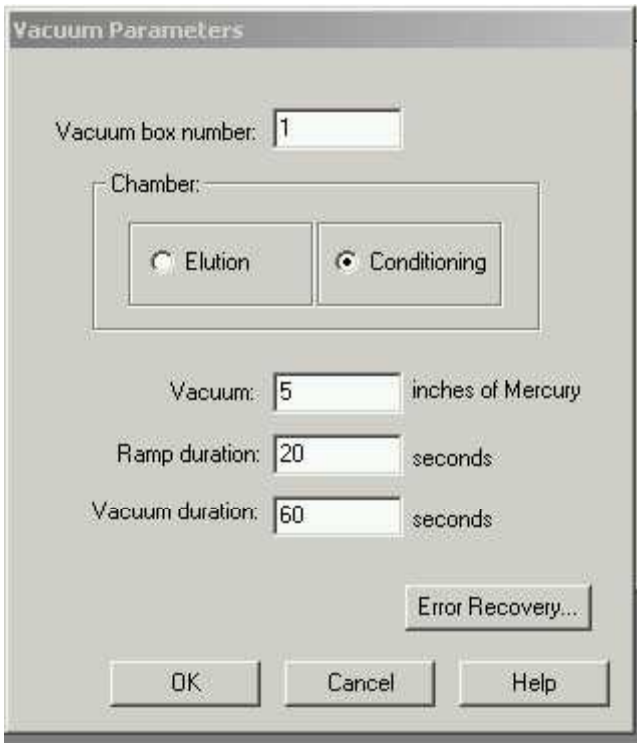
Move



This command moves the carriage from one chamber to the other. Select the chamber over which the carriage is to be moved. The conditioning chamber is in front and the elution chamber in rear. If the vacuum box is not yet initialized when it receives its first “Move“ command, it will initialize first and then move the carriage to the desired chamber. If it is already at the position specified in the “Move“ command, the carriage will not move.

Vacuum command:

Vacuum



This command runs the vacuum pump inside the vacuum box controller. The vacuum box number and the chamber have to be selected.

There are three vacuum parameters to be defined:

The vacuum, in inches of mercury (Hg) (1 inch Hg = 33.86 mbar), is dependent upon your protocol and the limits set by the filter plate manufacturer. The ramp duration is the time needed to reach the desired vacuum. This value may need to be determined by trial and error. The vacuum duration is the length of time that the desired vacuum is to be applied.

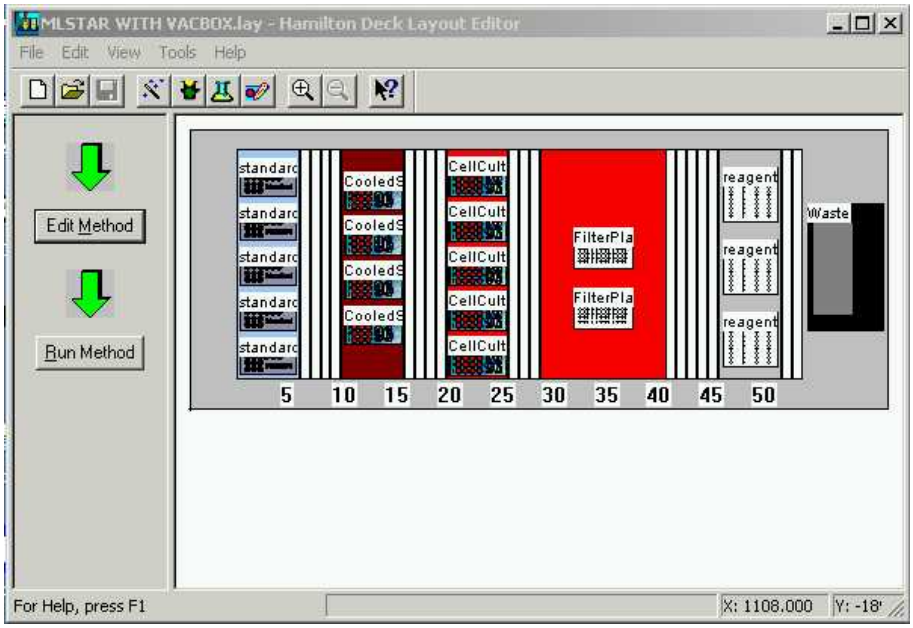
Variables can be used for Vacuum box number, Ramp duration, Vacuum duration and Vacuum. The vacuum pump will operate for a time equal to the ramp duration plus the vacuum duration.

In the event that the desired vacuum is not achieved during the ramp period, an error will appear during run time. Automatic error recovery is possible by defining recovery options in the method. If you click the “Error Recovery…“ button in the Vacuum Parameters dialog box, the “Vacuum Error Recovery“ window appears.

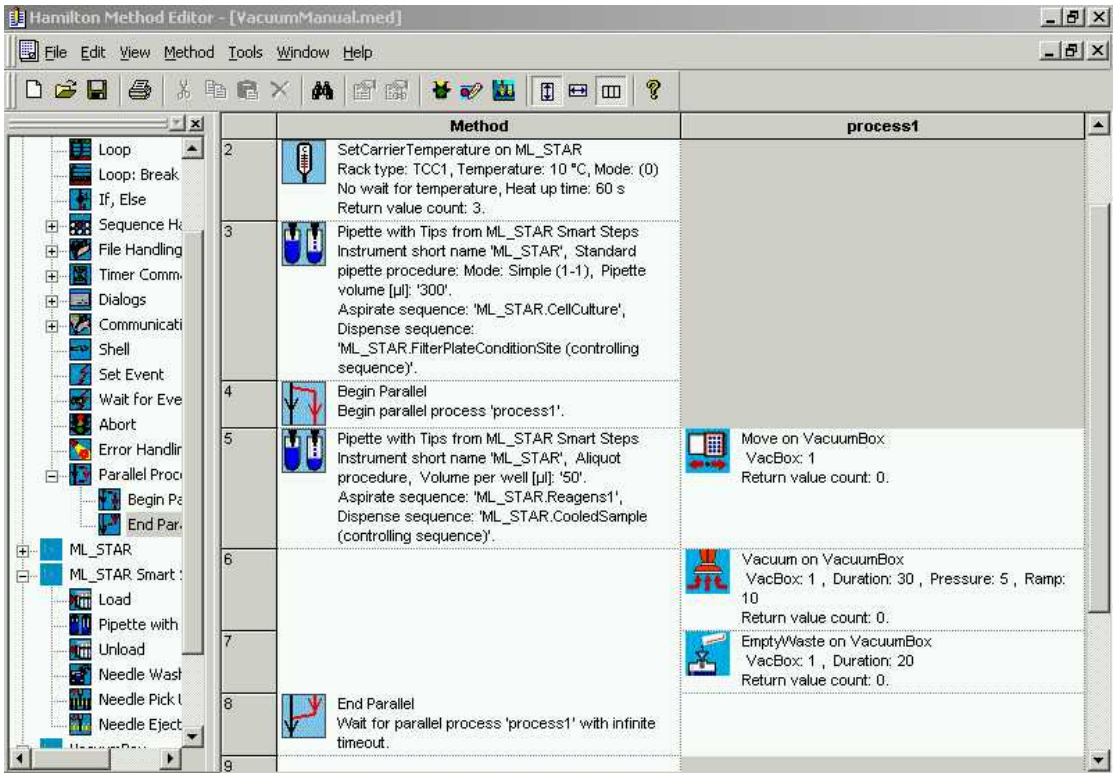
If you select “Invoke user recovery“, which is already selected as default, the operator can decide what to do at run time. “Ignore and continue“ and “Abort“, which will release the vacuum, are the other possibilities. After selecting an error recovery option, click OK.

12.3 Parallel Processing with the AVS

We now discuss a sample program to illustrate the use of the vacuum box software.



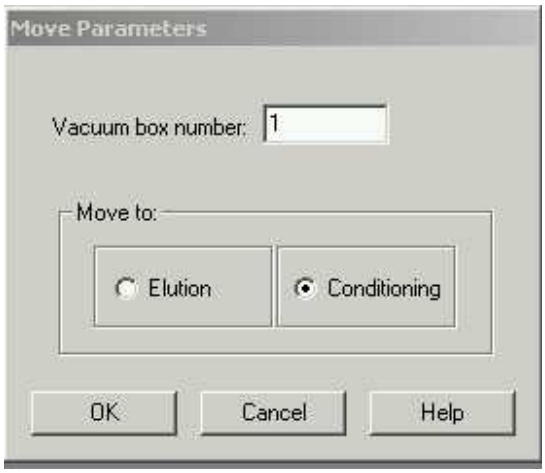
This could be a part of a normal plasmid DNA isolation protocol combined with a PCR setup. It is very useful to work with parallel processes using the vacuum box. During the vacuum procedures, the Microlab STAR robot goes on with pipetting and thus saves time. Accordingly, this section will also explain how to use the parallelism within the Microlab STAR User SW. The first step in our program is “initialize”. Then come the following steps:



Let's discuss the individual steps of this method now.

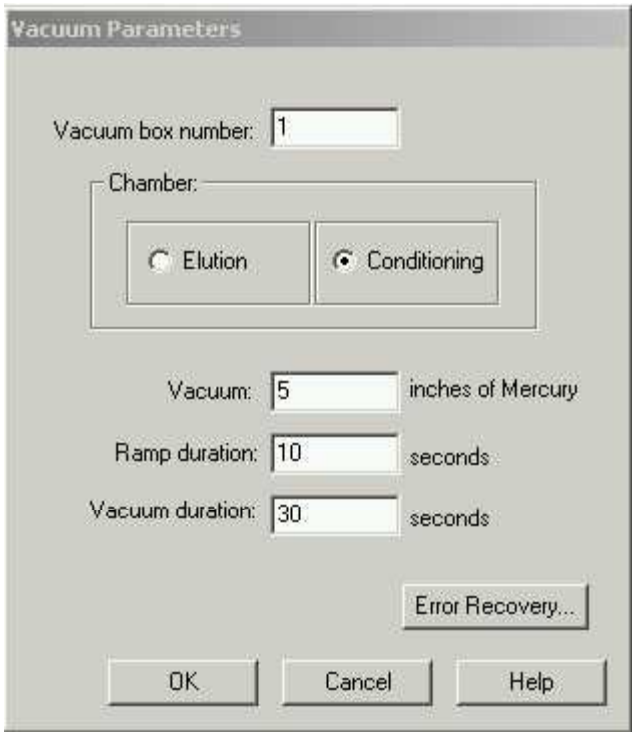
Drag the parallel process icon. A dialog appears to start parallel process No 1. Enter a name for the parallel process and click OK. The method editor now shows the bifurcation of the normal and the first parallel process. The STAR SW supports nested parallel processes.

Drag the Move Step of the vacuum box to the first line of the parallel process.



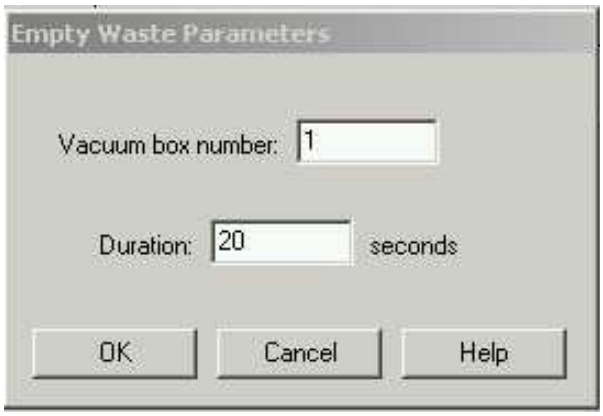
Move the carriage of vacuum box number 1 to the conditioning site. Click OK.

Now set the vacuum parameters:



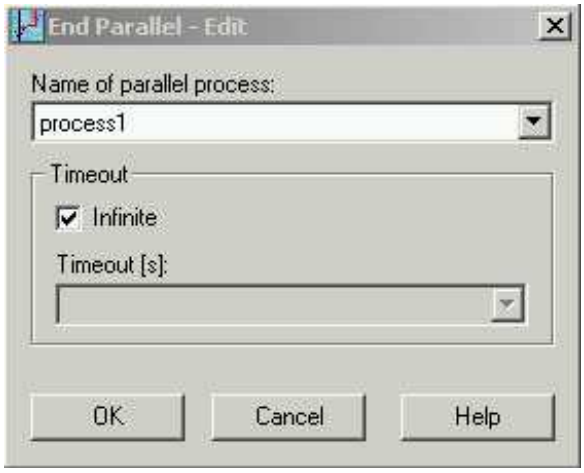
Apply a vacuum of 5 inches of mercury on the conditioning site for a total of 40 seconds.
10 seconds ramp duration plus 30 seconds vacuum duration.

Now empty the waste of the vacuum box:



Empty waste on the conditioning site for 20 seconds

Finally, end the first parallel process:



End of parallel process1 with all the vacuum box commands

The bifurcation within the method editor ends after this step.

To process a plate, for example, with the pipetting channels of the microlab STAR, add pipetting steps to the normal process (left side of the bifurcation).

13 Methods for Microlab® STAR

13.1 Overview

Most of the instrument's day-to-day operations will be driven by methods, so it is important that they are correctly defined and easy to operate. Programmed methods are stored as a linked set of ASCII files in the Methods sub-directory on the hard disk. They can be opened, edited and saved either in the text-like HSL Method Editor (9 *HSL* Method Editor, page 96) or in the Graphical Method Editor (*8 Graphical Method Editor*, page 66).

The present chapter takes a "cookbook" approach. It teaches you how to program a number of simple methods typically used in laboratories. By following these steps, you will become familiar with the layout and workings of the software and can then modify the suggested methods to suit your own particular requirements, or program new methods based loosely on those suggested. The scope of the complete command language is rather like that of common higher programming languages. For details, refer to the *Microlab STAR Reference Manual*, as incorporated in the online help.

NOTE
Sequence definitions have an important influence on programming methods.

ATTENTION
Ensure that all methods are tested first using deionized water.

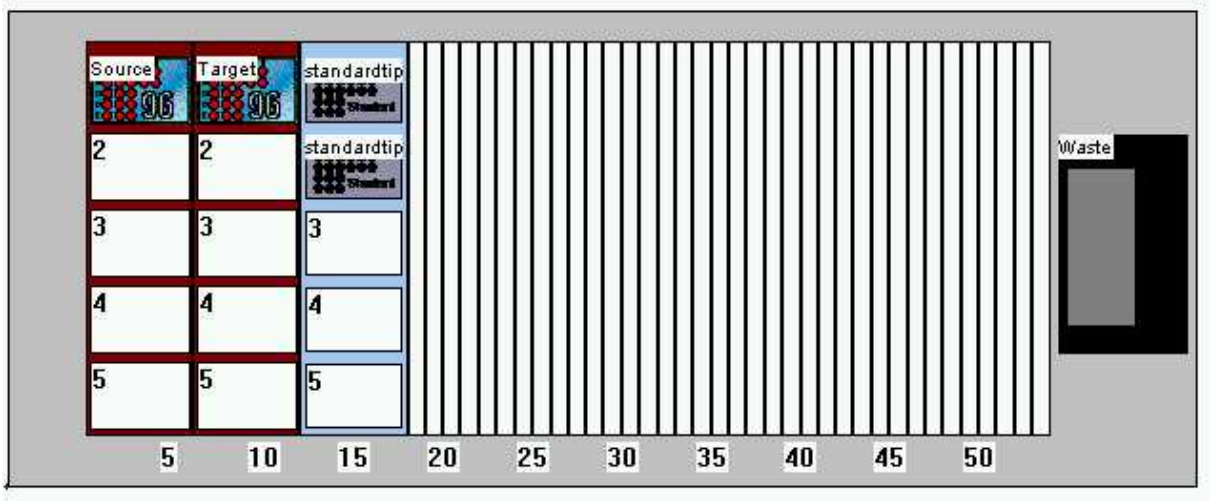
ATTENTION
The user is responsible for the validation of the method.

All sample methods explained in this manual are available in the "\DemoMethods_MLSTAR" directory following installation of the Microlab STAR User Software.

13.2 Create a Method to Copy from Plate to Plate Using SMART Steps

The method we are going to describe first "copies" wells, i.e. aspirates liquids from wells on a plate and dispenses them to the corresponding wells on another plate (A1→A1' ... H12→H12'). The transferred volume will be 50 µl. The method name is "OnePlateToPlate". The method uses new CO-RE tips for every well. We start with an empty target plate.

First, an appropriate deck layout has to be created and saved as "OnePlateToPlatePipette" (.lay). The deck layout for this method is shown in the picture on the next page.



Deck Layout for Method OnePlateToPlate

To create this deck layout, start the Deck Layout Editor by double-clicking on the “Microlab STAR Edit” shortcut on the desktop. Select “New” from the “File” menu. Select “ML_STAR” as the current instrument, if more than one instrument is installed. Click OK. Now you see the schematic view of the Microlab STAR deck.

Double-click on one of the tracks. A dialog box appears. Select a standard carrier for microplates PLT_CAR_L5MD from the ML_Star directory. You may assign a name to the carrier. Repeat this to place another plate carrier of the same type on to the deck. Now add a tip carrier TIP_CAR_480 to the deck.

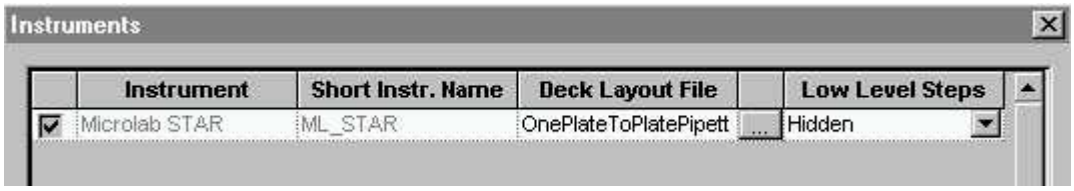
Double-click on one of the sites of the plate carrier. A dialog box appears. Type in “Source” for the source plate and click “Browse”. Select “nun_96_fl_l.rck” from the “Nunc” directory. Click OK. Now you have added a 96-well plate from Nunc to the carrier. Repeat this to place the target plate on the other plate carrier. Type “Target” in the dialog box. Now that this plate type has been specified within the deck layout, you may select it directly from the “Type” dropdown field.

Double-click on one of the sites on the tip carrier. A dialog box appears. Leave the name field blank and click “Browse”. Select “ standardtip_l.rck” from the “ML_Star” directory. Click OK. Note: In the example on the CD, a second tip rack is added.

Select “Save” from the “File” menu to store the deck layout under the name “OnePlateToPlatePipette”.

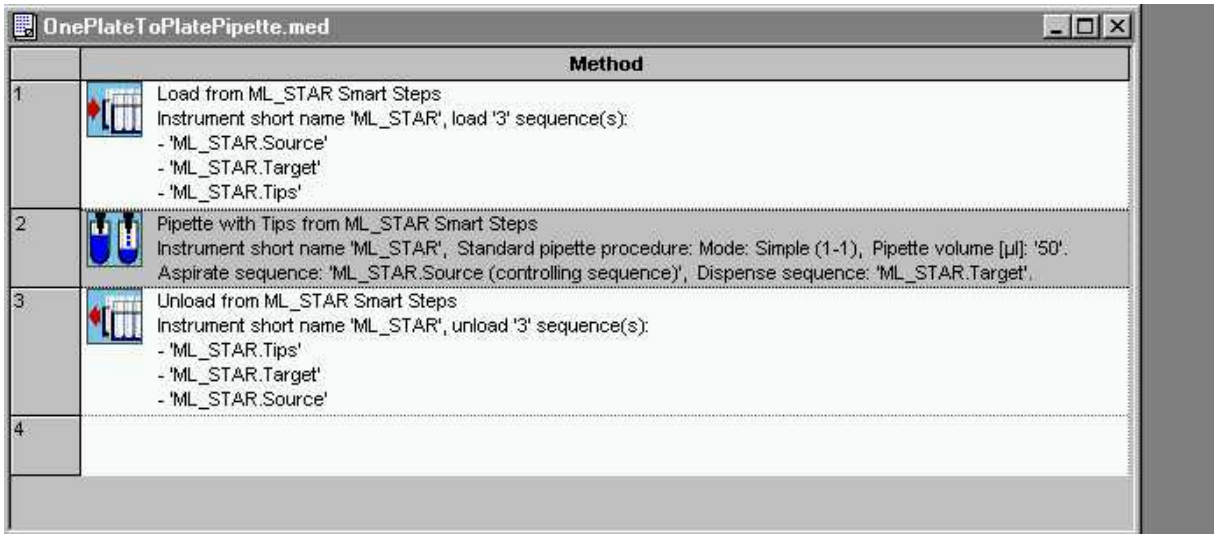
Click on “Edit Method” to open up the Graphical Method Editor.

From the method editor's "Method" menu, select "Instruments" to link the deck layout to the method as described in the chapter “The graphical Method Editor”. A window pops up:



Choose “OnePlateToPlatePipette.lay” as the deck layout (browse by clicking the “...” button) and click OK. Only now are the instrument-specific commands loaded into the method editor. They can be accessed by clicking on the “ML_STAR SMART Steps” toolbar in the toolbox window.

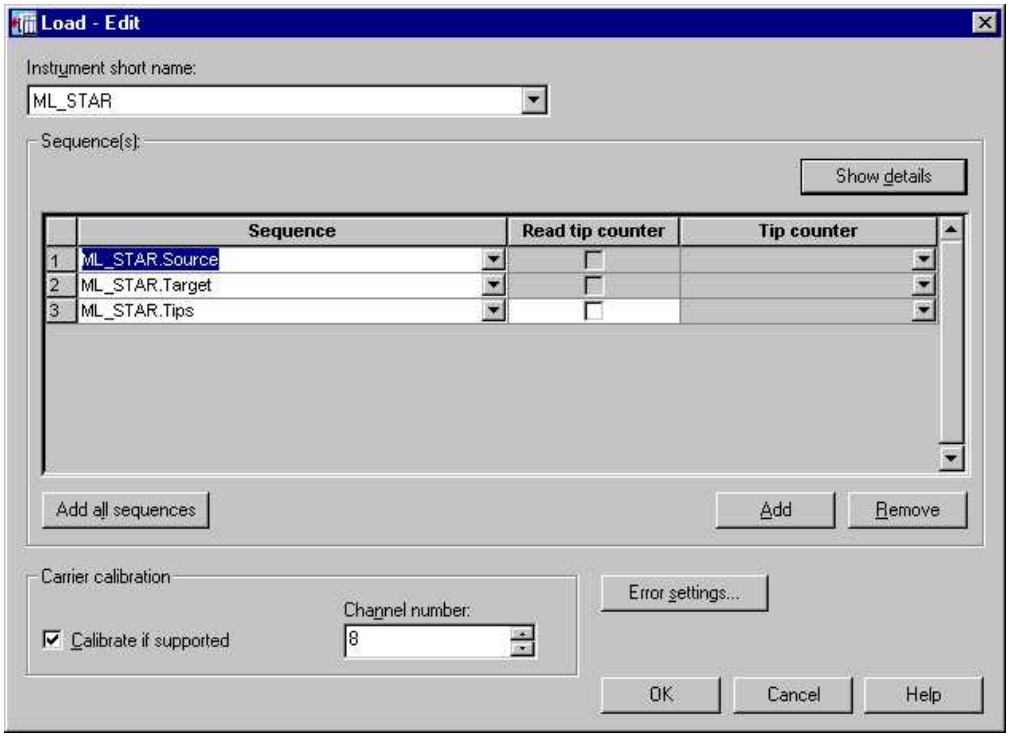
You can easily write the method by dragging icons from the toolbox on the left and dropping them in the method window on the right. The resulting method will look like this:



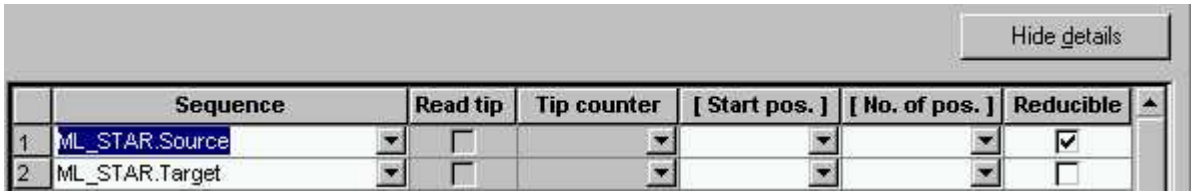
Note

Explicit loading (specifying loading commands within the method) is recommended for safety reasons, but is not mandatory. If no loading commands are specified, no check of the carrier positions is performed and the user has to ensure that all carriers are positioned manually on the correct tracks. This holds true for manual load and autoload Microlab STARs.

First, drag the SMART Step load into the method window:



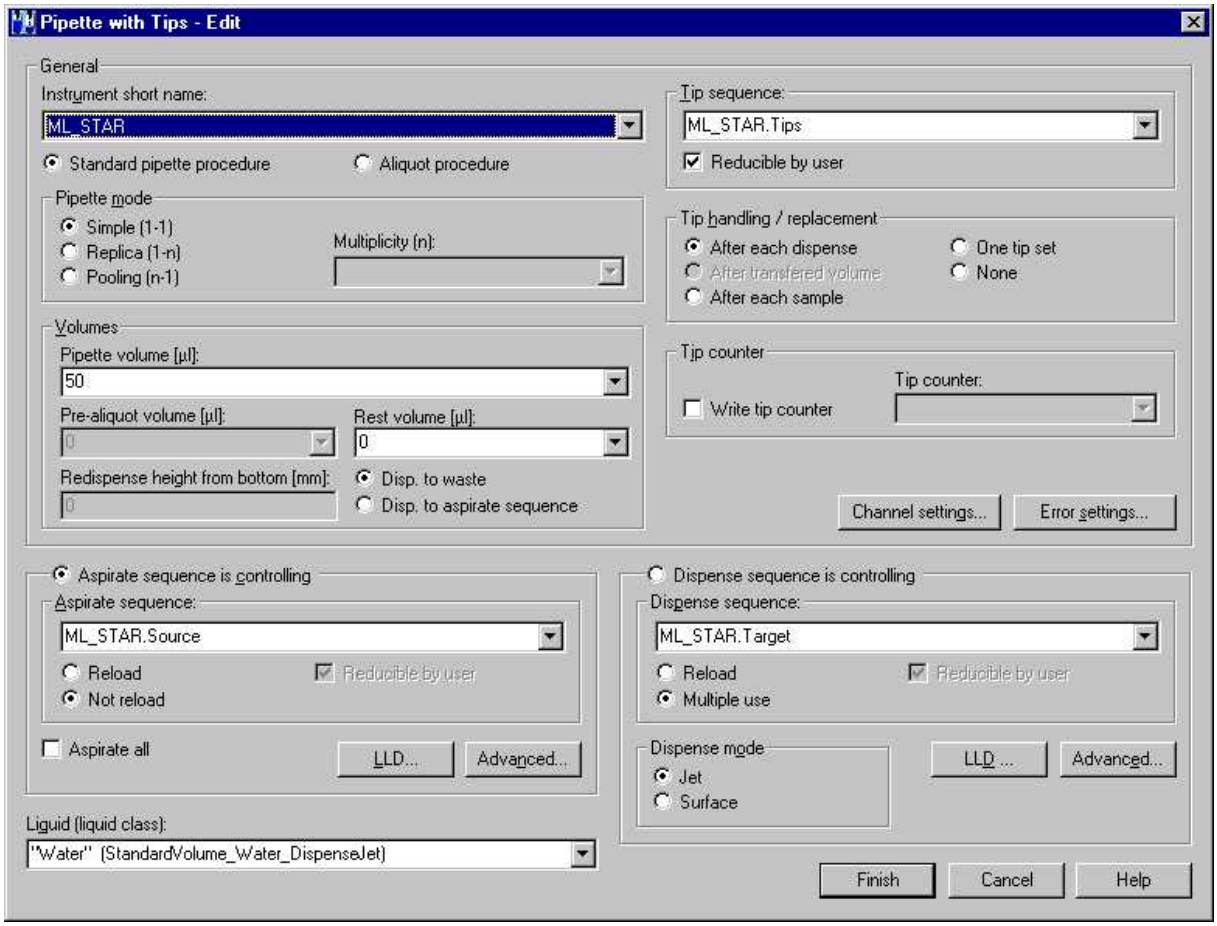
Click on “Add all sequences” to make sure all carriers are going to be loaded on to the instrument deck. Click OK. If you want to decide at runtime which or how many of the wells of the source plate are to be transfered to the target plate, click on “Show Details” and enable the checkbox “Reducible” for the source sequence:



Click OK again.

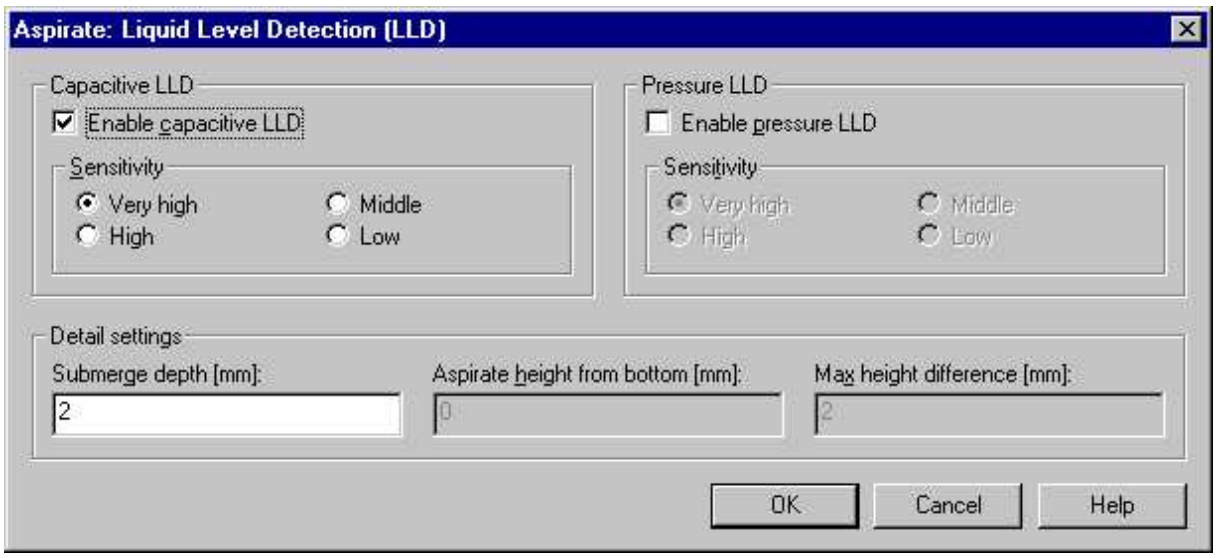
For an instrument with autoload option, this command loads the carriers automatically onto the instrument deck during runtime. For a manual load instrument, this command requests the user to load the carriers for runtime.

Now program your pipetting. Drag the SMART Step pipette to the line below the loading step:

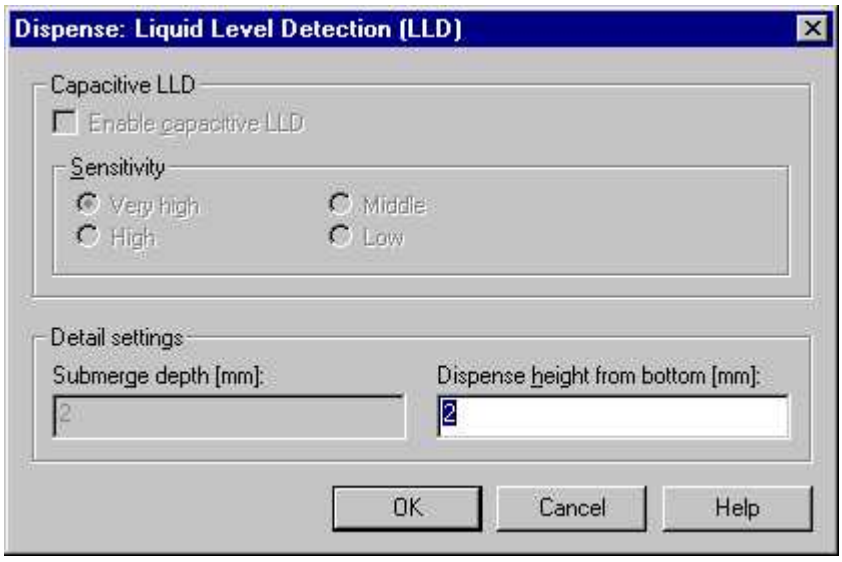


Choose “Standard Pipette Procedure” and “Simple” as a pipetting mode, because it’s a simple transfer from plate to plate. The volume to be transfered is 50 µl, the residual volume is 0. Select the tip sequence ML_STAR.Tips from the dropdown field. The tip handling chosen here is to take new tips for each sample (which is for 50 µl and standard tips, the same as “after each sample”). Now select the aspiration sequence as the controlling sequence. Even if both sequences have the same length initially, it makes sense to choose the aspiration sequence as the controlling one. Remember that on loading this sequence the sequence may be reduced to less than 96 positions. Then, only the current number of wells is transfered to the target plate. Both aspiration and dispense sequence are set as not reloadable. The dispense mode is to dispense in a ”jet”, because initially, the target plate is assumed to be empty. The liquid class used in this example is water.

On aspiration, you may use capacitance-based LLD. Click on the LLD button:

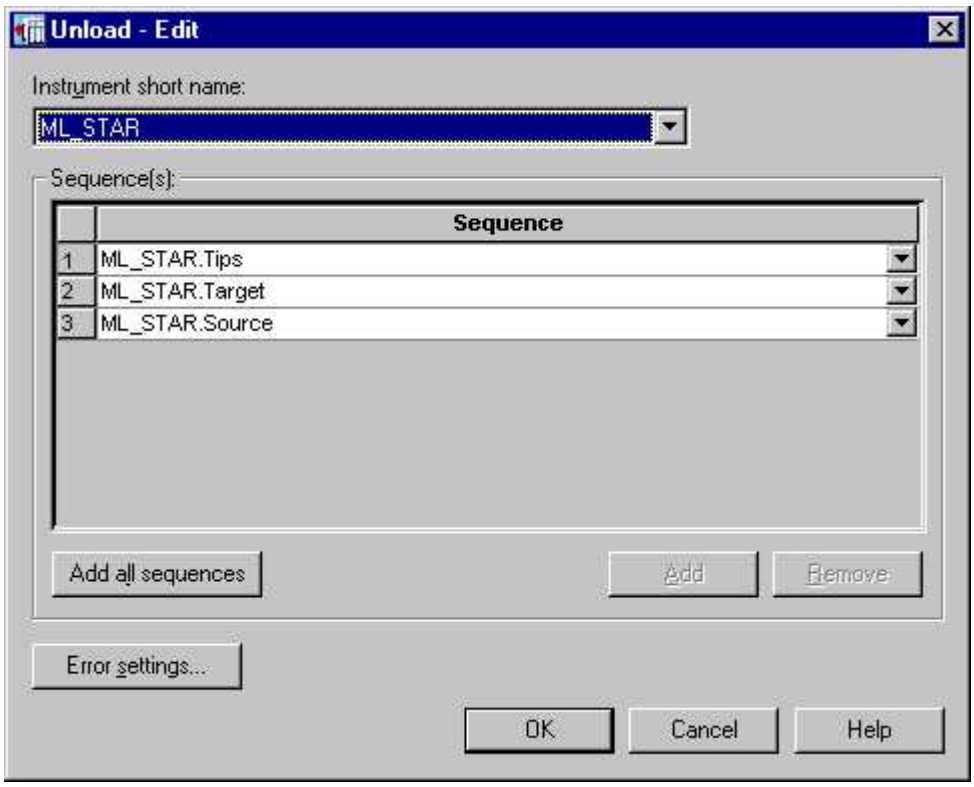


Set the settings according to this example. Click OK. On dispense, switch off the LLD and dispense to a fixed height (2 mm from bottom):



Accept the defaults for the settings under Advanced and for the error settings. Click OK. Click Finish in the SMART Step dialog. A window appears showing a summary of your settings. Click OK.

Finally, drag the SMART Step Unload to the line below the pipette step:



Click on “Add all Sequences” to add all sequences to the unload step. Click OK.

Within the method editor, click File->Save to store your method.

Your first method is ready to go. See the chapter about running the STAR to see how to run your method.

13.3 Create a Method to Copy from Plate to Plate with Single Steps

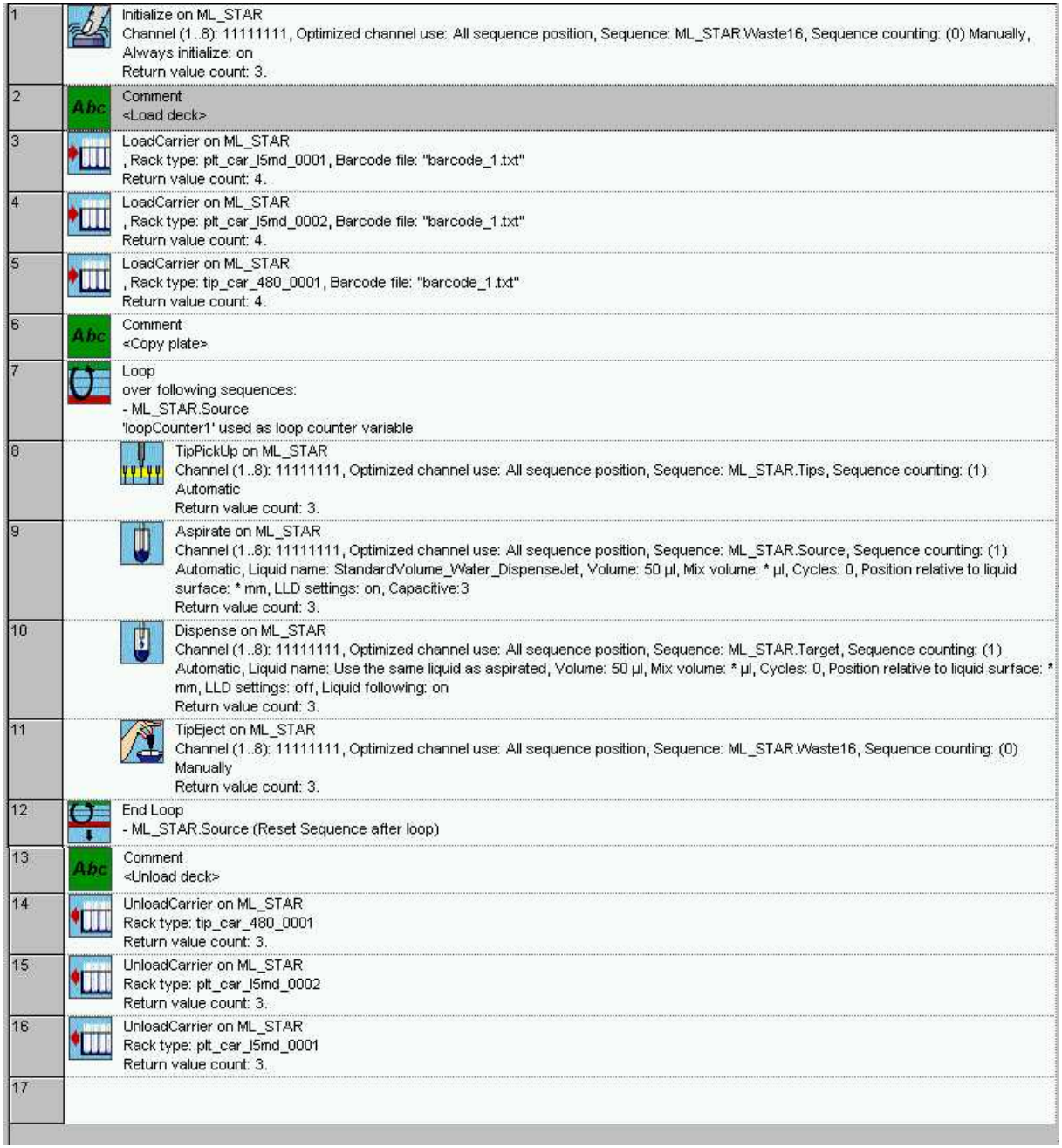
The method we are now going to describe does exactly the same as the method "OnePlateToPlatePipette" described in section 13.2. The only difference is that the method is now written using single steps, to illustrate the difference. No sample reduction is possible here.

First, create the deck layout in the same way as described for the method "OnePlateToPlatePipette". This time, save it under the name "OnePlateToPlate" (.lay).

Click on Edit Method. Link the deck layout "OnePlateToPlate" (.lay) to your new method, as described above.

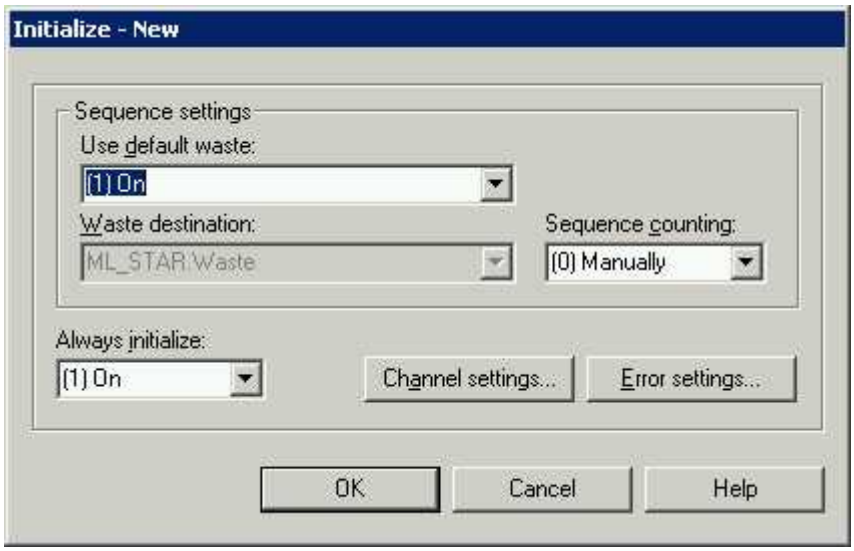
Only now are the instrument-specific commands loaded into the method editor. They can be accessed by clicking on the “ML_STAR” toolbar in the toolbox window.

You can easily write the method by dragging icons from the toolbox on the left and dropping them in the method window on the right. The resulting method will look like this:



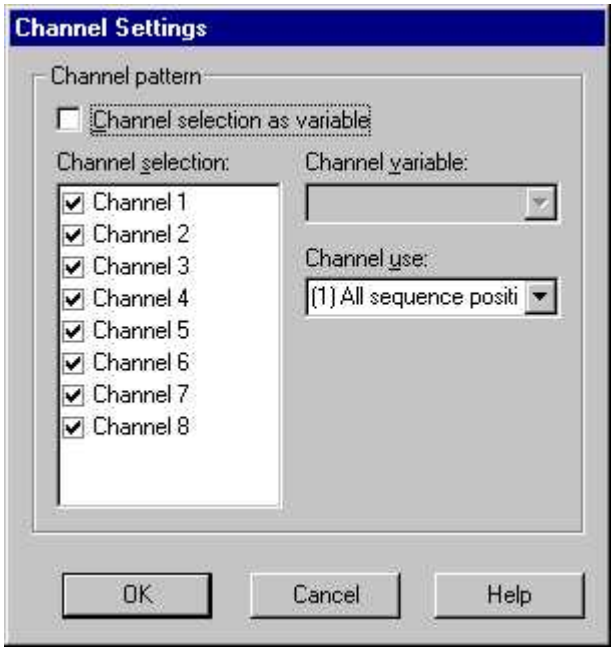
The Copy from Plate to Plate Method Using single steps.

Let's start to construct this method. Under the "ML_STAR" toolbar, drag "Initialize" to the main window. A window pops up:



Click OK to initialize the instrument in the first step. The waste sequence is selected to eject tips from the channel during initialization. Always use manual sequence counting for initialization (see below). Set the "Always initialize" switch to "On", to make sure the instrument is being initialized prior to each run.

Throughout all single steps, the channel pattern to be used may be specified manually. Clicking on "Channel Settings" opens the following dialog:

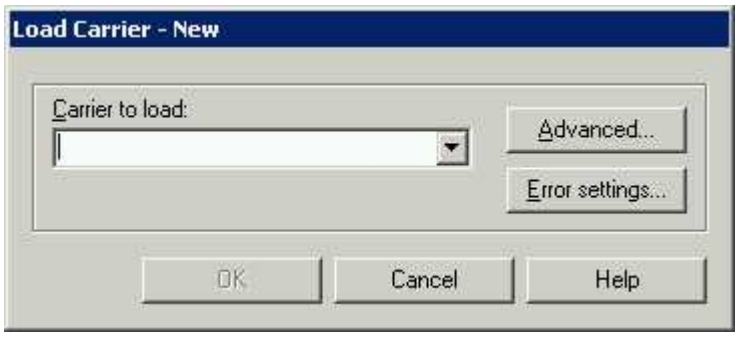


You may either deselect specific channels manually by clicking on the check boxes, or check the box "selection as variable". Then the channel pattern can be defined as a (string) variable having a 1 for each active and a 0 for each inactive channel, e.g. "11100011". Having deactivated a channel, the question arises whether to pipette all sequence positions with the remaining channels (select (1) All sequence positions) or to leave out the corresponding wells (select (2) channel pattern). Click OK to accept the settings.

NOTE

Before any other instrument-specific step can be carried out, the system has to be initialized.

Drag “Load Carrier” to the next line in the method. For an instrument with autoload option, this command loads the carriers automatically onto the instrument deck during runtime. For a manual load instrument, this command requests the user to load the carriers for runtime.

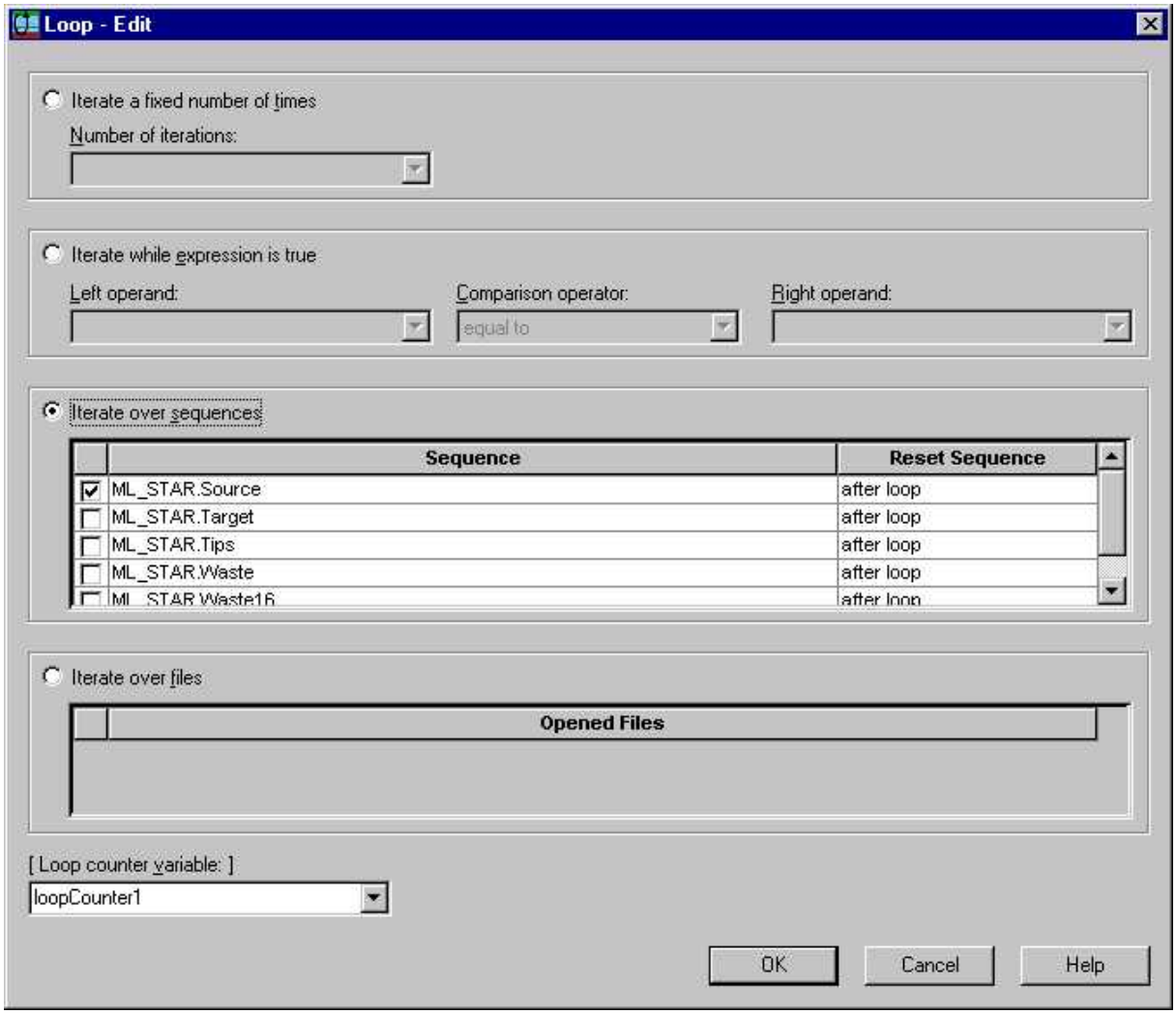


Specify the name of the carrier to be loaded. The plate barcodes are stored under the default file name “barcode_1.txt”. The positions on the deck are automatically retrieved from the deck layout on runtime. Click OK. (Note that the checkbox “barcode trace “ within the configuration editor must be checked to generate this file).

Repeat the Load Carrier command for the other plate carrier and the tip carrier.

To copy the whole source plate and not just the first 8 wells to the target plate, the tip pick-up, aspiration, dispense, and tip eject steps have to be performed 12 times. This can be achieved by a loop. Drag the Loop command to the next line of the method window. The loop statement consists of two lines, a “begin loop” and an “end loop” statement. Whatever code is inserted between these two statements will be looped.

The loop dialog window looks like this:



A loop can be performed looping over a fixed number of iterations, over an expression (repeat while the statement in the expression is true), a sequence, or a file (until the end-of-file is reached). Here, we loop over the source (plate) sequence. This means, that the loop will continue until all sequence positions (the 96 wells) of the source plate have been used. Then, the loop will stop. Choose the default “after loop” for the “Reset Sequence” option to reset the sequence “source” to the initial position (1) after the loop is done. If you pipette later to the same sequence (“source”), the sequence will then start at the first well again.

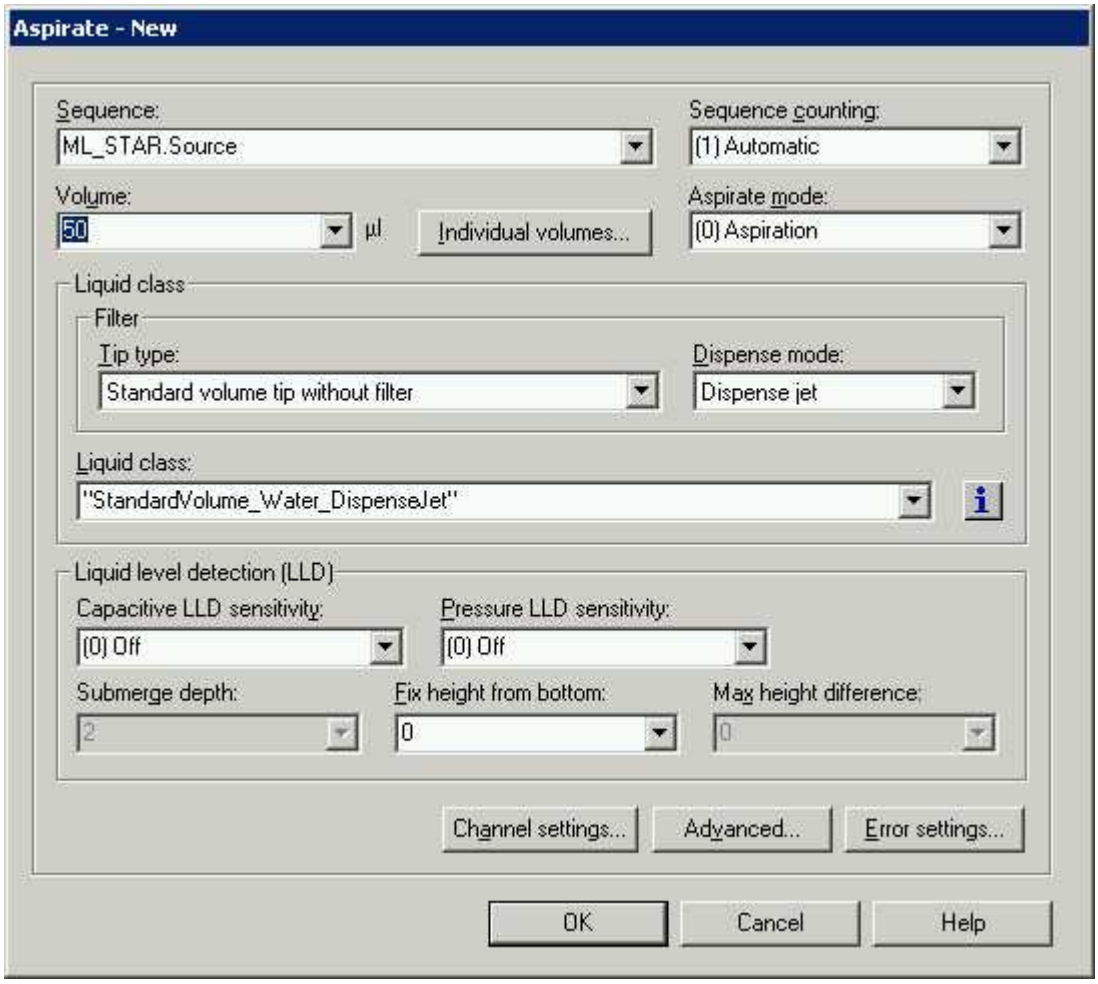
NOTE

Keep in mind that if you loop over a sequence, the sequence has to be incremented within the loop. If you loop over more than one sequence, the shortest sequence is will be taken as the relevant one.

Under the “ml_star” toolbar, drag “TipPickUp” to the position between the two loop statements.

A dialog box appears. Choose sequence “ml_star.Tips” and sequence counting "Automatic". Sequence counting “automatic” means that after pick-up takes place from the first eight sequence (tip) positions, the tip sequence is automatically incremented. Sequence counting “manually” means that the sequence used in this step will not be incremented automatically; during the next tip pick-up process, the positions within the tip rack already used will be used again. Therefore, we select “Automatic” to increment the sequence automatically by the number of channels used for the tip pick-up. Next time, tip pick-up will start with the next (unused) 8 positions in the same tip rack. Click OK.

Now drag Aspirate to the line below the tip pick-up step. A dialog box appears.



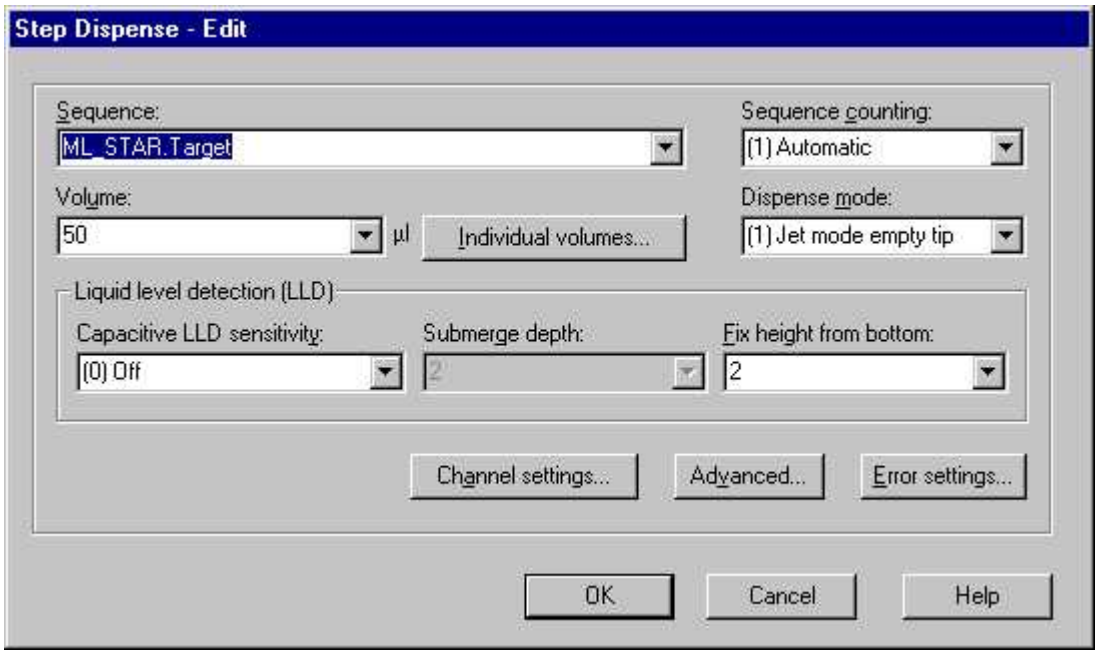
For sequence, choose “ml_star.Source” to aspirate from the source plate. Select "Automatic" as sequence counting. For volume, enter 50 µl. Select “Aspiration” as the aspiration mode. Choose “Standard volume tip without filter” for the tip class and “Dispense jet” as dispense mode. Then choose an item from the “Liquid class” dropdown list. (For questions about these parameters, refer Chapter 2 of this manual, “The Art of Pipetting”.) Select "medium" as the capacitive LLD sensitivity. Type in 1.0 (or 1) as additional submerge depth. Click "Advanced". A window appears:

Set “liquid following during aspirate and mix” to “on”, allowing the channel to follow the falling liquid level during aspiration. You may now enter the settings for mixing.

The “mix position” is the submerge depth used for mixing, moving the tip downwards from the current z-position.

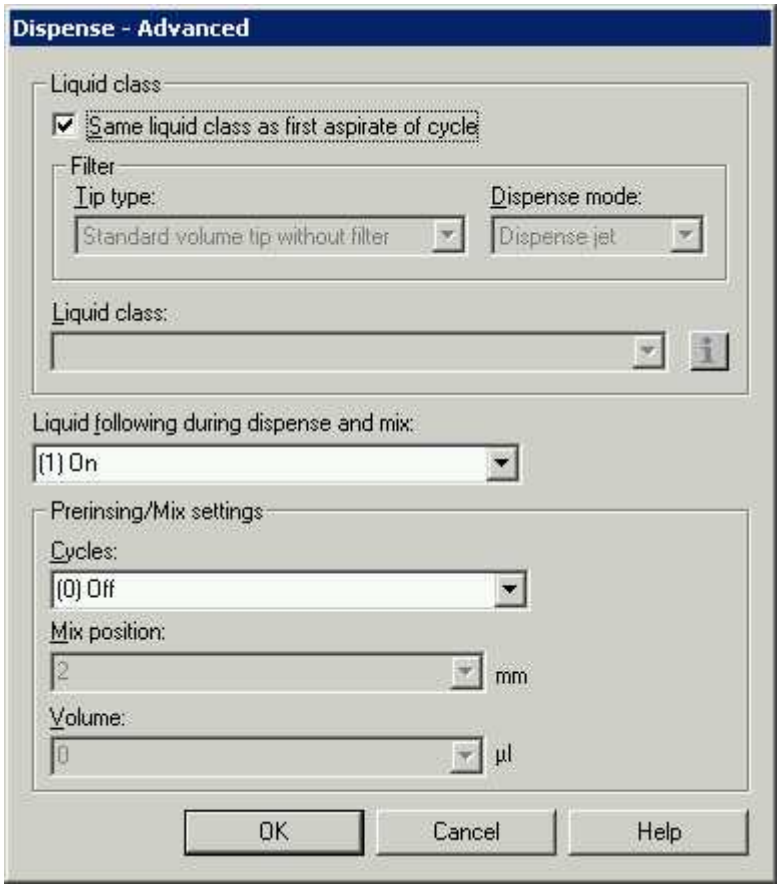
Click OK to accept the values. Click OK in the aspirate window.

Now drag Dispense to the position below the aspirate command. A dialog box appears:



Choose “ ml_star.Target” as sequence to dispense to the target plate. Choose sequence counting “automatic”. Enter 50 µl for the volume again. Select the dispense mode “Jet Mode Empty Tip”. Select “ off” as the capacitive LLD setting - because the target plate is empty - and enter 2 mm as “liquid level”, corresponding to a height of 2mm for the dispense, measured from the container bottom.

Click “Advanced”. A dialog window appears. The lower part of the dialog is exactly the same as for the aspiration step (liquid following and mixing settings).

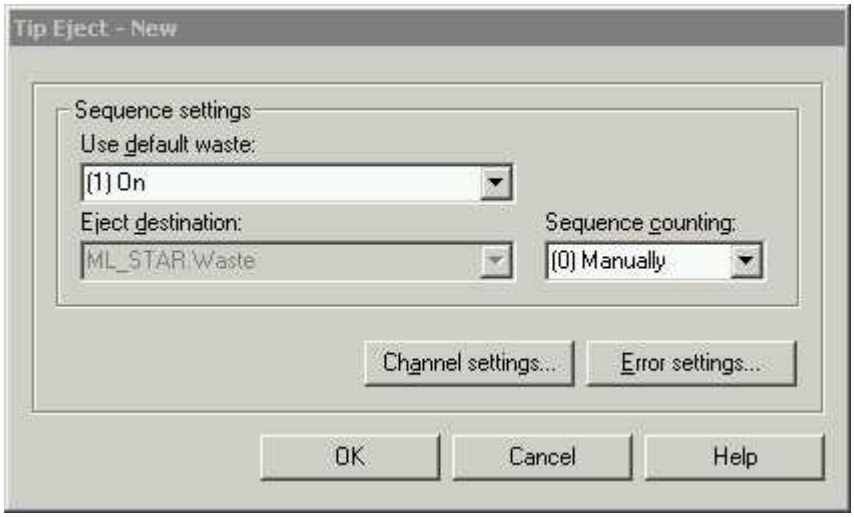


In the upper part, accept the default for using the same liquid class as in the aspiration step. Click OK to accept the values. Click OK in the dispense window.

NOTE

Using a different liquid class for aspiration and dispense is allowable, but not recommended.

Finally, drag TipEject. A dialog box appears.



Accept the defaults, and click OK.

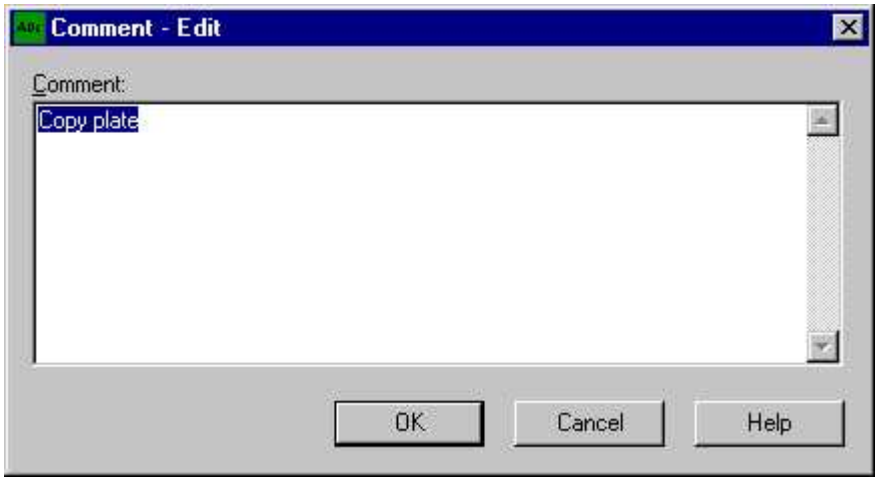
NOTE

Always use manual sequence counting for ejecting tips into the waste container. The waste position is itself a sequence having just 8 or 16 positions. Automatic incrementation would result in an error the next time tips are ejected into the waste (no positions left over).

Finally, the carriers have to be unloaded. This is done by the unload command, with the carrier name as a parameter.

Click OK. Repeat the unloading for all three carriers.

Within this method, some comment lines have been inserted. The dialog is simple:



To enter a new line hold CTRL and press Enter.

Now your method is complete. Exit the method editor by selecting File/Exit from the menu.

Chapter 15 “Running the Microlab STAR” explains how to run a method.

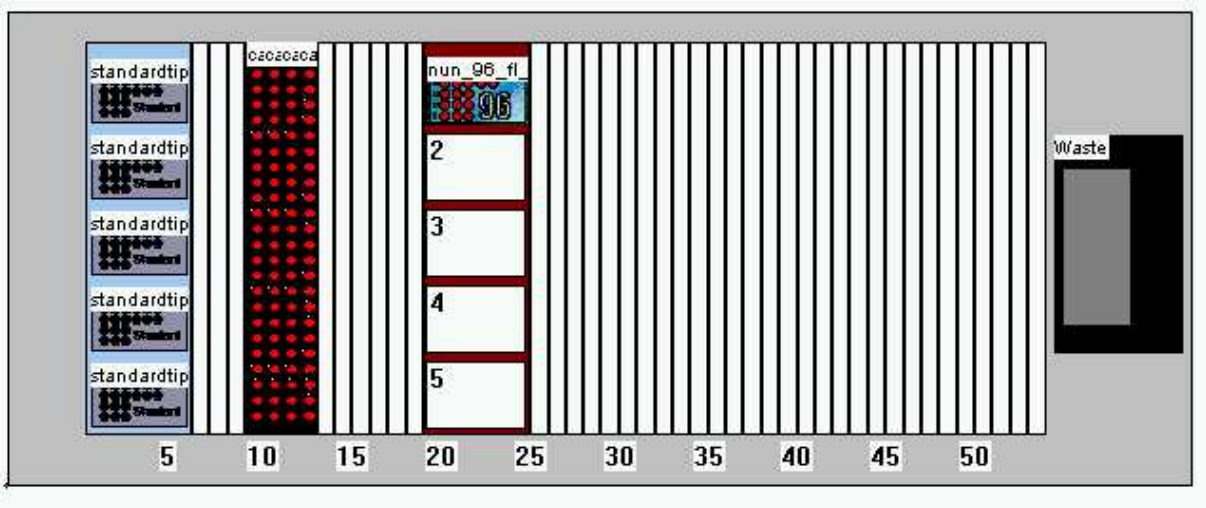
13.4 Create a Method to Copy from Tubes to Plates Using SMART Steps

This method copies tubes, i.e. aspirates liquid from tubes in a carrier and dispenses them to wells in a microtiter plate. The maximum number of tubes to be processed is 96, corresponding to a maximum of four 24-tube carriers (1T).

First, create an appropriate deck layout. In this case, it will be “TubesToPlatePipette (.lay)”.

To create this deck layout, start the Deck Layout Editor by double-clicking on the appropriate icon. Select “New” from the “File” menu. Select “Microlab STAR” as the current instrument (see method editor description). Click OK. Now you see the schematic view of the Microlab STAR deck.

Create a deck layout as shown below:



Deck Layout: TubesToPlate

Double-click on one of the tracks. In the pop-up window you may enter a name standing for the tube carrier, and click Browse. Select “Car24_cup15x100.rck” as rack type for a carrier holding 24 tubes of 15 mm diameter and a height of 100 mm from the “ML_Star” directory. Click OK. The carrier is added to the deck layout. Repeat this procedure three times more for the other tube carriers. Place them in adjacent positions on the deck. Now you have added the tube carriers.

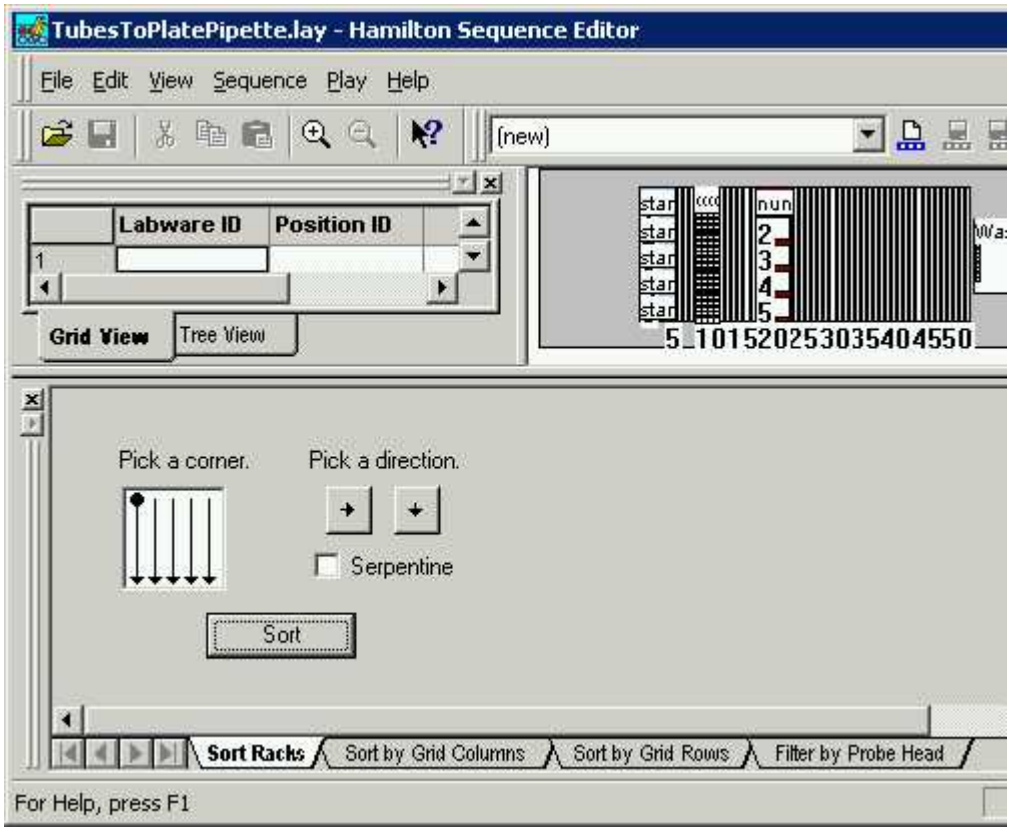
Double-click on another track to add a standard plate carrier. A dialog box appears. “Browse” for the PLT_CAR_L5MD in the ML_Star directory. Click OK to add the carrier. Double-click on one of the sites of the carrier. Select “Nunc_96_Flat_L.rck” from the “Nunc” directory. Click OK. Now you have added a 96-well plate to the carrier.

Double-click on another track to add a tip carrier. A dialog box appears. “Browse” for the TIP_CAR_480 within the ML_Star directory. Click OK. Double-click on one of the sites of the carrier. Select “ standardtip_l.rck” from the “ML_Star” directory. Click OK. Now you have added a tip rack to the carrier. Repeat this to add several tip racks to the carrier.

Save your deck layout under the name “TubesToPlatePipette” (.lay).

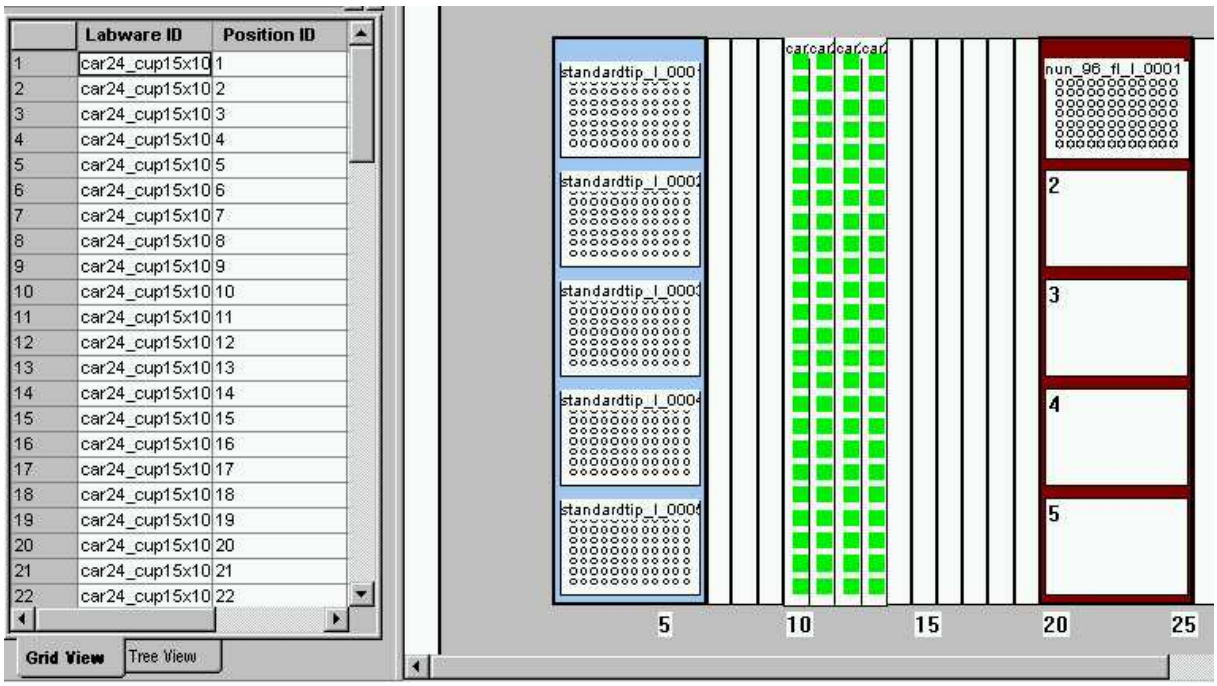
We will now define sequences which relate all samples on the one hand and all tip racks on the other hand.

Under the “Tools” menu select “Sequence Editor”. The Sequence Editor opens up.



Sequence Editor

Zoom in by clicking the (+) button on the tool bar. Rubber-band all four tube carriers with the left mouse button. You now see the 96 selected wells of the four carriers. The grid window (on the left) shows the generated sequence of 96 positions holding all tubes.

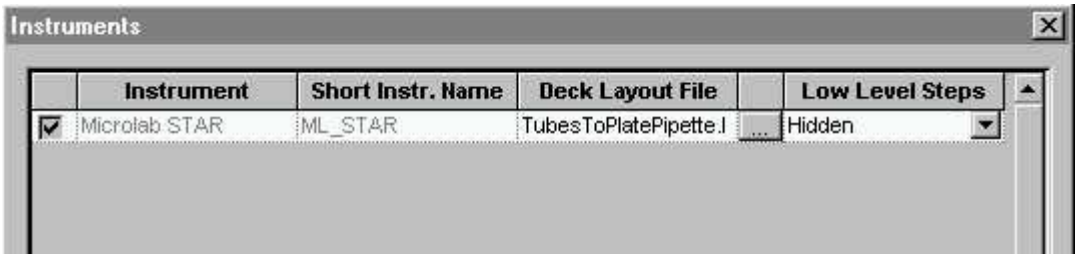


Select “Save Sequence” from the “Sequence” menu and enter the name “Samples” for your new sequence. Select “Save” from the “File” menu. Now the sequence linking all samples is stored with the deck layout. Select “New” from the “sequence” menu. Now create a sequence linking all tip racks, by rubber-banding the tip racks. Save the sequence under the name “Tips”. Now create a corresponding sequence for the plate, again by rubber-banding. Save this sequence under the name “Plate”. Select “Exit” from the “File” menu, then click “Yes” in the pop-up window to save the changes to the deck layout. (Technically, sequences are part of the deck layout).

Select “Save” from the “File” menu within the deck layout editor to save the changes to the deck layout under the name “TubesToPlatePipette” (.lay).

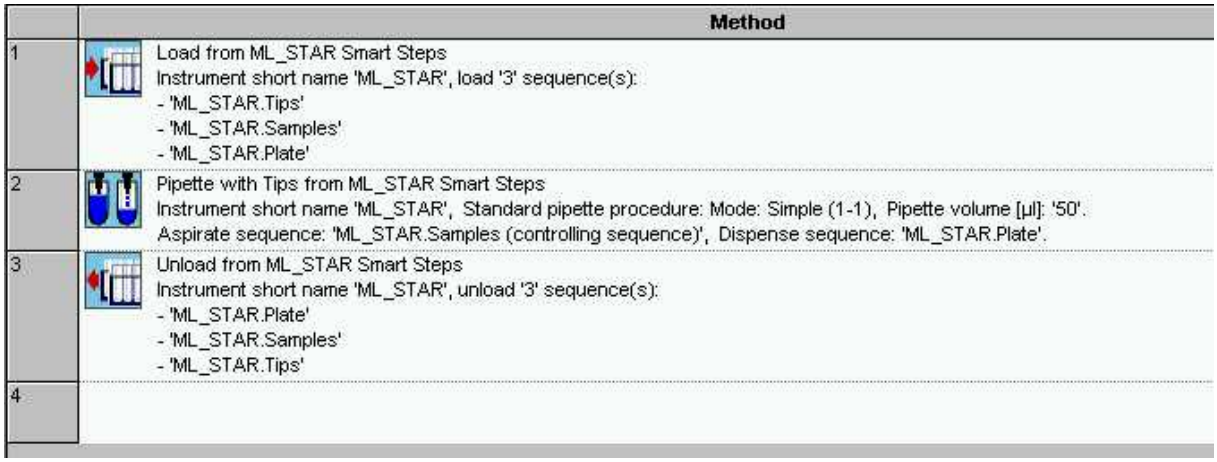
Click on the “Edit Method” button to open up the Method Editor.

From the editor's "Method" menu select "Instruments" to link the method to be written to the Microlab STAR deck layout “TubesToPlatePipette.lay“:



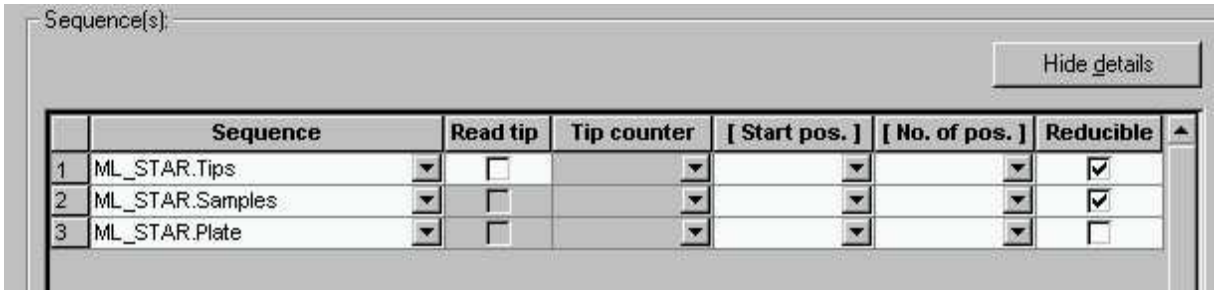
Click on Browse (...) to select the deck layout “TubesToPlatePipette”.

You can easily write the method by dragging icons from the toolbox on the left and dropping them in the method window on the right. Finally, your method should be as displayed in the next screen:



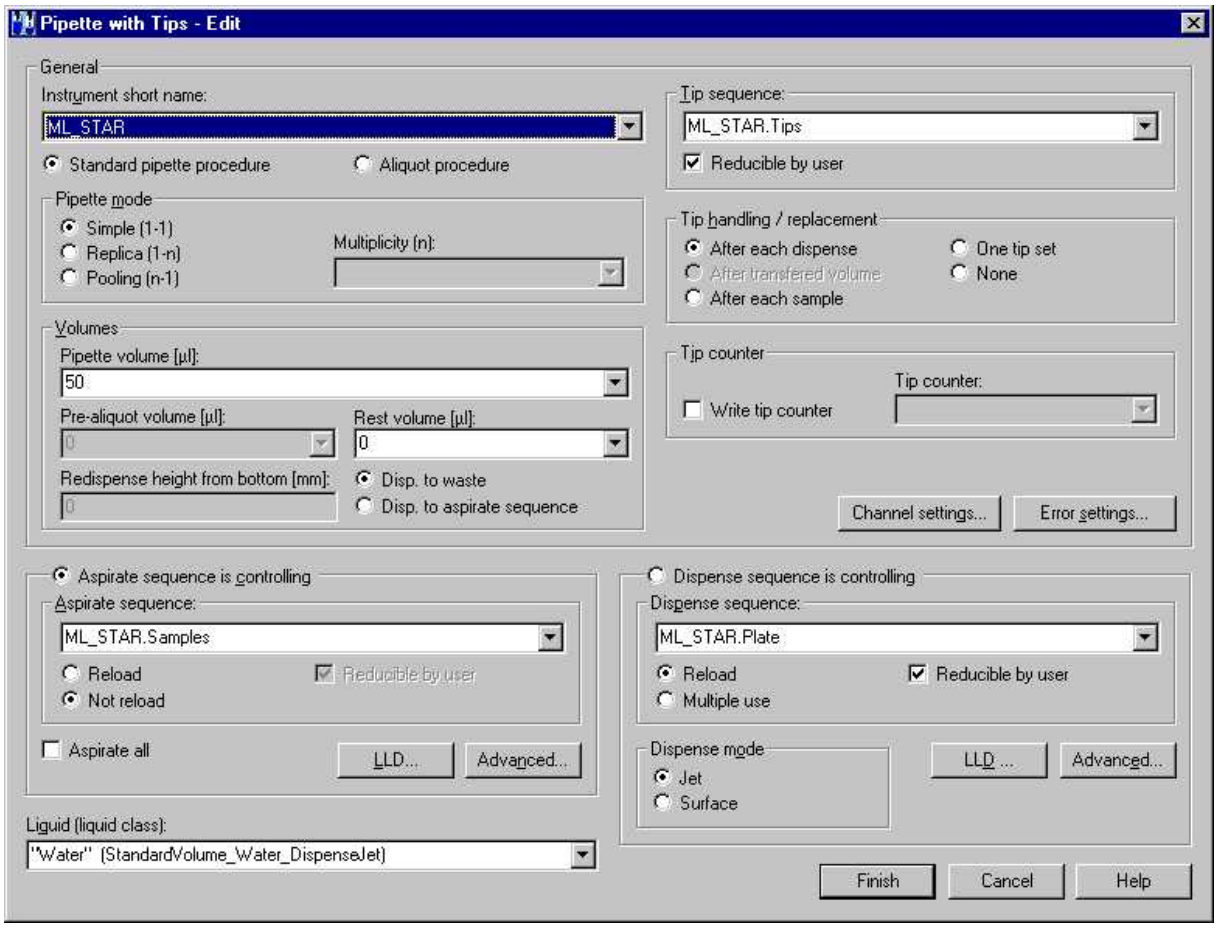
The method TubesToPlatePipette.

The deck is now loaded using the SMART Step load:



Click on “add all sequences” to load all sequences and corresponding carriers. Select the Samples sequence to be reducible by clicking on “show details”. Click OK.

Now add the pipetting step, by dragging it to the next line:

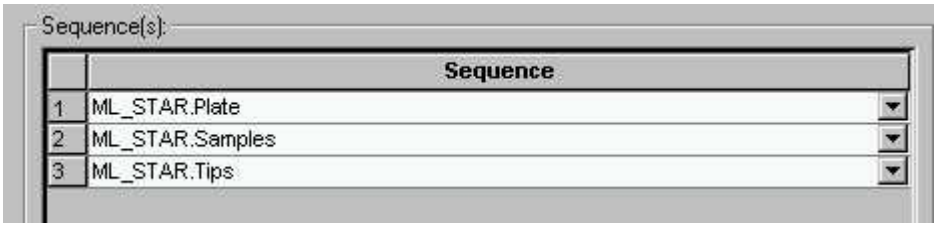


Select

- Standard Pipette Procedure,
- Simple Mode
- Tip Sequence: ML_STAR.Tips
- Volume = 50 µl, residual Volume 0
- Tip handling: After each dispense
- Aspiration sequence (ML_STAR.Samples) as controlling sequence
- ML_STAR.Plate as dispense sequence
- Choose “Not Reload” for the Sample Sequence
- Choose “Reload” or Multiple use for the Plate sequence. This is of no influence here, since the aspiration sequence is controlling and equally long or even shorter (by reduction on runtime) than the dispense sequence.
- Dispense mode is “Jet”
- Liquid is Water
- LLD settings are capacitance on aspiration (sensitivity low, submerge depth 2 mm) and fixed height (2 mm) on dispense, as in the example: “PlateToPlatePipette”.

Click Finish. Check the input on the summary that appears and click OK.

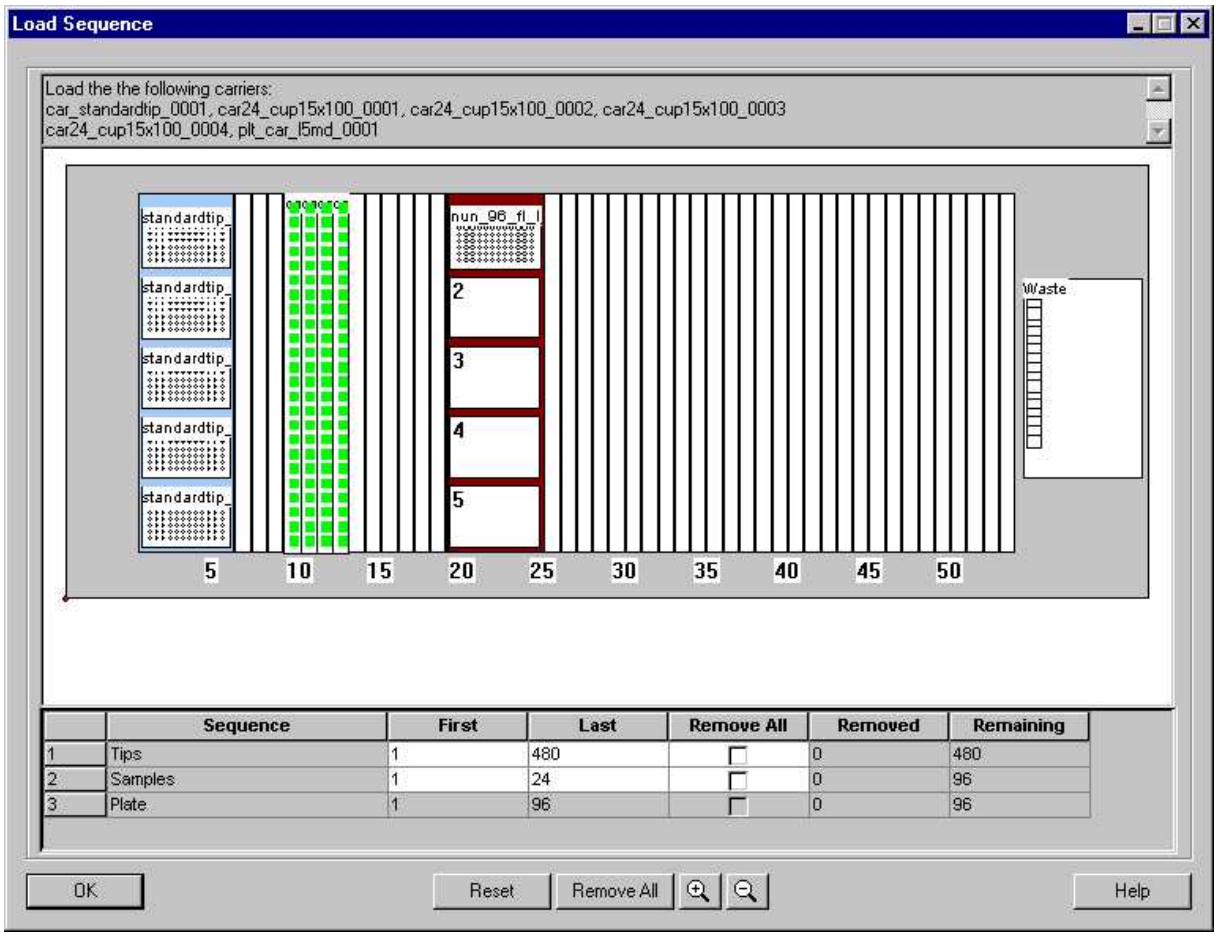
Now drag the SMART Step Unload to the next line:



Click on “add all sequences” to unload the complete deck. Click OK to add the step.

Now your method is ready to use.

What happens if you run this method? Within the loading step, the sample sequence was chosen to be reducible. This means that, at runtime, the user sees the following dialog, enabling reduction of the number of samples (from any position):



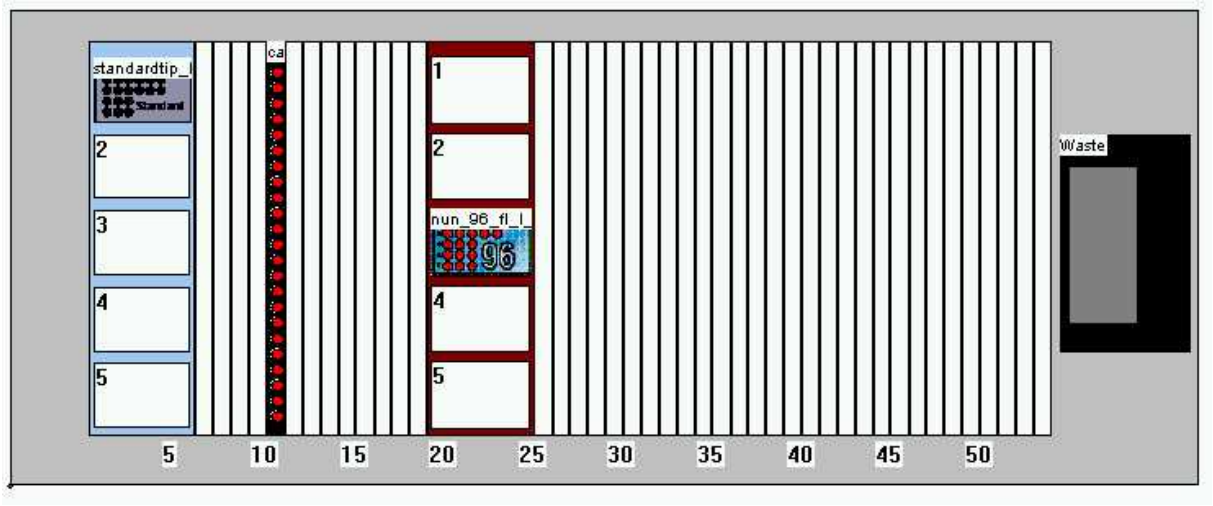
One may know to reduce the number of samples to 24. The method then copies 24 tubes (from the first carrier in this case) to the plate and stops. It is also possible to deselect distinct tubes from the sequence by clicking on the wells. A reset button is available to restore the original sequence.

An example of how to program this method using single steps is available on the user software CD.

13.5 A Method to Pipette Aliquots Using SMART Steps

In this example, a simple aliquoting procedure is decribed. The method aspirates 280 µl from a sample tube rack and dispenses the “pre-aliquot” as well as 12 aliquots of 20 µl each into an empty plate. The last aliquot is ejected with the tip.

Here’s the deck layout:



Place the following labware components on the deck: a tip carrier TIP_CAR_480 with one tip rack for standard volume tips, a sample tube rack of 24 tubes called “Sample”, and standard plate carrier PLT_CAR_L5MD with a 96-well flat-bottom microplate (e.g., the Nunc plate) called “Plate”. Save the deck layout under “Aliquoten” (.lay).

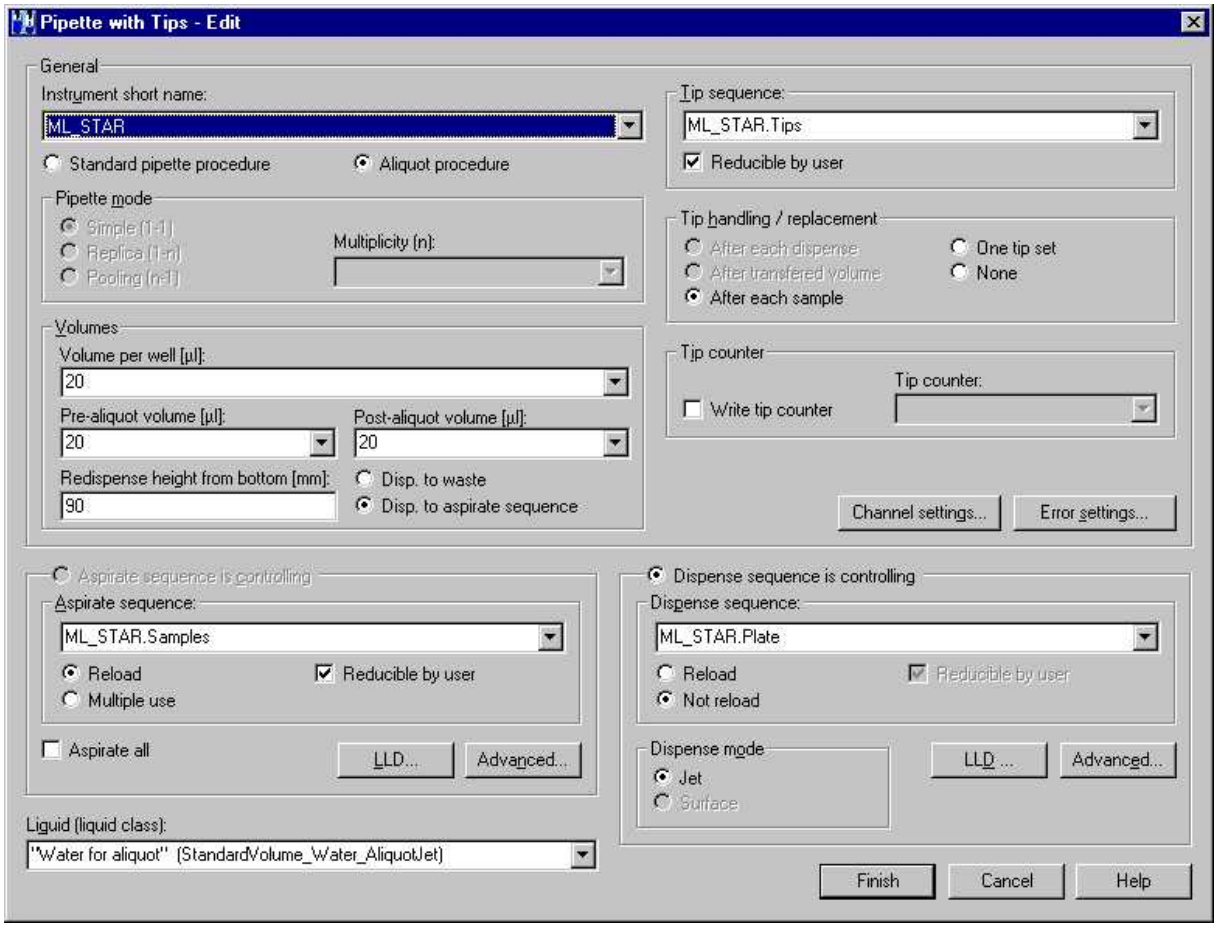
Open the method editor and select "Instruments" from the editor's "Method" menu to link the method to be written to the given deck layout “Aliquoten.lay”, as described in the previous examples.

Here’s the method:

Aliquoten.med

	Method
1	Load from ML_STAR Smart Steps Instrument short name 'ML_STAR', load '3' sequence(s): - 'ML_STAR.Tips' - 'ML_STAR.Samples' - 'ML_STAR.Plate'
2	Pipette with Tips from ML_STAR Smart Steps Instrument short name 'ML_STAR', Aliquot procedure, Volume per well [µl]: '20'. Aspirate sequence: 'ML_STAR.Samples', Dispense sequence: 'ML_STAR.Plate (controlling sequence)'.
3	Unload from ML_STAR Smart Steps Instrument short name 'ML_STAR', unload '3' sequence(s): - 'ML_STAR.Plate' - 'ML_STAR.Samples' - 'ML_STAR.Tips'
4	

The loading and unloading is very similar to the previous examples and may be skipped (see note in example “OnePlateToPlatePipette”). Let’s concentrate on the pipetting step. Drag the SMART Step pipette to the method:



The selections made here are

- Aliquoting procedure
- The pre- and post-aliquots are 20 µl, as well as the volume of the main aliquots
- The post-aliquot is dispensed back to the aspiration sequence
- The tip sequence is ML_STAR.Tips
- The tip handling is change “after each sample”, meaning that all aliquots (even if multiple repetitions are necessary) are performed with the same tips
- The aspiration sequence is ML_STAR.Samples and reloadable (this has no influence in this example, because the dispense sequence is controlling and one aspiration is sufficient to aliquot the whole plate).
- The (controlling) dispense sequence is ML_STAR.Plate and not reloadable
- LLD settings are as in the previous exampes: cLLD on aspiration (sensitivity low) and fixed height on dispense
- The dispense mode is now “Jet” (can’t be changed)
- The liquid is “Water for Aliquot” .

Click OK to accept the settings. Click OK to quit the summary.

Your method is now ready to go.

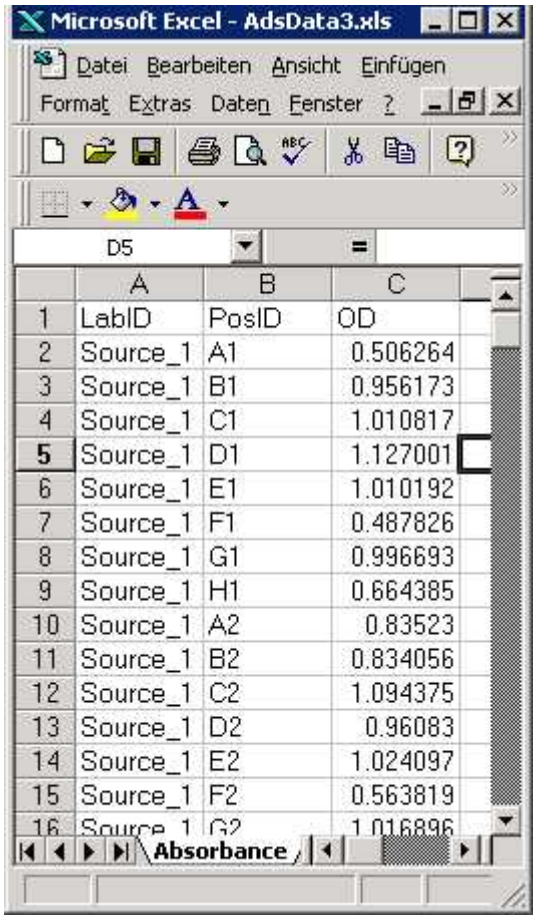
13.6 'Cherry Picking', or How to Change a Sequence Within a Method Using SMART Steps

Assume you have a source plate, and a photometer reads the optical absorbance of the wells of the plate. You now want to create a target plate with all the compounds in the source plate having an absorbance of A>1.0. The 'cherry picking' method does exactly this. The photometric results are retrieved from a file and a sequence of hits (A>1.0) is created 'on the fly' according to the absorbances read. Pipetting then occurs according to this sequence.

This method does not use 'Load Carrier' commands. The system therefore expects the carriers to be loaded manually onto the deck at the defined positions before the run is started. The method can, however, be easily adapted for automatic loading (with autoload option) by inserting the 'Load Carrier' commands after the 'Initialize' command.

For this method we need a database containing the absorbances of the 96 wells of the source microplate. The database can be an ASCII text file, a Microsoft® Excel file or a Microsoft® Access database.

In this case, our database is an Microsoft EXCEL file containing three columns: The "LabID" defining the plate name of the source plate (Source_1 for the first plate and Source_2 for the second plate), the "PosID" defining the position within the microplate alphanumerically (A1, A2, ... , H12), and the absorbance or optical density "OD". The work list therefore has193 lines, 1 header line and the entries from 2 plates with 96 wells.

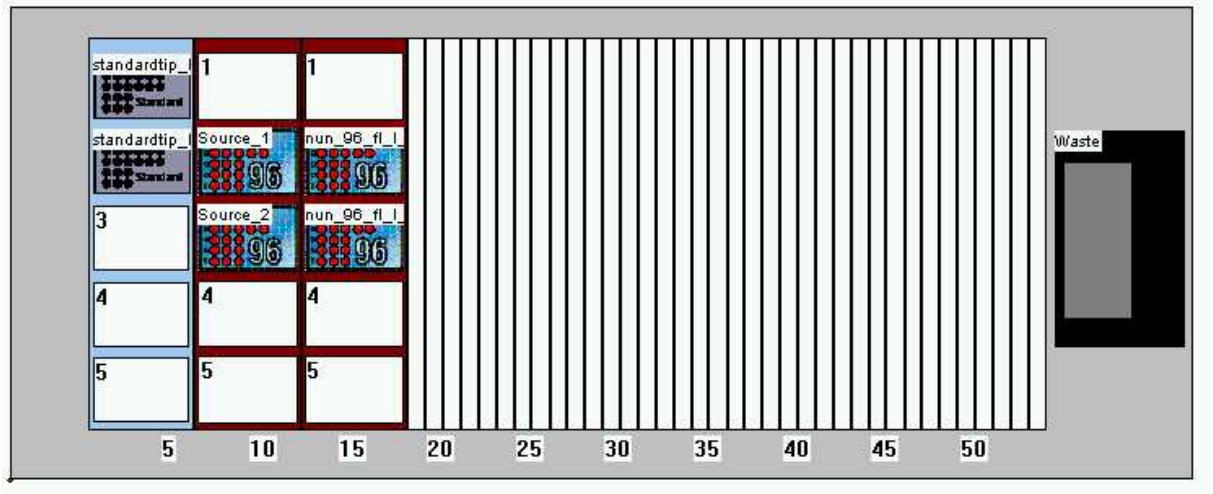


	A	B	C
1	LabID	PosID	OD
2	Source_1	A1	0.506264
3	Source_1	B1	0.956173
4	Source_1	C1	1.010817
5	Source_1	D1	1.127001
6	Source_1	E1	1.010192
7	Source_1	F1	0.487826
8	Source_1	G1	0.996693
9	Source_1	H1	0.664385
10	Source_1	A2	0.83523
11	Source_1	B2	0.834056
12	Source_1	C2	1.094375
13	Source_1	D2	0.96083
14	Source_1	E2	1.024097
15	Source_1	F2	0.563819
16	Source_1	G2	1.016896

The work list ADSData3.xls

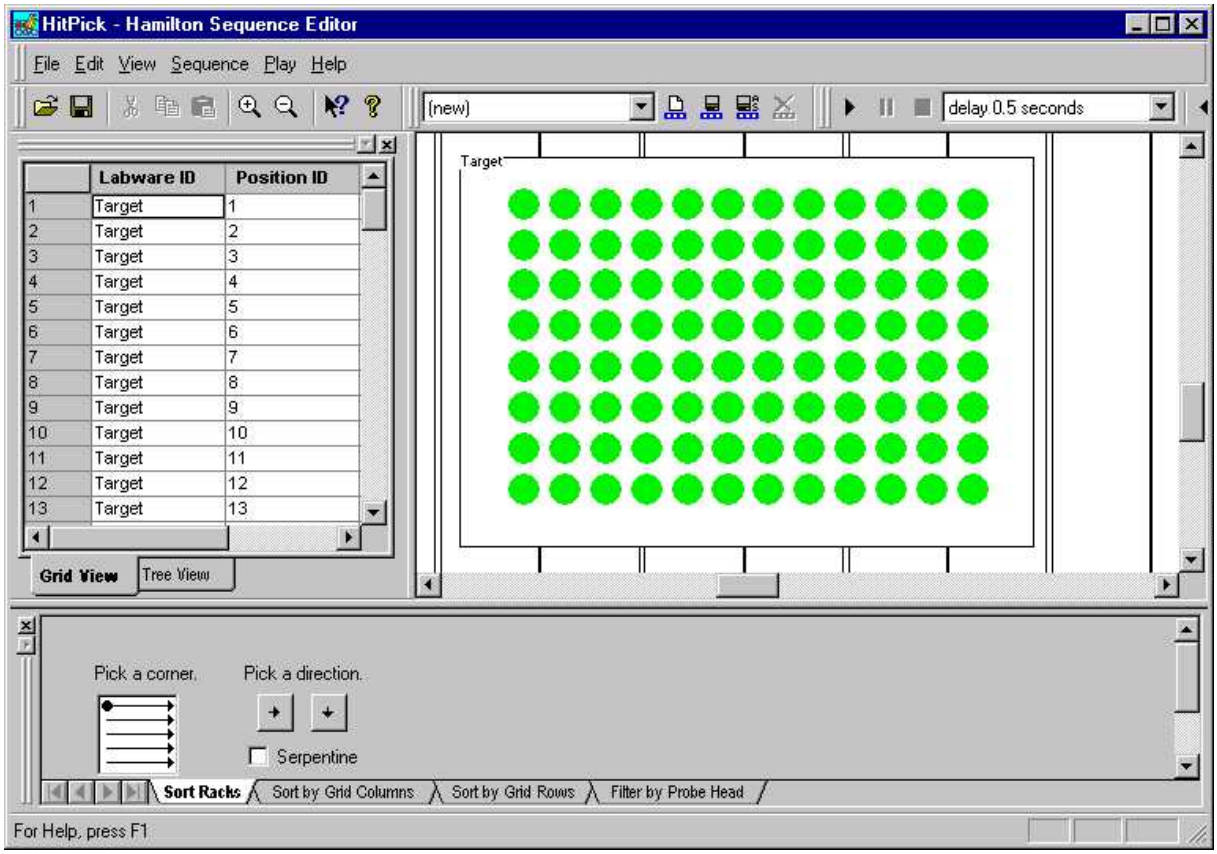
The name of the sheet is „Absorbance“. (Double-click on the text „Sheet1“ within your Excel file and edit it to „Absorbance“ (see the section on working with Microsoft Excel).

The deck layout looks like this:



For this method, place two plates called “Source_1“ and “Source_2” and two target plates (here without name) onto the deck. Two tip rack are needed as well. Save the deck layout under the name ‘HitPick’.

This method modifies a sequence (the hit sequence) on runtime. The next step is therefore to generate a sequence “hits” using the sequence editor. The sequence should span some or all of the wells in the source microplate(s). Start the sequence editor from the deck layout editor by selecting “Sequence Editor” from the “Tools” menu. Zoom in to view the source plate. Rubber-band the entire plate so as to add positions to your new sequence.

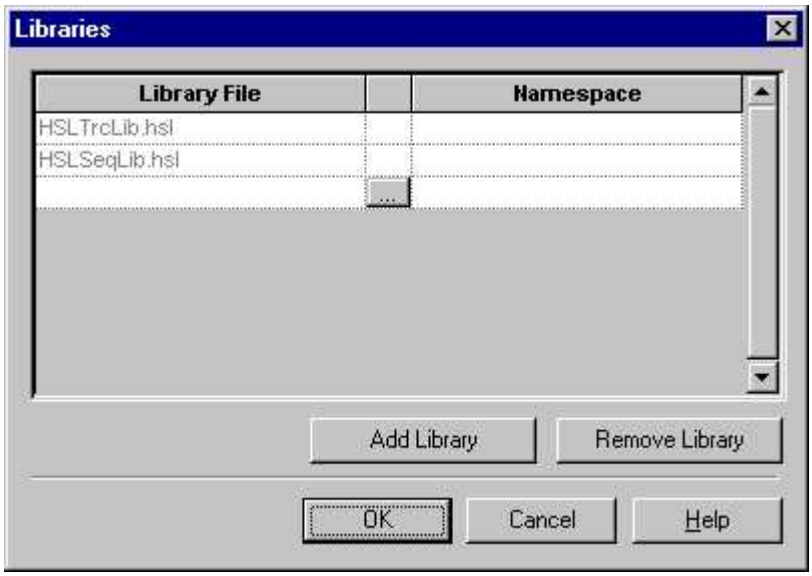


Save this sequence under the name “Hits”. Create another sequence spanning the two target plates and call it “Target”. The last sequence to be prepared is the “Tip” sequence holding both tip racks. Save the changes to the sequence editor on quitting.

Click the Edit Method Button.

From the editor's "Method" menu select "Instruments" to link the method to be written to the given deck layout “HitPick.lay”, as described in the previous examples.

This method uses library functions. To link the libraries to your method, select “Method” and “Libraries” from the menu. Click on the browse button and link the two libraries “HSLTrcLib” and “HSLSeqLib” to the method. Click OK.

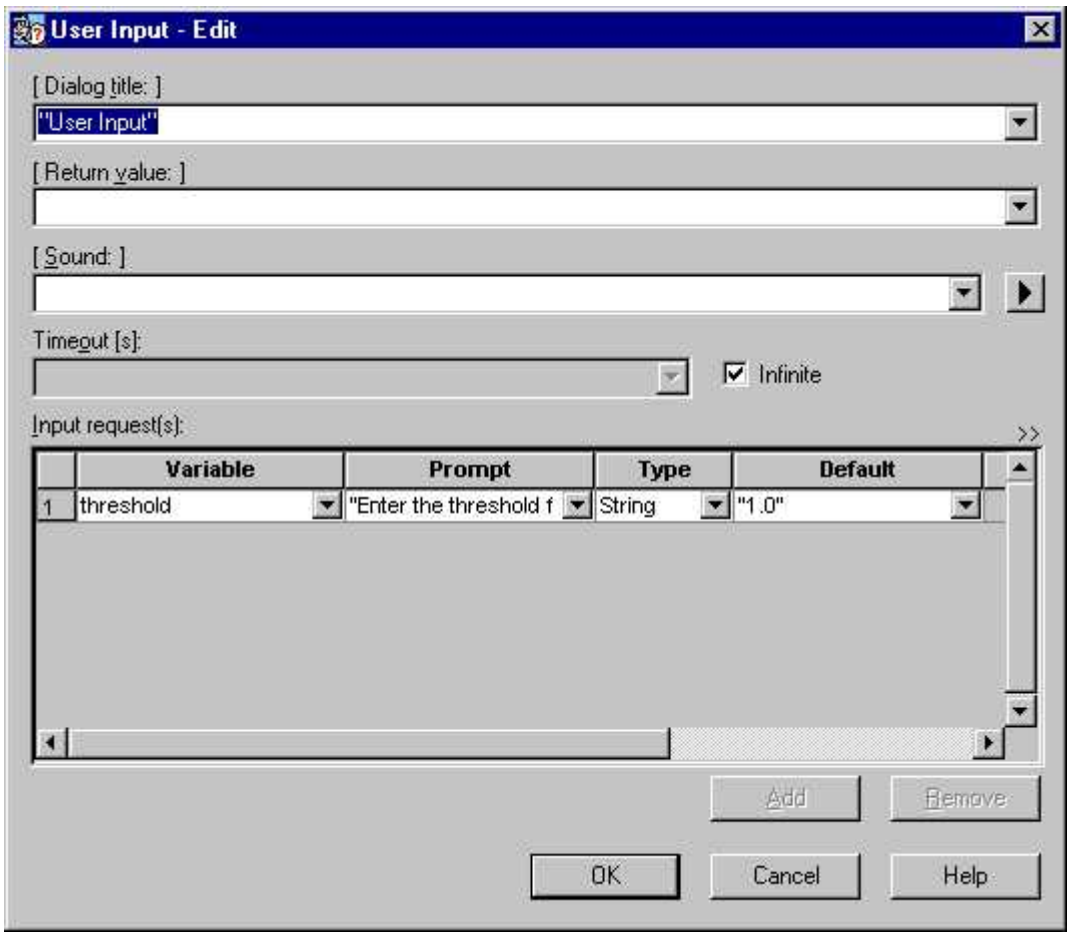


The functions available within these libraries are used to generate entries into the methods trace file, whenever a “Hit” is found, and to manipulate sequences in the appropriate way.

The items of this method in the graphical Method Editor are displayed on the next screen:

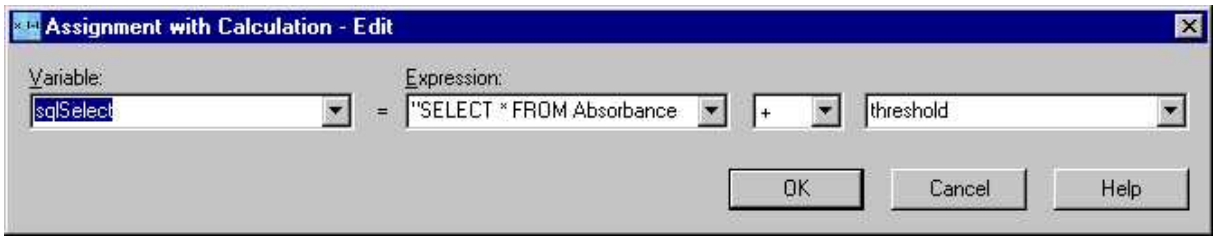
Method Editor : HitPick.med

The first step in this method is to request the user to input a threshold value for the absorption (“threshold”). This threshold – although numeric – is entered as a string. This is because, in the next step, a valid SQL database selection statement is created.



The dialog title is the text on the blue top bar of the dialog. The variable name is “threshold”, the text for the prompt is “Enter threshold for hits” (within quotation marks), the variable type is string, and the default value is “1.0” (within quotation marks). All other input fields are optional.

Within the next step, a calculation is made with the string variable read from the user input.

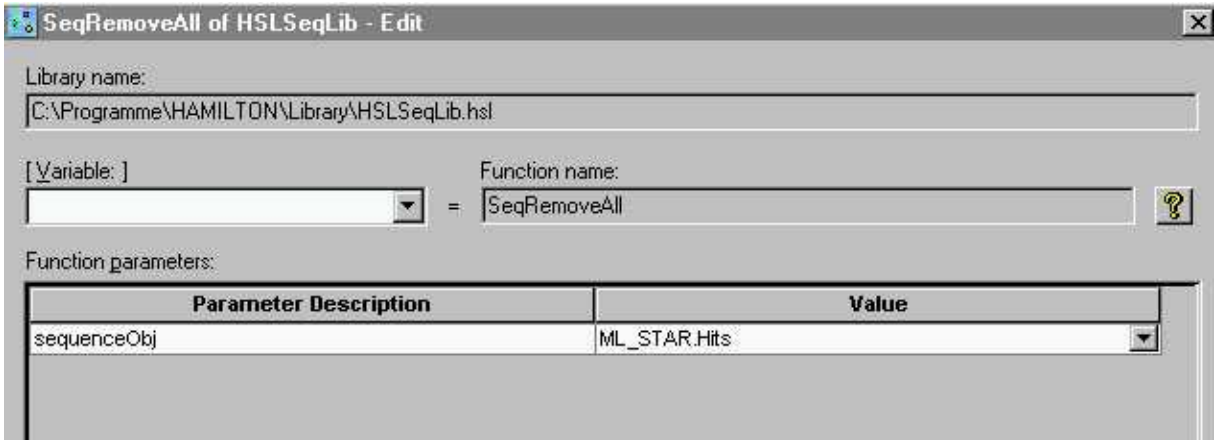


The variable “sqlSelect” is also a string. It is generated by adding the “sql selection statement” and the threshold value. This statement, if applied to the data set, selects only the records (lines) having absorbance values A>threshold.

The syntax of the SQL statement is: "SELECT * FROM [absorbance$] WHERE OD > "

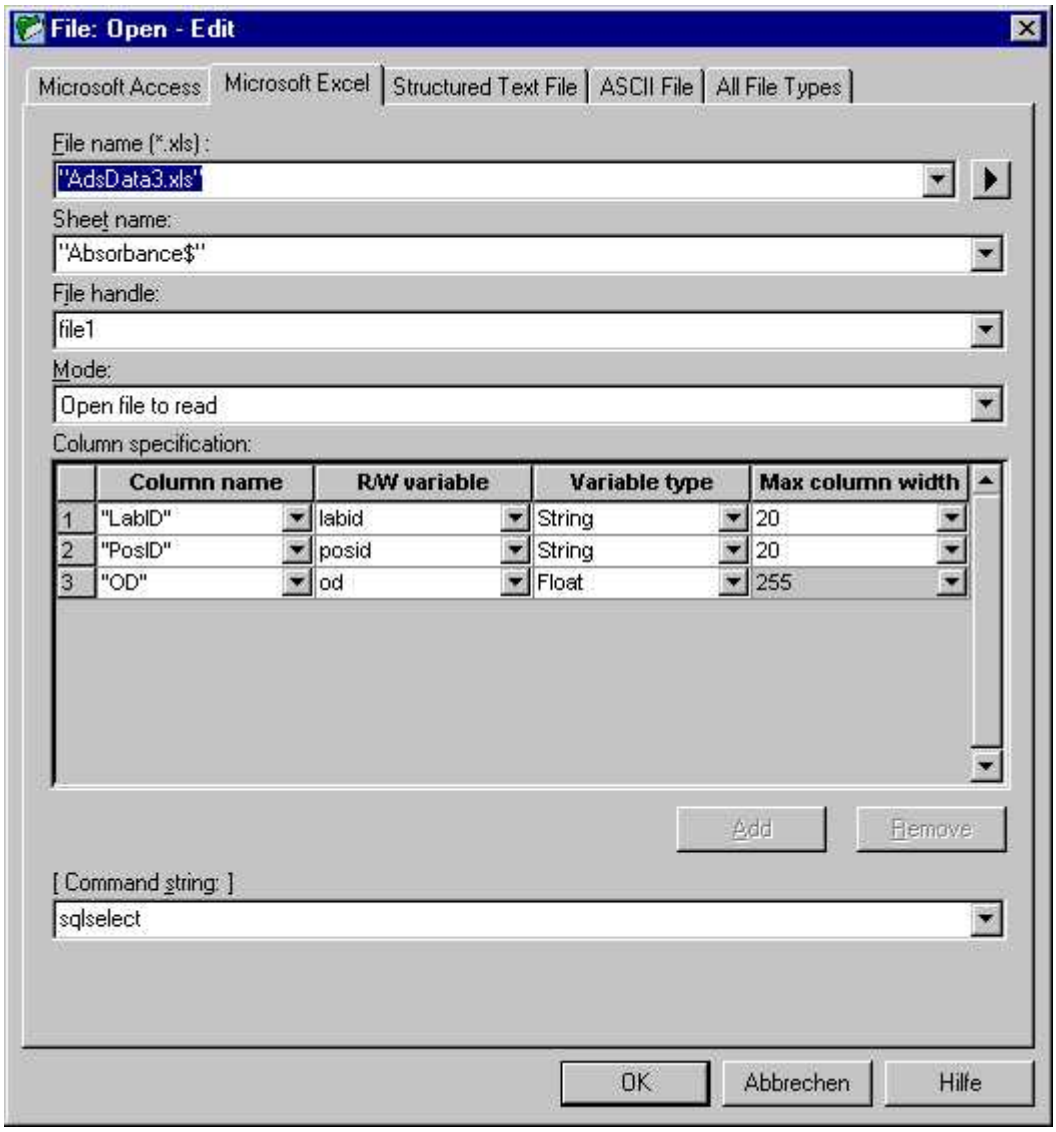
Here “absorbance$” refers to the name of the Excel sheet which is going to be opened during the next steps.

Now, the sequence “Hits” as generated within the sequence editor is reduced to 0 (all entries are deleted) using a library function call:



The parameter is the sequence ML_STAR.Hits.

Next, the file holding the absorbance information is opened. The file format is also defined within this step. Drag the green “Open File” icon to the method tree:



Select the tab for opening excel files. In the first input field, the file name is requested. Note that the file type is an Excel file (.xls) where the sheet name (sheet1 if not defined otherwise) and the $ sign must be added within the quotation marks. Define a file specifier (here the default: file1), which is just a name for the file used within your method. Data will later be read from this file, making reference to this file specifier. Select “Open File to Read” as a mode, since you want to read data from the file. Now define the file format. Here a variable is assigned to each column of the file. Later, for each reading step, one record (one line) is read from the file, and the numbers read are assigned to their corresponding variables automatically.

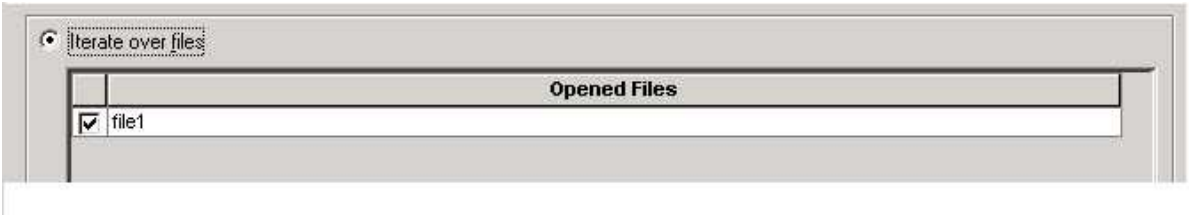
Now define the file structure. One line in the “column specification” of the file opening dialog represents one column in your file. Click on the “Add” button to add the next line to the dialog.

Enter the data as given in the foregoing screen. A header, a variable and a variable type are assigned to each column. The maximum column width is 20 for the string variables which are of interest here.

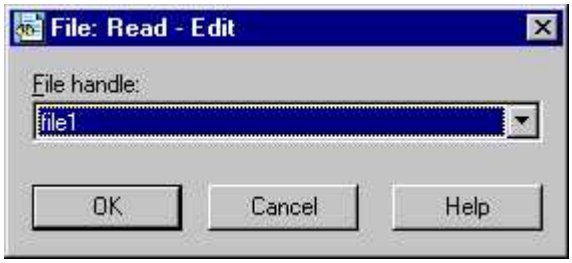
Select the variable “sqlSelect” from the dropdown list as a command string.

Click OK to finish the definition.

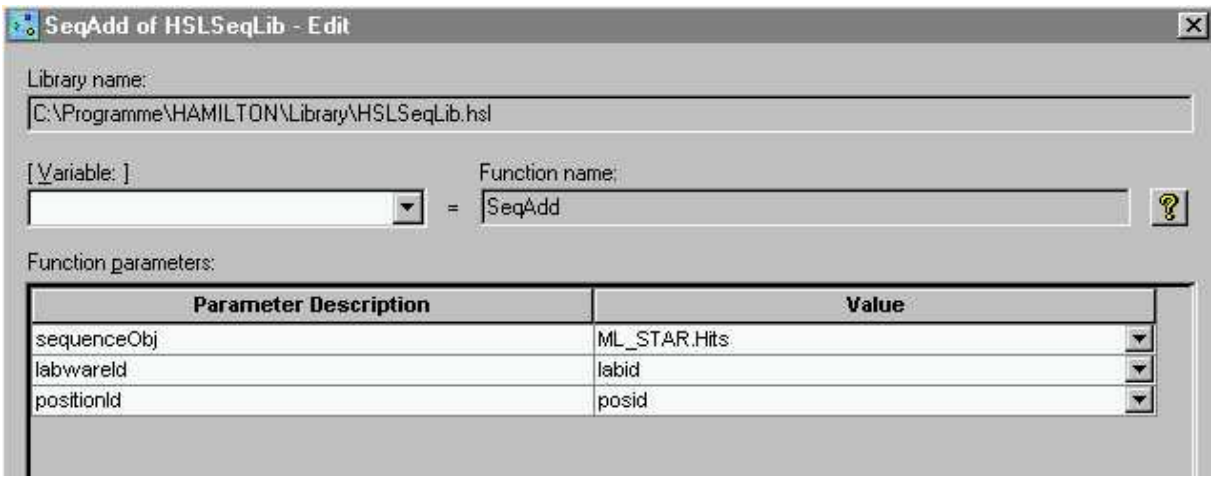
A loop is started, looping over the input file (until the file has been read completely):



Within the loop, the first record of the file is read:

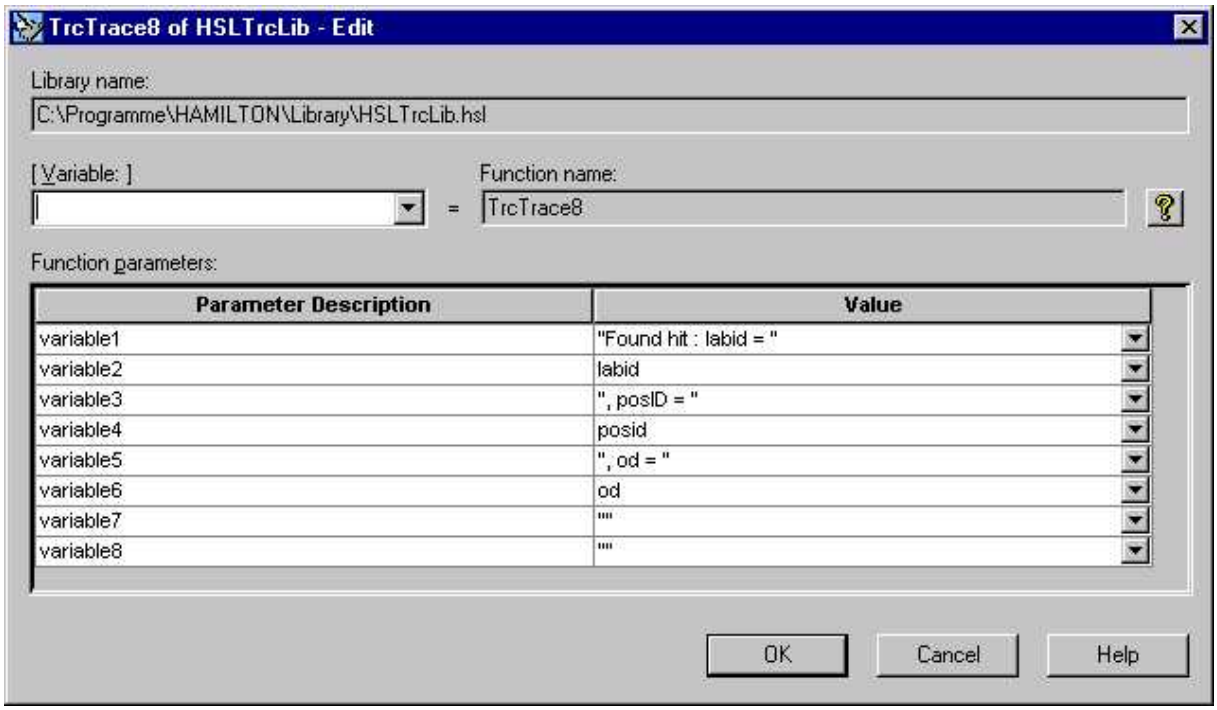


Next, using the library function “SeqAdd”, a sequence position is added to the hit sequence. No if-then is needed here, because the SQL selection string automatically skips all records with absorption values less than or equal to the value of the “threshold” variable.

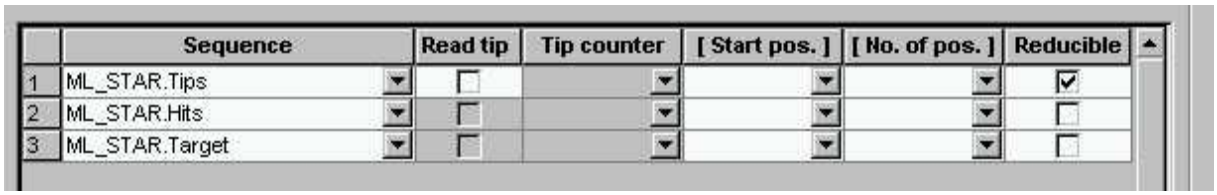


Then, an entry is made into the methods trace file (HitPick.trc) using the library function “TrcTrace8”, to inform the user.

This function adds a text of eight variables to the trace file.



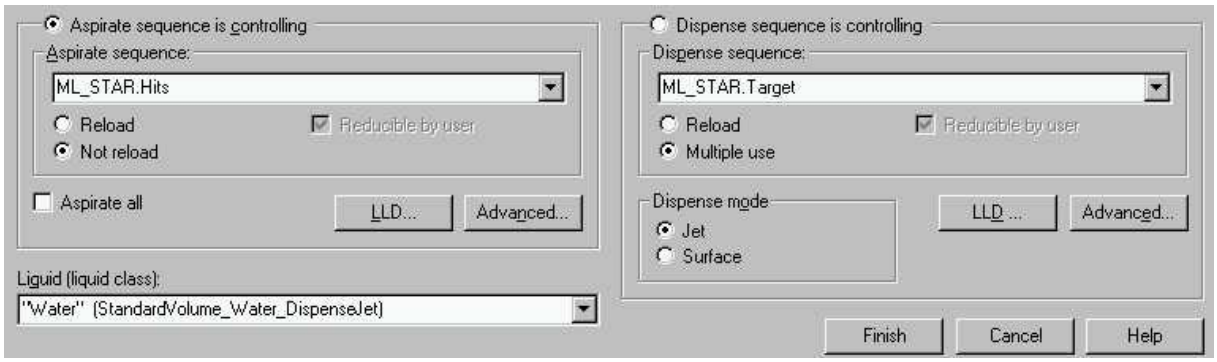
After the file has been analyzed and the Hit sequence has been generated, the loading of the sequences (carriers) to the deck may start. The load SMART Step looks like this:



NOTE

The sequence Hits, that has been freshly created and which has a current position of 0 (because it has reached its end position) is automatically set to 1 by the loading step. If there is no entry in the field "Start Pos", then the current position of the sequence is set to the first position.

The pipetting is then performed by the SMART Step pipette. The important sequence settings are shown in the next screen:



The aspiration sequence (the hits) is controlling here and not reloadable. The target sequence is not reloadable either. The rest of the settings is very similar to the previous example “OnePlateToPlatePipette”.

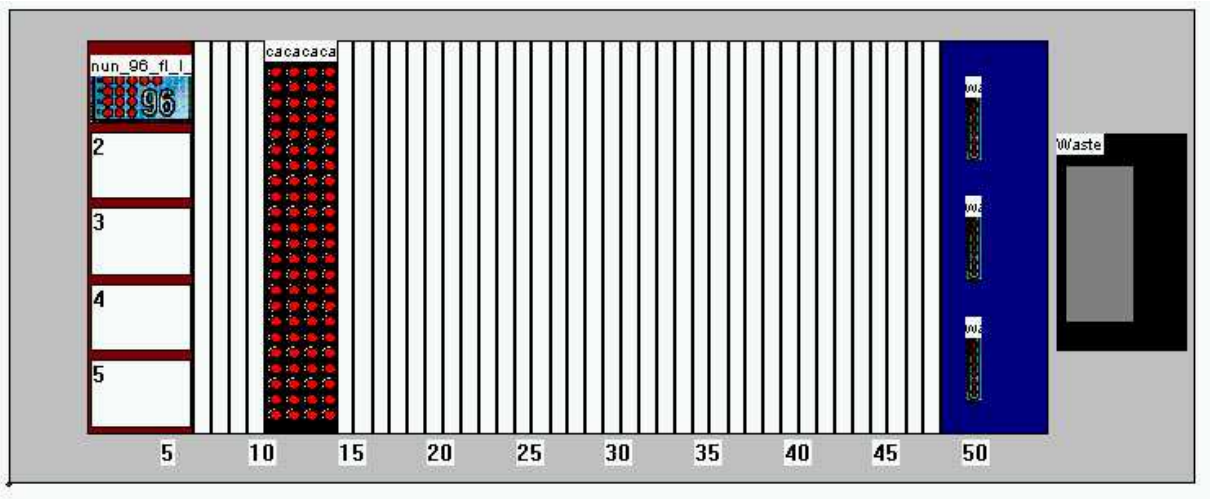
Finally, the unloading takes place in the usual way.

You will find additional examples, involving, for instance, the reading of a comma-separated file in MTP map format (HitPick_CSV), in your demo methods directory.

13.7 A Method Using Steel Needles, Single and SMART Steps Including Sample Reduction

The following method transfers liquid from tubes to a plate using steel needles. The aspirations and dispenses are done with single steps and for the washing of needles the SMART Steps are used. A reduction of the number of samples is possible.

The method uses the following deck layout:



A plate and a set of four tube carriers (of 24 tubes is used). Add the standard plate carrier (PLT_CAR_L5MD). Add a Nunc plate (nun_96_fl_l.rck) and name it “Plate”. as well as the tube carriers (car24_cup15x100.rck) as decribed previously. In addition, a wash station is added to the rightmost position on the deck. Select the carrier for the wash station “car_wash_1_standardneedle.tml” from the ML_Star directory.

Save the deck layout under the name “TubesToPlateWithNeedles(.lay)”.

Create a sequence holding all tubes called “Samples”, and a second sequence holding all wash modules and name it “WashStations”, using the sequence editor as described in the previous examples.

Click on Edit Method and link the deck layout to the method to be written.

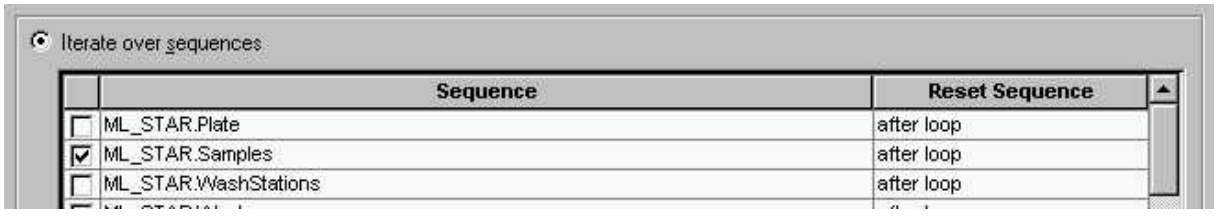
Here's what the method finally looks like:

A method using needles.

Load the "Plate" and "Samples" sequences. The "Samples" sequence is reducible (check the checkbox) to allow a runtime reduction of samples.

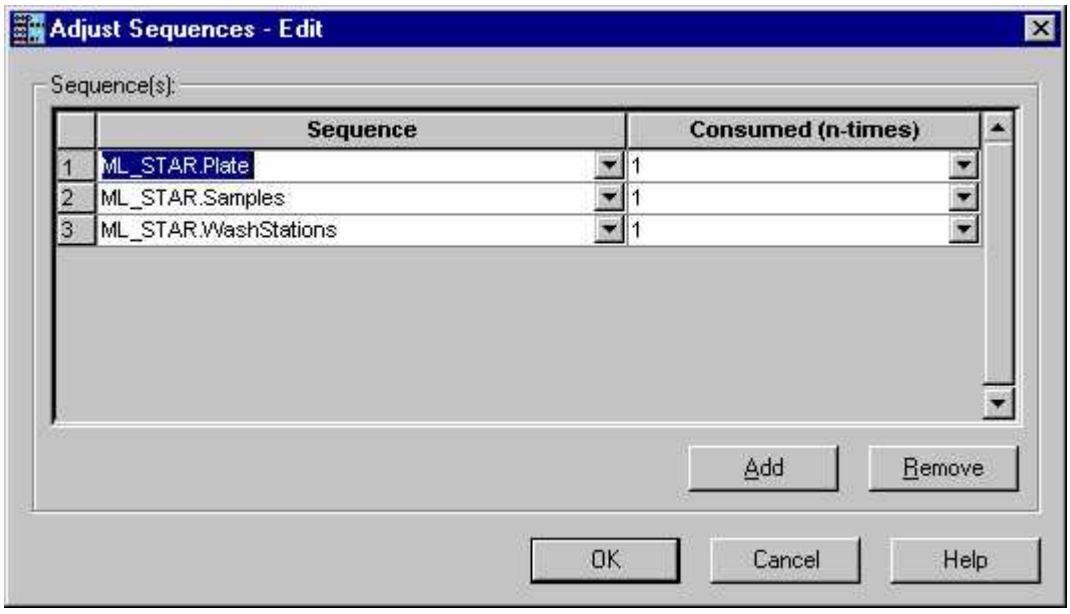
Drag the SMART Step "Needle Wash Settings" to the next line, accept the default settings and click OK. Select the sequence ML_STAR.WashStations.

Drag a loop from the general commands.



Loop over the Samples sequence.

The next step is the “adjust sequences” command. This command is used to automatically handle a reduction of samples when using single steps. If, say, only 4 samples are left over in the last step, “adjust sequences” ensures that the system will pick up only 4 tips, aspirate 4 samples, and dispenses into 4 plate positions, leaving the other 4 channels unused.



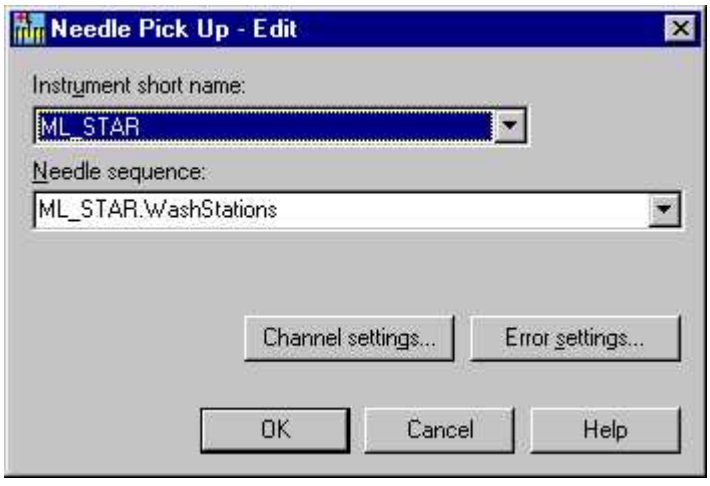
Multiple sequences may be added to the dialog by clicking on the “Add” button and selecting the sequence from the dropdown field.

NOTE

Every sequence that is used within the loop has to be added to the “adjust sequences” command, including the tip sequence.

The position of the “adjust sequences” command within the loop (line no.) is not important, as long as it is placed within the loop.

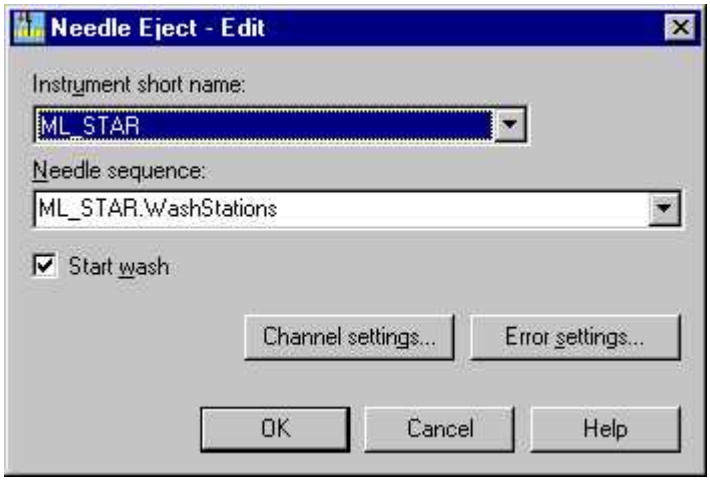
Now, pick up needles using the SMART Step:



Select the sequence ML_STAR.WashStations holding all individual wash modules. Click OK.

Aspirate from the “Samples” sequence and dispense into the “Plate” sequence with the settings as described in the example “TubesToPLate”.

Eject the needles with the SMART Step:



Again, select the sequence ML_STAR.WashStations and check the checkbox “Start wash”.

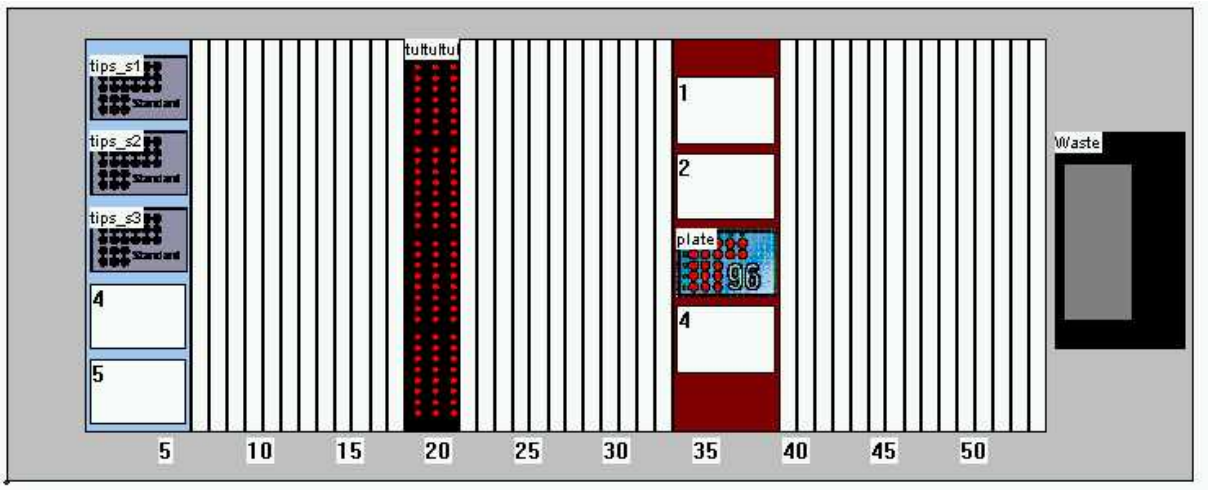
The SMART Steps “needle pick up” and “needle eject” will handle the washing within the alternating three wash modules automatically.

Finally, the carriers may be unloaded.

13.8 A General Example for User In- and Outputs, Sample Tracking, Sequence Manipulation, Tip Counter, and TCC Use

The following example gives an idea of the additional functionality of the Microlab STAR user software.

Create the following deck layout holding a tip carrier TIP_CAR_480 with tip racks (at least two racks), 3 tube carriers (car32_cup12x75.rck) for 32 tubes and a temperature-controlled carrier (car_tcc_1.tml) named “TempCarrier” with a standard nunc plate (nun_96_fl_l.rck), named “plate”.

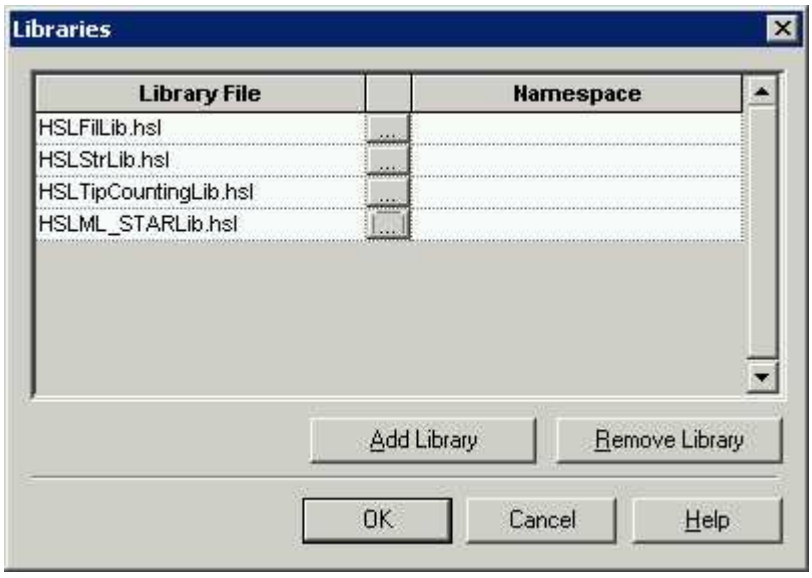


Save the layout under the name “Example” (do not use a name longer than 20 characters including extension).

Create sequences for all tips (“AllTips”) and for all tubes (“AllTubes”), using the sequence editor.

Open the method editor and link the deck layout to the method to be written.

Link the following libraries to the method (method->libraries):



NOTE

This method uses the sample tracker. Make sure the checkbox "sample tracker" within the configuration editor is checked. The name of a method using the sample tracker must not exceed 20 characters including the extensions.

Finally, the method should look like this, following the first step "Initialize" (the display of method steps continues overleaf):

Example.med

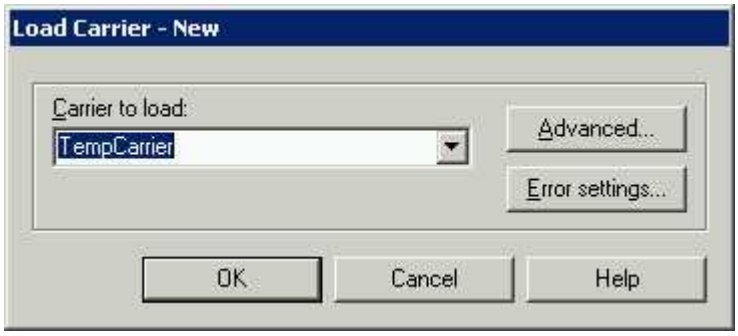
	Method
2	LoadCarrier on ML_STAR , Rack type: TempCarrier, Barcode file: "barcode_1.txt" Return value count: 4.
3	LoadCarrier on ML_STAR , Rack type: tubes1, Barcode file: "barcode_1.txt" Return value count: 4.
4	LoadCarrier on ML_STAR , Rack type: tubes2, Barcode file: "barcode_1.txt" Return value count: 4.
5	LoadCarrier on ML_STAR , Rack type: tubes3, Barcode file: "barcode_1.txt" Return value count: 4.
6	Assignment 'TipCounterVariable' = '"MyStandardTips"'
7	Comment <Set TCC>
8	User Input Dialog Title: '"Input"', Return Value: '', Sound: '', Timeout: 'infinite' Input: NoSamples ("How many samples today?", Integer, 96, 1, 96) CTemp ("Carrier Temperature in C?", Float, 10.0)
9	SetCarrierTemperature on ML_STAR Rack type: TempCarrier, Temperature: (1) Ambient °C Return value count: 3.
10	Comment <Read Tip Counter and Request for Reset>
11	Edit of HSLTipCountingLib TipCount::Edit(ML_STAR.AllTips, TipCounterVariable, ML_STAR, 7)
12	Comment <Reduce No of Samples to input value>
13	Sequence: Set End Position end position of sequence 'ML_STAR.AllTubes' = 'NoSamples'
14	Comment <Start Pipetting>
15	Loop over following sequences: - ML_STAR.AllTubes 'loopCounter1' used as loop counter variable

16	Adjust Sequences Adjust the following sequences: - 'ML_STAR.AllTips' ('1' times consumed) - 'ML_STAR.AllTubes' ('1' times consumed) - 'ML_STAR.plate' ('1' times consumed)
17	TipPickUp on ML_STAR Channel (1..8): 11111111, Optimized channel use: All sequence position, Sequence: ML_STAR.AllTips, Sequence counting: (1) Automatic Return value count: 3.
18	Aspirate on ML_STAR Channel (1..8): 11111111, Optimized channel use: All sequence position, Sequence: ML_STAR.AllTubes, Sequence counting: (1) Automatic, Liquid name: StandardVolume_Water_DispenseJet, Volume: 220 µl, Mix volume: * µl, Cycles: 0, Position relative to liquid surface: * mm, LLD settings: on, Capacitive:4 Return value count: 3.
19	Dispense on ML_STAR Channel (1..8): 11111111, Optimized channel use: All sequence position, Sequence: ML_STAR.plate, Sequence counting: (1) Automatic, Liquid name: Use the same liquid as aspirated, Volume: 220 µl, Mix volume: * µl, Cycles: 0, Position relative to liquid surface: * mm, LLD settings: off, Liquid following: on Return value count: 3.
20	TipEject on ML_STAR Channel (1..8): 11111111, Optimized channel use: All sequence position, Sequence: ML_STAR.Waste16, Sequence counting: (0) Manually Return value count: 3.
21	End Loop - ML_STAR.AllTubes (Reset Sequence after loop)
22	Write of HSLTipCountingLib TipCount::Write(ML_STAR.AllTips, TipCounterVariable)
23	Comment <Unload Tip Carrier on request>
24	User Output Dialog Title: '"Output"', Return Value: 'OutputReturn', Buttons: "Yes' and 'No' button', Default: 'No', Icons: 'Display information message icon', Sound: '', Timeout: 'infinite' Output: "Unload the tip carrier?"
25	If (OutputReturn is equal to 6)
26	UnloadCarrier on ML_STAR Rack type: tipcarrier Return value count: 3.
27	End If

28	Comment <Create a Microlab AT Barcode Format for plate>
29	CreateATBarcodefile of HSLML_STARLib HSLML_STAR::CreateATBarcodefile(ML_STAR, "plate")

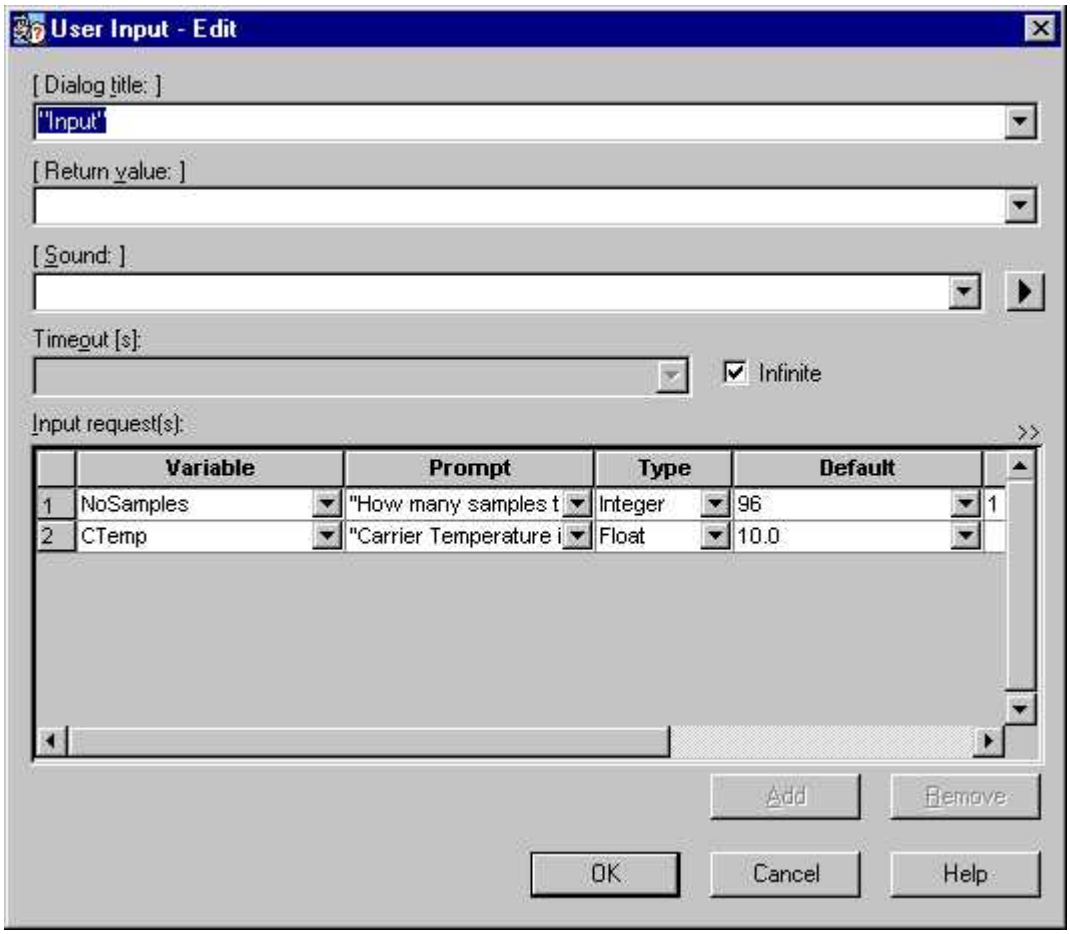
The sample method.

Firstly, initialize the system and load all carriers using the single steps:



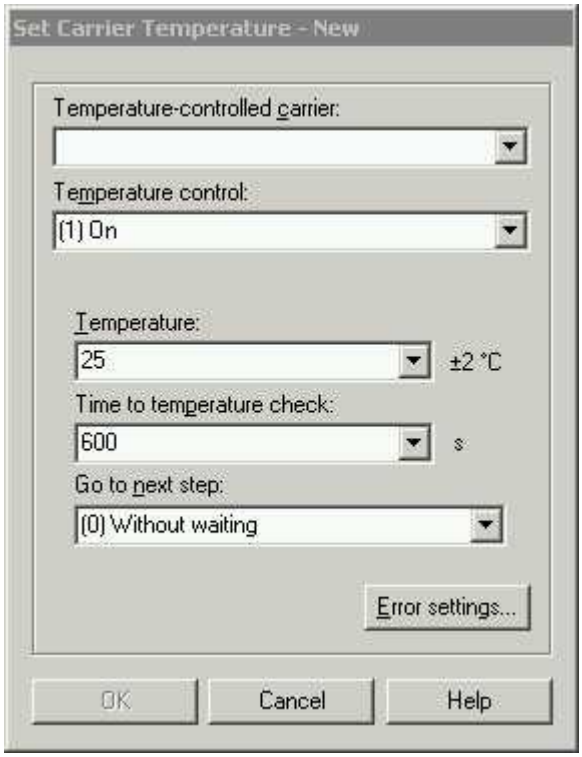
Assign the name of the tip counter (MyStandardTips) to be used throughout this method to the variable 'TipCounterVariable' (as a string, i.e. within quotation marks) :

Define a user input request for the temperature of the TCC as well as the number of samples to be transfered.



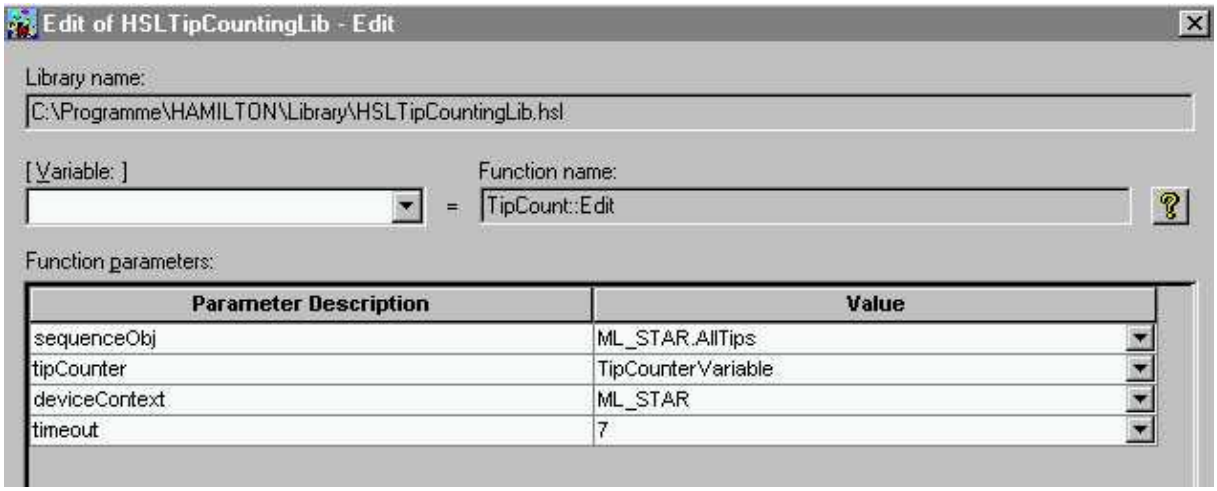
For every input, a variable name, a text to prompt for the variable (within quotation marks), the variable type, and a default value have to be given. Type in the inputs as shown in line 8 of the method overview.

Set the TCC settings according to the following dialog:



The temperature is now set to the variable CTemp, the value of which is to be typed in by the user at runtime.

Now, the tip counting is specified using functions from the tip counter library. First, the user will be requested to reset the tip counter (if required):

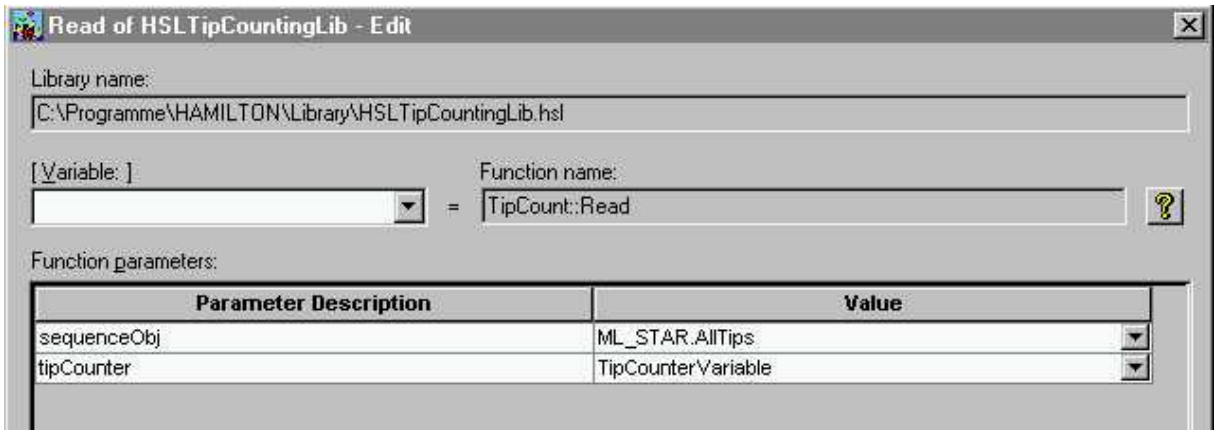


The “TipCountingEdit “ function opens up a dialog on runtime, showing the tip sequence and input fields for start and end positions. Setting the start position to 1 will reset the tip counter - then tips have to be reloaded manually. This function has parameters: the tip sequence, the name of the tip counter (which is stored in

the variable “TipCounterVariable” here), the device context (select the only choice “ML_STAR” from the dropdown field), and a timeout. This timeout is used to close the

dialog automatically without user intervention after 7 seconds (in this case) by defaulting to the current position of the tip counter.

Just for demonstration, and not part of this method - another step could be to simply read the tip counter:

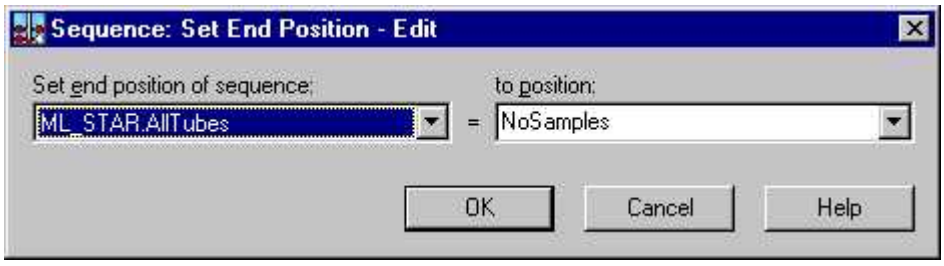


The parameters are the tip sequence and the variable, storing the name of the tip counter.

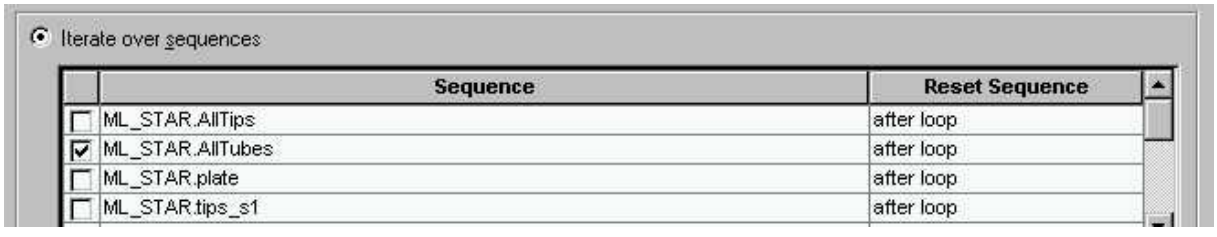
NOTE

Either one of the two steps involving the tip counter may be sufficent. The edit step also sets the counter to the position read. If no request for a manual reset is needed, the read step alone may be used.

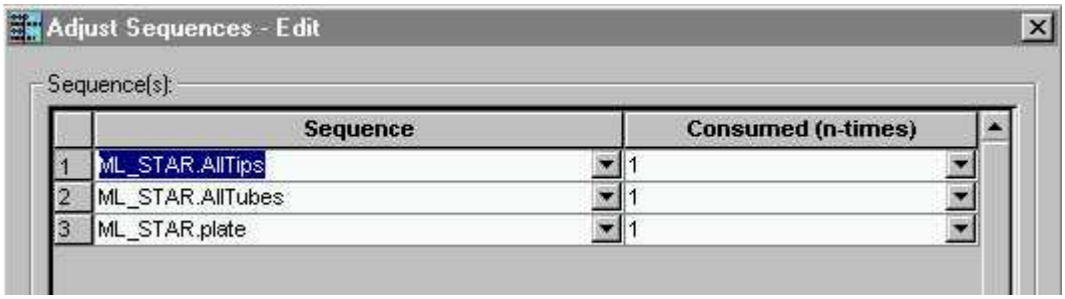
Then sample reduction from the user input is done by setting the end position of the sequence “AllTubes” to the number as input:



The pipetting loop thus loops over the sequence “AllTubes”:



Consequently, the “adjust sequences” step is used to handle sample reduction if less than the full number of channels are needed:



Add all the sequences shown in the dialog, and recall the notes in the previous chapter.

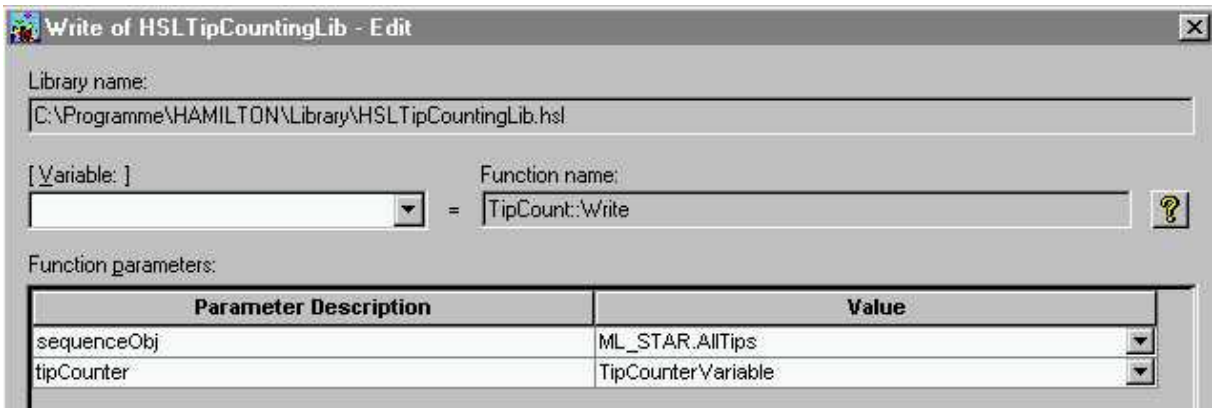
For the pipetting (single) steps, the settings are as in the other examples:

Tip Pick-up: Sequence “AllTips”, counting automatic

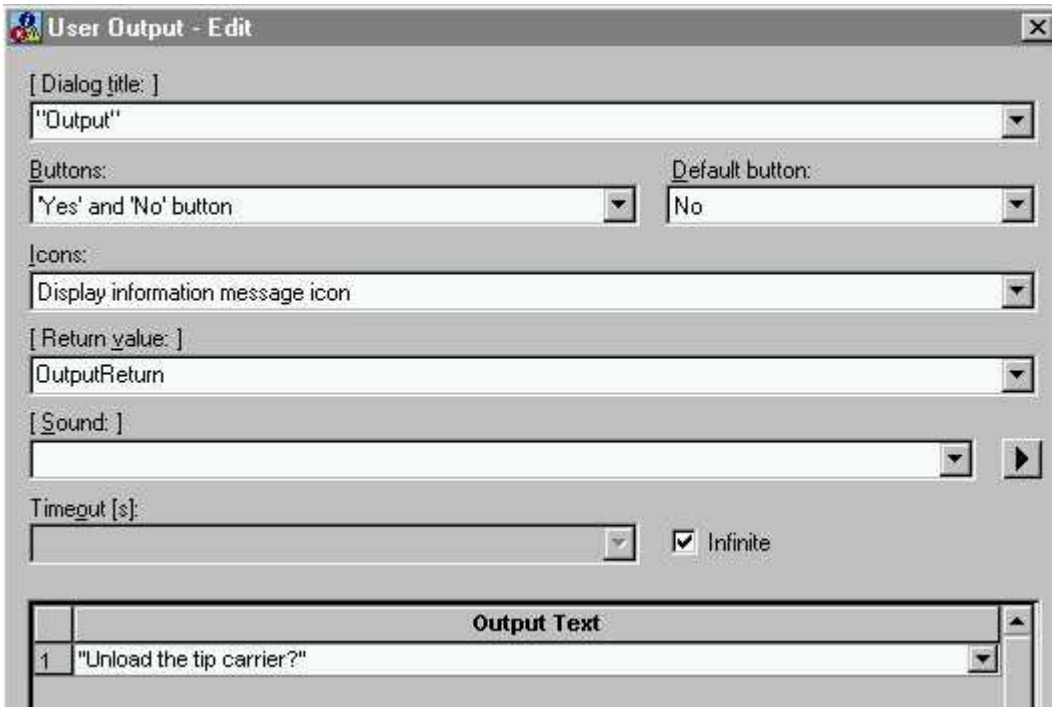
Aspiration: Sequence “AllTubes”, counting automatic

Dispense: Sequence “plate”, counting automatic

Then, the current position of the tip sequence is written into the tip counter:



Now a further possibility will be demonstrated: how to make decisions within the method. Suppose a user output is programmed:

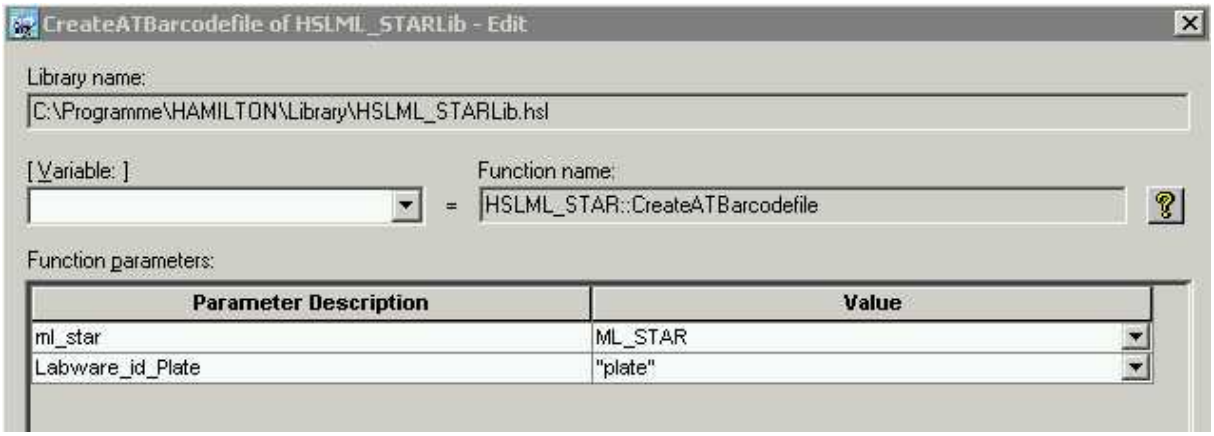


The dialog at runtime prompts for the tip carrier to be unloaded and shows a YES and a NO button. If the user clicks YES, the return value of this dialog will be 6, if he clicks NO, the return value will be 7. This value is stored to the variable “OutputReturn”, as specified in the dialog. The decision is made depending on the value of this variable.

The if – then construct compares the variable to the fixed value 6 (=YES clicked). Only then is the code between the if and the end-if statement executed:

This code is the unloading command for the tip carrier.

Finally, the AT Barcode Filter Program of the Microlab STAR user software is started by a library function from the HSLML_STAR.lib library. This function generates the Microlab AT-like barcode file for the specified plate within the c:\barcodes directory:



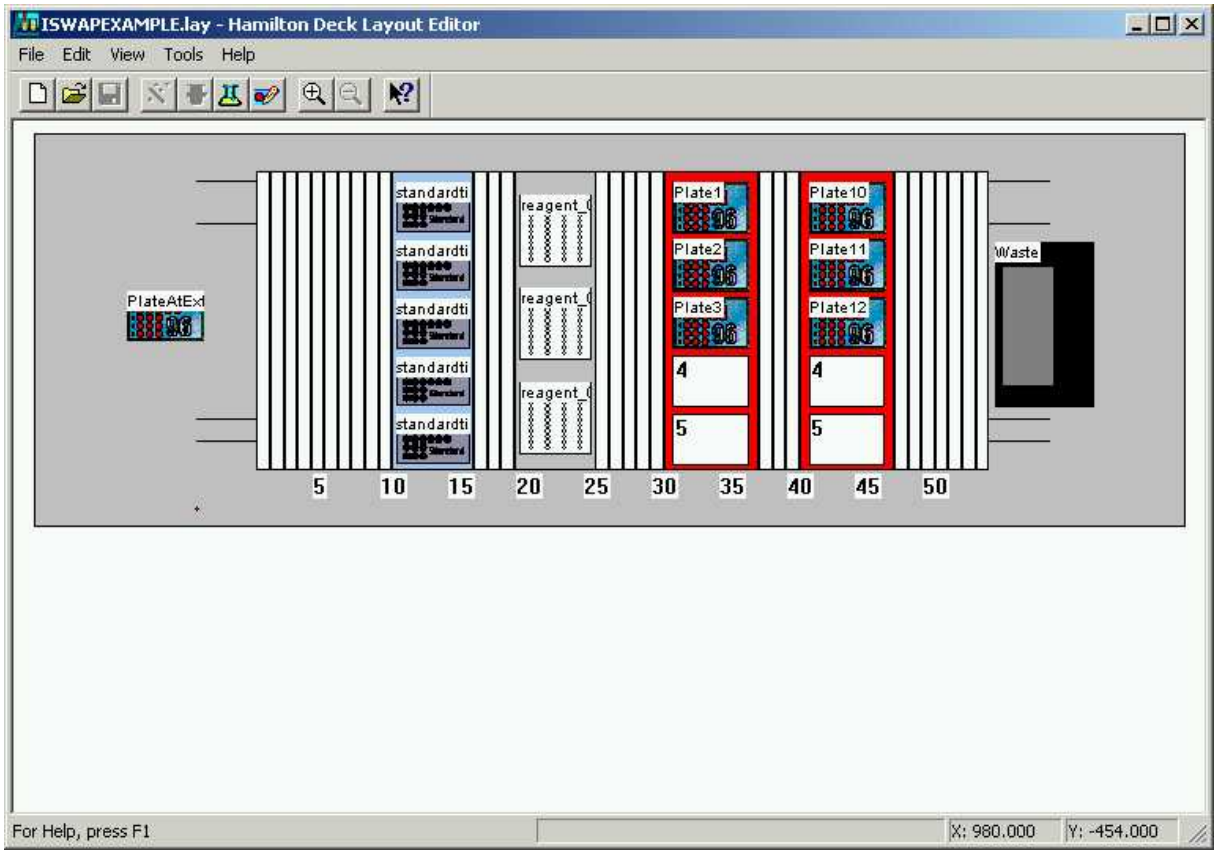
13.9 Using the iSWAP robotic plate handler

The following example demonstrates the use of the iSWAP with ML STAR. A set of plates is pipetted, transfered to a barcode scanner position without opening the robotic hand, and finally transferred to another carrier.

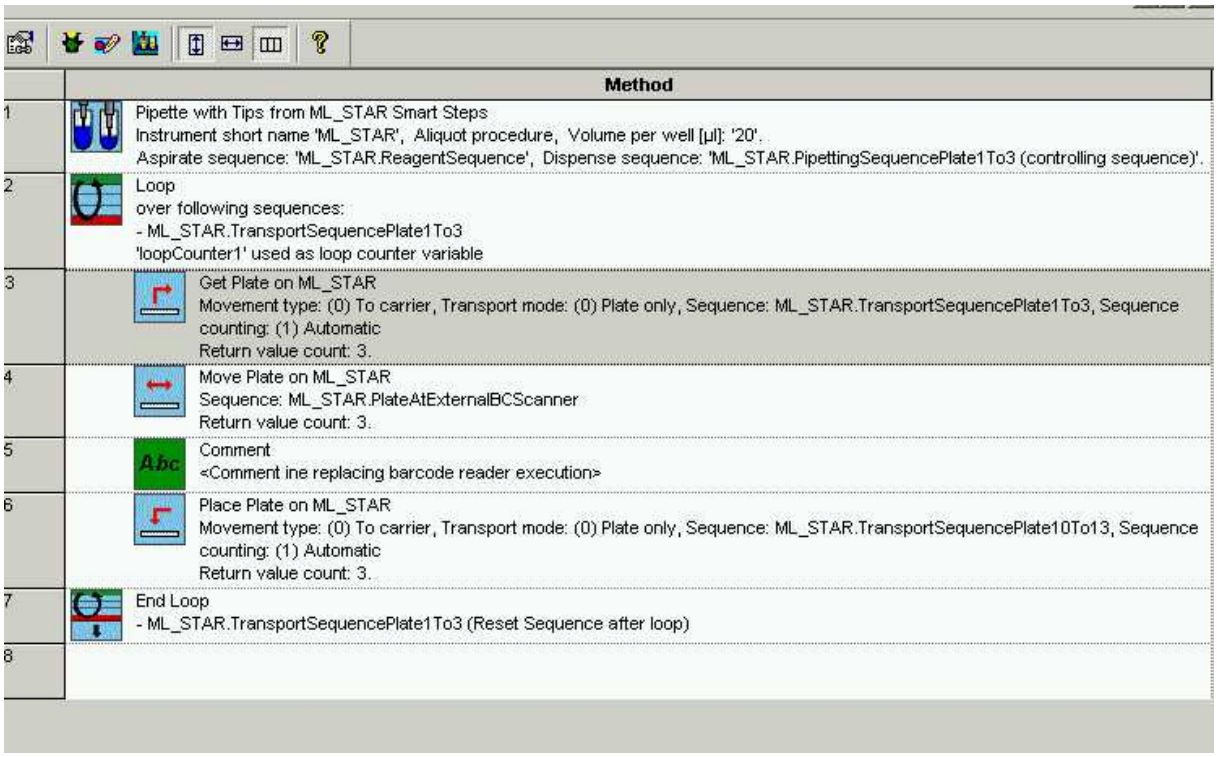
Note that the iSWAP works on the basis of sequences. Plates are moved from one sequence to another. The sequences remain fixed on the deck, but the plates change sequences. For this to happen, target and source plate position must be of the same labware type.

For this example, let's create the following deck layout using PLT-CAR-L5FLEX for the microplates. Use the "Add Labware" dialog (right click into the deck, where no labware is located) to add a plate position directly to the deck in front of an external barcode reader. Enter the coordinates

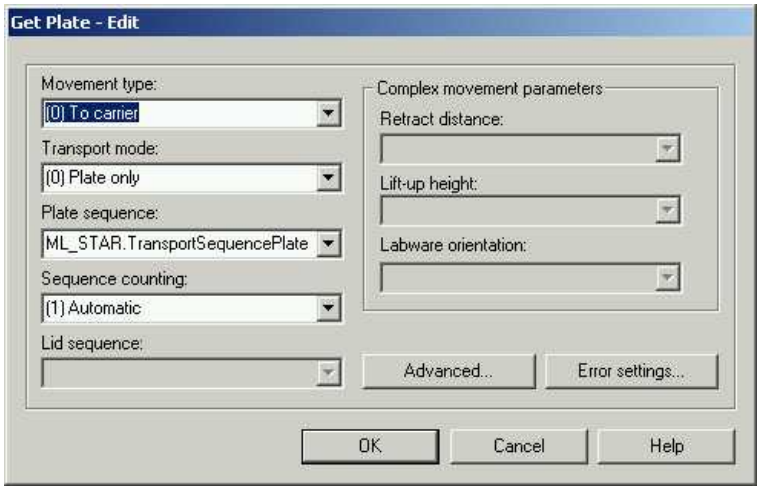
(x = -100mm, y=350mm, z=220mm). Click OK.



Create independent sequences for pipetting steps and transport steps, using the sequence editor. Both the transport and pipetting sequences span all 3 plates. The first step of the method is a simple aliquot step to transfer reagent to the plates, located at plate carrier 1 using the SMART Step “Pipette“.



Then, we loop over the transport sequence “TransportSequencePlate1To“ spanning all plates on the left-hand plate carrier. At the beginning of the method, the plates are located on this plate carrier. In step 3 we pick up the plate from the current sequence position of sequence “TransportSequencePlate1To“:



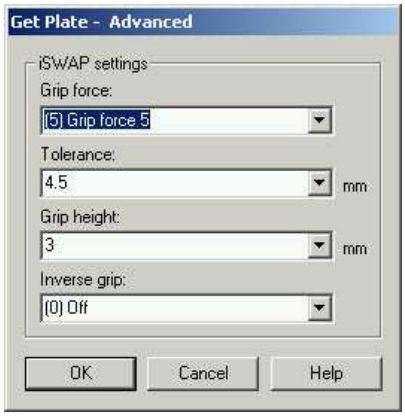
The “Movement Type“gives 2 options: either to pick up the plate from a carrier, which is a simple motion, or to pick up a plate from a reader, etc., which is a complex motion. For a complex motion additional inputs can be made, such as the “retract distance“ and the “lift-up height“ (see online help).

“Transport mode“ specifies whether the plate is to be picked up - with or without a lid - or just the lid itself.

Sequence counting again determines whether the iSWAP returns to the same or to the next plate of the given sequence next time.

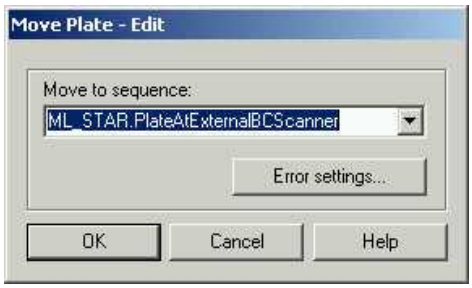
Note that a plate lid by itself has a sequence with 2 positions. Selecting the transport mode “Plate with Lid“ will increment the plate as well as the lid sequence.

Under “Advanced“, additional settings may be chosen:

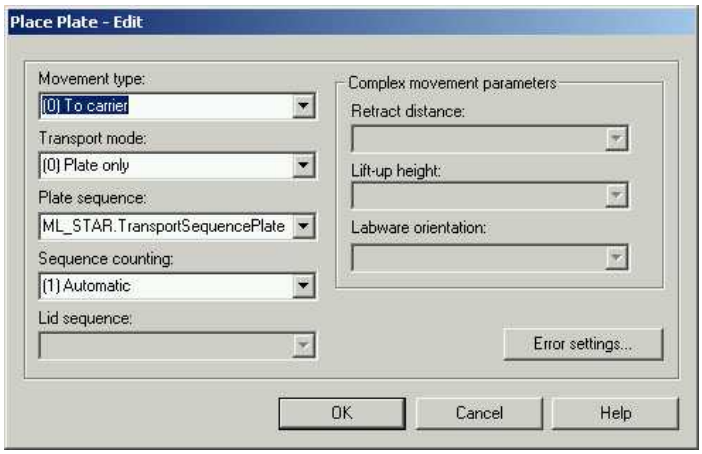


“Grip force“ determines the force that is used to grip the plate. The tolerance gives a bandwidth in mm for the closing mechanism of the robotic hand in which the plate must be gripped (torque sensor of iSWAP responds). The grip height is the distance that iSWAP moves down from the plate’s upper rim to grip the plate. Inverse grip instructs the robotic hand to pick up a plate with the opposite orientation of the iSWAP (this is not always possible).

The plate is now moved to the position of the barcode scanner (the position of the sequence “PlateAtExternalBCScanner“), without opening the hand:



The final step places the plate on the right-hand plate carrier:

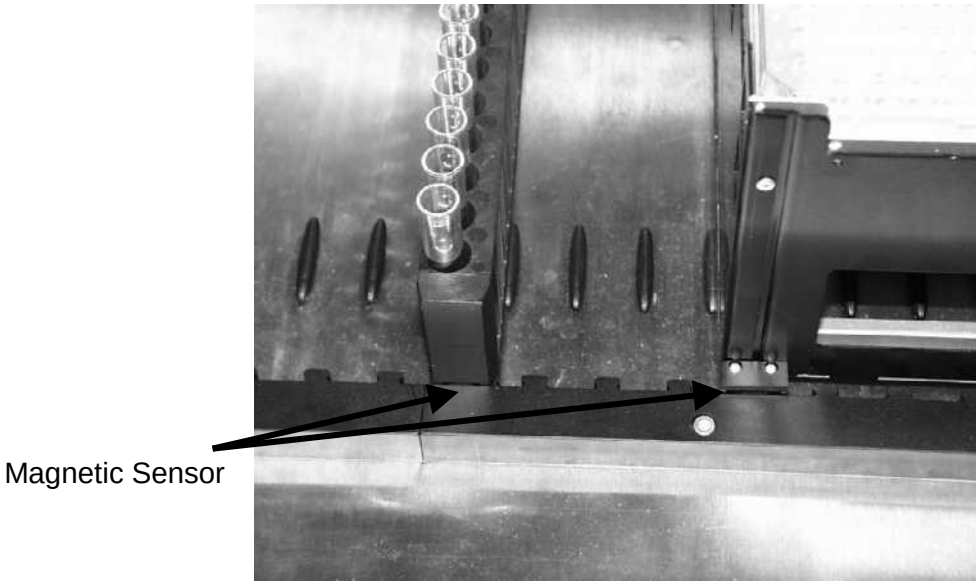


14 Loading the Microlab STAR

14.1 Manual Load

To load the Microlab STAR, first load the carriers with the appropriate labware.

.1) If you have incorporated **'LoadCarrier'** commands into your method, you can start the run and the instrument will prompt you to load the carriers manually onto the deck. Make sure the carriers are inserted completely, until they lock into the rear connectors. A magnetic detector checks whether your loading of carriers is correct.



.2) If you have **no 'LoadCarrier'** commands incorporated into your method, you must load the carriers manually into the positions on the deck defined in the deck layout **before** the run is started. Make sure that the carriers are inserted completely, until they touch the rear connectors.

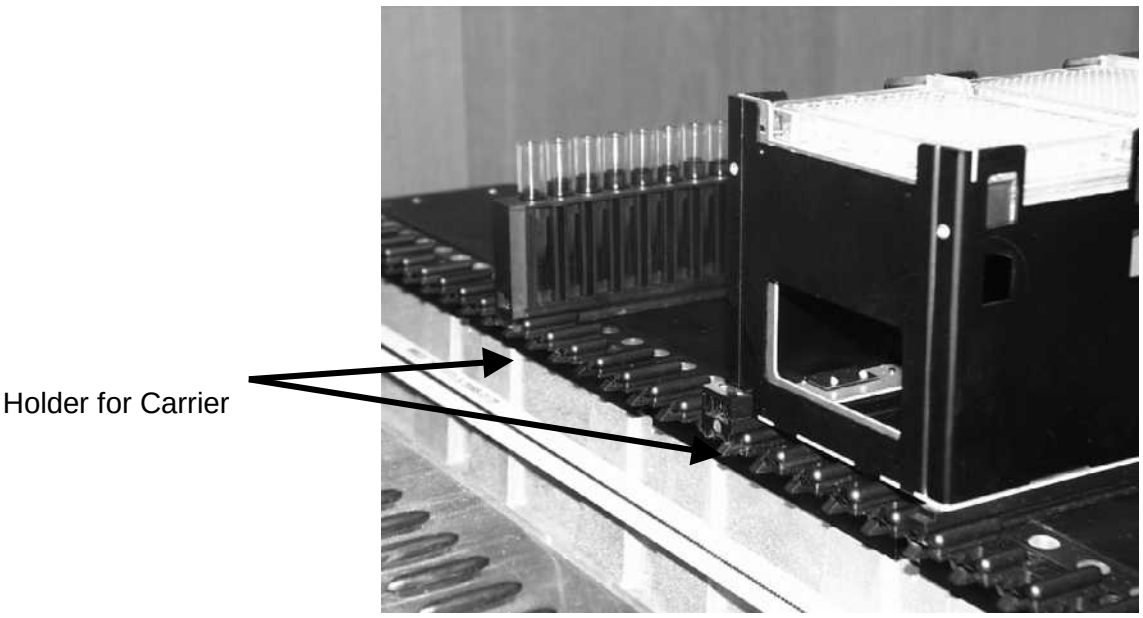
NOTE

If no load carrier commands are specified in the method, no check of carrier positions or correct loading is made.

Autoload

When using the Autoload option, first load the carriers with the appropriate labware.

.1) If you have incorporated **'LoadCarrier'** commands into your method: start the "Run Screen" as described in the following sections, and run the method. The correct positions for the insertion of carriers will be highlighted by the green LEDs. Insert the carriers into the tracks of the Autoload tray until they touch the holding pins on the far side of the tray.



Click "Load" in the dialog, and the carriers are loaded onto the deck automatically by the "load carrier" command in the method. At the same time, the barcodes of carriers and labware are read and stored in a file.

Alternatively, load the carriers on to the defined positions of the autoload tray before starting the method. Loading and barcode reading will then be performed without user input.

.2) To repeat what we said already: if you have no 'LoadCarrier' commands incorporated into your method, you must load the carriers manually into the positions on the deck defined in the deck layout before the run is started. Make sure that the carriers are inserted completely, until they touch the rear connectors.

NOTE

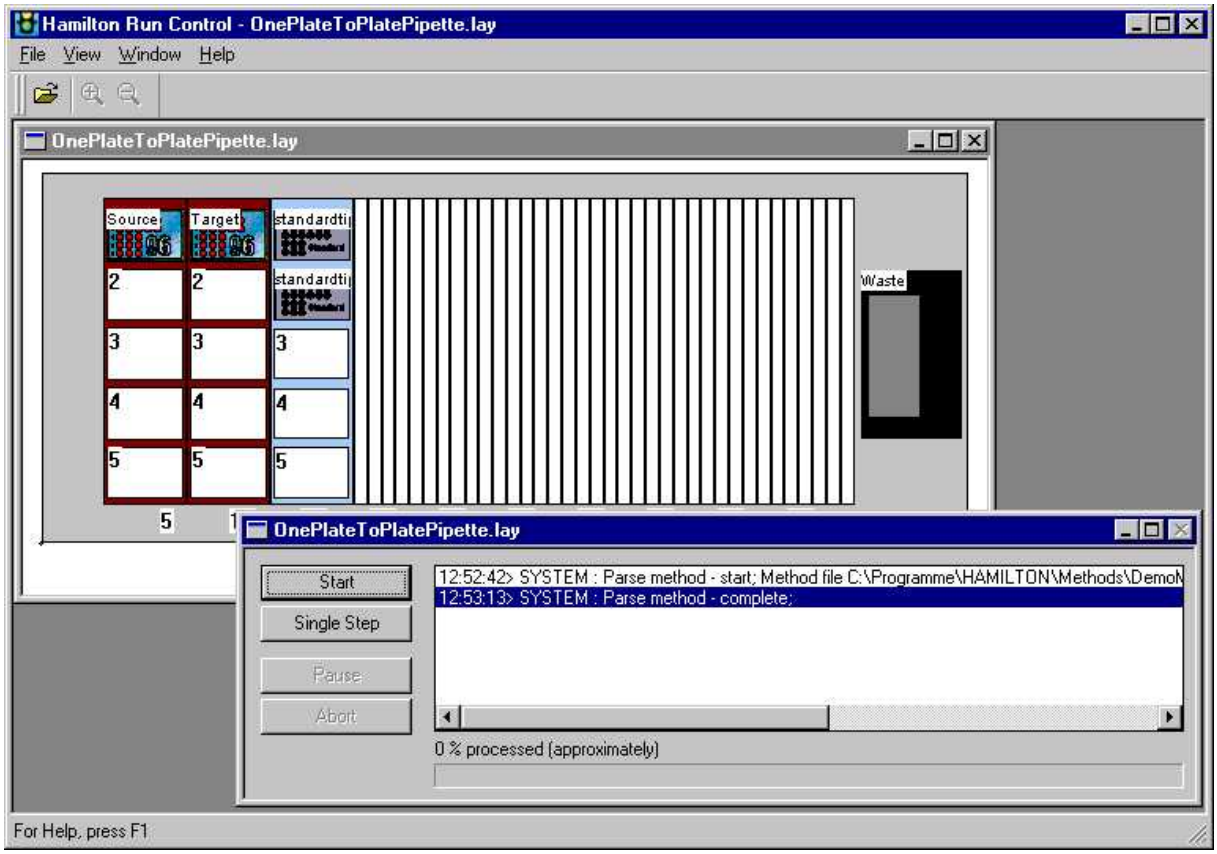
If no load carrier commands are specified in the method, no check of carrier positions or correct loading is made.

15 Running the Microlab STAR

15.1 Running a Sample Method with the Instrument

To run the method “OnePlateToPlatePipette” from the example section, you need to access the run control. Double-click the “Microlab STAR Run” short cut on the desk top:

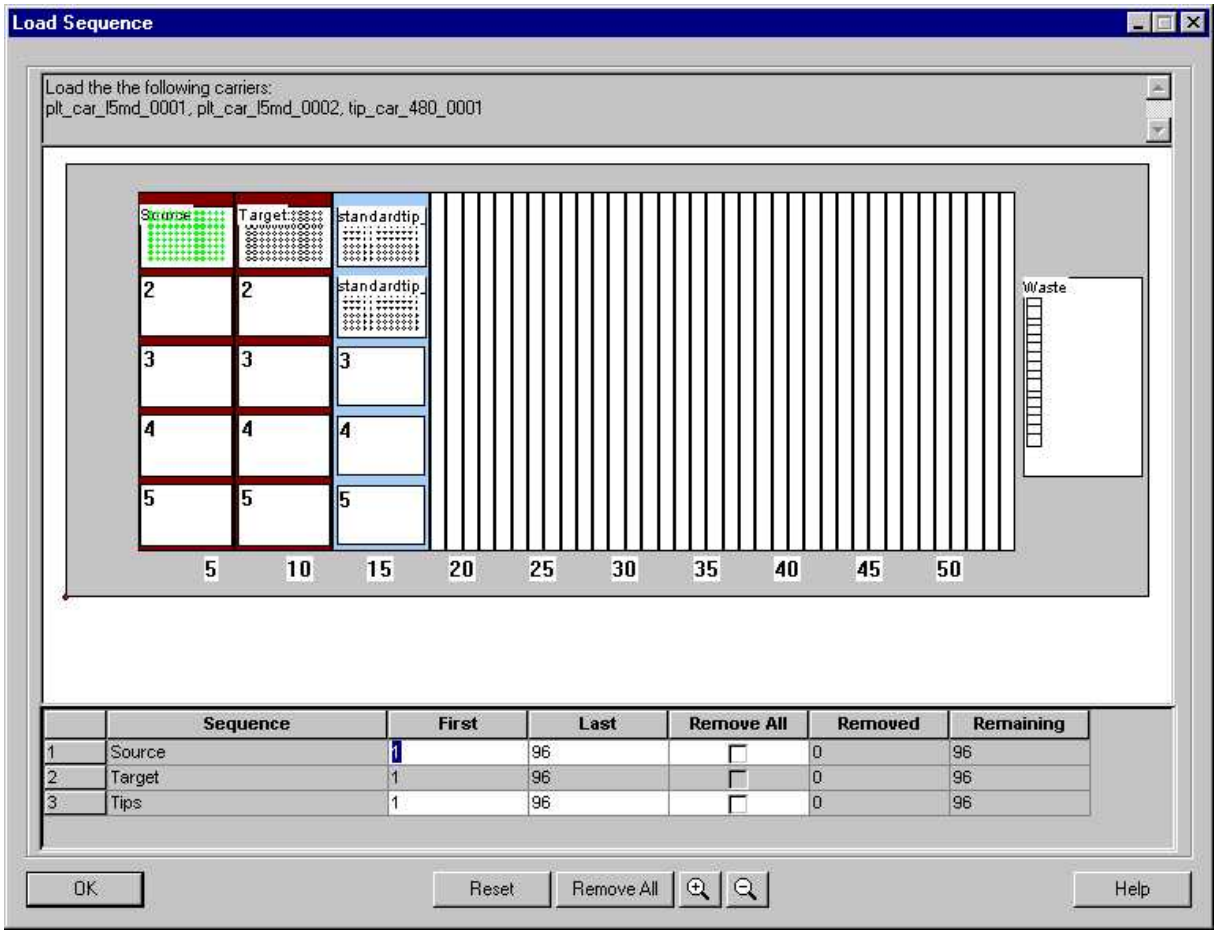
From the File menu of Run Control, select Open, and open the deck layout that was created for the method “OnePlateToPlatePipette” (directory ...\methods\DemoMethods_MLStar). Your method is now loaded:



Run Time Control window

The relevant deck layout appears in the frame in the upper half of the window. The lower half contains the Start button and another blank frame which will display the run method log (method trace file) as it generates.

Press the Start button to run the method. You will see that the steps in the method are traced to the log frame. A loading dialog appears, requesting a reduction of the number of positions on the source plate, as well as a start position for the tips to be picked up. Both pieces of information are optional:



You may enter the number of wells on the source plate for this run, delete wells graphically from the sequence or accept the default (copy the whole plate).

Manual Load Only:

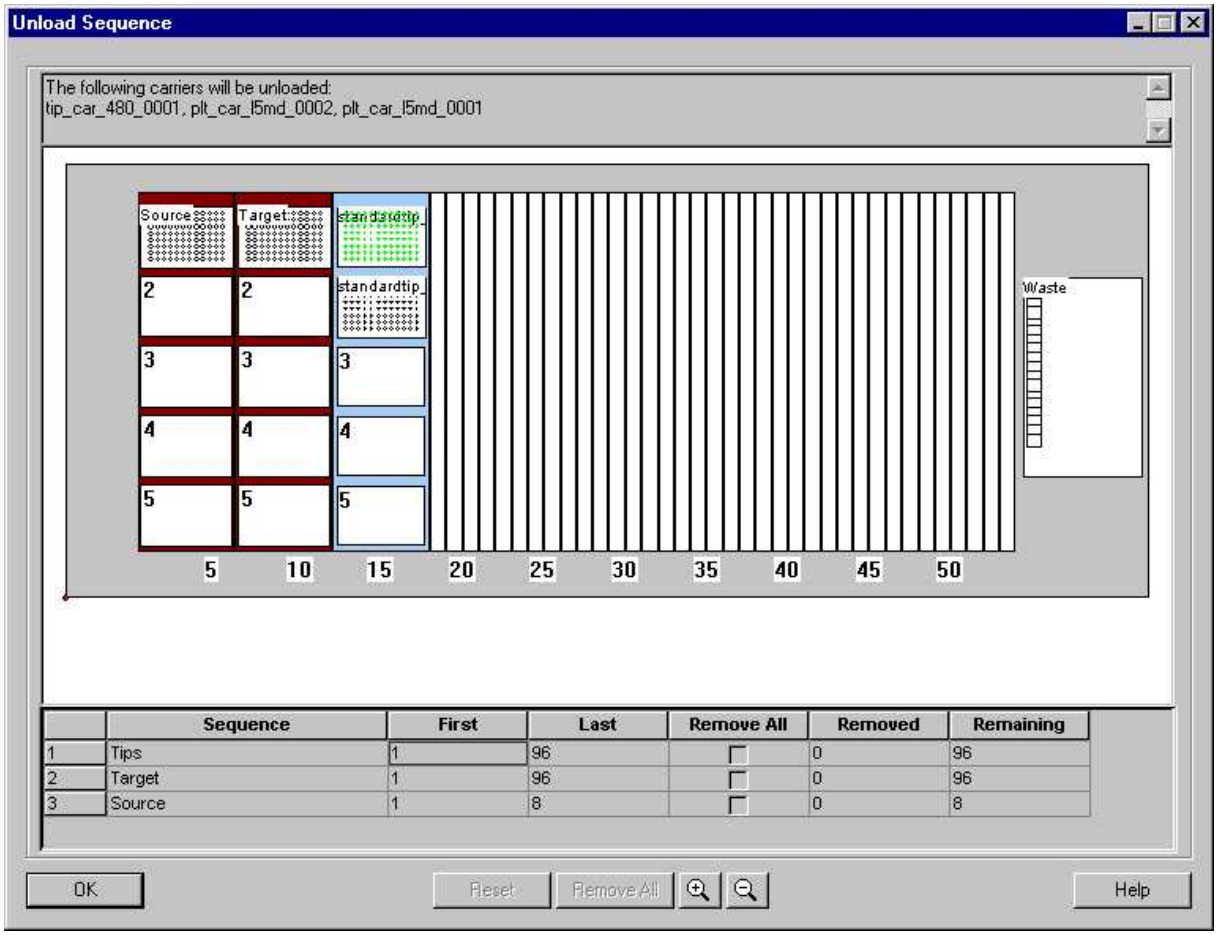
Load the deck with the carriers mentioned in the upper part of the dialog box (the 2 plate carriers and the tip carrier). Don't forget to place labware onto the correct positions.

Autoload Only:

Whenever the system finds a Load Carrier command in the method, the user is requested to feed the carrier holding the appropriate labware onto the autoload tray. The correct position is highlighted by LEDs. Alternatively, all carriers can be placed directly in their correct positions on the autoload tray.

Click OK in the dialog box to start loading.

At the end of the method, you are requested to unload the carriers from the deck. The following dialog box opens:



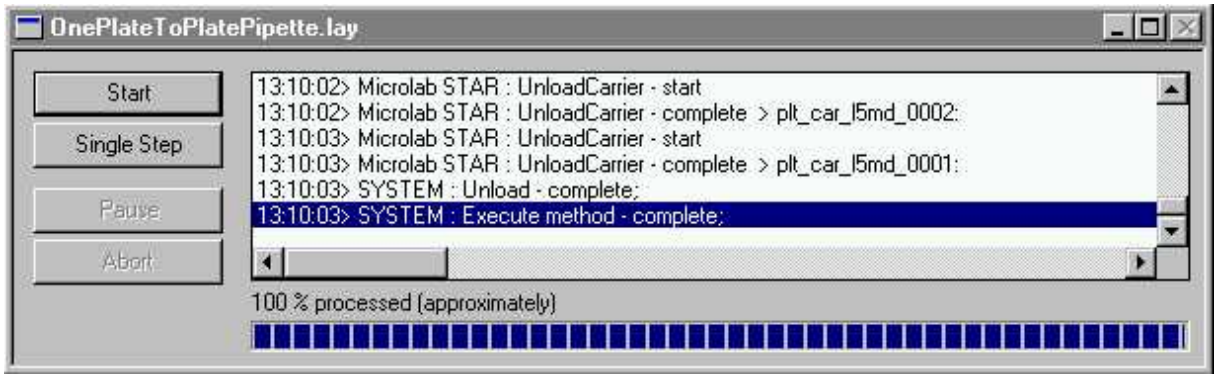
Manual Load Only:

Click OK within the dialog. Unload all the carriers manually. The unloading will be checked by the system.

Autoload Only:

Click OK within the dialog. The carriers are unloaded to the autoload tray.

The method is now finished. Within the method trace file, the method completion information is visible.



The method trace file is stored under “OnePlateToPlate.trc” within the ...\methods directory. Each method trace contains the date, the method name, and an index within the file name:

The method traces are not overwritten or appended.

NOTE

From time to time all unused method traces and com traces have to be deleted from the hard disk.

You may test your method using single steps. It is always possible to execute only the next single instrument step (like TipPickUp, Aspirate, Dispense, etc.) by using the Single Step button. After each step, the system will be paused and the pause screen appears.

A method can also be paused, clicking on the Pause button of Run Control:

Paused methods can be resumed and finished (**click on the pause dialog to stop the beeping**).

It is now possible (during the pause) to open the front cover of the Microlab STAR. Before continuing the method, make sure the cover is closed again. You now can continue or abort method execution.

To abort the method, click abort. You will be prompted to confirm the abort.

NOTE

An abort may cause the loss of data.

Note that aborted methods cannot be restored again.

A fast abort can always be done by opening the front shield of the STAR during run execution.

15.2 Run Simulations

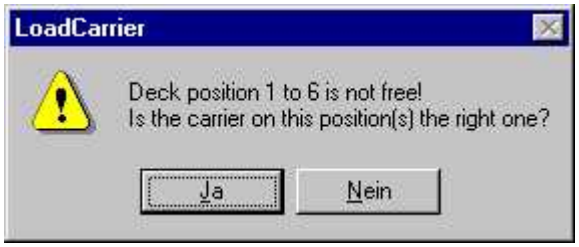
It is also possible to run a simulation instead of the instrument. It is recommended always to simulate a newly created method first, before running it on the instrument. The run simulation is switched on in the configuration editor. Access the configuration editor from the run control by clicking on the deck layout frame. Only now is the Tools menu visible. Select configuration editor.

Set the switch to simulation.

NOTE

FOR MANUAL LOAD INSTRUMENTS: Make sure the autoload checkbox is checked for the simulation. Click on advanced and select the tab "instrument configuration". Check the "autoload" checkbox. To run the instrument, set back the configuration.

On loading commands, the simulator responds for each carrier to be loaded (and unloaded) with the message:



Click Yes to continue.

15.3 Runtime Error Handling

Prior to runtime error handling being used, several types of problems causing errors have to be solved first. Among these are

- Syntax errors when programming in HSL (forgotten ";")
- Logical errors (tip eject before pick up, asp 10ul, disp 200ul)
- Semantic errors (wrong pipetting pattern)
- Method/deck interaction errors (dispense 100 µl into the first well of a 1536-well MTP)
- Liquid handling/application errors (droplets, foam, unpipetted wells)
- User-related errors (sample tubes not filled completely, wrong deck loading, barcodes unreadable)

These problems **cannot** be handled by any runtime error handling.

Problems that can be handled in runtime are

- Not enough liquid
- liquid level not found (if it occurs only rarely)
- No tip picked up
- Clot detected
- Barcode unreadable (if it occurs only rarely)
- Execution error (channel no. 1 has an error (e.g., not enough liquid), then channel nos. 2-8 have an execution error because they have been stopped before completion of the step)

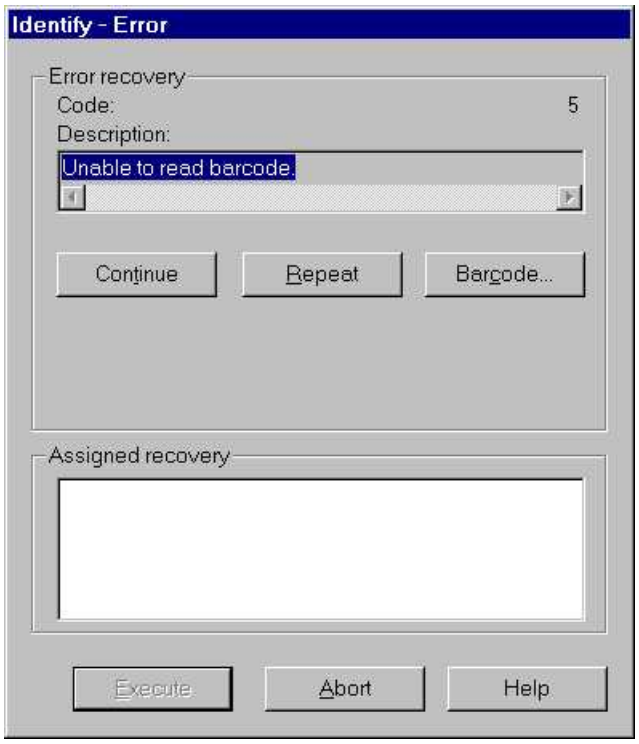
NOTE

In principle, each channel may have one or more different types of errors at a time.

If all channels have the same error at the same time, a colletive recovery can be made.

We now focus on some important examples. A detailed description is available in the online help. Click on Error Settings within the single step dialogs of the Microlab STAR-specific commands.

In the case of an error, the process may be continued using the error handling procedure. If, for example, a barcode of a carrier cannot be read, a dialog window opens up:



In this case, there are 3 options to handle the error:

- **Continue** Continue without reading barcode again.
- **Repeat** Read barcode again.
- **Barcode...** Enter barcode by hand.

A green dot stands for a tube where the barcode is read correctly.

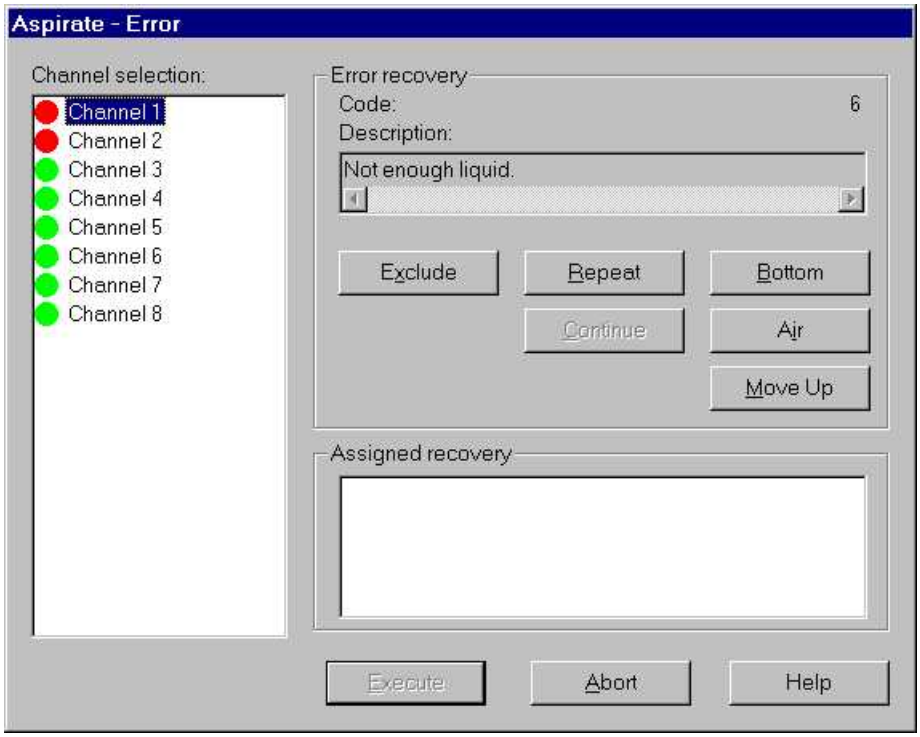
A red dot stands for a tube where the barcode has not been read correctly.

An orange dot stands for a position with no tube (not detected by the sensor).

After assigning a recovery option, the Execute button is activated and the selected option is displayed in the lower part of the window. Clicking on Execute causes the instrument to proceed.

If an error occurs while using pipetting channels, the displayed error dialog shows, for every single channel, its error state and its recovery options. (Different channels can have different errors.)

For example, in case of an LLD error such as no liquid in the container while aspirating, a window similar to the following pops up:



Red-coloured channel 1 and channel 2 failed to aspirate. To each of these two channels a recovery option has to be assigned. Only if all channels have the same error is the chosen recovery option assigned to all channels simultaneously.

Selecting channel 1 shows the error description and the available recovery options for this channel (buttons Exclude, Repeat, etc.). Invoke one of these options by clicking the appropriate button. Your selection is displayed in the lower part of the window.

In this case, there are 5 options to handle the error:

- **Exclude** The channel is excluded (no more aspirate, dispense, etc. with this channel) until next TipPickUp.
- **Repeat** Repeat aspirate command.
- **Bottom** Aspirate from bottom of container, without LLD.
- **Air** Aspirate air.
- **Move Up** Channel is moved up to dispense liquid into the container. After this action, aspiration can be repeated.

Note that the Continue button is disabled. This prevents any later dispense with insufficient volume.

Repeat the same procedure for channel 2. Note that the error and the associated recovery options may differ from those for channel 1.

When the last channel is processed, the Execute button becomes active and the system can proceed.

In any case the method can be aborted without further recovery options.

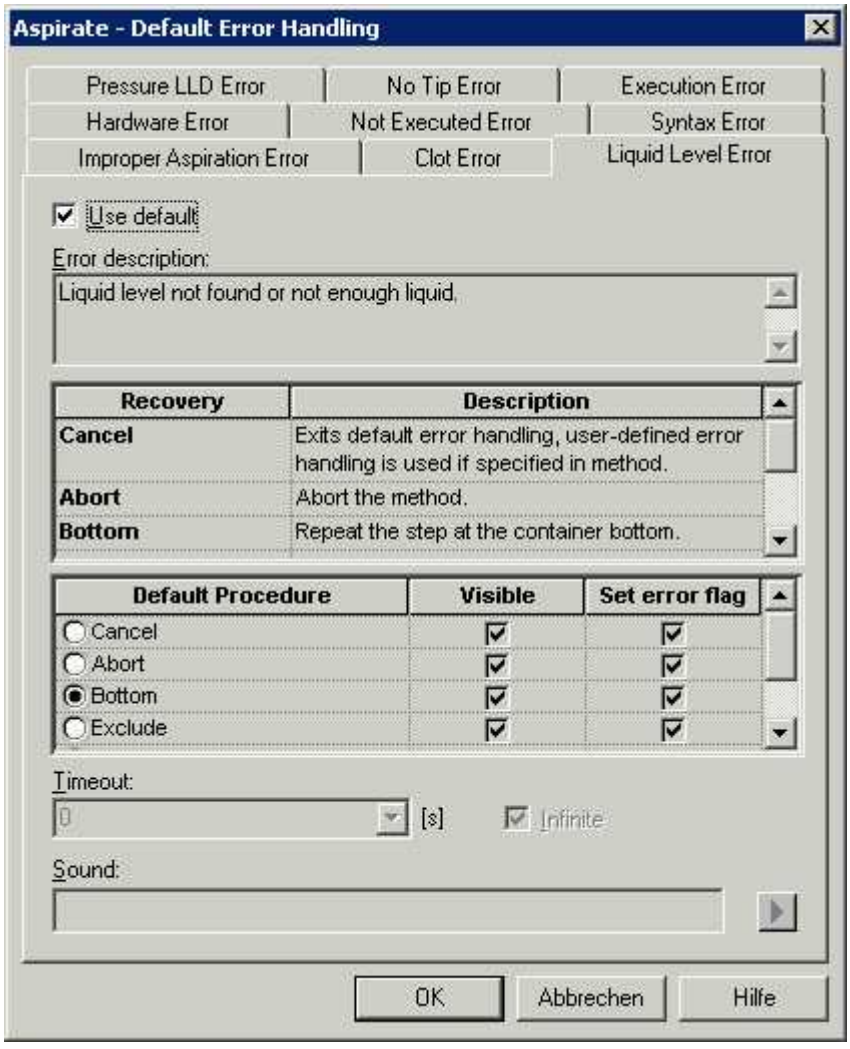
15.4 Walk-Away (Predefined) Error Handling

The user may define a walk-away error handling which uses predefined default settings for different error situations. These settings can be customized for single steps only. For SMART steps, the default error recovery is fixed.

For every instrument-specific single step of your method, an individual error recovery can be defined. You can configure

- the appearance of the error recovery dialogs (which buttons are available)
- the default procedure
- which error is flagged in the trace file
- a timeout, after which the default recovery is carried out (the dialog automatically closes down).

For this purpose, every instrument-specific single step has a “Error Settings” button. For the aspiration step, it looks like this:

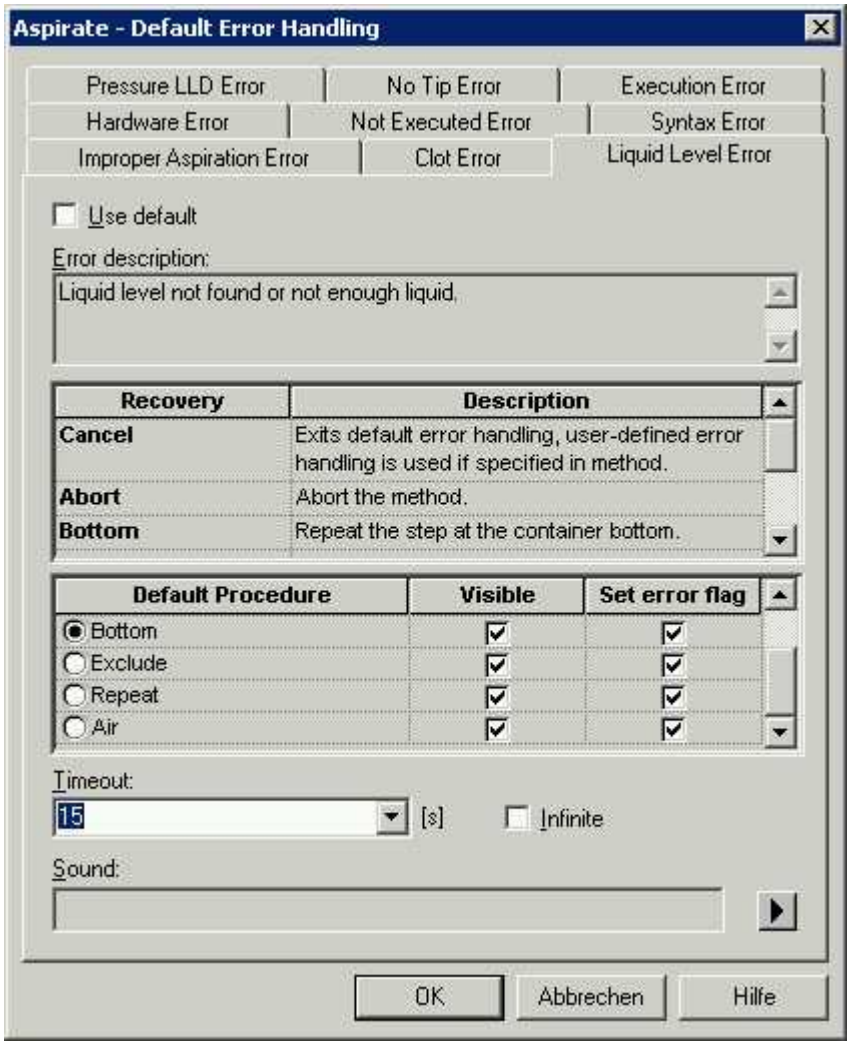


Among these various tabs, we have selected “Liquid Level Error”. The default settings are activated.

To customize the settings, disable the “Use Default” checkbox. A brief description of the error is given, followed by the available recovery options. Only one default procedure can be selected. Among the choices are:

- Cancel (quits the current step and starts the user defined error handling, if specified. If no user defined error handling is present, the method aborts).
- Abort (aborts the method).
- Bottom
- Exclude
- Repeat
- Air

The flag “visible” allows you to add the appropriate button to the error recovery dialog box. The flag “flag” allows you to flag the error to the trace file.



To enable walk-away handling of errors, disable the checkbox “infinite” and enter a timeout into the input field. The runtime error dialog then pops up, waits for the timeout, and closes to continue with the default error recovery chosen for this error.

If the user clicks on the error dialog during the timeout, the walk away will be stopped, and the user has to select a recovery and continue manually.

For a list of all errors and their recovery options, refer to the online help and the error settings dialogs. However, the most important errors are the ones listed in the foregoing chapter.

16 The Microlab AT Barcode File Filter

Along with the software comes a filter tool to generate a Microlab AT-like barcode file. This filter can be started manually, or from the shell command of a method (see example). The sample tracking works as follows:

- Check the check box “sample tracking” in the configuration editor.
- At runtime, one Access-based data base (*.mdb) will be generated within the ...\logfiles directory, storing all liquid transfers of one method.
- An additional register file HxRunIndex.mdb stores information about all runs performed.
- After the method (or at least the liquid transfers) are finished, the filter tool can be called to generate the Microlab AT-like barcode file from the data base. The filter tool is stored under ...\hamilton\bin.

NOTE

To run the AT filter tool, the method name must not exceed 20 characters including extensions.

The plate names used in the method must not exceed 20 characters.

Given that a method has been run with the sample tracking enabled and a 96-well plate has been prepared, the directory c:\barcodes now contains (at least) one barcode file (At_barco.nij) plus the two register files, containing information about all runs performed so far (for a description of *.reg files refer to the Microlab AT user manual):

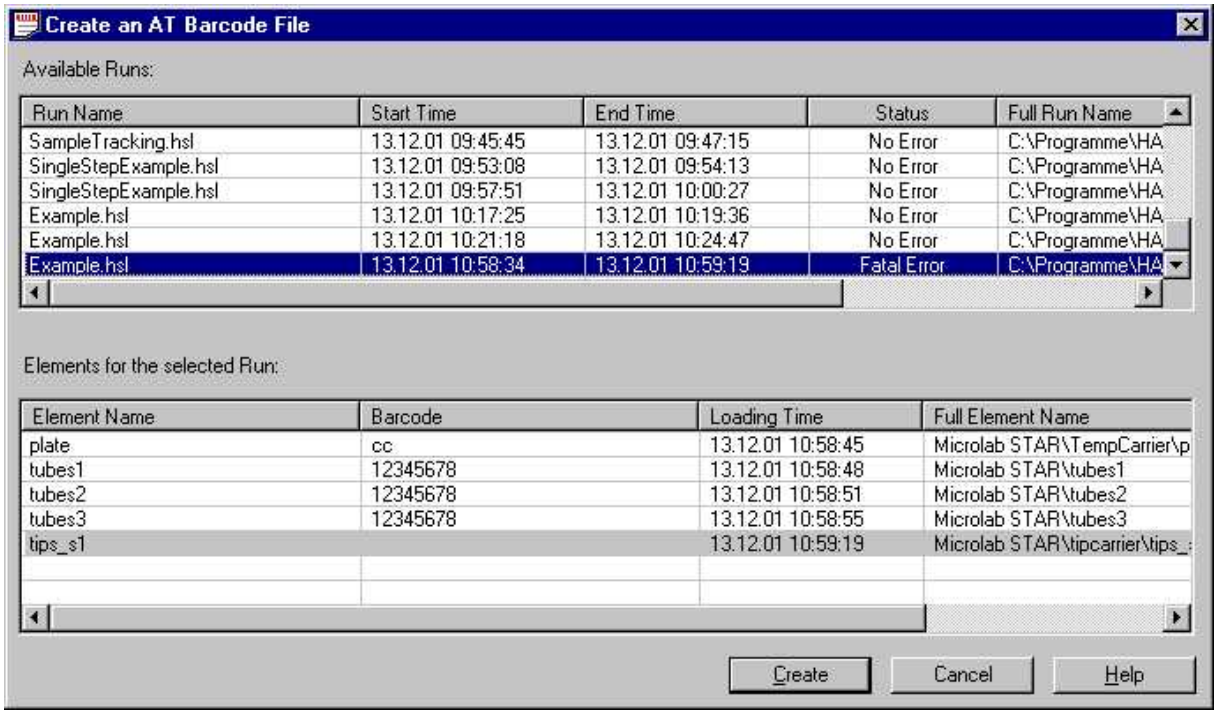
The barcode files can be directly used to couple the Microlab STAR and Microlab FAME.

Now, start the filter tool manually, by selecting

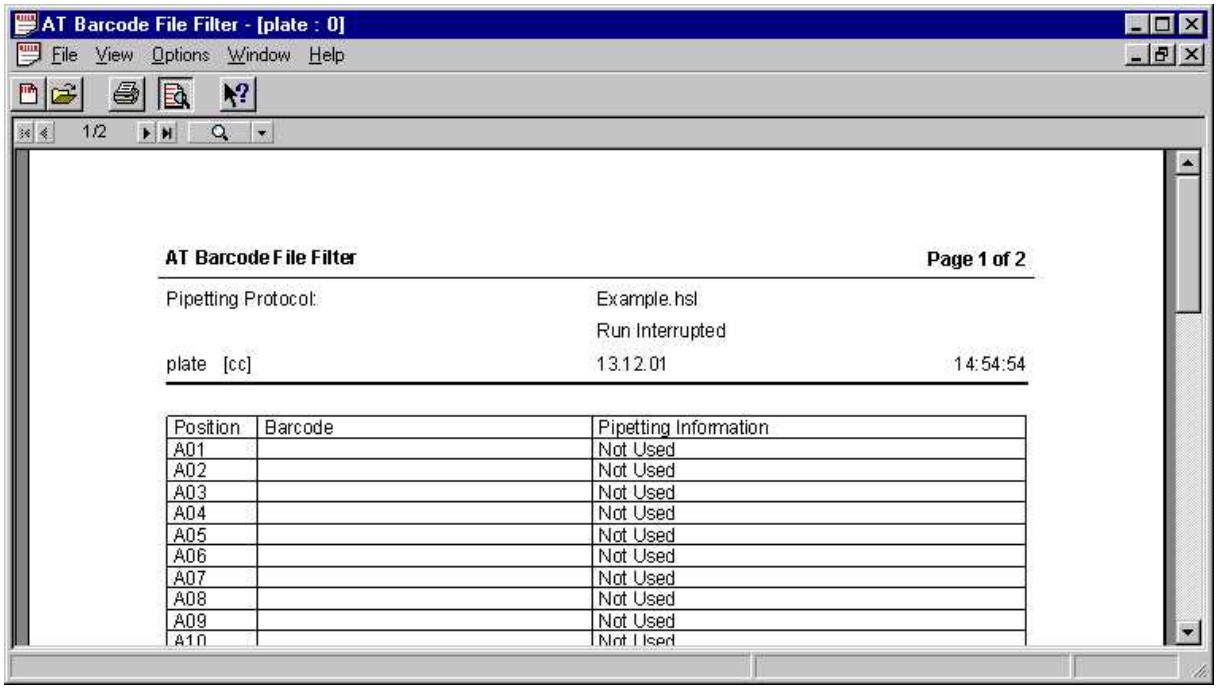
START->Programs->Hamilton->Micolab STAR->AT Barcode Filter

Click on the icon: 

A window opens up showing the available runs:



Select a run in the upper half to get a list of labware, processed during the run. Double-click on the plate for which a barcode file is to be generated. Another window opens up, displaying a table with the pipetting information of the plate in a convenient form:



The table may be printed from the menu.

To start the AT Filter automatically at the end of a method, see the example in chapter 13.7.

17 Reference Guide: Defining Labware

17.1 The Labware Editor

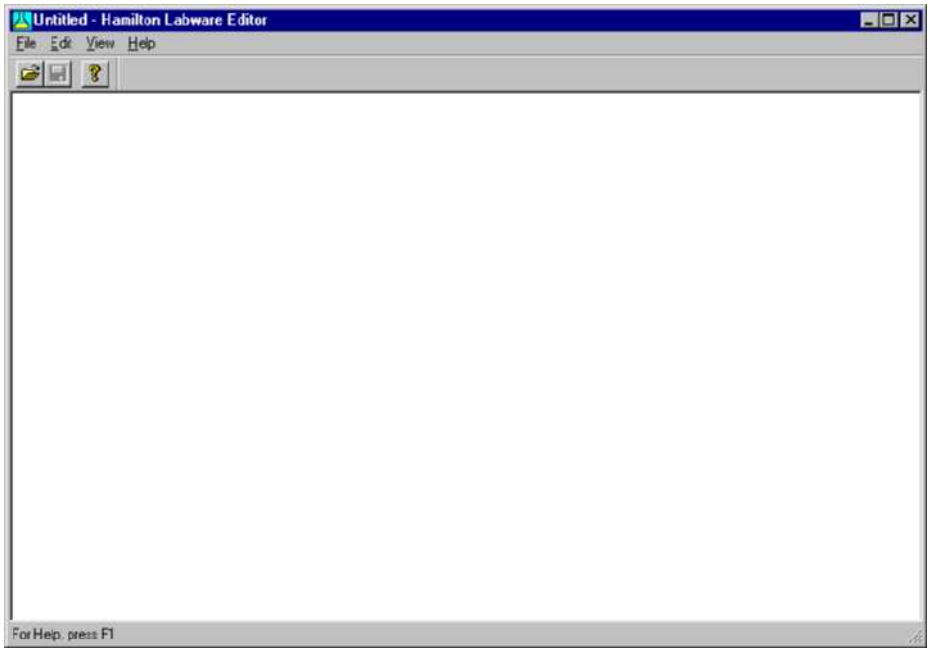
If your labware is not pre-defined, you can define custom racks and containers using the labware editor. Such custom labware can be used like any other pre-defined labware object from the library. The idea is that the labware object is a description of the real physical labware.

To start the Labware editor from the deck editor:

1. select 'Add Labware' from the 'Edit' menu or from the popup menu and click the 'Lab. Editor' button, or
2. select the Labware Editor from the 'Tool' menu, or
3. simply click on the 'Define Labware' icon under on the tool bar.

If you want to add the new labware directly after definition, choose the first procedure.

Whatever you choose, the labware editor starts with the following main window:



17.2 Types of labware

17.2.1 Rectangular Racks and Plates

Rectangular racks are specialized grids for holding either tips or containers in row and column order. A microtiter plate is a rack in this sense, and the wells represent the containers. The rack is therefore a template describing a discrete number of positions for holding containers or tips. Examples of racks include a tube rack, a microtiter plate, a microtiter strip, a deep-well plate, and a tip rack.

The filename has the extension ".rck".

NOTE

To change or define racks, always use the "Rectangular Rack" and not *the "microplate" definition within the labware editor.*

17.2.2 Containers

Containers are vessels holding liquids (e.g., the wells of a microtiter plate). Containers are usually placed within racks. Containers may be placed directly onto the carriers, which is the case e.g. with reagent containers.

The filename has the extension ".ctr".

NOTE

CO-RE tips and needles are also defined as containers.

17.2.3 Circular Racks

Circular racks are specialized grids for holding either CO-RE tips or containers in a segment of a circle.

The filename has the extension ".crk".

17.3 Example: Defining a Rectangular Rack with Containers

The following sections illustrate the procedures for defining labware using the example of a rectangular custom rack and containers.

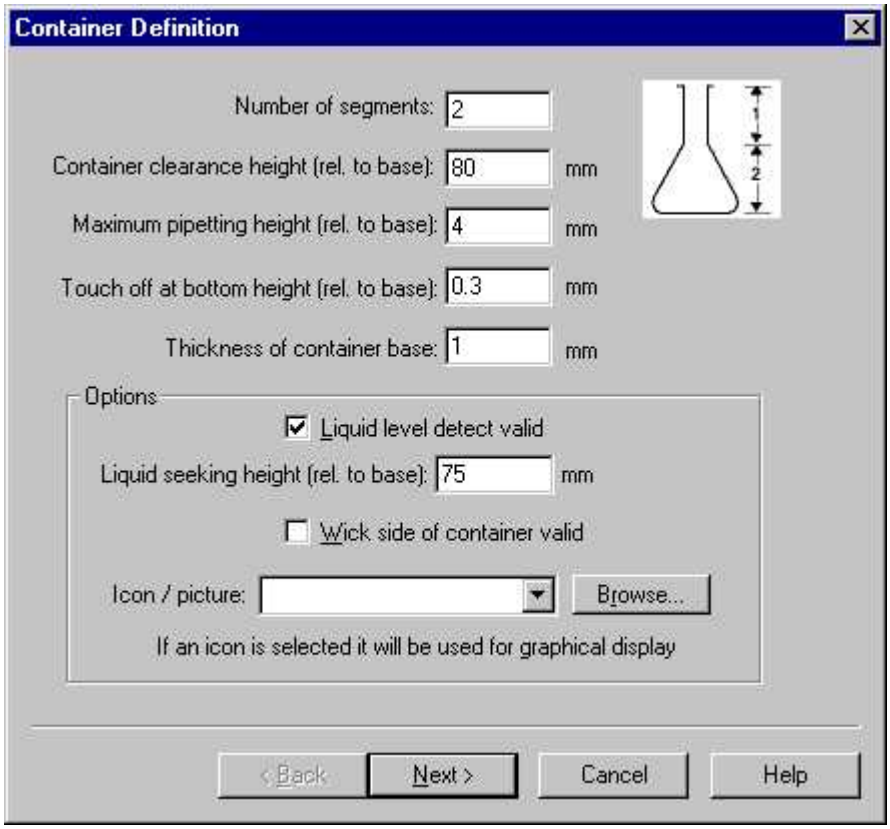
17.3.1 Defining a Container

Let's define a new container. At the end of the container definition we can use that container in a rack definition.

To start:

1. from the labware editor select 'New' and then 'Container' out of the 'File' menu, or
2. in the Rack definition dialog press the 'New' button.

In both cases the following dialog box is shown:



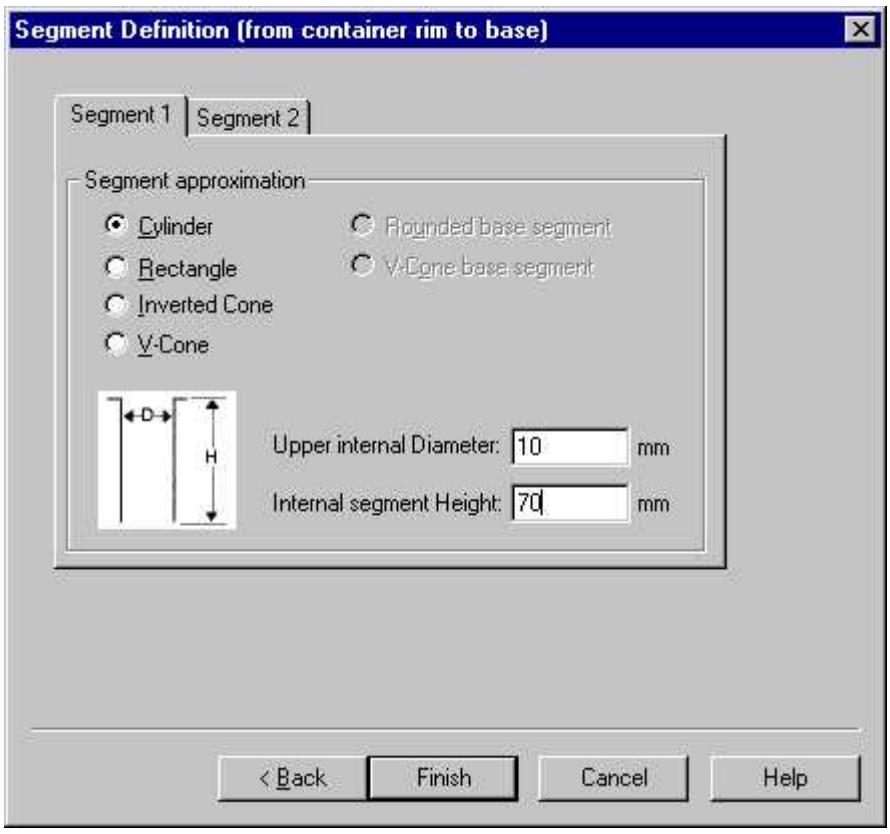
We define the containers as round-bottomed tubes of diameter 10 mm, with a total length of 75 mm. Indicate the number of container segments (here 2, because our tube has a cylindrical and a round-bottomed part) and the clearance height (here 80 mm) at which the pipetting arm can pass over the container without touching it, as measured from the container base. The maximum pipetting height counted from the container bottom is 4 mm, because we want to allow the tip to go down to a position of 4 mm above the tube bottom (this gives the “dead” volume).

The touch-off height means the position of the tip when dispensing with “touch off” into an empty container. It is set to 0.3 mm although this option is not available for all instruments (including the ML-STAR) at the present time. The same is true for the “wick side of container” checkbox, which in principle enables or disables touch-off at the sides of the containers but is also not available for all instruments.

The next important option is the “Liquid Level Valid” checkbox, which is activated here, and the value of 75 mm above the tube bottom to start the liquid level detection within the tube.

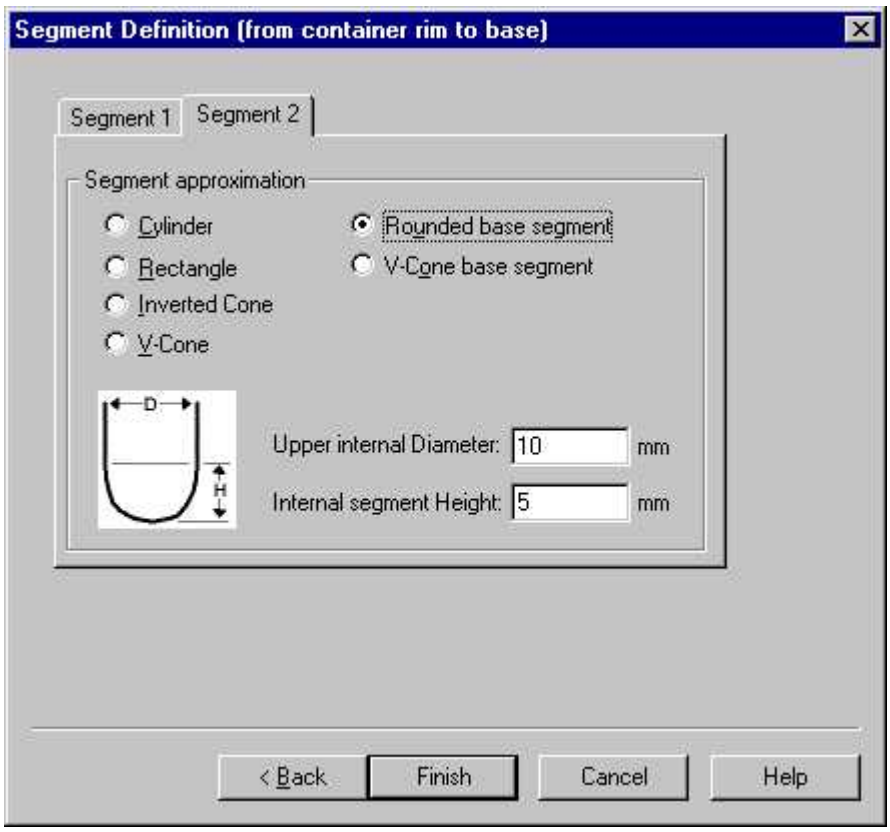
The 'Thickness of Container base' is used to place a rack filled with such containers in the correct Z position.

When the values are selected, click “Next” to open the next dialog box for segment definition:



Select “cylinder” as a shape for the upper segment and fill in the values for the inner diameter (10 mm) and the segment height (70 mm).

Click on the “Segment 2” tab.



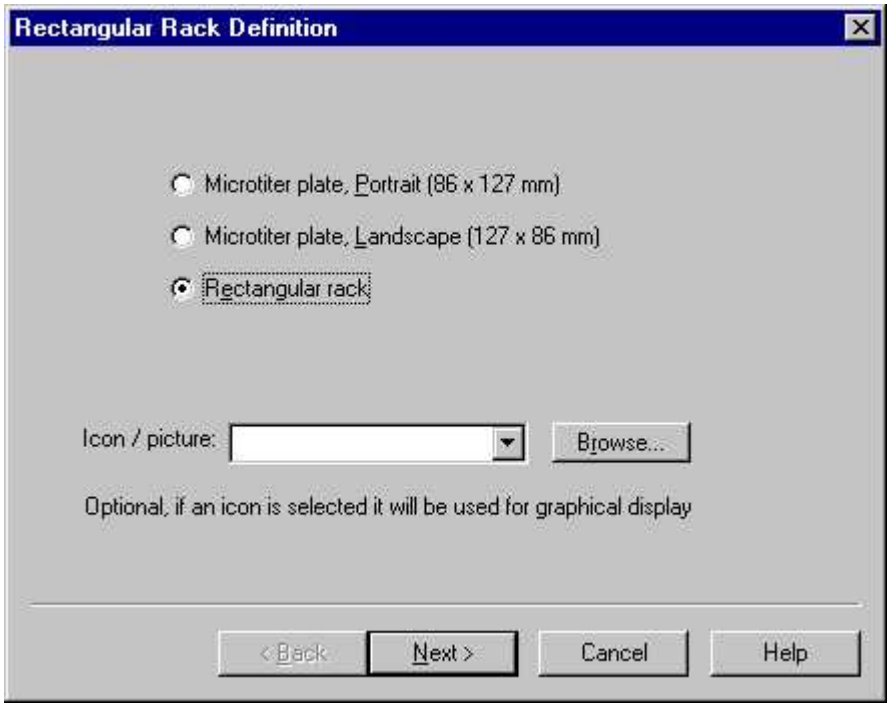
Select “Round base segment” and fill in the value for the upper inner diameter (10mm) and the segment height (5 mm). Click “Finish” to finish the container definition. You are prompted for a name for the newly defined container, e.g. choose “MyContainer” (.ctr) and click save.

17.3.2 Defining a Rectangular Custom Rack

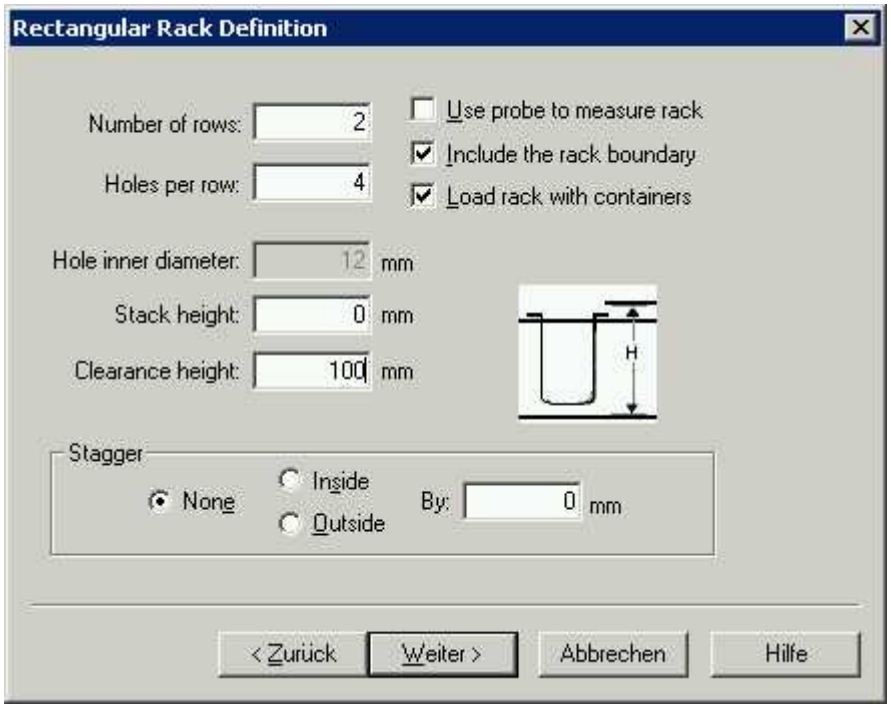
Now let’s define a rack for the containers.

Ensure all the racks and containers you define have distinct, obvious names for ease of use.

Select “New” and “Rectangular Rack” from the File menu of the main window. A series of query dialog windows starts, giving the user the opportunity to design the rack.



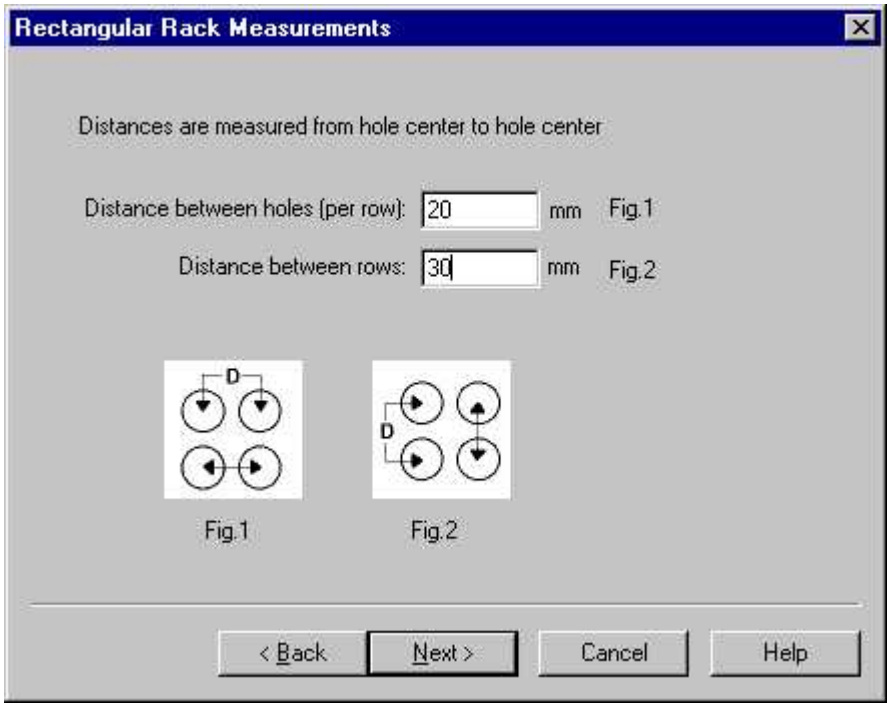
Choose always “Rectangular Rack” to define or modify rectangular racks and microplates. Choosing “microtiter plate” requests only a subset of the information relevant for the ML-STAR. Click “Next”.



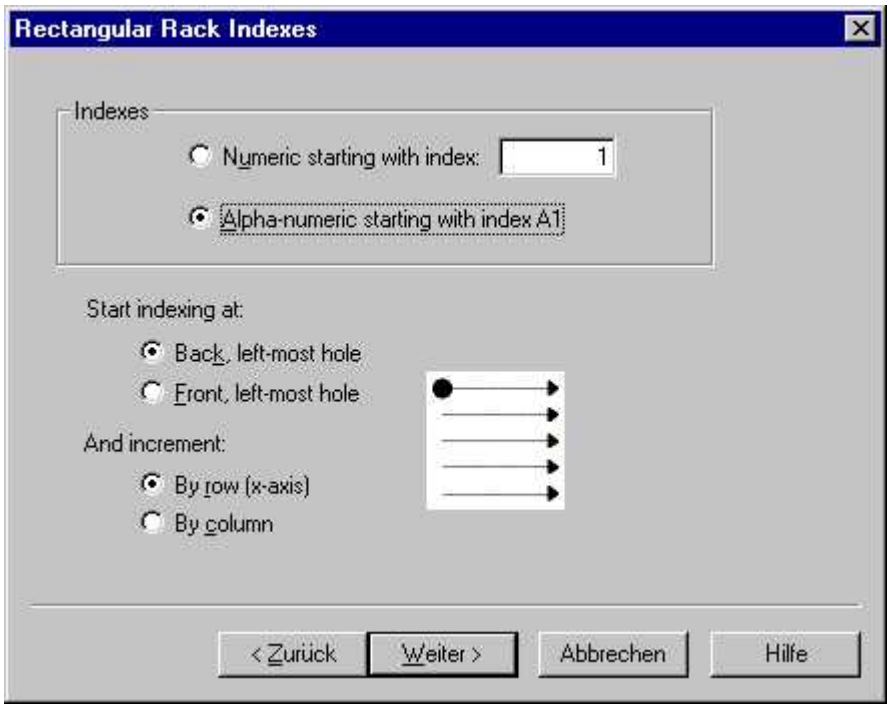
Specify the number of rows (here 2), the number of holes per row (here 4), and the inner diameter of the hole (here 12 mm) which will later receive the container. Check “Load Rack with Containers” and give the overall clearance height of the assembly (here 100 mm). Also check “Include Rack Boundary” to allow the boundaries to be set in the next steps. Accept

the default for “Stagger” (an option allowing you to shift the rows with respect to each other) and click “Next”.

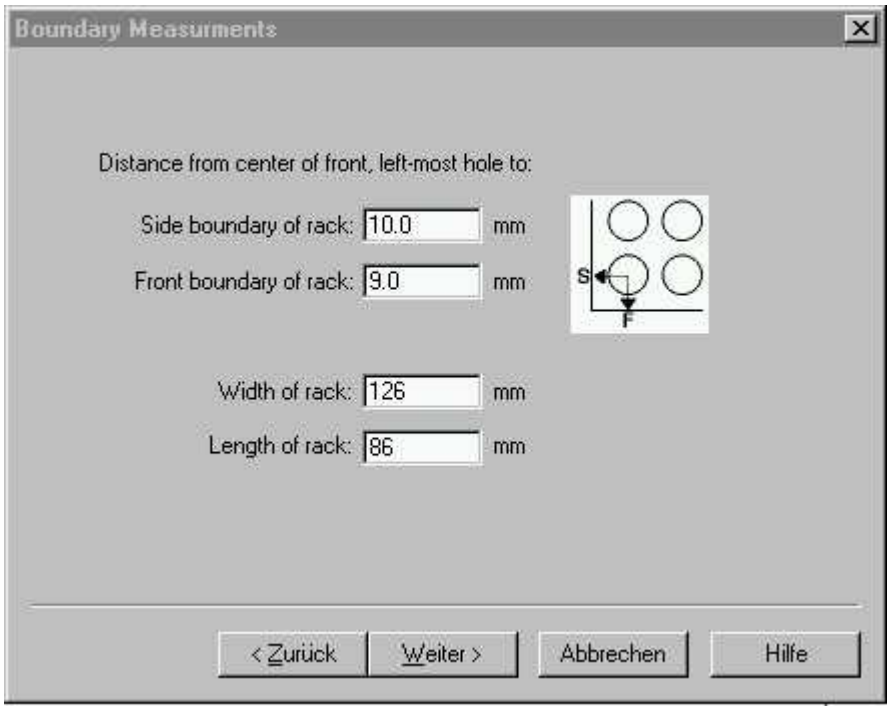
The next dialog appears:



Specify the distance between the holes in a row (here 20 mm) and the distance between the rows (here 30 mm). Click “Next”.



Accept the defaults for the indexing of the holes, for alpha-numeric indices ranging from A1 to H12, and click “Next”.

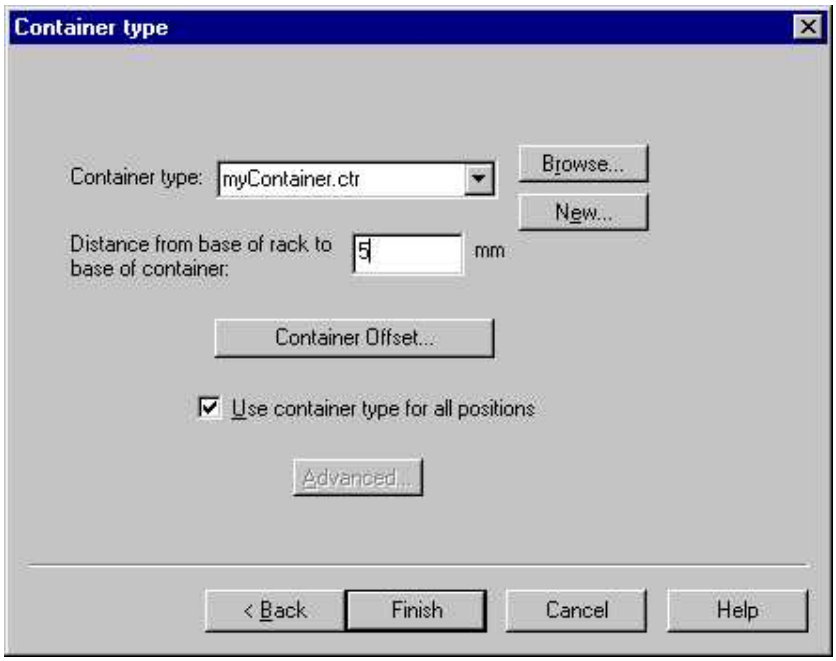


Type in the width and length of the rack (here, e.g. the outline of a microplate) and the rack boundaries. Click “Next”.

See section 17.5 for more information on these inputs.

Now the rack definition is finished. Since we checked “Load the Rack with Containers” in the second screen, we now have to define the containers which will be placed in the holes of the rack.

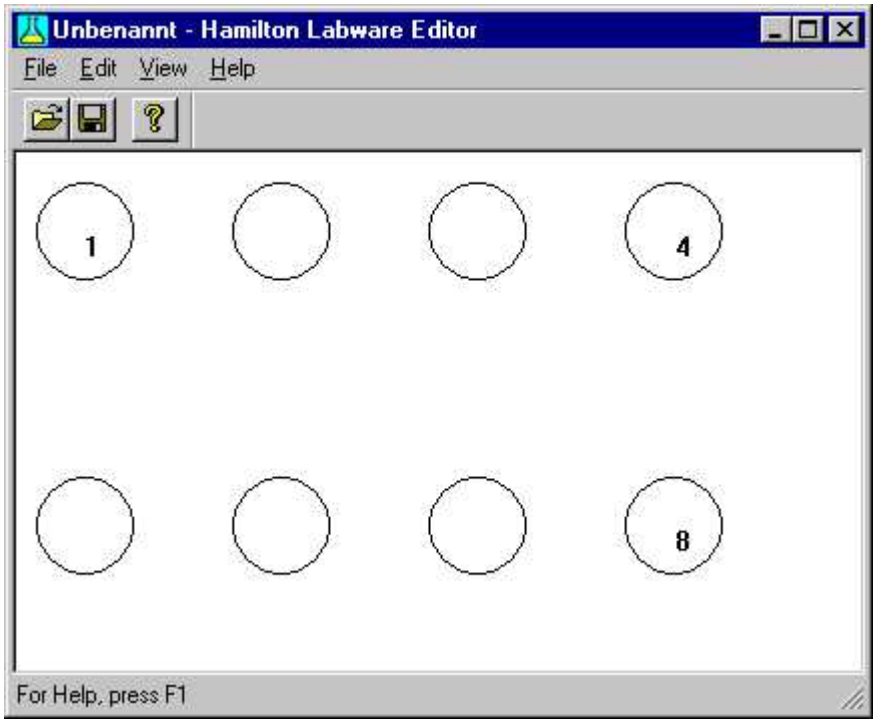
We have the choice either to browse the directories for defined containers (extension “.ctr”) to fill our new rack with, or to define a new container.



We now come to the end of rack definition, with the new container pre-selected.

Accept the defaults and click “Finish” to finish the rack definition.

You now see your newly defined rack in the Labware Editor:



Choose Save in the File menu to store the new rack under the name “MyRack” (.rck) in the labware directory. Select Exit from the File menu.

Once you are back in the “Add Labware” or in the "Deck Layout editor" window, you can put the newly-designed rack on the deck: under Type select “MyRack.rck”, and the new rack is visible in the “add Labware” Window.

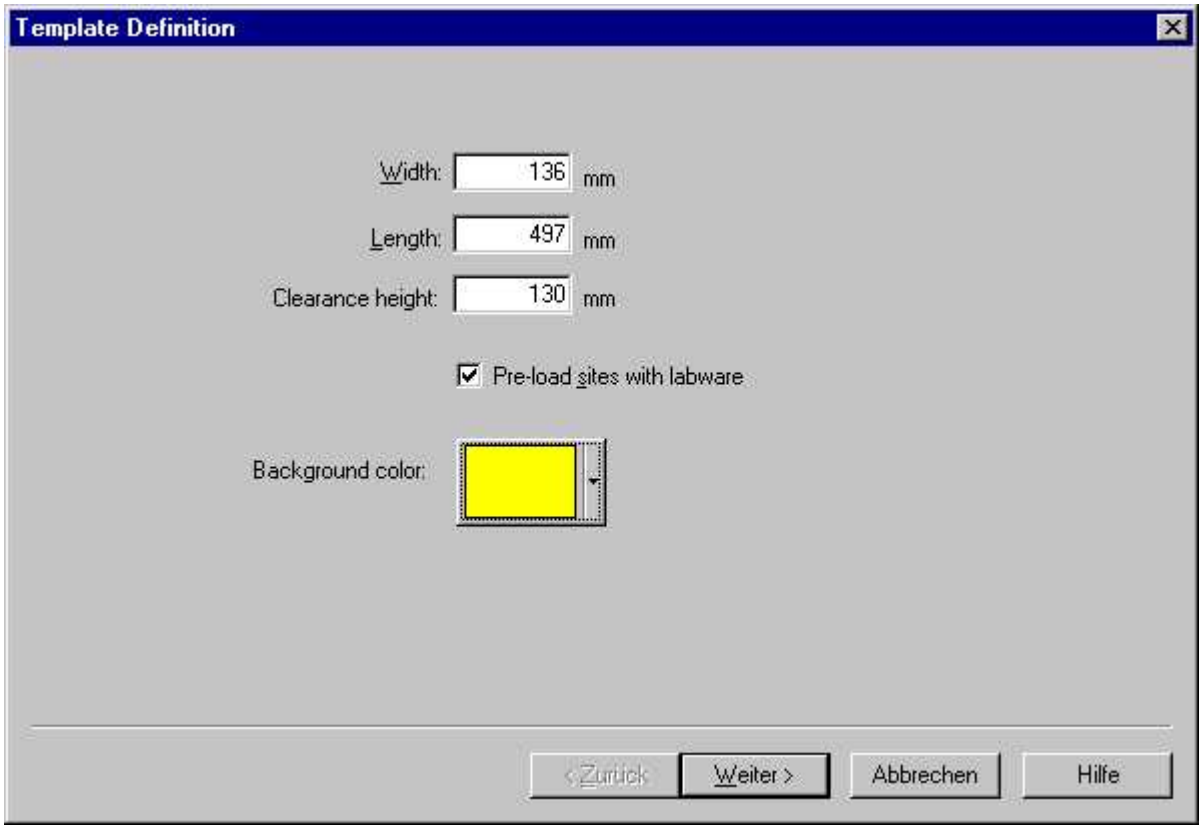
17.3.3 Defining a Carrier (Template)

Carriers are also pieces of labware. Carriers have sites to host racks such as microplates. Let us now define a carrier, preloaded with flat 96 well nunc microplates.

NOTE

A “tube carrier” in the sense of labware is a rack and not a carrier (a template). It is a rack, that directly fits the track geometry of the microlab STAR and therefore can be directly loaded on to the instrument deck.

To define a carrier, open the labware editor and select New->Template. A window pops up:



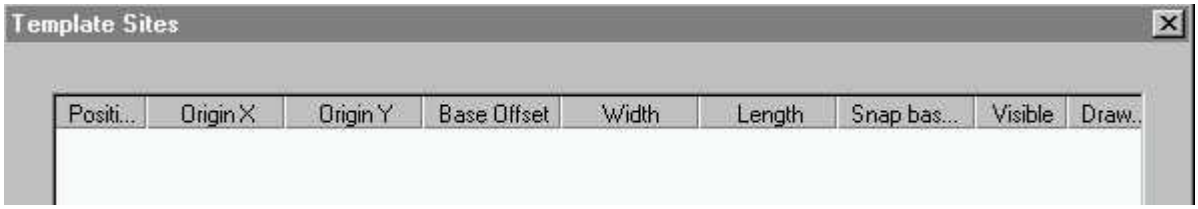
The width of the carrier is given by

width/mm="number of tracks" x 22.5 mm/track

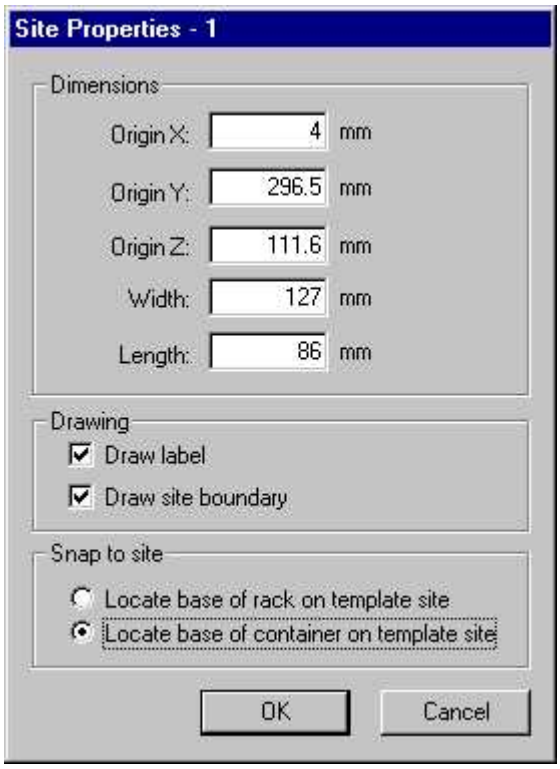
In the case of a plate carrier which is 6 tracks wide, the result is 136mm. The length of a Microlab STAR track is 497 mm. The clearance height for all carriers on the instrument is 136 mm.

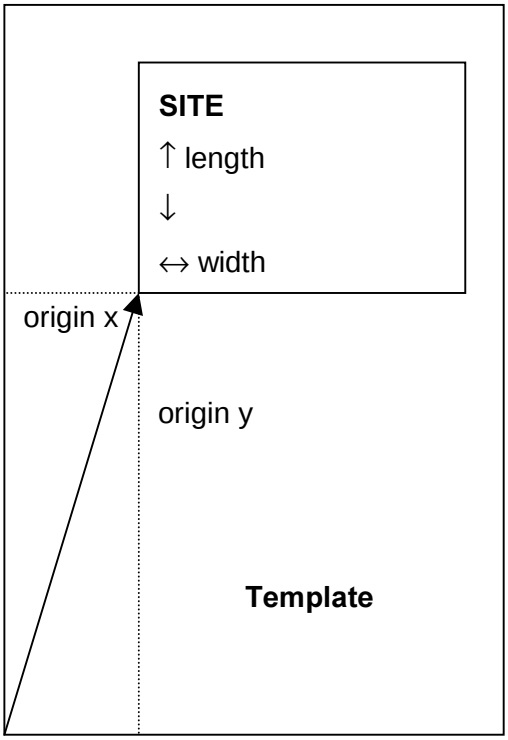
Select the "Preloaded" check box to let the carrier be preloaded with the microplates. Select a colour of your choice. Click Continue.

Within the next window, the sites hosting the plates have to be defined:



Click on the Add button and double-click the added line.





The drawing on the right illustrates the different coordinates. The values refer to a standard microplate. Drawing is only of “optical” influence; accept the defaults. For “Snap to site”, select the lower option for a microplate, because a microplate fits with its bottom on to the plate carrier (to enable a good electrical coupling for capacitance-based LLD).

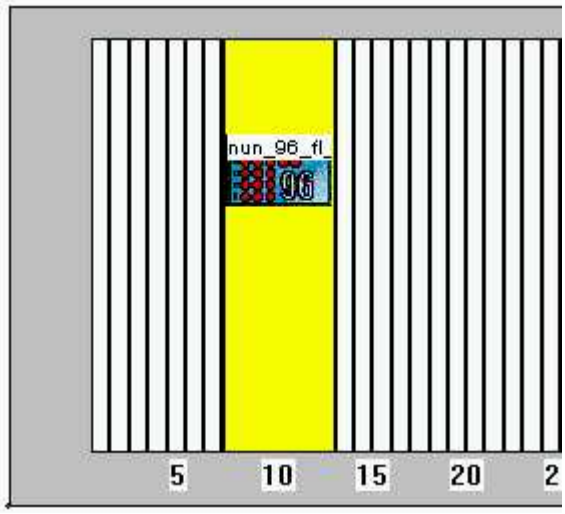
The decision whether a plate (or tip rack) fits on to a site of a carrier is made depending on the width and legnth of the site: all plates that have the same boundary measures (width and length) can be placed on the site.

Different plate types (96 flat and deep well, 1536 well, etc.) have the same boundary measures.

You may add additional sites to the same carrier. Click next. Now, the site can be preloaded:

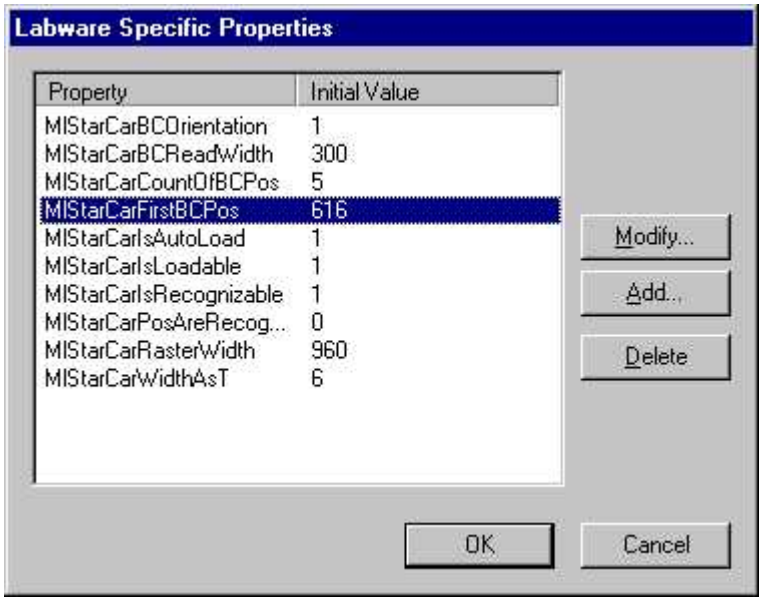
Click on Browse, to browse for the corresponding labware and click Add, to add the selected labware to the site. Click Finish. Save the new carrier within the labware editor.

Now open the deck editor and place the carrier onto the deck:



The carrier is now preloaded with a plate on its site.

Furthermore, a carrier has several properties, such as barcode positions, which may be specified. To do so, open your newly defined carrier in the labware editor and select Edit>Properties. A dialog opens up. Here, we open a standard plate carrier:



Properties of a PLT_CAR_L5MD.

Entries can be added by clicking on Add. The entry names are to be kept; they roughly explain the property (see chapter 17.4).

Add the entries and click OK to store them along with the carrier.

NOTE

Always start with the given settings of a standard carrier and apply changes step by step. Do not change the names or typing of the variable names. These variables are system variables.

17.4 System Flags for Labware Properties

Within the labware, some flags and settings (labware properties) are defined to determine handling of the labware elements. These properties should not be changed by the user. They are accessible just so that you can have the maximum of decision-making power in particular situations that call for it.

These properties can be divided up into the following groups:

- Information for the handling of the auto load unit
- Information for the special units like wash carrier, TTC, tips and needle handling, waste, etc.
- Information to support the reduction of selection during edit time

17.4.1 Structure

The properties are always combine a key name and a value. The key names are case sensitive (the distinction between capital and lower case is important).

All values are in integers (no decimal points).

17.4.2 Information for Handling the Autoload Unit

Key	Default	Range	Description
MlStarCarWidthAsT	-	1 .. (n)	Width of carrier in T
MlStarCarCountOfBCPos	-	0 .. (n)	Count of carrier barcode position How many barcodes positions are expected.
MlStarCarRasterWidth	-	0.1 mm	Distance middle of barcode position to middle of next barcode position.
MlStarCarBCOrientation	0	0 or 1	0 = Vertical, 1 = Horizontal barcode read direction.
MlStarCarFirstBCPos	-	0.1 mm	Distance between rear of carrier to middle of first barcode position.
MlStarCarBCReadWidth	-	0.1 mm	Width of barcode read window
MlStarCarIsRecognizable	0	0 or 1	The carrier has a magnetic bar so its presence can be detected. 1 = TRUE, 0 = FALSE
MlStarCarIsLoadable	0	0 or 1	The carrier can be loaded and unloaded. (A wash station, for example, is not loadable) 1 = TRUE, 0 = FALSE

Key	Default	Range	Description
MlStarCarIsAutoLoad	0	0 or 1	The carrier can be loaded and unloaded by the autoload. 1 = TRUE, 0 = FALSE
MlStarCarPosAreRecognizable	0	0 or 1	The presence of the elements loaded on the carrier is detected. (e.g. the containers on a sample carrier are detectable) 1 = TRUE, 0 = FALSE
MlStarCarNoReadBarcode	0	0 or 1	1 = Don't read barcode, 0 = read barcode

Notes:

- The position of the carrier barcode is not defined in the properties because this position is fixed.
- The barcode positions are ordered at regular intervals (one measurement for one carrier).
- On a carrier, all barcodes must have the same orientation, except the carrier barcode (one value for one carrier)
- The barcode read window is the same for all positions on a carrier. One window should not overlap the next window, otherwise in some cases the barcode cannot be assigned to the correct position.
- If a carrier is loadable with auto load (MlStarCarIsAutoLoad), the loadable property (MlStarCarIsLoadable) value must be set too.
- At edit time in the load step, the user can only select carriers (templates) with underlying carriers having the property "MlStarCarIsAutoLoad".
- At runtime, if "MlStarCarIsLoadable" and "MlStarCarIsAutoLoad" are not set, no load is required; the carriers are fixed on deck or already loaded. If "MlStarCarIsLoadable" is set, a load dialog is shown, and if "MlStarCarIsAutoLoad" is set, loading is executed by the instrument.

17.4.3 Information for Special Units

Properties for "calibrate carrier" single command (Carrier 1536):

Key	Default	Range	Description
MlStarCarCalibrateX	-	0.1 mm	Distance between left margin of carrier to middle of measuring hole.
MlStarCarCalibrateY	-	0.1 mm	Distance between carrier front to middle of measuring hole.
MlStarCarCalibrateZ	-	0.1 mm	Distance from deck to carrier top

NOTE

If a carrier can be calibrated, all values should be set.

The origin is the zero point of the carrier (front, left, down)

If the carrier cannot be calibrated, these keys and values should not be exist - remove key.

At edit time in the calibrate step, the user can only select carriers (templates) with underlying carriers having these properties.

Properties for Temperature-Controlled Carrier (TCC):

Key	Default	Range	Description
MlStarCarIsTemperated	0	0 or 1	The carrier can be temperature-controlled 1 = TRUE, 0 = FALSE
MlStarCarIncubatorNumber	-	1 or 2	Number of temperature incubation stations 1 or 2

NOTE

There are only two TCCs allowed on a deck.

Each TCC must have its unique identifying number

At edit time in the incubator step, the user can only select sequences out of the list of sequences with underlying racks having these properties.

Properties for tip and needle handling:

Key	Default	Range	Description
MlStarIsWasteRack	0	0 or 1	Into this rack tips or needles can be ejected 1 = TRUE, 0 = FALSE
MlStarTipRack	0	0..8	0 = Standard Vol Tip disposable 1 = Standard Vol Tip disposable with filter 2 = Low Vol Tip disposable 3 = Low Vol Tip disposable with filter 4 = High Vol Tip disposable 5 = High Vol Tip disposable with filter 6 = Low Vol Steel Needle 7 = Standard Vol Steel Needle 8 = High Vol Steel Needle

NOTE

At edit time the user does not have to define the tip type in the pick-up tip or needle. The information is taken from this property.

At edit time the user does not have to define the tip type in the eject tip or needle.

At edit time the user can only select sequences out of the list of sequences with underlying racks having these properties.

At edit time in the aspiration or dispense step the user can only select sequences out of the list of sequences having underlying racks without these properties.

Needles can only be ejected into a rack with the property MlStarIsWasteRack and the corresponding MLStarTipRack setting.

Tips can only be ejected into racks with the setting MlStarIsWasteRack (e.g. standard waste)

Properties for wash station:

Key	Default	Range	Description
MlStarNeedleWashRack	-	6 .. 8	6 = Low Vol Steel Needle, 7 = Standard Vol Steel Needle, 8 = High Vol Steel Needle
MlStarWashStationNumber	-	1 .. 3, 4 .. 6	Number of the wash rack Wash Station 1 Rack 1 .. 3 Wash Station 2 Rack 4 .. 6

NOTE

There are only two wash stations allowed on a deck.

Each wash station must have its unique identifying number

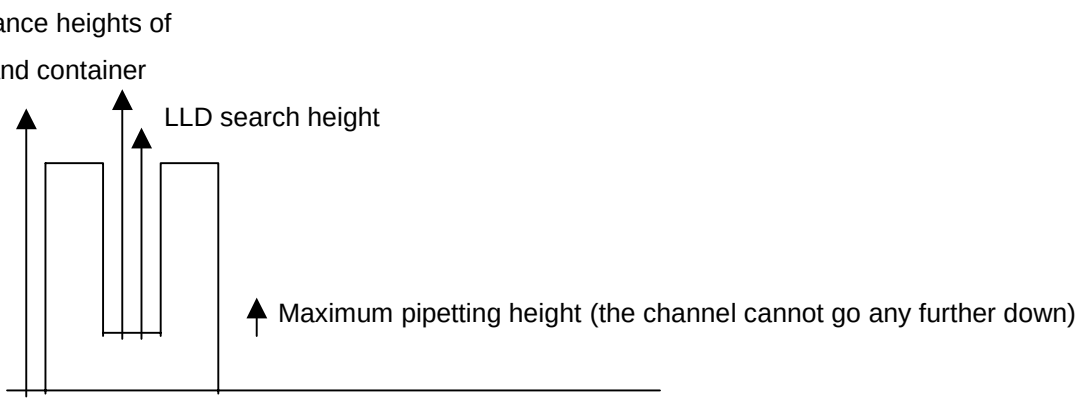
Each washer over both carriers should have a unique identifying number

At edit time in the wash step, the user can only select sequences out of the list of sequences with underlying racks having these properties.

17.5 Z Positions of Carriers, Racks and Containers

The aspiration and dispense dialogs always use the inner container bottom as a reference position (fixed height, liquid level = 0). The x,y,z values of the reference well (usually A1 or 1, labelled red in the move labware dialog) stored in the instrument's system of coordinates are shown in the "Move Labware" dialog. To access this dialog, right-click the labware item of interest in the Deck Layout Editor, and select "Move Labware".

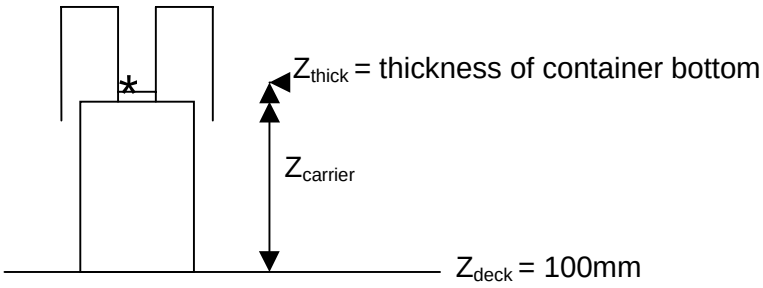
The rack and the container both have a clearance height – which means that the movement of the channels is not impeded if they pass above this height. The software automatically takes the highest clearance height. The maximum pipetting height is counted from the bottom of the container upwards and determines the dead volume of the container. The LLD search height is the height at which the speed of the channel is reduced to look for the liquid surface.



Clearance heights of rack and container

Regarding the **z position**, two different cases of rack placement on the deck can be distinguished in the Microlab Star:

1. A **"container-based" rack** is placed with the container bottom directly on the carrier (e.g. the microplates on a plate carrier):

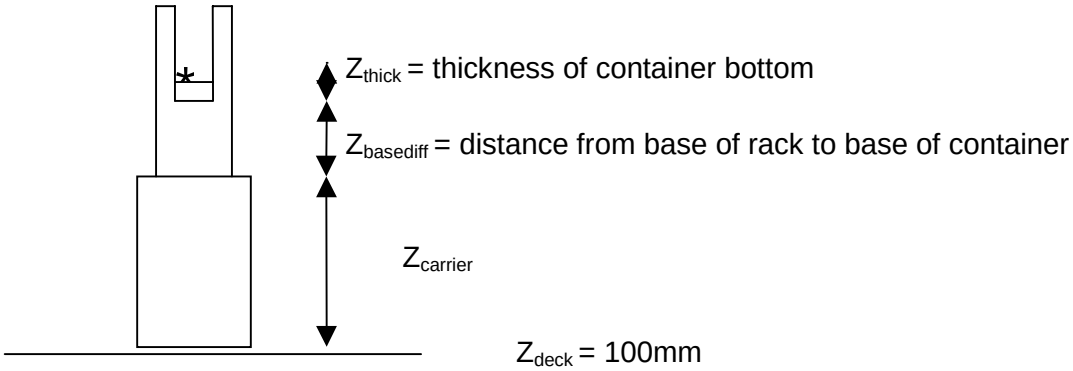


The reference position Z_0 is marked with the *. Here, the reference height is calculated from

$$Z_0 = Z_{deck}+Z_{carrier}+Z_{thick} ,$$

ignoring all other terms. Z_{deck} is a fixed quantity, Z_{thick} is defined in the labware, and $Z_{carrier}$ is defined in the carrier template definition.

2. A **"rack-based" rack** is placed with the frame on the instrument deck (e.g., a tube rack, where $Z_{carrier} = 0$, because the tube rack is used directly as a carrier)



The reference position Z_0 is marked with the *. Here, the reference height is calculated from

$$Z_0 = Z_{deck}+Z_{carrier}+Z_{basediff}+Z_{thick} .$$

Again, Z_{deck} is a fixed quantity, Z_{thick} and $Z_{basediff}$ are defined in the labware, and $Z_{carrier}$ is defined in the carrier definition. Note that here we have one more term in the equation, compared to the previous case.

Selection between the two types of racks (container-based rack or rack-based rack), and thus the decision which formula is used to calculate the reference z-height from the labware data, is done automatically by selecting the hidden grid for the rack. Thus the question arises how to define the hidden grid for a rack, i.e., how to define which hidden grid or carrier the rack should snap onto. For a rack on top of a carrier, this is done by selecting the appropriate switch "snap to base" in the carrier definition.

For a tube rack, which is placed directly on the deck (it snaps directly into the 1 track grid), the grid of the tracks automatically assumes a rack-based snap-on. When placing a plate directly on to the deck the user has to input the x,y,z coordinates of the reference well directly.

18 Reference Guide: Examples Using the HSL Method Editor

All examples shown in the following sections are identical to those in the previous sections, except that they are written using the HSL Method Editor. Therefore, the previously created deck layouts can be used here as well. Open a deck layout from the graphical method editor examples section and save it under a different name. We have chosen the same file names as those for the other sample methods, but with the notation “HSL_“ prefixed to the name. All these methods are also available from the “\DemoMethods_MLSTAR“ directory.

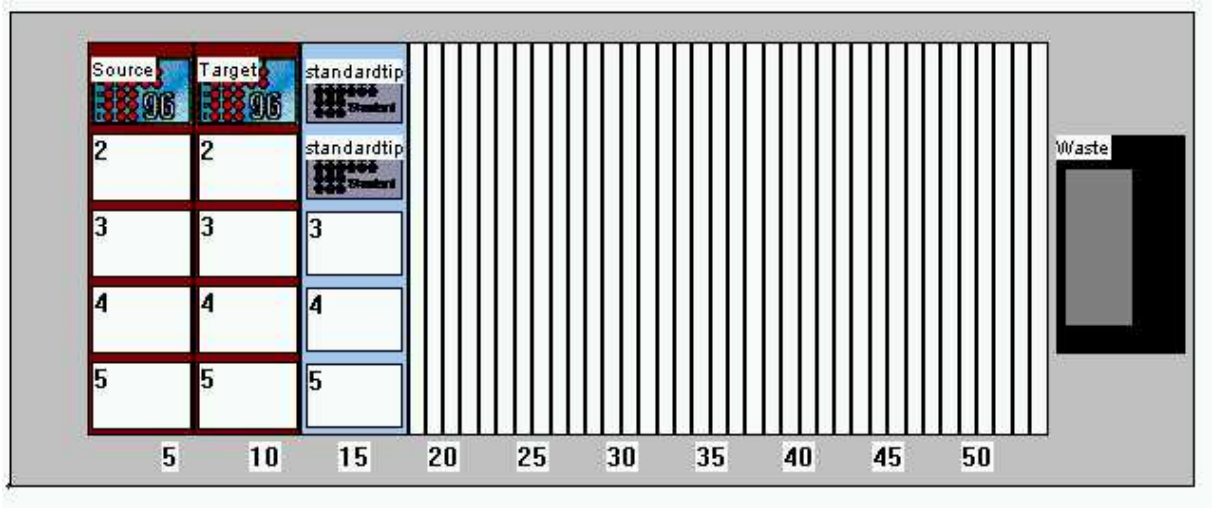
To link the Deck Layout Editor permanently to the HSL Method Editor, the value of the registry key *HKEY_LOCAL_MACHINE\SOFTWARE\Phoenix\Instruments\MicrolabSTAR\Method Editor* has to be changed from *HxMetEd* to *HxHSLMetEd*.

We recommend that you create a shortcut on the desktop to the HSL Method Editor. The editor is stored under ...\hamilton\bin\HxHSLMetEd.exe.

18.1 Create a Method to Copy from Plate to Plate

The method we are going to describe copies wells, i.e. aspirates liquids from wells on a plate and dispenses them to the corresponding wells on another plate (A1→A1’,...,H12→H12’). The transferred volume will be 50 µl. The method name is “HSL_OnePlateToPlate“.

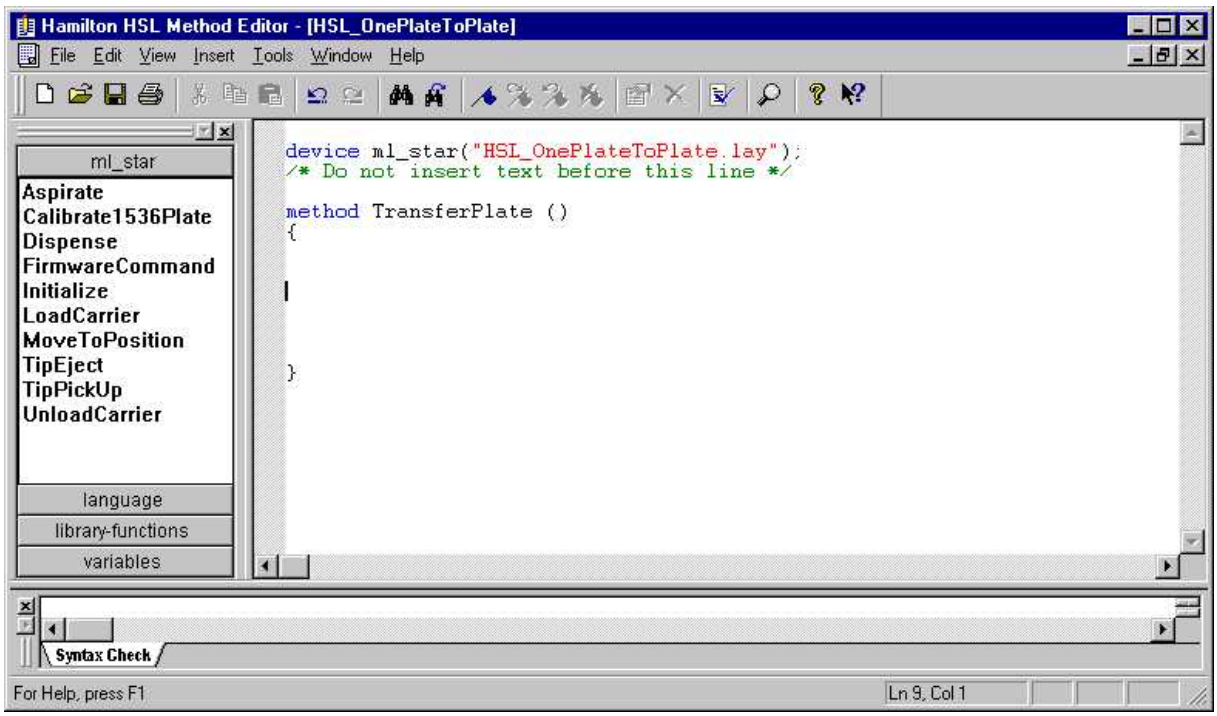
First, an appropriate deck layout has to be created and saved as “HSL_OnePlateToPlate” (.lay). The deck layout for this method is shown in the screenshot.



Decklayout HSL_OnePlateToPlate

To create this deck layout start the Deck Layout Editor by double-clicking on the appropriate icon. Select “Open” from the “File” menu, then select the deck layout from the example in chapter 13.3: OnePlateToPlate.lay. Save this layout under the name HSL_OnePlateToPlate.lay. Alternatively, create the new deck layout again by adding labware.

Open up the HSL Method Editor and load the “HSL_OnePlateToPlate” HSL file. Under the View menu on the menu bar, check “View All Stepdata” so that all the parameters we enter will be displayed on the screen.



HSL Method Editor

Save the method to be created under the name HSL_OnePlateToPlate.hsl. Type in the first line of our method

device ml_star("HSL_OnePlateToPlate.lay");

This command loads the Microlab STAR device driver and links the device to a concrete Microlab STAR deck layout context and the associated instrument.

The steps in the method we are going to write will appear between the curly brackets of the method skeleton. One HSL program must contain exactly one such method.

We enter a method name by replacing “oneplatetoplateMethod”:

method TransferPlate ( )

As a first step in the method we initialize the Microlab STAR. Under the sub-menu “ml_star”, drag Initialize to a line between the brackets {}. A dialog window appears. Click “OK” to accept the default values. The line in the programming window should now read

ml_star.Initialize (Channel (1..8): oooooooo);

If it reads only “ ml_star.Initialize();” select “View all step data” in the “View” menu. The parameters of the command are given between the parentheses. Here, all channels are initialized, the circles giving a graphic representation of the situation.

Now we enter the commands to load carriers onto the deck. If your instrument is equipped with the Autoload option, the carriers are automatically moved from the autoload tray into particular positions on the deck. If not, you will be requested to load the instrument manually.

In keeping with the deck layout, we will load three carriers, the first holding the source plate, the second holding the target plate, and the third holding the tip rack.

Under “ml_star”, drag “LoadCarrier” to a line below “ml_star.Initialize();”. A dialog window appears. For the first carrier, enter carrier position “6” and select rack type “PLT_CAR_L5AC”. Click OK. This selects a carrier (CAR) for 5 deep-well or archive (AC) plates (PLT) in landscape (L) orientation.

Again double-click or drag “LoadCarrier” to a line below the last “Load Carrier” command. A dialog window appears. For the second carrier, enter rack position “12” (these carriers are 6T wide) and select rack type “PLT_CAR_L5MD”. Click OK. This selects a carrier for 96-well or 384-well microtiter plates.

Again double-click or drag “LoadCarrier” to a line below the last “Load Carrier” command. A dialog window appears. For the third carrier, enter rack position “18” and select rack type “TIP_CAR_480” which is a tip carrier. Click OK. In the method frame we see:

ml_star.LoadCarrier (Position: 6, rack type: PLT_CAR_L5AC);

ml_star.LoadCarrier (Position: 12, rack type: PLT_CAR_L5MD);

ml_star.LoadCarrier (Position: 18, rack type: TIP_CAR_480);

Now we aspirate from one plate and dispense into another, using new tips. Under "language", double-click or drag “while” to a line below the last “load carrier” command.

In the method frame appears

```
while()
{
}
```

Position the cursor between the parentheses () and double-click “sequence” under “variable” in the command frame. In the dialog box select “ ml_star.Source” on the left and “GetCurrentPosition()” on the right and click OK. This results in:

```
while (ml_star.Source.GetCurrentPosition ( )  )
```

Now add by hand the condition “> 0”

```
while (ml_star.Source.GetCurrentPosition ( ) > 0 )
```

This means “as long as the position of the source well is greater than zero (or the operation is not finished), do the following”. When the position reads zero, the sequence has been worked through completely.

Now we enter the pipetting commands between the curly brackets.

Under the submenu “ml_star”, double-click or drag the single steps. Now double-click or drag tip pick up. The same dialog box as in the graphical method editor appears.

NOTE

Single steps are the same in the HSL and the graphical level of programming.

Choose the same settings as in the example with the graphical method editor. The same is true for the following aspiration, dispense, and tip eject steps.

We should now see the following lines in the programming window:

```
ml_star.TipPickUp (Channel (1..8): oooooooo , Sequence: ml_star.Tip ,
      Tip type: StandardVolumeTip);
```

This command picks up standard tips without filter on all used channels from the sequence of the tip rack, i.e. from the tip rack.

```
ml_star.Aspirate (Channel (1..8): oooooooo , Sequence: ml_star.Source,
                  Liquid name: StandardVolume_Water_DispenseJet,  Volume:  50 µl,
                  LLD settings: Capacitive: on - Pressure: off, Liquid following: on );
```

This command aspirates liquid on all used channels from the sequence of the source plate, i.e. from the source plate. The liquid name specified enables the software to use the appropriate parameters. Volume and the settings for liquid level detection are further parameters. Here we aspirate following at liquid level.

```
ml_star.Dispense (Channel (1..8): oooooooo , Sequence: ml_star.Target,
                  Liquid name: StandardVolume_Water_DispenseJet,  Volume:  50 µl,
                  LLD settings: off, Liquid following: on);
```

This command dispenses the given volume into the sequence “ml_star.Target”, i.e. into the target plate. The liquid name has to be the same as for the aspiration. Here, we dispense into an empty plate, therefore LLD is switched off and we dispense in a jet, following from 2 mm above the plate bottom.

```
ml_star.TipEject (Channel (1..8): oooooooo , Waste destination:
                ml_star.Waste);
```

This command ejects the tips to the sequence of the waste container “ml_star.Waste”, i.e. into the waste container.

We now need to get the program to keep count of the wells in the sequences, adding one to the total so far each time a well is copied. Create the following lines below the “ml_star.TipEject” command but above the closing bracket “}”. Under "variables" double-click "sequence" and select “ml_star.Source” and "Operator++" in the right frame. Click OK. Repeat this for the other sequences until you have written

```
ml_star.Source++;

ml_star.Target++;

ml_star.Tip++;
```

This tells the system to keep adding to the count of source plate wells, to the count of target plate wells, and to the count of tips. When 96 wells and tips have been counted, the counter will go back to zero, thus ending the program in accordance with the “while” statement we invoked at the beginning. The “++” command automatically adds to the sequence counters the number of pipettings done so far.

The last lines in the method frame you should see are

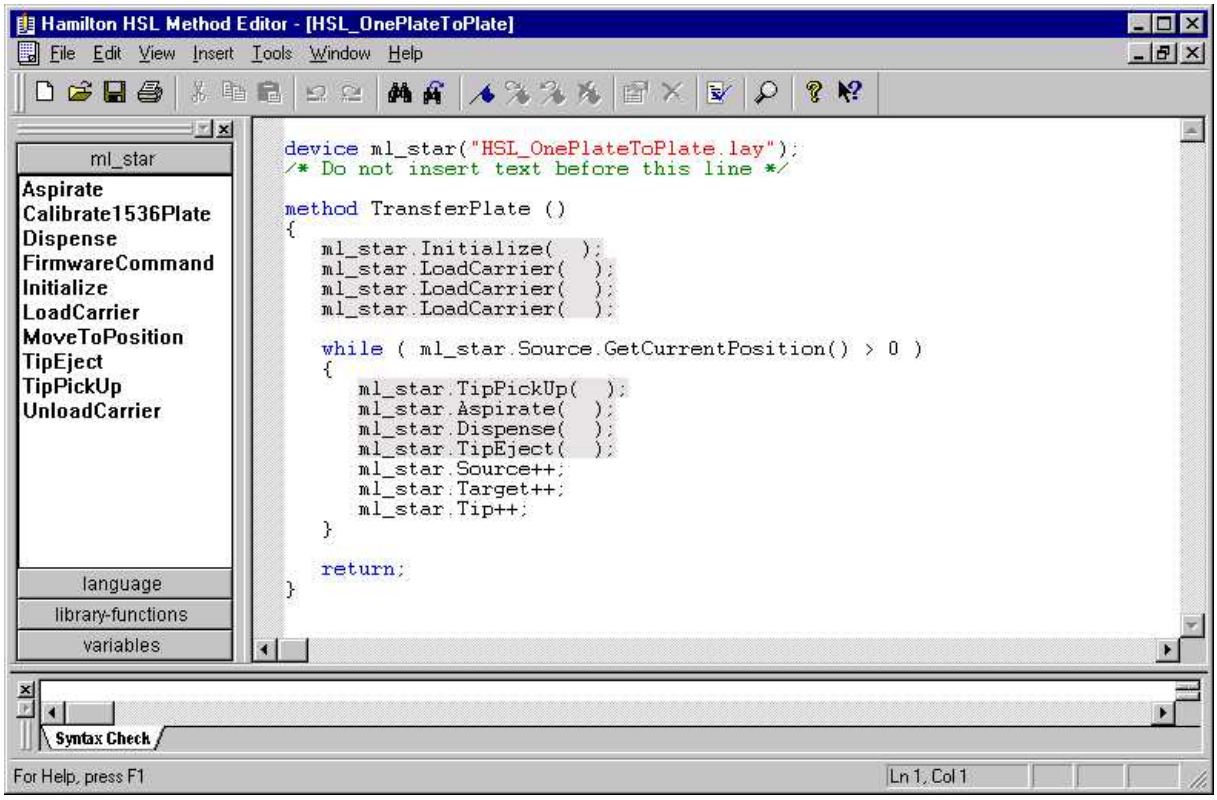
```
        }

        return;

}
```

The first bracket “}” finishes the loop of the “while” statement, “return;”, and the last bracket “}” ends the method “TransferPlate”.

Finally, your method should look like this:



Now save your work by selecting Save from the File menu. The Software prompts you to perform a syntax check. Click YES. The result of the syntax check is displayed in the bottom frame of the method editor. If you have typed in the commands correctly, the result should be “0 error(s)”.

Another example is available within your demo methods directory: “copy from tubes to plates”.

Appendices

A. Glossary

Adjustment

Detailed positional setting for the hardware

Air displacement tip

Commercial pipetting tip

Aliquot

Aliquots are identical small volumes of liquid.

Aspirate

To draw up liquid into a pipetting device.

Autoload

Option and hardware assembly that enables automatic loading of the Microlab STAR. It consists of a loading head movable in Y direction, which draws the carriers into the Microlab STAR and can read the barcodes on them.

AutoLoad tray

Hardware unit. On it the carriers can be placed and held outside the Microlab STAR. The loading tray is attached to the Microlab STAR, to support the automatic loading and unloading process.

Barcode Mask

The barcode mask defines the basic structure of a barcode. It is a pattern to which a barcode must conform. The assignment of a specific labware item can be done this way. The barcode mask can require a barcode to contain specific strings at fixed positions. It can contain wildcards, too.

Barcode Reader

Part of the Autoload Option.

Basic Microlab STAR

Basic parts of the Microlab STAR with pipetting arm and deck, to which the loading unit and the options can be added on.

Carrier

Manipulable unit for loading on the Microlab STAR. Hardware unit, in or on which elements stand, and which is transported when the Microlab STAR is loaded (cf. *Autoload*).

Container

Includes test tubes, reagent containers, and wells.

Container identification

Barcode for identification of containers. Serves for unambiguous identification of the vessel, e.g. a sample test tube.

Continuous loading

Refers to the loading of manipulable elements onto the Microlab STAR after processing has been started.

Front Window and Side Cover

Covering for the Microlab STAR. Option and assembly with which the work surface of the Microlab STAR is covered in such a way that it is shielded from user intervention and other outside influences (such as dust). At the same time, it protects the user from the movements of the Microlab STAR. It is transparent.

Deck

The work surface of the Microlab STAR. It presents at the same time the greatest possible area, cf. *Work Area*. The placing of the carriers on it is defined by the 55 tracks, as long as they are in the operating range of the pipetting area.

Dispense

To distribute quantities of liquid from a pipetting device.

Firmware

Programs (sequences of commands) which are carried out on the processors of the Microlab STAR.

Hardware error

Type of error which depends exclusively on faulty functioning of the hardware.

Instrument

Hardware of the Microlab STAR (mechanics, electronics, and firmware)

Instrument commands

The commands made available by the firmware for controlling the Microlab STAR.

Labware

Refers to movable items to be placed on the Microlab STAR deck, such as carriers, containers, or racks.

LIMS

Higher-level data processing system, generally known as Laboratory Information Management System, also LMS.

Liquid

Includes all kinds of liquids, among which are included reagents, controls, standards, wash fluids.

LLD (Liquid Level Detection)

Positive pipetting of liquid which may be achieved either by pressure, or capacitive signal detection and transfer.

Loading, unloading

The process by which carriers are brought on to the work surface of the Microlab STAR and taken off again. This can happen automatically by means of the Autoload Option, or manually.

Method

The method contains all instructions as to how the content of the source vessels is to be processed. The assignment of the vessels happens "virtually", however; they are positioned in the deck layout definition.

ML STAR User Software

Software running Microlab STAR.

MTP (Microtiter plate)

In general we assume a plate with 96 Wells (8 x 12) 9 mm wide.

There are also plates with 384 Wells (16 x 24 / 4.5 mm), or others with a different size.

Pause

Interruption of processing. The current processing steps are ended.

Pipetting

Transfer of liquids, usually a defined number of aliquots, from one container to another.

Pipetting arm

Assembly consisting of at least 8 Pipetting channels, as well as the common X-driving and arm casing.

Pipetting channel

Hardware part of the Pipetting arm, which can be moved horizontally in the Y direction.

Pipetting module

Firmware (-processor-program) which controls a pipetting channel, in which category are included the Y and Z pipetting movement, and the LLD.

Pooling

Pipetting of different liquids in one well:

1,2,3 .... to n, and n to 1,2,3,....

Processing step

Defines what, where, and with what has to happen on the Microlab STAR. It is defined in accordance with the methods, the loading, and the tasks. These are the foundation stones of Microlab STAR methods.

Rack

Grouping of containers in a mechanical, manipulable unit.

Rack identification

Barcode for Rack identification.

Random access

Means that every channel can access everywhere on the work area.

Run

Execution of the processing steps defined in the relevant method with the aim of processing one or more liquids and containers (e.g. MTP). The run is a series of timed commands, in order to carry out processing on the Microlab STAR according to the processing plan. The run can be interrupted to load more elements. Then processing goes on according to a newly calculated processing plan, with the run being started again. Loading is not a part of the run.

Run abort

Cancellation of a run by the user or by the Microlab STAR.

Run Visualisation

Visualization of the present work status of the Microlab STAR.

Sample

Refers to a liquid in an unambiguously identified container which is to be processed.

Stacker

Storage unit for racks

Tip

Disposable tip for dispensing

Tip rack

Frame that holds the tips together.

Tip waste

Container into which the tips are dropped.

Touch-off

Type of dispensing whereby the tip or needle approaches the bottom of the empty container so close as to allow the dispensed droplet to have simultaneous contact with the tip or needle and the container bottom.

Trace

Note of a status during a processing

Tube

A narrow container for liquid, usually having a circular cross-section, and a cylindrical length-section.

User

User of the product. Levels of user proficiency are not distinguished by the system.

Verification Kit

Option. Aid to check the functions of the Microlab STAR, including adjustment.

Waste Container

A device on the Microlab STAR deck to collect used disposable tips.

Well

The individual hollow in the MTP.

Well type

Geometrical shape of the well, such as U, V, or flat.

Wick side of container

Type of dispensing whereby tip or needle touches side of container and thus releases the droplet. Not possible with Microlab STAR.

Work area

The area on the Microlab STAR, to which access is provided during processing. Elements to be pipetted can be placed in this area.

Worklist

Information sent from outside the system, as to what method(s) is (are) to be executed on the Microlab STAR, and with what liquid.

B. Index

C. Regulatory Affairs

CE, CSA and UL conformity are maintained for Microlab STAR. See the Declaration of Conformity for the instrument reproduced on the next page.

Radio Interference (USA and Canada)

This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to both Part 15 of the FCC Rules and the radio interference regulations of the Canadian Department of Communications. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses and can radiate radio frequency energy and, if not installed and used in accordance with the present user manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference, in which case the user will be required to correct the interference at his own expense.

Pursuant to the Canadian Radio Interference Regulations, ICES-001 Notice for Industrial, Scientific and Medical Radio Frequency Generators, this ISM apparatus meets all requirements of the Canadian Interference-Causing Equipment Regulations. Please note that this requirement is only for generators which operate at over 10,000 Hz.

In Vitro Diagnostics

The Microlab STAR is **not** intended specifically to be used as an “In Vitro Diagnostic Device”. The following text defines an “In Vitro Diagnostic Device” [from: Directive 98/79/EC of the European Parliament and of the Council of 1998-10-27 on in vitro diagnostic medical devices]:

'[...] *in vitro* diagnostic medical device' means any medical device which is a reagent, product, calibrator, control material, kit, instrument, apparatus, equipment, or system, whether used alone or in combination, intended by the manufacturer to be used *in vitro* for the examination of specimens, including blood and tissue donations, derived from the human body, solely or principally for the purpose of providing information:

- concerning a physiological or pathological state, or

- concerning a congenital abnormality, or

- to determine the safety and compatibility with potential recipients, or

- to monitor therapeutic measures.

Specimen receptacles are considered to be *in vitro* diagnostic medical devices. 'Specimen receptacles' are those devices, whether vacuum-type or not, specifically intended by their manufacturers for the primary containment and preservation of specimens derived from the human body for the purpose of *in vitro* diagnostic examination.

Products for general laboratory use are not *in vitro* diagnostic medical devices unless such products, in view of their characteristics, are specifically intended by their manufacturer to be used for in vitro diagnostic examinations; [...]'

Declaration of Conformity

We Hamilton Bonaduz AG, CH-7402 Bonaduz/Switzerland confirm that the following product	Wir, Hamilton Bonaduz AG, CH-7402 Bonaduz/Schweiz bestätigen, dass das folgende Produkt	La société Hamilton Bonaduz AG, CH-7402 Bonaduz/Suisse confirme que l'instrument ci-dessous
Product name	**Microlab STAR**	
S/N	....	
meets the following EC directives (including all applicable amendments):	mit den folgenden EG-Richtlinien (einschliesslich aller zutreffenden Aenderungen) übereinstimmt:	est en conformité avec les directives CE suivantes (y compris leurs amendements, le cas échéant):
CE	EMC Directive	89/336/EEC
	Low Voltage Directive	73/23/EEC

Applied harmonised standards:

Safety	EN 61010-1 (1993) + A2 (1995) EN 60825-1 (1994) + A11 (1996)
Emission	EN 61326 (1997) + A1 (1998) class B
Immunity	EN 61326 (1997) + A1 (1998) (laboratory equipment)

Additional Information:

C US	Canada, USA	CAN/CSA-C22.2 No. 1010.1-92 UL Std. No. 3101-1 FCC, Part 15, class A

D. Ordering Information

A current checklist is available from HAMILTON.

E. Principles of Calibration

Statistical Definitions Used for Calibration

Calculated value in ml:

$$x = \frac{x_t(\mu l) \cdot x_m(mE)}{x_c(mE)}$$

where x_c = measured standard value
x_m = median of measured cuvette
x_t = target value

Mean ($\bar{x}$)

$$\bar{x} = \frac{\sum_{i=1}^{} x_i}{n}$$

Standard Deviation (s)

$$s = \sqrt{\frac{\sum_{i=1}^{n} (x_i - \bar{x})^2}{n-1}}$$

Precision (Coefficient of variation ($CV(\%)$))

$$CV = \frac{s}{\bar{x}} \cdot 100$$

Accuracy ($R(\%)$)

$$R = \frac{|\bar{x} - x_t|}{x_t} \cdot 100$$

where t = target value

F. Needle Wash Station: Chemical Compatibility

1. Chemical compatibility of standard wash station

Chemical	1.4310	EPDM	FPM	PE	NBR	PEEK	POM	PP	PTFE	PVC	PVDF	SI
Acetic acid, 20%	1	2	3	1	3	1	1	1	1	1	1	2
Acetic acid, glacial	1	4	4	1	4	1	4	1	1	4	1	2
Acetone	1	1	4	2	4	1	1	1	1	0	3	3
Acetonitrile	1	3	3	1	4	0	3	3	1	0	1	0
Ammonium hydroxide, 5%	1	1	2	1	2	1	1	1	1	0	2	1
Chloroform	1	4	3	3	4	1	4	3	1	4	1	4
Deionized water	1	1	1	1	1	1	1	1	1	1	1	1
Dimethyl formamide	1	2	4	1	4	1	1	1	1	4	4	2
Dimethyl sulfoxide	1	3	3	1	4	0	1	1	1	4	3	0
Ethyl acetate	1	3	4	2	4	1	1	1	1	4	3	2
Hexane	1	4	1	3	1	1	1	2	1	4	1	4
Hydrochloric acid, 20%	4	1	1	1	4	1	4	1	1	1	1	3
Isopropyl alcohol	1	1	1	1	3	1	1	1	1	4	1	1
Methanol	1	1	3	1	3	1	1	1	1	3	1	1
Methylene chloride	1	4	3	4	4	2	3	3	1	4	1	4
Nitric acid, 5-10%	1	2	1	1	4	1	4	1	1	1	1	2
Nitric acid, 70%	1	4	2	3	4	1	4	4	1	4	1	4
Phosphate buffer	1	1	1	1	1	0	1	1	1	0	1	4
Phosphoric acid, 85%	2	3	1	1	4	0	4	1	1	1	1	3
Potassium hydroxide conc.	1	1	4	1	3	1	3	1	1	0	2	3
Sodium acetate	1	1	3	1	3	0	1	1	1	3	1	4
Sodium borate	1	1	1	1	3	0	1	1	1	1	1	1
Sulfuric acid, 1-75%	2	4	1	1	4	2	4	1	1	1	1	3
Urine	1	1	1	1	1	1	1	1	1	1	1	1
Triethylamine	1	4	3	0	3	0	1	4	1	0	3	4
Toluene	1	4	1	3	4	1	1	3	1	4	1	4

Effects (Key to codes in above table):

1 = no effect, little or no noticeable change

2 = slight corrosion or discoloration

3 = Moderate corrosion or other change in physikal properties or dimensions; not recommended for continuous contact

4 = severe corrosion or physical change;prolonged contact not recommended

0 = No data

Materials

1.4310	Steel	POM	Polyoxymethylene
EPDM	Ethylene-propylene-elastomer	PP	Polypropylene
FPM	Fluoroelastomer	PTFE	Polytetrafluorethylene
NBR	Acrylnitril-butadiene-rubber	PVC	Polyvinylchloride
PE	Polyethylene	PVDF	Polyvinylidenefluoride
	Storage Containers for Wash Liquids	SI	Silikon
PEEK	Polyetheretherketone		

2. Chemical compatibility of higher resistance wash station

Chemical	1.4310	EPDM	FPM	PE	PEEK	PP	PTFE	PVDF	SI
Acetic acid, 20%	1	2	3	1	1	1	1	1	2
Acetic acid, glacial	1	4	4	1	1	1	1	1	2
Acetone	1	1	4	2	1	1	1	3	3
Acetonitrile	1	3	3	1	0	3	1	1	0
Ammonium hydroxide, 5%	1	1	2	1	1	1	1	2	1
Chloroform	1	4	3	3	1	3	1	1	4
Deionized water	1	1	1	1	1	1	1	1	1
Dimethyl formamide	1	2	4	1	1	1	1	4	2
Dimethyl sulfoxide	1	3	3	1	0	1	1	3	0
Ethyl acetate	1	3	4	2	1	1	1	3	2
Hexane	1	4	1	3	1	2	1	1	4
Hydrochloric acid, 20%	4	1	1	1	1	1	1	1	3
Isopropyl alcohol	1	1	1	1	1	1	1	1	1
Methanol	1	1	3	1	1	1	1	1	1
Methylene chloride	1	4	3	4	2	3	1	1	4
Nitric acid, 5-10%	1	2	1	1	1	1	1	1	2
Nitric acid, 70%	1	4	2	3	1	4	1	1	4
Phosphate buffer	1	1	1	1	0	1	1	1	4
Phosphoric acid, 85%	2	3	1	1	0	1	1	1	3
Potassium hydroxide conc.	1	1	4	1	1	1	1	2	3
Sodium acetate	1	1	3	1	0	1	1	1	4
Sodium borate	1	1	1	1	0	1	1	1	1
Sulfuric acid, 1-75%	2	4	1	1	2	1	1	1	3
Urine	1	1	1	1	1	1	1	1	1
Triethylamine	1	4	3	0	0	4	1	3	4
Toluene	1	4	1	3	1	3	1	1	4

Effects (Key to codes in above table):

1 =	no effect, little or no noticeable change
2 =	slight corrosion or discoloration
3 =	moderate corrosion or other change in physical properties or dimensions; not recommended for continuous contact
4 =	severe corrosion or physical change; prolonged contact not recommended
0 =	No data

Materials

1.4310	Steel	PEEK	Polyetheretherketone
EPDM	Ethylene-propylene-elastomer	PP	Polypropylene
FPM	Fluoroelastomer	PTFE	Polytetrafluorethylene
PE	Polyethylene	PVDF	Polyvinylidenefluoride
	Storage Containers for Wash Liquids	SI	Silicon