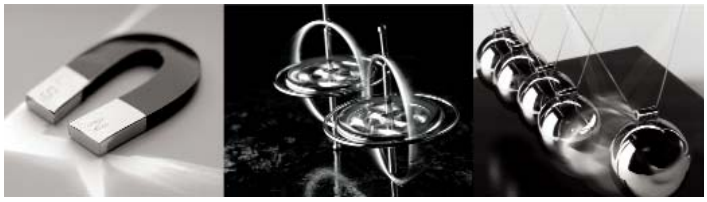

Binding Stability Kinetics

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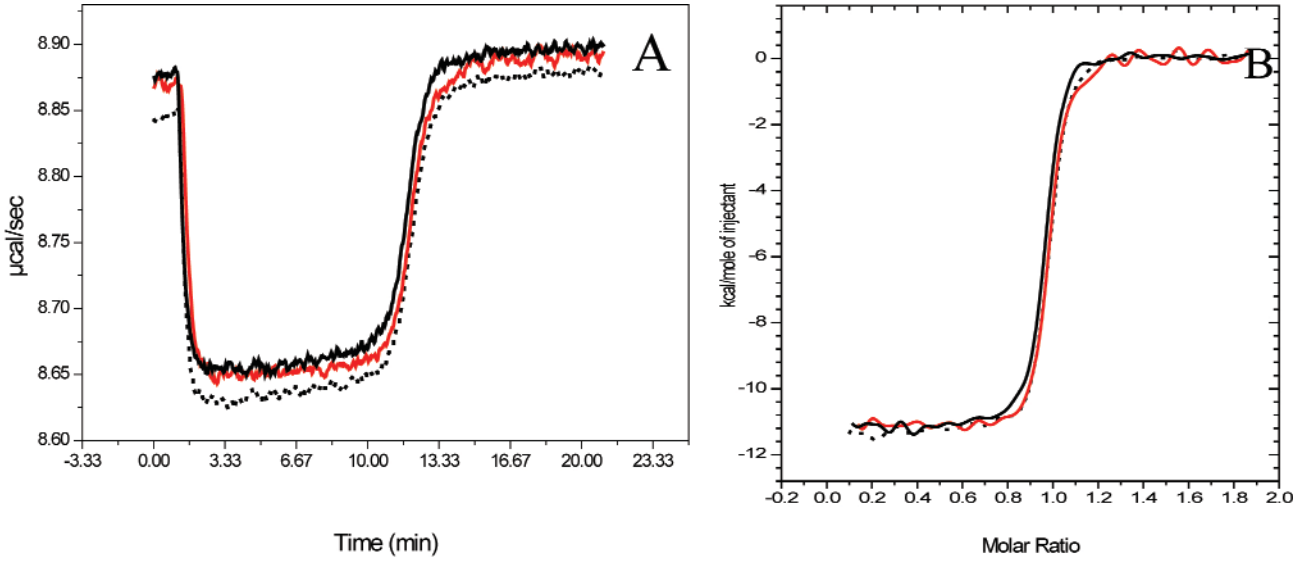
VP-ITC Single Injection Method

Hardware & Software Upgrade Instructions



MAU130040 Rev B

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Introduction

For a VP-ITC to accurately and reliably execute the Single Injection Method, hardware and software updates may be necessary. When necessary, the upgrades provide additional application flexibility in stirring speeds and more precision during the one continuous injection of ligand. The software upgrades for both VPViewer™ 2000 and Origin®, software support the hardware upgrades and provide the data analysis routine for easy experiment set-up and comprehensive data analysis.

Prior to embarking on the procedures described in this manual, it is important to confirm that all the correct parts have been received. If a problem should arise during the upgrade or additional information is needed, contact MicroCal Technical Support at 413-586-7720 Ext 25 or e-mail at support@microcalorimetry.com.

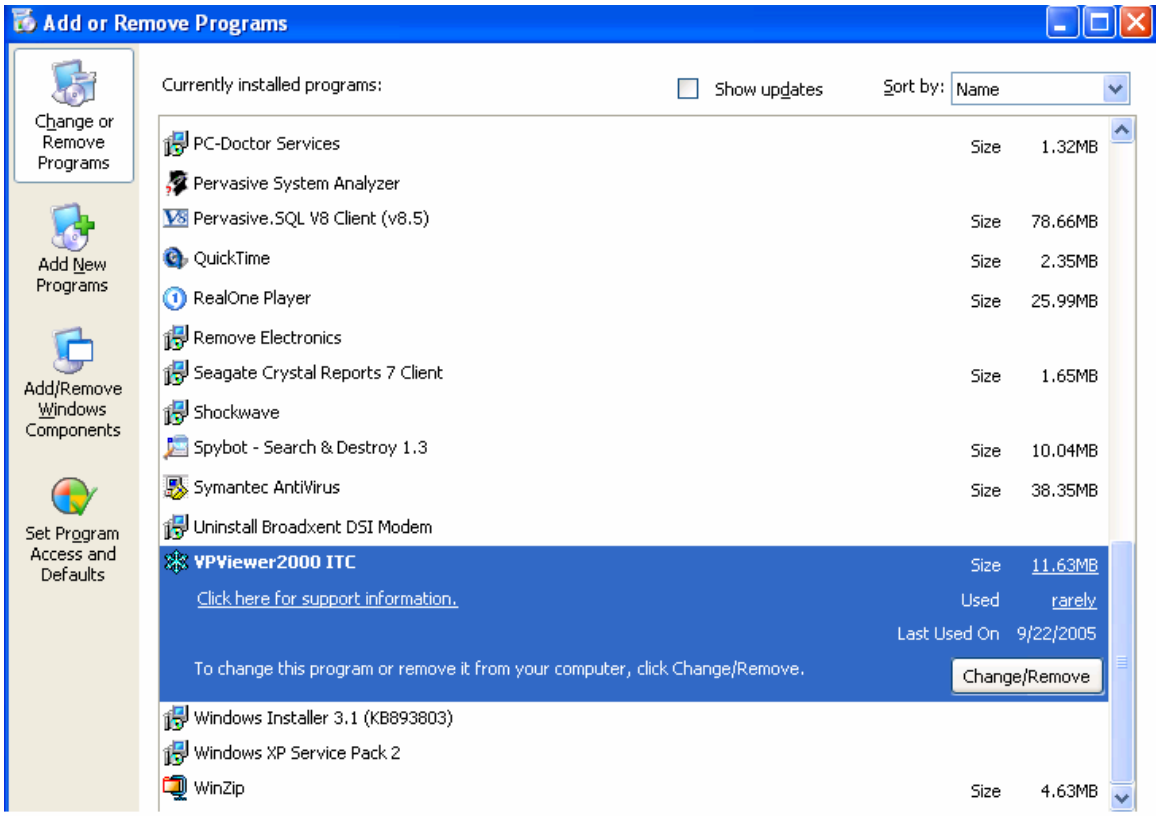
VPViewer™ 2000 Software Update

In order to utilize the Single Injection Method on your VP-ITC instrument you must first update your VPViewer 2000 software. Prior to installing the newest version of VPViewer 2000, you must remove the existing version using Control Panel. Follow the steps below to uninstall and then reinstall the VPViewer 2000 software. Uninstalling the VPViewer 2000 software will not delete any data files (or other files, folders, etc…) that you have generated and saved using the VP-ITC.

Uninstalling the existing VPViewer 2000 Software

1. Open Control Panel and then double-click on Add or Remove Programs from the list of Control Panel icons.
2. Scroll down the list of programs and select VPViewer 2000 ITC, as shown below.

Figure 1: Uninstall Window for VPViewer 2000

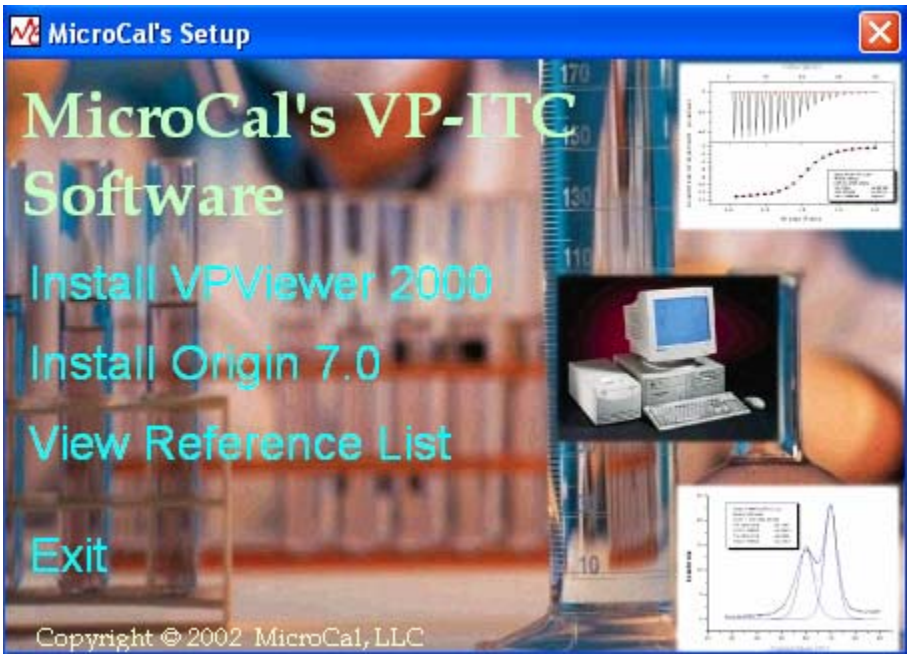


3. Click on Change/Remove and the removal process will begin.
4. When prompted with three choices of *Modify, Repair or Remove*, select *Remove* and then click on the Next button.
5. Accept all default choices until the removal is complete.

Installing New Version of VPViewer 2000 Software

1. Insert the new installation CD into the CD drive and wait for the front-end installation applet to load. The window shown below should appear. If it does not then you must invoke the VPSetup.exe program from the root directory of the CD.

Figure 2: MicroCal Software Install Window



2. Click on *Install VPViewer 2000* and the installation process will be invoked.
3. Accept all defaults during the installation process. The VPViewer 2000 software will be installed to the c:\vpitc folder.
4. When prompted about restarting your computer, select *No, I will Restart My Computer Later*.

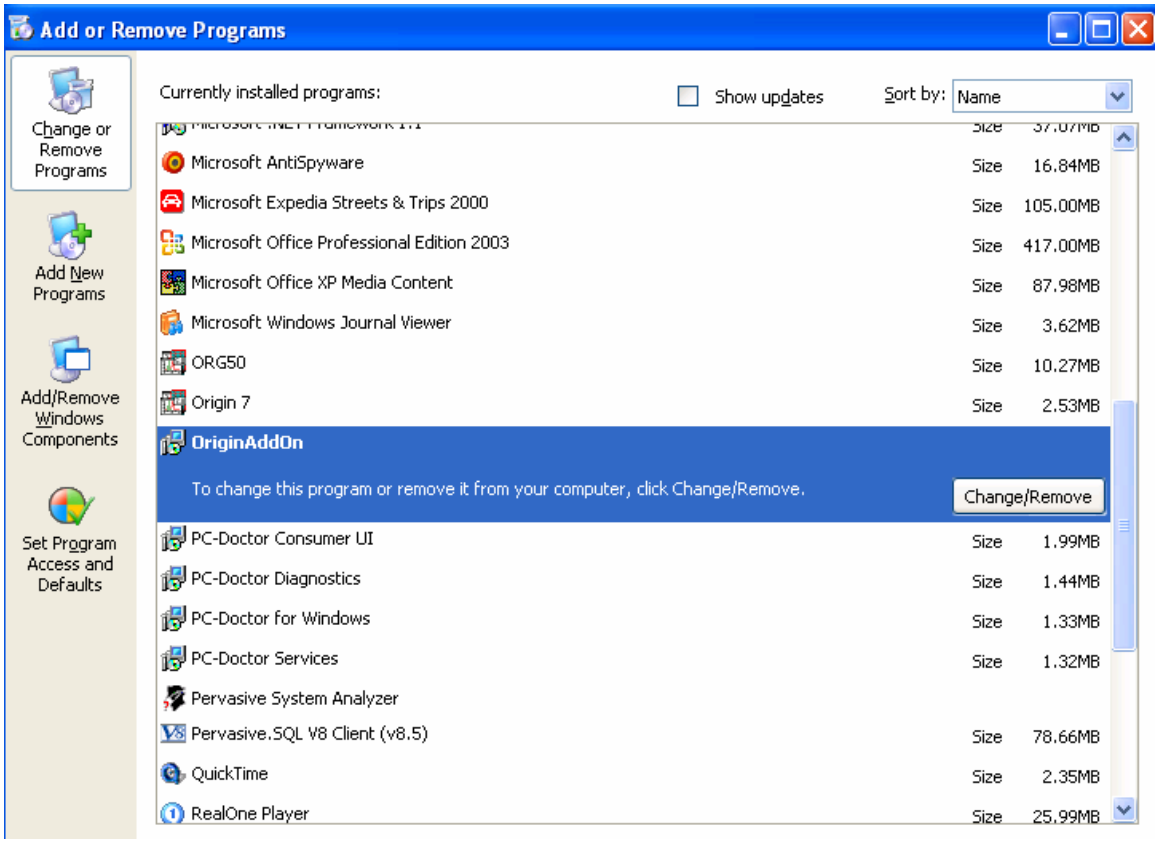
Origin® Software Update

In order to analyze data obtained from the Single Injection Method on your VP-ITC instrument you must first update your Origin software to v7.0. Prior to installing the newest version of Origin 7.0, you must remove the existing version using Control Panel. Follow the steps below to uninstall and then reinstall the Origin 7.0 software. Uninstalling the Origin 7 software will not delete any project files (or other files, folders, etc…) that you have generated and saved using Origin.

Uninstalling the Existing Origin Software

1. Open Control Panel and then double-click on Add or Remove Programs from the list of Control Panel icons.
2. Scroll down the list of programs and select *OriginAddOn*, as shown below.

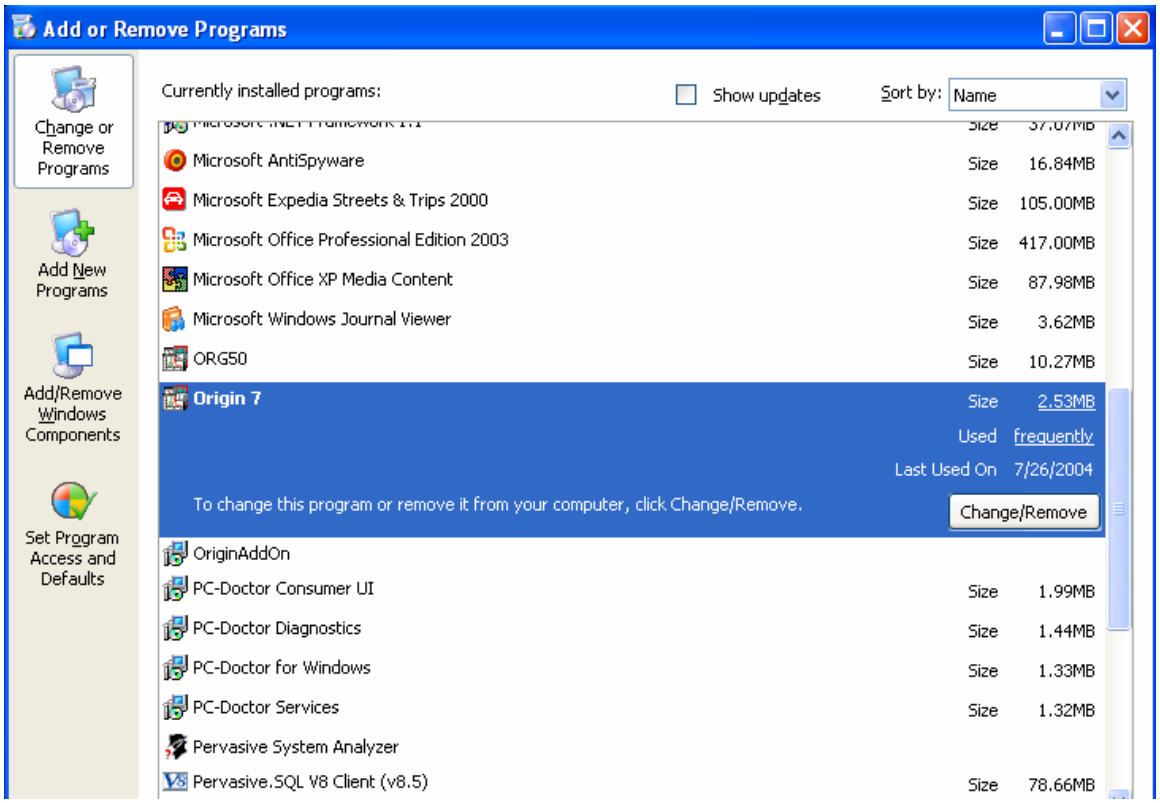
Figure 3: Uninstall window for OriginAddOn Software



3. Click on Change/Remove and the removal process will begin.
4. Accept all default choices until the removal is complete.
5. Open Control Panel and then double-click on Add or Remove Programs from the list of Control Panel icons.

6. Scroll down the list of programs and select Origin, as shown below.

Figure 4: Uninstall Window for Origin software



7. Click on Change/Remove and the removal process will begin.
8. Accept all default choices until the removal is complete

Installing New Version of Origin 7.0 Software

The installation program prompts you to type in your user information and the serial number for the software. This serial number is located inside your registration card in the Origin product package as well as on the CD case. (e.g. XXXXX-XXXX-XXXXXXX) There is also a License number and a Registration ID that will need to be entered the first two times you start Origin 7.0. If you do not know your serial number, license key or registration number then contact MicroCal for assistance.

1. Insert the new installation CD into the CD drive and wait for the front-end installation applet to load. The window shown below should appear. If it does not then you must invoke the VPSetup.exe program from the root directory of the CD.

Figure 5: MicroCal Software Install Window

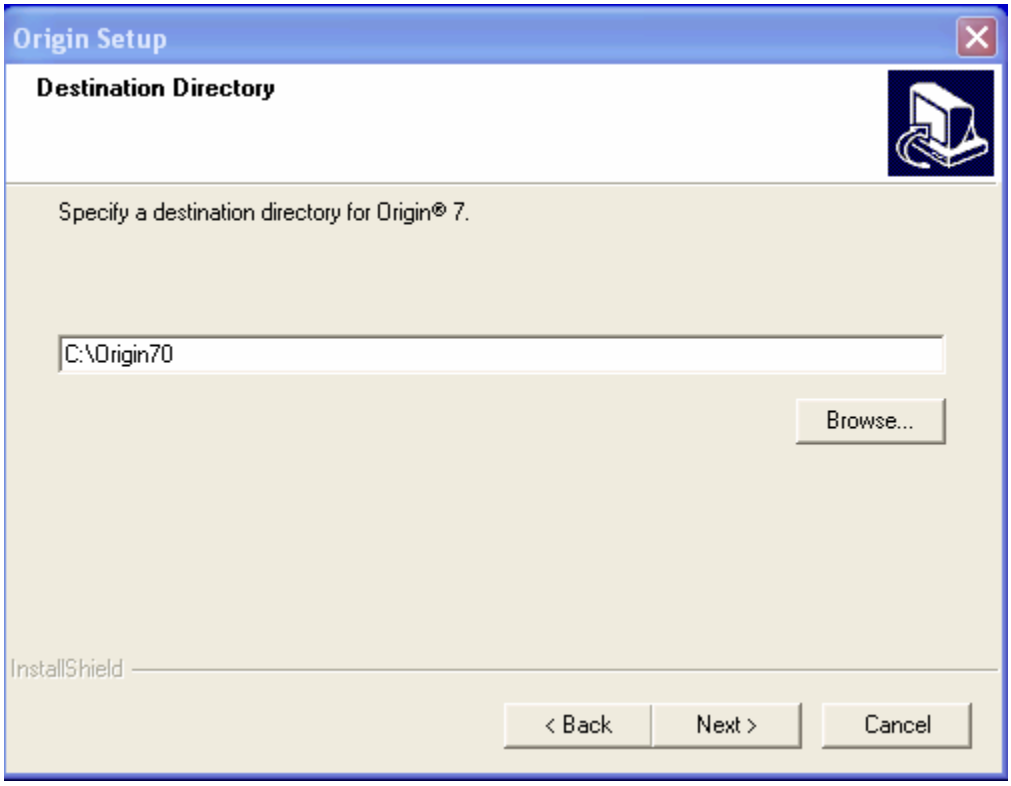
2. Click on *Install Origin 7.0* and the installation process will be invoked.

3. A 2nd window appears and you should click on Install Origin 7, as shown in the picture below.

Figure 6: Origin Software Install Window

4. Accept all defaults during the installation process, except for the default destination folder. **You must install Origin 7.0 to the c:\origin70 folder or the VP-ITC will not work properly.** The destination folder screen appear as shown below.

Figure 7: Origin Software Setup Window



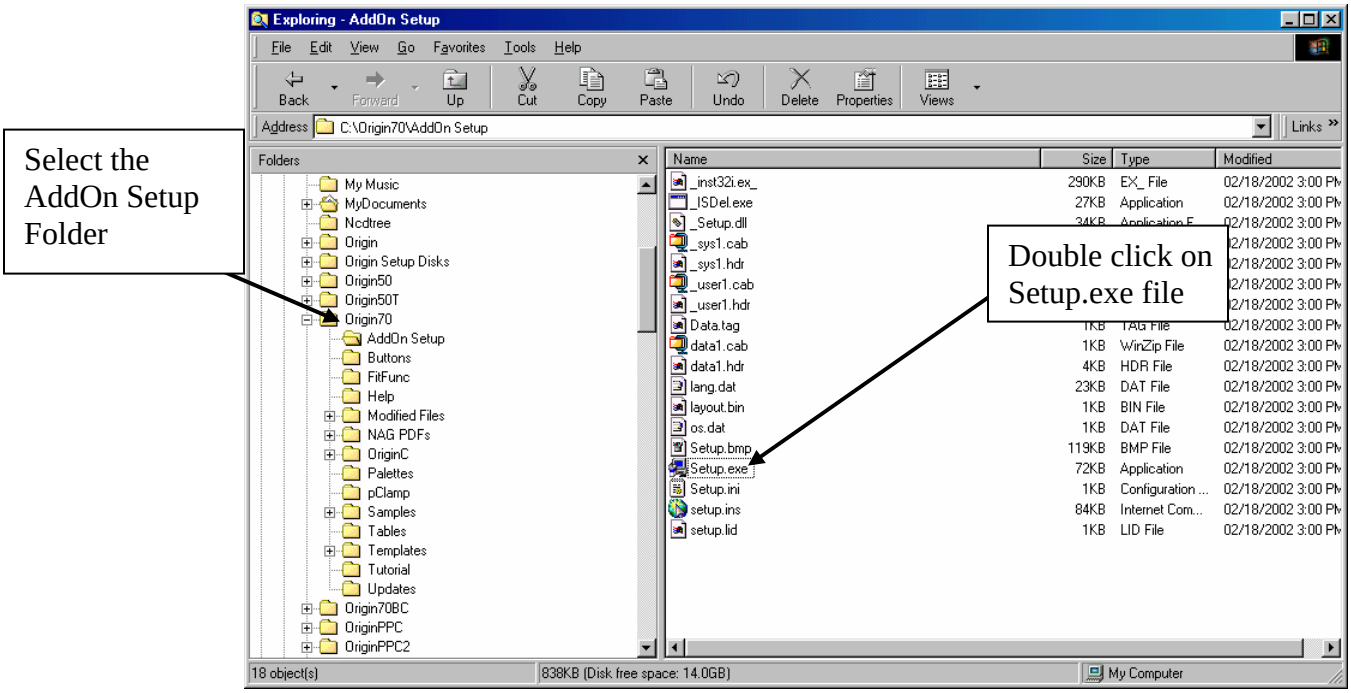
5. Continue to accept all default choices or options until the installation is completed.
6. Leave the installation CD in the CD drive since it will be needed in the next and final software installation step.

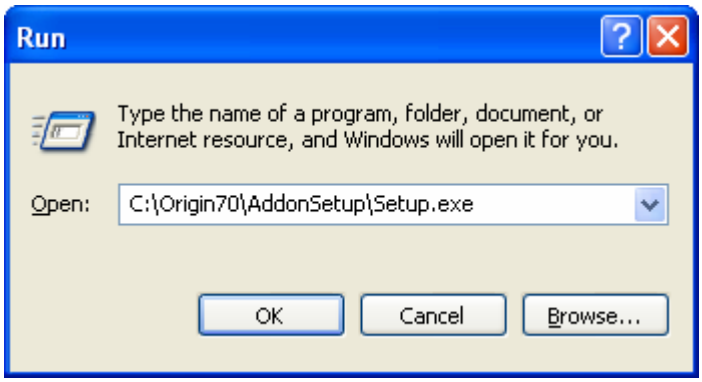
Installing The Origin 7.0 Add-On Disk

The "Add-On Disk" is actually a sub-folder that is contained on the installation CD and is not a separate disk as the name implies. Add-On disks must be installed properly for the ITC data analysis routines to work.

1. Using Windows Explorer, navigate to the c:\origin70\AddOnSetup sub-folder.
2. Double-click on the Setup.exe file listing, as shown in the picture below.

Figure 8: OriginAddOn Software Install Windows

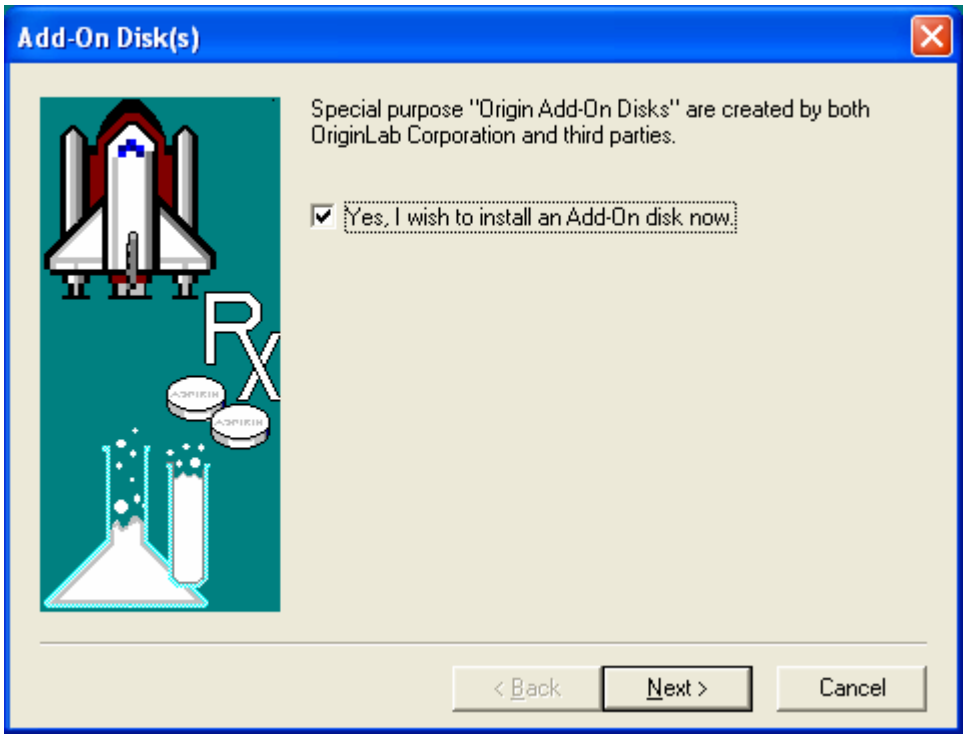




*Note: you may also launch the setup up program by selecting **Run** from the **Start** Menus then entering **c:\Origin70\AddOn Setup\Setup.exe** into the text box and clicking OK.*

3. Check the box to indicate that you want to install an Add-On disk.

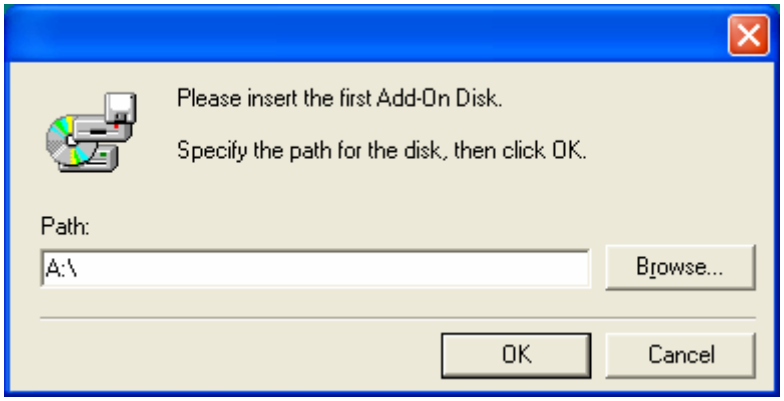
Figure 9: OriginAddOn Install Option Window



When the setup software loads you will be prompted with this screen. Make sure the "Yes, I wish to install an Add-On disk now." option is checked. Then click next.

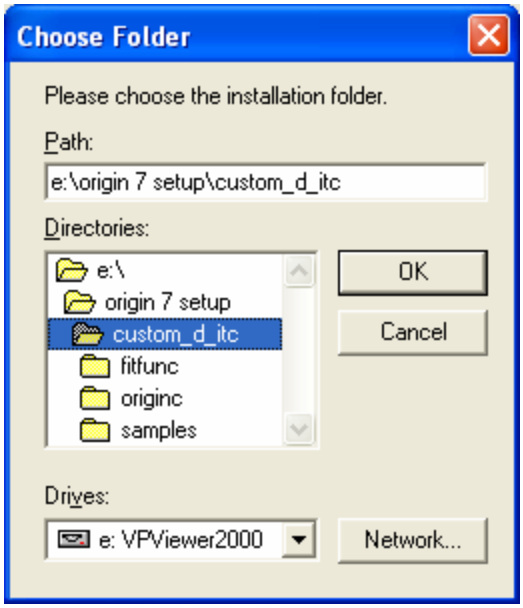
4. The software will now prompt you for an Add-On disk. All Add-On software is located on the Origin 7 CD and there is no need to insert any additional disk. Instead select browse and then the name of the CD drive with your Origin 7 disc in it. This is usually the D:\ or E:\ drive.

Figure 10: OriginAddOn Disk Prompt Window



5. Click on the *Browse* button and use the mouse navigate to the Add-On Disk path, as shown in the picture below, then click on the *OK* button.

Figure 11: OriginAddOn Folder Window

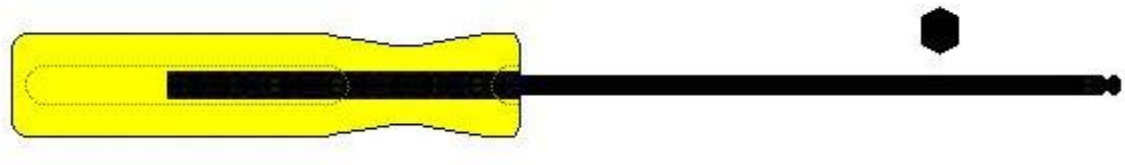


6. Allow the installation to complete, then restart your computer (because you opted not to restart it after completion of the VPViewer 2000 installation process).
7. If you wish to create a shortcut desktop icon you may do the following. Right-click the *MicroCal LLC ITC* icon and select *Copy* from the menu. Right-click anywhere on the desktop and select *Paste*.

Required Tools

1. 7/64” hex driver (shown in Figure 1 and provided with original instrument accessories)

Figure 12: 7/64" Hex Driver



2. 0.05” hex driver (shown in Figure 2 and provided with original instrument accessories)

Figure 13: 0.05" Hex Driver

Pre-Disassembly Steps

Prior to starting any disassembly, perform the following steps:

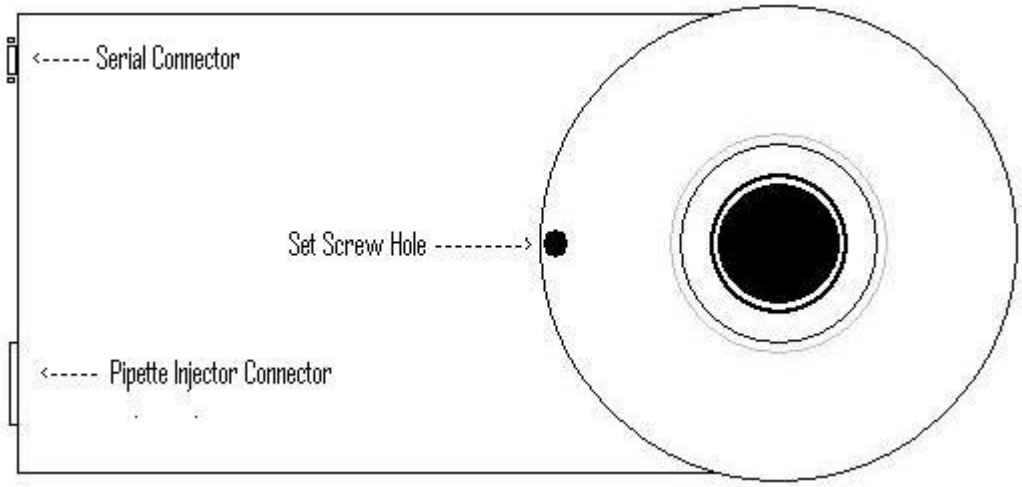
3. Place the pipette in its stand on the side of the instrument.
4. In VPViewer, open the ITC Controls Tab
5. From the pipette maintenance group click on the Open Fill Port button. Wait for the injector plunger to move up to its home position.
6. Turn off main power switch located on the back panel of the ITC cell unit.

VP-ITC Hardware Update

Removal of VP-ITC Stirring Base:

**** Special Note: This section is only required if you received a new Injector Base and Syringe Holder with your Single Injection Method upgrade package!**

Figure 14: Stirring Base Diagram

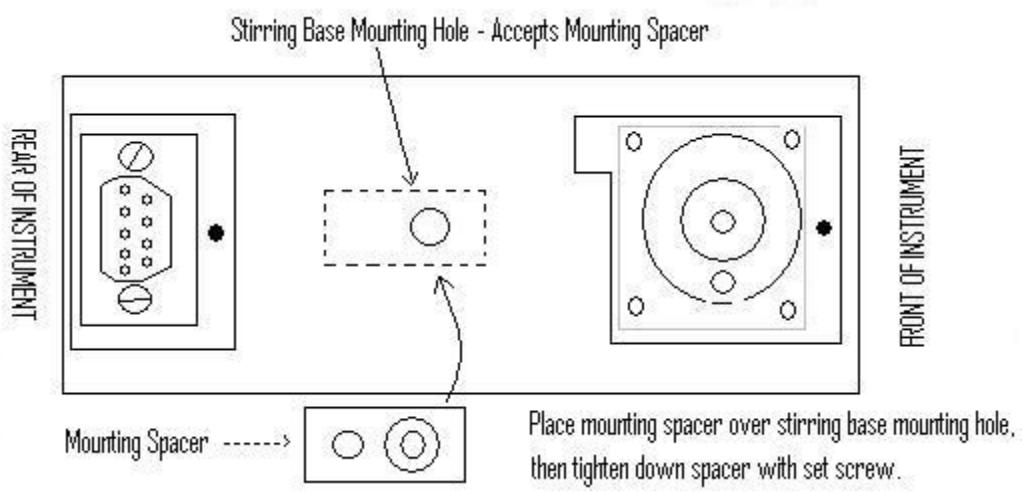


2. Unplug both ends of the serial cable, one at the back of the VP-Controller and the other at the back of the VP-ITC cell unit. The serial cable for the new stirring base will connect to the same port on the back of the VP-Controller.
3. Unplug the "phone-style" cable from pipette injector connector of the stirring base.
4. Insert 7/64" hex driver into the set screw hole that is approximately centered on top of the stirring base. Lower the tool until it catches the set screw head. Loosen the set screw all the way (CCW).
5. Lift stirring base straight up and off of the top of the instrument. It might require some gentle jostling, but not much force.

Installation of New VP-ITC Stirring Base

1. In the space vacated by the original stirring base, install the new stirring base mounting spacer, as shown below.

Figure 15: Stirring Base Mounting Diagram



2. Using the 7/64” hex driver, **install the mounting spacer set screw into the recessed hole of the Mounting Spacer and then into the Stirring Base Mounting Hole**. Tighten until snug. The vacant hole of the Mounting Spacer should be towards the rear of the instrument
3. Carefully lower the new stirring base into the slot on the top of the instrument and gently push into place.
4. Insert 7/64” hex driver into hole that is approximately centered on top of the stirring base. Lower the tool until it catches the set screw head. Tighten the set screw all the way (CW) until snug. Do not over-tighten!
5. Connect new serial cable to rear of new stirring base and to the rear of the Controller. **Note that the serial cable connection at the back of the VP-Controller is the same as with the original stirring base**. **The serial cable connection at the ITC is now at the back of the stirring base and no longer at the back of the cell**. It will not matter which end of the cable connects to the VP-Controller or stirring base.

Replacement of the Syringe Holder & Subsequent Syringe Height Adjustment

**** Special Note: This section is only required if you received a new Injector Base and Syringe Holder with your Single Injection Method upgrade package!**

! **Caution: At all times when handling the injection syringes you want to use care to prevent the long needle from any action that may cause it to bend. If the long needle is bent far enough it will become a permanent bend that will make the syringe unusable for any experiments.**

! **Caution: Never attempt to couple/decouple the pipette from the syringe assembly unless the pipette plunger is in the open port position (all the way up). Failure to follow this recommendation may result in a bend in the pipette screw. Even a slight bend will result in improper operation of the pipette.**

Replacing the Syringe Holder

1. **Separate the pipette injector from the syringe holder** by turning the Locking Collar on the syringe holder counter clock-wise. Lift the pipette injector up and off of the syringe and syringe holder assembly. Set the pipette injector aside.
2. **Remove the lower Syringe Clamp** using a 0.050" Ball Point Hex Driver. Set it aside.
3. **Remove the glass titration syringe** from the Syringe Holder by gently pulling it up through the top opening. Be very careful not to bend the long, metal needle of the titration syringe.
4. **Loosen the set screw on the upper white locking ring** so that the ring is free to slide up and down the glass of the injection syringe, **but do not remove it completely.**

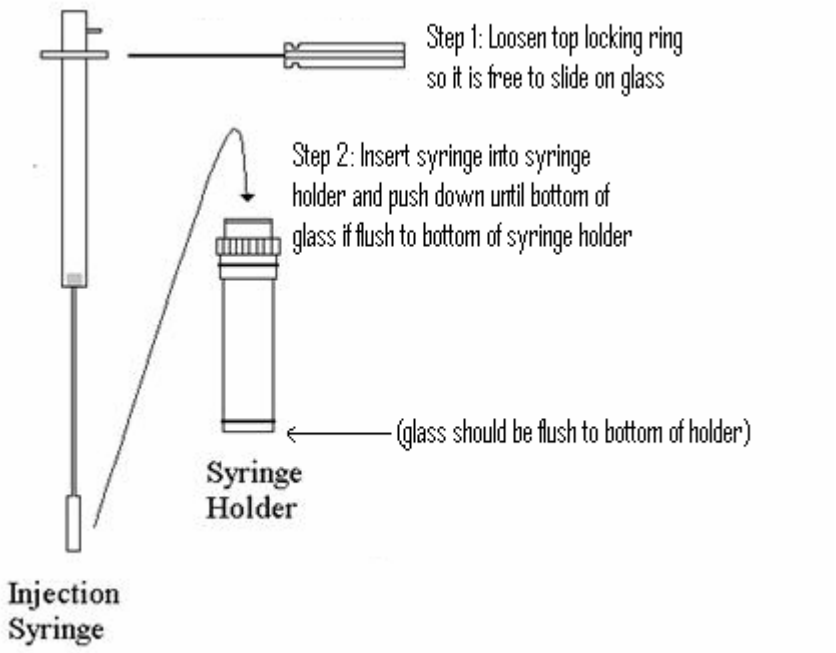
Syringe Height Adjustment

**** Special Note: This section is only required if you received a new Injector Base and Syringe Holder with your Single Injection Method upgrade package!**

Each titration syringe comes with a round 'height disk' (upper Syringe Clamp) that will ultimately determine your syringe height once the syringe and syringe holder is inserted into the ITC sample cell. Prior to using your titration syringes in the ITC the 'syringe height' will first need to be properly set. The syringe height is the distance between the bottom of the syringe needle and the bottom of the cell, and is a critical setting for ITC performance. The proper setting for the ITC syringe height is 3 mm off the bottom of the cell.

1. **Position the syringe into the new syringe holder** so that the tip of the syringe will not hit the bottom of the cell when the syringe/holder assembly is inserted into the sample cell. This can be easily accomplished **by positioning the glass of the syringe so that it is flush to the bottom of the holder, but not lower**.

Figure 16: Injection Syringe & Syringe Holder Diagram



2. Holding the syringe holder only, insert the syringe/holder assembly into the sample cell compartment and properly seat the assembly into place. The syringe should still be flush to the bottom of the syringe holder and not lower.
3. **Gently push down on the top of the syringe until you feel it hit the bottom of the cell**. If you are unsure then double-check it by lifting the syringe up and lowering it again until the bottom of the cell stops it.
4. **Lower the upper Syringe Clamp** so that it is flush to the top of the syringe holder.

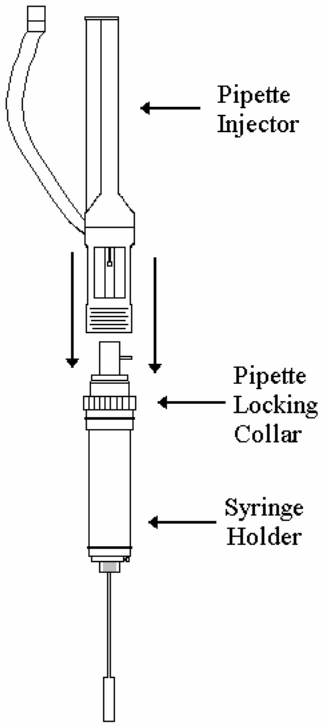
5. **Tighten the set-screw** of the upper Syringe Clamp so that the disk is fixed to the syringe glass.
6. **Raise the syringe** by carefully prying up on the upper Syringe Clamp until it is **approximately 3 mm** higher than the top of the syringe holder.
7. **Without moving the syringe, loosen the set-screw** of the upper Syringe Clamp so that the clamp is free to slide up and down on the outer diameter of the glass bore. **Lower the Syringe Clamp** all the way down so that it is again flush to the top of the syringe holder.
8. **Tighten the set-screw** of the upper Syringe Clamp. The syringe height is now fixed (for this syringe only) at 3 mm from the bottom of the sample cell.
9. Remove Syringe Holder assembly from the instrument.
10. Repeat the procedure with all injection syringes so that they are all fixed to be 3 mm above the bottom of the sample cell.

! **Use care not to over tighten the set screw, as too much pressure will cause the glass to break. You want to tighten the screw just enough to prevent the bottom syringe clamp from moving freely.**

Figure 17: Pipette Injector & syringe Holder Assembly Diagram

Installing the Pipette Injector

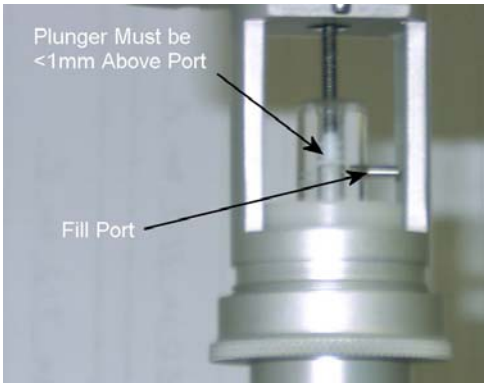
1. Insert the syringe holder into the pipette holder (attached to the side of the VP-ITC).
2. Bring the pipette injector directly above the syringe holder, start bringing the injector down while observing the plunger tip of the pipette injector to insure that it is being inserted into the hole in the glass barrel of the injector syringe, then mate the screws of the pipette injector to the locking collar of the syringe holder.
3. With your fingers, turn the locking collar until it is fully tightened on the pipette injector. When the locking collar is fully tightened there should be only a couple threads of the pipette injector visible.
4. Plug the connector of the pipette into the back of the pipette controller to complete the pipette assembly.



Pipette Open Port Position Adjustment

****Special Note: This section is only required if you received a new injector base and syringe holder with your single injection method upgrade. This calibration must be done only after the new stirring base has been installed and all syringe heights have been properly set!**

Figure 18: Pipette Plunger & Fill Port



The open port position is the resulting pipette plunger position after the *open port* button is depressed in VPViewer 2000. A properly set open port position allows the titrant to be drawn up into the syringe by manual vacuum. After the the new stirring base is installed and all syringe heights are properly set, the open port calibration must be completed.

Calibration of the open port position is accomplished in VPViewer 2000 by first selecting the *ITC|Pipette Calibration* menu. This will open the *Pipette Setup* window. Perform this calibration by completely assembling the syringe and pipette mechanism and then selecting the *open port* button in the *Pipette Setup* window. The plunger should move just above (<1mm) the filling port on the upper part of the syringe. If the Plunger is not positioned approximately 1 mm above the port then the position will need to be adjusted using the controls within the *Pipette Setup* window. Use the Up/Down buttons together with a distance of 0.025" to position the plunger to the appropriate open port position, 1 mm above the filling port. Once the plunger is properly positioned, click on the *Calibrate Open Fill Port* button and the calibration is complete. Click on the Close button once and confirm that the plunger is positioned juts below the filling port. Finally, click on the Open button and confirm that the plunger goes to the appropriate open port position.

Figure 19: ITC/Pipette Calibration Menu

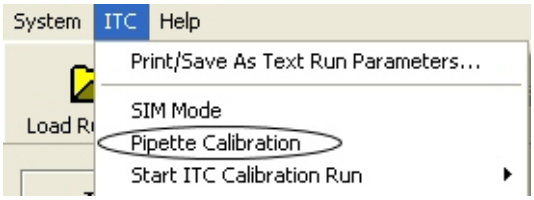
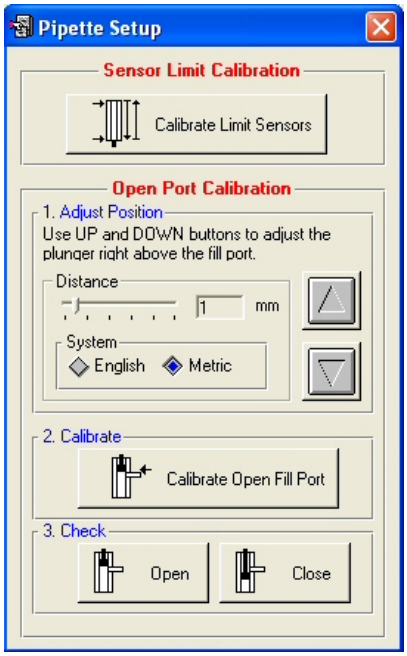


Figure 20: Pipette Setup Window

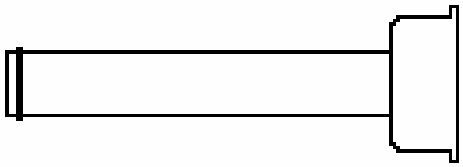


Initiate the limit sensor calibration by selection the *Calibrate Limit Sensors* button. This will move the pipette plunger to the upper and lower limits and record these limits in the calibration file. Once the pipette plunger has finished moving, the calibrations are complete.

****IMPORTANT NOTE: After the SIM upgrade has been completed the original software disks previously shipped with the instrument are no longer usable and should be discarded. The newly installed hardware is not backwards compatible with old software. Any attempt to reinstall the original software after the SIM upgrade is completed will result in the instrument becoming completely inoperative. Please contact MicroCal Technical Service at 413-586-7720 Ext 25 or e-mail at support@microcalorimetry.com for further information.**

Cleaning Device Adaptor

Figure 21: Cleaning Device Adaptor



A new cleaning device adaptor is provided with the Single Injection Method upgrade. Discard the original adaptor supplied with the instrument. When sample cell cleaning is required, refer to the VP-ITC User's Manual, Section 3.7 for proper use of the cleaning device adaptor.