

# LEAP IFC User Manual

Document Revision 1.7

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#### **Overview**

The Agilent-LEAP purification system consists of an Agilent 1200 prep LC system (pumps and detectors) controlling a CTC IFC fraction collector/Injector PAL.

The IFC sample injection functions are handled within ChemStation using the standard CTC PAL driver. The fraction collection functions of the IFC are controlled using an additional addon software package called "FractPAL". FractPAL is a license software package from LEAP Technologies. This software must be installed separately after ChemStation has been installed. The software is launched automatically when ChemStation is started after both software packages are installed and configured correctly.

The FractPAL software handles communications between ChemStation, the Collector PAL and the Agilent 1200 Automated Fraction Collector (AFC), which sends a signal whenever a fraction is to be collected.

#### The IFC PAL

The CTC Integrated Fraction Collector (IFC) is an autosampler and fraction collector. It is a Twin PAL mounted on a single horizontal beam. Both PALs have their own electronic boards and control panel along with separate i/o panels.



#### The Injector PAL

The CTC PAL autosampler on the right hand side of the IFC behaves as an independent unit and may be controlled either with Cycle Composer (linked to ChemStation) or the internal CTC PAL driver within ChemStation.

There are no significant changes required to the software for autosampling. The autosampler can handle both vials and well plates. It can access all positions on the IFC including the collected fractions.

#### **The Fraction Collector**

The left hand side of the IFC is the fraction collector, which has a modified injection head to accommodate the side-port syringe and collection tubing. It has a special needle guide for collection. It does not handle raw sample and does not have a wash station. Fraction collection is initiated by raising the plunger of the side-port syringe

When the plunger is raised above the lower inlet port, flow is directed downward to the collection tube. When the plunger is below this position, flow is direct to waste via the upper port. There is an O-ring seal at the top the plunger to prevent leakage.

Fraction collection is triggered by a switch closure signal from the Agilent 1200 AFC. The Agilent 1200 AFC is operating normally if it is collecting into a 40x50mL tube tray with a tube volume matched to the IFC tube.

After each run, the AFC tray will be reset in software, so that the tray is never filled.



IFC TTL signal #1 input



In this mode, samples are injected onto the Prep Valve and signal is monitored by the detectors via the active splitter. Fractions are collected by the IFC PAL directed by ChemStation method and the Agilent 1200 AFC.



In this mode, samples are injected onto the Analytical Valve. The active splitter is turned off all flow goes to the detectors. There is no fraction collection.

#### System configuration: A2Prep Mode



Samples may be run either analytically or preparative and, because the autosampler can access all positions of the fraction collector, fractions may be analyzed analytically after collection.

Agilent can supply A2PREP software which can automatically complete:

- 1. Pre Analytical run, which queues qualifying samples for the Prep phase:
- 2. Prep run, which purifies the samples according to the pre-selected purification method.
- 3. Fraction re-analysis, where fractions are re-injected to the analytical system for confirmation.

#### Prep Applications – Higher Flows, up to 100ml/minute

Wider bores are required for all liquid paths. Everything on the PAL in the Prep flowpath is upgraded to 19gauge

- Valve inlets, ports and rotor grooves
- 19g inlet sleeves (blue)
- 19g syringes (1ml, 2.5ml and 5ml)
- Injection head comes with drilled out needle guides
- Larger bore glass capillaries on wash station.

#### Fraction Collection adaptations to the collector PAL:

- "Dilutor" type Side Port syringe assembly acts as a valve
- Needle never touches raw sample no wash station
- Special lower needle guide design
- Trays are defined with no penetration, needle must clear vials
- Grounding wire is essential for safety with high flow rates
- Delay sensor on Waste station wired to Agilent fraction collector

#### System Requirements

#### **Computer Workstation**

The computer workstation is the central interface for the user. The FractPAL software will be loaded onto it, and will control the configured component methods via 9-pin RS-232 ports. The computer should be a PC and have all of the following features as minimum requirements:

- 1G MHz Pentium Processor or equivalent
- 64 MB of RAM
- -60MB Hard disk Memory for application, additional needed for files
- CD-ROM Drive
- -2x 9 pin COM Ports
- Windows 2000 or XP Operating System

#### Software

The ChemStation software is supplied by Agilent and must be B.01.01 or later. The FractPAL software is incompatible with earlier ChemStation versions,

Agilent ChemStation controls the Agilent 1200 system and the injection PAL. If below ChemStation Version B 03.01use the appropriate CTC driver installed in ChemStation along with the CTC ICC (Instrument Control Components, included with the IFC PAL) or in later version B 03.01 or later the Agilent CTC Control Software (purchased from Agilent)

Fraction collection is controlled by FractPAL, which is from LEAP Technologies. While FractPAL integrates tightly with ChemStation, it is a separately licensed package and is also provided with the IFC PAL.

#### Installing IFC hardware

The IFC PAL should be installed in accordance with the CTC Manual. The system may be installed in 2 possible configurations:

Analytical to Prep:	This system has 2 injection valves; Valve 1 is for prep injections (large volumes). The fast wash station is mounted to the front of thi valve drive. Valve 2 is for analytical injections.	
Prep only:	This system has only 1 injection valve and is dedicated to Prep runs only.	

#### Interface connections:

For either configuration there are 2 interface cables required: (see Appendix)

• Injector:

Connect interface 1 of the Injector PAL to the Remote input of any Agilent module. (Standard Sync cable for Agilent 1200/PAL system).

• Collector:

Connect the interface 2 connector of the PAL to the BCD Connector on Agilent 1200 BCD board.

• Com ports:

Each PAL requires its own RS232 (null modem) cable which is supplied with the system. The default settings expect the Injector PAL to be on Com1 and the collector PAL to be on Com2. These can be changed in the software configuration for Com 1-4.

#### **IFC PAL Setup**

There are some special considerations when setting up the IFC PAL for the LEAP-Agilent applications. The CTC manual covers basic hardware installation issues, this section describes the proper arrangement of the configured objects on the cross rail so as to meet the special requirements of the application.

Objects which must be accessible by the Injection PAL:

- Prep Injection valve
- Analytical Injection valve (if using)
- Fast Wash Station
- Sample Tray(s)
- Fraction collection trays

Objects which must be accessible by the Collection PAL:

- Fraction collection trays
- Collector Waste station

Note that both the sample trays and the fraction collection trays must be within the working range of the injection PAL. This requires careful placement of the support legs beneath the cross-rail in order to allow sufficient room for all the accessible components.

Signal settings on the Collection PAL: (these are not default)

- **TTL-In1** Active State **Low** (is active when slider is fully forward)
- **TTL-In2** Active State **Low** (is active when slider is fully backward)
- **TTL-In3** Active State **High** (Fraction collect signal from ChemStation)

Signal settings on the Injection PAL: (these are not default)

- TTL-In1 Active State High
- TTL-In2 Active State Low

There is only about 1cm of slack on the rail to meet these requirements. In order to help in the positioning of the support legs (which are the first step in the hardware installation) use the following pictures as guides.



#### Collector side.

Collector side support leg should line up with the screw to the left of the power connector. This will leave room on the injector side for the following arrangement of mountings.



Injector side.

Note that the above picture shows only a Prep valve. The independent wash bottle bracket should always be mounted between these legs (otherwise the Prep valve cannot be included in the working range of the PAL). If an analytical valve is added, it must be

mounted between the support legs of the Sample tray. You will then find that the valve interferes with the rear most positions on the Tray holder. This problem is resolved by installing a forward extension to the holder as shown below.



Sample Tray holder extension

Install the extension by first removing the tray holder support legs from the tray platform, then reassembling with the extension inserted between the tray legs and the tray platform as shown above.

#### Installing FractPAL software

Before doing this installation, it is necessary to first remove any preexisting versions of FractPAL. To do this, click 'Start'  $\rightarrow$  'Control Panel'  $\rightarrow$  'Add/Remove Programs'. Select FractPAL from the list, and remove the program.

Next, Insert the FractPAL CD into the disk drive. If the setup does not start automatically, using Windows Explorer, locate the disk icon and double-click 'setup.exe' to launch the installer.

After the FractPAL splashscreen, this will be the first window you see. Type in the license key found in your CD case and Click 'OK' to continue. (Note that the key is Case Sensitive).

FractPAL - InstallShield Wizard
Customer Information
Please enter your information
User Name:
Organization:
Serial Number:  Install this application for:
Anyone who uses this computer (all users)
O Only for me (Peter Smith)
stallShield
< Back Next > Cancel

The following screen is then displayed which allows the user to select the operating mode of the software. For the Agilent-LEAP IFC system you *MUST* select the second option. (This is not the default selection).

🙀 FractPAL - Ins	tallShield Wizard			×
Setup Type				1
Choose the se	tup type that best suits y	our needs.		
Please select a	a setup type,			
O FractPAL				
1	Install standalone Fract	tion collector softw	vare	
r IFC PAL	Special version of fracti	ion collector integr	ated with ChemSta	tion.
		< <u>B</u> ack	<u>N</u> ext >	Cancel

The first option is used for a standalone version of the software which is adapted for analytical fraction collection, and requires different hardware.

#### Launching FractPAL

The program will load when ChemStation is started. It may also be launched from the "PAL - Start FractPAL" pull-down menu in ChemStation.

The program will initially search for the collector PAL on COM2. If it cannot establish communication it will prompt the user to select another COM port or to run Offline. When connected, it will download all the Tray objects from that PAL and display them in the main screen (below).



FractPAL main screen – Trayholder View

The position colored blue is the next collection position. Previously collected fractions are shown grouped with different colors for each sample. If any trays are excluded from the collection sequence (see later) they are grayed out as shown in the screen below.



FractPAL main screen when some trays are excluded

If FractPAL is running in Offline mode, then the last available tray set (or a default set for firsttime connection) will be displayed. The lower right corner of the screen will show "Simulation" mode is active.

When in normal operation the LED on the lower right of the status line will say "Connected" (indicating the presence of a PAL), and the Status line will say "Wait CS" which indicated a FractPAL method is running and the program is waiting for instructions from ChemStation

#### **Configuring FractPAL**

Tray collecting sequence can be changed when in the "Method View". The sequence of collection trays depends on the list on the right side of the "Fraction Destination Tray" boxes. To re-order this list or remove trays from the collection sequence use the buttons between the boxes. Only trays on the right side will be used (in the sequence they are listed).

FractPAL	
File Method Login Utilities Tray Help	
Mode: Dual Signal Controlled, with ChemStation         Fraction Destination Tray         Tray01[FC21-55]         Tray03[FC21-55]         Tray08[FC21-55]         Tray09[FC21-55]         Remove         Tray09[FC21-55]         Remove All         Tray12[FC21-55]         Tray09[FC21-55]         Tray09[FC21-55]         Remove All         Tray12[FC21-55]         Tray13[FC21-55]	Tray06
Flush time between samples (m.ss) 0:03 Default maximum well volume (ul) 100	TTL-IN1       TTL-Out1       SW-Out1       Pwr-Out1       FlushVIv       Fract         [11:06:10]Log displace on.1       [11:06:10]Working thread running.1       [11:06:10]Getting PAL Objects Info1       [11:06:15]PAL Objects loaded!!         [11:06:15]Changing status to: TRAYS1       [11:06:27]Changing status to: TRAYS1       [11:06:27]Changing status to: WAITING1         [11:08:01]Changing status to: WAITING1       [11:08:01]Changing status to: WAITING1       [11:08:01]Changing status to: WAITING1         [11:08:18]SeqName=5, SampleName=, StartPos=, 31       Image: Start Star

"Flush time between samples" is the length of time to open the flow to waste after finishing the collection of the last fraction of a sample. This cleans out any residual sample from the syringe and needle of the collecting syringe.

It is possible to change the collection mode for the fractions. Using the "Tray Setting" button available in the Tray pulldown menu: (selection here affects all trays).

Multiple Fill will permit you to collect fractions into the same vials when you have made multiple injections of the same sample.

The Penetration in mm is the distance that the needle will penetrate into a vial. The default of -2mm will leave the needle above the receiver. If your receiver vial has a cap or

#### Tray Setting



Skip first well(s) of row	0 *
Skip first well(s) of column	0 📩
Multiple Fill	1 🔹
Penetration (mm)	·2 🔹
ОК	Cancel

cap mat that needs to be penetrated then the Penetration distance in mm will have to be increased to a desired penetration depth.

There are a number of Pull-down menus items in FractPAL which allow you to change the configuration and way the program works. There are TWO basic views in which FractPAL can operate (Display Modes). Access these from the "Tray" pull-down menu.

#### **TrayHolder View**

This is the default view shown above, which shows the user the full bed layout with all configured trays. There is a status line and status indicator at the bottom of the screen. Only essential information is provided.

#### Method View

This view is mainly used for troubleshooting, and provides additional information about the status of the program. It is also the only place where certain configuration options are available.



FractPAL main screen – Methods View

Primary configuration functions can be accessed directly from the main pull-down menu in either view. There are two:

#### **Utilities - Config**

Config is selected:

This dialogue is displayed when

Config is where communications settings are set. These should normally not be modified from their default values (except for selecting the appropriate COM port for the Collector PAL.

I Require user login			
Lock PAL panel while running			
Data log file name			
FractPALD ata.fcd			Broswe
New data file for each method RU	N		
Carial Bart	СОМЗ	<b>T</b>	
Sellar Full.	COMO		
Sample start input signal	None		
Semain Font. Sample start input signal Fraction control input signal	None TTL-In3		
Senai Foit. Sample start input signal Fraction control input signal Stop/Cancel input signal	None TTL-In3 None		OK

**FractPAL Configuration dialogue** 

#### **Utilities - Options**

These are advanced settings and should only be configured by LEAP Personnel.

"Quick off" and "Full off" option determine whether the collector plunger is pushed all the way down between fractions or just far enough to stop flow. The former mode ensures all fraction is deposited in the tube, but is slows down the move between fractions.

Iptions	x	Options	
ChemStation Calibration port DelaySns Dilute Syringe Quick off O Full off Wide open needle guide Using maldi plate	Tray holder access Holder Slider-R None By path By motor BackRest Distance (mm) OK Cancel	Options       Itray holder access         Calibration pot       Itray holder access         Iver Dilute Syinge       Ouick off         Iver Quick off       Full off         Iver Vide open needle guide       Distance (mm)         Iver Using maldi plate       Itray holder access	erF v path C By motor
		OK Can	icel Apply

#### FractPAL Options dialogue

In cases were the collector bed has a forward and backward position, the options under Tray Holder options need to be set accordingly for both positions and saved. Access by path will enable the PAL to move the bed with the injection head. By motor, requires a special motorized tray holder. None indicates the bed is fixed.

Once all of the Trays are defined and the settings are confirmed it is essential to save the FractPAL default method - FCCHEM.fcc. Otherwise there will be messages that the trays are not configured properly at startup.

#### **Delay Calibration**

This calibration step should be done by the Agilent engineer at installation and again by the customer whenever the liquid path to the Collector is modified. It is a way for ChemStation to determine with high accuracy the time required for flow from the column to the collection point. This measurement is important to assure accurate and efficient collection of peaks. It is important that after the delay calibration is done that Chemstation be closed and reopened to allow all of the settings to be updated for error states. A special Agilent sensor is mounted near to the Collector waste station on the PAL. This must be defined as a new object in the PAL firmware.



This should be under the class of objects called "INJECTORS" and have the name "DelaySns" (case sensitive). It is easy to copy the "Waste" object and rename it then re-teach the position. Set the Z-Tolerance parameter to 0.

Adjust the XY position and penetration depth very carefully, as the needle must make a good seal inside the detector at quite high flow rates.

Test the seal with the prep pumps running, by selecting "Move FC to Calib Pos" in the ChemStation PAL menu. This moves the PAL to this point and opens the flow.

To stop the test, select "Move FC to Park" in the ChemStation PAL menu.

#### Setting up the ChemStation Calibration Method

There are two steps to setting up delay calibration using the LEAP IFC.

1. Modify the "Run Time Checklist" in the "DGCALPS.M" method. Enter the pre and post-run macros as shown in the dialogue here.

2. Modify the sequence "AFCDelay.s" with the location of the vial/well containing the calibration dye sample.

course noy i	Running						1
Line:	Method	t [			Loc	ation:	ni:
ampleInfo	for Vial 1:						
							4
							<b>*</b>
Line I	ocation	Sample Name	Method	Name	Inj/Locatio	n Sample Type	Cal Level
1	P1-A01	Delay Calibrant	DEF_LC			1 Sample	
			<u>.</u>				
Insert	Cut	Сору	Paste	Append Line	Undo All Un	ndo Wizard	

Run Time Checklist: LEAPprep1	×
Check Method Sections to Run	
Pre-Run Command / Macro	
FCP_MoveFCtoCalbPort	
✓ Data Acquisition	
Standard Data Analysis	
🔲 <u>C</u> ustomized Data Analysis Macro	
☐ <u>S</u> ave GLP Data	
Post- <u>R</u> un Command / Macro	
FCP_MoveFCtoPark	
Save Method with Data	
OK Cancel Help	

#### **Performing the calibration:**

LEAPprep1 (online): method & Ru	n Control			_	
The RunControl Instrument Metho	Sequence View Abort Help	PAL			
Method and Run Control 👱	PDS_LC.M	DEF_LC.S	Run Method		
Not Ready	Last Run 0.0	Method: PDS_LC.M	Sequence: DEF_LC.S		
Start Stop	4	2			
the second	0 ba 10.00 µl			🗩 🚧 🏹 🧹	
					off
	90 - 80 - 10 -		j no De	etans 💽	
Stack: Slider-F     Tray: Tray14     Plate: FC21-55	50 40 - 30				
	20-				
C:\data\112904\TBTEST000022.D		1 2	,, min		
	[F1=Help] [F3=Be all] [F5=S	tartBun] [E6=StartSeqBun] [E9	Sector: IF11=NextWindow	1	
Displays method information. Click t	p edit.	tarananj ji o-stattseqnunj ji u	-stopj [i i i -itext#iild0#]		

Select the Diagnostics view from ChemStation. When within Diagnostics, select delay Volume calibration.



ChemStation will then complete the calibration procedure.

The PAL fraction collector (using the pre-run macro, FCP\_MoveFCToCalbPort) will move to the DelaySns position and raise the plunger to allow flow to pass to the sensor and to waste. At the end of the ChemStation calibration procedure, the post-run macro, FCP\_MoveFCToPark, will lower the PAL fraction collector plunger to send the system flow directly to waste, and will then move the PAL fraction collector arm back to the park position on the left hand side of the instrument.

It is important to close ChemStation to update all of the configurations for error states.

#### **Fraction Reanalysis**

teaching positions.

Access to fraction tubes by the Injector head requires that this PAL be taught the reference point to the Trayholder called "Slider-G" (or R/F), and that the collector Tray Types be included in the object set. This PAL cannot reach the normal Slider reference point (it is too far t the left), so an arbitrary reference point has been selected at the rear right corner of the drip tray.

CTC will include a central reference point on the drip tray itself in future instruments.

#### Teaching objects with the Long needle.

- 1. When checking positions, Clear the Z-axis parameter (F2) when not sure if it will be too deep.
- 2. Z tolerance settings for all objects must be set to zero.
- 3. Z Position for all objects should be set to the tip of the needle (imagine the needle guide at its normal position.)
- 4. Z Retract must be a value which will ensure the long needle is retracted enough into the injection head as to not hit any objects. Z Retract = 16.00 is a good starting point.

After this setup has been established, it is best to create a special firmware backup of the Injector PAL (using the Loader program) to allow easy switching between long and short needle configurations. When completed there should be a firmware backup of the fraction collection PAL and two firmware backups of the injector side (one for each needle length).

#### **Fraction collection into Stacks**

If it is required to do fraction collection into PAL Stacks, then it is necessary to ensure that the injection does not take place until the collection head is in place over the appropriate plate. Otherwise, if the injection took place immediately, the time required to open a drawer and position the head may be too long and the first fraction may be missed. In order to achieve this there are some special configuration changes which force the Injector PAL to wait at the valve before injecting. (Supported in FractPAL Version 2.2 and above)

#### **Special Injection cycle in Chemstation**

There is a special cycle required called "IFC LCInj" and is supplied on the FractPAL CD. This cycle contains some extra synchronization commands, and requires some sync signal settings to be changed on *both* PALs. (It is not necessary to change the interface cable, the pathways are already provided in the existing cable).

#### Signal settings on Collector and Injector PALs.

Collector:	Event TTL-In2 will be used (Active State Low)
	Out Signal "Injectd2" will be used and is set to TTL-Out2.
Injector:	Sync Signal "Inject2" is set to TTL-In2 (Active State Low)

In the FractPAL Configuration you must select **"TTL-In2**" as the Sample start input signal instead of **None** (See screen below).

	×
	Broswe
N	
СОМ2 💌	
TTL-In2	
TTL-In3	<u></u>
None	OK
Dilute 👻	Carbol
	N COM2 TTL-In2 TTL-In3 None

Note: When PAL signal settings are changed, it is necessary to close and re-open FractPAL

### **Appendix 1 - Installation Troubleshooting Notes**

#### Notes on PAL Hardware Setup

- 1. Sample Tray extender is very tight when mating to the Tray holder platform. It is sometimes necessary to file the edge a little to allow it to fit.
- 2. Delay sensor board needs to be drilled for mounting This will be done at the LEAP office if assemblies can be obtained by LEAP ahead of time.
- 3. Tray types may need to be uploaded to the PAL firmware (using Object manager)
- 4. The Home position of the Injector PAL needs to be made safe so that if power is turned off the long needle will not fall onto any objects and be damaged.
- 5. For the same reason as in 4, the Change syringe position for both PALs needs to be made safe.
- 6. The Collection PAL must contain a Wash1 object in the firmware for Cleanup Step purposes. There will be an error when opening FractPAL that looks *PAL ERROR: Err Code=2, RetCode PALreturn error:[@11:Object not found]*
- 7. If using a Fixed Gilson Rack and the Collection PAL still tries to move the bed then remove the **PATH: Back Rest** from the firmware on the Collection PAL.
- 8. Minimum Default Syringe volume to large for Delay calibration for larger syringes. Agilent requires 50ul – 100ul range. Lower the minimum syringe volume to 50ul on the Injector PAL.
- 9. If the Collection PAL is diverting fractions to waste and waste to fraction tubes then go into the Collection PAL firmware and confirm that the **TTL-In3** Active State is set to **High** (Fraction collect signal from ChemStation)(*this is not default in firmware*)

#### ChemStation Issues:

There is now a patch available from Agilent which fixes the noted problems.

1. Delay calibration fails – the AFC reports Delay sensor is not detected.

Consider downgrading the Product type of the Agilent fraction collector from type B to Type A to void sensing delay sensor. This effectively changes the way the AFC is recognized by ChemStation, making it behave like an old style G1364A which didn't have a delay sensor and hence would not look for it during the delay calibration process. Although this step is not essential, not doing it requires that the **AFC delay sensor must be in place for the calibration to complete**. (Or a rigid object such as a rolled up business card or vial with caps taped to it in its place!)

To make this change, type in at the command line:

print sendmodule\$(lafc1, "TYPE G1364A" (case sensitive)

Close ChemStation, power cycle AFC and restart ChemStation. You will need to

2. <sup>\*</sup>Error messages such as the one shown below are seen during the ChemStation startup sequence:

CTC Driver X
Tray Type [FC50-12] is not configured!
OK

This means that the reported PAL Tray types are not defined in the "CTC.ini" file. Most common tray types are defined, but a few are not, and any custom tray types will provoke this error.

Workaround: Edit the "*CTC.ini*" file. To do so, find the file in the ChemStation PALDATA folder and open it in Notepad.

Find the section in the file related to configuring custom tray types which will look like the column on the left. Edit it with the appropriate definition for the unrecognized tray type. Don't forget to change the "Plates=n" line to reflect the total number of plates after your additions:

Note: The last parameter in the lines (underlined) refers to the graphic representation of the tray type to show on the screen when the tray type is selected in ChemStation.

## 3. \*ChemStation Tray mapping changes for unexplained reasons or Trays are not mapped in logical order.

It has been determined that ChemStation maps trays in ascending sequence depending on the physical location of the trays. Sort order is from front left to rear right. If tray positions are changed, then mapping will change. There is currently no way to work around this.

4. When sampling for injection, ChemStation has been seen to aspirate sample from incorrect locations.

This has been determined to be caused by the way ChemStation converts PAL tray positions into alpha-numeric coordinates. This conversion is only done correctly if the PAL Tray-types are set up so that the PAL "Rows" are in the X direction. (left-right). Some PAL Tray-types e.g. VT32 and Fraction trays have the "Row-length" defined in the Y direction with numbering of positions front to back. Currently, ChemStation cannot handle these correctly. The workaround is to make sure all sample trays are defined with rows in the same manner as a microtiter plate (Row length is in the X direction). Note, this only affects the injection PAL.

5. Delay calibration does not move the Collector PAL to the Delay sensor.

Remember to modify the "Run Time Checklist" in the Delay calibration method (normally "DGCALPS.M"). Enter the pre and post-run macros as shown here: Pre – FCP\_MoveFCtoCalbPort Post - FCP\_MoveFCtoPark

6. Delay calibration fails with an out of range error:.

The installed version of the Sequence File used for delay calibration is bad. It is installed as a 16-bit file. It is necessary to recreate it before it can be run. To do so find the file in explorer c:\chem32\1\sequence\afcdelay.s First change it from "Read Only" then delete it. From within CS go NEW SEQUENCE, add a single line with location of delay sample (check P number is correct!) and sample name "Delay Calibrant" and save as AFCDELAY.S

7. \*The following error has sometimes been seen when ChemStation is launching:



When the further information screen is selected, the following message appears:

HPCORE C	hemStation	
Error sign AppNa ModVe	ature me: hpcore.exe AppVer: 32.1.83.0 ModName: hplctc00.dll r: 32.0.0.0 Offset: 0000535c	
To view t	echnical information about the error report, click here.	<u>C</u> lose

This error causes ChemStation to abort, and leaves the CTCSinglID process running, requiring a reboot of the PC. It has been determined that this is caused by a limitation imposed in the CTC.ini file for a maximum of 3 Trayholders, and the message is triggered when a 4<sup>th</sup> Trayholder as been created on the PAL. The only workaround currently is to avoid having this many tray holders.

8. If the collection head continues to dispense fractions when stopping a Chemstation method it is most likely due to a Delay Calibration being performed and Chemstation closed and re-opened.

#### Other Possible problems:

1. FractPAL does not see end of run and is still waiting for next fraction signal.

It is possible there is an error message related to another device – Look under other screens for such error messages (e.g. active splitter).

Workaround – Stop the FractPAL method and restart it again. Worst case, close and restart FractPAL

2. Message that other COM objects are running, or system hangs at launch.

When ChemStation aborts or crashes, the CTC PAL driver is usually not unloaded correctly, and will prevent re-launching of ChemStation. Workaround: In the Task manager, look to see if the CTCSingle ID process is still running, and if so, stop it. Alternatively, re-boot the system.

#### FractPAL Installation issues:

1. The "User.mac" file which is located in the "Core" folder under ChemStation is important in launching FractPAL with ChemStation. This file is used by ChemStation to launch optional startup macros, and may or may not exist depending on whether there are any other applications being handled by the ChemStation application.

This file must be present and contain the following line to launch FractPAL properly with ChemStation:

*Macro ''C:\Program Files\LEAPTEC\FractPAL\FractPAL23.mac'', go* (Where the Path points to the currently installed version of FractPAL)

A default version of this file is present on the FractPAL installation CD, which may be copied and used as required. Do not use this file if there is an existing

version in place. Instead, add the above line to the existing file using notepad. (Remember that files copied from CD's have "read only" attributes and this must be unchecked in the file properties after it is copied).

Note: If there is no User.mac file, it is possible to check if FractPAL will launch correctly from within ChemStation, by typing in the above line directly into the command-line of ChemStation.

2. If upgrading FractPAL at an existing site, first go through the Windows Control Panel to uninstall any previous versions. (You may wish to backup or rename the original FractPAL folder before doing so).

Do not simply copy individual files, unless specifically told to do so by LEAP. There are several files which must work together and doing so incorrectly may result in file incompatibilities.

#### PAL Interface Cable:

**IFC Collector** 

The cable shown below connects the IFC to the Agilent system, carrying signals which are used for timing the fraction collection as well as the sample injection.

The settings for events which are required on each PAL are shown on the drawing.

#### DB25M TTL-In 3 3 white Agilent 1100 AFC BCD Bd. TTL-In 3 ground 16 ---black -DB15F TTL-In 2 ground 15 -5 BCD 0 TTL-In 2 2 -9 Ground TTL-Out 2 5 -TTL-Out 2 ground 18 -TTL-In 2 11 SW-Out 2 5 Agilent 1100 Remote DB9M Common 12 RED 3 START 3 GROUND 4 WHITE 7 READY 7 GREEN Common 8 1 GREEN / BLACK DB15M **IFC Injector** RED UNTERMINATED BLACK Note: Set PAL Events as follows: Active State Collector PAL: TTL-In2 Low PART NAME IFC PAL COMBO Cable TTL-In3 High FILENAME IFC PAL COMBO Cable rev2.bmp Injector PAL: TTL-In1 High 100.IFCCOMBO 8/5/05 S3 TTL-In2 Low