

MultiQuant User Manual

MultiQuant User Manual (software release version v5.0)
V5.0 June 2006

Note: It is recommended that users access the CD-ROM or USB drive supplied with the instrument for the latest version of this manual.

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Section 1 - Introduction

Instructions to Basic Operators

Please read this section carefully

The MQC and MARAN Ultra series are designed for prolonged use in harsh industrial environments. However, the following points should be noted:

- Never, under any circumstances, insert metallic objects into the sample access hole (the round hole in the top of the magnet).
- Never force sample tubes into the sample access hole. If a glass tube breaks inside the sample access hole, contact your local representative of OIMBL for advice.
- Do not place any magnetic media (floppy disks, videotapes etc) in close proximity to the magnet box.
- If the instrument's magnet box power supply is switched off at any time, the instrument requires up to 24 hours to stabilise.
- For information on basic maintenance of the MQC and MARAN Ultra (including changing fuses), please refer to the appropriate installation guide.

MultiQuant Software Suite

The MultiQuant software suite is a software toolbox designed to facilitate the development and use of NMR methods for routine analysis. It comprises of a calibration software section (**RI EasyCal** and **RI Calibration**), a routine analysis software section (**RI Analysis**), and additional tools for multiple analyses using an autosampler (**RI Autosampler** and **RI Tareweight**).

RI Easycal is user-friendly, programmable calibration software aimed at simplifying the acquisition of NMR data, their integration into a calibration table and the production of a calibration file. Although the **EasyCal** executable file used by **EasyCal** applications is part of the **MultiQuant** installation, **EasyCal** application templates are required to operate **RI EasyCal**. These are provided separately on either floppy disks or CD ROMs for each specific application on the MARAN-Ultra, and preinstalled on the MQC. The templates include all the operating parameters to produce a calibration to standard method conditions. Most standard NMR methods are now available as **EasyCal** applications. Should you require a standard **Easycal** application please contact your local representative of OIMBL.

RI Calibration is Oxford Instruments' Windows-based calibration software for the MQC, MARAN and MARAN Ultra series. RI Calibration provides the facility to generate calibrations from calibration standards of known concentrations that are then used in RI Analysis to measure unknown samples routinely. RI Calibration is designed to be intuitive to use by users with little expertise in NMR, but assumes some user familiarity with MS Windows and, basics of statistics and treatment of

calibration curves. Unskilled operators that are only trained to perform routine measurements on unknown samples are not required to learn more than the basics of RI Analysis software, and therefore do not need the knowledge of RI Calibration.

Note to former users of MARAN MS-DOS calibration software:

The main change between RI Calibration and the standard MARAN MS-DOS software lies in the way data is handled. In the MS-DOS software, data is acquired within the calibration software so, once the data has been acquired, it is processed and reduced to a single number that is then used in subsequent calculations. Using MultiQuant, the NMR data has to be acquired first in the data acquisition software RINMR (or using RI Easycal whenever it is applicable), and then imported into RI Calibration. RI Calibration retains all the NMR data so that calculation ranges and processing options can be altered without the need for re-acquiring data, which leads to a considerable amount of time saving. Re-calculation of the calibration curve following the alteration of calculation ranges is done in real time, allowing the user to optimise the processing parameters with minimum effort.

RI Analysis is the routine analysis environment of MultiQuant that can be accessed to basic users. The calibration file generated from either **RI EasyCal** or **RI Calibration** is loaded into **RI Analysis** software to perform routine measurements. It contains information specific to the method, i.e. the experimental conditions, calibration and operational options. It is then used for each NMR analysis. **RI Analysis** is equipped with an Auto-tune function that allows tuning of the instrument on resonance and also performs a diagnostic test.

RI Autosampler is organised in a table format to program a series of automated routine analyses using a Duratech autosampler, along with the possibility of bar code reading and sample mass measurement with a Mettler balance. Like RI Analysis, it allows repeated measurements with a choice of multiple calibrations and the possibility of auto-tuning within series of automated analyses.

RI Tareweight is a software utility that allows the weight taring of a series of empty tubes to be automated against a bar code or a tube position using a Duratech autosampler. **RI Tareweight** creates an editable list that is loaded into **RI Autosampler** so that each sample can be associated with a pre-defined tare weight against its allocated sample tube, prior to the start of automation.

System Requirements

MultiQuant requires the following minimum PC system specifications:

- Pentium or Celeron 200 MHz processor.
- Windows 98 SE/NT/2000/XP operating system.
- Minimum 64 megabytes of RAM.
- Minimum 20 megabytes space on 1 GB hard drive.
- SVGA video card supporting at least 800 by 600.

- A 10 BaseT Ethernet network card (i.e.: not required for MARAN internal hardware).
- RS232 serial ports.

Note: one extra serial port for external balance connection, one extra serial port required for Duratech autosampler, one extra serial port for RI's Variable Temperature control unit.

Users should note that **RI Calibration**, **RI Analysis**, **RI autosampler** and **RI Tareweight** are stand-alone software tools of the MultiQuant software suite. If multiple copies of **MultiQuant** are needed, users should contact Oxford Instruments Molecular Biotools or their local distributor regarding the terms and conditions of purchase.

1.4 Software Installation

Installation of MultiQuant software suite (version 3.3 and later)

- To install MultiQuant from a CD-ROM or USB drive:
- Place the software installation CD-ROM into the CD-ROM drive (MARAN or MARAN Ultra), or USB drive into USB port (MQC).
- From Windows Explorer, select the drive (e.g. D:\) to start installation. Alternatively, select Start > Run and type D:\Setup.exe if the drive is D:\.

The **Setup.exe** program activates the installation. The first window indicates that the system is ready to start the installation procedure (Figure 1.1). At this point, the user can proceed by mouse clicking on the **Next** button in this window, or abort the installation by clicking on the **Cancel** button. If the latter option is chosen a small dialog box appears that enables the user to abort the cancellation of the installation (Figure 1.2). If the **Yes** button on this box is clicked the installation is aborted. If the **No** button is clicked the installation will continue.

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Figure 1.1 – First window of the MultiQuant installation program

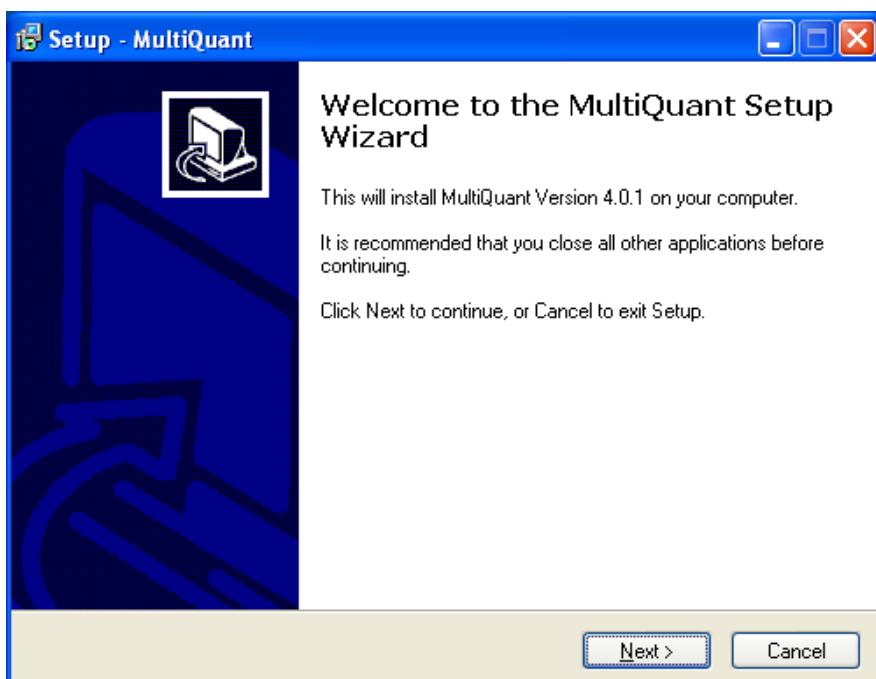
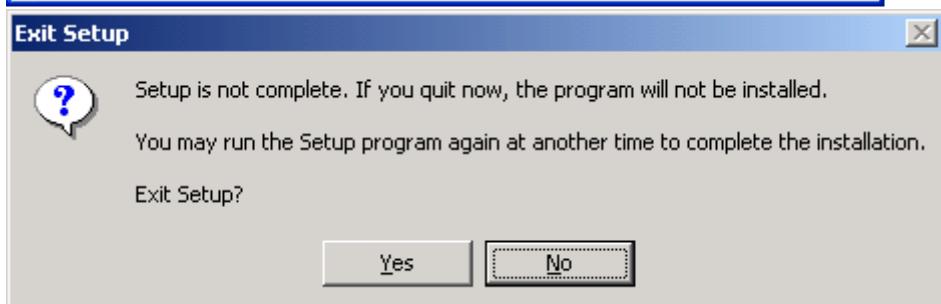


Figure 1.2 – Dialog box to confirm exit from MultiQuant installation program



The user is requested to select the directory where MultiQuant should be installed (Figure 1.3). Unless you wish to proceed otherwise, select the Default directory \Program Files\Resonance\Ricalib. Select **Next** to start the installation or **Cancel** to abort the installation. The installation program will then commence installation of the software (Figure 1.4).

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Figure 1.3 – Prompt to choose the installation directory for the MultiQuant software

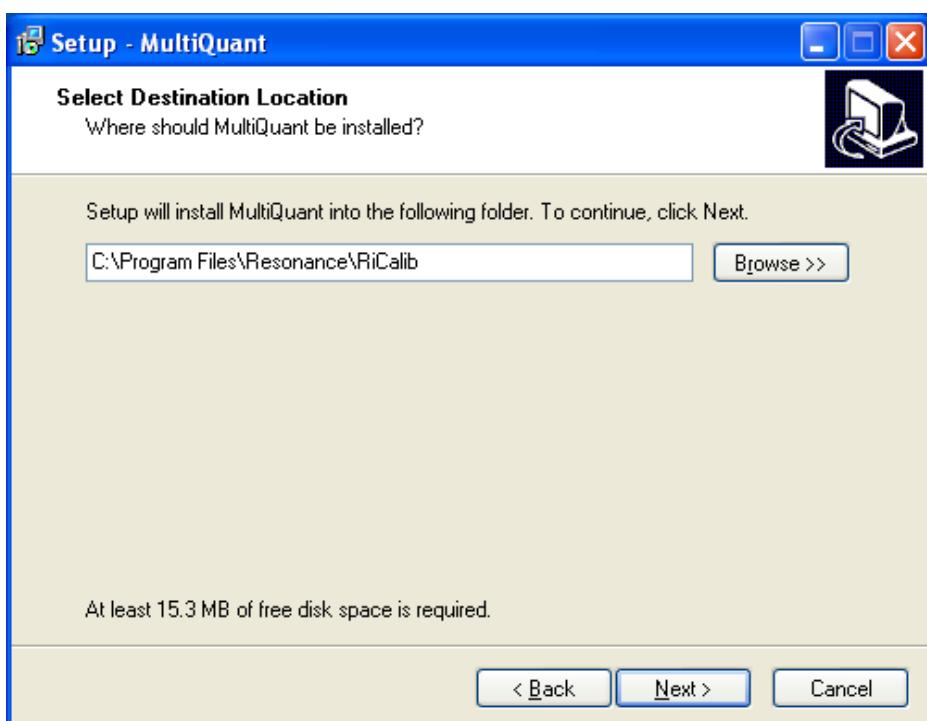
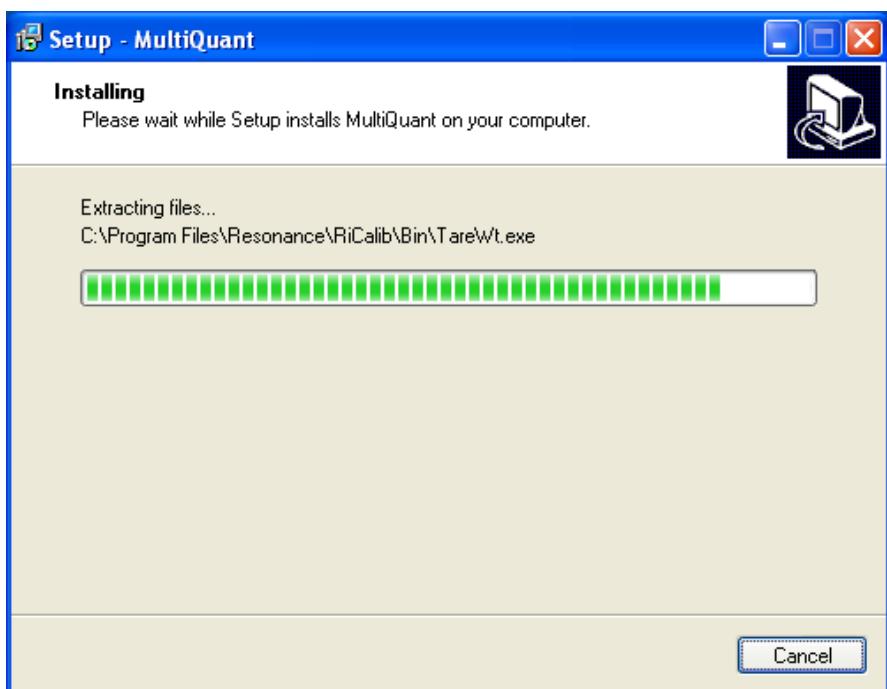
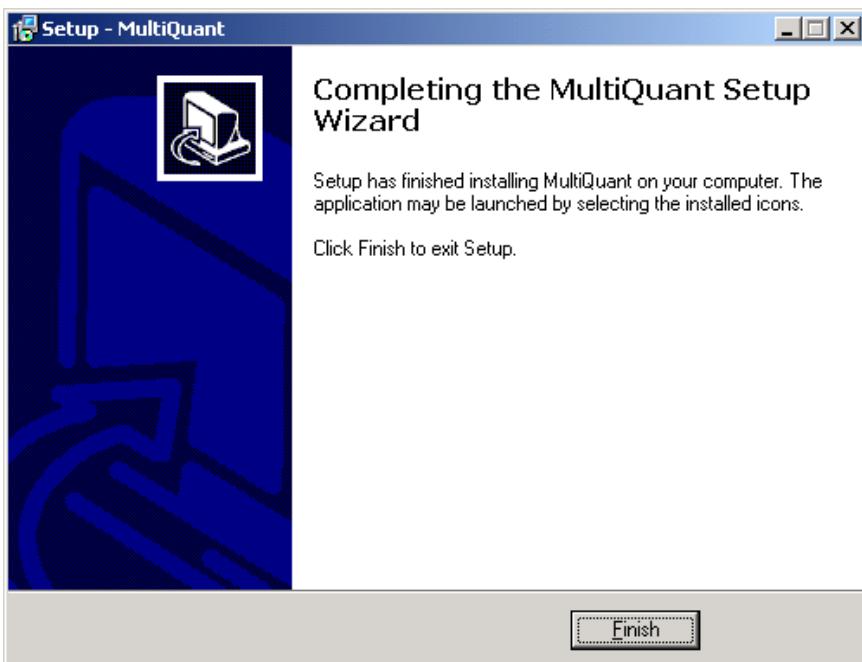


Figure 1.4 – Information on the current status of the installation



- The installation shield informs the user on the status of the installation (Figure 1.4) until the end of the process when the installation of **MultiQuant** has been successful (Figure 1.5).

Figure 1.5 - Prompt to inform the user that the installation has been successfully completed



- At the end of the installation process, a desktop directory that contains shortcuts to RI Calibration, RI Analysis, RI Autosampler and RI Tareweight should be created.
 - To enable the use of all MultiQuant facilities, double-click on RI Calibration icon or select RI Calibration from the *Start > Programs > MultiQuant* menu.
- Note:**
- For communication of MultiQuant software with an external balance (Mettler only), refer to Appendix A.2 Balance Settings for further details.
 - For communication of MultiQuant software with Duratech autosampler software (MARAN Ultra only), refer to Appendix A.3 Autosampler Settings for further details.
 - Mettler balance interface and RINMR (version 4.2 and later) should be installed prior to the installation of MultiQuant (version 3.2 and later) and Easycal application files. Consult the respective instructions and manuals from Mettler and Duratech before installing MultiQuant software. To enable the use of the autosampler, some default options have to be checked in the appropriate initialisation files (see Appendix A – Interface and connections).
 - Installation of EasyCal applications: Easycal applications containing individual, pre-programmed methods are supplied separately by Oxford Instruments Molecular Biotools. Please contact your local representative of the company for further details.

1.5 How to use this manual

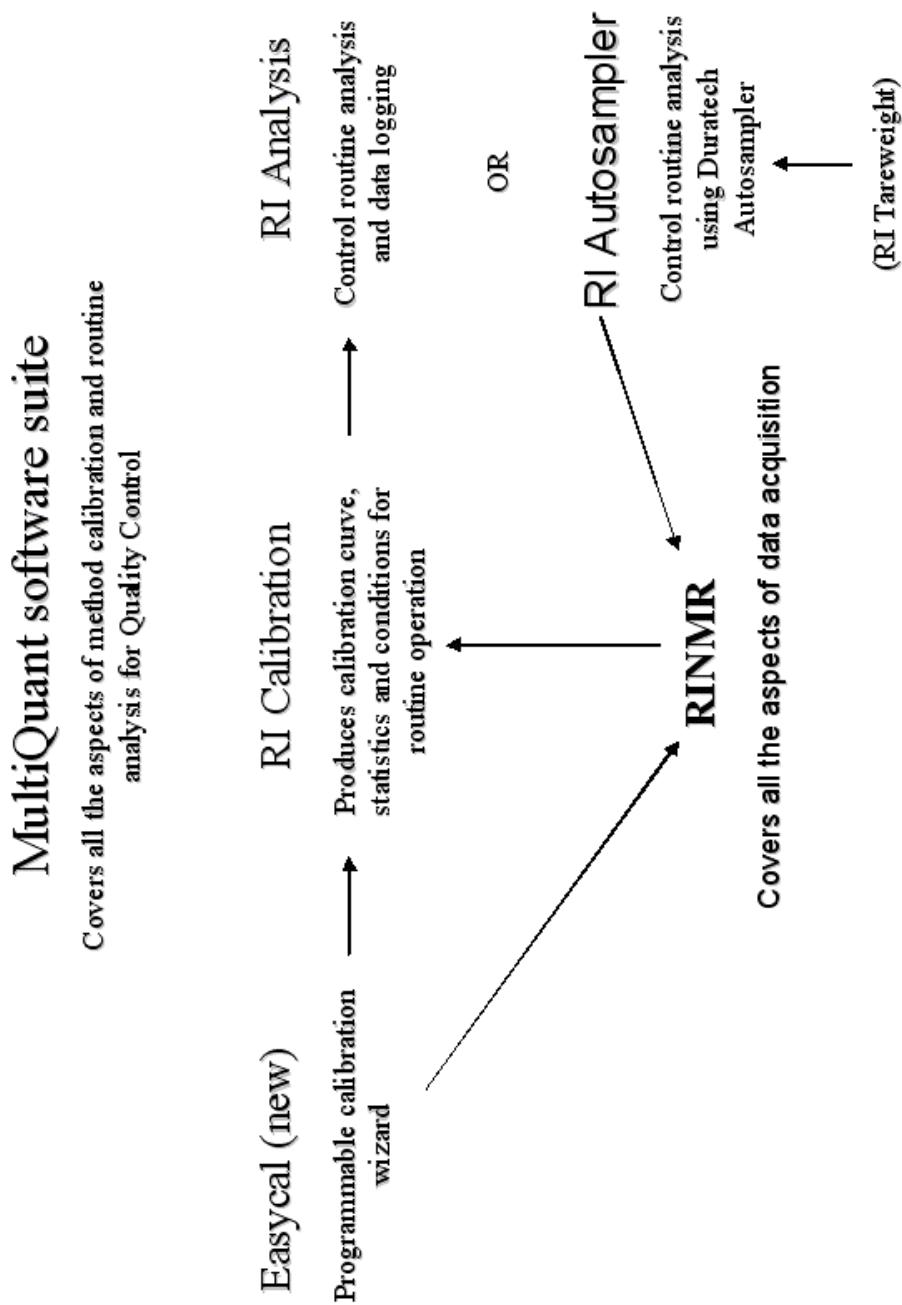
- This manual is aimed at providing guidance to laboratory personnel regarding the use of MultiQuant software for Quality Control (QC).
- Users unfamiliar with creating NMR calibrations should first read Chapter 2 Calibration Process, in particular the Basics of Method

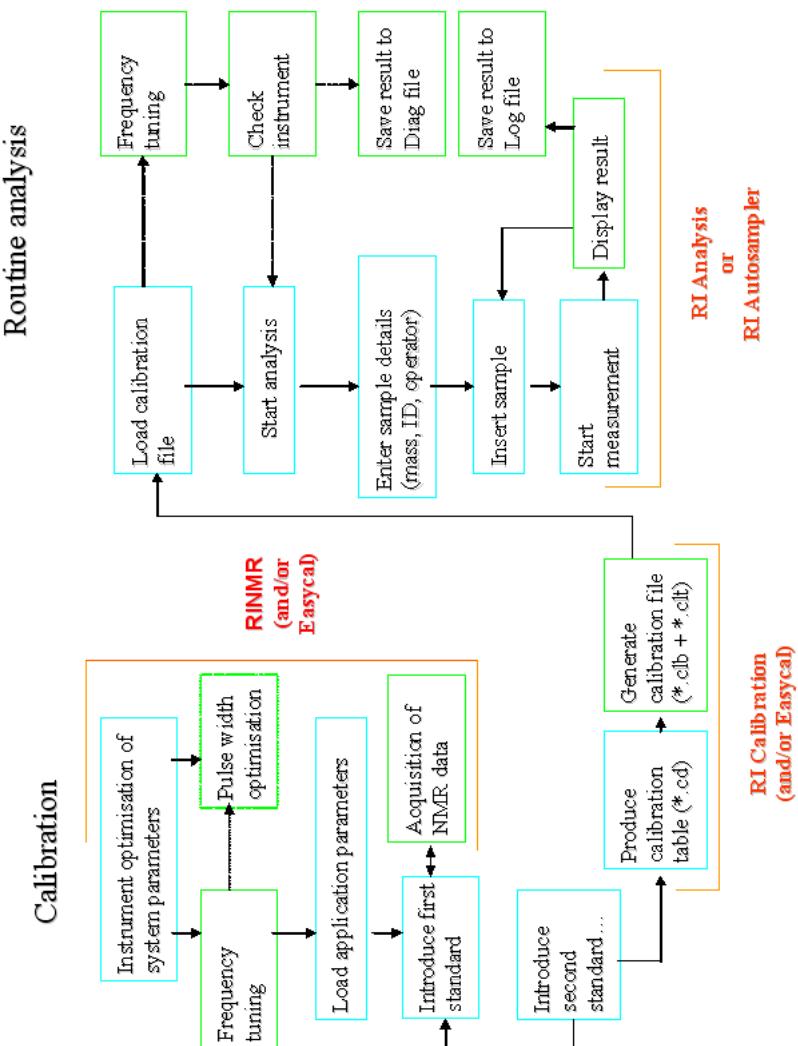
Calibration section, to familiarise themselves with the standard calibration procedures; proceed to section 2.2 for an overview of the calibration software.

- RI Calibration has many similarities with the former MARAN MS-DOS calibration software described in preceding MARAN software manuals. Users familiar with the original MS-DOS software and calibration concepts can to proceed directly to Chapter 2, section 2.4.16, where a step-by-step tutorial describes how to create calibrations from a demonstration data set (this set is included as part of the installation of MultiQuant software suite).
- MultiQuant also provides the executable file for **RI EasyCal** applications, a user-friendly utility designed to help laboratory personnel produce the raw NMR data required for calibration (see section 2.3 of this manual). Contact Oxford Instruments Molecular Biotools for details of existing EasyCal applications. For information on how to change password access used by both MultiQuant and Easycal, consult Chapter 3.
- Routine users of a MQC, stand-alone MARAN or MARAN Ultra analyser and those who do not need knowledge about the calibration procedure should refer to Chapter 4 - **Routine Analysis**.
- Users of a MARAN Ultra analyser equipped with a Duratech autosampler should refer to Chapter 5 - **Use of an autosampler for Routine Analysis**.

1.6 Flow Chart

Description of
MultiQuant





2 Calibration process

2.1 Basics of Method Calibration

2.1.1 Basic Concept

NMR is not usually available as an absolute measurement technique, i.e. one cannot simply place a sample in an NMR analyser and obtain a result for % oil content in seeds, % spin finish or any other quantity. To perform an NMR analysis, a set of calibration standards (which have a known concentration of the property we are interested in, this may be % oil in seeds, % spin finish, % degree of crystallinity, parts per million of F in dental creams etc.) must first be measured. A record of calibration is generated from the calibration standards. Users may then measure unknown samples and compare them to the calibration produced from the calibration standards.

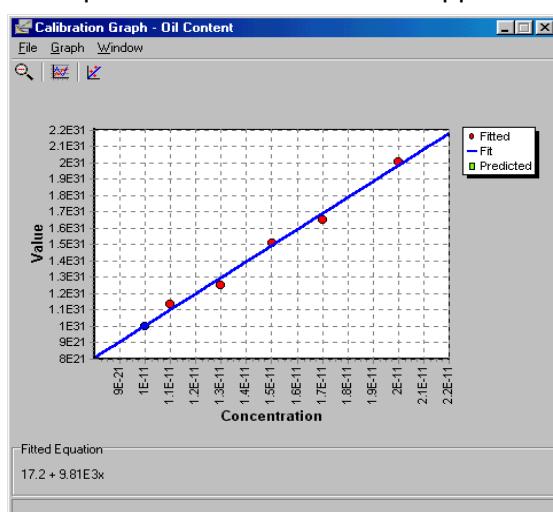
For example, an NMR experiment may yield the following values for 10 calibration standards (Table 2.1):

*Table 2.1 - Example of data series
Y=NMR/mass value against series
X=known concentration (%)*

Concentration (%)	NMR Value
0.10	998
0.11	1130
0.13	1250
0.15	1510
0.17	1650
0.20	2003

If the NMR values (Table 2.1) are plotted against the % concentration of the calibration standards, the graph displayed in Figure 2.1 is produced. The data points marked with the red circles are fitted using linear regression to produce a calibration fit as a solid line. The fit may be a straight line or a polynomial curve. Unknown samples may be measured by establishing where they lie on the fit. In the following graph, for example, an unknown sample producing an NMR value of 1800 would correspond to a concentration of approximately 0.182%.

*Figure 2.1 – Calibration curve
NMR versus Oil content*



2.1.2 Sets of Calibration Standards

- A good calibration set should have the following properties:
 1. It should ONLY vary in the property associated with the concentration (for example, in spin finish measurements only % spin finish, NOT the type of textile).
 2. The calibration is only valid by correlating the NMR value of standards belonging to the same family or category (if % spin finish measurement of two different textile types is required, in principle a separate calibration must be performed for each textile). If you are in any doubt, consult an expert.
 3. The set of standards should span regularly over the whole range of concentrations and include the lowest and highest possible concentrations.
 4. The set should contain duplicates for testing reproducibility and repeatability.

Finally, it is important to note that all calibration standards (and any subsequent measurement of unknown samples) MUST be measured at the temperature specified by the experimental protocol. In some methods, the amplitude of a particular NMR signal is highly dependent on the sample temperature, as variations in the temperature at which the calibration standards are measured can lead to a significant increase of the standard deviation in the calibration statistics.

2.1.3 NMR Data, Calculation Ranges and dividing Signal by Sample Mass

NMR data is acquired in the time following a radio-frequency pulse (cf: basic Nuclear Magnetic Resonance handbooks). The signal amplitude of the NMR data varies with the evolution of the magnetisation. A typical NMR signal may look like the curve displayed in Figure 2.2 where the signal decreasing as a function of time represents the NMR signal; it is called a Free Induction Decay (FID). Another type of NMR signal is shown in Figure 2.3 where the signal increases to a maximum value when the time is 50000, then decreases again; it is called a spin-echo (or Hahn echo).

Figure 2.2 – Free Induction Decay NMR signal

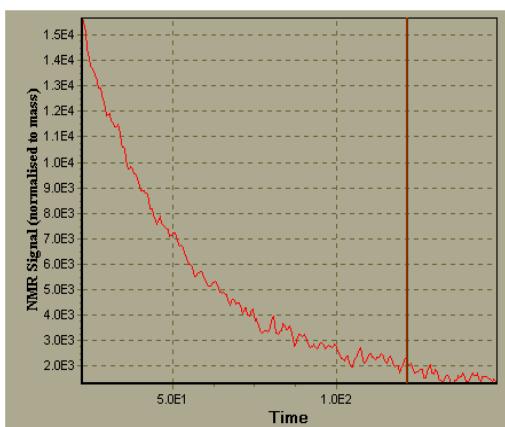
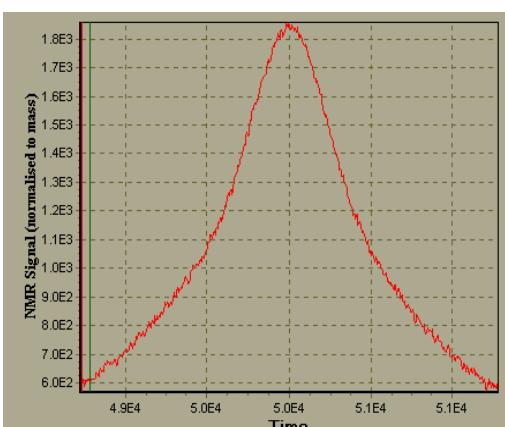


Figure 2.3 – Spin echo NMR signal



Some areas of the NMR data contain information that we are interested in. Other areas do not. In the first example of NMR data shown in Figure 2.2, we are not interested in data from over 125 μ s following the start of the acquisition (time zero) as this contains only noise. Only the data from approximately 0 μ s to 125 μ s contains valuable information that we may be able to correlate to the calibration standards concentration.

Once NMR data has been acquired, a sensitive region or calculation range must be selected for use in the calibration. For example, it may be that the data from 50 to 100 μ s in Figure 2.3 can be averaged and then correlated with the percentage of spin finish present in the sample. The position of the calculation range necessary to produce a good calibration varies depending on the application, and may be found either by experience, by trial and error (by varying the values at which the calculation range starts and finishes and observing a change in the quality of the calibration) or by referring to information supplied by Oxford Instruments Molecular Biotools.

Finally, the amplitude of the NMR signal following the excitation pulse is also proportional to the sample mass. For example, if a sample of mass 1g produces an NMR signal of amplitude 100 at 50 μ s, a sample of mass 2g will produce an NMR signal of amplitude 200. Most calibration values must be divided by the sample mass or ‘normalised for mass’ before the calibration takes place.

- Users should note that signal normalisation is achieved by dividing two NMR signals (A/A_0 , where A_0 is the initial NMR signal at the beginning of the NMR experiment), removing the need to weigh the samples. However this can lead to an increase in the standard deviation of the results.

2.1.4 Calibration Procedure

- The creation of a calibration may be summarised as follows:
 - a) Acquire NMR data from a set of samples that have known values (or concentrations) of the property that we are interested in.
 - b) Divide this data by the sample mass (if applicable).
 - c) Define a calculation range of the NMR data that relates to the concentration.
 - d) Average the data in the calculation range to produce a single data value.
 - e) Correlate this value with the concentrations of the calibration standards.
 - f) Perform a curve fit to produce a calibration fit, usually with least squares minimisation.

The equation obtained from the fitted curve can then be used to measure unknown samples.

Note: Although restandardisation was part of MARAN MS-DOS calibration software, it is no longer a feature of **MultiQuant** software suite. In practice, it is more reliable and traceable to recreate a calibration curve than operate a restandardisation. If a large number of calibration standards are required, then the calibration curve may be reproduced using two or more synthetic standards read from the original calibration curve.

2.1.5 Common questions regarding calibrations

How often should I perform a calibration?

Initially, calibrations should be performed on a routine basis to check instrument stability. Users may wish to perform a calibration once per month to test the stability of the calibration over the long term. From then onwards, the user may wish to reduce the frequency of the calibration once confidence has been established to match local guidelines. Many calibrations show little drift even after several years of use.

How many calibration standards should I use in a calibration?

The chance that a calibration fit may be weighted by an unsuitable sample is dramatically increased if a small number of calibration standards are measured. However, increasing the number of calibration standards above a certain level gives little gain in accuracy (in terms of statistical confidence) to the calibration. Users should use their discretion or refer to local guidelines for establishing how many calibration standards are

necessary. RI Calibration can accept a calibration table up to 200 rows.

Which parameters have the greatest effect on the reproducibility of a calibration?

Variations in sample temperature have been found to produce strong variations in reproducibility for NMR measurements, although, when little signal is available it is the thermal noise in the measurement that predominates. To ensure that accuracy is maintained, all samples (both while the calibration is taking place and during the measurement of unknown samples) must be measured at the same temperature as that the calibration was performed at. Variations in sample composition can also affect the quality of calibrations. Some changes in sample composition have little or no effect on the calibration quality. Others, however, may invalidate the calibration completely. It is difficult even for NMR experts to predict exactly what changes in sample composition are necessary to invalidate a particular calibration. For example, a calibration to measure the percentage of plasticiser in PVC may no longer be valid when the plasticiser type is changed. Users are advised to re-calibrate whenever significant changes in sample composition take place.

2.2 Overview of Calibration Software

- As was discussed in chapter 1, there are two calibration programs that can be used in conjunction with the MultiQuant software suite:

EasyCal is designed to provide a simple procedure for calibrating pre-programmed methods used in routine quality control applications. The software is designed to be easy to use and builds on the previous experience of OIMBL staff in developing these applications. The EasyCal engine is provided as part of MultiQuant. However an application template tailored to the specific application involved is required to use the EasyCal software. These templates are provided separately from the general MultiQuant software. Please ask your local OIMBL representative for further information on EasyCal templates for your specific application. EasyCal is discussed in more detail in section 2.3 of this manual.

RI Calibration is a more sophisticated calibration routine that gives the user greater control over the calibration process. The software is designed for users of a higher technical level who are more familiar with the calibration process than EasyCal users. RI Calibration is an integral part of MultiQuant and can be used as installed without the need for additional application templates. RI Calibration is discussed in more detail in section 2.4 of this manual.

2.3 EasyCal Calibration Software

2.3.1 Installation of EasyCal Applications

EasyCal is the generic name used to describe a number of simplified calibration and analysis packages used for routine applications on the MQC and Maran Ultra instruments. Although the general form of the software is similar for each package there are significant differences. The configuration of the software for a specific package is configured by Oxford Instruments applications developers to suit the specific application. EasyCal applications are not provided as part of the standard MultiQuant software package. Specific applications are provided on a separate floppy disk or USB drive when the customer purchases an instrument for a specific application. An appropriate version of MultiQuant must be installed on the instrument PC before EasyCal is

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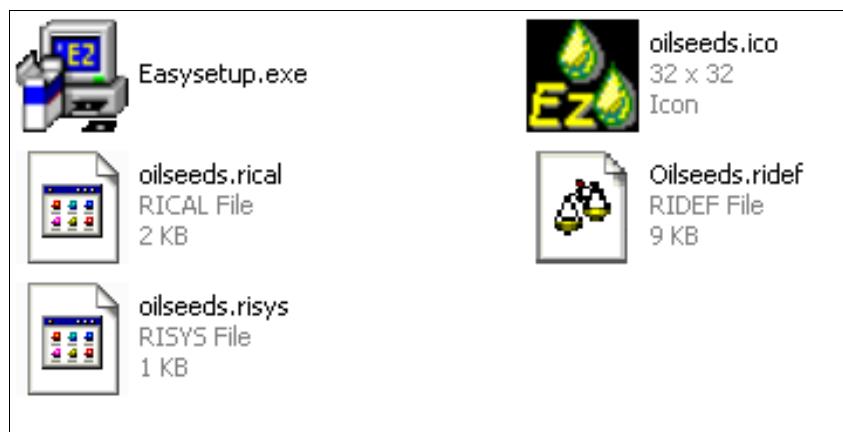
Section 2 – Calibration Process

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installed as the EasyCal engine is installed as part of the MultiQuant package. EasyCal applications are installed as follows:
Insert the floppy disk or USB drive into the instrument PC.

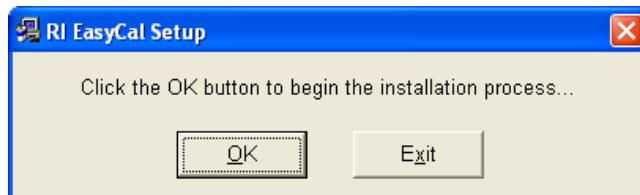
- Open the A:\ floppy or D:\ USB drive folder using Windows Explorer. The files contained in a typical folder are shown in Figure 2.4 below:

Figure 2.4 - Typical Example of Files on an EasyCal Install disk



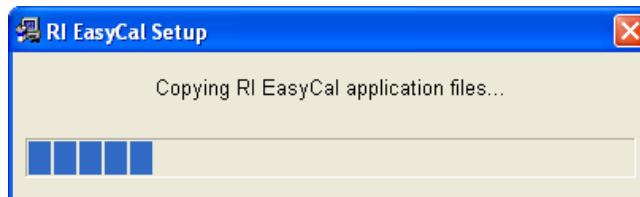
- Double click on the Easysetup.exe on the floppy disk to install the software.
- The first screen is a dialog box asking the user to confirm that they wish to install the EasyCal application (Figure 2.5). Click on the **OK** button to proceed.

Figure 2.5 - First dialog box of EasyCal installation process



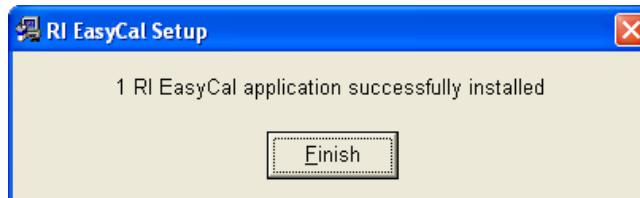
The installation process will start and the dialog box shown in Figure 2.6 will be displayed:

Figure 2.6 - Second dialog box of EasyCal installation process



Once the installation is complete a final dialog box will be displayed confirming a successful installation (Figure 2.7).

Figure 2.7 - Final dialog box of EasyCal installation process



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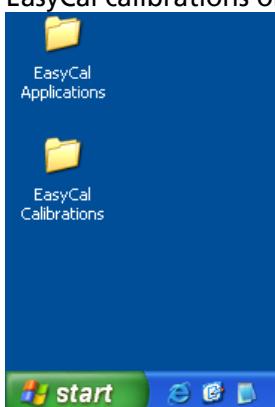
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5. Click on the **Finish** button to complete the installation process

2.3.2 Opening an EasyCal Application

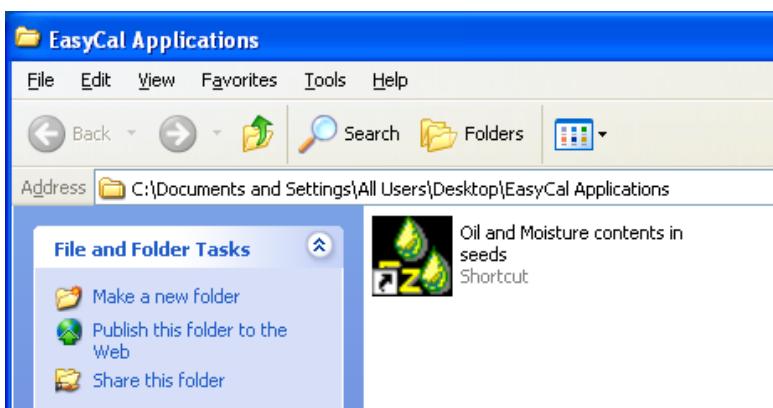
On installation the software creates two folders EasyCal applications and EasyCal calibrations on the desktop of the PC (Figure 2.8):

Figure 2.8 - Section of Windows Desktop showing EasyCal folders following installation



Within the EasyCal applications folder an icon is created that can be copied to the desktop of the PC for ease of use if required (Figure 2.9):

Figure 2.9 - Section of Windows Explorer window showing an EasyCal application shortcut



Double click on this icon to launch the application. The application will ask for a password. This is the administrative password set up on installation of MultiQuant on the instrument PC. Once this is entered correctly the splashscreen of the EasyCal software will appear (Figure 2.10) followed by the main screen of the software (Figure 2.11).

Figure 2.10 - EasyCal
Splashscreen



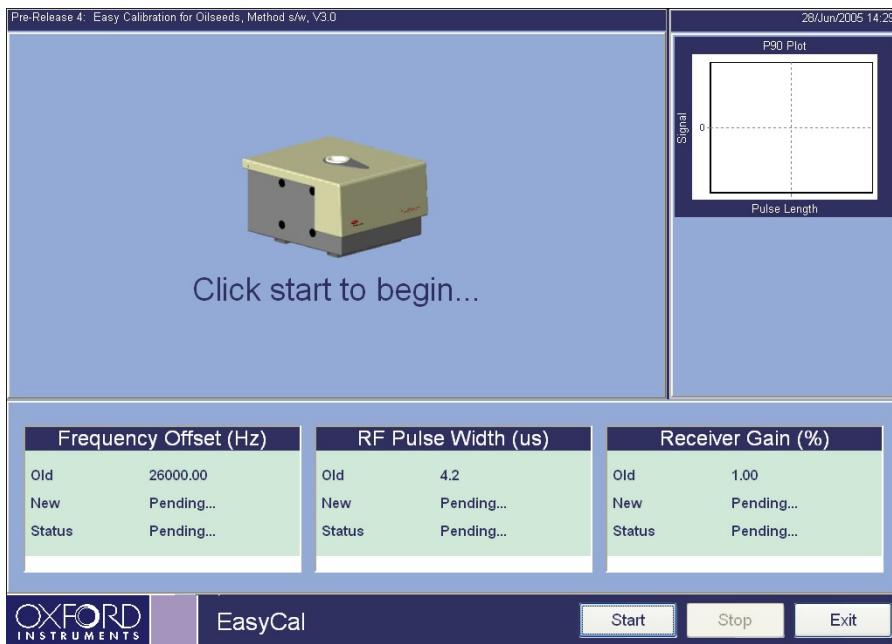
The configuration of the main screen of the EasyCal software is application dependent but the outline of the configuration is similar for all applications and is as follows. At the bottom of the main screen is a task bar (the Action Bar) used to initiate actions within the software. Initially, it contains 3 buttons labelled **Start**, **Stop** and **Exit**. The Start and Stop buttons start and abort the operation currently being performed and the Exit button allows the user to exit the software. Above the task bar is a frame (the Parameters Frame) containing the status of three instrument parameters, the Frequency Offset, RF Pulse Width and the Receiver Gain. The first two parameters are instrument parameters and depend on the configuration of the instrument whereas the Receiver Gain is application dependent. Each of these parameters can be optimised by the user using in built optimisation routines within the software. Whether these functions need to be optimised regularly is application dependent and this is determined by the configuration of the EasyCal application software. This configuration is determined by the Oxford Instruments Application Developer and is not available to the user. In the example we are looking at here all of the optimisation routines are available to the user. These will generally need to be run on entering the software for the first time following installation of the instrument. Further optimisations will be required on a regular basis. The frequency of these optimisations is application dependent. Please consult the documentation provided with your EasyCal application for further details. As can be seen in Figure 2.11 the status of all three parameters is "Pending" as these have not been optimised since the software was installed.

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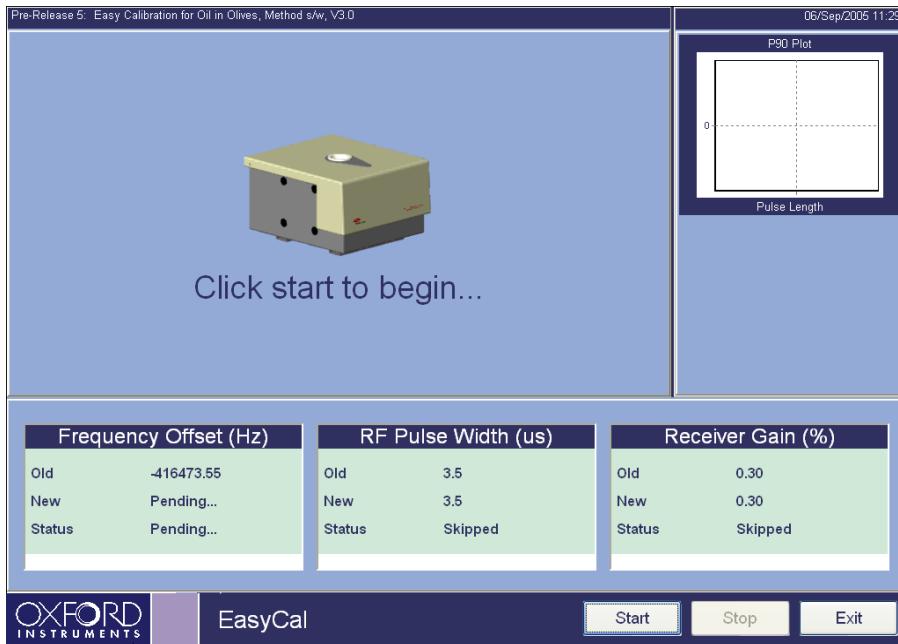
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Figure 2.11 - Start window of a typical EasyCal application



If some of these parameters do not require to be optimised for a certain application, the status will be shown as "skipped" and these parameters will not be optimised as is shown in the example below (Figure 2.12)

Figure 2.12 - Start window of EasyCal application with two optimisations skipped



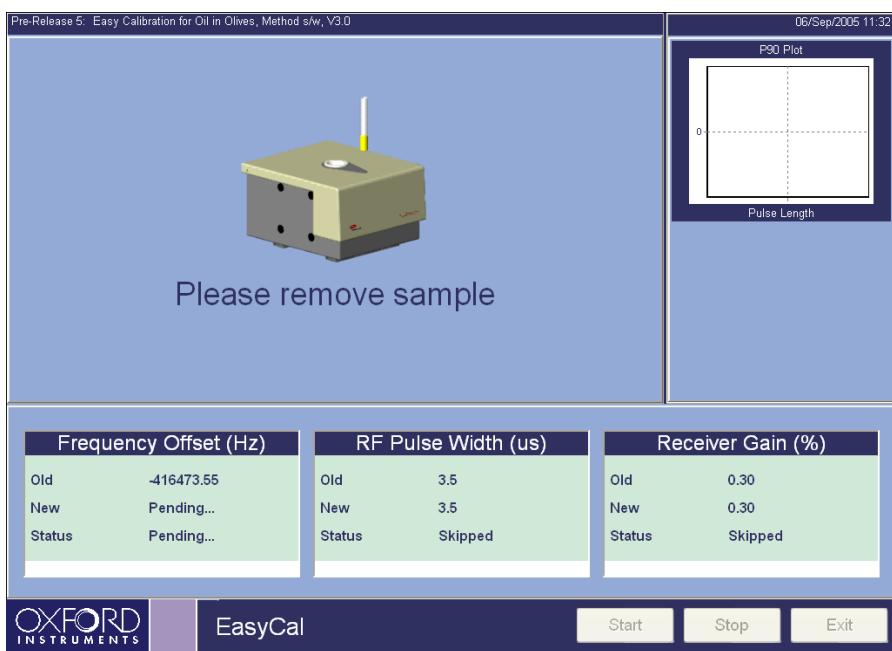
Above the Parameters Frame, the main screen is divided into two parts. On the left hand side of the screen is a large frame (the Status Frame) containing an icon representing the instrument plus a message describing the current status of the application or giving instructions on what to do next. In the example shown in Figure 2.11 above the user is asked to click on the start button to commence the application. On the right hand side of the main screen is a smaller frame (the Graph Frame) containing a

graphical representation of the current operation being performed by the software.

2.3.3 AutoSetUp of an EasyCal Application

The first operation that needs to be performed on the instrument is the optimisation of some or all of the parameters displayed in the Parameters Frame. This is done automatically using the Automatic Set Up routine within the software (abbreviated to AutoSetUp). To start the AutoSetUp routine click on the **Start** button in the action bar on the main screen of the software. The main screen will change and a request will appear in the Status Frame to insert the Tuning Sample into the bore of the magnet (Figure 2.13). The Tuning Sample is either a test sample provided by Oxford Instruments, or a suitable oil sample, used to optimise the instrument. Note that if a sample is already present in the magnet of the instrument at this time a message will appear requesting that the user removes it (Figure 2.13)

Figure 2.13 - Start window of EasyCal showing request to remove sample from magnet



Once the Tuning Sample has been inserted into the magnet, the software will automatically start to optimise the frequency offset of the instrument to bring the instrument on resonance. The time this will take is again application dependent but can be up to several minutes. During this optimisation process the Status Frame will show a message indicating that the resonance frequency is being set (Figure 2.14) and the status displayed in the frequency offset box in the Parameters Frame will change from Pending to Setting. In addition, a blue progress bar indicates the progress of the optimisation.

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Figure 2.14 - EasyCal main screen at the start of AutoSetUp

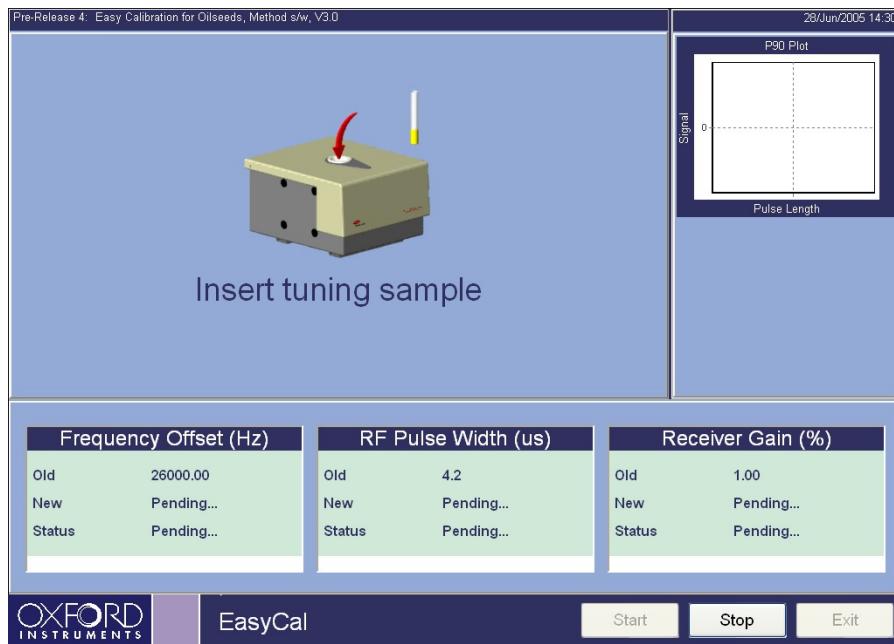
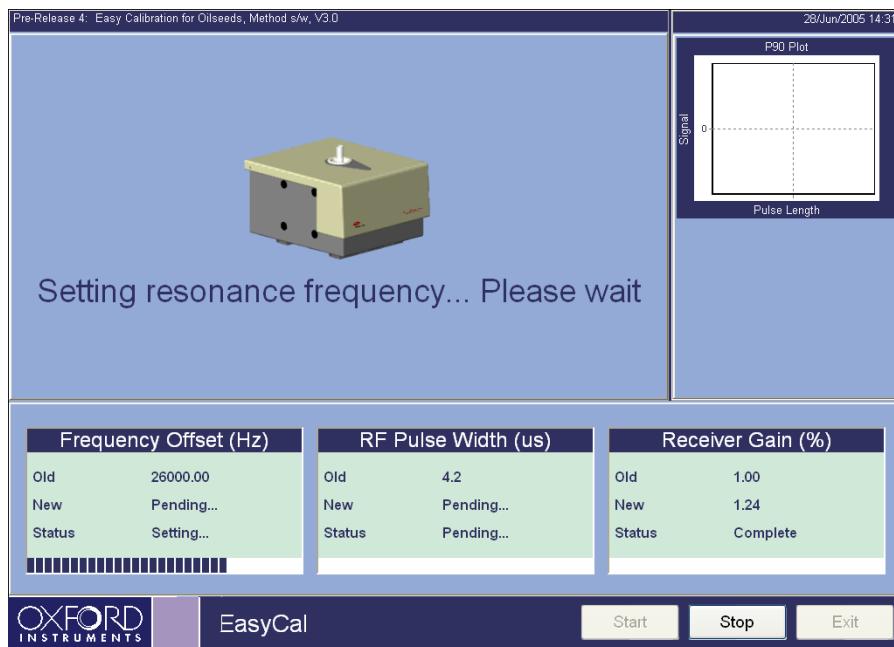


Figure 2.15 - EasyCal main screen during setting of frequency offset



- After the frequency offset optimisation is complete the new value of the frequency will be displayed in the Frequency Offset box and the status in the frequency offset box will change to **Complete**. The AutoSetUp routine will then begin to perform the next optimisation in the sequence provided this is required for the application involved. Usually this is the RF pulse width optimisation. This is also performed on the Tuning Sample and this optimisation will progress in a similar fashion to the Frequency Offset optimisation with the status in the RF pulse width box in the Parameters Frame changing to **Setting** and a progress bar appearing to monitor progress. In addition the setting of the RF pulse width can be followed graphically in the graph frame

(Figure 2.). This shows a plot of the variation in the signal strength for various RF pulse width values and is normally approximately parabolic in shape.

Once the RF pulse width optimisation is complete, the AutoSetUp performs the final optimisation of the routine, the Receiver Gain. This is again performed using the Tuning Sample and progress can be monitored in the Receiver Gain box in the Parameters Frame as for the previous two optimisations. Once this is complete, the AutoSetUp routine requests the user to remove the Tuning Sample (Figure 2.16). When this is done the AutoSetUp is complete and the Status Frame of the main screen displays a message to reflect this (Figure 2.17). The instrument is now ready to calibrate.

Figure 2.16 - EasyCal main screen after RF pulse optimisation

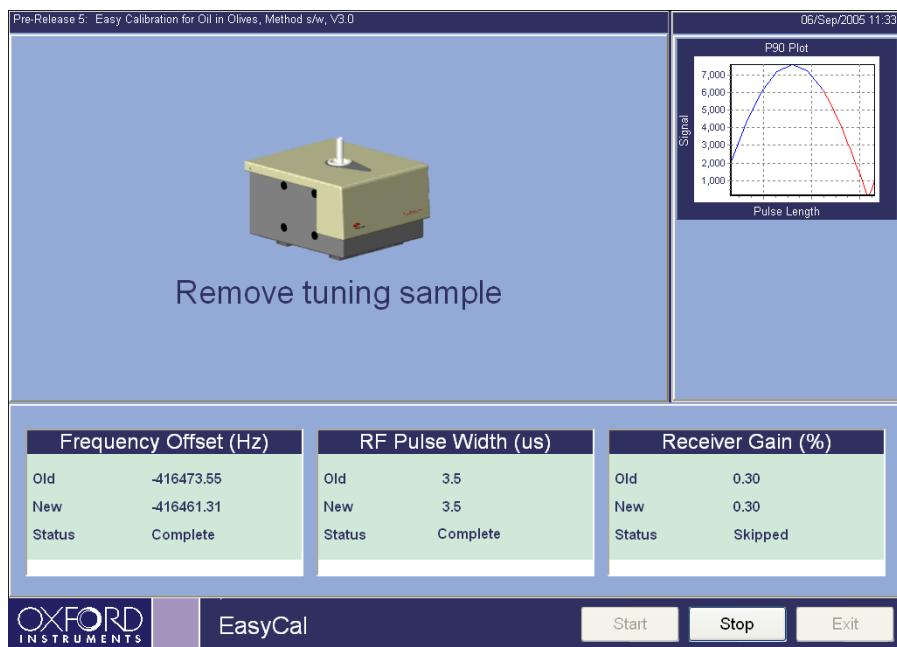
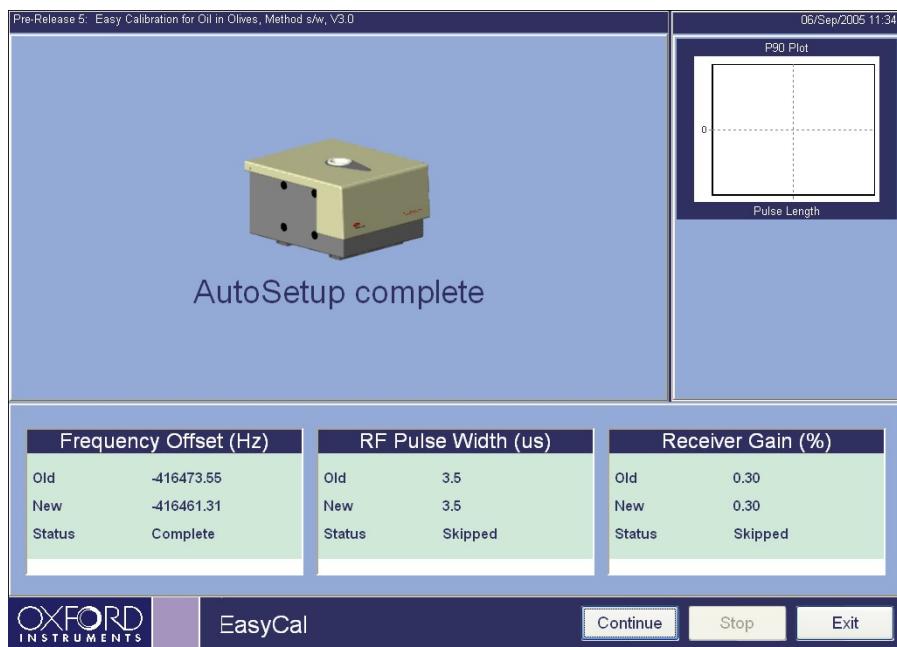


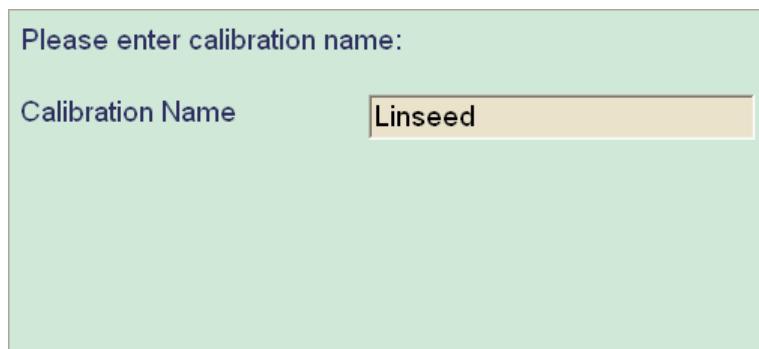
Figure 2.17- EasyCal main screen following completion of AutoSetup



2.3.4 EasyCal Calibration Procedure

Following the end of the AutoSetup routine the instrument can now be calibrated. This is initiated by clicking on the Start button in the action bar at the bottom of the main screen. The first thing that will appear is a dialog box asking the user to name the calibration that is to be created (Figure 2.18).

Figure 2.18 - Dialog box for calibration name



Once a suitable name has been entered, hit the **Continue** button in the action bar to enter the calibration name. The dialog box will now disappear and a new version of the main screen of the software will appear that is more suited for instrument calibration. This is illustrated in Figure 2.19.

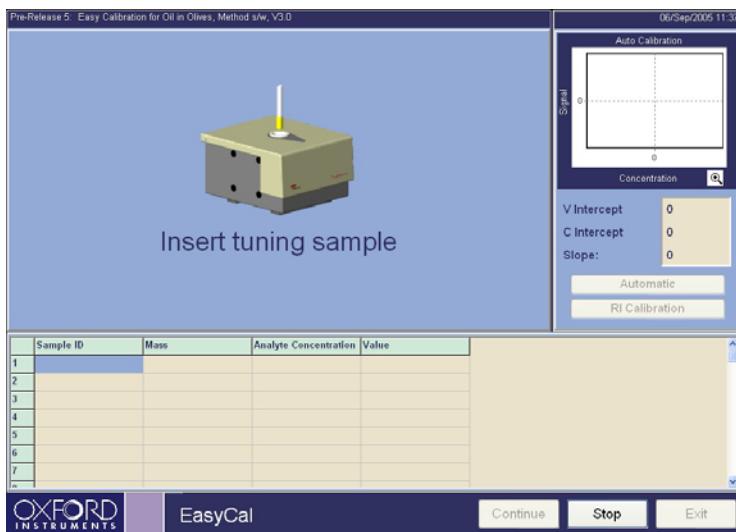
The main difference compared with the main screen in the AutoSetup mode is that the parameters frame has been replaced with a table that will contain data from the measurements used to create the calibration. In addition the graph frame has also changed. It now contains a graph that represents the calibration in its current state and also displays three parameters that characterise the calibration. These are the slope of the

graph plus two intercepts that characterise the line (see section 2.4.10). Below the display of these parameters are two buttons labelled

Automatic and **RI Calibration**. The function of these two buttons will be discussed later in this section.

As can be seen in Figure 2.19, initially the software requests that the tuning sample is re-inserted to perform some of the optimisation routines again. This usually consists of optimising the frequency offset only as this parameter requires frequent optimisation. However, this may vary for other applications. Once the optimisation routines are performed the status frame displays a message prompting the user to remove the tuning sample. The first analyte can now be analysed by clicking on the **Continue** button in the action bar.

Figure 2.19 – EasyCal main screen in calibration mode



Initially a dialog box is displayed requesting the user to enter details of the sample that has been analysed (Figure). The sample name, mass and analyte concentration need to be entered here. If required the sample can be weighed on a balance connected to the instrument PC (see Appendix A.2 Balance Settings for details of setting up a balance to interface to the instrument PC). The sample is weighed by placing it on the balance and pressing the “Weigh” button on the dialog box. Once all the required information has been entered, the experiment can be started by clicking on the **Continue** button in the action bar. If an entry of the wrong format is typed into one of the boxes (for example a floating point number representing the mass in the Sample ID box) this is refused and the typing will have no effect. If one of the entries is left blank, the empty box will turn red indicating an error once the continue button is clicked (Figure 2.21).

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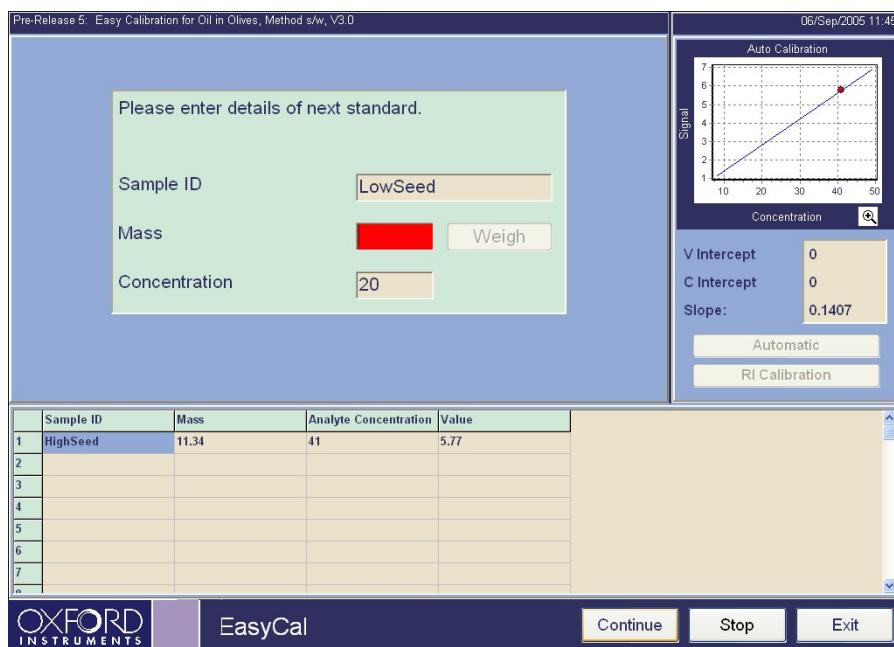
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Figure 2.20 - Sample details dialog box

Please enter details of standard with highest analyte concentration:

Sample ID	SeedHigh
Mass	10.56
Concentration	40

Figure 2.21 - Main screen showing error state when sample mass is not entered



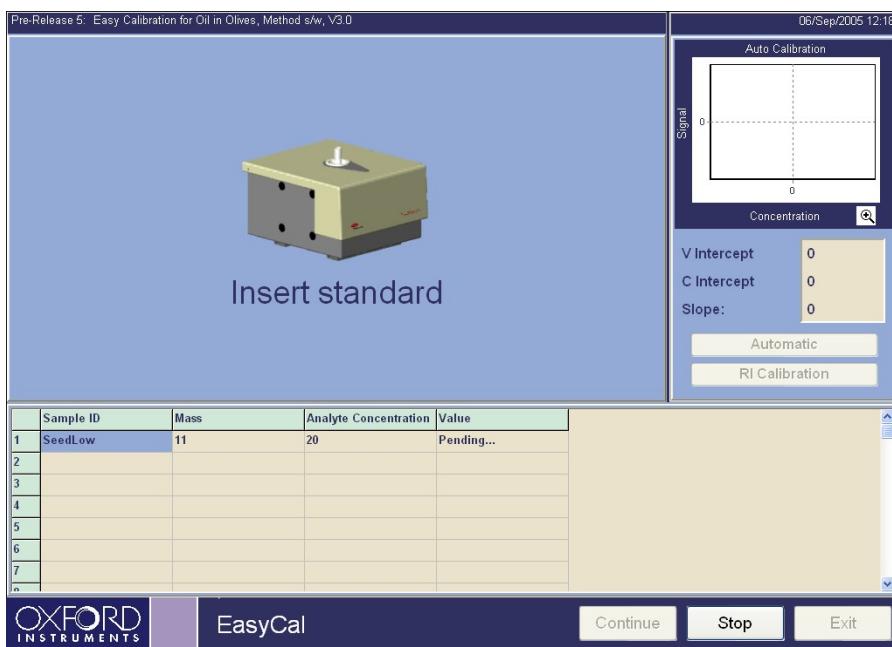
Once the sample details have been entered correctly, clicking on the **Continue** button will start the sample analysis. If a standard has not been entered a message will appear in the status frame requesting that the sample is inserted (Figure 2.21). The sample insertion is detected by the sample in detector and analysis commences automatically.

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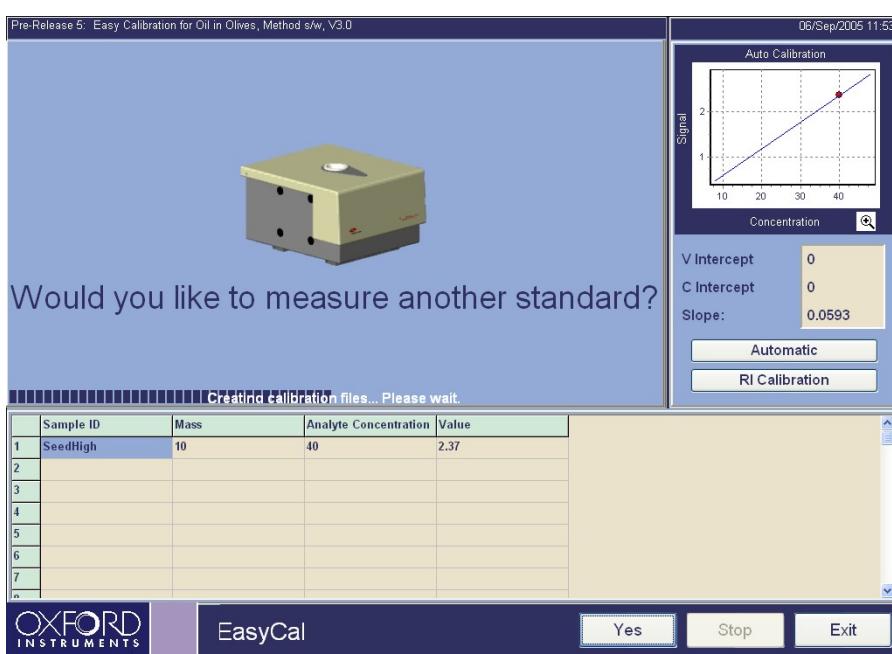
Figure 2.22 - Main screen of software showing sample prompt message



2.3.5 Single Point Calibration

At this point a single point calibration can be created if desired. This will use the single analyte measurement plus the (0,0) point of the coordinate system to produce a linear calibration for this measurement. This is implemented by clicking on the **Automatic** button in the graph frame of the main screen (Figure). Clicking on the button causes the calibration data to be written to a file on the hard disk of the PC. By default this file is written to the EasyCal calibrations folder created when the EasyCal application was installed. This file has extension .RICal and can be read by the RICalibration software that is part of the MultiQuant suite.

Figure 2.23 - EasyCal main screen during creation of a single point calibration file

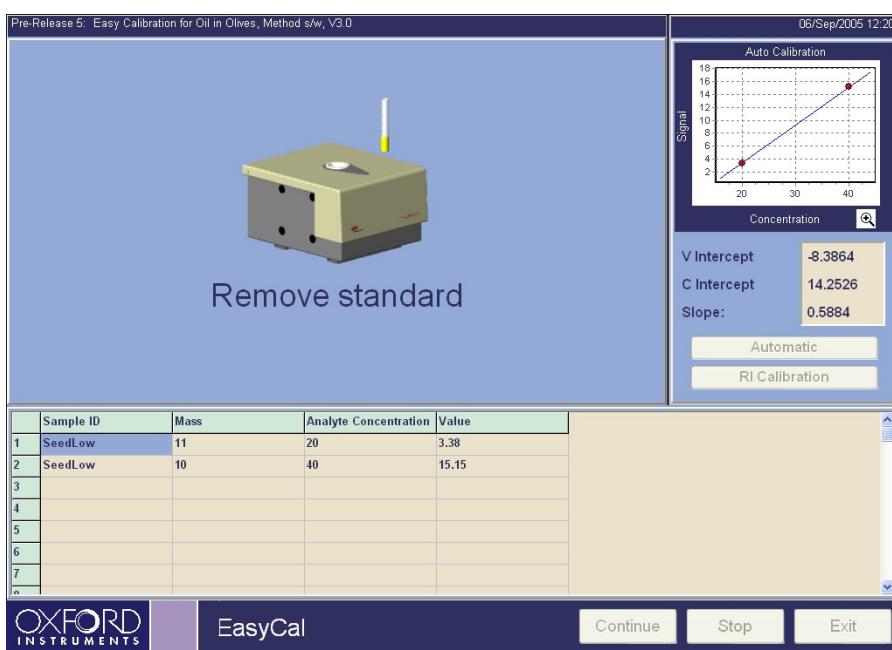


N.B. single point calibrations are often only of use in very simple applications and care should be taken when using this facility. Please consult the application literature or Oxford Instruments Molecular Biotools local representative for advice on the suitability of a single point calibration for a specific application.

2.3.6 Two Point Calibrations

Most of the calibrations created using EasyCal contain two calibration points. Such calibrations are created as follows. Once a single calibration standard has been measured the status frame will prompt the user to measure a second standard. If the user presses the **Yes** button the sample details dialog box will appear for this sample (Figure 2.20). When this has been filled in the second sample can be analysed by clicking on the **Continue** button. When the analysis is complete the status frame will again contain a prompt asking the user to remove the standard (Figure 2.24).

Figure 2.24 EasyCal main screen after analysis of second calibration standard



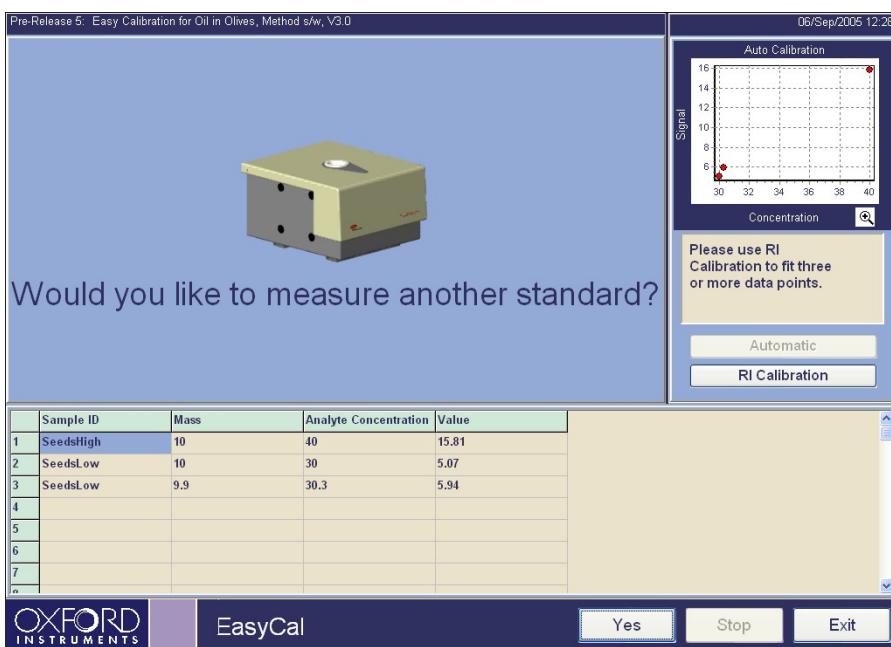
Once this is done the details for the two calibration samples will be displayed in the table at the bottom of the main screen and the graph plus slope and intercept values for the two point calibration will be displayed in the graph frame. At this point, as with the single point calibration, the calibration can be accepted and written to a calibration file by clicking on the Automatic button in this frame. Alternatively, RI Calibration can be used to optimise the application by clicking on the RI Calibration button.

2.3.7 Calibrations with more than two points

EasyCal is primarily designed to create one or two point calibrations. For calibrations containing more than two calibration points RI Calibration should generally be used. However, it is possible to start to create calibrations containing more than two points using EasyCal. Once the

second point has been measured the status frame will display a prompt asking the user if they wish to measure another sample. If the answer to this is yes, the software will follow the same procedure as was used for the second calibration standard until the measurement has been made and the calibration needs to be displayed. At this point, the software will inform the user that RI Calibration will be required to generate this calibration (Figure 2.25) and this software will automatically be opened. The user will now have to use RI Calibration to generate the calibration for this application. Further details on the use of RI Calibration can be found in section 2.4 of this manual.

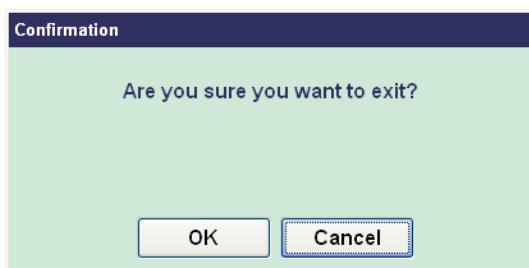
Figure 2.25 EasyCal main screen showing screen prompt to use RI Calibration



2.3.8 Exit of EasyCal Software

Once a calibration of some type has been created using EasyCal the user can exit the software using the **Exit** Button. This will produce the Exit prompt (Figure 2.26).

Figure 2.26 – EasyCal exit dialog box



Press **OK** to exit the software and **Cancel** to return to EasyCal if required.

2.4 RI Calibration Software

2.4.1 Overview of RI Calibration Software

2.4.1.1 Start-up RI Calibration Main Window

RI Calibration behaves as any standard Windows application. The windows may be moved, minimised and restored as for most Windows software. Users unfamiliar with Windows operation should refer to Microsoft Windows manual for further information. Start RI Calibration by selecting *Start > Programs > MultiQuant > RI Calibration* from Windows menu bar.

2.4.1.2 The RI Calibration Main Window

- The **RI Calibration** main window allows the user to select menu options, enter the data for the samples and to customise the visual aspects of the calibration software.
- The menu options are situated towards the top of the screen, and the ToolBar lies below them. The spreadsheet, containing the data entry cells, resides in the white box in the middle of the window. The signal function box at the bottom of the window allows the user to specify how the variables (A, B, C...) defining the processing of signal regions are combined to produce the final NMR result for the calibration (Figure 2.27). Each page contains a different calibration table, therefore up to 4 multiple calibrations can be produced simultaneously.

Figure 2.27 - Main RI Calibration window

2.4.1.3 Using the Spreadsheet

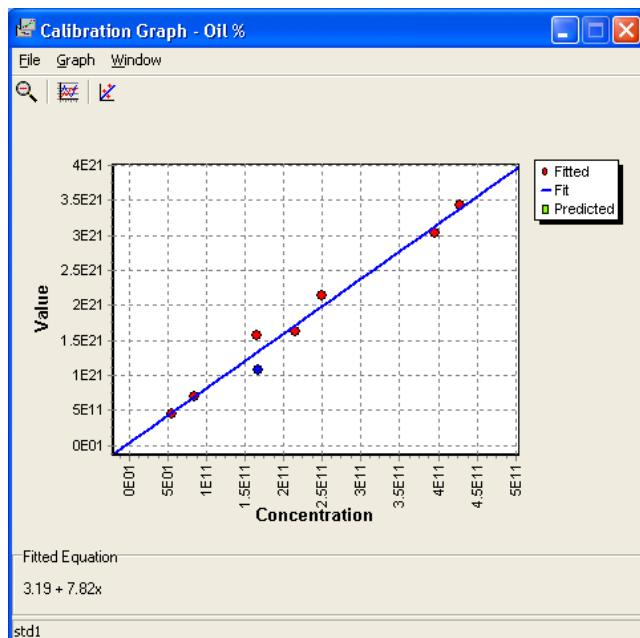
- RI calibration spreadsheet provides columns and a default number of rows that can be extended; each cell should be filled with the appropriate information for each standard. To select a cell in a particular row, left click with the mouse on the required cell. An outlined border surrounds the active cell. Clicking on the row cell on the left selects the row by highlighting it in blue. Rows may be cut, copied or pasted using the **Edit** menu or by right-mouse click on the row cell highlighted in blue. Note that when the row is pasted, it is can be inserted immediately after or before the currently highlighted row. The column to the left of the **SampleID** also indicates the currently active row with an arrow (the row indicator column). Rows can be deleted or inserted by right clicking on the row indicator column and selecting the appropriate option. Right clicking on the name of the column allows changing the name of the column denomination and the number of decimal places if applicable.
 - Place the mouse arrow on the dividing line between two columns to alter the width of the columns, then left click the mouse and drag the line left and right until the desired location is found.
 - **Sample IDentification** - Enter a name for each sample here. Note that this column defaults to the name of the data file that is attached to the row if the cell is empty.

- Conc. - The concentration for the calibration standard.
- Mass - Sample mass in grams.
- **Signal** - Value of the signal function for that particular data set. NMR data files are attached to the signal cells by right clicking on the appropriate signal cell. Numbers can also be written instead of data obtained by file attachment, only if none of the rows contains a file attachment. Therefore, the file attachment must be removed from each row by right clicking the mouse on the Signal column or selecting File > Detach All Files, before numbers can be manipulated in the Signal column for demonstration purposes. Note that new rows to be added with a number in the Signal Column must first be specified with a Sample ID.
- ? - Sample selector enabling the use of a standard in the calibration curve. Left click the mouse to highlight the row relating to this standard. Data point is used when the (*) sign is shown. Click space to make the (*) sign disappear, disable the use of the data point and empty the Value and Calculated result cells.
- Raw - Allows the user to specify whether or not the data should appear in the NMR Data Window (Y or N).
- Value – NMR value used for the calibration. This may be the NMR result divided by the sample mass when the Use Mass box in the right mouse click menu of the sample mass column is ticked off.
- Calc - Calculated value of concentration obtained from the fitted curve.
- Diff - Difference between calculated value of concentration and reference concentration for a given standard.
- Signal Function - The Signal Function box in the bottom left hand corner of the main RI Calibration window displays the signal function and allows the user to type in a formula containing up to seven data ranges (A to F). Users should refer to section 0 for more information on data ranges.
- Page 1, Page 2, Page 3, Page 4 - Users may select one of four spreadsheet pages. Different pages may be selected by left clicking on the page tabs at the top of the spreadsheet. The pages exist to enable the user to create several calibrations from the same data set, each with different calculation ranges or signal functions. When the calibration is generated and used with RI Analysis, the measurement software reports a value for

each spreadsheet page. For example, if a user wishes to create a calibration for measuring oil and water contents in seeds, the first page can be used to produce the oil content, the second page the water content. Right click on the page tab to change a page name and enter the new name (for example **Page 1** could be changed to **Oil** and **Page 2** to **Water**). Note that the Calibration Graph window, the NMR Data window and the Statistics window are updated automatically when a new page is selected.

2.4.1.4 Calibration Graph Window

Figure 2.28 – Calibration graph window

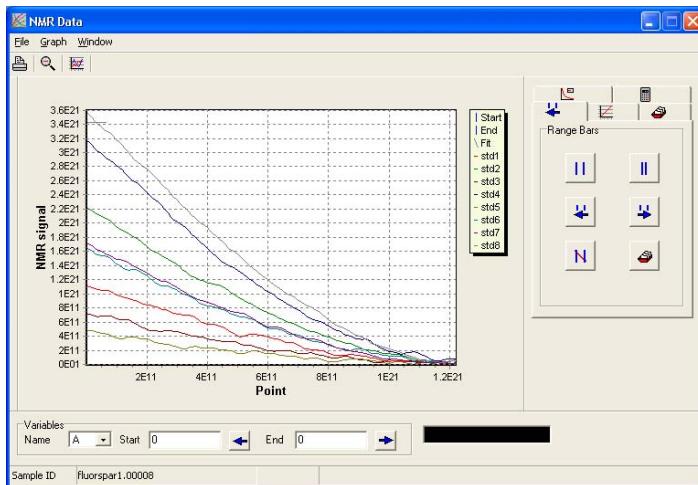


- The Calibration graph window (Figure 2.28) displays the current curve fit and data points with the value from the main calibration screen vs. conc. from the main calibration screen.

2.4.1.5 NMR Data Window

- The NMR data window displays the raw NMR data that has been loaded into the calibration software (Figure 2.). The window allows the user to specify data ranges for evaluation and range fitting options. Information on the options available using the NMR data window may be found in Section 2.4.7.

Figure 2.29 – NMR data window



2.4.2 ToolBar Icons

Figure 2.30 – RI Calibration tool bar

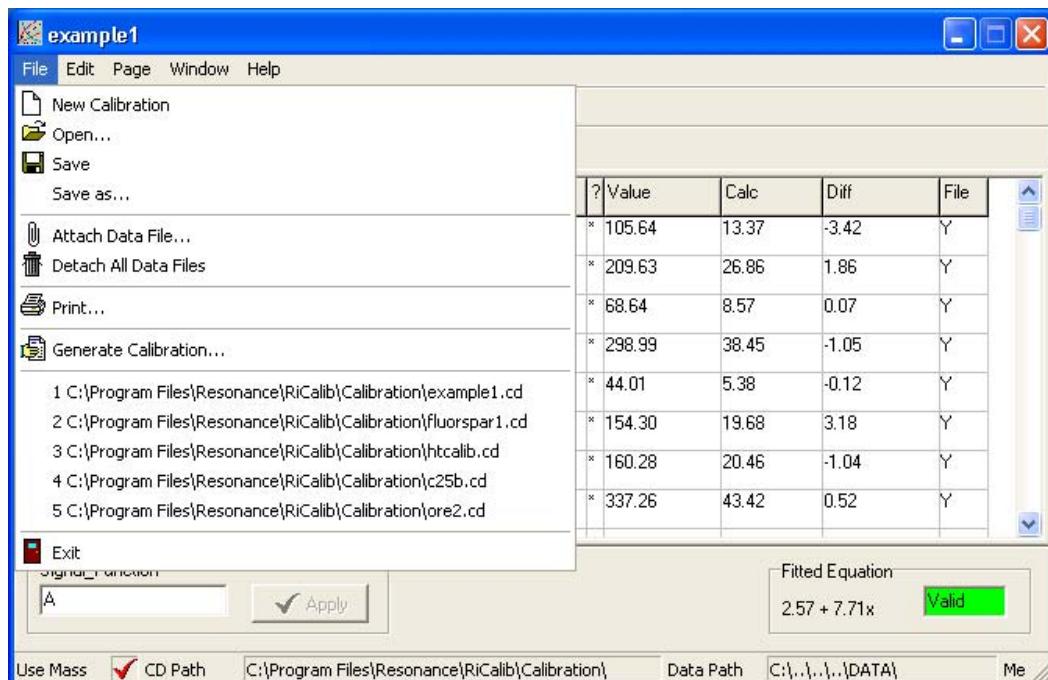


The ToolBar icons provide shortcuts to the most frequently used menu options (Figure 2.). A description of the available shortcuts can be obtained by placing the mouse pointer over each icon in turn. Thirteen menus may be accessed using the calibration software. Some of the options contained within the menus can be reached via the ToolBar. Click on the relevant icon in the ToolBar to select a menu option. Note that a description of the function of each icon can be obtained by holding the mouse pointer over the icon.

2.4.3 Menus

2.4.3.1 File

Figure 2.31 - File manager to open/save a calibration table

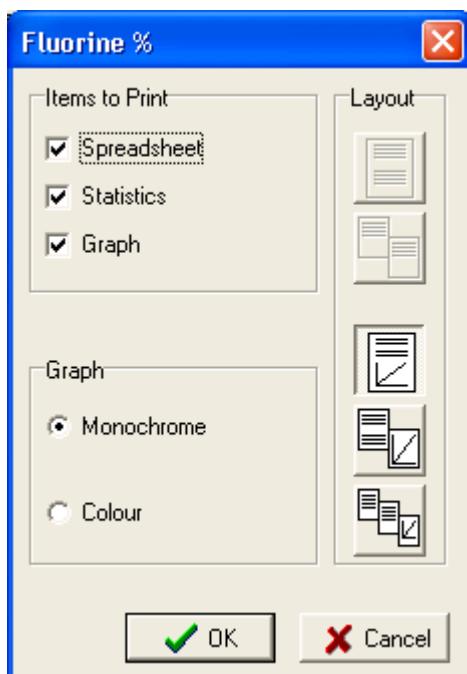


The file manager (Figure 2.31) allows users to create, open and save calibration tables, to attach or detach data files from each row, print the results of a calibration and generate a calibration file for use with the RI Analysis software.

The **New** option allows the user to start a new calibration. The user is prompted to save any existing calibrations before the new calibration is started.

The **Open** option allows the user to open an existing calibration table. Save allows the user to save the current calibration table for use at a later date. Calibration tables are saved by default under the filename ***.cd** in the default calibration directory.

Figure 2.32 - Print Preview options



Print (Figure 2.32) allows the user to print out information regarding the calibration. When the print option is selected, several options may be specified. These allow the user to select which windows and also which pages of the calibration are printed. Note that the page highlighted is the page that will be printed.

Under the **Items to Print** heading:

- Spread Sheet tick box provides a print of the calibration table content.
- Statistics tick box prints out a summary of the calibration statistics.
- Graph enables a printout of the calibration curve.

Under the **Graph** heading:

- **Monochrome** indicates that the print out will appear using a greyscale (option for non- colour printers).
- **Colour** indicates that all colours will be used for the print out on a colour printer.

Using the **Layout** icons:

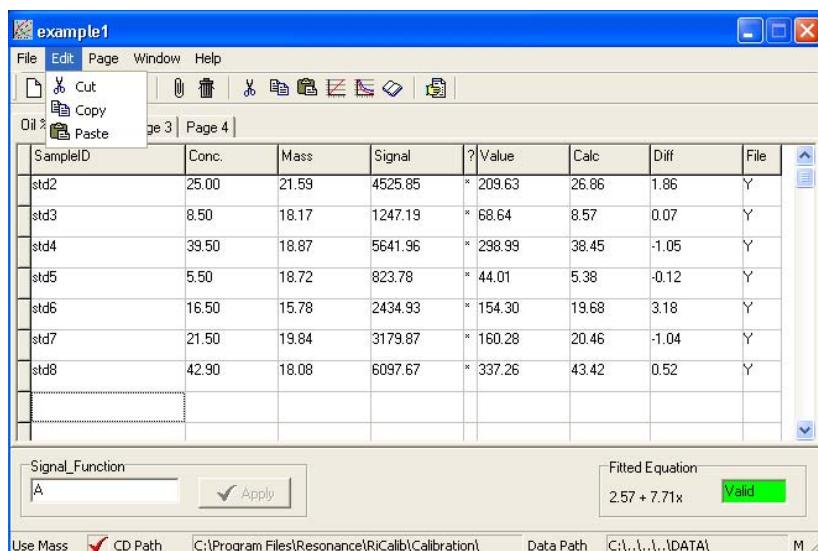
- Different icons options also allow collating the results on the same page or on different pages.
- The **OK** option allows the user to proceed with the printing of the selected information; select **Cancel** to abort the printing

selection.

- Finally, select **Exit** from the File menu to exit the **RI Calibration** software, or left click the mouse on the cross button in the top right hand corner of the screen.

2.4.3.2 Edit

Figure 2.33 – Edit Menu



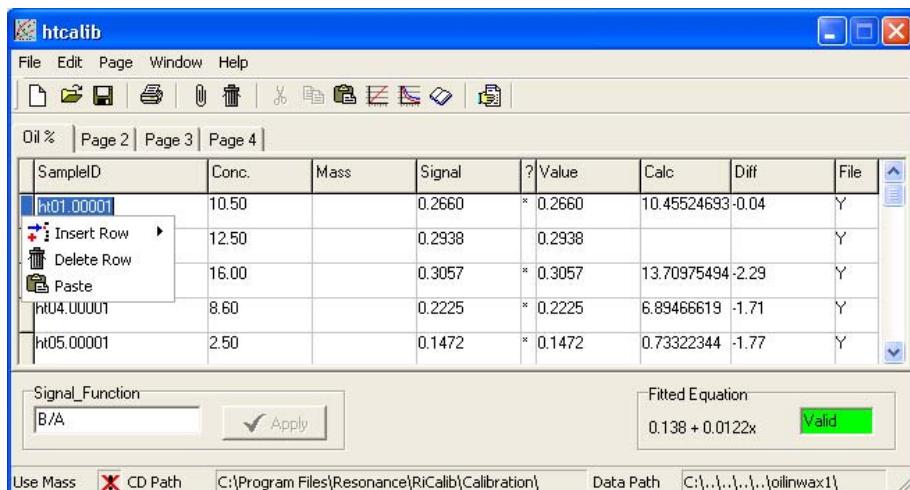
This menu shown in Figure 2.33 allows users to cut, copy and paste rows on the spreadsheet. Highlight in a required row by applying a left mouse click on the appropriate location (i.e. the case on the first column is then highlighted in blue); select **Cut** from the **Edit** menu to delete, cut, paste, or insert a row. Select **Copy** from the edit menu to copy the selected data to the clipboard. Select **Cut** to remove the content of a row. Select **Paste** to re-insert the row at the desired location. Alternatively, use the right mouse click on the beginning of each row to activate an equivalent menu. The right click mouse menu also allows the insertion of additional rows by selecting **Insert > Below** or **Insert > Above** to respectively insert a row below or above the highlighted row (Figure 2.34).

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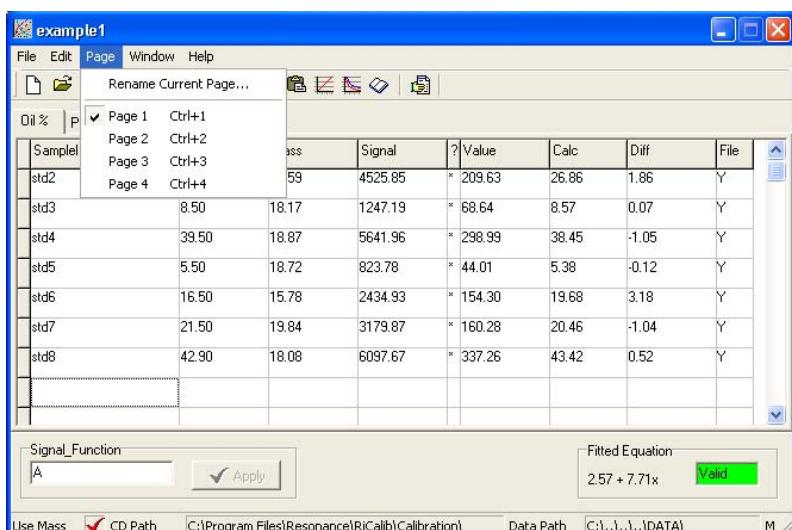
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Figure 2.34—
Insert/Delete options
by clicking the right
mouse button on the
highlighted row



2.4.3.3 Page

Figure 2.35 – Page
Menu



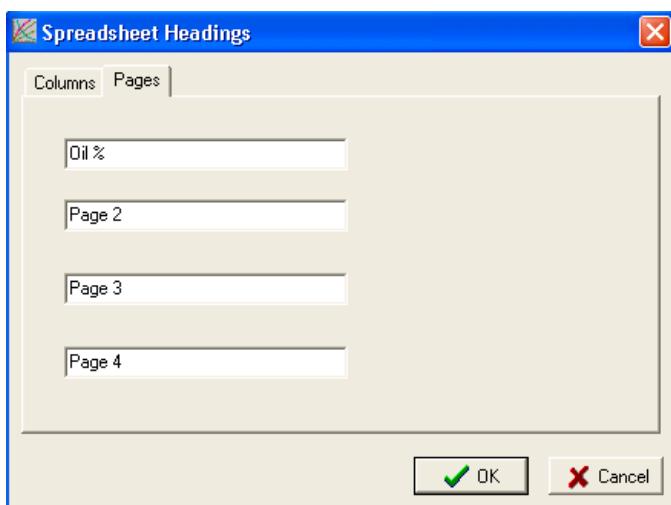
The Page option of the RI Calibration menu allows the user to select a Page and customise the title of each page (Figure 2.35). An alternative approach consists in left clicking on the mouse to bring forward the **Page** label to activate a page. Right clicking the mouse on a **Page** label (**Page 1**, **Page 2**, **Page 3** or **Page 4**) allows the user to rename the current page (Figure 2.3636). Note that the pages may also be selected by clicking on the relevant tabs in the calibration window. Calibration pages may also be renamed by selecting the **Rename Current Page** option from the page menu. Click on the column tab to rename the title of individual columns (Figure 2.36). The same menu also appears by right clicking with the mouse on the cell of each column title and selecting the **Rename column** option.

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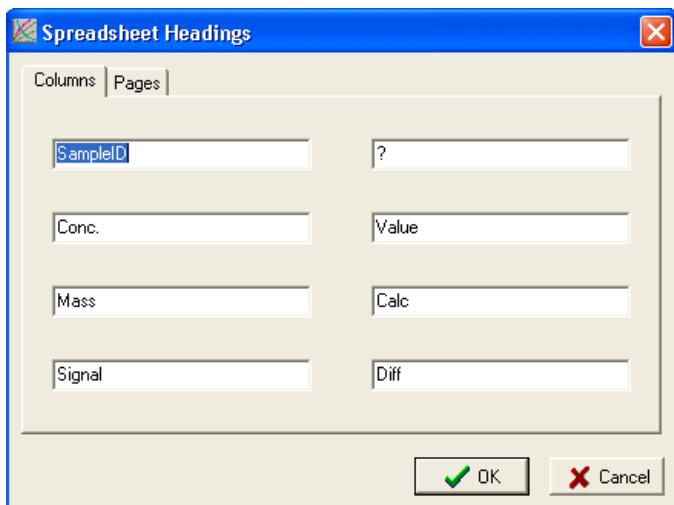
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Figure 2.36 – Rename Page



Left clicking the mouse on a **Page** label allows the user to select an active page. Right clicking the mouse on a **Page** label (**Page 1**, **Page 2**, **Page 3** or **Page 4**) allows the user to rename the current page (Figure 2.36). Note that the pages may also be selected by clicking on the relevant tabs in the calibration window. Calibration pages may also be renamed by selecting the **Rename Current Page** option from the page menu. Click on the column tab to rename the title of individual columns. The same menu also appears by right clicking with the mouse on the cell of each column title and selecting the **Rename column** option (Figure 2.37).

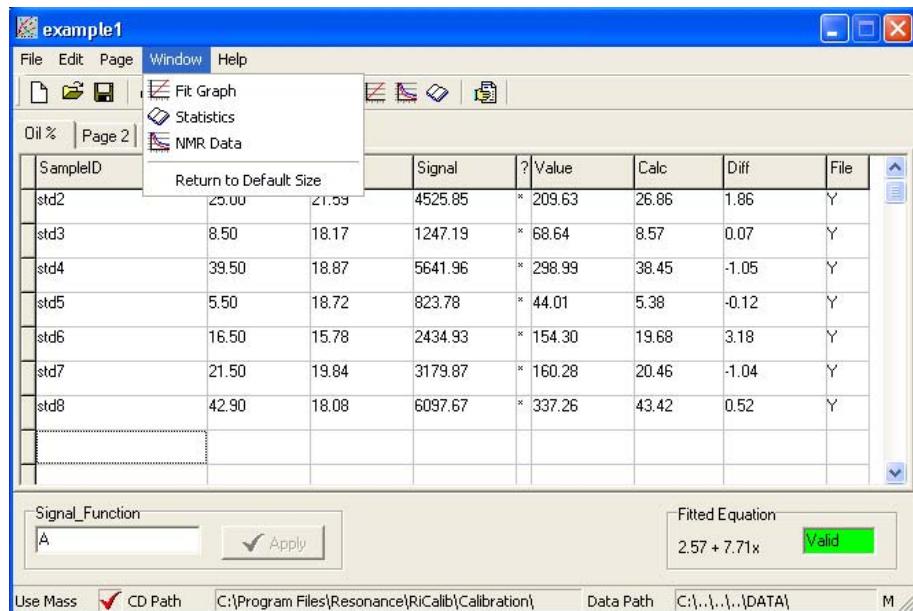
Figure 2.37 – Rename Column



2.4.3.4 Window

The Window option allows the user to select which of the three supplementary windows (**Calibration Graph**, **Statistics** and **NMR Data**) are displayed on the screen (Figure 2.3838). The option **Return to Default Size** allows the window to resize automatically to the **RI Calibration** window size default.

Figure 2.38 – Window Options



2.4.3.5 Help

Help displays information pertaining to the software version number and the address for Oxford Instruments Molecular Biotools and main distributors (Figure 2.39). The software version number should be provided during queries addressed to OIMBL support team (e-mail: helpdesk.nanoscience@oxinst.co.uk).

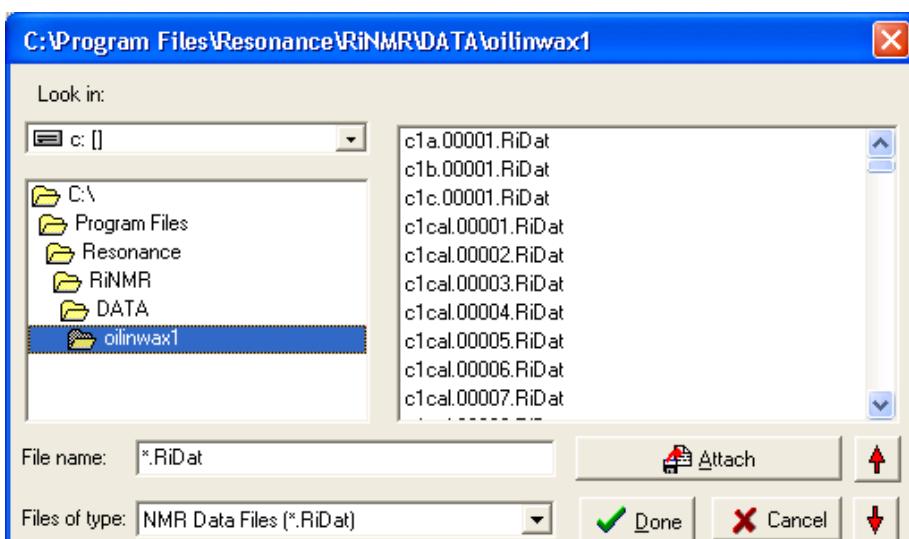
Figure 2.39 – Help Information



2.4.4 Attachment of Raw Data Files

To attach NMR data from an RINMR file, right click on the sample column of the desired cell. A pop-up box appears which allows the user to attach an RINMR data file to that particular row of cells. Highlight the **Attach NMR file** pops up the file attachment manager and browse the hard drive for the required file (Figure 2.40).

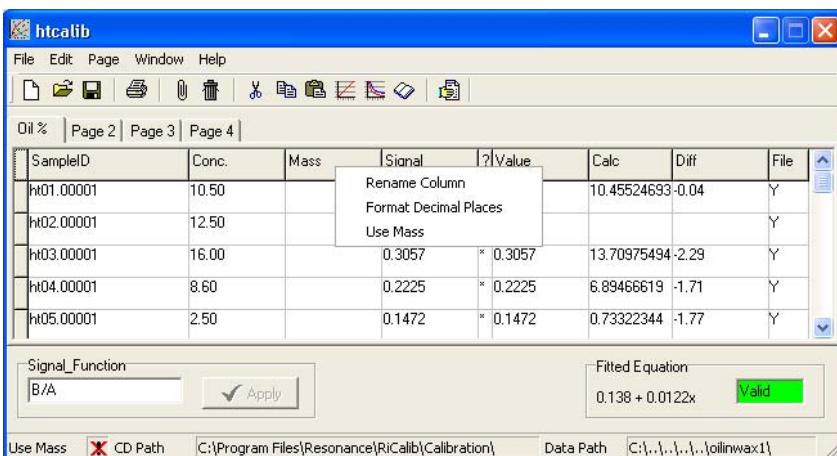
Figure 2.40- File attachment to a row in RI Calibration spreadsheet



Once a file of a given prefix has been loaded further files in the same series can be loaded by simply clicking on the **Attach** button. The file indicator will then increment automatically down the list. Click on the up or down arrow to choose a different row of the calibration table to attach the *.ridat file. If a **Sample ID** has not been entered in the spreadsheet, the software will assign the filename of the data file to the **Sample ID**. Click on **Done** to exit the file attachment menu after a file has been attached to each row, or **Cancel** to return to the main menu.

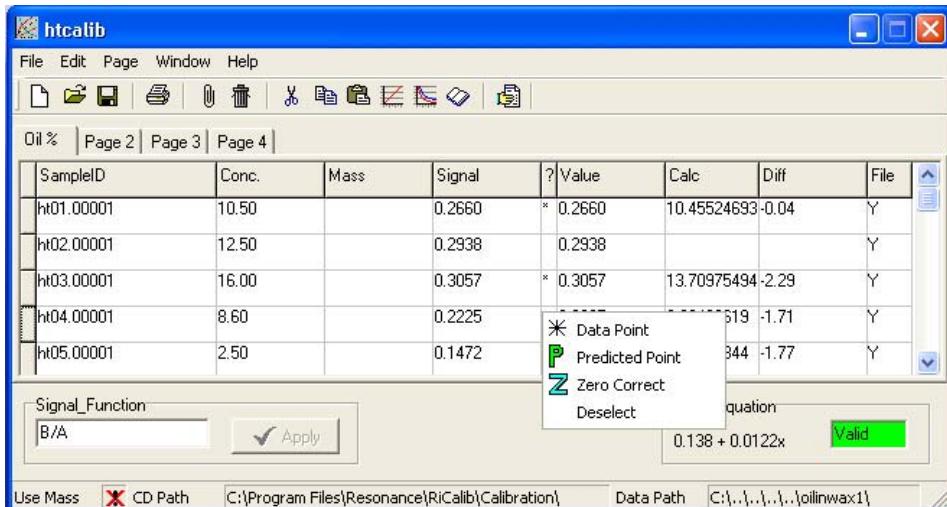
2.4.5 Concentrations, Masses and Data Type Specification

Figure 2.41 – Access to Rename Column, Format Decimal Places and Use Mass options by right click action of the mouse on the mass column



Once the NMR data files have been attached it is necessary to assign a known value (or concentration) and, if the **Use Mass** tick box is selected in the right mouse click menu of the **Mass** column (Figure 2.41), assign a sample mass to each row. To assign concentrations, type in the known values of the calibration standards in the **Conc.** column of the spreadsheet. In a similar manner, type the mass of each sample in the **Mass** column for mass normalisation.

Figure 2.42 - Options for data point selection by clicking the right mouse button on the (?) column



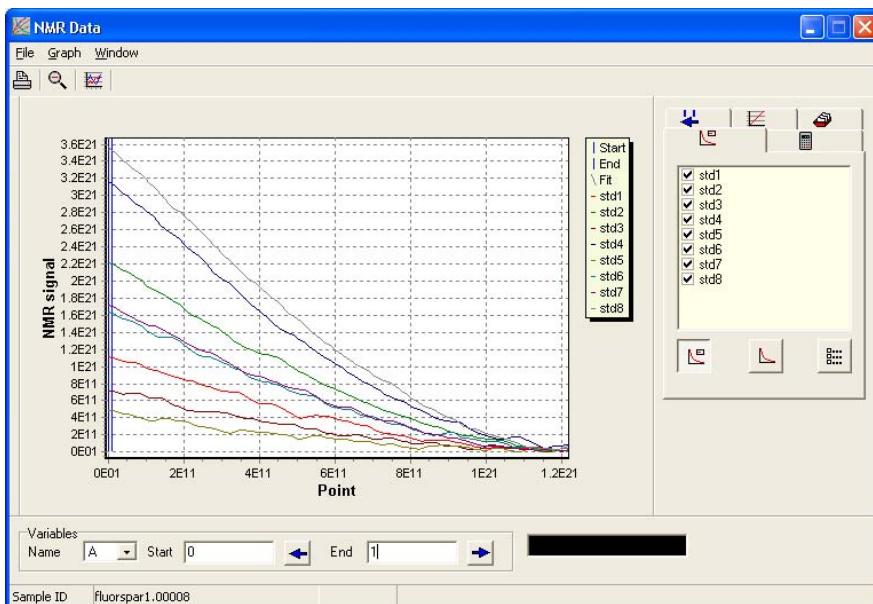
The Data Type Specification column (?) informs the program on how to handle the data. Various data selection types can be selected by clicking the right mouse button against the cell referring to the (?) column (Figure 2.42). The data types are as follows:

- The option '*' indicates that the data will be used in the calibration.

- The option '(space)' indicates that the data will not be used in the calibration (i.e. it can be used to remove outlying points or bad results).
- The option 'Z' indicates that the data should be used to zero-correct the calibration; data cells are highlighted in blue.
- The option 'P' indicates that the data is used to predict the result from the calibration, so that the data point is NOT part of the calibration; data cells are highlighted in green.
- Right click with the mouse also provides the option of using the data point, selecting the predicted point option or using the standard for zero correction.

2.4.6 Viewing NMR data

Figure 2.43 - NMR Data Window



Once the data file has been attached to the cells, click on the NMR Data window to view the raw data (Figure 2.43). The NMR Data window contains a plot of the acquired NMR data vs. Time on the left, and a menu containing a list of the *.RiDat files loaded with display activation options, a data selection range manager, a set of calculation range specification options, a data range archive and a data zooming window.

The NMR Data window graph shows the NMR data that has been loaded into the calibration software and activated on display. The legend on the right of the graph allows identification of each data set. To zoom to an area of the graph, left click on the graph and drag the mouse to the right and down to define a zoom window. To un-zoom the graph, left click on the graph and drag the mouse to any position to the left and up from the original point. Alternatively, click on the un-zoom icon (central icon, top left of the data display). The graph should return to its original settings.

To move the graph along the x or y axes, right click on the graph and drag the mouse in the desired direction.

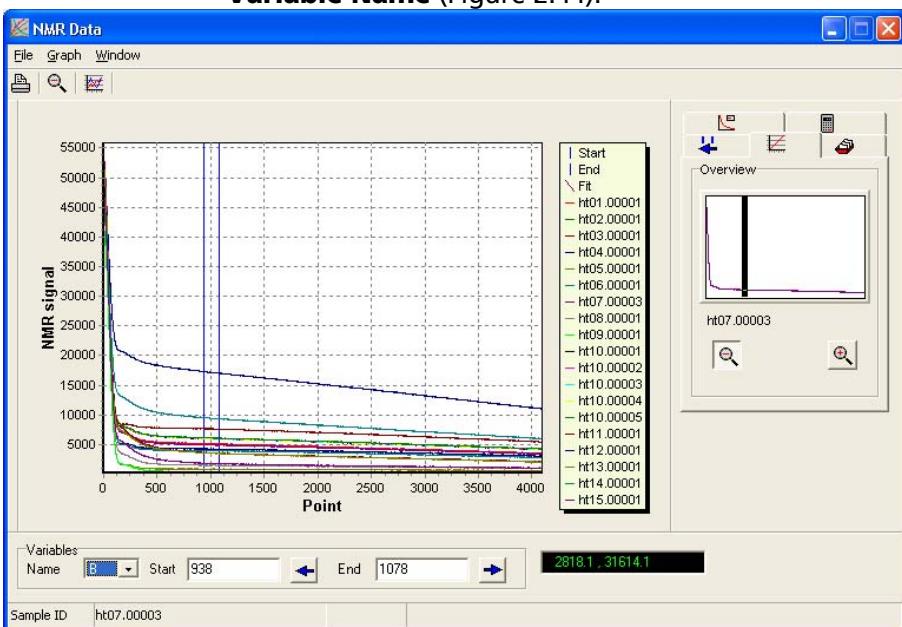
- Note that in the case of the NMR Data window, the graph may also be returned to its original settings by left clicking on the Unzoom icon (second from the left in the icon bar). Users should note that the same zooming scheme applies to both the **NMR Data** and the **Calibration Graph** windows.

2.4.7 The NMR Data Window

2.4.7.1 Specification of the Calculation Range

- The specification of the calculation range may be performed in a number of different ways. First click on the arrow in the bottom left hand corner of the data display window to allocate a letter to the **Variable Name** (Figure 2.44).

Figure 2.44 – NMR Data Window showing the variable data range selection (Start, End and vertical lines)

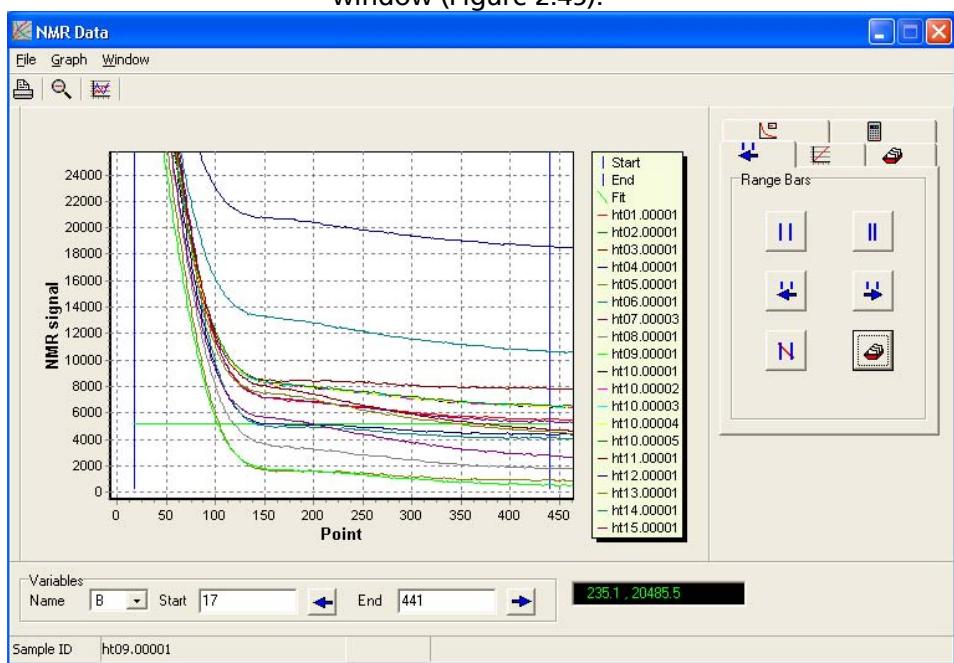


- The current **Start** and **End** points of the data range may be found in the two boxes to the right of the Variable Name. The **Start** and **End** points of the ranges can be specified in three ways:
 1. Select the **Start** and **End** blue vertical lines by pressing [**Ctrl + left mouse click**] simultaneously and dragging the mouse left and right of the screen. Note that the position of the vertical lines can be swapped.
 2. Specify the **Start** and **End** values for the data range in the two boxes to the right of the calculation range label display. The two blue vertical lines that indicate the beginning and end of the calculation ranges will move to reflect the

new values.

3. Select the page with the blue left arrow icon in the top right hand menu of the NMR Data window (Figure 2.45).

Figure 2.45 – Range Bars page in the right hand menu of the NMR data window



2.4.7.2 Range bars

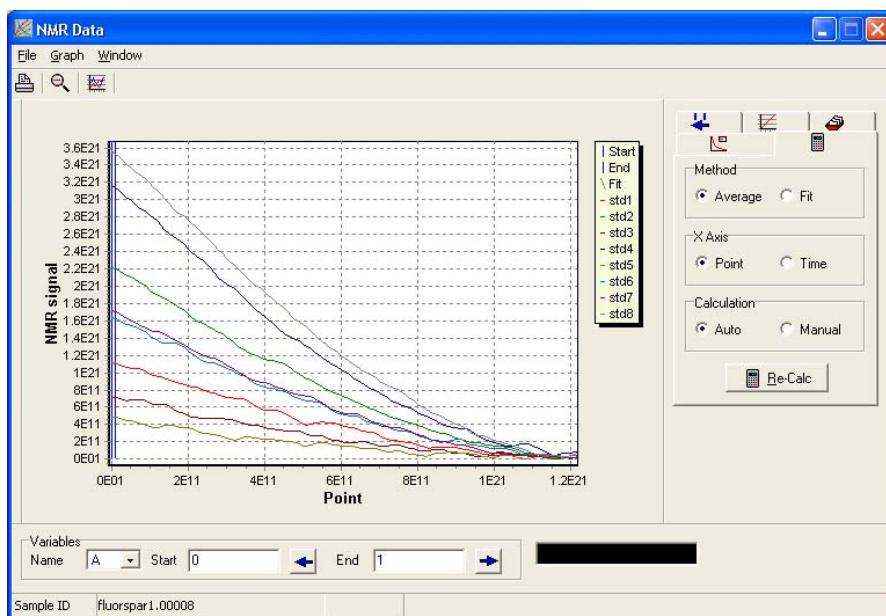
The range bars page in the NMR data menu offers several tools to facilitate the manipulation of the data selection range determined by the Start and End positions of the vertical lines. Here is a description of the function against each icon:

- Fits the two vertical lines inside the zoomed window.
- Reduces the size of the data range about its centre, by reducing the gap between the two vertical lines.
- Increases the size of the data range about its centre, by increasing the gap between the two vertical lines.
- Moves the data range to the left.
- Moves the data range to the right.
- Transfers the data range selection to the archive so it can be recalled at a later stage.

2.4.7.3 Method mode (Method, X-axis and Calculation Page)

One of two calculation modes may be specified from each calculation range. The first, **Average**, calculates the average value from all the points in the specified data range and transfers it to the main calibration program. The second option, **Fit**, fits a straight line to the selected data range and extrapolates the line back to time zero. The value of the y-intercept is then transferred to the NMR value displayed in the RI Calibration table.

Figure 2.46 – Fit option from the Method page in the right hand menu of the NMR data window



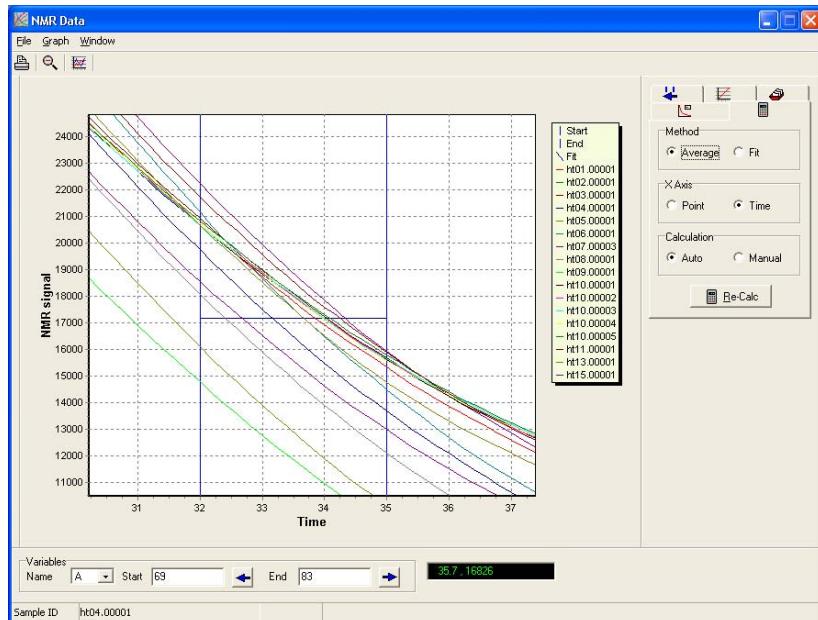
- Once the data ranges have been specified, a fit line appears between the **Start** and **End** limits on the data display graph. The line either reflects the average value of the data between **Start** and **End** limits (Figure 2.46), or the straight line that the data has been fitted to (Figure 2.47).

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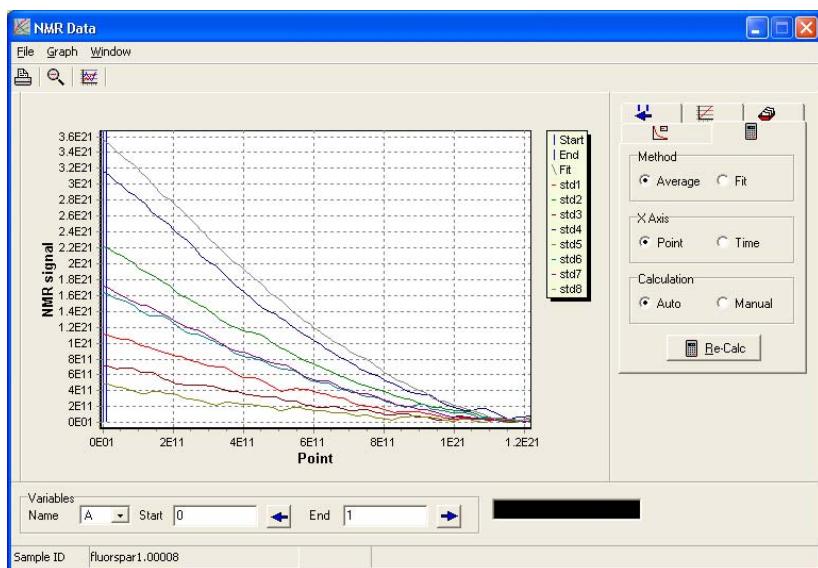
Figure 2.47 – Average option of the Method page in the right hand menu of the NMR data window



2.4.7.4 Point and Time X-axis domains (Method, x-axis and Calculation Page)

- The raw data in the NMR data window may be displayed in Point or Time domain. In Point domain, the x-axis displayed the data point by point in the order of their acquisition, regardless of the time reference at which they were acquired (Figure 2.48). In Time domain, the X-axis display the true time for each point from the zero time reference in the NMR pulse sequence (Figure 2.49).

Figure 2.48 – Point domain displayed on the x-axis of the NMR data window

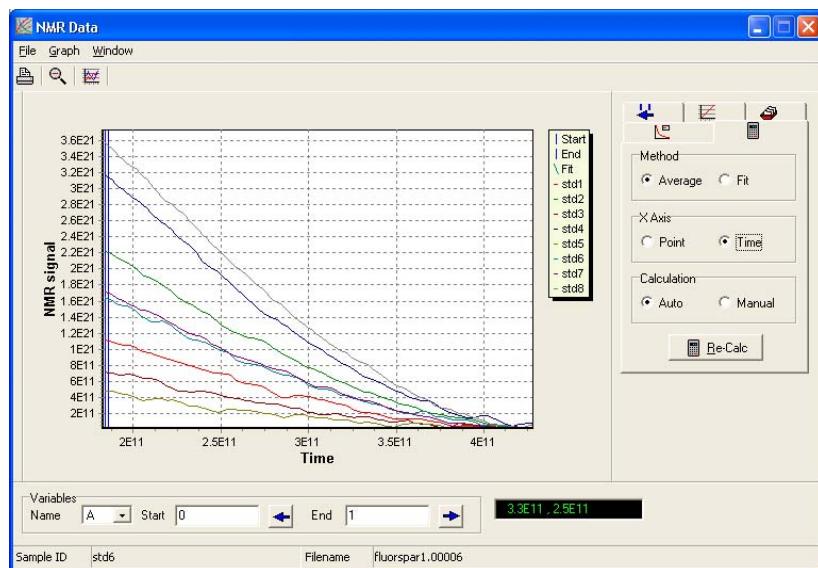


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Figure 2.49 – Time domain displayed on the x-axis of the NMR data window

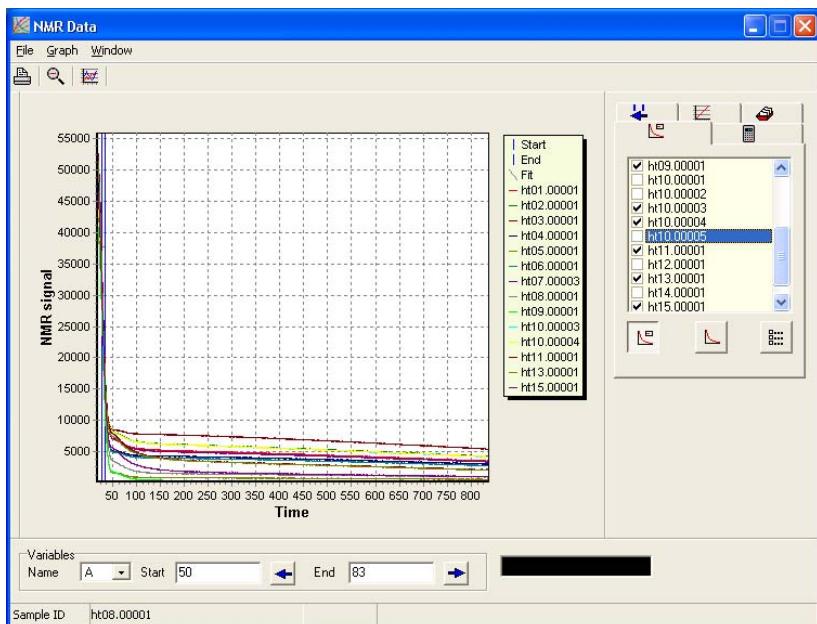


2.4.7.5 Calculation mode (Method, X-axis and Calculation Page)

The function **Recalc**, recalculates the calibration based on any new range that has been specified (Figure 2.49). Note that if the **Auto** radio button is checked, recalculation occurs automatically every time a calculation range is altered or a new calculation range is specified. If the **Manual** radio button is checked, the user needs to left click on the **Recalc** button before recalculation takes place.

2.4.7.6 Selective display of NMR data sets

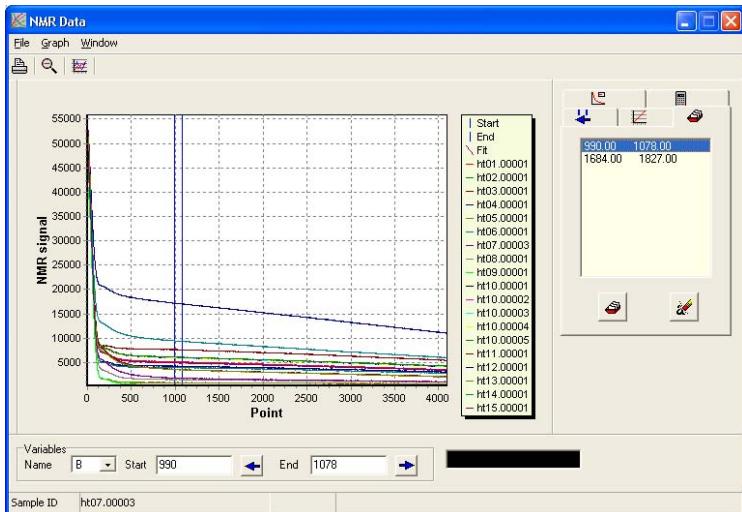
Figure 2.50 – Tick box options to activate or de-activate the display of data sets



The data display feature (Figure 2.50) allows the selection of data sets for display when the display of many data sets at a time complicates their visualisation. This is achieved by ticking the box

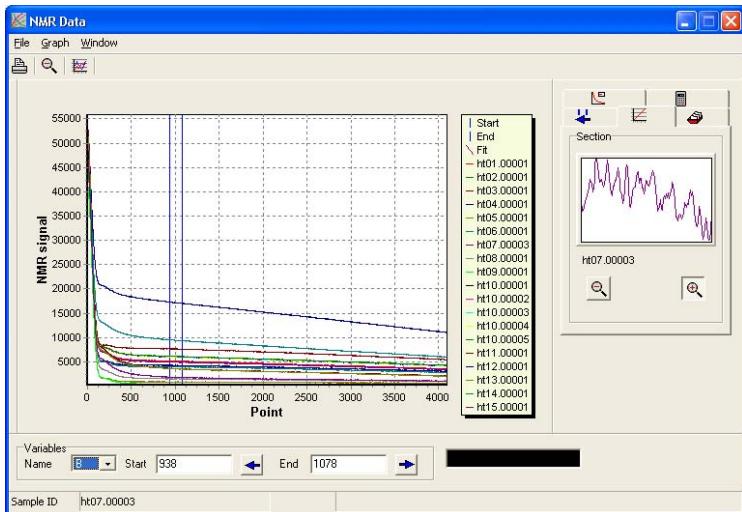
allocated against the name of each data set. Note that, even though the data name may be ticked off, the data is still present and can be potentially used for the calibration. Click on the left bottom icon of the data name page to show the legend. Click on the central bottom icon to hide the legend. To remove all the data sets from the display except the active one, click on the icon on the bottom right.

Figure 2.51 – Archive showing the possibility of saving a data selection range



2.4.7.7 Data range archive

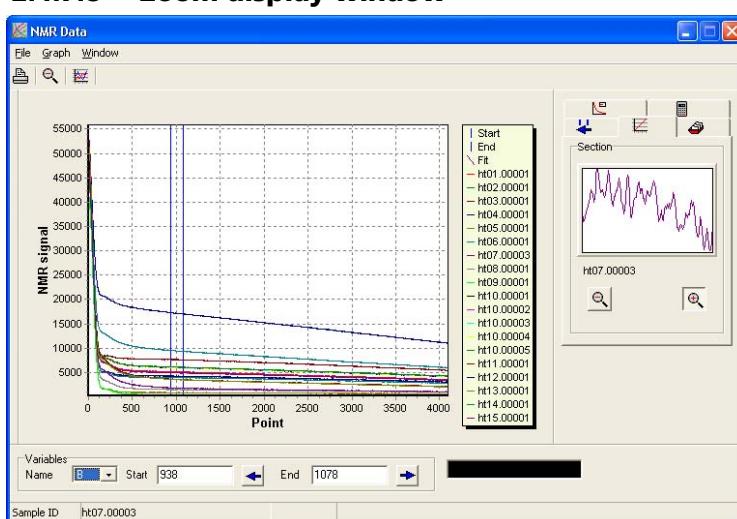
Figure 2.52 – Archive showing the possibility of saving a data selection range



The data range archive page allows a data range selection to be saved so it can be recalled and the effect of a data range compared with another data range at a later stage (Figure 2.51). Click on the bottom left icon of the page to add the data range selection (**Start** and **End** values) to the archive. Click on the bottom right icon of the data range archive page to clear the whole content of the archive. Note that the archive is automatically cleared after closing RI Calibration.

2.4.7.8 Zoom display window

Figure 2.52 – Archive showing the possibility of saving a data selection range



- The NMR data menu offers the facility to expand the selection of data between the two vertical lines without the need to zoom the NMR data display window. Select the Section page of the NMR data menu on the right of the NMR data window (Figure 2.52) to display the specific data region between the Start and End data points. The zoom icon on the left indicates the option to display the whole dataset while a zoom may be applied in the NMR data window. Click on the bottom right icon to zoom automatically between the two vertical lines.

2.4.7.9 Editing the raw data graphic display

The NMR data window also offers a tool to customise the information displayed on the raw data graph. Click on the top right icon to display the **Edit Graph** window. The first page (Labels) provides the possibility to input or change the Graph title, X-axis label or Y-axis label (Figure 2.53). The second page (Background) allows the user to choose a different background colour than white, the default setting (Figure 2.54). The third page allows the display of the numbers indicated on each axis using a Scientific or General format (Figure 2.55).

Figure 2.53 – Choice of Graph title, X-axis label and Y-axis label

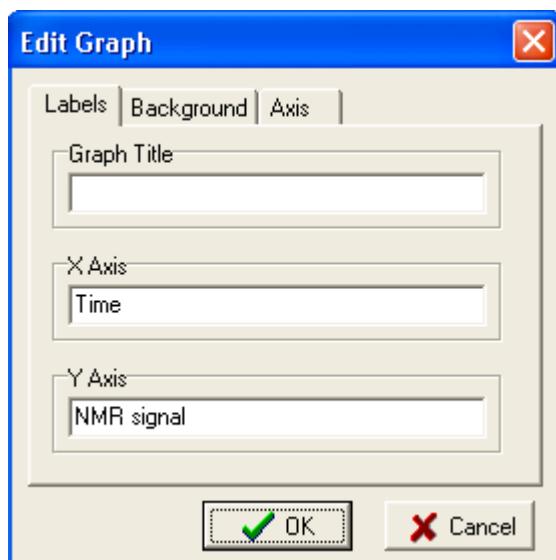


Figure 2.54 – Choice of background colour on the NMR data display window

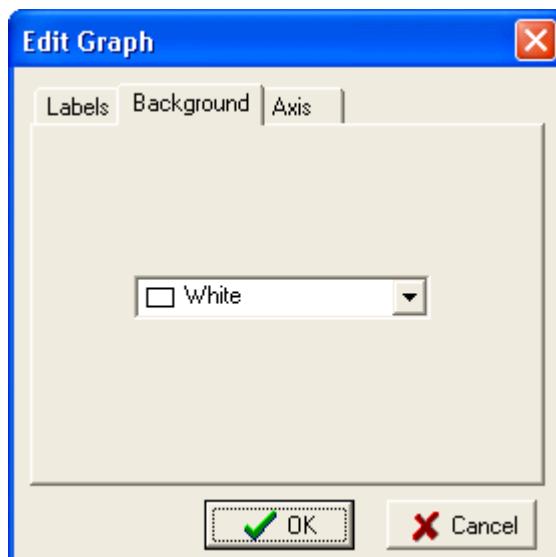
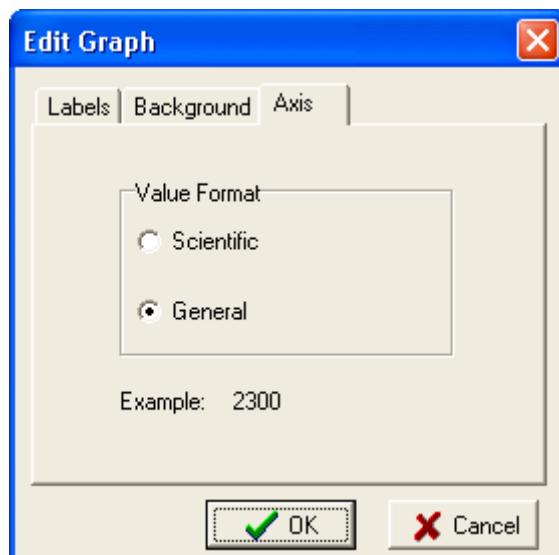
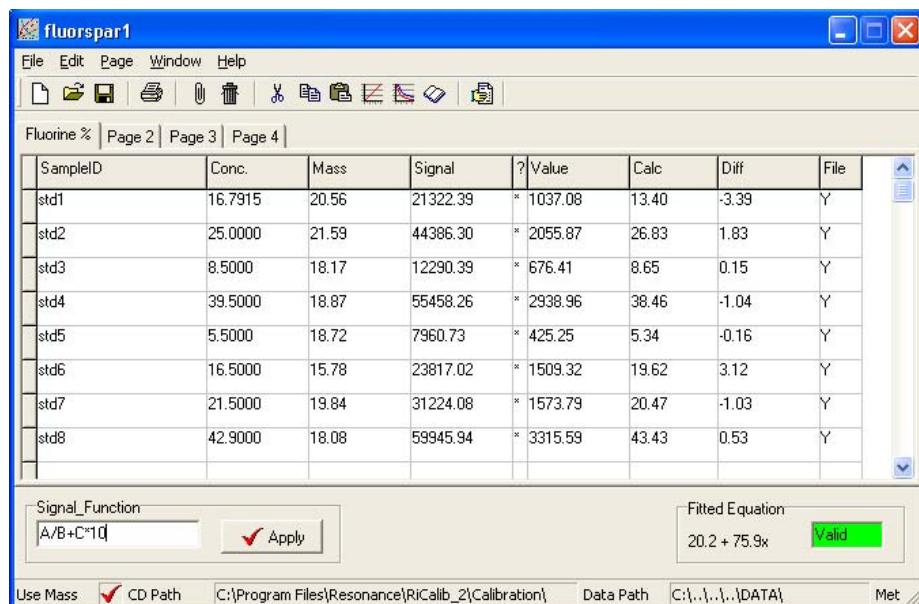


Figure 2.55 - Display of axis values in Scientific or General format



2.4.8 Specifying the Signal Function

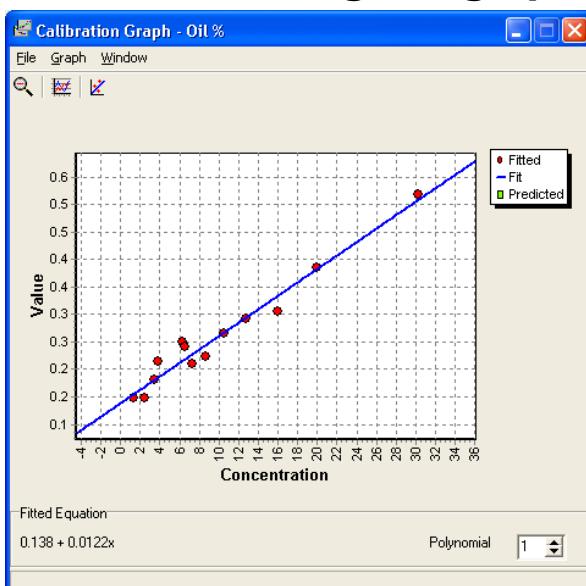
Figure 2.56 - Example of signal function used to calculate the NMR value ($A/B+C*10$)



- Once the calculation ranges have been specified, the way in which the variables are combined to form the final value for the calibration is specified in the signal function box that is located in the main RI Calibration window (Figure 2.56).
- Up to 10 calculation variables may be specified and can be combined with the four operators +, -, * and /. The priority of operation is (), / and *, - and +. For example, A+B, A+(B/D), and 1/(A)*A+C/(D-F)+100 are all valid expressions for the signal function.

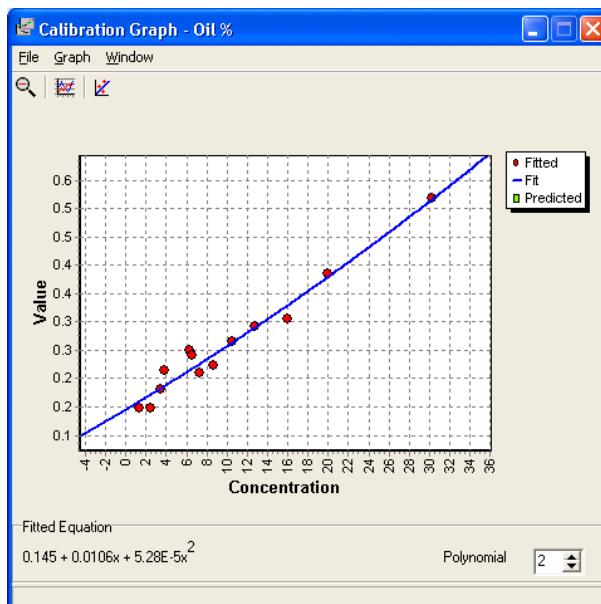
2.4.9 Curve fitting and graph window

Figure 2.57 - Calibration curve using a least-square fit (Polynomial = 1)



Once the signal function has been specified the calibration curve can be viewed in the Calibration Graph window. Click on the Calibration Graph icon from the main RI Calibration menu to display the calibration graph window (Figure 2.57). This window shows a plot of NMR value (after mass normalisation, if the Use Mass option has been ticked) vs. Concentration. Position the mouse and hold on the right button to show the data point name at the bottom right of the Calibration Graph window. Data that does not follow a straight line can also be fitted using a polynomial, up to ninth order (Figure 2.58).

Figure 2.58 - Calibration curve using a quadratic function (Polynomial = 2)



2.4.10 Checking the Calibration

Figure 2.59 - Statistical information from the calibration curve fitting

Line Fit Statistics	
Fitted	Predicted
	Format Decimal Places
Number of points	8
Slope	7.71
Value intercept	2.57
Conc. intercept	-0.33
Correln. coeff.	0.99
Standard deviation	1.86
Variance	3.47

- To view the statistics associated with the calibration curve, click on the Display Statistics icon from the main RI Calibration window (Figure 2.59). The data displayed in each row is as follows:
- **Num Points** - the number of points of each type present in the calibration.
- **Slope** - **The slope of the line resulting from the linear regression.**
- **Value Intercept** - The y-intercept of the line resulting from the linear regression.
- **Conc. Intercept** - The x-intercept of the line resulting from the linear regression.
- **Correlation Coeff.** - Coefficient of linear correlation (cf: section 0).
- **Standard Deviation** - Standard Deviation (cf: section 0).
- **Variance** – Variance (cf: section 0)

Users can use the data type column ‘?’ in the spreadsheet to remove outlying or suspect points from the calibration by selecting ‘?’ for the data type instead of ‘*’. Note that if the data ranges are altered (and the **Recalc** option is set to **Auto**) or the signal function is changed, the effect of the changes on the calibration curve and statistics will be updated automatically. This allows the user to study the effect of varying the data ranges on the calibration and the quality of the results in real time, which significantly facilitates the process of identifying the optimum calculation

range for the calibration.

2.4.11 Generating the Calibration File

Figure 2.60 - Analysis Parameters: entry of customised parameter options needed to generate the calibration files (.clb and *.clt files) for routine analysis*

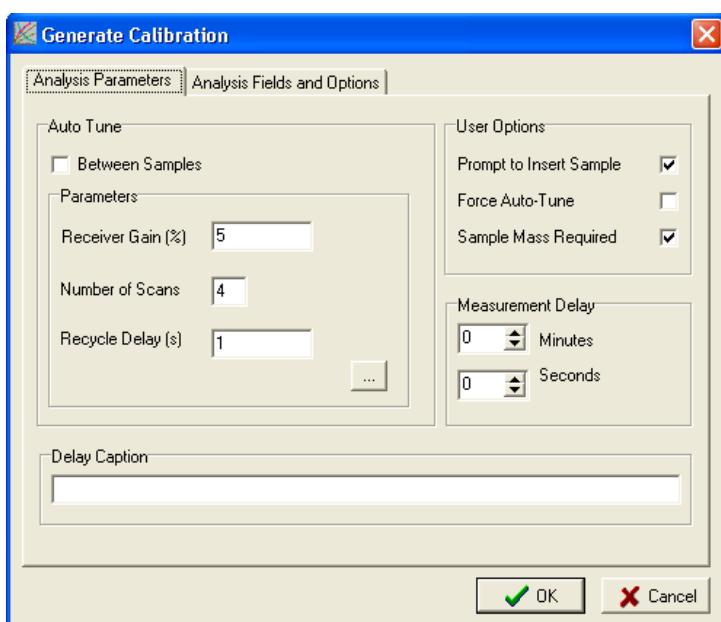
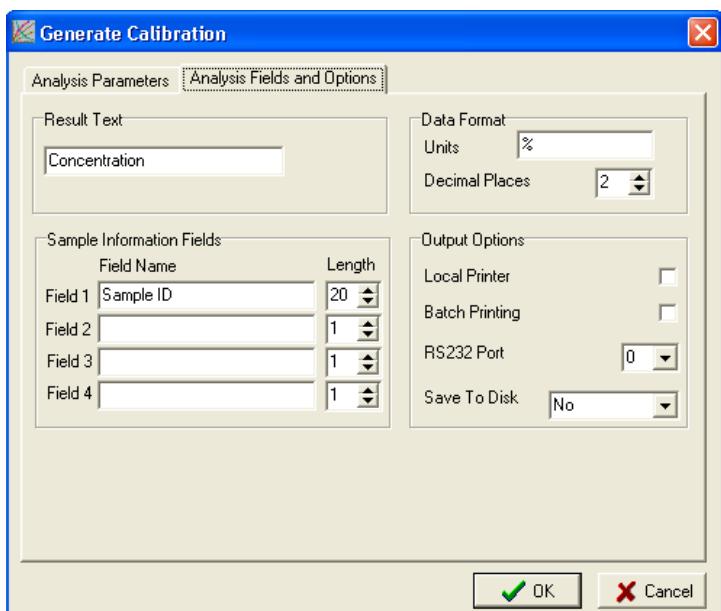


Figure 2.61 - Analysis Fields and Options: data handling options needed to generate the calibration files (.clb and *.clt files) for routine analysis*



- Once the user is satisfied with the quality of the calibration, the calibration may be used to generate a file for use with **RI Analysis**. **RI Analysis** allows non-expert users to perform routine measurements on unknown samples.

For **RI Analysis** to function correctly it requires details of routine operation, including the NMR experiment, the NMR parameters used, the values of the calculation ranges, the slope of the calibration graph etc. All these details

are contained within a calibration file that is generated from the RI **Calibration** software. When satisfied with the results of the calibration software, select the **Generate Calibration** option from the **File** menu of the **Main Calibration** window. The screen contains a list of custom options available for routine analysis.

2.4.12 Analysis Parameters

- Page 1 of the Generate Calibration window (Figure 2.60) describes customisable parameters for routine analysis (Analysis Parameters section).

2.4.12.1 User Options

These options allow the user to specify details for the routine operation. The option **Prompt to Insert Sample** box enables a prompt to insert a sample every time a new measurement takes place. The option **Force Auto-tune** forces the operator to auto tune the instrument. The option **Sample Mass Required** forces the operator to auto tune the instrument. Check the box to validate any of these options.

2.4.12.2 Auto Tune Definition

This section defines how the system auto tunes the instrument at the resonance frequency, and if necessary, forces the insertion of the tuning sample between each analysis (Autotune ... between samples). The user may specify an RINMR data file by clicking on the attach file radio button, from which the tuning parameters are extracted. Alternatively, enter the appropriate values for RG (Receiver Gain), NS (Number of scans) and RD (repeat delay between scans) in the RG, NS and RD boxes.

2.4.12.3 Measurement delay and delay caption

Those fields are used to set a pre-analysis delay and screen message explaining the reason for waiting before the beginning of the analysis (e.g. 03:00 minutes of sample conditioning).

2.4.13 Analysis fields and Options

Page 2 (Analysis Fields and Options, Figure 2.61) describes the list of data handling options for Quality Control.

2.4.13.1 Result Text

This option allows the user to specify the name of the measured property (e.g. oil in seeds, % spin finish etc). Where possible, this text should confirm that the correct calibration is being used. For example, if the calibration is set to measure spin finish in polyester, a good choice for the result test would be: '% spin finish (Polyester)'.

2.4.13.2 Data Format

Data format allows the user to specify what unit the measurement is displayed in (e.g. %, ppm, p.u.). The option **Decimal Places** allows the user to specify how many decimal places are displayed in the measurement readout.

2.4.13.3 Sample Information Fields

These fields allow the user to specify whether the operator should enter extra information associated with the sample to be analysed. A data field might be required for sample batch number, sample code number and operator name. The length parameter specifies the number of characters in each data field.

2.4.13.4 Output Options (Save to disk)

The result of the measurement is displayed to the screen as default. In addition the user may specify that the output results log is sent to the local printer (continuous page printer), to a batch file for printing after all measurements have been completed (standard printer), or to the RS 232 port (specify which port by clicking on the down arrow next to the RS 232 box; the output may also be sent to disk).

2.4.13.5 Creating the Calibration files (*filename.clb* and *filename.clt*)

Click OK after filling in the fields and options for the Analysis Parameters and Analysis Fields and Options. The software then requests a filename for the calibration file. The filename has the *.clt* extension. The calibration file *filename.clt* is saved by clicking OK to save the file. While the file *filename.clt* contains the calibration details and analysis options, RI Calibration saves another file *filename.clb* containing the experimental parameters. Although RI Analysis requests the file with the **.clt* extension, both files must be present, and RI Analysis uses the *filename.clb* to define the experimental conditions to apply during routine analysis.

2.4.15 Saving the Calibration Table

Left click on **OK** to generate the calibration file for use with the measurement software. Note that each time the operator uses **RI Analysis** software a calibration must be loaded. Labelling the calibration file with a name such as *Polyester_1.cd* is more likely to ensure correct usage than *12_09_AB4D.cd*.

Note that when the calibration is generated and used with **RI Analysis**, the measurement software will report a value for each individual page that has been specified. If test calibrations are generated on different pages they must be removed before the calibration is generated.

2.4.16 Calibration Tutorial

2.4.16.1 Introduction

This section illustrates the use of **RI Calibration** in creating calibrations. It takes the user through a step-by-step tutorial in creating a calibration for use with the **RI Analysis** software.

2.4.16.2 How to Produce a Calibration

The following steps are necessary to produce a calibration:

1. Acquire NMR data using RINMR (or alternately using **Easycal** if an appropriate application is available).
2. Specify sample details using the calibration screen.
3. Load in acquired NMR data.
4. Specify ranges for the processing of NMR data (average, extrapolation back to zero).
5. Check calibration curve and modify data ranges where necessary.
6. Generate the calibration file for use with **RI Analysis**.

2.4.16.3 Example of Data Set

The example data set consists of five FID files acquired with RINMR. Four of the samples have a known concentration; the remaining sample is referred to as 'unknown'. For the masses, concentrations and filenames refer to Table 2.2.

2.4.16.4 Attachment of Raw Data Files to a New Calibration Table

First select a page to introduce the data. Next right click on the Value column of the first row of cells. Move the mouse so that the Attach file box is highlighted. A file browser will appear. Attach the first demonstration file (poly.00001) to the first row of cells. Click again on the **Attach data file** button to repeat the process for all five data files.

Next the sample masses and concentrations must be specified. Enter the following details in the mass and concentration columns:

Table 2.2 - Raw data files (.dat) ready for attachment, sample mass and concentration value for each standard*

File Name	Mass (g)	Concentration
poly.00001.RiDat	4.33	935.4
poly.00002.RiDat	4.28	926.2
poly.00003.RiDat	4.28	Unknown
poly.00004.RiDat	4.16	920.6
poly.00005.RiDat	4.18	919.5

The final table after all the data has been entered (Table 2.2) should look like the table displayed in Figure 2.62.

Figure 2.62 - Example of calibration table using the information provided in Table 2.2.

The screenshot shows the RI Calibration software window. At the top is a menu bar with File, Edit, Page, Window, Help. Below the menu is a toolbar with various icons. The main area contains a table with five rows of data. The columns are SampleID, Conc., Mass, Signal, ?Value, Calc, Diff, and File. The data rows are:

SampleID	Conc.	Mass	Signal	?Value	Calc	Diff	File
poly.00001	935.4	4.33	5791.37	* 1337.50	935.34	-0.06	Y
poly.00002	926.2	4.28	8382.99	* 1958.64	926.14	-0.06	Y
poly.00003		4.28	8846.11	2066.85	924.54		Y
poly.00004	920.6	4.16	9425.00	* 2265.63	921.60	1.00	Y
poly.00005	919.5	4.18	10307.75	* 2465.97	918.63	-0.87	Y

Below the table are two input fields: 'Signal_Function' containing 'A' and 'Fitted Equation' containing '6.44E4 - 67.4x'. A green 'Valid' button is next to the fitted equation. At the bottom left are buttons for 'Use Mass' and 'Data Path' (with a checked checkbox). The path 'C:\Program Files\Resonance\RICalib\Data' is displayed.

Load in the data by right clicking on the first cell in the 'Signal' column. A file browser will appear in the default \RICALIB\DATA directory. Select the file 'poly.0001' and left click on the attach button. Left click again to attach the next data file to the second 'Signal' cell (the highlighted cell and the highlighted filename increment automatically when files are saved using the RINMR auto number procedure). Repeat the process until all five demonstration files have been attached.

2.4.16.5 Specification of the Calibration Range

Once the data has been loaded into RI Calibration, select the View NMR Data icon (third icon from the right on the ToolBar) to view the data.

All five data sets should be displayed on the graph, along with two vertical blue lines. These lines define the start and end of the calculation range. All points within the calculation range may be either averaged or fitted to produce a final value that is used with the calibration. Data ranges may be specified in several ways.

Either left click on the graph (after selecting either the Start or End radio button) or enter new ranges in the boxes below the graph. Ensure the following modes are set:

- Method – Average (averages all points in the calculation range to produce a final value).
- X-axis – Point (averages in point domain).
- Calculation – Auto (automatically recalculate, statistics every time a range is altered).

Up to 10 calculation ranges may be specified for use in a calculation; a letter is associated with each range. Ensure that the 'A' calculation range is specified. Enter the following values for the start and end of the A calculation range:

- Start = 50
- End = 100

Once the calculation range has been specified, return to the main

calibration screen and type 'A' in the signal function box.

2.4.16.6 Inspection of the Calibration Graph and Modification of the Data Range

The **Calibration Graph** window shows the data points and the straight line fit. The details of the fit may be found in the **Calibration Statistics** window. It can be seen from the **Calibration Statistics** window that the standard deviation of the measurement is quite large. This may be reduced by optimisation of the calculation ranges. Returning to the NMR Data window, alter the values of the start and end parameters of the A calculation range.

Figure 2.63 – Calibration statistics following the instructions and using the data in Table 2.2

Line Fit Statistics	
	[Fitted] Predicted Format Decimal Places
Number of points	4
Slope	-67.55
Value intercept	64518.59
Conc. intercept	955.14
Correln. coeff.	-1.00
Standard deviation	0.66
Variance	0.44

Use the variation in the calibration graph and the change in the calibration statistics (which automatically update when the start and end ranges vary) to optimise the calculation range. For example, a standard deviation of 0.20 is achievable if the start and end parameters are set to 5 and 10 respectively. When satisfied with the quality of the calibration return to the Main Calibration window.

Select the unknown sample (poly.00003) and enter a 'P' in the '?' column. The calibration will now predict the values of the unknown sample (the green point on the calibration graph) from the other four data points. The concentration value of the unknown sample should be approximately 924.

2.4.16.7 Generation of the Calibration File for Use with RI Analysis

The final step is to generate the calibration file for use with the **RI Analysis** software. Select **Generate Calibration** from the **File** menu of the calibration screen and select the appropriate options (for a full description of the options, refer to section 0). Finally, save the calibration file. This file can now be used with **RI Analysis**.

2.4.17 Statistical Definitions

Correlation coefficient - Linear correlation coefficient r defined by Equation 2.1 where x_i and y_i are the values of the i th data point.

Equation 2.1 - Coefficient of linear correlation

$$r = \frac{\sum (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum (x_i - \bar{x})^2} \sqrt{\sum (y_i - \bar{y})^2}}$$

Standard Deviation - Standard Deviation defined in Equation 2.2 where N is the number of data points and x_j is the j th point.

Equation 2.2 - Definition of standard deviation

$$\sigma = \sqrt{\frac{1}{N-1} \sum (x_j - \bar{x})^2}$$

2.4.18 Keyboard Short Cuts

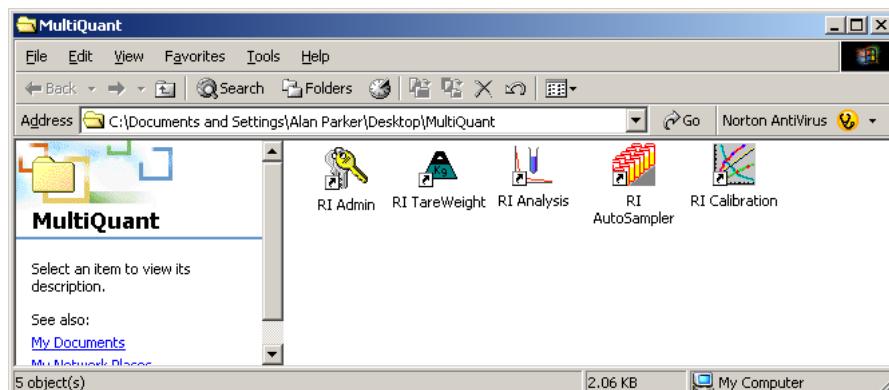
- <ALT>-<F6> Brings an active hidden window to the foreground.
- <ALT>-<F4> Exits the RI Calibration software.

3 Instrument Administration

For this latest version of MultiQuant there has been a significant change in the top level entry software compared with previous versions. In the past MultiQuant was supplied with a tool to produce password protection. This has been expanded for the new version to create an administration tool. This not only controls passwords, but also allows the user to configure the language version of MultiQuant and the format of the output to the printer. The administration tool is called RI Admin and is installed automatically with the rest of the MultiQuant software suite. RI Admin is used in particular with RI Easycal to prevent non-expert users from producing calibration curves without authorisation. By default, the password is set to 'MARAN'.

From Windows Start menu, select RI Admin in the Start > Programs > MultiQuant directory to start the RI Admin tool. Alternatively, access the Change Password tool from the MultiQuant desktop directory (Figure 3.1).

Figure 3.1 - Access to RI Admin facility from the MultiQuant Desktop directory



The Administration tool consists of three tabs in a small dialog box. Each tab deals with one of the separate functions of the tool:

When the tool is first opened, the front tab is that which determines the language version of the software. This is shown in Figure 3.2 below. The language version is chosen using the radio buttons within the frame labelled "Language Files". At present, there is only one language option, English. If this is the only change that is required click on the Exit button to leave the Admin Tool.

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Figure 3.2 - Language Version Tab of the Admin Tool



The second tab deals with changing the passwords used to enter the MultiQuant software. RI Analysis, RI Calibration and EasyCal are all password protected. Passwords from previous versions of MultiQuant may work with the new version but this is not guaranteed. It is best practise to reset passwords following installation of the new version of the software. The password tab is shown in Figure 3.3 below. It consists of three text boxes for password entry, a status frame which displays the current status of the password for the instrument and an OK button to enter any changes that have been made.

First enter the old password in the first entry line, then enter the new password in the second entry line and confirm it in the third entry line (Figure 3.3). Click **OK** to validate and the new password will be acknowledged (Figure 3.4). Passwords are not case sensitive and can be made up of any alphabetic and numeric characters. If the password change that has been implemented is not valid (e.g if the entries in the new and confirm new text boxes do not match) the two text boxes will be coloured red (see **Error! Reference source not found.**) and a message describing the reason for the invalidity in the password change will be displayed in the status frame of the tab. If the password change is the only change required, click on **Exit** to exit the admin tool.

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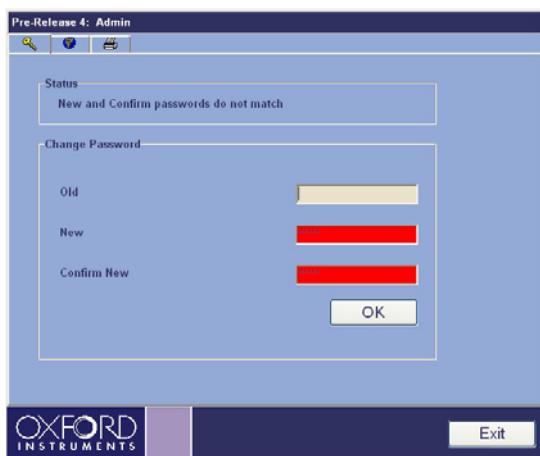
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Figure 3.3 - Change Password facility



Figure 3.4 - Screen to confirm password has been set.



The final tab in the Admin tool is shown in Figure 3.5 below and is used to configure the sample in detector and the output to the printer. As can be seen in Figure 3.5, the screen contains two frames. The Print Orientation frame contains two radio buttons that can be used to set the orientation of any data or graph sent to the printer. The Sample In Detector frame contains a checkbox that determines whether the sample will be automatically detected by the sample in detector to start analysis. This option is only available for instruments fitted with a sample detector.

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Figure 3.5 - Printer Screen in Admin Tool



Once the printer and sample detection options have been set, click on the **Exit** button to exit the RI Admin tool.

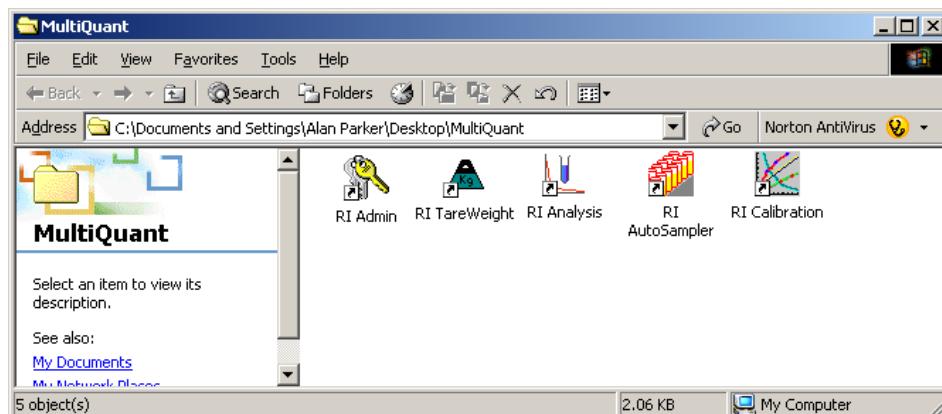
4 Routine Analysis

4.1 Introduction

RI Analysis software has been designed to provide easy handling of routine analyses by basic operators with the appropriate level of control from the laboratory manager. In addition, it reflects some built-in quality control (QC) features of MultiQuant, such as data logging and LIMS interface (*.rilog results log file, send data to RS232 port), caption of customised measurement details (sample ID, sample mass, operator's name, etc.), instrument's tuning and diagnostics. Furthermore, there is no risk of introducing unwanted errors in the calibration while using RI Analysis, as one major concept of MultiQuant software toolbox is to treat calibration and routine analysis as two different processes, using two different software tools. A basic user could not alter the calibration file without generating the calibration file from RI Calibration.

4.2 Starting up RI Analysis

Figure 4.1 - MultiQuant directory with shortcut to RI Analysis and RI Calibration



To start RI Analysis, left click on the Start button in the bottom left hand corner of the Windows screen. Select MultiQuant>RI Analysis from Windows Start menu. It is also possible to set up a shortcut to the RI Analysis software that resides on the Windows desktop (Figure 4.1). To do this, refer to the Windows menu or discuss it with your local system administrator.

4.3 Entering the RI Analysis Software

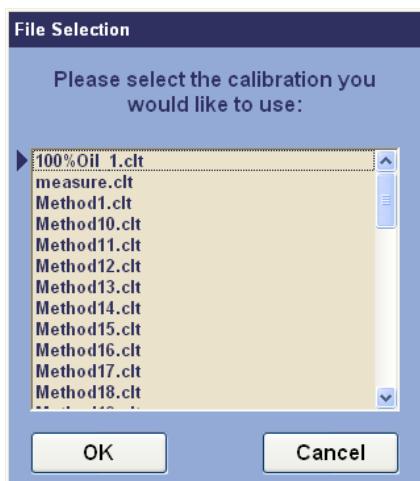
A new feature in the latest version of the RI Analysis software is that entry to the software is password protected. Whichever method you use to activate the RI Analysis software you can only gain entry if a password has been set up for you using the Admin Tool (see chapter 0). When you start the software the password screen will appear and you will need to enter your password (Figure 4.2).

Figure 4.2 - Password Dialog Box for RI Analysis



4.4 Specifying a Calibration

Figure 4.3 - Dialog box to select calibration to be used with RIAnalysis



Click on the calibration to be used using the mouse and then click on the OK button to accept this calibration as the one to be used with RI Analysis Click on **Cancel** to exit the software.

Note: each calibration requires a set of two files *filename.clt*, containing the equation and routine options, and *filename.clb* containing the experimental parameters. Tampering with the name of those files (such as changing the prefix of one of those files) will result in erroneous operation of RI Analysis.

4.5 The RI Analysis Screen

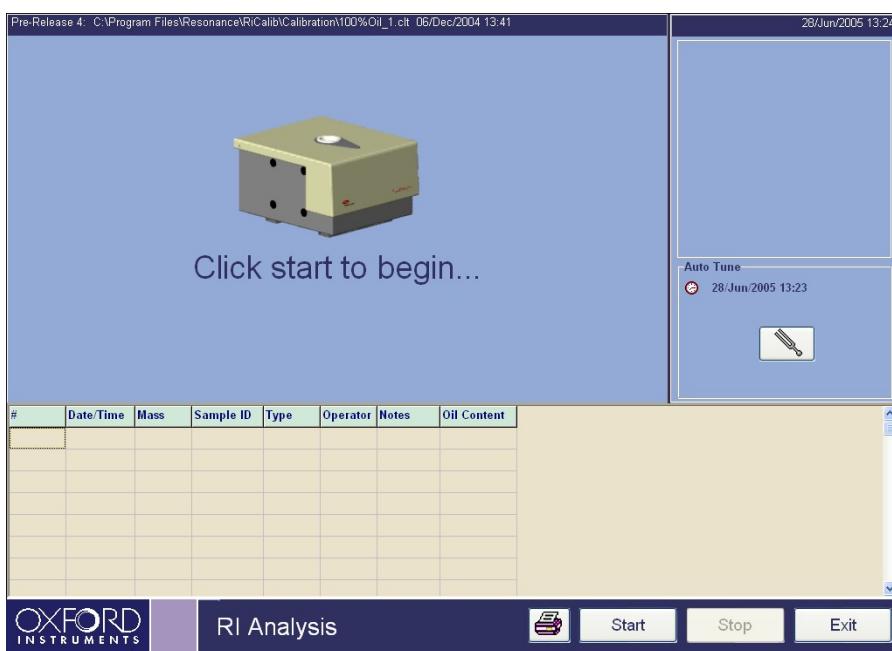
Figure 4.4 - Splashscreen for RIAnalysis Software

Once the calibration has been accepted a splashscreen for the software appears (Figure 4.4)

The splashscreen is followed by the start page of the Analysis software (Figure 4.5).



Figure 4.5 - RI Analysis main screen



Once the calibration is selected the RI Analysis main screen will appear (Figure 4.5). The screen is similar to the main screen of the EasyCal software and consists of three sections. At the top left hand side of the screen is the **status frame** that has a similar function to the same frame in the EasyCal software. It contains a picture of the MARAN Ultra analyser and displays messages to the operator, prompts for information regarding sample masses

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and ID's and reports the progress of current measurements. The central results window (in white) contains a list of all previous measurements made in the current session. The bottom tool bar (**action bar**) offers command button options to start an experiment, stop an experiment, print data and exit RI Analysis.

4.6 Auto-tuning

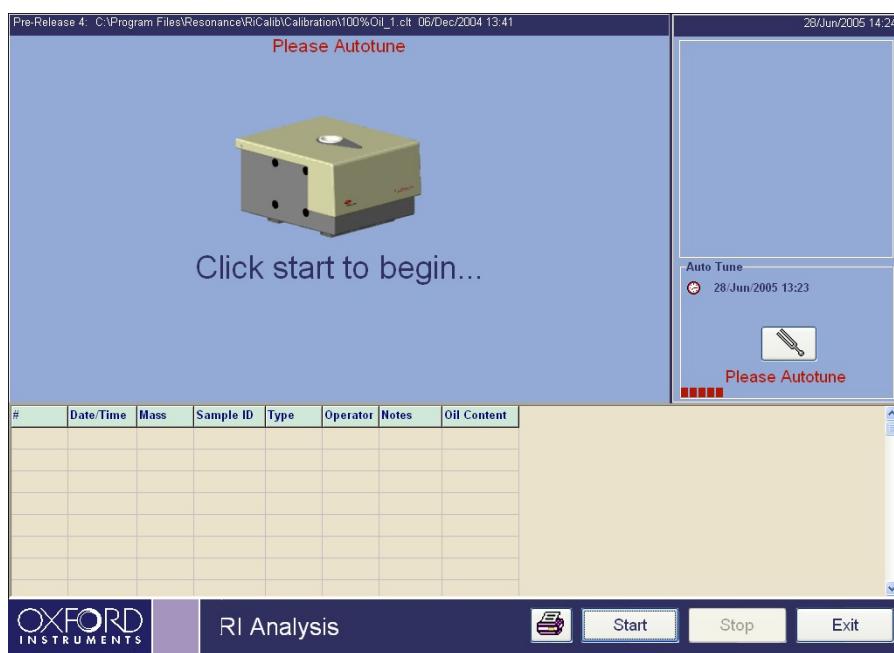
Figure 4.6 – AutoTune Button



Once RI Analysis has been started, an auto tune must be performed to tune the instrument. Click on the AutoTune button (Figure 4.6) and follow the on screen instructions to set the instrument on resonance.

The instrument should be auto tuned at least once per day on the supplied sample. Operators should refer to local guidelines for details on how often AutoTune measurements should be performed. Auto-tune may be followed by a routine diagnostic test that qualifies the good working condition of the instrument. If an auto-tune is not performed for a set period of time the software will prompt the user to perform an auto-tune (Figure 4.7 below). This is voluntary and the warning can be ignored. However, it is recommended that these warnings are heeded for continued good operation of the instrument.

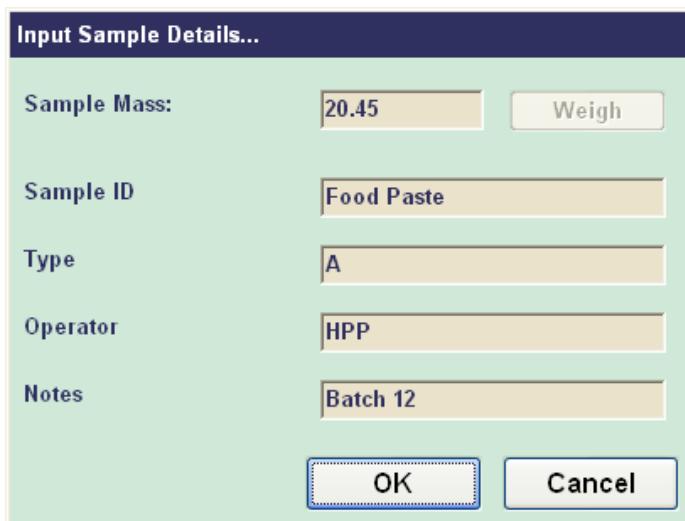
Figure 4.7 - Prompt for auto-tune of instrument



4.7 Routine Analysis

To start an analysis mouse click on the Start Analysis button in the task bar of the screen. A dialog box will appear to prompt the user to enter the sample reference information (Figure 4.8). The reference information that needs to be entered includes the sample name and mass, any information on the type of sample if required, the operator name and user notes if required. Apart from the sample name and mass, the other entries are optional. The sample can be weighed at this time using a balance attached to the instrument PC (Appendix A.2 Balance Settings). Once the required information has been entered click on the **OK** button to start the analysis. If a sample detector is fitted with the instrument, the software will then prompt for the sample if it has not been placed in the magnet. Once the sample is in the magnet the analysis will commence.

Figure 4.8 – Dialog Box showing Sample Information



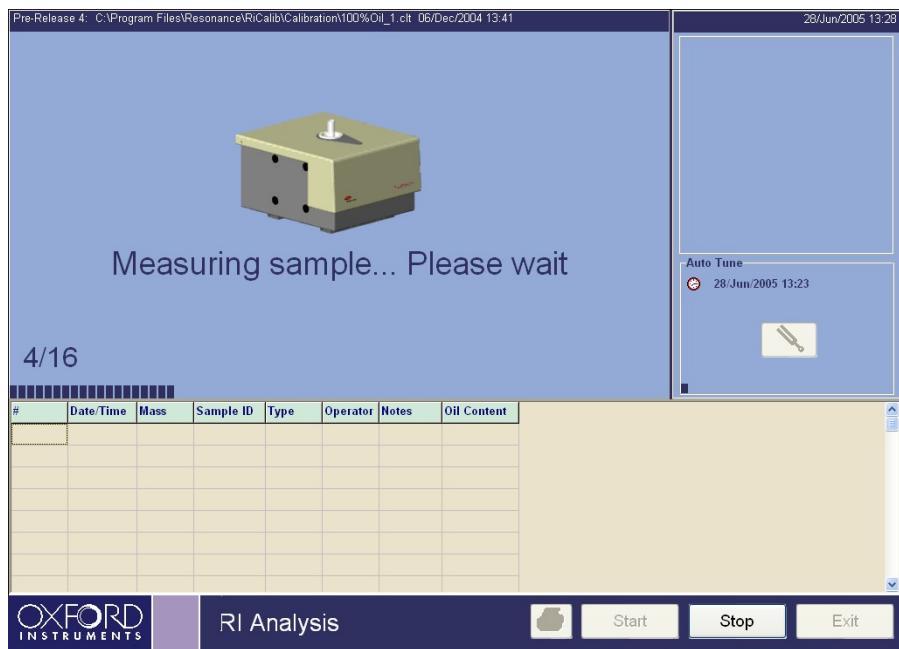
The software displays the message **Measuring sample ... Please wait** (Figure 4.9) whilst the analysis is occurring. The progress of the analysis can be monitored using the blue progress bar on the screen (Figure 4.9) and also by monitoring the scan count placed just above this. The scan count reads **4 / 16** in Figure 4.9 indicating that 4 scans have been performed from a total of 16 scans in the experiment.

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Figure 4.9 – RI Analysis main screen



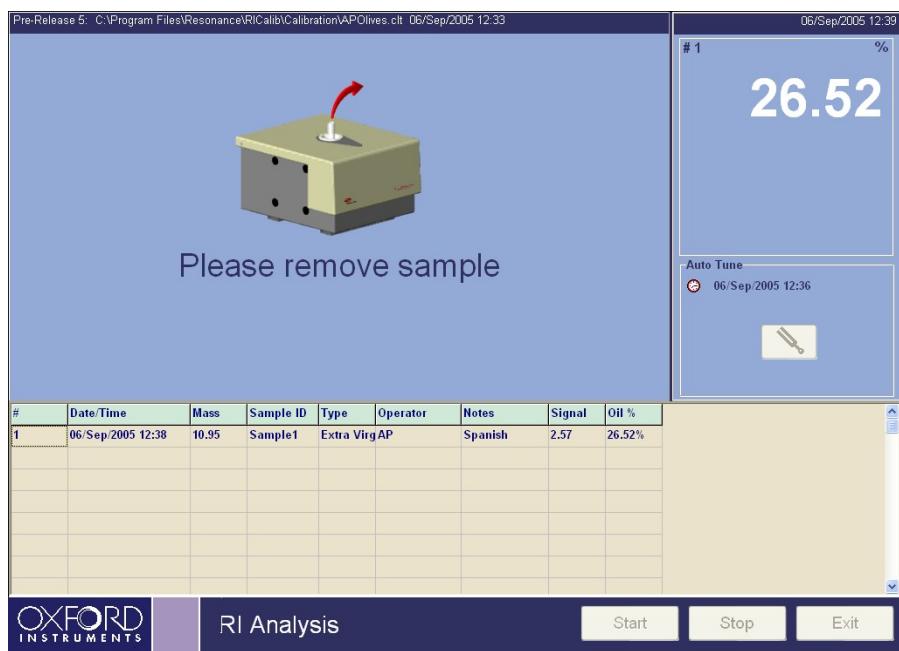
Once the measurement has finished the operator will be prompted to remove the sample (Figure 4.10). The result will be displayed in a large font in the top right hand corner of the main screen and will also be added to the results table at the bottom of the screen. Once the sample has been removed, a message will appear in the status frame asking the user if they wish to analyse another sample. If another sample is to be analysed click on the **Yes** button in the action bar. If not, click on the **Exit** button.

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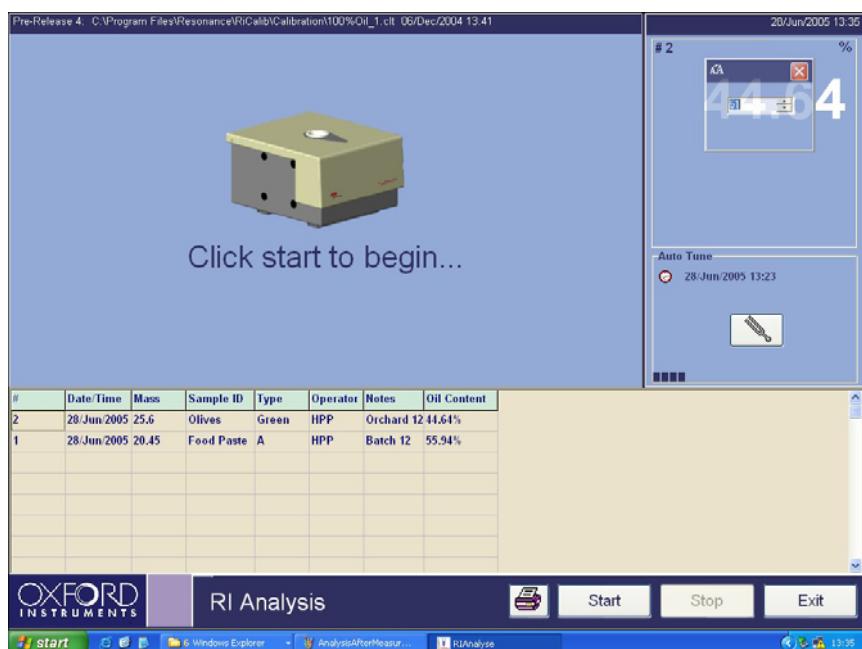
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Figure 4.10 – RI Analysis main screen with prompt to remove the sample



Finally the font size of the results display can be altered by right mouse clicking on the result. This will create a small dialog box that can be used to adjust the display font (Figure 4.11).

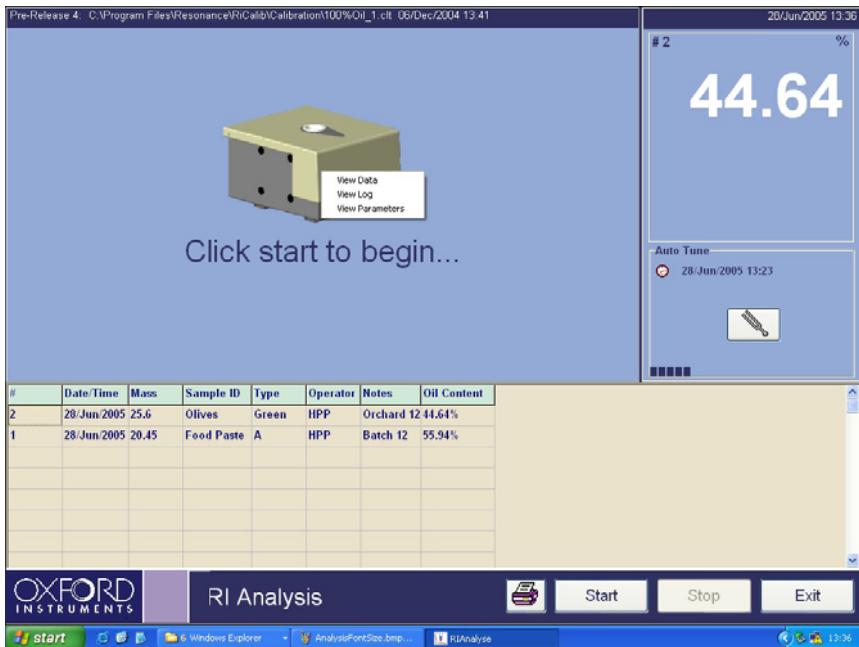
Figure 4.11 - RI Analysis main screen showing font size adjustment



4.8 Configuration Options

The configuration options are provided for expert users to examine the NMR data, NMR parameters and the current calibration file settings. It is not necessary for non-expert users to have familiarity with the configuration options. The configuration options menu may be accessed by right clicking with the mouse on the instrument magnet icon in the centre of the screen.

Figure 4.12 - Main Screen of software showing menu options following right mouse click of Maran

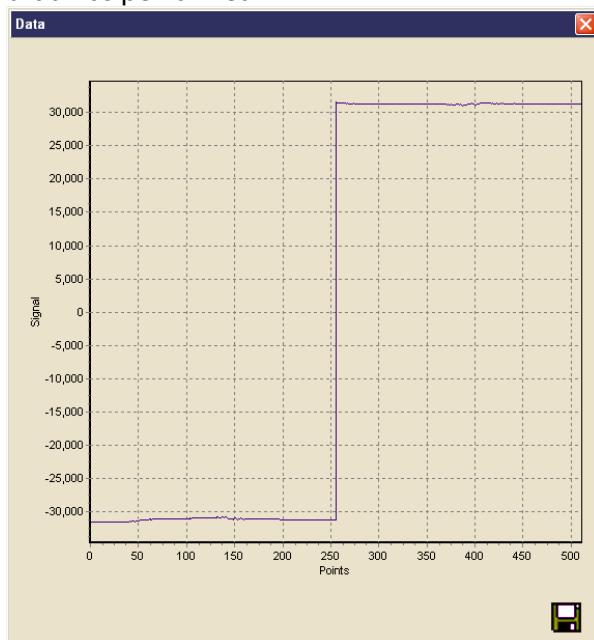


Three options are available from the menu produced by right clicking on the Maran icon: View data, view log and view parameters

4.8.1 View Data

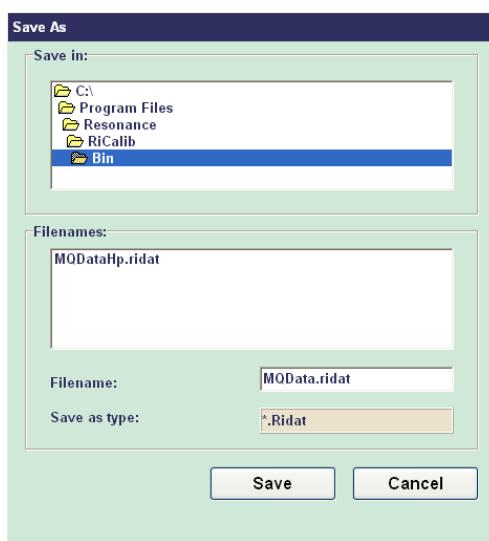
The View data window displays the NMR data from the last analysis that was performed.

Figure 4.13 - Display of Raw Data



The data can be saved by clicking on the save data icon in the bottom right hand corner of the data window (Figure 4.13). This will generate another explorer type window (Figure 4.14) which will enable a filename and path for the data to be saved in to be entered. The data is stored in ASCII format.

Figure 4.14 - Save data dialog box



4.8.2 View Parameters

The Instrument Parameters section displays a list of the current NMR acquisition parameters.

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Figure 4.15 – Parameters

Parameters	
Slope	39.286
Intercept	1.049
Aquisition Start Pt	354
Aquisition End Pt	446
Analysis Method	Average
Sequence	fid_hahn
DW (us)	0.20
Dead1 (us)	10.00
Dead2 (us)	2.80
SI	256
HS	16
RD (s)	2.00
RG (%)	8.00
SF (Hz)	23400000.00
O1 (Hz)	-81567.46
FW (Hz)	1000000.0
P90 (us)	8.10
P180 (us)	16.20
Tau (ms)	2.00
Tuning Scans	2
Tuning Gain (%)	0.30
Data Smoothed?	No
SMP	0

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4.8.3 View Log

This menu displays a log of recent analyses that have been made on the instrument (Figure 4.16).

Figure 4.16 - Table

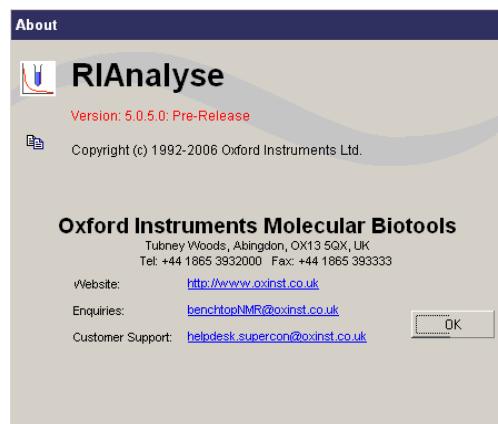
#	Date	Time	Mass	Sample	Operator	Team	Line	Result	Result 2	Result 3	Result 4	01	Signal
1	28/06/05	13:27:53	10	Food P/A		HPP	Batch 1	114.46	No Anal	No Anal	No Anal	81567.44497.60	
6	07/12/04	12:02:50	9.602	R1				9.95	No Anal	No Anal	No Anal	87655.1391.77	
5	07/12/04	12:01:55	9.631	O2				24.85	No Anal	No Anal	No Anal	87655.1977.47	
4	07/12/04	12:01:02	9.656	O1				24.81	No Anal	No Anal	No Anal	87655.1975.86	
3	07/12/04	12:00:04	9.497	G2				39.73	No Anal	No Anal	No Anal	87655.11561.89	
2	07/12/04	11:58:52	9.436	G1				39.73	No Anal	No Anal	No Anal	87655.11561.78	
1	07/12/04	11:57:37	7.835	100%Oil				99.00	No Anal	No Anal	No Anal	87655.13890.45	
27	06/12/04	14:48:42	7.835	100%oil				99.55	No Anal	No Anal	No Anal	86068.93912.20	
26	06/12/04	14:47:42	9.631	O2				24.95	No Anal	No Anal	No Anal	86068.9981.40	
25	06/12/04	14:46:42	9.497	G2				39.88	No Anal	No Anal	No Anal	86068.91567.97	
24	06/12/04	14:45:44	9.602	R1				9.92	No Anal	No Anal	No Anal	86068.9390.90	
23	06/12/04	14:44:49	9.656	O1				24.93	No Anal	No Anal	No Anal	86068.9980.28	
22	06/12/04	14:43:50	9.436	40%				39.88	No Anal	No Anal	No Anal	86068.91567.81	
21	06/12/04	14:22:32	7.835	100%Oil				99.68	No Anal	No Anal	No Anal	85734.83916.97	
20	06/12/04	14:21:16	9.631	O2				25.03	No Anal	No Anal	No Anal	85734.8984.56	
19	06/12/04	14:20:13	9.497	G2				39.95	No Anal	No Anal	No Anal	85734.81570.72	
18	06/12/04	14:19:15	9.602	R1				9.98	No Anal	No Anal	No Anal	85734.8393.09	

Close

4.8.4 About

This option provides information on Oxford Instruments' contact details and the version number of the software (Figure 4.17). Please quote the version number when contacting OIMBL.

Figure 4.17 - RIAnalyse Help page



5 Use of an Autosampler for Routine Analysis

5.1 Introduction

The use of an autosampler greatly increases the throughput of NMR analyses by completely removing human intervention (e.g. measurement batch of up to 120 tubes of 26mm diameter for a complete set-up). For series of routine measurements, samples often have to be weighed before the analysis can take place. To achieve this, the MARAN Ultra is set up together with a multi-rack Duratech autosampler system equipped with the option of a barcode reader and a Mettler balance (both barcode reader and balance connections can be disabled or enabled directly from RI Autosampler software).

Programming of the autosampler is achieved on the MARAN Ultra using **RI Autosampler** software that is part of **MultiQuant** software suite for routine analysis. Measurement calibrations are first produced using **RI Calibration** (cf: MultiQuant), then loaded in the table environment of **RI Autosampler** software (Figure 5.1). For use of the system with a balance, **RI Autosampler** software offers a **Tareweight** text utility that loads a series of pre-defined sample weights against a sample ID (i.e. barcode, tube position or user-defined). To help with the process of taring the weight of empty tubes, **MultiQuant** software suite also includes **RI Tareweight** software that is designed specifically to create a Tareweight file automatically. Finally, **RI Autosampler** offers a choice of multiple calibrations and programmed autotuning steps between analyses.

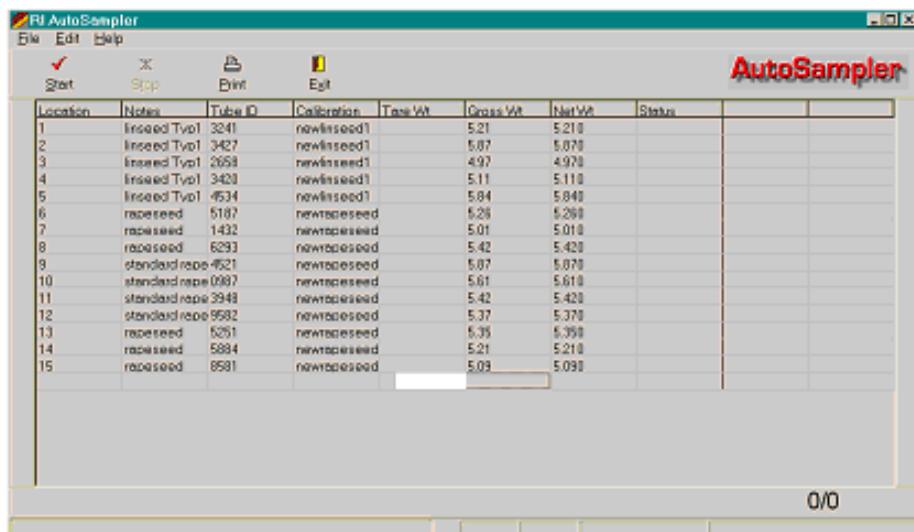
Note: if no tube is found in a rack position during the course of an autosampler run, the *no tube* message will appear in the Status column and the software will abort the autosampler run, assuming by default that an error in the rack position has been made.

5.2 Overview of RI Autosampler Software

Note: This section is specific to the use of RI autosampler software with a Duratech autosampler.

MultiQuant is supplied with RI Autosampler, a Routine analysis table environment designed to complement RI Analysis with the optional use of an autosampler. From the Start button under MS Windows, select Programs> MultiQuant > RI Autosampler or double-click RI Autosampler inside the MultiQuant desktop folder. The main window of RI autosampler then appears on the screen (Figure 5.1). RI autosampler can be used to analyse sample batches with or without autosampler. To find out how to set up the autosampler operation from RI Autosampler, refer to Appendix A.3 Autosampler Settings.

Figure 5.1 – Main screen of RI Autosampler

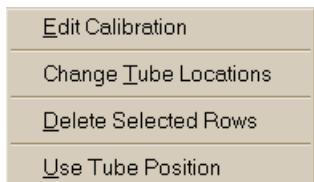


RI Autosampler contains a menu with various options (File, Edit or Help), an icon bar and an analysis table. As RI Autosampler is designed to facilitate sample batch measurements, it requires a table of sample information in the preparation for routine analysis.

5.2.1 Analysis Table

Location	The tube Location is assigned according to the Oxford Instruments' rack positioning scheme specific of the autosampler configuration (it is read from the file as.tab provided with the autosampler). The location of the tube is proposed by RI autosampler as part of its measurement scheme although it can be edited manually to allow multiple repeats of the same sample.
Notes	The Notes should contain information relevant only to the sample measured.
Tube ID	Provides the sample identifier as written on the tube. The tube identifier is picked up from the barcode reader when it is used. The sample identifier can be edited manually in the absence of a barcode reader.
Calibration file	The calibration file must be specified to satisfy the measurement of a given sample. Calibration files are produced from RI Calibration using adequate calibration standards.
Tare wt	The tube Tare weight is obtained from the file Tare.tab when an external balance is used to provide the direct transfer of the sample mass to RI software. This column is greyed out in absence of an external balance.
Gross wt	The Gross wt is the total weight sample+tube if an external balance is used. It becomes the Net sample weight in the absence of an external balance connection.
Net wt	The sample weight is the result used by RI autosampler for the calculation of NMR Signal/Mass.

Figure 5.2 - Right-click menu of the analysis table



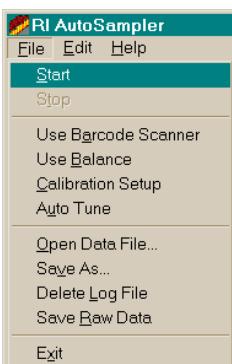
The analysis table also provides equivalent shortcuts by right-clicking the mouse on the column header. The options are shown in Figure 5.2. By clicking on the column header, the option **Edit calibration** allows changing the name of the calibration file. The option **Change Tube Locations** allows changing the tube location for one or more successive rows. The option **Delete Selected Rows** allows the deletion of a series of measurements in the sample batch. By right clicking on the Tube ID header, the option **Use tube position** forces the tube position to be used as sample ID.

5.2.2 Menu Options

The menu options are summarised under the headings File, Edit and Help.

5.2.2.1

Figure 5.3 – File menu



File

The file menu allows the start and end of analysis, the activation of various options (**Use barcode reader**, **Use balance**, **Calibration Setup** for each row, and **autotune** between analyses), and the management of data files and data logging (**Open Data File...**, **Save As** data file, **Delete Log File**, **Save Raw Data**). Select **Exit** to exit RI Autosampler.

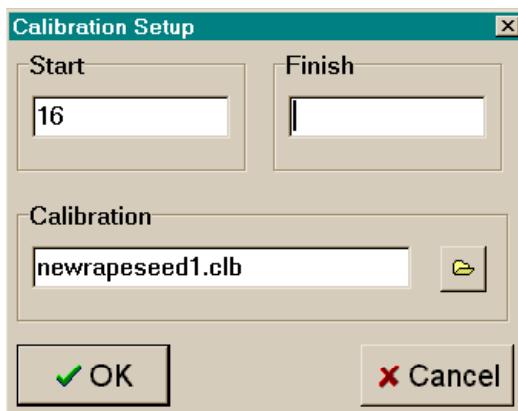
Simply tick on the relevant option to activate Barcode reading and Balance communication. When the tick is not visible against the option, then the option is not active. To allocate a calibration file against a row or a series of rows defined by their tube position, click on **Calibration Setup** for the **Calibration Setup** menu to pop up (Figure 5.). In the **Calibration Setup** menu, choose the start and end of the tube location and select the calibration file (*.clb) to be used for the measurement in each row.

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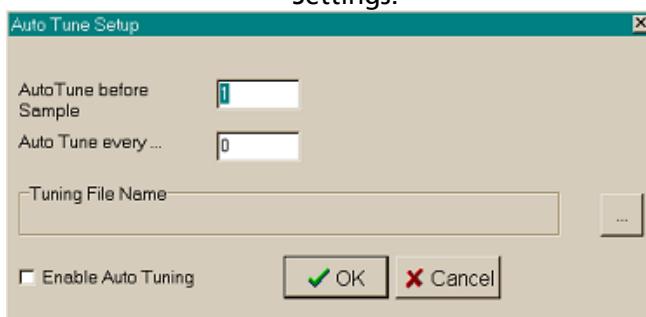
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Figure 5.4 - Calibration Set-up menu



To adjust the tuning of the instrument, select the option Autotune to bring up the **Autotune Setup** menu (Figure 5.4). Tick the **Enable Auto Tuning** box to activate the autotuning facility. In the **AutoTune before Sample** entry box, select the row to define the first Autotune routine in the series, knowing that Autotune always operates before the measurement for the row. Choose the interval between Autotune routines by entering the spacing between rows in the **Auto Tune every...** entry box. Choose the tuning file name to be used by clicking on the ... box. The autotuning sample has a specific tube location shown in Figure A.1 and A.2 of Appendix A.3 – Autosampler Settings.

Figure 5.5 – Auto Tune Setup menu

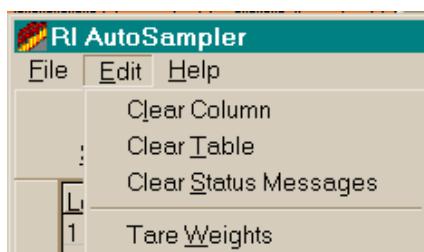


Select **Open Data file** to open a previous table. Select **Save As...** to save the current table for later use. As for RI Analysis and according to the definition in the calibration file, a log file is produced and updated automatically after each analysis. Select **Delete log file** to empty the data log file. Select **Save Raw Data** to enable subsequent reprocessing of the raw data rather than keep only the final result.

5.2.2.2

Edit

Figure 5.6 - RI Autosampler
Edit menu



The series of options available under **Edit** is shown in Figure 5.6. **Clear Column** can be used to remove the content of a whole column. **Clear Table** allows reducing the content of the whole table to a blank sheet. **Clear Status** messages simply deletes the messages from the **Status** column. **Tareweight** activates the text editor as shown in Figure 5.7. The Tare.tab file should contain a series of tare weights associated with each tube. When a large series of tubes have to be tared before batch analysis, it allows a whole list to be read in one go instead of typing each weight individually for each row. The Tare.tab file can be created automatically using the **RI Tareweight** software tool offered as part of the MultiQuant software suite.

Figure 5.7 - Tare Weight text editor



Important: The tube position should not be assumed as the number of the RI Autosampler row in the absence of a bar code reader; the tube position or ID must be defined from the tareweight file. If the tareweight file does not contain the tube position or ID (e.g., in the absence of a barcode reader), then the mass of the tube filled with sample (Gross Wt) will be assumed as the sample mass (Net Wt).

5.2.2.3

Help

Select **Help** to find out how to contact Oxford Instruments.

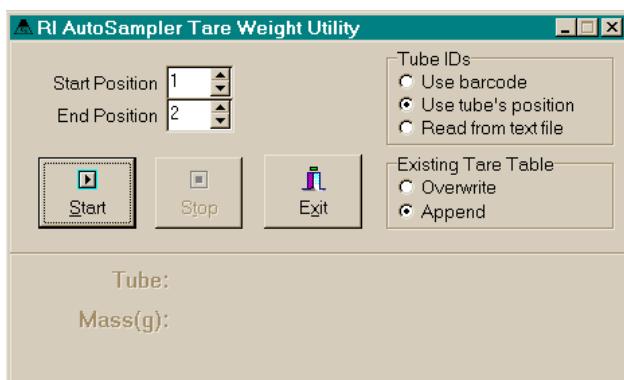
5.2.3 Icons

RI autosampler offers additional options accessible by clicking on the icon bar. As an alternative to the same option in the **File** menu, **Start** icon can be clicked to start analysis. To stop analysis, click on the **Stop** icon. Click on **Print** icon to activate the Print manager and produce a print out of the Analysis table. Select **Exit** to exit RI Autosampler.

5.3 RI Tareweight

To activate **RI TareWeight**, select the option from the Start Menu of MS Windows under Programs < MultiQuant, or double click on **RI TareWeight** from MultiQuant desktop folder. **RI TareWeight** is used to predefine the **Tare.tab** text file readable under **RI Autosampler** software. Using a Duratech autosampler and providing the autosampler configuration file **as.tab**, **RI Tareweight** produces the tare mass of each tube against an identifier (Barcode or tube position) and transfers the result into a **tab** list. When activating RI Tareweight tool, the window shown in Figure 5.8 appears on the screen.

Figure 5.8 - RI Tare Weight menu



In the RI Tareweight menu, the **Start position** and **End position** entry box define the tube position encompassing the tubes to be tared. Under the **Tube IDs** heading, the tare mass can be associated either with a barcode, a tube position or the tube identifier as read from the Tareweight text file, by simply activating the corresponding radio button.

In the event a bar code reader is fitted on the autosampler, select the **Use Barcode** radio button. Before proceeding any further, check the initialisation file setting (cf: Appendix A1) for the software to communicate with the bar code reader.

In the event no bar code reader is available, it is possible to use the tube position for taring the weight of each tube in a series. Select the **Use Tube's position** radio button. Remember that each tube will have to be used in exactly the same position during an autosampler run. Alternatively, the tube IDs may be read from a text file (**Read from Text File** radio button).

Under the **Existing Table** heading, the option **Overwrite** is selected to create a new **Tare.tab** file by deleting any previous record. The option **Append** is used to add more tare weights; however, **RI Tareweight** does check that the same **Tube ID** is not associated with two tare masses. Click on the **Start** button to choose the destination of the tab file

Note: the file must be called **Tare.tab** and be located under the **Program files/Resonance/Ricalib/bin** directory) before starting the Tareweight process (Figure 5.9). If the option **Read from text file** has been selected, then the file manager will also ask for the text file name before starting the tareweight process (Figure 5.10). Finally, the **Stop** button is used only to abort the **Tareweight** process.

Figure 5.9 - Save Tare Weights
File... manager

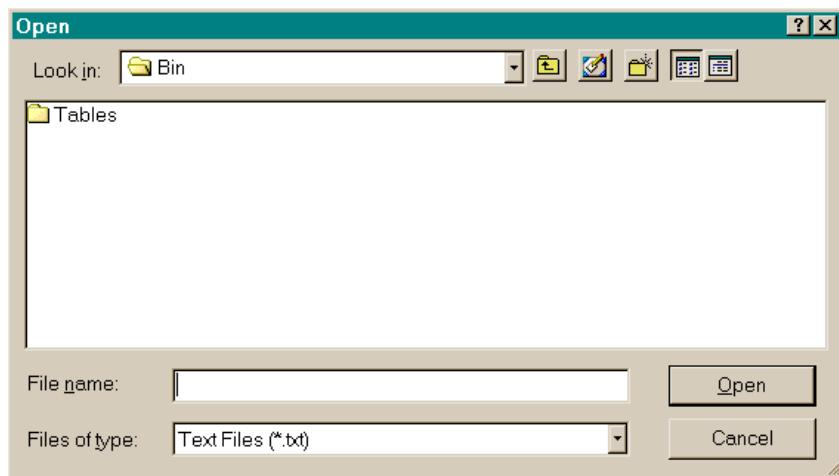


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Figure 5.10 - Open [text file] manager



Appendix A Interface and connections

A.1 Calibration Settings

- In RICALIB.ini, section [NMR data] should include an acceptable tolerance limit O1 tolerance to allow retuning and therefore a change of the O1 value between calibration measurements, if the method requires frequency retuning between individual calibration measurements.
- RICALIB.ini
- Location: Program files/Resonance/Ricalib/bin/RINMR.ini
- [NMRData]
- O1 Tolerance=20000

A.2 Balance Settings

- Please ensure that the interface menu of the Mettler balance is correctly set-up for external communication and that the interface cable is connected to an appropriate serial port in the back of the PC (port 2 recommended). Consult RI instruction sheet IS001 for further information. The initialisation file RINMR.ini located under Program files/Resonance/RINMR/bin directory is needed to contain the following information for the balance to communicate externally with RI Analysis:
- In RINMR.ini
- File location: Program files/Resonance/RINMR/bin
- In the [hardware] section, choose the correct BalancePort (1, 2 etc.) corresponding to the PC serial port connector to the RS232C interface cable from the balance. BalanceType must be set to Mettler.
- [Hardware]
- BalancePort=2
- BalanceType=METTLER

A.3 Autosampler Settings

- To ensure proper recognition of the autosampler commands, specific options should be inserted in the following initialisation (*.ini) files.
 - For all standard operations of the autosampler functionalities from RINMR, the command options are defined in the RINMR.ini file. In the [AS Setup] section of RINMR.ini, ReadBarcode is set to Yes if a barcode reader is likely to be used, No to indicate the absence of a barcode reader. Balance must be a METTLER BD type balance fitted with RS232C interface (cf: Appendix A.2 for installation). Autosampler is usually set-up to the PC RS232C serial link, port 1. For this reason, the balance PC serial link should be set to port 2. Check the PC installation manual for the identification of each serial port.
 - When RI Autosampler software is used, the command options predefined in the file Autosamp.ini override the options set in RINMR.ini. In the [AS Setup] section of AUTOSAMP.ini, various options are decided for use with the Autosampler. Set Usebalance to Yes to enable the use of the external balance with the autosampler, No to disable this option. Set Readbarcode to Yes to enable the use of the bar code reader with the autosampler, No to disable this option. For the option BalancePort, choose the correct balance port (1, 2 etc.) to connect the balance to the allocated serial port. BalanceType must be set to Mettler.
 - Examples of standard set-up:
- a) Duratech autosampler with bar code reader and Mettler balance.
- RINMR.ini
- Location: Program files/Resonance/Rinmr/bin/RINMR.ini

- [Hardware]
- HardwareType=3
- LanaID=0
- Lana=3
- Usebalance=YES
- balanceport=2
- balancetype=METTLER
- {...}
- [AS Setup]
- UseBalance=Yes
- ReadBarcode=Yes
- BalancePort=2
- BalanceType=METTLER

- AUTOSAMP.ini
- Location: Program files/Resonance/Ricalib/bin/AUTOSAMP.ini

- [AS Setup]
- ReadBarcode=YES
- UseBalance=YES
- MassDecimalPlaces=2
- autosamplerport=1
- balanceport=2
- balancetype=METTLER
- ASType=1
- XYSpeed=80
- ZSpeed=40
- ResultCols=2
- SaveDir=C:\Program Files\Resonance\...
- RawData=0

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- Auto=1
- AutoFirst=1
- AutoEvery=1
- RawSaveDir=C:\Program Files\Resonance\Rinmr\DATA\...
- AutoMulFile=C:\Program Files\Resonance\RICalib\Bin\...
- ResultCols=2

b) Duratech autosampler without bar code reader, but with a Mettler balance.

- RINMR.ini
- Location: Program files/Resonance/Rinmr/bin/RINMR.ini
- [Hardware]
- HardwareType=3
- LanaID=0
- Lana=3
- Usebalance=YES
- balanceport=2
- balancetype=METTLER
- {...}
- [AS Setup]
- UseBalance=YES
- ReadBarcode=NO
- BalancePort=2
- BalanceType=METTLER
- AUTOSAMP.ini
- Location: Program files/Resonance/Ricalib/bin/AUTOSAMP.ini
- [AS Setup]
- ReadBarcode=NO
-
- UseBalance=YES
- MassDecimalPlaces=2
- autosamplerport=1
- balanceport=2
- balancetype=METTLER
- ASType=1
- XYSpeed=80
- ZSpeed=40

resultcols=2

Oxford Instruments Molecular Biotools Ltd., Tubney Woods,
Abingdon, Oxfordshire, OX13 5QX, United Kingdom SaveDir=C:\Program Files\Resonance\...
Tel: +44 1865 393 200. Fax: +44 1865 393 333. <http://www.oxford-instruments.com>

- rawData=0
- Auto=1
- AutoFirst=1



- MassDecimalPlaces=2
- autosamplerport=1
- balanceport=1
- balancetype=METTLER
- ASType=1
- XYSpeed=1
- Zspeed=40
- ResultCols=2
- SaveDir=C:\Program Files\Resonance\...
- RawData=0
- Auto=1
- AutoFirst=1
- AutoEvery=1
- RawSaveDir=C:\Program Files\Resonance\Rinmr\DATA\...
- AutoMulFile=C:\Program Files\Resonance\RICalib\Bin\...
- ResultCols=2

- **Note:** the options XYSpeed and Zspeed are set to values that suit the particular Duratech autosampler configuration. The values of XYSpeed=80 and Zspeed=40 are realistic examples.
- Finally, to operate the autosampler, MultiQuant must be provided with a specific autosampler configuration file **as.tab** that translates the Duratech tube position into the OIMBL order code (Figure A.1 and Figure A.2, overleaf, for the example of a 26mm diameter tube rack) and should have been provided together with the autosampler. Note that the reserved position of the auto-tuning sample is **3** of rack **0**.

Here is the content of a typical **as.tab** conversion file (four racks of 32 positions):

0	3
0	7
0	11
0	15
0	19
0	23
0	27
0	31
0	2
0	6
0	10
0	14
0	18
0	22
0	26
0	30
0	1
0	5
0	9
0	13
0	17
0	21
0	25
0	29
0	0
0	4
0	8
0	12
0	16
0	20
0	24
0	28
1	3
...	

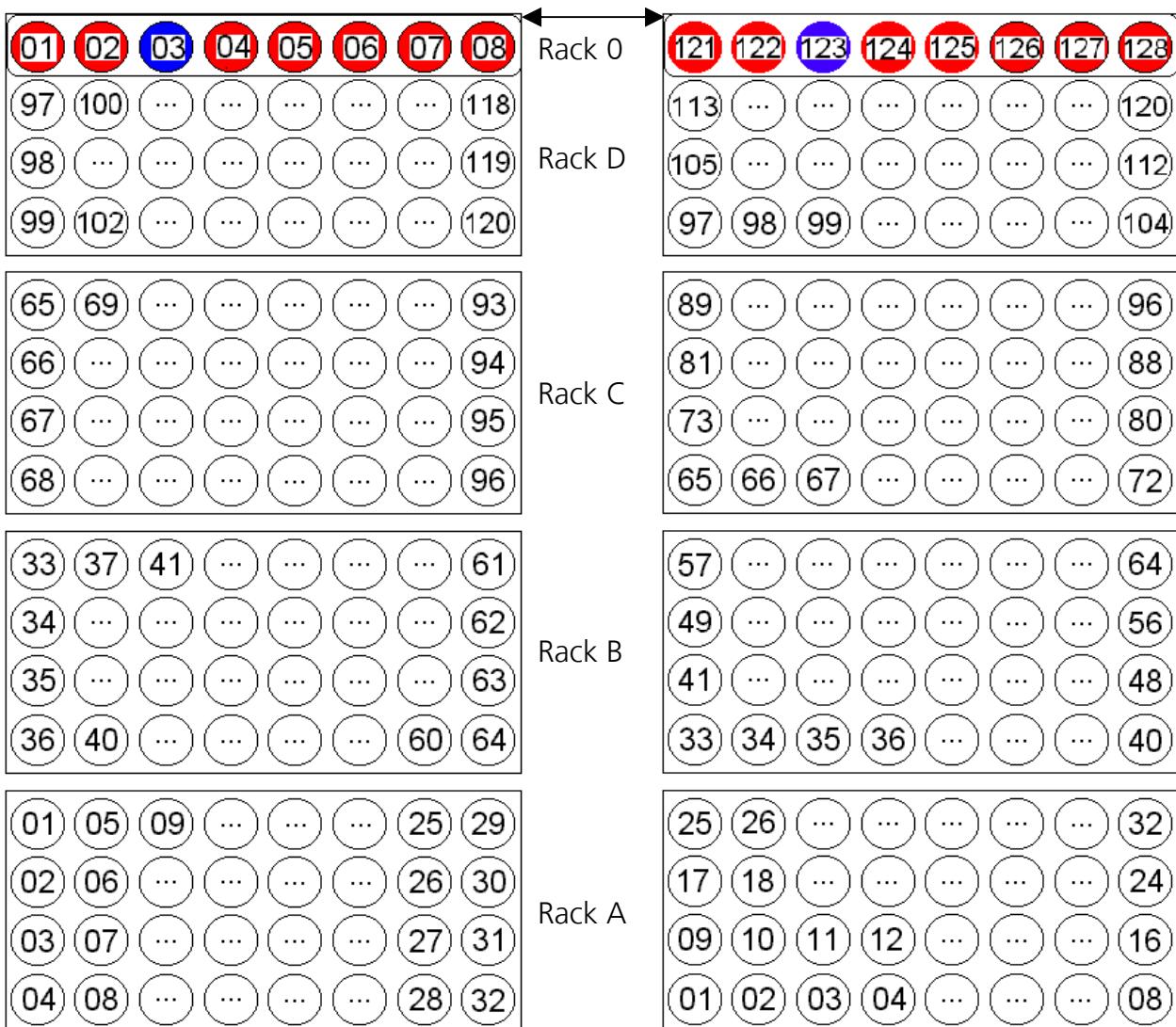


Figure A.1 – Duratech tube numbering for 26mm diameter tube racks

Figure A.2 – OIMBL tube numbering for 26mm diameter tube racks