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BIACORE® probe Handbook

Edition April 1997

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BIACORE®probe Handbook, edition April 1997 (version AA)

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Biacore AB reserves the right to make changes to the design and specification of BIACORE®probe and to the content of the Handbook without prior notice.

Warranty

Biacore AB guarantees that the product delivered has been thoroughly tested to ensure that it meets the published specifications. The warranty included in the conditions of delivery is valid only if the product has been installed and used according to the instructions supplied by Biacore AB.

The technology in this product was developed in collaboration with EBI Sensors Inc., who were in part supported by the U.S. Defense Advanced Research Projects Agency (contract #DAAL01-95-C-3503).

Read the manual before using the product.

An understanding of the English language is required.

Text and layout: Biacore AB

Illustrations: LeBlanc Illustrations, Stockholm, Sweden and Biacore AB

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Important user information

Safety information

Before operating or maintaining the system, **you must read the handbook.** Follow the instructions provided to avoid personal injuries or damage to the equipment. Do not use the equipment in any other way than described in this manual.



Warnings and cautions are marked in the manual with the warning symbol shown here. Be sure not to proceed until the instructions are clearly understood and all stated conditions are met.

Warnings
Warnings call attention to the necessity of following an instruction in detail to avoid personal injury and/or damage to the product or other equipment.

Cautions
Cautions call attention to instructions or conditions that shall be followed to avoid damage to the product or other equipment.

Conformance with standards

BIACORE®probe meets the following directives:

- 89/336/EEC, European directive concerning electromagnetic compatibility.
- 73/23/EEC, European directive concerning low voltage devices.

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1. Introduction

BIACORE®probe is designed for rapid routine determination of the presence and active concentration of biomolecules in solutions, at any stage from expression and extraction to final purification. Measurement is based on the ability of the biomolecule in question (the *analyte)* to bind to a chosen interactant (the *detecting molecule*). No label is required. Operation is simple and each measurement takes typically less than 5 minutes, making BIACORE®probe well suited to method development and other application areas where the design of the next step in a process is directly dependent on the results of the previous step. Approximately 100 µl of sample is required, and the measurement is non-invasive so that sample can normally be used for further processing.

The detection principle exploits the optical phenomenon of surface plasmon resonance (SPR), which measures the surface concentration of solute at a gold-liquid interface (see Appendix B for a technical description). In BIACORE®probe, SPR occurs at the gold-coated tip of an optical fibre (the *sensor probe*) coated with detecting molecule. When analyte binds to the detecting molecule, an SPR response is measured which is a direct measure of the amount of functionally active analyte in the sample. ("Functional activity" is defined in terms of the ability of the analyte to bind to the detecting molecule – BIACORE®probe will not detect analyte molecules which have lost this ability.) Typical detecting molecules are high affinity antibodies for specific antigens, although other molecules may be appropriate in specific cases.

Once the detecting molecule has been attached to the sensor probe, determining the presence or concentration of analyte with BIACORE®probe involves four simple steps (Figure 1-1):

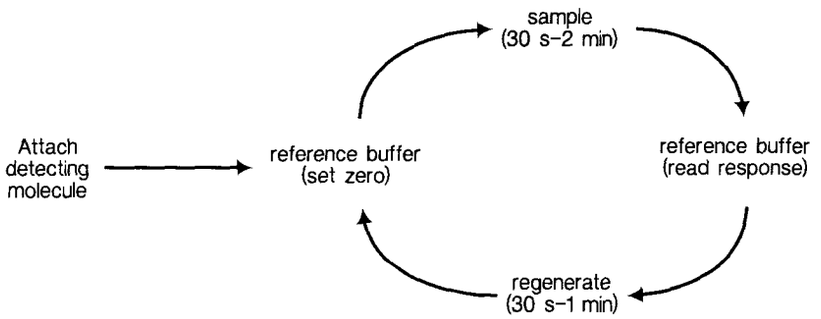


Figure 1-1. *Basic analysis cycle with BIACORE® probe.*

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1. Set a zero response with the sensor probe in a suitable reference buffer.

2. Bring the sample into contact with the sensor probe incubate for 30 seconds – 2 minutes.

3. Return the sensor probe to reference buffer and read the response (which is a measure of the amount of analyte bound to the sensor probe).

4. Incubate the sensor probe with regenerating solution for 30 seconds – 1 minute to remove bound analyte in preparation for the next measurement. This step can be skipped if no analyte has bound.

The detection principle used in BIACORE®probe offers major advantages over conventional methods for biospecific determination of molecules such as ELISA:

- BIACORE®probe uses a label-free technology: neither the analyte nor the detecting molecule needs to be labelled, saving time, effort and money. In addition, all steps in an assay are revealed with the same detection principle, providing valuable intra-assay controls (e.g. attachment of the detecting molecule to the sensor probe is monitored in the same way as binding of analyte).

- BIACORE®probe uses a non-invasive technology: since the light used to generate the response does not penetrate the sample, measurements can be made on turbid or opaque samples (e.g. cell culture supernatants, fermentation broth, crude cell extracts).

- BIACORE®probe uses an interactive technology: each measurement takes typically less than 5 minutes, so that decisions can be made on the basis of each measurement (e.g. chromatogram fractions can be rapidly scanned to localize the active peak for immediate follow-up processing).

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2. Description

BIACORE®probe consists of the following main components (Figure 2-1):

- the detection unit with touch screen display
- the sensor probe
- the optocable connecting the sensor probe to the detection unit
- the pipette which serves to bring the sample to the sensor probe

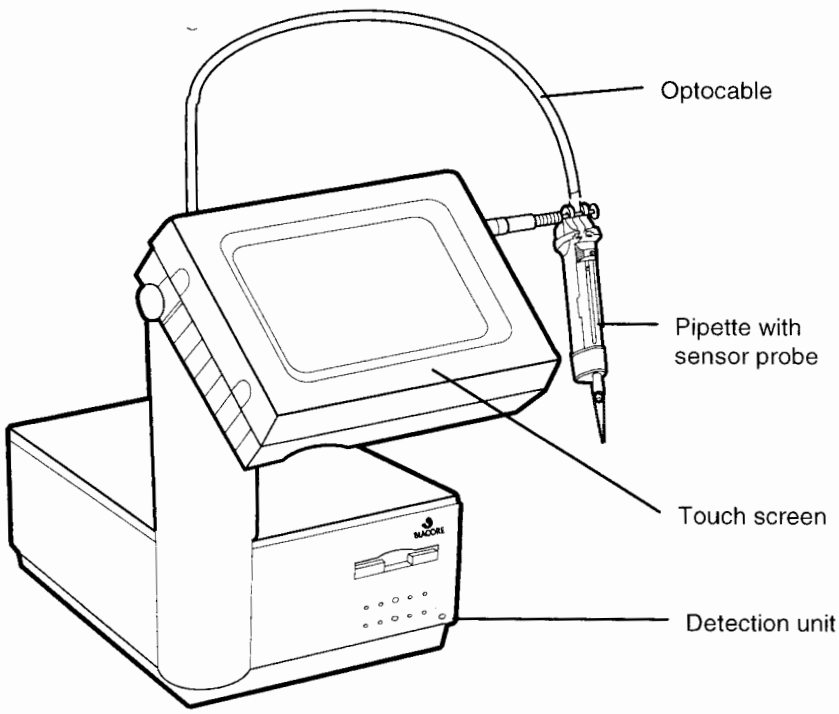


Figure 2-1. *BIACORE® probe.*

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2.1 The detection unit

The main body of the detection unit houses the light source (a low temperature halogen lamp), detector and software for registering and interpreting the signal from the sensor probe, and a 3.5" diskette drive for copying data to PC-formatted computer diskettes (see Section 4.7). A green lamp on the front panel indicates that the power is on.

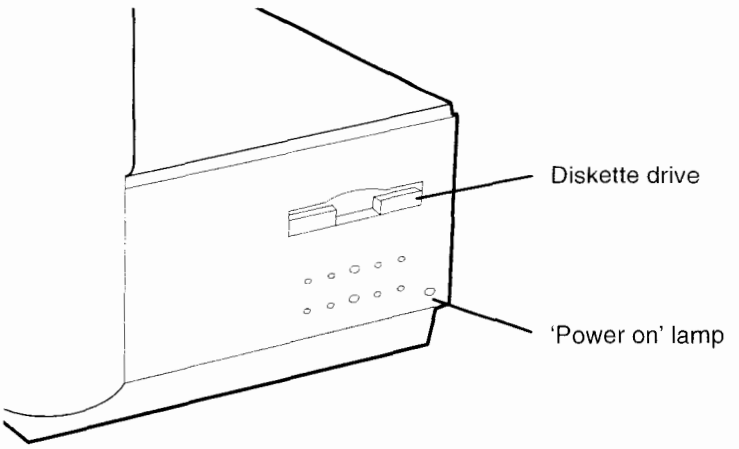


Figure 2-2. *BIACORE® probe front panel, showing the diskette drive and the power on indicator.*

The rear panel of the detection unit has the mains power switch and connectors for mains power and a printer (25-pin computer connector). There is also a 9-pin computer connector which is used for service purposes.

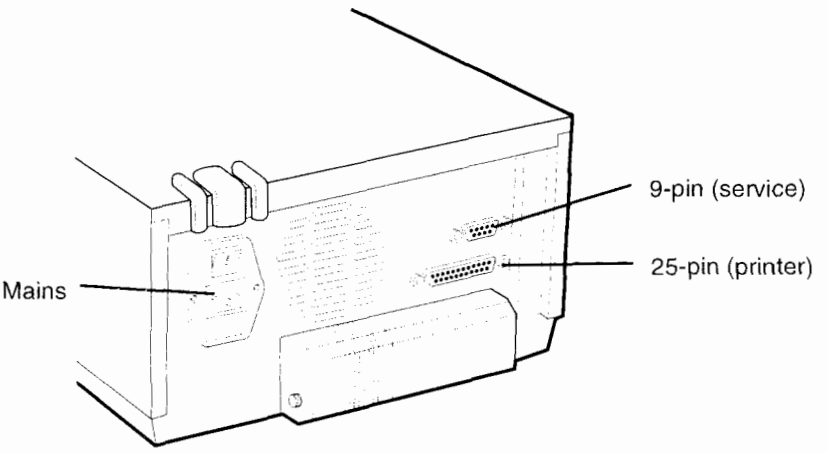


Figure 2-3. *Connections on the rear panel.*

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The touch screen provides the interface for entering instructions and data to the instrument, as well as displaying the results of measurements. Buttons are displayed on the screen for the functions available at any given time. To issue an instruction, touch the screen lightly on the appropriate display button.

The screen can be turned on its supporting column and tilted from the vertical for adjustment to standing or sitting working positions (Figure 2-4). The red buttons on the top and side of the screen column release the screen for turning and tilting adjustment respectively. The screen locks automatically in the chosen position. Take care not to trap your hand between the screen and the supporting column when you adjust the screen angle.

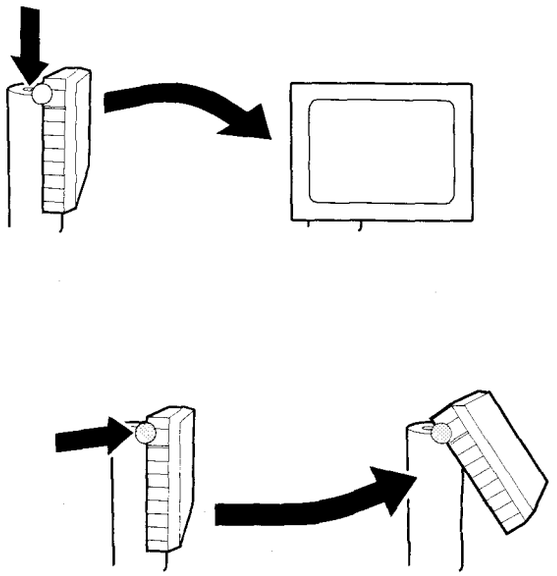


Figure 2-4. *Adjustment of the touch screen angle.*

Pipette holder

A holder for the pipette is fitted on either the left or right side of the screen, according to which side you prefer to work (see Figure 3-1). The pipette should hang on the holder during measurements as well as when not in use.

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2.2 The sensor probe

The sensor probe, where the actual detection takes place, is an optical fibre coated at the tip with a gold layer (Figure 2-5). The detecting molecule is immobilized directly or indirectly on the surface to provide a biospecific sensor probe. A description of the surface characteristics and full instructions for immobilizing the detecting molecule and using the sensor probe are provided in a separate Sensor Probe Handbook.

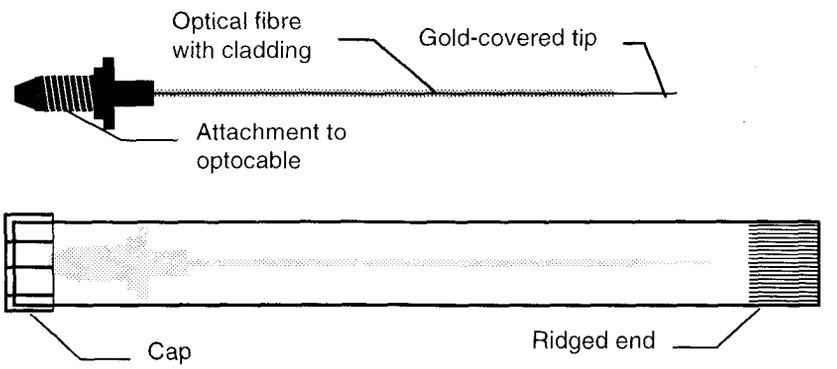


Figure 2-5. *Construction of the sensor probe (top) and protective tube (bottom) with the position of the sensor probe marked in grey.*

The sensor probe is supplied in a protective tube. The tube is used for handling the sensor probe during fitting to and removal from the pipette, and for storing the sensor probe when it is removed from the pipette (see Section 4.4).

2.3 The optocable

The optocable is an optical fibre cable that transmits both the incident and reflected light between the detection unit and the sensor probe. The optocable is flexible, but should not be bent tightly since this may damage the optical fibre in the cable. The minimum diameter of curvature for bending the cable is about 13 cm.

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2.4 The pipette

BIACORE®probe uses a specially designed pipette to hold the sensor probe. The sensor probe protrudes into the pipette tip. Measurements are made on sample aspirated into the pipette tip.

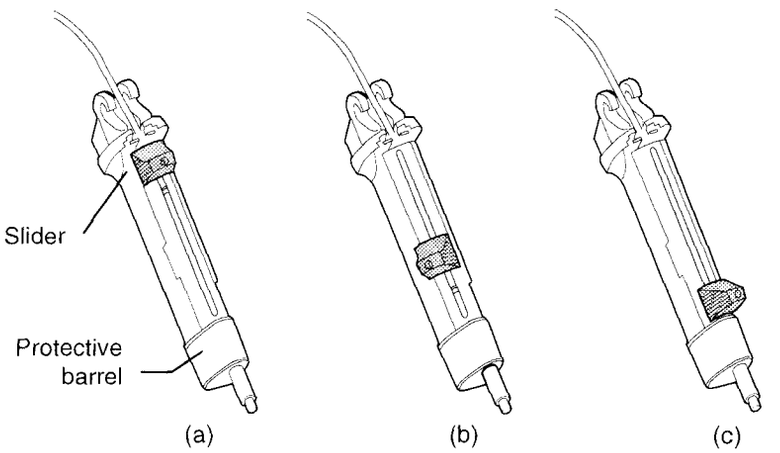


Figure 2-6. *The three positions for the slider on the pipette. (a) Top position: for changing the sensor probe. (b) Middle position: for changing the pipette tip. (c) Bottom position: for aspirating and dispensing solutions.*

The red slider on the pipette has three positions (see Figure 2-6):

- The top position is used when fitting or removing the sensor probe (see Section 4.4).
- The middle position (locked by turning the slider) holds the tip of the sensor probe in the protective barrel, and is used when fitting or changing the pipette tip.
- In the bottom position (locked by turning the slider), the sensor probe tip is extended into the pipette tip. This position is used when making measurements.

With the slider in the bottom position, the plunger at the top of the pipette aspirates and dispenses a fixed volume of about 100 µl. This is sufficient to cover the tip of the sensor probe when it is extended in the pipette tip. The pipette will only aspirate and dispense liquids when the slider is in the bottom position.

With the slider in the middle position, the plunger releases the pipette tip from the pipette.

Recommended pipette tips are 200 µl micropipette tips from Gilson.

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3. Unpacking and installation

3.1 Unpacking

Unpack the instrument carefully and check the contents against the packing list included with the instrument.

When unpacking or moving the instrument, do not lift the instrument by the touch screen. Hold the instrument under the detector unit, bearing in mind that the detector unit is heavier at the front than the back.

Inspect each item for damage that may have occurred during transit.

3.2 Installing the instrument

When placing the instrument on the laboratory bench, make sure that the fan inlet (under the instrument) and outlet (on the back panel) are not obstructed. Do not put papers on the bench under the instrument: these can easily be sucked up against the fan inlet, blocking the air intake.

Avoid placing the instrument on the same bench as vibrating equipment (e.g. bench centrifuges and shakers).

Pipette holder

The pipette holder can be fitted on the left or right side of the screen, in either the top or bottom position (Figure 3-1). Remove the plug from the appropriate position and screw in the pipette holder.

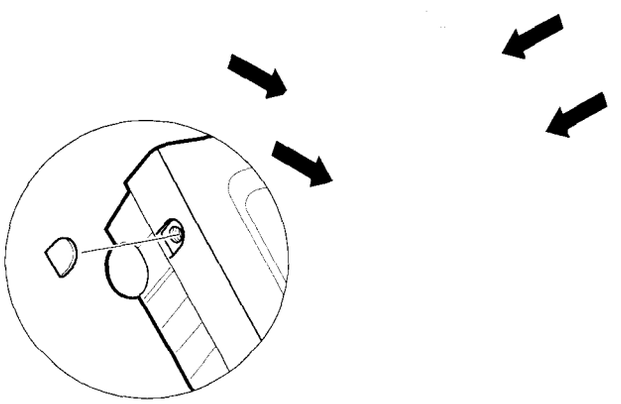


Figure 3-1. *Fittings for the pipette holder.*

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Optocable and pipette

As supplied, one end of the optocable is connected to the probe holder. Follow the steps below to connect the other end to the instrument.

Caution!
Do not bend the optocable more than is necessary, since this may damage the optical fibre in the cable. The minimum diameter of curvature for bending the cable is about 13 cm.

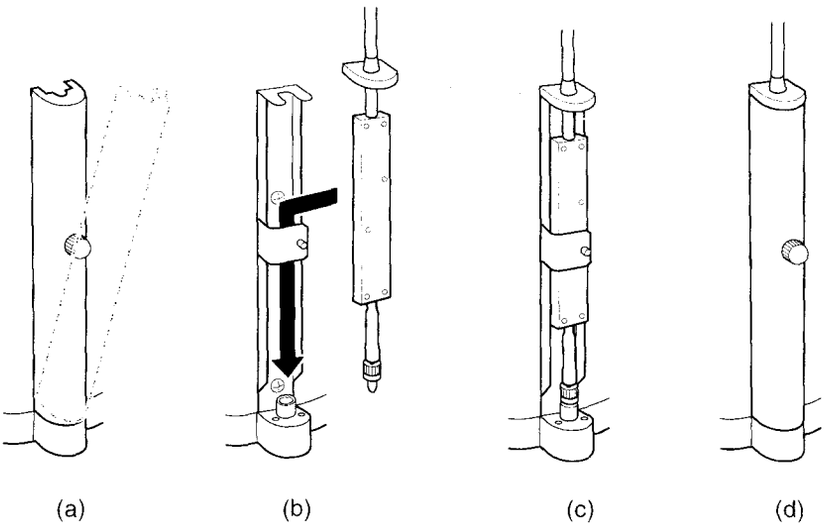


Figure 3-2. *Fitting the optocable.*

1. Loosen the retaining screw on the back of the column that supports the screen and remove the rear casing from the column (Figure 3-2a).

2. Open the optocable package and hang the pipette on the pipette holder.

3. Remove the protective cap from the optocable connection socket at the bottom of the column on the instrument. Fit the free end of the optocable into the socket, making sure that the rubber cover that fits over the top of the column has the flat side facing the screen. This will ensure that the optocable bends in the right direction (forwards over the touch screen). Tighten the retaining nut by hand (Figure 3-2b,c).

4. Replace the back of the column (Figure 3-2d).

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Sensor probe

If you do not intend to use BIACORE®probe immediately, fit a dry pipette tip on the pipette and move the red slider to the bottom position. This will protect the optical connection for the sensor probe.

Instructions for fitting a sensor probe are given in Section 4.4.

Electrical connections

Connect BIACORE®probe to a properly earthed power source using the mains cable provided.

A printer (optional) may be connected to the parallel (25-pin) port on the back of the instrument. The printer should be compatible with the IBM Proprinter. Turn the power to BIACORE®probe off when you connect or disconnect a printer.

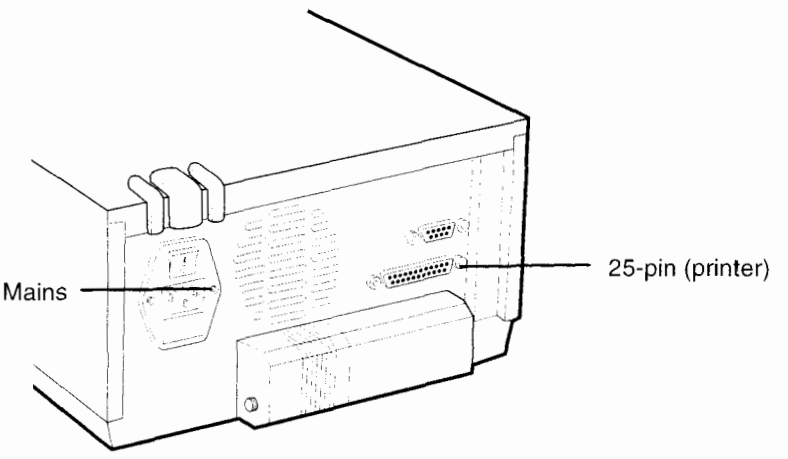


Figure 3-3. *Rear panel showing the mains and printer connectors.*

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3.3 BIACORE® probe Software

BIACORE®probe is delivered with software installed. You are provided with two diskettes, one containing the software and the second containing instrument-specific data. Keep these diskettes in a safe place in case you need to re-install the software after an instrument failure. See below for instructions on software re-installation.

If you wish to make a copy of the diskettes for safety purposes, do this on an IBM PC-compatible computer. Do not simply copy the files to new diskettes: use the disk copy function on your PC to ensure that all disk information is faithfully copied (see your computer software documentation for instructions on how to copy diskettes). Label the diskette copies clearly.

Note: The instrument-specific diskette applies only to the instrument with the identification number specified on the diskette label, and cannot be used with any other BIACORE®probe instrument.

Re-installing BIACORE® probe Software

If you need to re-install the software, follow the steps below:

1. Turn the power to BIACORE®probe off.
2. Insert the software diskette in the disk drive.
3. Turn the power on again and follow the instructions on the screen.

Note: BIACORE®probe Software is provided on a bootable PC-formatted diskette. Do not use this diskette in a desktop computer.

Setting the date and time

The first time BIACORE®probe is turned on after delivery or software re-installation, you will be asked to enter the current date and time. These settings are used to enter a time-stamp in result files (see Section 4.2). Instructions for re-setting the date or time (e.g. for daylight saving time adjustments) are given in Section 6.6.

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4. Operation

This chapter describes the basic operation of BIACORE®probe. For details of how to use a specific type of sensor probe, refer to the Sensor Probe Handbook. For a hands-on tutorial to help you learn how to use the instrument, see Getting Started for BIACORE®probe.

4.1 Starting BIACORE® probe

The mains power switch is located on the back of the detection unit at the right hand side (see Figure 2-3). The green lamp on the front of the instrument is lit when the power is on (see Figure 2-2).

Adjust the touch screen to the desired angle for best access and legibility (see Figure 2-4).

Allow 10 minutes for the instrument to stabilize before making measurements.

4.2 Screen display and button functions

The main screen is displayed at start-up and when an analysis is not in progress:

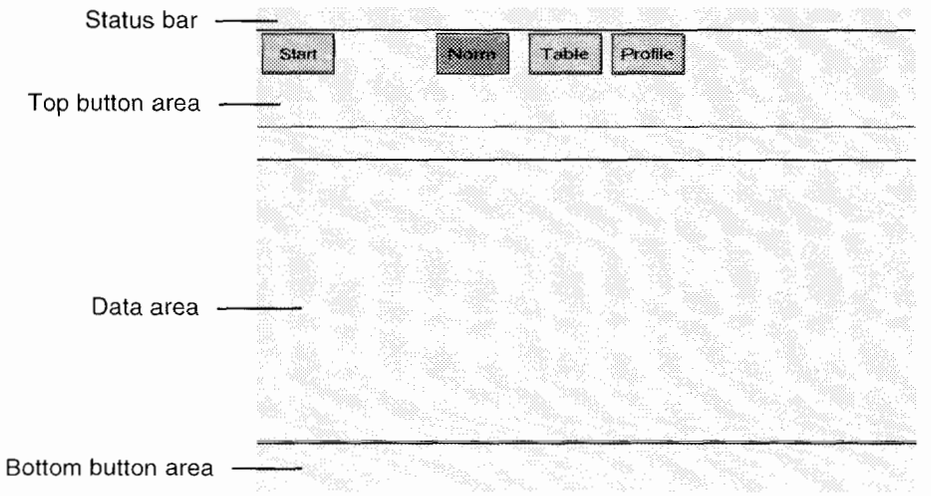


Figure 4-1. *BIACORE® probe Software screen at startup.*

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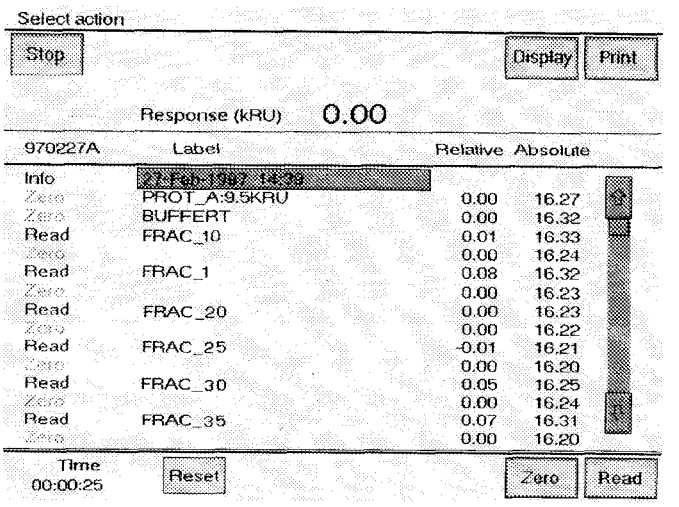


Figure 4-2. *BIACORE® probe Software screen at startup during analysis.*

The screen display is divided into 4 main areas (Figures 4-1 and 4-2):

- The status bar displays short status messages.
- The top button area displays buttons for main software functions and the current response.
- The data area displays data during analysis.
- The bottom button area displays timer information and buttons used during analysis (see Sections 4.6 and 4.8).

Top button area

Start/ Stop Starts and stops an analysis (see Section 4.6).

Save Saves the results of the analysis (see Section 4.7). This button only appears after an analysis is stopped.

Norm Starts the normalization procedure (see Section 4.5). This button is green when the instrument is first turned on to indicate that normalization should be performed before starting an analysis.

Table Used for managing stored result tables (see Section 4.7).

Profile Used for managing user profiles (see Section 4.8).

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Display Switches between table (numerical) display of the results and graphical (histogram) display of the zero and response readings (see below).

Print Prints the results (available only in table display).

Data area

When an analysis is started, the data area displays a table of numerical values for zero and response points. Each point is identified by a label that you can edit. By default, zero readings have a blank label and response readings are labelled with a sequence number starting with **No:1** at the beginning of the analysis.

To select a label to edit, either touch the **Relative** or **Absolute** value in the appropriate row or use the vertical scroll arrows at the right of the table. The selected label will be highlighted. To edit the label, touch the label: this opens a keyboard display where you can enter the text for the label.

The first line in the table records the date and time when the analysis was started. The second line is an automatic zero reading made at the start of the analysis. This reading initially has no label: you may use the label as an identifier for the analysis.

The table shows both absolute and relative response values for each reading. Relative values are obtained from absolute values by subtraction of the most recently registered zero value. Relative values for zero readings are always 0.00.

If the table of values is too long to fit on one screen, a scroll bar appears between the vertical scroll arrows at the right of the table. When the selected line is at the top or bottom of the display, touching the arrows scrolls the display 5 lines up or down.

Graphical display

Touch **Display** in the top button area to switch the display to a histogram of relative response values for response points. Touch **Display** once more to switch to a histogram of absolute response values for zero points. Horizontal scroll arrows appear on the histogram displays if the histogram is too wide to fit on one screen. Touch **Display** once more to return to the table display.

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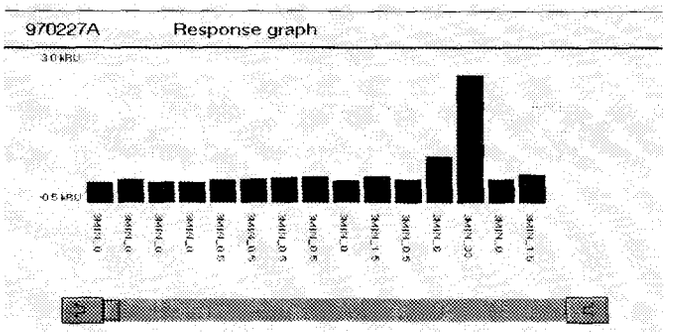


Figure 4-3. *Graphical display of response data.*

Bottom button area



Figure 4-4. *The bottom button area during an analysis..*

Time Shows the elapsed time since the start of the analysis or the most recent **Reset**.

Reset Touch this button to reset the timer to zero.

<timer> During profile operation, special profile timer buttons also appear in the bottom button area (see Section 4.8).

Zero Touch this button to record a zero reading and set the zero for the relative response in subsequent readings.

Read Touch this button to record a response reading.

4.3 General liquid handling

Reference buffer, sample and other solutions are brought into contact with the sensor probe tip by aspirating solution into the pipette. Bear the following considerations in mind in handling solutions with BIACORE®probe.

Avoiding carry-over between solutions

When a solution is dispensed from the pipette tip, a thin film of liquid will remain inside the pipette tip. It is important, particularly for quantitative determinations, to minimize carry-over between solutions both to maintain precision in measurements and to ensure defined

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solution conditions at the sensor probe tip. There are two ways to minimize carry-over:

- Wash the sensor probe tip by aspirating and dispensing solution (usually buffer) 2–3 times.
- Change the pipette tip. Do this when it is important to avoid contaminating the solution to which you are changing (e.g. before you aspirate valuable sample, and when you change between solutions of very different composition).

How to change the pipette tip

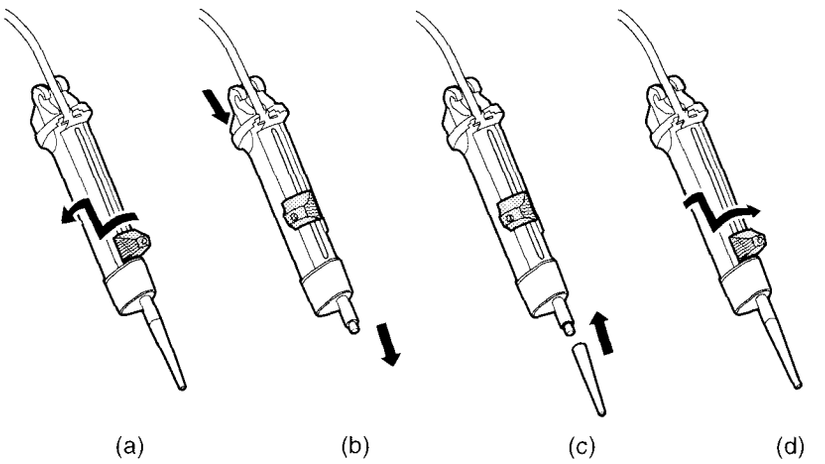


Figure 4-5. *Changing the pipette tip.*

1. Dispense the contents of the pipette tip if it is not already empty.
2. Move the red slider to the middle position and lock it, in order to retract the sensor probe into a protected position (Figure 4-5a).
3. Press the pipette plunger to displace the used tip (Figure 4-5b).
4. Fit a new tip on to the pipette (Figure 4-5c).
5. Move the red slider to the bottom position and lock it (Figure 4-5d).



Warning!
Do not remove the pipette tip without first retracting the sensor probe tip by moving the red slider to the middle position. The sensor probe tip is both sharp and delicate, and may cause injury and/or be damaged if it is not protected by a pipette tip.

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The pipette plunger will only displace the pipette tip when the red slider is in the middle position. Do not remove the pipette tip by hand: always use the pipette plunger so that the sensor probe is protected when the tip is removed.

Controlling the measurement temperature

The response in BIACORE®probe is dependent on the temperature at the sensor probe tip. Provided that room temperature is reasonably constant, this effect can usually be ignored (a change in temperature of +1°C results in a change in response of approximately -0.1 kRU). Beware however of using BIACORE®probe where large temperature fluctuations can occur (e.g. in direct sunlight or close to an air conditioner or cold-room door).

Between measurements

Do not allow the sensor probe to dry out between measurements. Either keep reference buffer in the pipette tip with the sensor probe extended (red slider in the bottom position) or remove the sensor probe for storage (see the Sensor Probe Handbook) when the instrument is not in use.

4.4 Changing the sensor probe

The sensor probe is supplied in a protective tube, which is also used for storing the sensor probe after detecting molecule has been immobilized.



Warning!
Do not remove the sensor probe from the protective tube except by fitting it into the pipette. The sensor probe tip is both sharp and delicate, and may cause injury and/or be damaged by incorrect handling.

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Fitting the sensor probe

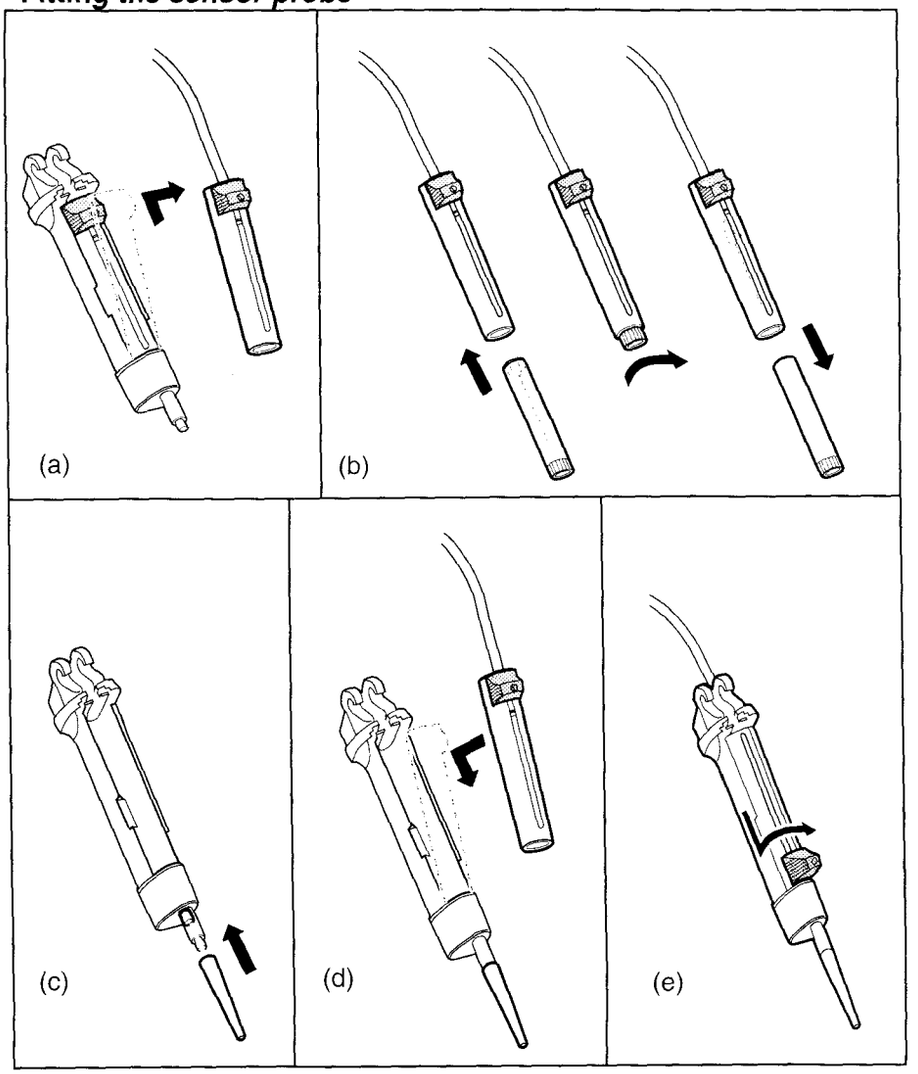


Figure 4-6. *Fitting the sensor probe.*

1. Move the red slider on the pipette to the top position and lift the probe holder out of the pipette (Figure 4-6a).

2. Make sure there is no sensor probe in the probe holder.

3. Remove the protective cap from the sensor probe tube. Hold the sensor probe tube at the ridged end and push the other end firmly into the probe holder. Screw the sensor probe tube clockwise into the holder until it stops, then pull the tube out leaving the sensor probe in the holder (Figure 4-6b). Do not discard the tube: you will need it for removing and storing the sensor probe after use.

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4. Fit a pipette tip on to the pipette (Figure 4-6c), then replace the probe holder in the pipette (Figure 4-6d).

5. Move the red slider to the bottom position and turn it into the locked position (Figure 4-6e). The sensor probe tip should be visible inside the pipette tip.

6. Wash the sensor probe tip 3–4 times with reference buffer and allow to stand with reference buffer in the pipette tip.



Warning!
Do not move the red slider to the bottom position unless a pipette tip is fitted on the pipette. The sensor probe tip is both sharp and delicate, and may cause injury and/or be damaged if it is not protected by a pipette tip.

Removing the sensor probe

If the sensor probe is to be stored for any length of time, particularly using wet storage (see the Sensor Probe Handbook), wash the protective tube and cap with 90% ethanol to sterilize it. Dry the tube with nitrogen or oil-free air before using it to remove the sensor probe.

To remove the sensor probe from the pipette:

1. Move the red slider on the pipette to the top position and lift the probe holder out of the pipette.

2. Check that the sensor probe tube you are using is empty. Hold the tube at the ridged end and push the other end firmly into the probe holder (see Figure 4-7).

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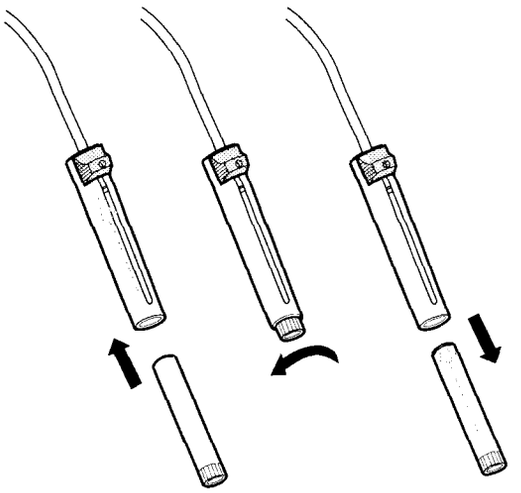


Figure 4-7. *Removing the sensor probe from the probe holder.*

3. Screw the sensor probe tube anticlockwise out of the holder, taking the sensor probe with it.

4. Put the cap on the protective tube at the non-ridged end to protect the optocable connector surface.

Storing the sensor probe

For recommended storage conditions, see the Sensor Probe Handbook.

4.5 Normalizing the signal

Normalization corrects for optical characteristics of the sensor probe tip, optocable connection and light source and is necessary for reliable measurements. You should normalize the signal:

- when the instrument has just been switched on
- at the beginning of each working day (even if the instrument has been left on overnight)
- each time you fit a sensor probe into the pipette (even if the sensor probe has been used and normalized previously).

Normalization is done by measuring a signal with the sensor probe in BIAnormalizing solution for BIACORE®probe (available from Biacore AB), which provides reference data for automatic signal adjustment.

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Make sure that the BIAnormalizing solution you use is at room temperature.

If you have just turned the instrument on, allow 10 minutes for the signal to stabilize before normalizing. Follow the steps below to normalize the signal.

1. Touch **Norm** on the screen.

2. Wash the sensor probe 3 times with reference buffer.

3. Change the pipette tip, then aspirate BIAnormalizing solution into the pipette tip and touch **Continue** on the screen. This will start a 3-minute timer to ensure that the sensor probe is fully equilibrated with BIAnormalizing solution.
   If you abort the normalization procedure by touching ***Cancel*** *on the timer display, the signal will not be normalized.*

4. At the end of the equilibration period, dispense and discard the BIAnormalizing solution. Change the pipette tip, wash the sensor probe 3 times with reference buffer, aspirate reference buffer and then touch **Continue** on the screen.

5. If the normalization is successful, the display returns to the start-up screen and you can continue with attachment of the detecting molecule or measurement (Section 4.6). If the normalization is not successful, a message to that effect is displayed. See Section 7.2 for steps to take if you cannot normalize the signal successfully.

Notes: BIAnormalizing solution is viscous. Make sure the pipette tip is properly filled when you aspirate the solution. Change the pipette tip after normalization to avoid carry-over of BIAnormalizing solution to reference buffer.

Do not perform measurements if you have not been able to normalize the signal successfully. The reliability of such measurements cannot be guaranteed.

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4.6 Performing measurements

A requirement for measurements with BIACORE®probe is that the sensor probe is functionalized by attachment of a detecting molecule. Attachment may be covalent or through high affinity capture (see Section 5.1). Techniques available for attaching the detecting molecule depend on the type of sensor probe, and are described in detail in the separate Sensor Probe Handbook.

BIACORE®probe determines the presence of analyte by measuring the amount of analyte that binds specifically to the detecting molecule on the sensor probe. When the sensor probe is in contact with the sample solution, other solutes in the sample will contribute to the measured response. For this reason, always transfer the sensor probe from sample to reference buffer before touching **Read** on the screen to record the response.

When a sample response has been detected, the sensor probe is regenerated with a solution which removes the analyte from the detecting molecule without damaging the detecting molecule itself. Typical regeneration procedures involve brief exposure to high or low pH. The exact conditions for regeneration will depend on the type of sensor probe used and the nature of the detecting molecule (see the Sensor Probe Handbook for further discussion of regeneration procedures). After regeneration the sensor probe is ready for measuring the next sample. Regeneration can be skipped if no response is observed (i.e. no analyte has bound to the sensor probe).

Steps in typical sample measurement are as follows (Figure 4-8). These steps can be programmed in a user profile for convenience and reliable repeated operation (see Section 4.8). During incubation of the sensor probe with reference buffer, sample, regeneration solution etc, allow the pipette to hang on the pipette holder on the side of the screen.

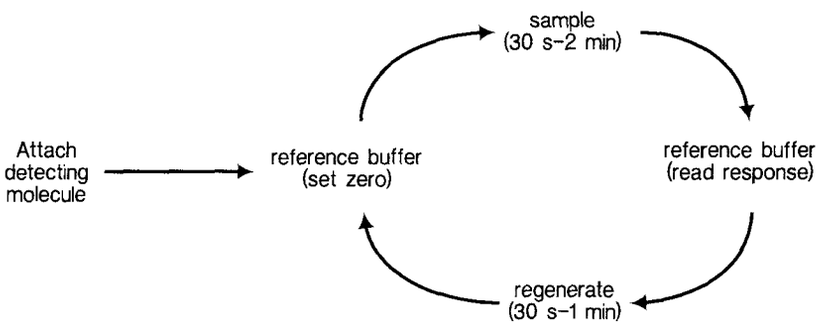


Figure 4-8. *Analysis cycle using directly coupled detecting molecule.*

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1. Aspirate reference buffer and touch **Zero** on the screen to set the relative response to zero.

2. Change the pipette tip to avoid carry-over of reference buffer to the sample, aspirate sample and touch **Reset**.

3. Wait for a fixed time interval (typically 30 seconds–2 minutes) to allow analyte to bind to the detecting molecule.

4. Dispense the sample and wash the sensor probe twice with reference buffer.

5. Aspirate reference buffer, wait 5–10 seconds for the signal to stabilize, then touch **Read** to record the sample response.

6. If the response is negligible (i.e. there is no analyte in the sample), regeneration is not necessary. Go to step 1 for the next sample.

7. Dispense the reference buffer, then follow the procedure established for optimal regeneration of your sensor probe.

8. After regeneration, wash the sensor probe with reference buffer, aspirate reference buffer and check the response. The value should be close to zero indicating complete regeneration. If it is not, review your regeneration procedure.

9. Go to step 1 for the next sample.

10. Touch **Stop** to stop the analysis when your measurements are complete.

11. Save the results (Section 4.7).

4.7 Saving and loading results

You can save the results of an analysis in memory in BIACORE®probe or on a PC-formatted 3.5" diskette. Results saved in BIACORE®probe memory are retained even when the power is turned off: you are however advised always to save results to diskette as a back-up copy. You also save results to diskette when you wish to display or evaluate the results in a personal computer.

Saving results

To save results to BIACORE®probe memory, touch the **Save** button and use the keyboard display that appears to name your result file. File names may be up to 8 characters long and must be unique within the destination (memory or diskette). If you enter the name of an existing

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file, you are given an opportunity to overwrite the existing file or enter a new name.

When you have entered the file name, touch either **Diskette** or **Memory** to save the file to a diskette or BIACORE®probe memory respectively. Diskettes must be formatted for an IBM-compatible PC and must not be write-protected.

If power to the instrument is lost during an analysis, the results are saved automatically to a file called RESCUE. This file can be loaded to recover the results.

Managing result files

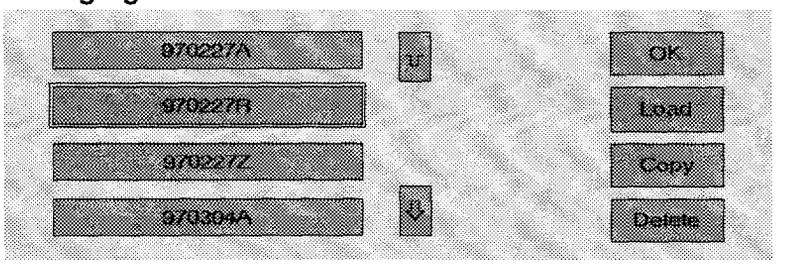


Figure 4-9. *Managing result tables.*

Loading results from memory
You can open result files saved in BIACORE®probe memory to inspect or print the results. You can also edit labels in the loaded file.

Touch **Table** on the main screen. Scroll the display until the desired result file is shown in the buttons to the left, then touch the file name to select the file. The selection is marked by an extra border around the file name button. Touch **Load** to load the file.

Note: You cannot load files from diskettes.

Printing results
To print the results shown on the screen, touch **Print**. To print results from a saved file, load the results into memory first.

Copying results to diskette
To copy result files to a diskette, touch **Table** on the main screen, select the file, then touch **Copy**. This will copy the file to the diskette using the same file name. Diskettes must be formatted for an IBM-compatible PC and must not be write-protected.

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Alternatively, you can load the file from BIACORE®probe memory, touch **Save** and save the file to diskette. This allows you to specify a different file name for the file saved to diskette.

Deleting results
To delete a result file from BIACORE®probe memory, touch **Table** on the main screen, select the file, then touch **Delete**. You will be asked to confirm the deletion.

> *Caution!*
> ***Result files deleted from BIACORE®probe memory cannot be recovered. Make sure that you have documented your analysis properly before deleting results.***

Opening results in computer programs
To transfer results to a personal computer for display or evaluation, save the results first to diskette. The file on diskette is saved with the file name extension '.txt' (e.g. saving the results of TEST creates a file called TEST.TXT). The contents of the file are in tab-separated text format, which can be opened by all common spreadsheet and similar programs. The file contains a header row identifying the columns followed by the results of the analysis. An example is listed below.

Type	Label	RelResp(kRU)	AbsResp(kRU)
Info	1996-08-13 04:27	0.00	24.24
Zero		0.00	24.24
Zero		0.00	24.30
Resp	7	0.14	24.44
Zero	REG	0.00	24.13
Resp	8	0.10	24.23
Zero	REG	0.00	24.08
Resp	9	0.19	24.27
Zero	REG	0.00	24.08
Resp	10	0.32	24.40

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4.8 Recording and using analysis profiles

Analysis profiles help to automate the timing of measurements and standardise the way you work, and also help to keep track of which solution is in the pipette tip by guiding you through the steps in an analysis cycle. A profile defines a set of up to three timers for different steps in the analysis, and can for example record the value at a fixed time after you change from sample to buffer. BIACORE®probe is supplied with a predefined profile called AMINECPL with recommended times for functionalizing Sensor Probe CM5 using covalent amine coupling procedures (see the Sensor Probe Handbook for details).

Example of a profile

Below is an example of a profile as defined in BIACORE®probe:

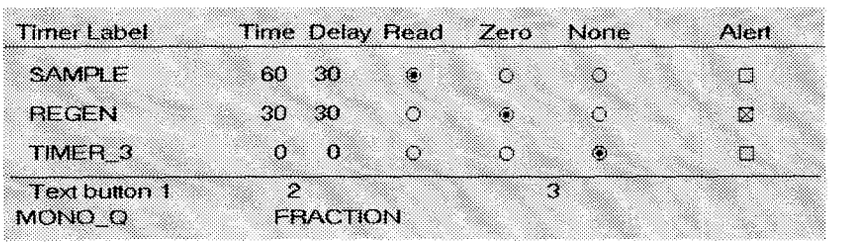


Figure 4-10. *Example of a profile definition.*

This profile uses two predefined timers, one for sample measurement and one for regeneration. Up to three timers can be defined for a given profile.

SAMPLE The sample timer sets a contact time of 60 seconds followed by a delay time (after transferring the sensor probe to reference buffer) of 30 seconds. The response is recorded automatically at the end of the delay time.

REGEN The regeneration timer sets a contact time of 30 seconds followed by a delay time (after transferring the sensor probe to reference buffer) of 30 seconds. A new zero level is set automatically at the end of the delay time. This timer also has an alert set, which means that a signal will sound during the delay period. This can be useful as a reminder – in this case a signal that the regeneration solution must be dispensed as soon as the alert begins to sound.

The profile also has two text buttons. These appear in the keyboard display for response labels, and provide a one-touch way of entering a pre-defined text. In this example, text button 1 (**MONO_Q**) can be used to enter an identifier for the analysis as the label for the first zero point, and text button 2 (**FRACTION**) to enter the word 'FRACTION'

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for each sample response point. You can edit labels added with text buttons in the same way as ordinary labels: defining a text button as **FRACTION** thus saves the effort of entering the word each time while still allowing you to enter the fraction number ('FRACTION 10', 'FRACTION 20' etc).

When an analysis is run using a profile, the profile name is displayed at the top right of the screen and new timer buttons appear in the bottom button area. The buttons are identified by the timer names and the respective times.

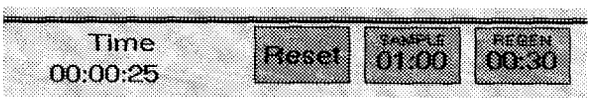


Figure 4-11. *Timer buttons displayed during operation of the profile illustrated in Figure 4-10.*

To measure a sample using this profile:

1. Set the zero reading if this has not already been done.

2. Aspirate sample and touch the **SAMPLE** button. The timer display will start to count down from the preset timer value (in this case 60 seconds).

3. When the timer reaches zero, a single signal will sound to indicate the end of the preset time period. Dispense the sample and wash the sensor probe as soon as you hear the signal (when the timer reaches zero).

4. The delay timer will start immediately, counting upwards from zero. The preset delay time is shown in brackets after the timer display. At the end of the delay time, the response will be recorded and a signal will sound to indicate that the instrument is ready for the next step.

5. To regenerate the sensor probe, wash once with regeneration solution aspirate regeneration solution and touch the **REGEN** button. This starts a timer as defined by the **REGEN** timer in the profile (30 seconds contact time, 30 seconds delay time, set new zero level). During the delay period, a signal will sound continuously (**Alert** function) to remind you to transfer the sensor probe from regeneration solution to reference buffer.

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Managing user profiles

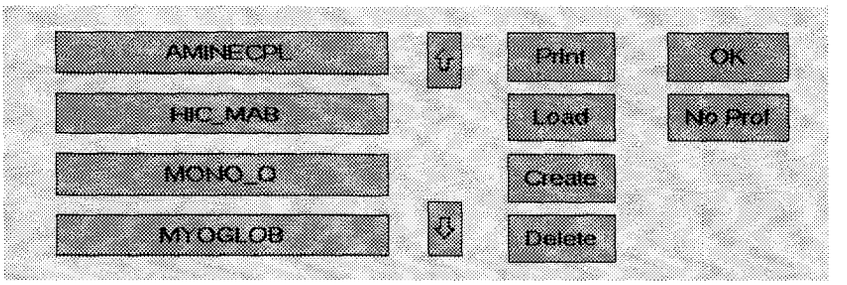


Figure 4-12. *Managing user profiles.*

Creating a profile
To create a profile, touch **Profile** on the main screen to open the profile management panel.

Touch **Create**. The profile definition panel appears with the first timer name selected. Touch ← on the keyboard to erase the default name **TIMER_n** and enter a new name for the timer if desired. Use the **Next** and **Previous** buttons to move forwards and backwards through the display, defining settings as required. Timer and delay times are entered in seconds. Touch **OK** when you have defined all settings.

If you choose to save the profile, it will be available for use on later occasions (see below, loading a profile). If you do not save the profile, it will be loaded in current memory and can still be used, but will be lost when the instrument is turned off or when you load or create another profile.

Documenting a profile
To print a copy of the currently loaded profile definition, touch **Profile** on the main screen and then touch **Print**. The profile definition will be printed as it appears on the screen.

Using a profile
To use a profile, it must first be loaded into current memory. The name of the profile currently loaded (if any) is shown in the top right-hand corner of the screen. To load a profile, touch **Profile** on the main screen to open the profile management panel. Select the profile to be loaded, then touch **Load**. Once loaded, a profile remains in current memory until a new profile is created or loaded or BIACORE®probe is turned off.

When you perform an analysis using a profile, timer buttons corresponding to the pre-set timers in the profile appear in the bottom button area (see Figure 4-10). How you use these buttons is illustrated in the example at the beginning of this section.

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Removing a profile from memory
If you want to remove a profile from current memory to perform an analysis without a profile, touch **Profile** on the main screen to open the profile management panel, then touch **No Prof**.

Editing an existing profile
To edit an existing profile, touch **Profile** on the main screen to open the profile management panel. Select the profile to be edited, then touch **Edit**. Make any changes you require and touch **OK** on the keyboard display to save the profile.

Note that the profile is loaded in memory and will be used for subsequent runs unless you load a different profile or remove the profile from memory.

Deleting a profile
To delete a profile, touch **Profile** on the main screen to open the profile management panel. Select the profile to be deleted, then touch **Delete**. You will be asked to confirm that you want to delete the chosen profile.

A deleted profile cannot be recovered.

4.9 Shutting down

Stand-by
Leave the instrument switched on during the working day to avoid repeating the 10-minute equilibration period at start-up. Leave the pipette:

- *either* with a sensor probe fitted, the red slider in the bottom position, and with buffer in the pipette tip
- *or* with the sensor probe removed, the red slider in the bottom position and with a dry pipette tip fitted.

This will protect the optical contact surface at the end of the optocable.

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Shut-down

When you turn off power to the instrument:

- Remove any diskette from the diskette drive to reduce the risk of starting up with a diskette in the drive (see Section 7.1).
- Store the sensor probe as described in the Sensor Probe Handbook.
- Protect the optical contact surface at the end of the optocable as described for stand-by above.

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5. Assay development and techniques

This chapter considers development of analysis tools and protocols for working with BIACORE®probe, and covers

- functionalizing the sensor probe
- optimizing and performing the analysis
- regenerating the detecting molecule

5.1 Functionalizing the sensor probe

Choosing a detecting molecule

The choice of detecting molecule for BIACORE®probe is governed primarily by the following requirements:

- high ***affinity*** for the analyte for efficient detection with short incubation times
- high ***specificity*** for the analyte to ensure selectivity in detecting analyte in complex mixtures
- high ***specific activity*** to give high analyte binding capacity on the sensor probe

Antibodies (either polyclonal or monoclonal) are often suitable as detecting molecules, and in general antibodies that can be used as reagents for ELISA or other immunoassay techniques work well in BIACORE®probe. Other detecting molecules can also be used, such as specific ligands for detecting receptor molecules or vice versa. An advantage of using antibodies however is that protocols for functionalizing and regenerating developed for one antibody can often be applied at least in broad terms to a range of other antibodies regardless of specificity. Use of other detecting molecules such as ligands or receptors generally entails development of conditions for attachment and regeneration that are specific for each experimental system.

If you have several candidates for a detecting molecule (e.g. a series of antibodies), compare the suitability of the candidates using a sensor probe functionalized with a capturing molecule e.g. rabbit anti-mouse (RAM) IgG1 or Protein A. Follow the steps below to test the

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candidates. The analyte used should be as pure as can be obtained to reduce the risk of possible interference from other components.

1. Set a zero reading in reference buffer. Check that the analyte does not bind to the sensor probe in the absence of detecting molecule, by performing a blank measurement with a standard solution of analyte. If the blank response is more than 0.5 kRU, you should consider changing the buffer conditions or using a different capturing molecule.

2. Reset the zero response in reference buffer. Capture the candidate molecule on the functionalized probe (typical contact time 30 seconds-1 minute). Wash the sensor probe twice with reference buffer, wait 30 seconds for the signal to stabilize and record the response to measure the amount captured. Most antibodies bind tightly to Protein A or RAM IgG1: if you are using a different capturing molecule you may want to take readings at intervals of e.g. 10 seconds in reference buffer to check that the level of detecting molecule captured is stable. Set a new zero reading.

3. Aspirate a standard solution of analyte and incubate for 1 minute.

4. Dispense the analyte solution and wash the sensor probe with reference buffer. Aspirate reference buffer, wait 30 seconds for the signal to stabilize and then take readings at 0, 10 and 20 seconds thereafter.

5. Divide the analyte response readings by the amount of detecting molecule captured to obtain comparable values.

6. Repeat steps 1-4 for each detecting molecule candidate. Try to capture comparable amounts of the different detecting molecules (use solutions at the same concentration and with the same capturing time).

7. Choose the detecting molecule that gives the highest response which is stable over the 10-30 second period. Detecting molecules that show significant dissociation of analyte on this time scale should be avoided if possible.

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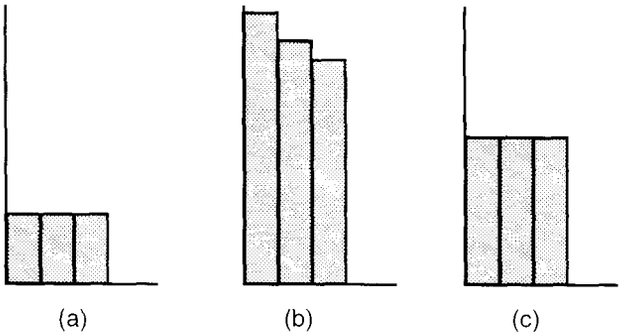


Figure 5-1. *Suitable and unsuitable detecting molecules. For each panel, the three bars represent the sample response obtained at 10-second intervals after the sensor probe has been transferred to reference buffer (step 4 above).*
(a) The response is stable but low.
(b) The response is high but falls off progressively indicating a significant dissociation.
(c) The response is intermediate and stable.
In this example, (c) represents the most suitable detecting molecule.

Attaching the detecting molecule to the sensor probe

Two strategies can be used to attach the detecting molecule to the sensor probe (Figure 5-2):

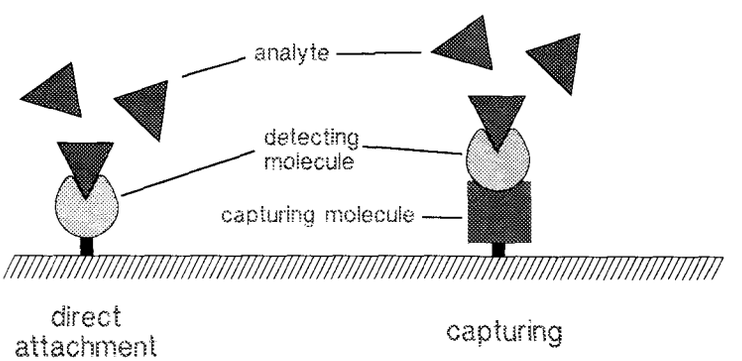


Figure 5-2. *Two ways of functionalizing the sensor probe.*

Direct covalent attachment

For sensor probes with a derivatized surface matrix (e.g. Sensor Probe CM5), the detecting molecule can be covalently linked to the matrix on the sensor probe surface. Coupling methods are described in detail in the Sensor Probe Handbook.

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Direct attachment is the method of choice provided that:

- the detecting molecule is available in a sufficiently pure state at a sufficiently high concentration (at least 100 µg/ml in coupling buffer),
- covalent attachment to the sensor probe surface does not impair the binding activity for analyte, and
- conditions for regenerating the sensor probe can be established (see the Sensor Probe Handbook).

Capturing

For capturing techniques, the sensor probe is first functionalized with a capturing molecule (e.g. RAM IgG1 or Protein A for antibodies), and the detecting molecule is captured on this molecule with a non-covalent interaction. This capturing interaction must be stable so that the detecting molecule does not dissociate significantly from the sensor probe surface during measurements.

The capturing approach is useful in the following situations:

- When the detecting molecule is not available in a pure state. The capturing step then represents a selective extraction of the detecting molecule from the crude preparation. For example, capture on RAM IgG1 will efficiently select monoclonal antibodies from unfractionated hybridoma culture medium.

  The value of affinity capture as a selective extraction step depends of course on the specificity of the capturing interaction. For example, Protein A will extract a crude Ig fraction from immune serum, which may not always give a sufficiently high specific activity for the antibody of interest. In such cases, purification of the antibody by affinity chromatography on column-bound antigen would be recommended as a first step.

- When one sensor probe is to be used for a range of different detecting molecules (e.g. in scanning candidate antibodies for the most suitable for use as a detecting molecule). In such situations, a non-covalent capture approach to attaching the detecting molecule may be used.

- When the detecting molecule does not withstand regeneration conditions. With a capturing approach, regeneration involves removal of both the analyte and the detecting molecule, leaving the capturing molecule on the sensor probe surface. This can be done using standard conditions developed for the general capturing interaction, and can prove useful if the specific binding of analyte to detecting molecule is difficult to regenerate.

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Disadvantages of the capturing approach are:

- Extra steps are introduced in the measurement cycle, since new detecting molecule must be bound after each regeneration (Figure 5-3).
- The sample used for measurement should be discarded since it may become contaminated with small amounts of detecting molecule which dissociates from the surface.
- The detection limit with capturing methods is often higher than with direct coupling methods, since the amount of detecting molecule that can be attached to the surface is usually lower.

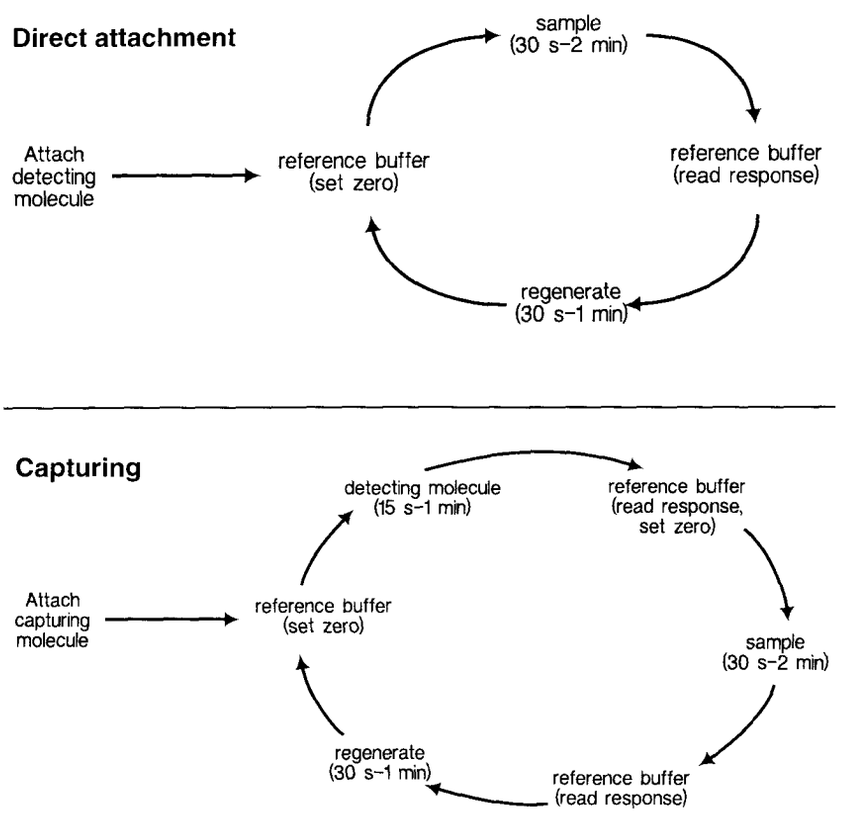


Figure 5-3. *Steps in analysis when the detecting molecule is attached to the sensor probe by direct coupling (top) and capturing (bottom).*

Optimizing the sensor probe functionalization

For best results, the amount of detecting molecule attached to the sensor probe should be as high as possible, to maximize the sensitivity

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for analyte. Recommended amounts for antibodies are 10-15 kRU. Lower levels will give lower sensitivity, particularly for smaller analytes since measurement is based on mass detection. Significantly higher levels (>25 kRU) may lead to a reduction in the dynamic range of the detection, resulting from steric hindrance of access of analyte to the detecting molecules.

The Sensor Probe Handbook gives recommended conditions for attaching detecting molecules to the sensor probe and indicates ways in which the amount of detecting molecule attached can be controlled.

5.2 Detecting and measuring analyte

BIACORE®probe is designed primarily for detecting the presence of analyte and obtaining a semi-quantitative indication of the active concentration. Standard analysis conditions generally suffice for this purpose. If accuracy in concentration determinations is required, it is important to optimize the analysis conditions and procedure carefully. Quantitative measurements also require a standard curve with known concentrations of analyte under the same conditions as the sample.

This section considers the factors which can influence the reliability of measurements made with BIACORE®probe (assuming that a suitable detecting molecule has been attached to the sensor probe as considered in Section 5.1 and the Sensor Probe Handbook).

Reference buffer

Since the detection principle in BIACORE®probe is based on refractive index changes at the surface of the sensor probe (see Appendix B), the measured response is influenced by the composition of the solution in contact with the sensor probe as well as by the binding of analyte to the sensor probe surface. This is why the zero response and sample response are always measured with the sensor probe in reference buffer (see Section 4.6). To minimize carry-over of the bulk refractive index contribution between sample and reference buffer, always wash the sensor probe twice with reference buffer between transferring from the sample and recording the response. Using a reference buffer with the same composition as the sample buffer can also help to eliminate bulk refractive index effects.

Sample composition

The detection principle and construction of the sensor probe allows measurements to be made in turbid or opaque samples and in a wide range of media, including extremes of pH and high salt concentration. Limitations on measurement conditions are generally imposed by the

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properties of the interaction between detecting molecule and analyte. Ionic strength and pH are generally the most important factors to consider in when performing measurements in samples of different composition.

For non-hydrophobic samples prepared in low ionic strength buffer, addition of salt to a physiological concentration (150 mM Na^+ or K^+) or higher can help to reduce non-specific binding of components in the sample to the sensor probe. This is particularly relevant in analysis of complex samples such as fermentation broth or crude extracts. The reference buffer recommended for general use in BIACORE®probe contains 150 mM NaCl.

If you intend to analyse a series of samples of varying ionic strength or pH (e.g. fractions eluted from ion exchange or hydrophobic interaction chromatography or from chromatofocusing), either adjust the salt concentration in the samples or check the response of BIACORE®probe if possible over the whole range of conditions using purified analyte. If this is not feasible, be aware that the response obtained from the different fractions may not be quantitatively comparable.

Contact times for sample and reference buffer

Standard analysis procedures allow a contact time of 30 seconds – 2 minutes between the sample and the sensor probe before the sensor probe is transferred to reference buffer to measure the amount of analyte retained. For high affinity detecting molecules and a high level of detecting molecule on the sensor probe, this time will usually be sufficient to reach a stable response level. If you suspect that the interaction is slow so that a stable response is not reached on this time scale, increasing the contact time can improve both the sensitivity and the consistency of measurements.

The response is measured 15 – 30 seconds after transferring the sensor probe from sample to reference buffer (see Section 4-6). Loss of analyte through dissociation in buffer should not be significant on this time scale if the detecting molecule has a high affinity for analyte (see Figure 5-1). If you are unable to find a detecting molecule with a sufficiently stable interaction, standardise the time between transferring to buffer and reading the response as carefully as possible.

Standard curves

Establish a standard curve for the assay over the required concentration range using analyte in the buffer conditions that will be used in the assay. You should repeat selected points on the standard curve for calibration each time for each measurement session. A new standard curve must be determined whenever a new sensor probe is functionalized.

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If you are measuring samples in different buffer environments (e.g. fractions from ion exchange, hydrophobic interaction or chromatofocusing separations), either check the validity of the standard curve under the range of buffer conditions used or make sure that all samples are adjusted to the same conditions before measurement.

Standardized analysis procedures
For reproducible measurements it is important that the analysis procedure is standardized, in particular the time of contact between sensor probe and sample and the time between transferring the sensor probe to reference buffer and reading the response (see above). Performing analyses with user profiles (Section 4.8) can help to standardize analysis procedures. Using longer times can reduce the relative errors in times of measurement.

In developing assays for quantitative use, perform a series of replicate measurements on known analyte samples to obtain an indication of the reproducibility of the assay.

Avoiding carry-over

Carry-over between samples or between sample and reference buffer can impair the reproducibility of the assay. The following points are important for reducing carry-over:

- Remember to wash the sensor probe with reference buffer when transferring from sample to measure the response, and discard the buffer used for washing.
- Change the pipette tip at critical points in the assay procedure.
- Use a relatively large reservoir of reference buffer (at least 50 ml) for washing the sensor probe after sample incubation and regeneration.

Controlling the temperature

It is important for reproducible measurements that the temperature at the sensor probe is kept constant. Avoid placing BIACORE®probe in a situation exposed to sudden temperature changes (e.g. direct sunlight, draughts, close to a refrigerator or cold-room door). Use a relatively large reservoir of reference buffer (at least 50 ml) equilibrated to the measuring temperature to minimize fluctuations in buffer temperature.

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Regeneration

Consistent regeneration of the sensor probe surface is essential for reproducible quantitative measurements. For qualitative detection of antibody, efficient regeneration will help to extend the usable life of the functionalized sensor probe. Regeneration is discussed in Section 5.3 below.

Interpreting the response

Selective detection and measurement of analyte is dependent in the first place on a specific interaction with the detecting molecule. Aspects of selectivity which should be considered in interpreting the response include non-specific binding of material to the sensor probe (false positive responses) and lack of analyte binding (false negative responses).

Non-specific binding

Test for non-specific binding to the sensor probe surface by measuring the response with a sensor probe to which no detecting molecule is attached. The measured response should be close to zero.

Avoid making measurements in buffers of low ionic strength (less than 50 mM). Use of physiological ionic strength buffers (monovalent cation concentration 150 mM) generally reduces non-specific binding. Higher salt concentrations may be used if the problem is persistent.

Unwanted binding of material to the attached detecting molecule can also present a problem, particularly if the sample contains so-called "sticky" proteins (proteins with binding activity for a wide range of other molecules). This binding may be genuinely "non-specific", like the general aggregation tendency of hydrophobic proteins in aqueous solution, or it may be a specific but unwanted interaction with the detecting molecule. Unwanted binding can be tested by using a functionalized sensor probe together with sample depleted of analyte.

The identity and/or activity of material which binds to the sensor probe from the sample can often be checked using a secondary interactant specific for the analyte. This principle is illustrated in Figure 5-4.

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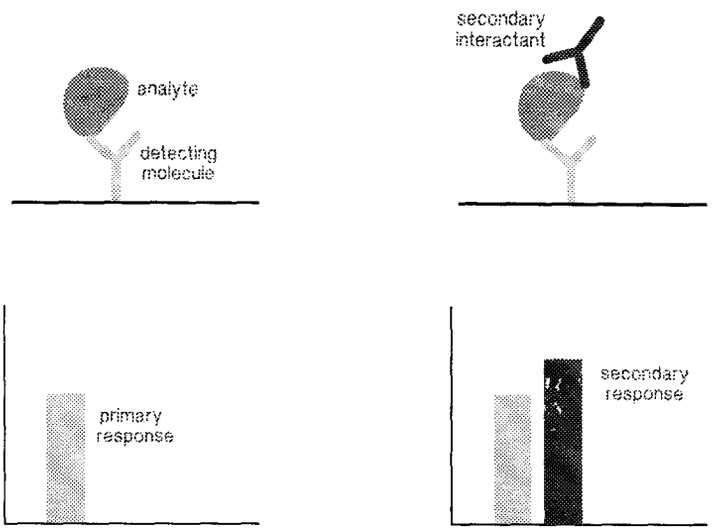


Figure 5-4. *Schematic illustration of the use of a secondary interactant to confirm the identity and/or activity of detected analyte.*

Limits of detection
The detection limit using BIACORE®probe is determined primarily by the signal-to-noise ratio in the measuring system. The instrument characteristics allow confident detection of a response of 0.1 kRU, although this figure may in practice be higher since experimental conditions can introduce additional uncertainties. Typically, responses of ≥0.2 kRU may be regarded as positive for yes/no detection of analyte. In a test assay for myoglobin (molecular weight 17,000 daltons), the a detection limit was about 1 µg/ml using a directly attached antibody and 2-5 µg/ml using a capturing approach. In molar terms, the detection limit is higher for small molecules since the detection is based on mass concentration.

5.3 Regeneration

Regeneration refers to the procedure of removing bound analyte (and also detecting molecule when the capturing approach is used, see Section 5.1) from the sensor probe surface in preparation for analysis of the next sample. Regeneration is necessary when a response has been detected, but can be skipped if the response from a sample is negligible indicating that no analyte remains bound to the sensor probe.

Conditions for regeneration are designed to dissociate analyte from detecting molecule without impairing the binding capacity of the detecting molecule, and have to be established for each individual combination of analyte and detecting molecule. The sensor probe itself

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withstands exposure to harsh conditions: it is generally the properties of the detecting molecule that set the limits for conditions that can be used for regeneration.

When antibodies are used as detecting molecules, regeneration can usually be achieved by lowering the pH to 1.5-3 using dilute HCl or a 10 mM glycine-HCl buffer. The time of exposure of the sensor probe to regeneration solution should be kept short to minimize turnaround time between measurements. Provided that regeneration is efficient and does not damage the detecting molecule, a functionalized sensor probe can typically be used for at least 100 measurements.

Procedures for establishing optimal regeneration conditions are described in the Sensor Probe Handbook.

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6. Maintenance

This chapter describes maintenance procedures that can be performed by the user. *Maintenance and service procedures not described in this chapter may only be performed by authorized service personnel.*

6.1 Cleaning

External surfaces on the instrument and pipette may be cleaned by wiping with water or a mild detergent solution, or with 70% ethanol if contamination with infectious organisms is suspected. Always wear appropriate protective clothing in the event of suspected contamination.

Avoid getting liquids in the diskette drive on the front panel. If liquids are spilt or splashed into the diskette drive, call your local Biacore service representative for advice.

6.2 Replacing the optocable

The optocable may be replaced if it becomes damaged. Replacement optocables are supplied with the probe holder attached. Follow the steps below to replace the optocable:

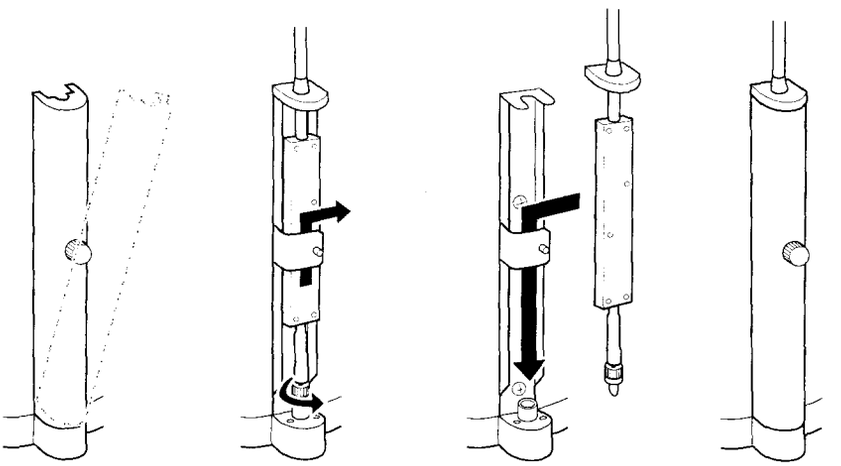


Figure 6-1. *Replacing the optocable.*

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1. Loosen the retaining screw on the back of the column that supports the screen and remove the rear casing from the column (Figure 6-1a).

2. Unscrew the retaining nut at the bottom end of the old optocable and remove the optocable (Figure 6-1b).

3. Hang the pipette on the pipette holder. Fit the free end of the new optocable into the socket at the bottom of the column, making sure that the rubber cover that fits over the top of the column has the flat side facing the screen (Figure 6-1c). This will ensure that the optocable bends in the right direction. Tighten the retaining nut by hand.

4. Replace the back of the column (Figure 6-1d).

6.3 Replacing the lamp

The light source in BIACORE®probe is a halogen lamp with an expected life time of at least 7000 hours (2-3 years of constant use). Replacement lamps are available from Biacore AB. Follow the steps below to replace the lamp:

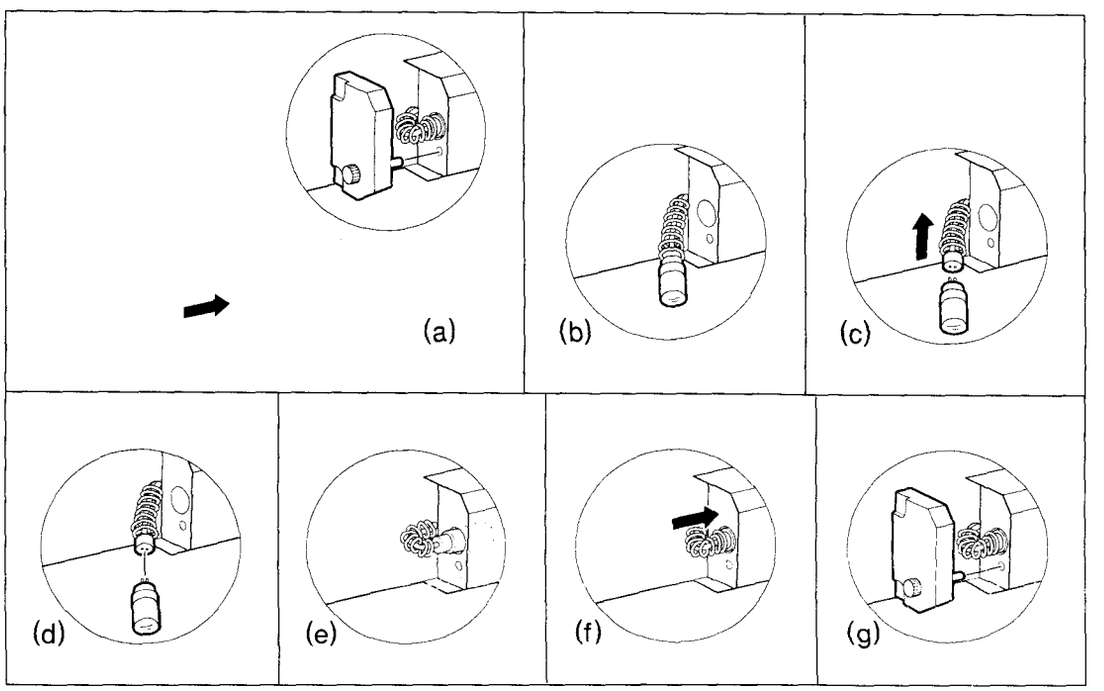


Figure 6-2 *Replacing the lamp.*

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