SOFTWARE NOTE

Biacore™ Insight Software version 5.0.18

This document describes Biacore™ Insight Control Software version 5.0.18.22405 and Biacore™ Insight Evaluation Software version 5.0.18.22102, Cytiva Software Licensing Server, and Biacore™ Insight Database version 2.2.0.110 in terms of new functionality, resolved and known software issues and limitations.

Biacore™ Insight Control Software version 5.0.18.22405 was released soon after version 5.0.18.22102 and resolves issues in sample positioning, report point placement during immobilization, and recovery of data after database connection loss.

! Upgrading from version 4.0.8 to 5.0.18 will require an update of the database. Details on the installation and upgrade process are covered in:

- Biacore™ Insight Installation and Upgrade instructions (29729572)
- Biacore™ Biacore Insight Database Installation and Management Guide (29287249)
- Biacore™ eLicensing Guide (29287250)

Updating Biacore™ Insight Control Software from version 5.0.18.22102 to version 5.0.18.22405 does not require any other updates and will not preclude the use of the older version 5.0.18.22102.

I.   Operating systems

Biacore™ Insight Control Software v 5.0.18 and Biacore™ Insight Evaluation Software v 5.0.18 are compatible with the following operating systems:

- Microsoft Windows 10 (Professional or Enterprise, 64-bit) with English System Locale

II.   Functionality

The functionality is described in detail in the following manuals:

- Biacore™ 1 series User Manual (29706293)
- Biacore™ 8 series User Manual (29287247)
- Biacore™ Insight Evaluation User Manual (29287248)
- Biacore™ Insight GxP User Manual (29312548)

The manuals, guides, instructions and software installers can be downloaded from the Cytiva eDelivery portal using the activation ID(s) you received via email after purchase or by visiting and log-in with your Cytiva account on: www.cytivalifesciences.com/support/software/Biacore-downloads.

III. Biacore™ Insight Control Software version 5.0.18.22405

New functionality compared to version 5.0.18.22102.

- No new functionality has been introduced.

New functionality compared to version 4.0.8

General

- The name is changed from Biacore™ 8K Control Software to Biacore™ Insight Control Software since the software now supports both Biacore™ 1 series and Biacore™ 8 series SPR systems.

- A new workspace called *Action history* replaces *Activity history*. It includes more information, such as
  - Creation and updates of evaluations.
  - Sensor chip usage.
  - Handling of prediction models (requires Biacore Intelligent Analysis™ software).
  - Possibility to view performed service runs (Service mode is still required to open them).
  - Improved filtering possibilities.

- Scientific notation is allowed for variables (e.g., 1E3).

Support for multiple systems

- Supported instrument types can be defined in the new *Preferences* workspace and the software is tailored accordingly. Information and features related to non-selected instruments remains hidden.

- Separate pre-defined methods tabs if instruments from both Biacore™ 1 series and Biacore™ 8 series are selected in *Preferences*.

- Possibility to convert a *Method Builder* method from Biacore™ 1 series and Biacore™ 8 series.

- Method compatibility for Biacore™ 1 series and Biacore™ 8 series is visible in the user interface and database view.

Interactive run

- *Interactive run* provides means to interactively control the instrument, add commands, and make decisions based on the obtained results. It includes:

  - Support for most *Method builder* commands.
  - Possibility to *pause*, *resume* and *stop* commands.
  - Possibility to enter information such as solution name and concentration.
  - Drag and drop to change command order (for not started commands).
  - Copy of individual commands and entire cycles.
  - Eject tray.

- Alignment and response rulers, with the possibility to save response readouts in a *Readout table*.

- New graphical injection markers with additional injection information presented in tooltip.

Immobilization

- Aim for target immobilization level is now available for Biacore™ 1 series and Biacore™ 8 series.

- Possibility to pause, resume and stop ligand injection, to manually control ligand level in detail.

- *Immobilization checkpoint* is added to the activity queue options. It compares the chip immobilization levels with acceptance criteria entered by the user and pauses the activity queue if criteria are not met.

- Full sensor chip information is available when docking an already used sensor chip.

Method builder

- Setting of flow cell and sample compartment temperature are now handled as a separate assay step.

- New functionality for setting of concentration units in *Method builder* and handling of the same in the Biacore™ Insight Evaluation Software. It is now possible to set different concentration units for different types of commands. Two new concentration units have been added to the list: vp/mL and cells/mL.

- *Poly* command is available in *Method builder*, for Biacore™ 1 series methods. With *Poly*, three to five solutions are injected in direct sequence. No extension is needed to set up and evaluate runs with *Poly*.

- Biacore™ 1 series:

  o New user interface control for selection of flow cells to use.

  o New dialogue for selection of flow cells and reference, allowing more referencing options than before.

  o Software support when moving a method between flow cells and when increasing or decreasing the number of flow cells.

- Variable table now has a new empty row at the end. Writing or pasting into this row automatically creates new cycle(s).

- Possibility to select and drag and drop multiple positions in the plate/tray view by pressing the left mouse button and dragging a rectangle around the positions of interest.

- Possibility to select multiple positions and then click *Exclude selected positions* so that they are not used by the positioning algorithm.

- The user interface in *Variables and positioning* has been improved, e.g., for positioning settings and steps without variables.

Resolved issues compared to version 5.0.18.22102

- If the system controller computer has issues with unexpectedly slow USB communication, tools runs might be unable to finish correctly. A "NullReference" error message appears, and the user interface may get out of sync. Streaming video or using a mass storage device over USB during an ongoing run increases the risk of encountering this error.

- For Biacore 8 series methods that contain multiple sample series and have the "Keep series together" functionality enabled, an unhandled positioning error can occur. The user interface in Variables and positioning then becomes corrupt. Two prerequisites for the issue to occur are:
  o The method contains more than eight different sample series, i.e., two different series are run in the same channel.
  o The Biacore Insight software is responsible for assigning positions.

  Long series with many samples, pooling of the Analyte command and manual locking of non-series positions (e.g., regeneration positions) increases the risk of encountering the error.

- When the database connection is lost during the run and not reestablished before the run ends the automatic recover feature that uploads the data to the database will not upload all variables for all cycles. This affects the following variables:
  - Concentration Unit, Dilution Factor, Pause/Resume/Stop
  - Locked/Unlocked status of all variables.
  - Saved Response readouts, performed with the response ruler in the Biacore Insight Control software.
  - Reference flow cell settings

- Opening a run method with **Auto Populate** disabled and a solution assigned to the reagent bottle caused an unhandled exception.

- The "After ligand" report point is placed incorrectly in immobilization methods where the ligand command is the last command in the cycle. For methods that lack a deactivation command - such as e.g., SA-biotin capture - the "After ligand" report point is placed too early. This in turn affects the immobilization result **Response Bound**, which uses the "After ligand" report point.

Resolved issues compared to version 5.0.16.

- Under certain circumstances, trays were not handled correctly by the instrument in version 5.0.16. If two or more trays were used in a run and the trays were moved downwards in the sample hotel, trays were not switched and the instrument – incorrectly - continued to use the previous tray. The defect could arise in Biacore™ 8K, Biacore™ 8K+, Biacore™ 1K+ and Biacore™ 1S+ - under the following circumstances when setting up a run in the software:

  - the user re-arranged trays manually in a 6-shelf hotel.
  - the user manually switched tray positions in a 2-shelf hotel.
  - several runs were queued up and the instrument changed tray positions automatically.

There had to be 2 or more trays in the run for the problem to occur.

Resolved issues compared to version 4.0.8.

- When using **Save as** for a Biacore™ 8 series immobilization method updates were not correctly saved in some cases and the wrong method version was displayed.
- Scrolling in the sample table in **Variables and positioning** could in some rare cases cause locked positions to become unlocked.
- When adding a **Single cycle kinetics** command, molecular weight was selected as variable by default although it should not be.

Known issues / limitations

- **Immobilization**: If injection markers are shown for an **Aim for target level** immobilization, a cascade of error messages is thrown. The issue is related to the fact that the **Aim for target level** command has several sub-injections.

  - Workaround: Under Chart display settings (cog wheel menu), do not check **Show injections** for **Aim for target level** immobilizations.

- **Immobilization:** For immobilization methods such as e.g., SA-biotin coupling, where the ligand is the last command in the cycle, the "After ligand" report point is placed incorrectly if the ligand command is paused and resumed, or manually stopped. The report point will still be placed at the predetermined location, based on the contact time in the method. This affects the Response bound result, which uses the "After ligand" report point.

  - o Workaround: If the ligand command is paused and resumed, keep the pause short (< 30 sec). This way, the report point will be placed close to where it is supposed to be.

- **Immobilization**: If an Aim for target level immobilization fails because the test injection is too slow, the surface wash with NaOH is not executed before the cycle is aborted. This means that electrostatically bound ligand might remain on the surface and potentially interfere with the next immobilization attempt.
  - o Workaround: Run a short method with a Regeneration command before starting the next immobilization. Note that it is not enough to add a Wash command since it only washes the flow system and does not pass over the sensor chip surface.

- **Immobilization:** In the pre-defined immobilization method "Immobilization low levels", EDC and NHS are mixed in with ratio 20:80 to achieve the low levels of immobilization. If another amine step is added via the **Add step** button, the mix ratio is set to standard 50:50 mix.
  - o Workaround: Copy the existing step instead of adding a new step via the **Add step** button. Then the 20:80 ratio is preserved. Alternatively, the mix ratio can be modified in custom mode.

- **Immobilization:** In the pre-defined immobilization method "Immobilization low levels", EDC and NHS are mixed in with ratio 20:80. But if the Immobilization procedure is changed from **Specify contact time** to **Aim for target level,** the mix ratio is re-set to standard 50:50 mix.
  - o Workaround: The mix ratio can be modified in custom mode.

- **Immobilization:** When restoring an immobilization run after the connection with the database has been lost, the new chip information is not directly available in the Biacore Insight Control Software Control panel until the next run is started. Restarting the software also solves the issue.

- Biacore™ 1 series immobilization: In some cases, especially when there are many different solutions in each step, repositioning via drag and drop can cause the other existing positions to become scrambled in an unintuitive way.
  - o Workaround: Open the Positioning settings panel and uncheck the checkbox **Auto populate position upon manual reposition** to get existing positions to stay in place.

- *Method builder*: The **Positioning settings** panel has been re-designed and assignment of positioning direction and whether a positioning group shall be taken from the left or right microplate in a Biacore™ 8 series method is done differently than before. As a result of this, it is no longer possible to start positioning from the top down (from the row with the highest row number). When opening an old Biacore™ 8K or Biacore™ 8K+ method with positioning groups that uses positioning direction **Top right** or **Top left** in Biacore™ Insight Control Software version 5.0.18, the positioning order will be changed automatically. The user is informed via a message stating "Positioning direction for some solutions have changed".

- *Method builder*: Positive numbers in scientific format (e.g., 1E4) that are entered in Variables and positioning are not kept when proceeding to Cycle overview or Plate layout. They are automatically translated to general number format (10000).

- **Method builder**: For Biacore 8 series methods: If sample series are run in duplicate, with pooling activated, the **Keep series together** functionality does not work as intended in the positioning.
    - Workaround: Use multi-select drag and drop to position the sample series as intended.
- **Method builder**: If solution names are replaced by a numerical ID, e.g., from a barcode reader, sample import removes initial zeros from the IDs. This corrupts the IDs which can cause problems when exporting the results for further processing.
    - Workaround: Paste the IDs into the sample table instead of importing them.
- **Method builder**: In the sample import workspace, 96 well plates are the default plate type. If there are too many samples, a dialogue box with the option to change plate type is presented to the user upon clicking the "Import into method" button. However, if the alternative "Change to 384 well plates" is chosen, an unhandled error occurs.
    - Workaround: If you know that a 384 well plate is needed, change the plate type in the import settings before clicking the **Import into method** button. It is also possible to select to keep the 96 well plate in the dialog message and change to a 384 well plate later in Variables and positioning in Method builder.
- When starting an **Interactive run**, it takes some time before the sensorgrams appear and the user is not notified that something is ongoing. The same applies when you click **End run** and **Abort run**, the software and instrument are working but not presenting status clearly.
- When running an **Interactive run**, with the cycle event log displayed, new events are not visible unless the view is manually scrolled to the bottom.

IV. Biacore™ Insight Evaluation Software version 5.0.18

New functionality compared to version 4.0.8

General

- A new workspace called **Action history** has been introduced. It includes information such as
    - Creation and updates of evaluations.
    - Sensor chip usage.
    - Handling of prediction models (requires Biacore Intelligent Analysis™ extension).
    - Possibility to view performed service runs (Service mode is still required to open them).
    - Improved filtering possibilities.
- Scientific notation is allowed for variables.
- The same set of pre-defined evaluation methods support both Biacore™ 8 series and Biacore™ 1 series runs. Additional methods for Parallel Kinetics™, 2D Kinetics™ and parallel concentration assay formats are available for Biacore™ 8 series.
- In the **Column calculator** in **Plot**, it is possible to repeat a value from a specified row on a set number of rows. When hoovering over functions in the **Column calculator**, more informative tooltips with examples have been added.

Support for Biacore™ 1 series

- Possibility to customize reference settings and save in evaluation methods.

- Biacore™ 1 series adaptation of evaluation items with adapted availability of functions.
- Support for capture in multiple flow cells e.g., for epitope binning applications.
- Support for evaluation of **Poly** command in **Sensorgram**, **Plot** and **Epitope binning** items.

Support for Biacore™ T200 and Biacore™ S200

- Possibility to customize reference settings and save in evaluation methods.
- **Control type** definitions can be changed for Biacore™ T200 and Biacore™ S200 runs containing controls.
- Improved possibility to evaluate when using capture in multiple flow cells e.g., in **Epitope binning**.

New functionality compared to version 5.0.16

- None

Resolved issues compared to version 4.0.8

- In some situations, the software did not show **Sensorgram** items after a report point definition had been modified or added.
- **Kinetics/Affinity**: It was not possible to save evaluations containing a **Kinetic/affinity** item when either a KD chart or an On-off rate chart had a data label originating from a calculated column.
- **Kinetics/Affinity**: Blank settings and fit models were reset after removing all curves and restoring them in the filter.
- **Kinetics/Affinity**: Changes in **Variables** table locked the fitting function and initial value setting in **Kinetics** for GxP regulated evaluation.
- Curve markers were not visible in sensorgrams in exported data.
- JSON/XML export: For the "EC50 Settings and Parameters" table, the exported ID was not a unique identifier which may confuse users of the export.
- **Epitope binning**: When changing the binning definition, the **Bin no** column in the table were not updated.
- **Remove ranges at start and end of injections** setting was not applied correctly when using an evaluation method. The state of the checkbox was not saved as intended within the method.

Resolved issues compared to version 5.0.16

- None

Known issues / limitations

- There is no restriction on the number of runs that can be selected and opened for co-evaluation. However, evaluation software performance will be slow when handling too large data sets.
- An evaluation item created while the **Curve markers** workspace has pending changes gets curve markers definitions that have not been applied.

o   Workaround: Apply changes in the **Curve markers** workspace before creating any new items.

- When run names and/or ligand names are long, the layout of detailed thumbnails in **Kinetics/Affinity** is negatively affected. E.g., fit parameters are no longer visible in the thumbnail and the thumbnail chart becomes very narrow upon export to pdf.

- The affinity lines in On-off rate charts are not supported in exports to presentation due to limitations in Microsoft PowerPoint.

- The software does not support special characters that do not exist in standard western fonts, such as smileys or non-Latin characters. When such characters are used in the software they may be ignored or shown as empty squares. Problems are known to occur in PDF exports and database exports but may also occur in other situations.

    o   Workaround: Do not use special characters in any text entered in the software.

- **Plot**: Calculated columns using referencing functions may generate different results on computers with different regional settings, if the table is sorted on a text column containing characters that are sorted differently in different regions (for example ß, Å, Ä and Ö).

    o   Workaround: Do not sort the table on text columns when using calculated columns with referencing functions.

- Search in database view: When the search result is sorted by **Type**, the hits are not sorted alphabetically.

- Search in database view: When scrolling the list of search results the header row disappears.

V.   Biacore™ Insight extensions

New functionality compared to version 4.0.8

Biacore™ Insight Extended Screening Extension

- Name changed from "Extended Screening and Characterization" to "Biacore Insight Extended Screening Extension". The characterization content has been moved to the basic package. All kinetics models are now available without any extension.

Biacore™ GxP Extension

- Support for electronic signatures

    o   When a regulated evaluation is completed and saved

    o   When a regulated procedure is created or revised

    o   The **GxPadministrator** role configures usage of electronic signatures.

- Possibility to save **Regulated evaluations** in draft versions.

- Improved **Audit trail** functionality

    o   More events are included, such as usage of curve markers and solvent correction,

    o   More details are included for logged events (value before and after)

- GxP users get more actions logged in Action history.

    o   User login and logout

    o   Applied signatures

- o Changes in signature settings
- o Exports of database objects
- o Creation, revisioning and withdrawal of Regulated procedures
- o Possibility to manually add free text entries to the log.
- Profound user interface updates in some GxP-related workspaces

New functionality compared to version 5.0.16

- None

Resolved issues compared to version 4.0.8 or 5.0.16

- None

VI. Cytiva Software Licensing Server

New functionality compared to the licensing server used for version 4.0.8 or 5.0.16

- None

Resolved issues compared to the licensing server used for version 4.0.8 or 5.0.16

- None

VII. Biacore™ Insight Database

New functionality compared to version 4.0.8 or 5.0.16

- The database has been upgraded. As with previous releases, it is required that all computers connecting to a Biacore™ Insight Database must use the same version of Biacore™ Insight Control Software and Biacore™ Insight Evaluation Software. This means that once one computer with Biacore™ Insight Software version 5.0.18 has connected to the database, it is no longer possible to connect with older software versions from any computer.

Resolved issues compared to version 4.0.8

- Local Database Log4j vulnerabilities CVE-2021-44228 and CVE-2021-45046 fixed through cumulative update KB5017593 (previous KB5011644 fixes the vulnerability).

Resolved issues compared to version 5.0.16

- Microsoft SQL Server Management Studio is no longer included in the installation of Biacore™ Insight Local Database. It can be downloaded free of charge from Microsoft.

Known issues / limitations

- There is no GxP support for imported runs or evaluations, neither from Biacore™ T200 system nor from Biacore™ 1 series or Biacore™ 8 series systems.

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